EFFECTS OF ACTINOMYCIN D AND PUROMYCIN ON THE ACTH-INDUCED ULTRASTRUCTURAL TRANSFORMATION OF MITOCHONDRIA OF CORTICAL CELLS OF RAT ADRENALS IN TISSUE CULTURE

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ABSTRACT

The ultrastructure of the mitochondria of the cultured cortical cells of rat adrenals was studied. In vivo it was found that the zona fasciculata mitochondria have vesicular internal structure. 600-A vesicles appear free in the matrix or as protrusions of the inner mitochondrial membrane. In tissue cultures of the fetal and newborn rat adrenal cortex it was seen that ACTH induces transformation of the tubulo-vesicular internal structure of the mitochondria to 600-A vesicles. Actinomycin D and puromycin inhibited this transformation if they were added with ACTH. When added alone, these inhibitors of protein synthesis induced no change in the ultrastructure of the mitochondria in cultured cortical cells of rat adrenals.

INTRODUCTION

Numerous observations have been published concerning the fine structure of the adrenal cortex in the rat (1–17). The existence of differences in the internal structure of the mitochondria in the cortical zones of the mammalian adrenal cortex has been emphasized. It has been demonstrated that the internal structure of the mitochondria is tubular in the zona glomerulosa and tubulo-vesicular or vesicular in the zona fasciculata and zona reticularis. As has been shown in tissue-culture studies (18), ACTH plays a major role in the transformation of the internal structure of the mitochondria from the tubular to the vesicular form. The development of mitochondrial vesicles and the organization of these microvesicles in the mitochondria in which they occur has been very poorly analyzed. The purpose of the present study is to throw further light on the genesis of the internal structure of the mitochondria in the rat adrenal cortex in vitro and to ascertain whether suppression of protein synthesis by actinomycin D and puromycin would influence the action of ACTH on the internal structure of the mitochondria in the cortical cells of rat adrenals in tissue culture.

MATERIALS AND METHODS

Rats of the Sprague-Dawley strain served as the source of the experimental material. Adrenals of 19–21-day-old fetal and newborn rats were used for tissue culture and in vivo studies. The fetal and newborn animals were sacrificed by decapitation without ether anesthesia.

A tissue culture method suitable for long-term cultivation (18) was used. The medium consisted of 50% Melnick's solution A (Hanks' balanced salt solution + 0.5% lactalbumin hydrolysate), 25% calf serum and 25% amino acid Parker (Pharmaceutical Manufactures, Orion Oy. Finland).

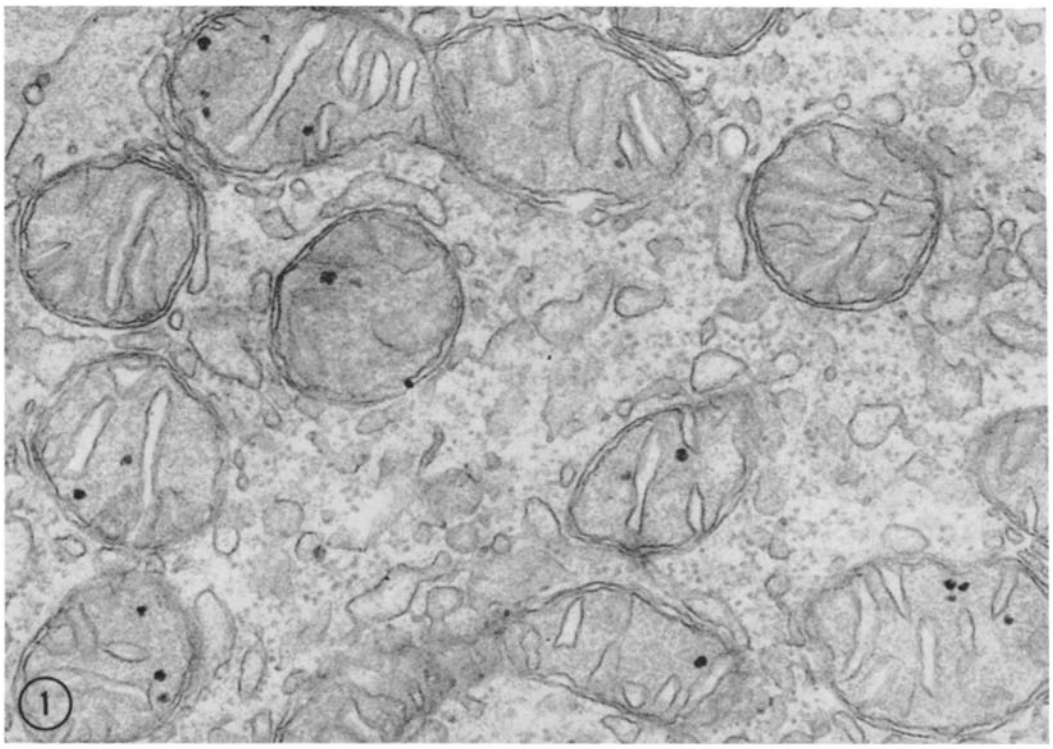


FIGURE 1 Mitochondria with tubular or tubulo-vesicular internal structure in the zona glomerulosa cells in the adrenal cortex of the newborn rat. There are numerous highly electron-opaque mitochondrial granules in the matrix. × 42,000.

0.1–0.2 IU/cc of ACTH (Cortrophin ACTH, pig ACTH, Organon, Inc., West Orange, N. J.) was added to the medium of the tissue cultures of the adrenals every day for 7 days, from the 15th day of cultivation up to and including the 21st day. 0.002 μg/cc of actinomycin D (Cosmegen, Merck, Sharp & Dohme, West Point, Pa.) and 0.1 μg/cc of puromycin (puromycin dihydrochloride Nutritional Biochemical Co., Cleveland, O.) were added, both separately and with ACTH. Final concentrations of 0.002 and 0.1 μg/cc and total doses of 0.07 μg/5 cc/7 days and 3.5 μg/5 cc/7 days were chosen on the basis of previous work concerning the effect of actinomycin D and puromycin on the activity of Δ^5-3β hydroxysteroid dehydrogenase in cultured cortical cells (Kahri, Data to be published.)

The fragments of adrenals and isolated colonies of cultured cortical cells were fixed in 2.5% glutaraldehyde in water with phosphate buffer at pH 7.2 and postfixed in 1% osmium tetroxide (19), or in 1% osmium tetroxide in 4.5% aqueous sucrose solution buffered to pH 7.4 (20) for 2 hr at +4°C, or in the solution prescribed by Rhodin (21) and Zetterquist (22). The cell colonies, which were studied by phase microscopy before fixation, were carefully detached, after fixation, with a splinter of wood. The specimens were then dehydrated in a graded series of ethyl alcohol and embedded in Epon 812 (Shell Chemical Corp., New York) (23). Thin sections for electron microscopy were cut with a Porter-Blum MT-2 microtome fitted with glass knives. The sections were poststained with 1% lead citrate for 5 min (24). Electron micrographs were made at original magnifications of 2,000–14,000 with a Siemens Elmiskop I.

RESULTS

Zona Fasciculata Mitochondria in Vivo

The mitochondria in the cells of the zona fasciculata and internal part of the adrenal cortex of the newborn rat are spherical (Fig. 2) and enveloped by a double membrane. From the inner limiting membrane small buds protrude into the mitochondrial matrix. Small microvesicles with an average diameter of about 600 A lie free in the matrix of the mitochondria. In all mitochondria the size of the microvesicles is the same. In the

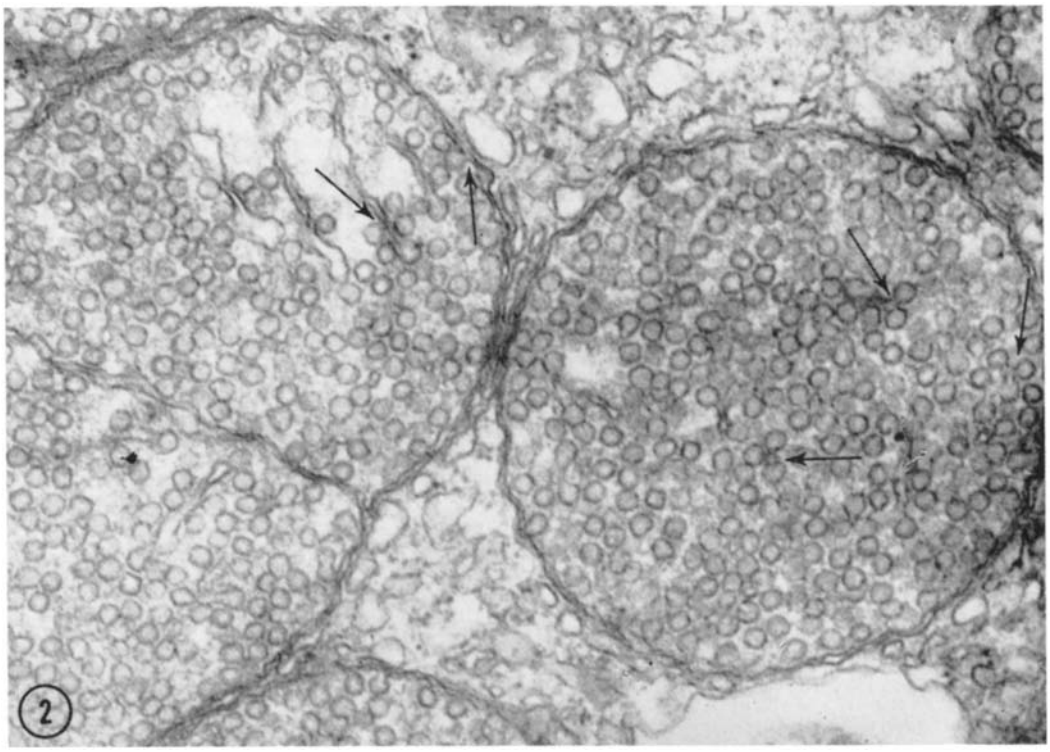


FIGURE 2 Spherical mitochondria with vesicular internal structure in the zona fasciculata in the adrenal cortex of newborn rat. Note the 600-A vesicles free in the matrix. A few ~300-A microtubules and ~200-A microtubules can be seen also (arrows). No mitochondrial granules can be found. × 42,000.

matrix a few tubular or lamellar membranes are found which might be remnants of protrusions of the inner mitochondrial membrane. The average diameter of these tubules is about 300 A. From these narrow tubules protrude also small microvesicles. Between them is found occasionally a narrow microtubule with an average diameter of about 200 A.

Effect of ACTH on the in Vitro Genesis of Cortical Mitochondria

In tissue culture the cortical cells of rat adrenals consist of two types: zona glomerulosa cell, in which the mitochondria have a tubular internal structure, and a zona intermedia cell in which the mitochondria have a tubulovesicular internal structure (Figs. 3 and 4). ACTH did not induce any change in the internal structure of the cells which had mitochondria of tubular structure. By contrast, the cells which had mitochondria both with tubular structures in the matrix and with a few 600-A microvesicles closely associated with the inner limiting membrane were transformed by the hormone to typical zona fasciculata cells (Figs. 5 and 6), (see also reference 18). In the latter cells the internal structure of the mitochondria consisted of a mass of 600-A microvesicles similar to those found in mitochondria of zona fasciculata cells in vivo. 300-A tubules, possible remnants of tubular structures in mitochondria of untreated cells, and 200-A microtubules were also observed. In some ACTH-treated cortical cells the microvesicles were seen to be concentrated in the periphery of the mitochondria, while the central area of the mitochondria was almost devoid of them.

Effect of Actinomycin D on the ACTH-Induced Genesis of Mitochondrial Internal Structure in Tissue Culture

Actinomycin D inhibited the development of mitochondria with vesicular internal structure

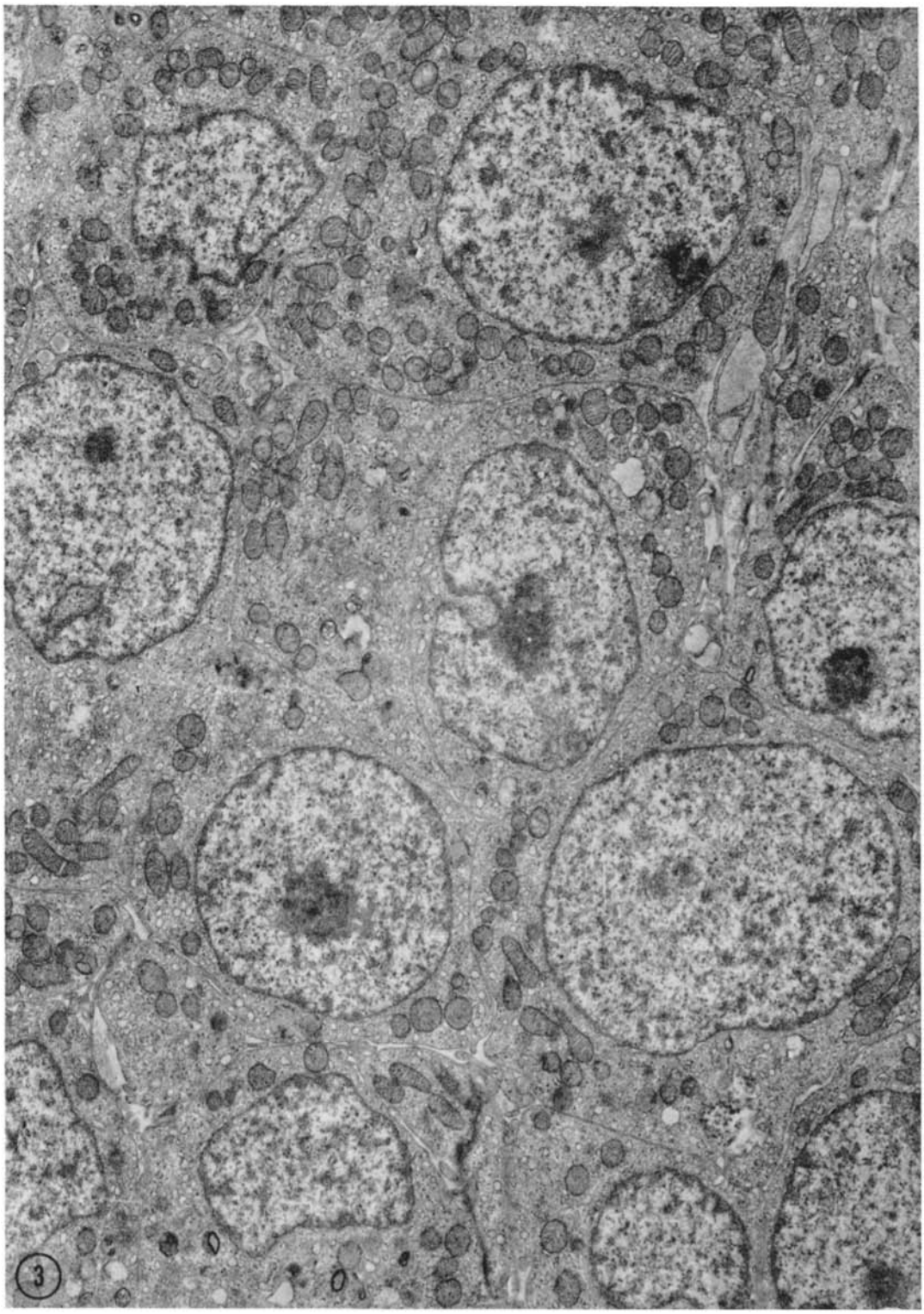


FIGURE 3 Cortical cells in monolayer colony in tissue culture of fetal rat adrenals. Cultivated 22 days. × 6,900.

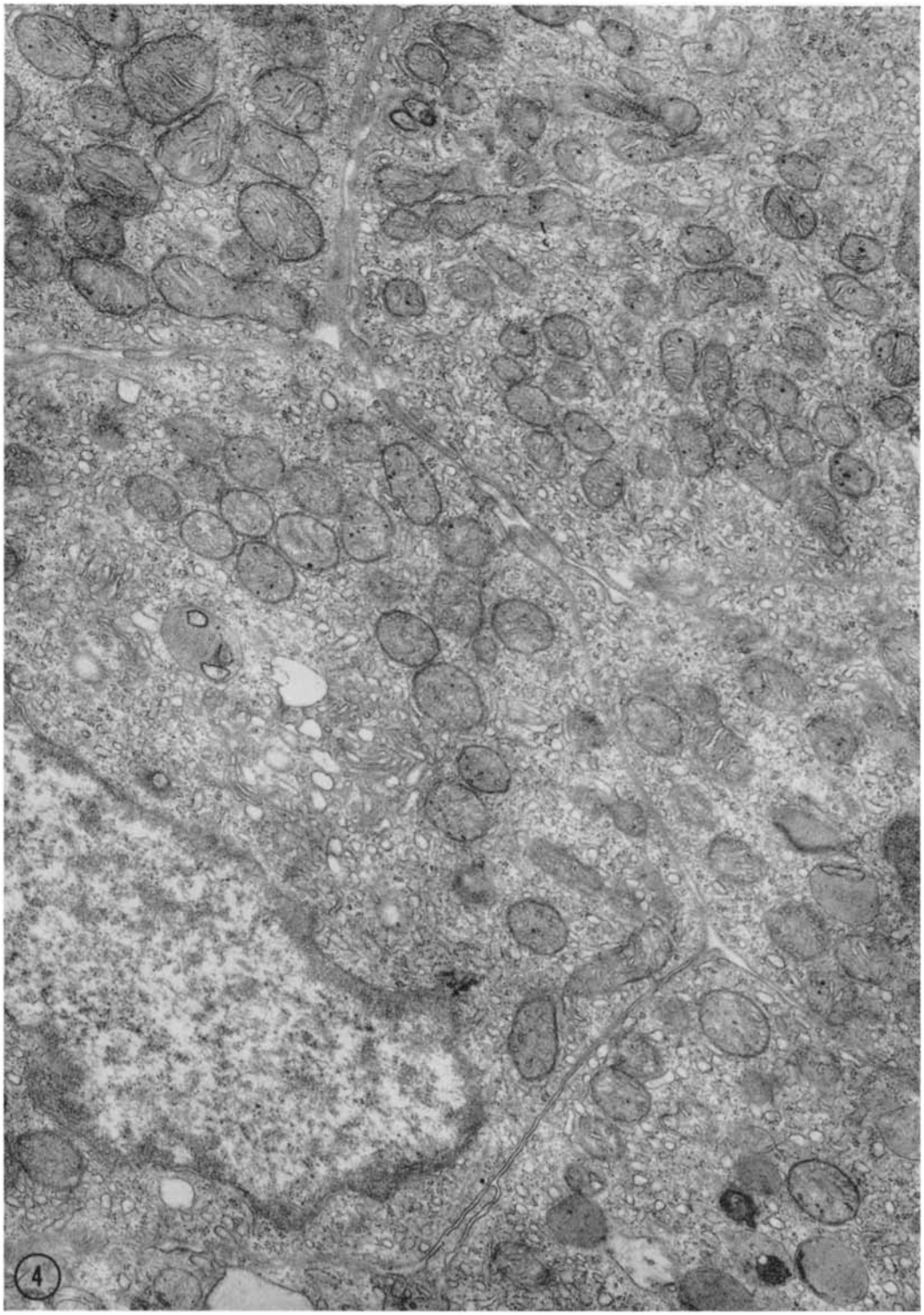


FIGURE 4 Mitochondria in the cortical cells in tissue culture of the fetal rat adrenals cultured 22 days. Note the tubular or tubulo-vesicular (600-A vesicles) internal structure of the mitochondria and the mitochondrial dense granules. × 15,000.

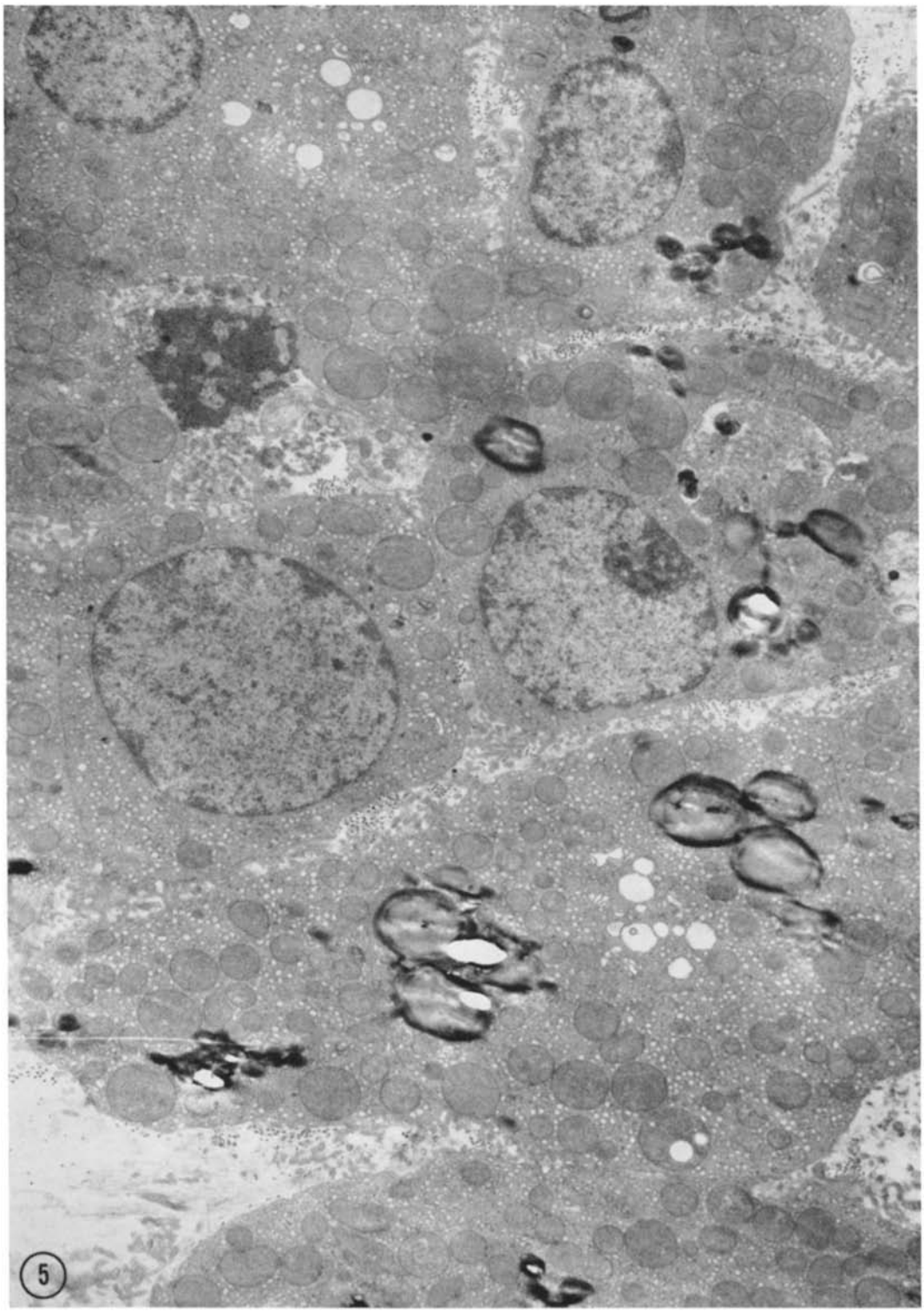


FIGURE 5 Cortical cells in monolayer colony in tissue culture of fetal rat adrenals treated with ACTH. Cultivated 22 days. × 6,000.

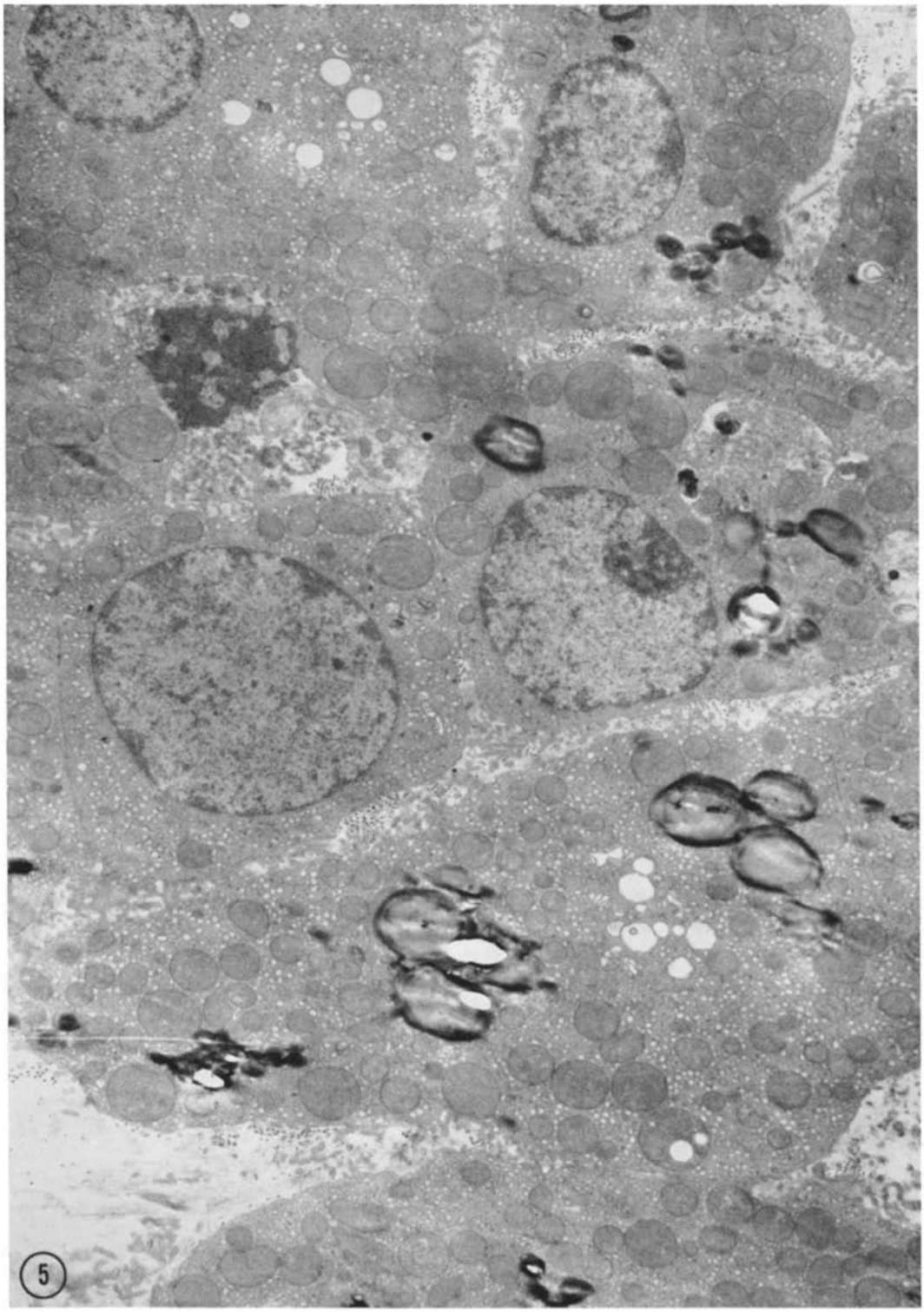


FIGURE 6 Mitochondria in cortical cells in tissue culture of the fetal rat adrenals cultured 22 days and treated with ACTH. 0.2 IU/ml ACTH was added to the medium every day for 7 days, from the 15th to the 21st day of cultivation. The size of the mitochondria is increased, the tubulo-vesicular internal structure has been transformed to vesicular, and the mitochondrial matrix is filled now with masses of 600-A vesicles. Mitochondrial granules have disappeared. × 42,000.

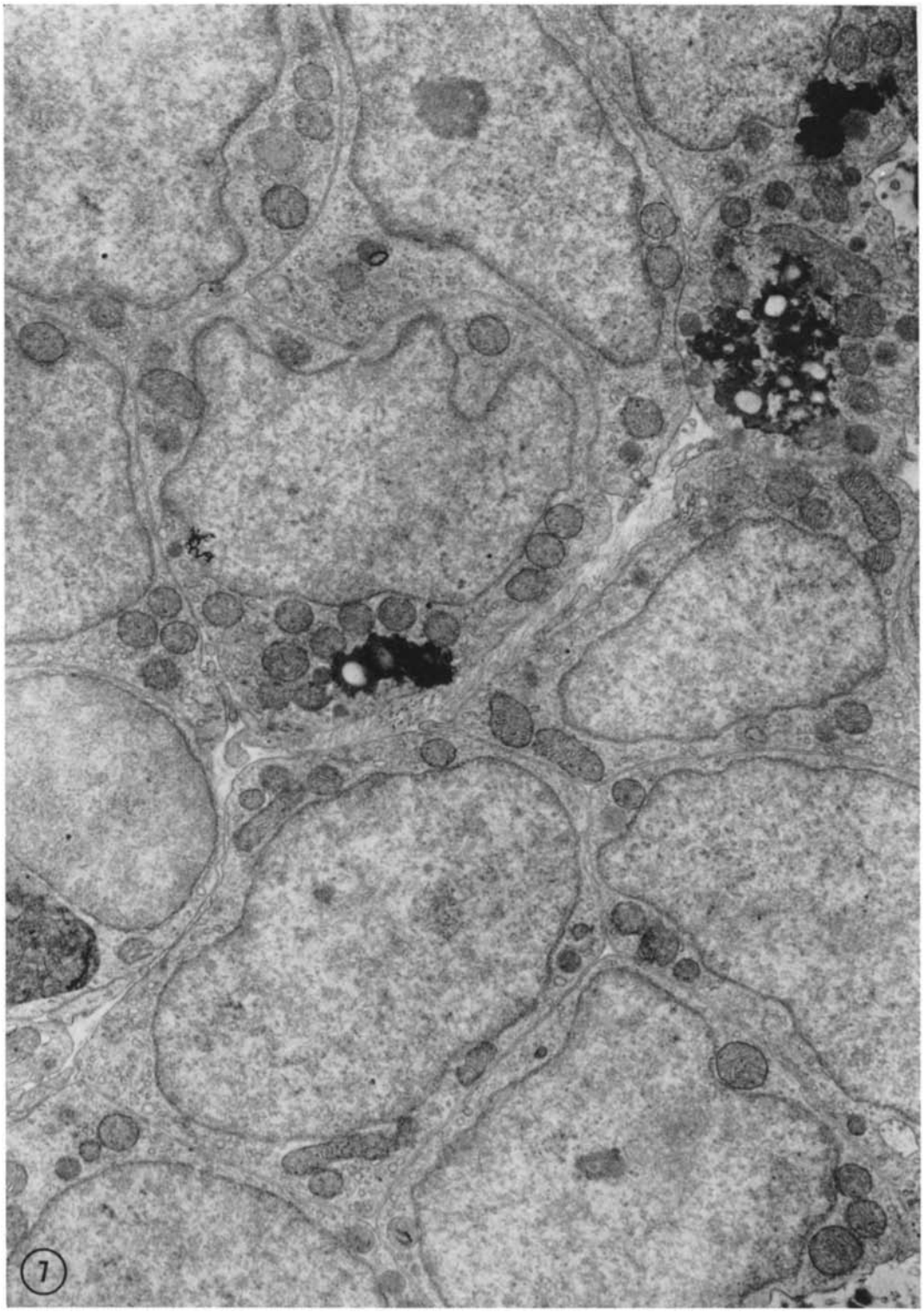


FIGURE 7 Cortical cells in monolayer colony in tissue culture of foetal rat adrenals treated with actinomycin D. Cultivated 22 days. × 6900.

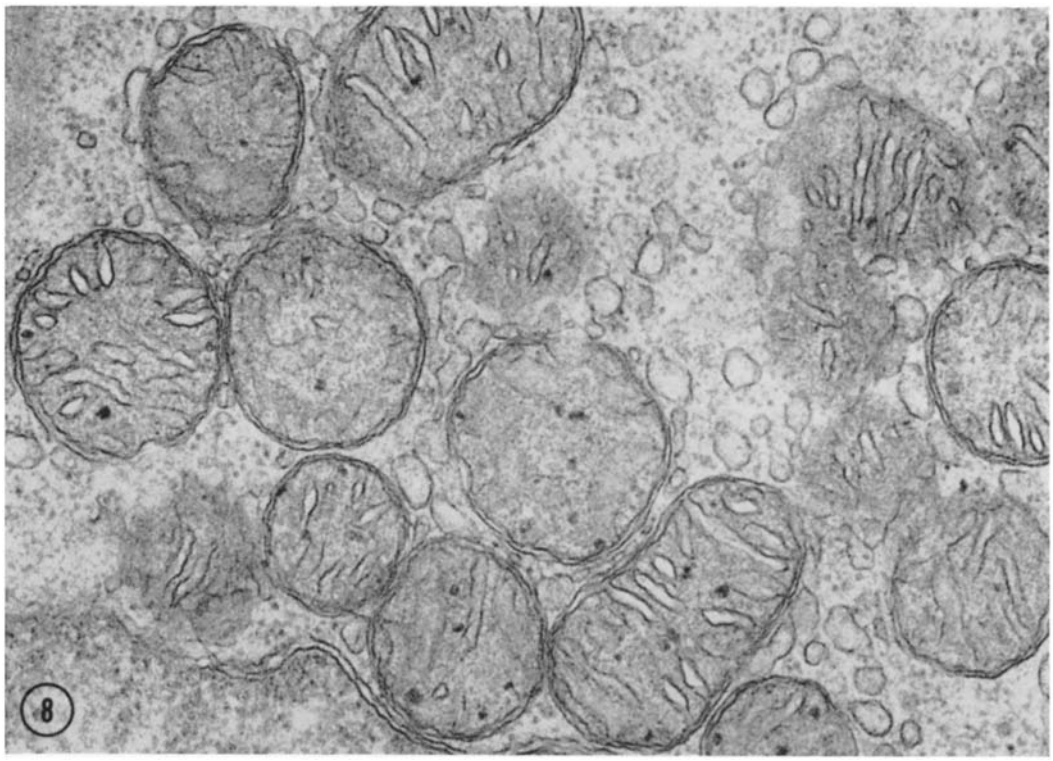


FIGURE 8 Mitochondria in cortical cell in tissue culture of the foetal rat adrenals cultured 22 days and treated with actinomycin D. 0.002 μg/ml actinomycin D was added to the medium every day for 7 days, from the 15th to the 21st day of cultivation. Actinomycin D induced no change in the ultrastructure of the mitochondria. Small, rounded, or short, rod-shaped mitochondria still have a tubular or tubulo-vesicular internal structure. × 42,000.

(Figs. 9 and 10). Enlargement of the spherical mitochondria occurred. The internal structure of these mitochondria is tubular or almost structureless. Only a few protrusions of 600-A microvesicles could be seen near the inner limiting membrane or free in the mitochondrial matrix. The fine structure of the mitochondria of fibroblasts did not change. Actinomycin D in a concentration of 0.002 μg/cc alone induced no change in the ultrastructure of the mitochondria of cultured cortical cells (Figs. 7 and 8).

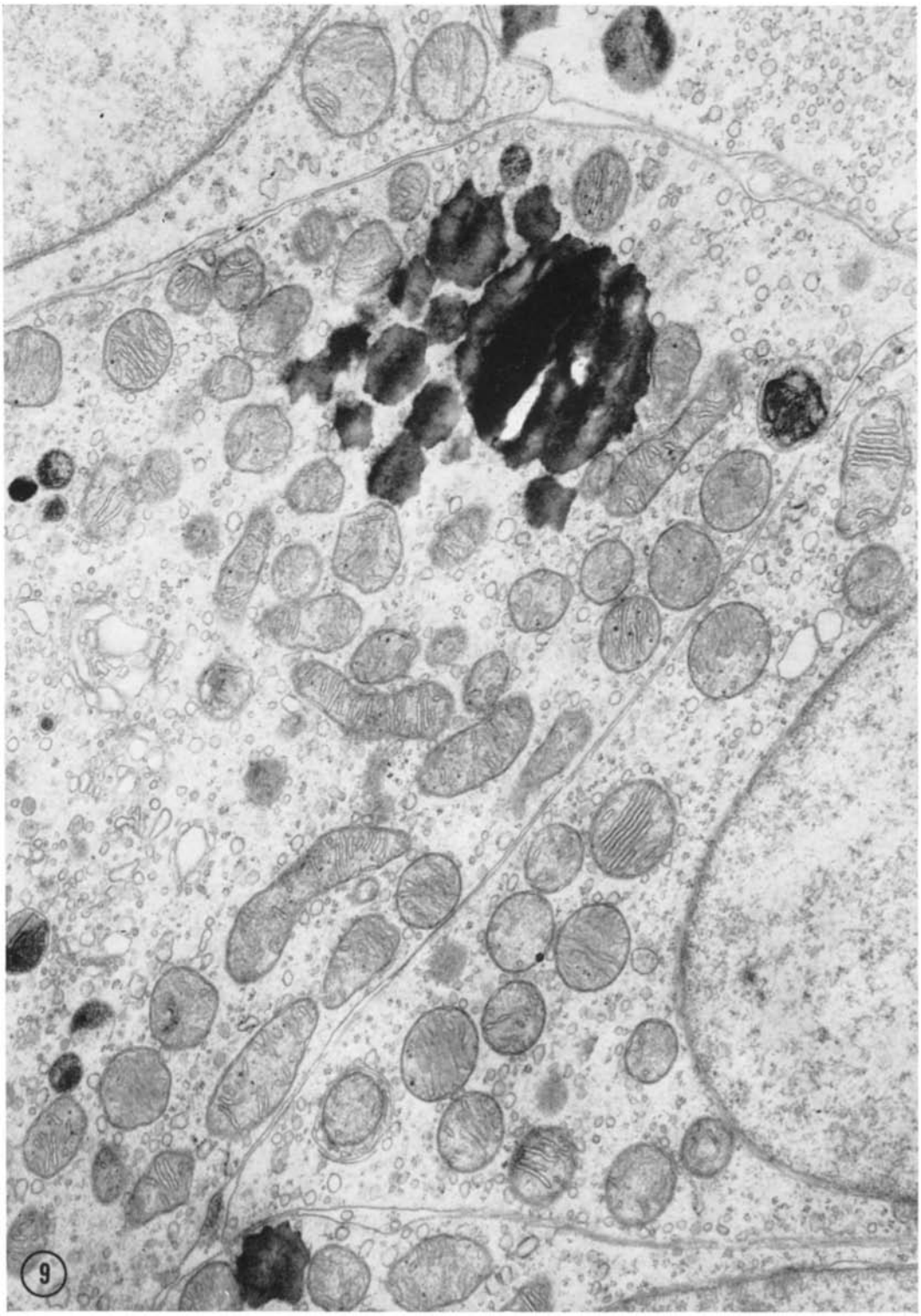
Effect of Puromycin on the ACTH-Induced Genesis of Mitochondrial Internal Structure in Tissue Culture

Puromycin in a concentration of 0.1 μg/cc inhibited the ACTH-induced development of mitochondria with vesicular internal structure (Fig. 12). The mitochondria increased somewhat in size, but they continued to have tubular or tubulovesicular internal structure. The number of tubular structures increased, and near the inner mitochondrial membrane a few 600-A vesicles were seen. In the mitochondrial matrix one lamellated highly electron-opaque granule was often visible. No change was observed in the mitochondria treated with puromycin alone (Fig. 11). Likewise, in the mitochondria of the fibroblasts no change was seen after simultaneous ACTH and puromycin treatment.

DISCUSSION

Genesis of the Zona Fasciculata Mitochondria

The present study disclosed that a typical feature of the zona fasciculata cells of the rat adrenal cortex in vivo is the existence of 600-A vesicles in the mitochondria. Such mitochondria are not present in the cortical cells in tissue cultures of rat



FIGURES 9 and 10 Mitochondria in cortical cells in tissue culture of the fetal rat adrenals cultured 22 days and treated with actinomycin D and ACTH. 0.002 μg/ml of actinomycin D and 0.2 IU/ml of ACTH were added to the medium every day for 7 days, from the 15th to 21st day of cultivation. Actinomycin D inhibited the ACTH-induced transformation of the mitochondrial internal structure. The size of the mitochondria is somewhat increased but the internal structure is still tubular and/or almost structureless. Fig. 9, × 15,000; Fig. 10, × 42,000.

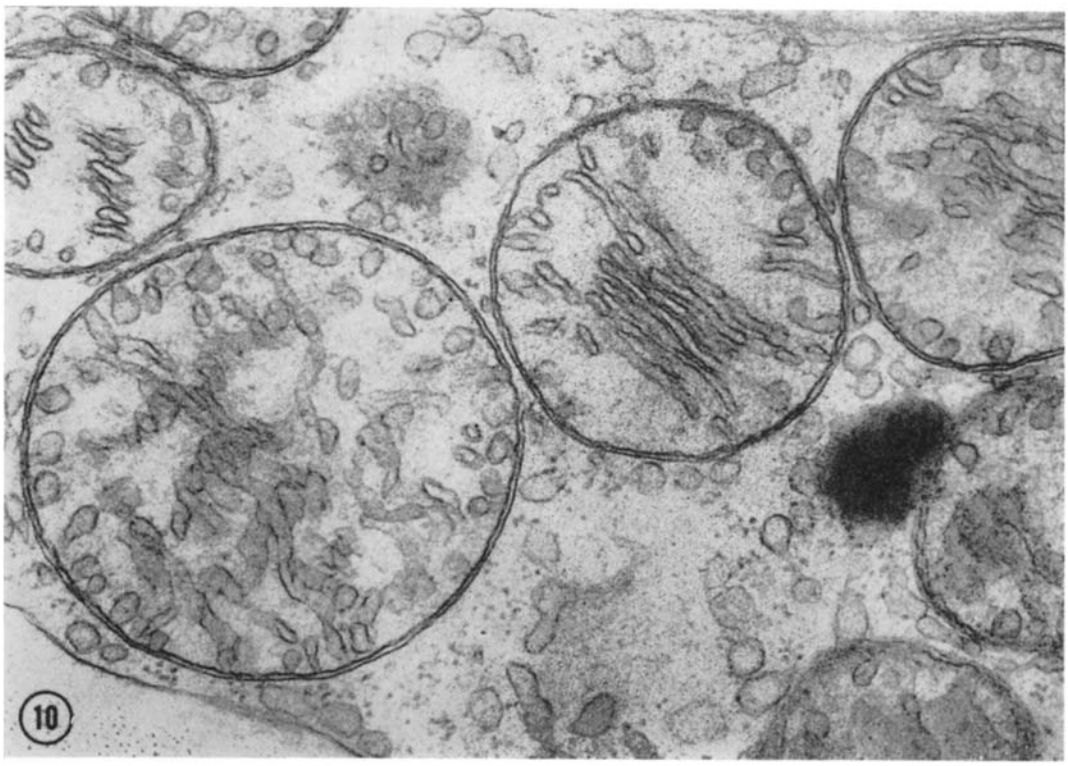


FIGURE 10 See legend under Fig. 9.

adrenals. The small mitochondria in cultivated cortical cells have a tubular or tubulo-vesicular internal structure resembling that of the mitochondria of the zona glomerulosa (Fig. 1) and zona intermedia cells in vivo. Only a few vesicles 600 A in diameter were found in the mitochondria with tubulo-vesicular internal structure. In vitro ACTH treatment induced transformation of these mitochondria with tubulo-vesicular internal structure into typical zona fasciculata mitochondria with 600-A vesicles in the matrix. This is in agreement with observations made in vivo. It has been shown that after hypophysectomy ACTH induced reversion of the degenerated fasciculata mitochondria to the vesicular form (12, 15, 25). In the light of these observations it seems that ACTH plays a primary role in the genesis of the zona fasciculata mitochondria.

Effects of ACTH on Protein Synthesis in the Cortical Cells

In the present work it has been found that actinomycin D, which inhibits the DNA-dependent RNA polymerase (26, 27), and puromycin, which inhibits the transfer of amino acid from the aminoacyl-t RNA to the growing polypeptide on the ribosomes (28) and causes release of incomplete polypeptide chains from the t-RNA on the ribosomes (29, 30), inhibited the ACTH-induced transformation of the internal structure of the mitochondria from tubulo-vesicular to vesicular form (Fig. 13). These findings and the reports that chloramphenicol and actinomycin D inhibit the stimulation of corticosterone synthesis by ACTH (31, 69) and that chloramphenicol inhibits amino acid incorporation by mitochondria of the adrenal cortex (32) prompt the speculation that ACTH stimulates the uptake of amino acid by the adrenal cortex (33), the incorporation of amino acid into rat adrenal protein in vivo and in vitro (34, 35), the synthesis of adrenal nucleic acid (36–42), and the synthesis of nuclear RNA (43) as well as the synthesis of some proteins in the mitochondria.

Steroidogenic Activity of the Cortical Mitochondria

The ultrastructural internal organization of the mitochondria may be very closely related to the

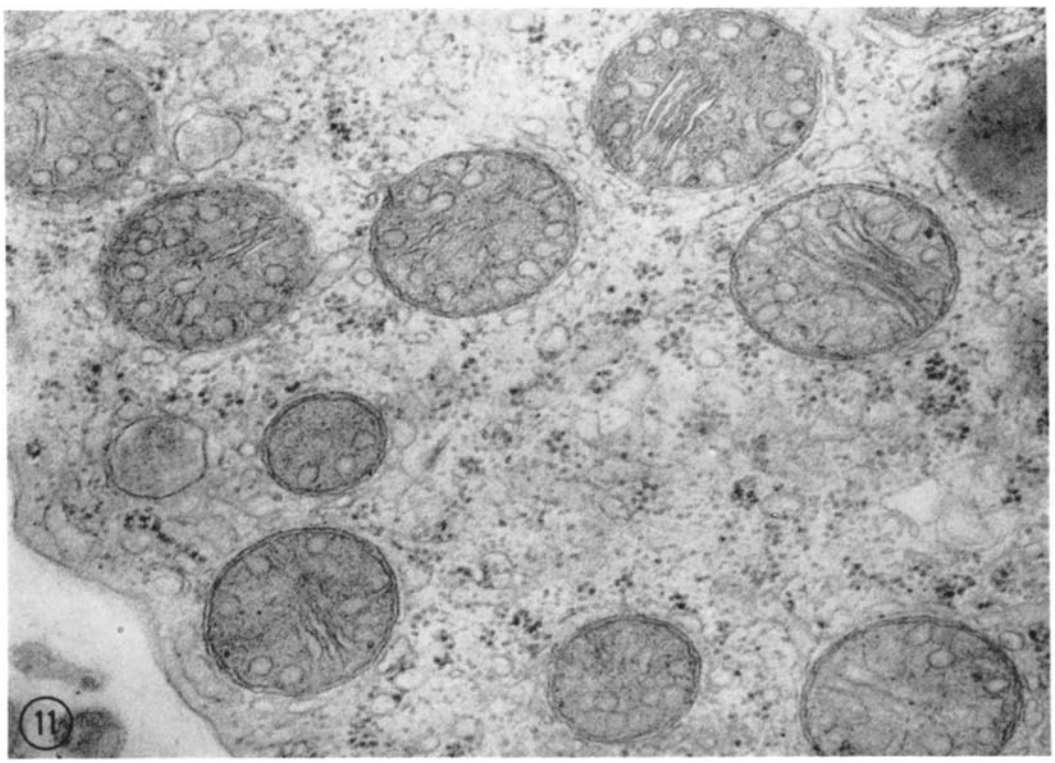


FIGURE 11 No change is seen in the mitochondria in tissue culture of the fetal rat adrenals cultured 22 days and treated with puromycin. 0.1 μg/ml of puromycin was added to the medium every day for 7 days, from the 15th to 21st day of cultivation. The internal structure of the mitochondria is still tubulovesicular (600-A vesicles) and numerous mitochondrial dense granules can be seen. × 42,000.

functional differentiation of these organelles in the cortical cells in the course of ACTH treatment, and an investigation on the metabolic activity of the cortical cells in tissue cultures of rat adrenals is in progress. In preliminary observations (44), it was found that, in the course of ACTH treatment of tissue cultures of rat adrenals, the enzyme systems of 18- and 11βhydroxylases were activated. This is in agreement with earlier reports concerning the enzymatic pathways of steroid biosynthesis in the rat adrenal cortex, in which it was shown that the adrenal cortex is capable of synthesizing corticosterone, 11-deoxycorticosterone, 18-hydroxy-11-deoxycorticosterone, and 18-hydroxycorticosterone (45–61) and that 17αhydroxylase is absent from the albino rat adrenal cortex (62–63). Of the hydroxylases in steroid biosynthesis, 11β- and 18-hydroxylating enzymes were found in the mitochondrial fraction (64–68). The relationships between mitochondrial ultrastructure and mitochondrial distribution of functionally active 11β- and 18-hydroxylases are unknown. However, there is lack of information concerning the effect of actinomycin D and puromycin on the different steroid metabolites formed after ACTH stimulation, although it has been shown that in rats actinomycin D inhibits ACTH-induced corticosterone production in vivo (69). By contrast, in vitro actinomycin D was not found to exert any effect on ACTH-induced corticosteroid production during incubation of rat adrenal gland quarters (70), although it completely abolished the in vitro steroidogenic effect of ACTH during incubation of cow adrenal slices. Until we have information on function to compare with these actinomycin D- and puromycin-induced structural inhibitory changes in the mitochondria of the cortical cells in

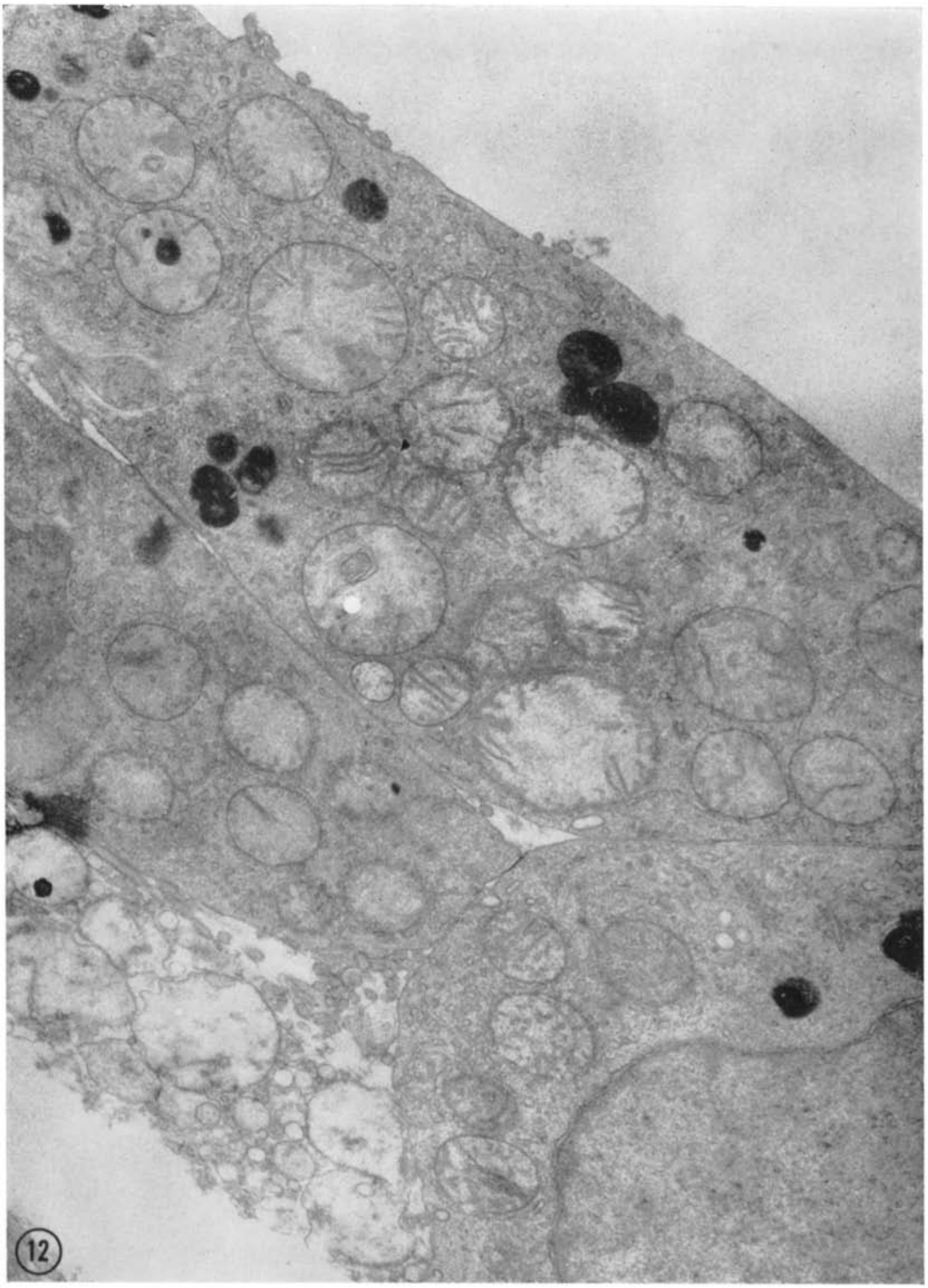


FIGURE 12 Puromycin-induced inhibition of the ACTH-induced transformation of the mitochondrial ultrastructure in cortical cells in tissue culture of the fetal rat adrenals cultured 22 days and treated with puromycin and ACTH. 0.1 μg/ml of puromycin and 0.2 IU/ml of ACTH were added to the medium every day for 7 days, from the 15th to 21st day of cultivation. Only a few tubular structures and vesicular protrusions from the inner mitochondrial membrane can be seen. The dense mitochondrial granules have disappeared. One lamellated highly electron-opaque granule can often be seen in the matrix of the mitochondria after puromycin inhibition. × 15,000.

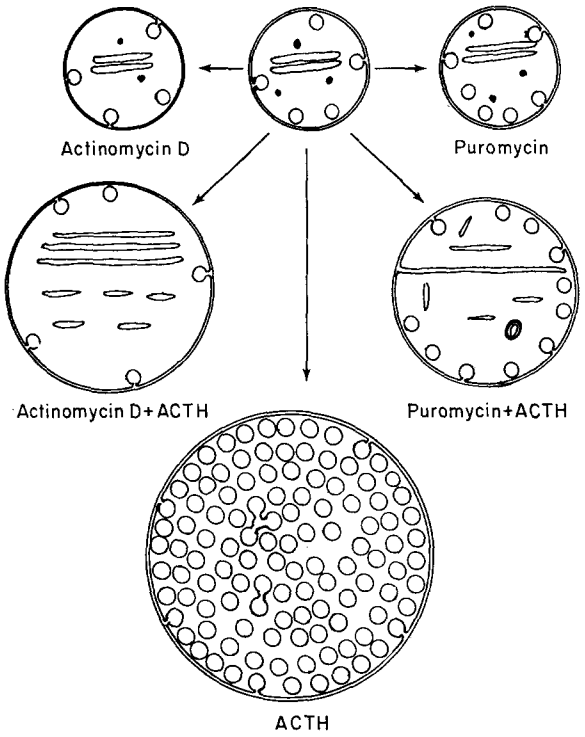


FIGURE 13 Schematic representation of the organization of zona fasciculata mitochondria in tissue cultures of the rat adrenals and of the effects of actinomycin D and puromycin on the genesis of zona fasciculata mitochondria in vitro.

tissue cultures of rat adrenals, we can say nothing about the relationships between mitochondrial hydroxylases and 600-A vesicles.

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