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Further Observations on the Relationships between the SCO and the Adrenal Gland of *Lacerta s. sicula* Raf.: Effects of Oestrogen Administration during Summer[1]

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Abstract. In order to verify the relationships between the SCO and the adrenal, specimens of *Lacerta s. sicula* were treated with oestrogens during summer, when the adrenal is very active and the SCO cells are filled with secretory material. After oestrogen treatment the interrenal cells appear reduced in size and large blood vessels appear between the interrenal cords. The SCO cells during the first period of treatment show a clear decrease in the amount of secretory material; subsequent treatment restores the levels of secretory material in the SCO cells cytoplasm, which shows an appearance identical to that exhibited by the control specimens of the same period. The results of these experiments point out that adrenal steroidogenic tissue is affected by the action of oestrogen, whose administration causes inactivation of the interrenal tissue with consequences on the SCO activity confirming the existence of a relationship between adrenal gland, interstitial tissue of the testis and SCO, already pointed out by preceding studies carried out in our laboratory.

Introduction

The subcommissural organ (SCO) has been described in all Vertebrates as an ependymal specialization of the diencephalon at the level of the posterior commissure, at the caudal limit of the third ventricle (Sterba, 1969; Ziegel, 1976, for earlier works). More recently, papers have been published, dealing with the structure and function of the SCO in several vertebrates: in amphibia (Diederen, 1970; Rodriguez, 1970a, b; Diederen, 1972, 1973, 1975; Diederen and Vullings, 1980; Wake et al., 1974; Wakahara, 1974) in reptiles (Ziegels, 1974; D'Uva and Ciarcia, 1976; D'Uva et al., 1976, 1977, 1978, 1979; Varano et al., 1978; D'Uva, 1980), in Birds (Ziegels, 1979a, b), in mammals (Wenzel et al., 1970; Schuette, 1971; Murakami et al., 1972; Milin et al., 1971; Attila and Talanti, 1973; Chen et al., 1973; Czewzyk, 1974, Czewzyk and Juraniec, 1980; Siziakina, 1975; Ziegels and Devecerski, 1976).

[1] Dedicated to Prof. Mario Galgano in occation of his 75th birthday.

As far as Reptiles are concerned, D'Uva et al. (1976, 1977) have pointed out in the SCO an annual secretory cycle which is characterized in June by the presence of huge quantities of secretory material in the cells. The quantity of secretory material decreases progressively and reaches a minimum in December, then it increases constantly during spring to reach its maximum at the beginning of summer. D'Uva et al. (1977) have distinguished four stages in the secretory cycle of the SCO cells. The first stage, found in December, is characterized by a reduced quantity of secretory material. The cells contain 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 middle 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 middle September, is characterized by an increase in the A and B granules in the apical region, and by the appearance of the RER cisternae filled with secretory material (type C material) also in the supranuclear region. In the fourth stage, which is reached in June, the quantity of secretory material is at its maximum and large amounts of all types of secretory material, without a preferential location, are evident.

Subsequent works carried out on the same species, have pointed out a correlation among the activities of the SCO, the adrenal gland and the testicular interstitial tissue. All the experimental treatments that reduce the activity of these steroid-producing glands affect also the SCO, causing a depletion of the SCO cells, while the treatments that enhance their steroid hormone production cause an accumulation of secretory material in the SCO cell cytoplasm.

Particularly, in a preceding paper on the relationships between SCO and adrenal gland, the authors have pointed out that, after ACTH administration during winter, when the adrenal is not active and the SCO is depleted of secretory material, the activity of the adrenal is restored, and the SCO shows an increase in the secretory material in the cells. In order to verify these relationships between the SCO and the adrenal, we have treated specimens of *Lacerta* with oestrogens, whose inhibiting action on the corticosteroid synthesis is well known (Varano and Laforgia, 1976), during summer, when the adrenal is very active and the SCO cells are filled with secretory material.

Materials and Methods

A total of 40 adult male *Lacerta s. sicula* Raf. captured in the Naples area (Arzano) during the second half of June 1980 were kept in terraria under temperature and humidity conditions similar to those of external environment. They were fed on larvae of *Tenebrio molitor* L. and fresh fruit ad libitum. Ten specimens were sacrificed at the beginning of the experiment in order to control the state of the SCO and the adrenal gland. 20 specimens received injections of 100 γ of oestradiol benzoate in almond oil on

alternate days and were sacrificed in groups of ten after 6 and 10 injections. Together with the experimental groups, 5 specimens were sacrificed after receiving the same number of injections of almond oil.

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 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 chromalumhematoxylin according to Gomori and Bargmann. The adrenals were stained with a trichromic Mallory stain or with a Giemsa solution according to Pearse (1960).

In 40% of the specimens of each group the brain and one of the adrenals were processed for electron microscopy and serial sectioned. The adrenals and the SCO region, removed from the brain and trimmed into small pieces, were fixed with 2.5% glutaraldhyde in phosphate buffer, postfixed in 1% O_sO_4 and embedded in Epon 812. The thin sections were stained with uranyl acetate and lead citrate and observed with a Siemens Elmiskop 1A electron microscope ("Centro di Studio di Microscopia Elettronica" of the Science Faculty, University of Naples). The following descriptions apply to all the specimens of each group, since no important morphological differences were found between single specimens.

With the aid of an interactive image analysis system IBAS I was measured the total area of the SCO and of the interrenal cells in the normal and the oestrogen treated specimens and also the area occupied by the SCO vacuoles containing secretory material and that occupied by the lipid droplets in the interrenal cells estimating the percentage. The measurements were carried out on micrographs all with the same enlargement for a minimum of 100 sections and a maximum of 230 chosen at random for the single groups of animals. The estimated percentages were statistically processed, calculating the average, the standard error and the t of Student.

Results

Light microscopy

1. *Normal and oil injected specimens of June*

a) *The 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, the nucleus is prominent with an evident nucleolus and is displaced in the basal part of the cell; the cytoplasm is rather well stainable and does not show large vacuoles (Fig. 1).

b) *The SCO cells.* The SCO is typically constituted by cells at stage 3-4 exhibiting large amounts of secretory material in the basal region with some granules in the apical region (Fig. 2).

2. *June specimens with oestrogen-inhibited adrenal (6 injections)*

a) *The interrenal (steroidogenic) tissue of the adrenal gland.* The gland volume on the whole diminishes; the interrenal cords appear narrower than in normal specimens and are separated by large blood vessels (Fig. 3); the cell nucleus is smaller than in normal specimens and is more stainable; the cytoplasm is reduced and at high resolution appears spongy, sometimes featuring large vacuoles.

b) *The SCO cells.* The SCO cells of these specimens contain numerous small granules of secretory material in the basal region. In most cases no large secretory granules can be detected in the supranuclear region (stage 2-1) (Fig. 4).

3. *June specimens with estrogen-inhibited adrenal (10 injections)*

a) *The interrenal (steroidogenic) tissue of the adrenal gland.* The interrenal tissue of these specimens still shows clear signs of inactivation. The blood vessels between the cords appear very large and sometimes reach a very considerable size. The interrenal cords show sign of degeneration with small cell cytoplasms and picnotic nuclei.

b) *The SCO cells.* After 10 injections of oestrogens, the SCO cells do not show any difference when compared with the SCO cells of the control specimens. Large amounts of secretory material of all types fill the cells, without any preferential location.

Electron microscopy

1. *Normal and oil injected specimens of June*

a) *The interrenal (steroidogenic) tissue of the adrenal gland.* The interrenal cells of this season show large, clear nuclei containing little heterochromatin and an evident nucleolus. The smooth endoplasmic reticulum (SER) appears very developed, arranged as narrow anastomosing tubules. The mitochondria, showing the typical tubular cristae, are very numerous and of variable sizes. The lipid droplets are small-sized and usually very few, and may even be absent in some cells (Fig. 5), occupying $1.96\% \pm 0{,}39$ of the cells areas.

b) *The SCO cells.* The SCO cells of these specimens show an apical region rich in secretory granules of A and B types and few microvilli at the free cell surface (Fig. 7). In the basal region RER cisternae appear enlarged and filled with a finely granular material. Sometimes the cisternae surround the nucleus and reach the supranuclear region (Fig. 9). The area occupied by the cysternae is $42.7\% \pm 2.85$.

2. *Specimens with inhibited adrenal*

a) *The interrenal (steroidogenic) tissue.* The interrenal cells of the specimens inhibited with estrogens appear at the E.M. rather different from the normal ones. The SER is reduced and is prevailingly made up of enlarged vesicles. Mitochondria are less numerous and often small, even if still retaining the typical tubular cristae. The lipid

droplets are numerous and may reach a considerable size (Fig. 6). The area occupied by the lipid droplets is 26.7% ± 2.53 of the total cell area in the specimens receiving 6 injections and 28.3% ± 2.21 in those receiving 10 injections with no statistically significative differerences.

b) *The SCO cells*. The SCO cells of inhibited specimens show in the apical region the same kind of granules as observed in normal specimens, but they are greatly reduced in number. The microvilli at the free cell surface are, instead, more numerous (Fig. 8). The basal region of these cells, when compared with that of normal June specimens, shows also irregularly dilated cisternae of RER containing finely granular material of low electron density, but they are rather flattened and never reach the supranuclear region (Fig. 10). This description applies to specimens receiving 6 oestrogen injections which show 5.9% ± 0.6 of the total cell areas occupied by vacuoles containing secretory material, while after 10 injections the cell area occupied by vacuoles is of 39.6% ± 2.28 with no significative differences when comparing with normal specimens of June.

Discussion

The results of this experiment point out that:

1) As already reported in mammals (McKerns and Bell, 1960; Vogt, 1955) and also in *Lacerta* (Varano and Laforgia, 1976) the interrenal steroidogenic tissue is affected by oestrogens which cause a block of the corticosteroid synthesis. This action is morphologically evidentiated by the appearance of picnotic nuclei, the reduction in the size of the cell cords and the appearance of large blood vessels, which occupy the volume of the gland left free by the reduced parenchima. At the E.M. the cells show a reduction in the SER, few mitochondria and often large lipid vacuoles.

The block of the corticosteroid synthesis both after 6 and 10 injections of oestrogens is clearly confirmed by the percentages of cell areas occupied by lipid droplets, 1.96% in normal specimens and 26.7% and 28.3% after 6 and 10 injections respectively, with statistically significative differences between normal and oestrogen treated specimens with $P < 1\%$.

2) The SCO cells of the specimens which received 6 injections show a clear decrease in the amounts of secretory material stored in the cytoplasm. From the typical stage 3-4 shown by the control specimens in this period of the year, the treated specimens pass to stage 2-1 which is, instead, typical for late winter and early autumn. After 10 injections the SCO cells appear again filled with secretory material with an appearance identical to that exhibited by the control specimens of the same period. Quantitative data confirm morphological finding because while the cell area occupied by vacuoles in normal specimens is 42.7%, it becomes 5.9% in the SCO of specimens receiving 6 injections with a statistically significative difference between the two findings with $P < 1\%$. Instead, after 10 injections, the area occupied by vacuoles is 39.6%, very close to 42.7% of normal specimens and not statistically significative.

In a long series of experiments undertaken in order to clarify possible relationships between the SCO activity and the activity of the steroid-producing glands (D'Uva, 1980; D'Uva et al., 1977, 1978; Varano et al., 1978), we always found a correspondence between the behaviour of the SCO and that of the gland investigated. The correspondence was in that all the treatments stimulating steroid hormone production (ACTH, FSH, LH, temperature increase), caused an increase in the secretory material stored in the SCO cells. On the contrary, the treatments inhibiting steroid synthesis or their action on the target organs (castration, adrenalectomy, cyproteron), caused a decrease in the stores of secretory material in the SCO. The decrease in the stores was promptly corrected by the administration of substitutive steroid hormones (corticosteroids, testosterone). In this study, oestrogen administration, performed in order to block corticosteroid synthesis in the adrenal gland, has turned out in an apparently contradictory result. In fact, 6 injections of oestrogens induced changes both in the adrenal gland and in the SCO consistently with the preceding experiments; instead, after 10 injections this trend is no longer maintained. The interrenal tissue is still clearly influenced by oestrogens, showing marked signs of inhibition, while the SCO does not seem to be affected by this further treatment and shows a morphology similar to that of the controls. These findings might be interpreted in the terms that during the first part of the experiment the decrease in corticosteroids due to the inhibiting action of oestrogens on the adrenal leads to a decrease in the SCO stores of secretory material, while the subsequent administration of oestradiol affects directly the SCO, restoring the levels of secretory material contained in the SCO cells.

Recently it has been reported that, in the rat, stereotaxic destruction of the SCO causes atrophy of the adrenal glomerulosa (Siziakina, 1975) and a lowering of the blood levels of FSH and LH (Limonta and Piva, 1980). These findings lead us to consider that the SCO is involved in the regulation of the activity of steroid hormones producing glands.

By correlating these data with all those obtained in our laboratory, which show that steroid producing glands affect the SCO secretory material, increasing it when the blood levels of steroids are high, lowering it when the quantity of circulating steroids is low, one could hypothesize that the SCO plays a role in the mechanism controlling the steroid hormones secretion of the testis, adrenal gland and possibly the ovary, similar to that played by the hypothalamic regions.

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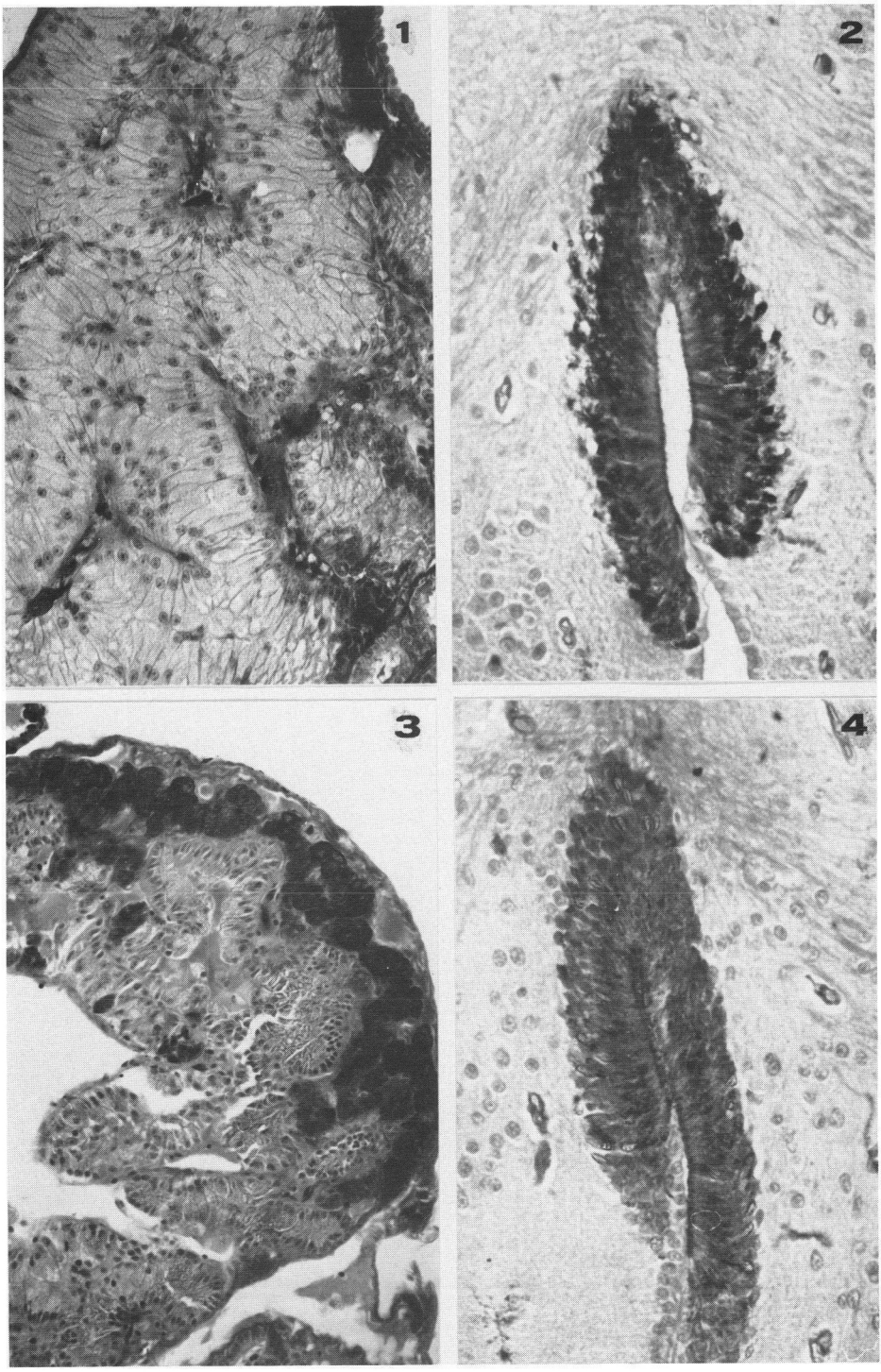
Fig. 1. Longitudinal section of the adrenal gland of a control specimen. Mallory stain, x 200. Notice the interrenal cords made up of two strata of elongated cells, featuring a clear nucleus with one or two evident nucleoli. The blood vessels are very narrow.

Fig. 2. Transverse section of the SCO of a control specimen. Gomori-Bargman stain, x 320. Notice the SCO cells filled with secretory material in the basal region and the small secretory granules in the cell apex.

Fig. 3. Longitudinal section of the adrenal gland of an oestrogen treated specimen (6 Injections) x 200. Notice the reduced size of the gland, the narrow interrenal cords, whose cells often show picnotic nuclei and the large blood sinuses. On the right the dorsal ribbon of chromaffin cells.

Fig. 4. Transverse section of the SCO of a specimen treated with oestrogens (6 injections). Gomori and Bargman stain, x 320. Comparing with Fig. 2, notice the reduced quantity of secretory material. The cells are devoid of large Gomori-positive masses both in the basal and supranuclear region.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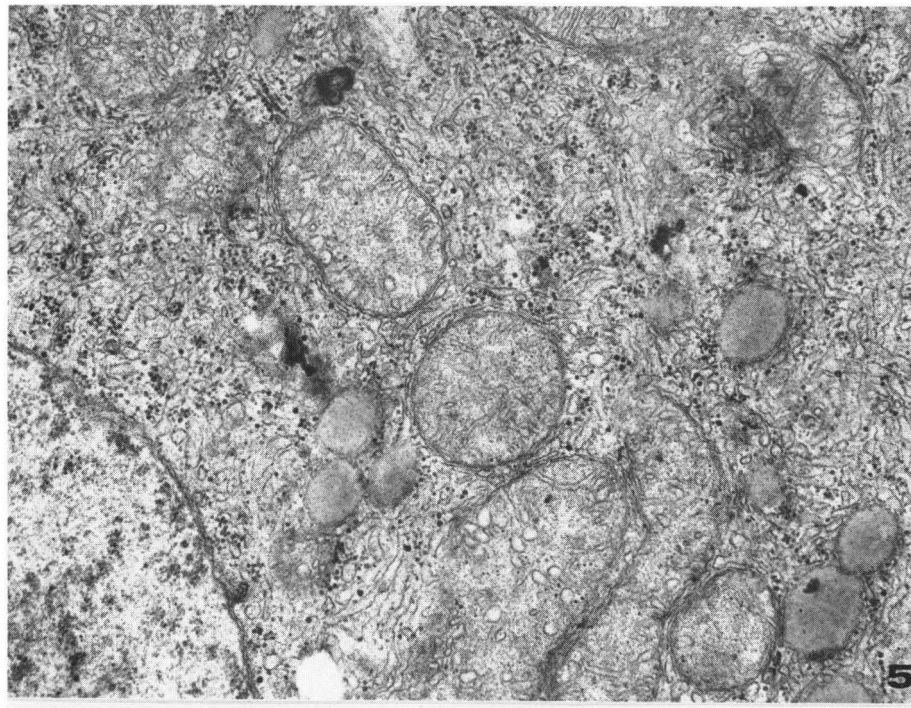

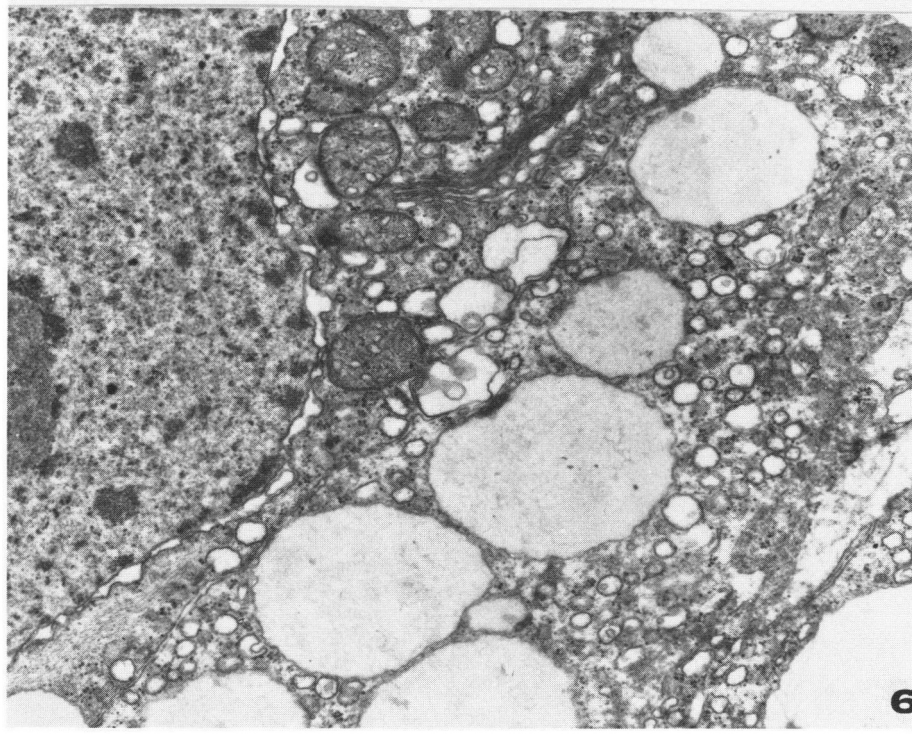

Fig. 5. Interrenal cells of a normal specimen of June. x 26000. Notice the development of the SER in form of narrow anastomosed tubules, the polymorphic mitochondria and the small lipid droplets.

Fig. 6. Interrenal cell of an oestrogen-inhibited specimen of June. x 17500. Notice the reduced SER, here present in form of enlarged vesicles, the small mitochondria and the large vacuoles occupying most of the cytoplasm.

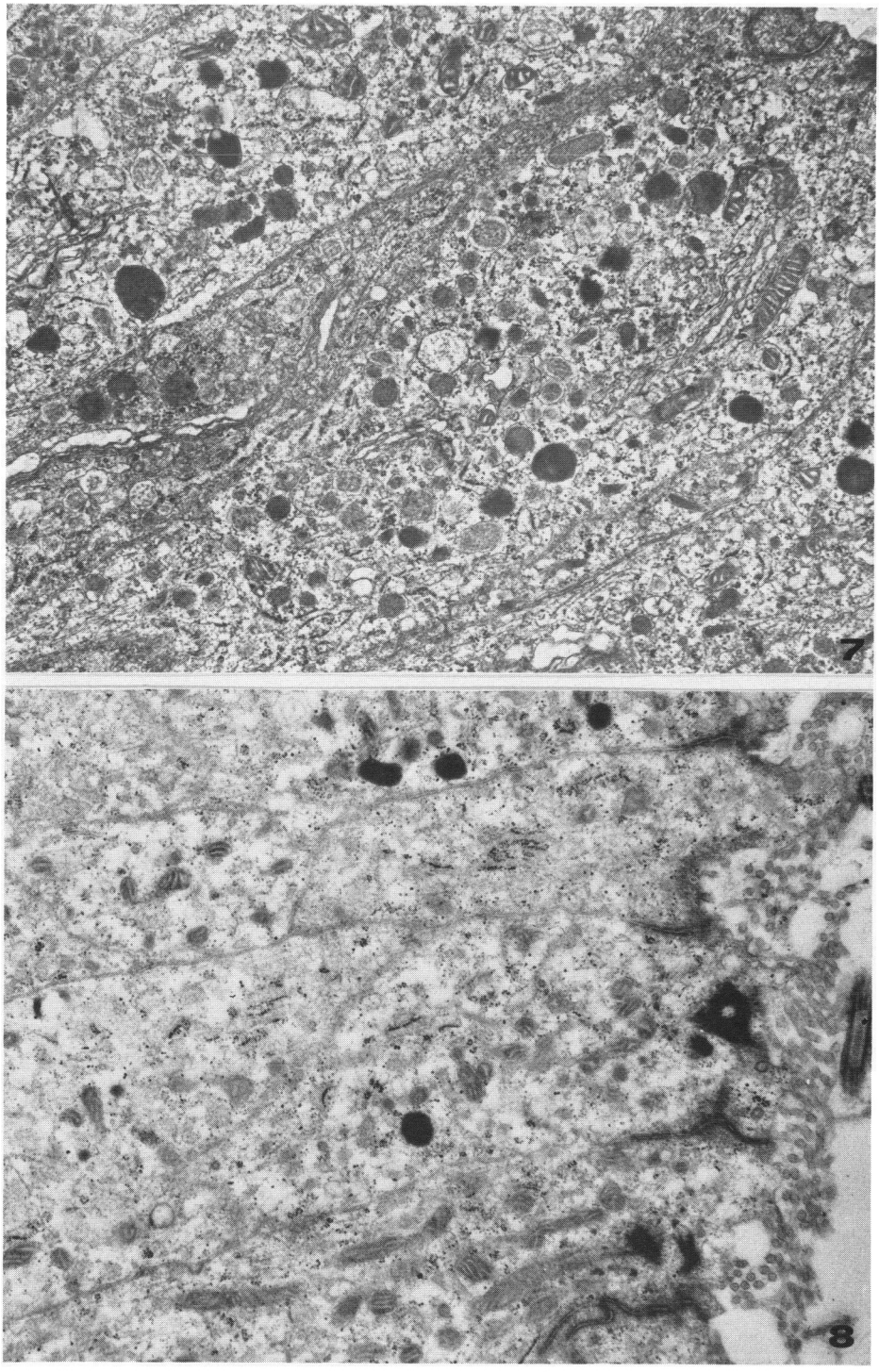

Fig. 7. Apical region of the SCO cell of a normal specimen of June. x 1200. Note the numerous granules of A and B type filling the supranuclear region.

Fig. 8. Apical region of the SCO cell of an adrenal-inhibited specimen. x 12000. A comparison with Fig. 7 shows a complete absence of granules in this region. Notice also the numerous microvilli at free cell surface.

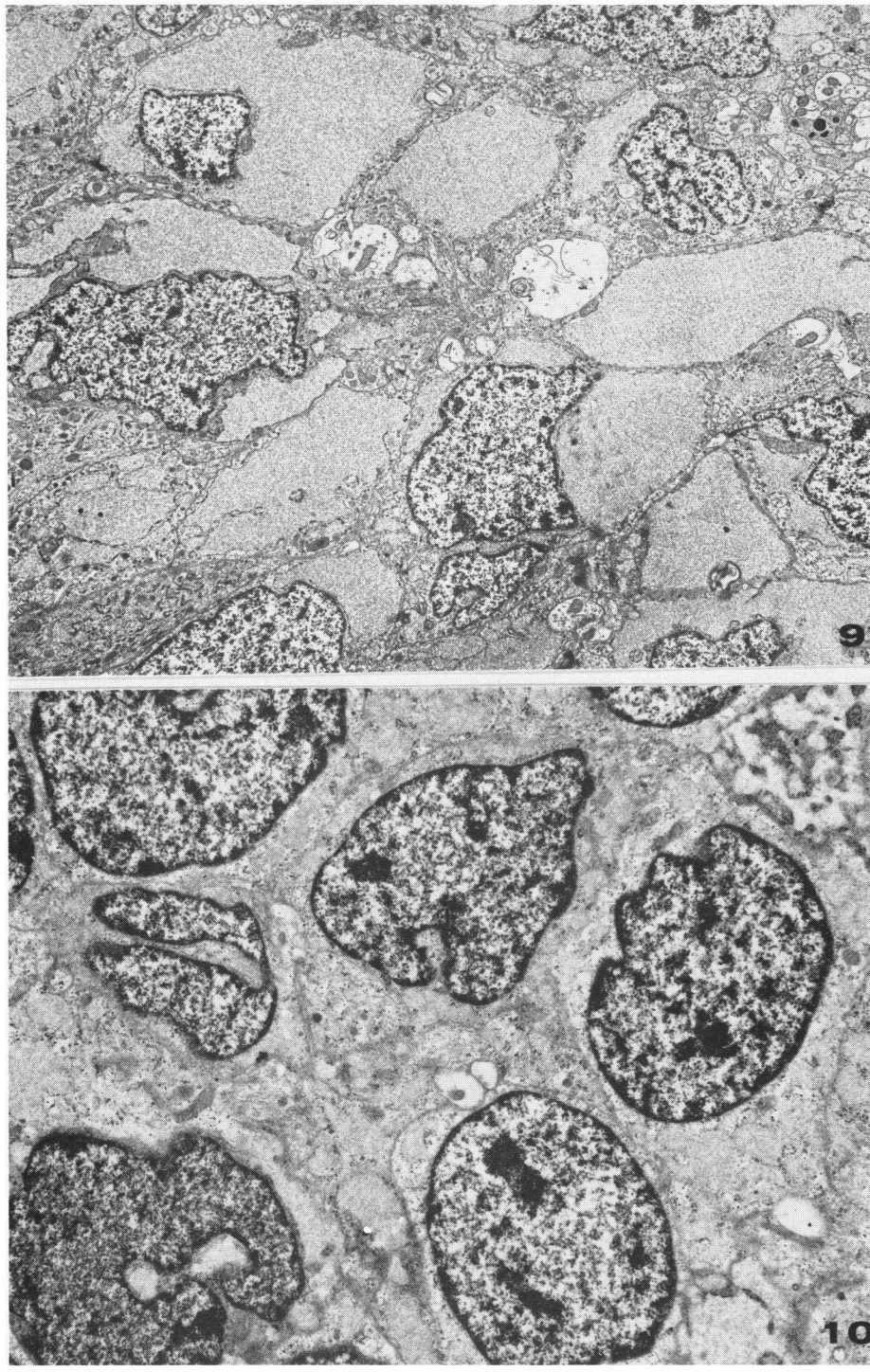

Fig. 9. Basal region of the SCO cell of a normal specimen. x 4000. Notice the large RER cisternae filled with secretory material that sometimes reach the supranuclear region.

Fig. 10. Basal region of the SCO cell of an inhibited specimen. x 5500. A comparison with Fig. 9 shows the reduced size of the RER cisternae which appear flattened and filled with lower amounts of elecrondense material.