Selective Inhibition by Chloramphenicol of ACTH-Induced Reorganization of Inner Mitochondrial Membranes in Fetal Adrenal Cortical Cells in Tissue Cultures [1]

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ABSTRACT Tissue cultures of fetal rat adrenals were used to study effects of chloramphenicol on the ACTH-induced synthesis of mitochondrial inner membranes in the cortical cells. Chloramphenicol alone added to the culture medium in concentrations of 0.003, 0.03, 0.3, and 0.6 μ mole/ml/6 days induced no changes in the ultrastructure of cortical cells. Chloramphenicol in concentrations of 0.3 and 0.6 μ mole/ml/6 days given together with 100 mu/ml/6 days of ACTH inhibited completely the ACTH induced changes of mitochondrial inner membranes (formation of 600 Å vesicles). Chloramphenicol in concentrations of 0.003 μ mole/ml/6 days caused no inhibition of the ACTH effects. In concentration of 0.03 μ mole/ml/6 days chloramphenicol resulted in incomplete inhibition of ACTH-induced formation of mitochondrial vesicular cristae. None of these doses of chloramphenicol affected other ACTH-induced changes in the fine structure of the cells such as increase of smooth surfaced endoplasmic reticulum, hypertrophy of Golgi apparatus, and development of microvillous processes of plasma membranes. Chloramphenicol also caused no inhibition of the ACTH-induced accumulation of lipid in the cytoplasm. In cultivated cortical cells of Charles River strain albino rats small groups of annulated lamellae are commonly observed.

The present observations suggest that: (1) the development of mitochondrial inner membranes is dependent, at least in part, on mitochondrial protein synthesis; (2) ACTH stimulation of mitochondrial protein synthesis in cortical cells is independent of ACTH-induced stimulation of nuclear-DNA-dependent protein synthesis; (3) doses of chloramphenicol which inhibit specialization of mitochondrial inner membranes of fetal adrenal cortical cells are comparable to those which inhibit protein synthesis in bacteria.

It has been shown in an earlier study (Kahri, '66) that ACTH induces differentiation of cultured cortical cells of the zona intermedia type into typical zona fasciculata cells and causes reorganization of inner mitochondrial membranes into 600 Å vesicles. Actinomycin D, which inhibits DNA-dependent RNA polymerase (Reich et al., '61, '62), and puromycin, which inhibits the transfer of amino acid from the aminoacyl-tRNA to the growing polypeptide on the ribosomes (Yarmolinsky and de la Haba, '59) and causes release of incomplete polypeptide chains from the RNA on ribosomes (Morris and Schweet, '61; Nathans and Lipman, '61), both inhibited the ACTH-induced reorganization of the internal membranes of the mitochondria in cultured cortical cells (Kahri, '68). These findings left open the question as to whether the ACTH-induced synthesis of structural proteins in mitochondria is dependent on nuclear or on mitochondrial protein synthesis. The present study was undertaken to throw further light upon the possible role of mitochondrial protein synthesis in the ACTH-induced changes of mitochondrial fine structure. To this end we have studied effects of chloramphenicol which is believed to be a specific inhibitor of mitochondrial protein synthesis (Huang et al., '66; Clark-Walker and Linnane, '66, '67; Linnane, '68; Vasquez, '66a, b; Kroon, '63) to determine whether the previously demonstrated effects of ACTH on mitochondrial structure would be inhibited.

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MATERIALS AND METHODS

Twenty-one day old fetal rats of the Charles River strain were sacrificed by decapitation and their adrenals were explanted as tissue cultures. A total of 288 fetuses have been used in 80 cultures. The average amount of tissue per culture vessel was 7.2 adrenals. The culture method employed was found in earlier studies (Kahri, '66) to be suitable for long-term cultivation of adrenals. The medium consisted of 50% Monkey Kidney Medium A (0.5% lactalbumin hydrolysate in Hank's balanced salt solution and 2% calf serum); 25% calf serum ultrafiltrate, and 25% Minimum Essential Medium, Eagle (Microbiological Associates, Bethesda, Maryland, U. S. A.). Adrenocorticotrophic hormone (ACTH, Corticotrophin Injection, U.S.P., Parke-Davis and Co., Detroit, Michigan, U. S. A.) was added to the culture medium in the amount of 100 mu/ml every day for six days, from day 19 of cultivation up to and including day 24. Chloramphenicol (chloramphenicol-succinate, Parke-Davis and Co., Detroit, Michigan, U. S. A.) was added in the amounts of 0.003, 0.03, 0.3, and 0.6 μ mole/ml both separately and with ACTH every day for six days from day 19 of cultivation up to and including day 24. Total doses of chloramphenicol were 30, 300, 3000, and 6000 μg/5 ml/6 days. The culture medium was changed every fifth day of cultivation.

Tissue cultures were carefully studied before fixation by phase contrast microscopy. Cultures were fixed in a solution containing 1% formaldehyde and 1.25% glutaraldehyde adjusted to pH 7.4 with 0.2 M cacodylate buffer (Karnovsky, '65) and 0.01% of 2, 4, 6-trinitrocresol (Ito and Karnovsky, '68). Tissues were post-fixed in 1% osmium tetroxide with phosphate buffer (Millonig, '62). After fixation they were dehydrated, and the cortical cell colonies were carefully detached from the bottom of the culture vessels with a splinter of wood. Dehydration was completed in proplyene oxide and the tissue embedded in Epon 812. Some cultures were embedded *in situ* in the culture vessel directly after alcohol dehydration.

Thin sections were stained with 2% lead citrate for 20 seconds (Venable and Coggeshall, '65). Electron micrographs were made at original magnifications of 2000–30,000 with an RCA-3F electron microscope.

RESULTS

Ultrastructure of cortical cells of cultured fetal rat adrenals

The ultrastructure of cultured fetal rat adrenals has been described in detail in an earlier publication (Kahri, '66, '68) and will be reviewed here only briefly as a basis for comparison with the experimentally altered cultures which are our principal concern in the present study.

In the absence of hormonal stimulation the cells remain relatively undifferentiated but mitotic figures are common. The cells are loosely adherent with desmosome-like specializations on their areas of contact and a few small irregularly oriented microvilli on free portions of their surface. The cells have a small oval or irregularly shaped nucleus and little cytoplasm. The mitochondria are small but quite numerous. They are spherical or short rods with an internal structure usually consisting of lamellar or tubular cristae, but some are of mixed type containing some tubular cristae and a few 600 Å vesicles which do not appear to be fixed to the inner mitochondrial membrane. The mitochondrial matrix is moderately dense and contains numerous dense matrix granules (fig. 1). In addition to the mitochondria the cytoplasm contains occasional elongate profiles of rough surfaced endoplasmic reticulum and a few tubular elements of smooth surfaced reticulum. There are a few scattered lipid droplets and single free ribosomes are abundant in the cytoplasmic matrix. The Golgi complex is well developed and consists of an assemblage of membranous profiles of both flat saccular and vesicular elements. Associated with these are occasional membrane-bounded dense granules. The centrioles also tend to be localized in the vicinity of the Golgi apparatus. Irregular myelin-like figures are frequently encountered in the cytoplasm.

Ultrastructure of cultured cortical cells after ACTH-induced differentiation. As previously described (Kahri, '66, '68) addition of ACTH to the culture medium

results in an increase in overall size of the cells and an increase in the number of lipid droplets. The microvilli become more numerous, the desmosome-like thickenings of the plasma membranes disappear, and the cells tend to migrate from the explant more than formerly. The Golgi apparatus hypertrophies with some of its elements becoming notably dilated. There is a striking increase in the tubular and vesicular profiles of the smooth endoplasmic reticulum but no obvious change in the rough reticulum. Ribosomes are predominantly in the form of polysomes. The mitochondria of ACTH-treated cultures increase greatly in size and become spherical. Their internal structure consists predominantly of 600 Å membrane-limited vesicles with only a few tubular cristae remaining (fig. 2).

Mitoses also occur in ACTH-treated fetal adrenal cultures if the medium is changed every fifth day. However, the dividing cells appear less differentiated than the others, with scant smooth endoplasmic reticulum, poorly differentiated internal structure in the mitochondria, few lipid droplets, and ribosomes generally single rather than in polysomal clusters (fig. 3). In cultivated cortical cells of Charles River strain albino rats small groups of annulated lamellae are commonly observed (fig. 4).

Effects of chloramphenicol on the ultrastructure of cultured cortical cells. Chloramphenicol in concentrations of 0.003, 0.03, and 0.3 μ mole/ml has no effect upon the ultrastructure of the cultured adrenocortical cells. In a concentration of 0.6 μ mole/ml it causes a small decrease in size of the mitochondria but their internal structure is similar to those of control cultures (fig. 5). Chloramphenicol also had no effect upon the ultrastructure of mitochondria in fibroblasts, macrophages, adrenal medullary cells, or liver cells in the same cultures. Higher concentrations resulted in appearance of greater numbers of small granules in the mitochondrial matrix. These were smaller but otherwise similar in appearance to cytoplasmic ribosomes (figs. 6, 7).

Effects of chloramphenicol on ACTH stimulated cortical cells. The ACTH-induced differentiation of cortical cells *in vitro* proceeds normally in the presence of 0.003 μ mole/ml chloramphenicol. The cells increase in size, the Golgi apparatus and smooth reticulum hypertrophy, lipids are enhanced, and migration of the differeniated cortical cells proceed as usual. In this low dosage chloramphenicol caused no inhibition of the ACTH-induced transformation of the cristae to 600 Å vesicles. The ribosomes were, as usual, in polysomal configuration and annulate lamellae were as common as in control cultures.

In a dosage of 0.03 μ mole/ml chloramphenicol caused no structural alterations in cytoplasmic organelles other than the mitochondria (fig. 8). These, however, clearly show effects of the chloramphenicol treatment. Although they respond to ACTH by increase in size, and enspherulation, the development of vesicles in their interior is to some extent inhibited. The mitochondria contain a smaller number of 600 Å vesicles than usually form in response to ACTH treatment, and may show scattered tubular cristae (fig. 8). Cortical cells undergoing mitosis during combined chloramphenicol and ACTH treatment sometimes contain mitochondria with a few 600 Å vesicles and an inner membrane that seems incomplete in certain areas. Some of these altered mitochondria appear to have a single limiting membrane around most of their circumference (fig. 9).

In a dosage of 0.3 and 0.6 μ mole/ml chloramphenicol continues to have no effect upon ACTH-induced differentiation of other cytoplasmic organelles, but with these higher concentrations, its selective inhibitory effect on the mitochondrial response to trophic hormone was more obvious (figs. 10, 11). Interestingly enough the formation of the outer and inner limiting membranes is apparently not significantly affected, for the mitochondria undergo the expected change of shape and increase in size (figs. 12, 13). Many mitochondria are, however, entirely devoid of internal membrane structure and appear to be a spherical mass of homogeneous matrix of low density limited by a pair of membranes (fig. 14). Other mitochondria show a few vesicular cristae adjacent to the inner limiting membrane, while the interior of the mitochondrion appears empty. In thick sections examined with oil immersion microscopy, these mitochondria appear as

rounded empty vacuoles. There seems also to be an increased tendency for the appearance of tubular rather than vesicular cristae and for the occurrence of granular bodies of high electron density in the matrix (fig. 15).

In these same cultures the combined treatment with ACTH and chloramphenicol induced no structural alterations in the mitochondria of fibroblasts, macrophages, adrenal medullary cells, or liver cells.

DISCUSSION

Structural-functional relationships in the mitochondria of the adrenal zona fasciculata. It has now been demonstrated *in vivo* both after hypophysectomy (Sabatini et al., '62; Nishikawa et al., '63; Idelman, '66) and in tissue cultures of fetal adrenals (Kahri, '66, '68) that ACTH induces changes in the structure of cortical cell mitochondria, which involve transformation of lamellar or tubular cristae of the fetal or hypophysectomized animal into a population of free vesicles of uniform diameter (600 Å) which is the internal structure typical of mitochondria in the zona fasciculata of the intact adult rat. These specific mitochondrial changes in cultured fetal rat adrenals can also be induced with a synthetic β-ACTH peptide containing 24 amino-acids, but not with shorter peptides consisting of amino acids 1 to 10 or 11 to 24 (Kahri and Halinen, '67). The minimal concentration of ACTH that induces maximal differentiation of mitochondria *in vitro* is 2 mu/ml (Corticotrophin, Organon) and the time required for development of the effects is 48–72 hours (Kahri and Halinen, '68). Slight changes are detectable after as little as 0.2 mu/ml, and the most sensitive indicator of ACTH effect is the formation of vesicles in place of the usual cristae.

The current concept of the biosynthetic pathway leading to formation of adrenal steroids suggests the 11β and 18-hydroxylases (Harding et al., '65; Harding and Nelson, '66; Peron et al., '66; Guerra et al., '66; Kimura and Suzuki, '67) and enzymes involved in hydroxylation and side-chain cleavage of cholesterol (Lynn et al., '54; Saba et al., '54; Constantopoulos and Tchen, '61; Tchen, '68) are all located in the mitochondria and more specifically in a subfraction derived from the inner membranes of cortical mitochondria (Yago and Ichii, '69). It has been claimed that side-chain cleavage is the main target of ACTH in steroid biosynthesis (Stone and Hechter, '54; Karaboyas and Koritz, '65; Bransome, '68). Stimulation of 11β-hydroxylation in zona fasciculata-reticularis border in the rat adrenal cortex has also been demonstrated (Griffiths and Glick, '66). In monolayer cultures of mouse adrenocortical tumor cells ACTH is reported to induce a progressive increase of 11β-hydroxylation after 12–72 hours (Kowal, '67). In tissue cultures of fetal rat adrenals the mitochondrial changes induced in 48-72 hours are followed by a significant increase of corticosterone and 18-OH-desoxycorticosterone synthesis after about 72 hours of stimulation (Kahri et al., '70). Consideration of these findings brings us to suggest that there is a direct relationship between the specific vesicular internal structure of cortical mitochondria and the acquisition or activation of the enzymes responsible for cholesterol hydroxylations, side-chain cleavage and 11β- and 18-hydroxylations in the biosynthesis of adrenal steroids.

Effects of ACTH on protein synthesis in adrenocortical cells. Previous studies on the effects of ACTH on protein synthesis and growth of the adrenal cortex have reported an increase in adrenal weight, total protein, and nucleic acid content (Bransome and Reddy, '61; Farese, '64a; Farese and Reddy, '63; Symington et al., '56; Fiala et al., '56) and enhanced incorporation of amino acid into protein of the rat adrenal has been found both *in vivo* and *in vitro* (Farese, '64b, '65a, b, '66a, '68; Bransome and Reddy, '63a, b; Scriba and Reddy, '65; Scriba and Fries, '67; Ganis et al., '55; Reddy and Streeto, '68). An opposite effect has been reported by others (Koritz et al., '57; Ferguson, '63; Halkerston et al., '64). ACTH is also reported to increase adrenal RNA synthesis (Bransome and Chargaff, '64; Farese and Schnure, '67; Bransome, '67a, b). These findings have also been contradicted (Ferguson and Morita, '64). Numerous studies employing inhibitors of protein synthesis such as actinomycin-D, puromycin, cycloheximide, and chloramphenicol also suggest a stimulatory effect of ACTH on pro-

tein synthesis in the adrenal cortex (Farese, '64c, '66b; Ferguson, '62, '68; Ferguson et al., '67; Garren et al., '65; Ney et al., '66). All of these studies have been interpreted as indicating that ACTH acts as an inducer of DNA-dependent protein synthesis. There has been little or no work implicating ACTH in activation of mitochondrial protein synthesis.

It has been shown in the present study that chloramphenicol, which is generally accepted as a specific inhibitor of mitochondrial protein synthesis, inhibits the transformation of mitochondrial structure normally induced by ACTH. This selective inhibition of synthesis of structural proteins of mitochondria strongly suggests that ACTH may be the specific inducer of mitochondrial-DNA-dependent protein synthesis during normal differentiation of adrenocortical cells. The absence of an effect of chloramphenicol on ACTH-induced proliferation of other cytoplasmic membranes (Golgi and smooth reticulum) lends credence to the view that ACTH may have a dual action, inducing both nuclear DNA-dependent and mitochondrial DNA-dependent protein synthesis.

In addition to the present findings on chloramphenicol inhibition of ACTH-induced membrane synthesis in mitochondria, it has been shown that this substance inhibits amino acid incorporation into adrenal mitochondria (Garren and Crocco, '67). It has been calculated (table 1) that the change from small mitochondria with foliate cristae to large spherical ones filled with vesicles must involve a very great synthesis of structural proteins associated with the 20- to 100-fold increase in their membrane surface area. If this synthesis takes place within the organelle it suggests that ACTH is a very strong inducer of mitochondrial DNA-dependent synthesis of structural and enzyme protein.

If it be accepted that the ACTH-induced changes in surface area of the mitochondrial membranes are associated with a corresponding increase in the enzymes involved in biosynthesis of steroids, then the significance of the vesicular form of cristae is more readily understood. It would appear that the primary effect of ACTH on protein synthesis is to increase the nuclear and mitochondrial DNA-dependent synthesis of enzyme and structural protein rather than to activate existing enzymes. Thus the effect of ACTH on protein synthesis during differentiation evidently serves to increase the steroid-synthesizing capacity of relatively undifferentiated cells transforming them into typical highly differentiated cells of the zona fasciculata. On the other hand, the rapid effect of ACTH on fully differentiated cells is probably mediated by activation of some energy yielding mechanism, e.g., activation of cyclic 3′, 5′-AMP (Haynes and Berthet, '57; Haynes et al., '59) or activation of NADP-linked glucose-6-phosphate dehydrogenase (McKerns, '64), or possibly induction of changes in membrane permeability (Matthews and Saffran, '68; Cortes and Peron, '66) rather than via induction of rapid protein synthesis.

Biogenesis of mitochondrial membranes. Chloramphenicol in concentration of 0.3 μ mole/ml suppressed ACTH-induced changes in the internal organization of mitochondria but did not inhibit their enlargement. This observation supports the view that the internal membranes may be dependent upon mitochondrial protein synthesis, but that the outer membranes may not be. The absence of an effect of chloramphenicol on the structure of mitochon-

TABLE 1

Theoretically calculated data on the increase of surface area of mitochondrial cristae during transformation from lamellar cristae to 600 Å vesicles

Mitochondria	Shape of cristae	Diameter of mitochondria	Number of cristae	Membrane surface area of cristae
		μ		μ^2
Spherule	lamellar	1.5	29	78
Spherule	lamellar	0.75	3	2.4
Spherule	600 Å vesicles	1.5	a. 7000 [1]	a. 300 [1]

[1] The number of vesicles in one mitochondria is calculated approximately and does not represent the absolute maximum number.

dria in fibroblasts, macrophages, or liver cells, supports the idea (Weisberger and Wolfe, '64) that chloramphenicol is effective specifically in those situations such as cellular differentiation where there is a rapid turnover of mRNA. The present findings are consistent with the assumption that changes in the biogenesis of internal mitochondrial membranes are dependent on two factors: first the existence of specific enzymes or proteins coded by mitochondrial DNA, and second the existence of a specific inducer which has as its target mitochondrial DNA-dependent protein synthesis.

The inability of chloramphenicol to prevent ACTH-induced increase in size of mitochondria is consistent with findings on the biogenesis of mitochondrial inner membranes in glucose-repressed yeast. Chloramphenicol, which strongly inhibits amino acid incorporation into isolated mitochondria (Mager, '60; Wintersberger, '65), inhibits the formation of cristae of yeast mitochondria but does not affect the development of the outer mitochondrial membrane (Clark-Walker and Linnane, '67). There is also some indication from studies on action of cycloheximide on synthesis of soluble mitochondrial protein that the outer membrane of yeast mitochondria may be a product of cycloheximide-sensitive cytoplasmic protein synthesis (Yu et al., '68). Studies of incorporation of amino-acids into the outer and inner membranes of isolated rat liver mitochondria also provide suggestive evidence that the outer membrane is not a product of mitochondrial protein synthesis (Neupert et al., '68). The observations reported here provide no evidence in support of the suggestion that the outer mitochondrial membrane is derived from the endoplasmic reticulum (Neupert et al., '68; Parsons et al., '67) but instead favors the view that the site of protein synthesis is located within the inner limiting membrane (Neupert et al., '68).

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PLATE 1

EXPLANATION OF FIGURES

1 Mitochondria in the cortical cells in tissue culture of fetal rat adrenals cultivated 25 days. Note the tubular or lamellar cristae of the mitochondria and numerous mitochondrial dense granules in the matrix. × 15,900.

2 Mitochondria in the cortical cells in tissue culture of fetal rat adrenals treated with ACTH, 0.1 I. U./ml of ACTH was added to the medium every day for six days, from the nineteenth day of cultivation up to and including the twenty-fourth day. Note the ACTH-induced modification cristae into 600 Å vesicles. Cultivated 25 days. × 40,600.

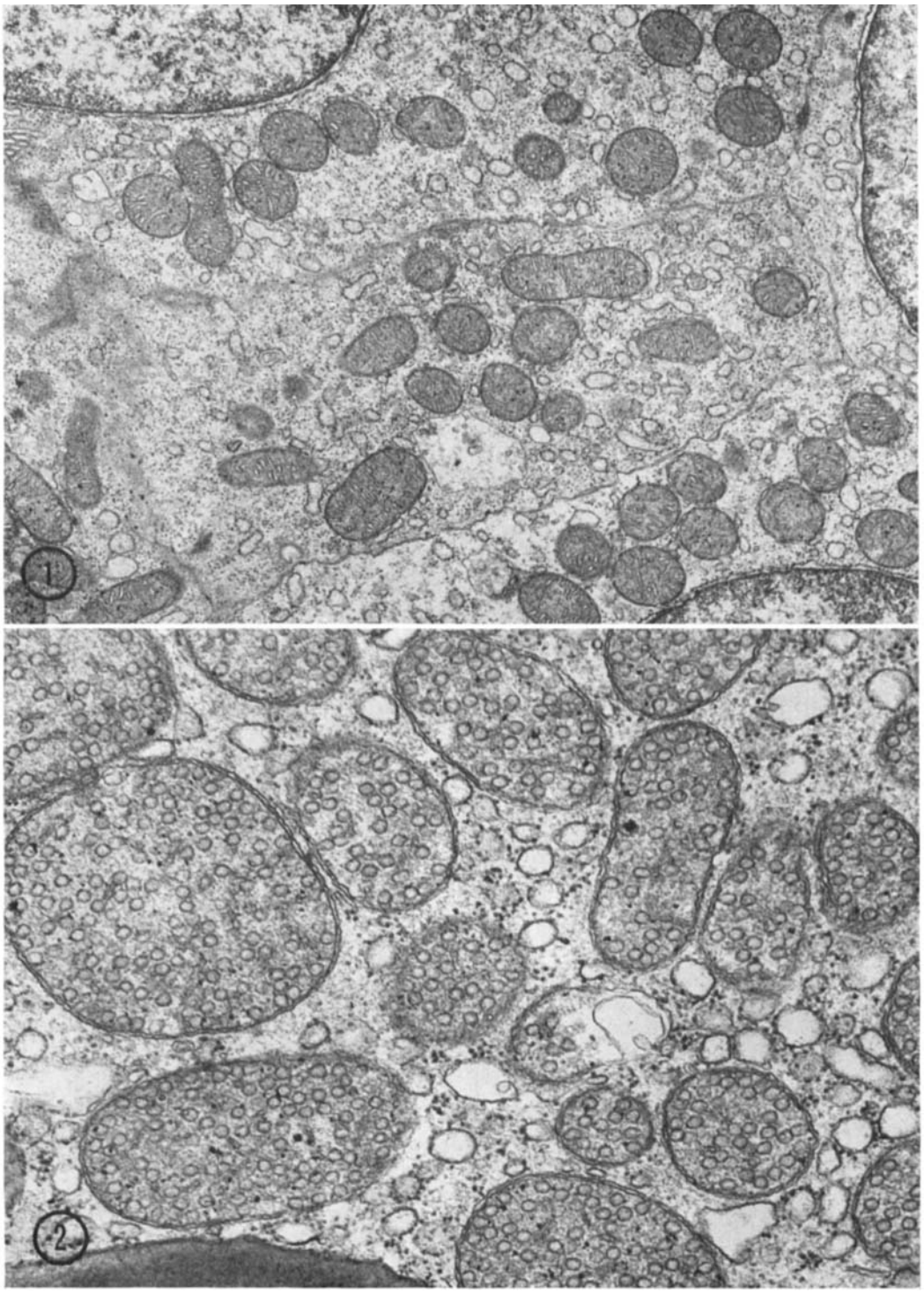
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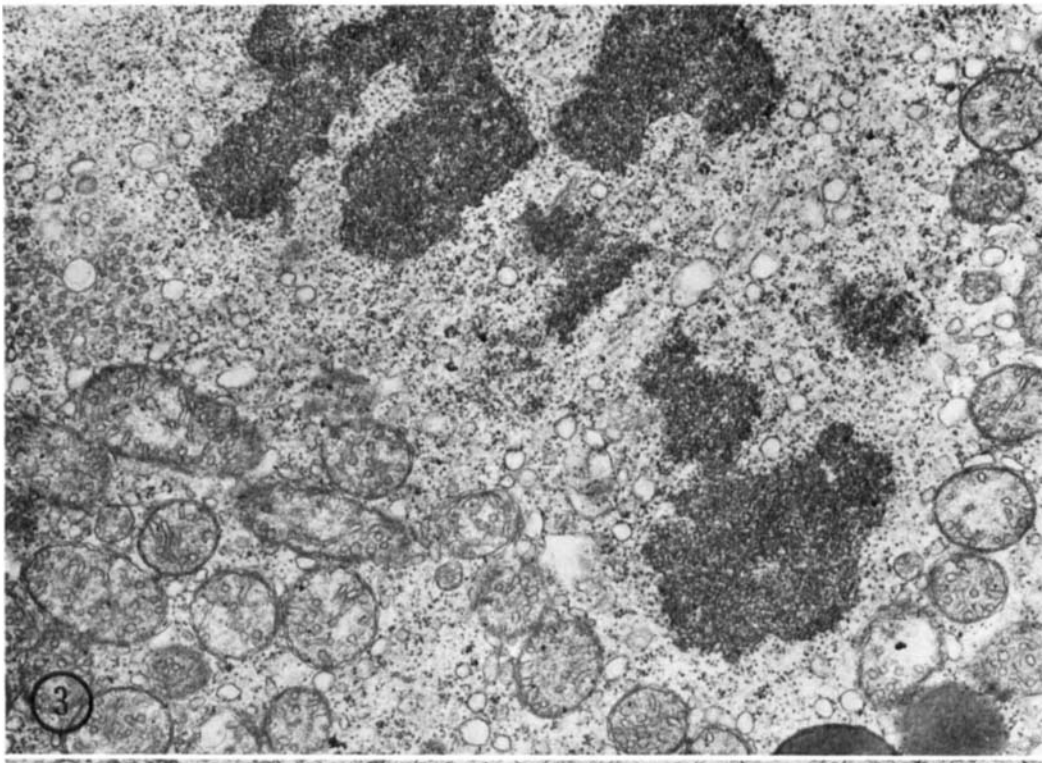
PLATE 2

EXPLANATION OF FIGURES

3 A cortical cell in mitosis during ACTH treatment. Note the high amount of single ribosomes and poorly developed smooth surfaced endoplasmic reticulum membranes and inner membranes of mitochondria. Cultivated 25 days and treated with ACTH. × 20,000.

4 Annulated lamella as shown here are very frequently seen in cultivated cortical cells of fetal albino rats of the Charles River strain. Cultured 25 days and treated with ACTH. × 40,600.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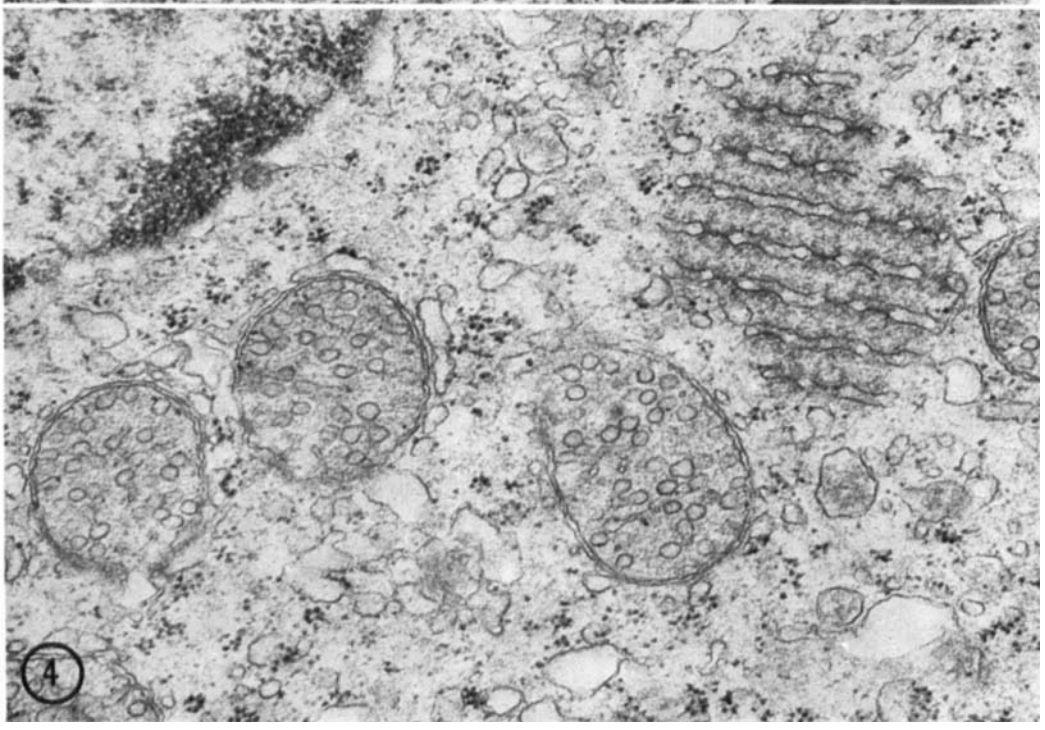
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PLATE 3

EXPLANATION OF FIGURE

5 Cortical cells in monolayer colony in tissue culture of fetal rat adrenals, treated with 0.6 μ mole/ml of chloramphenicol for six days from the nineteenth day of cultivation up to and including the twenty-fourth day. Mitochondria are slightly decreased in size but still contain tubular or lamellar cristae and numerous intramitochondrial dense granules. Cultivated 25 days. $\times$ 13,650.

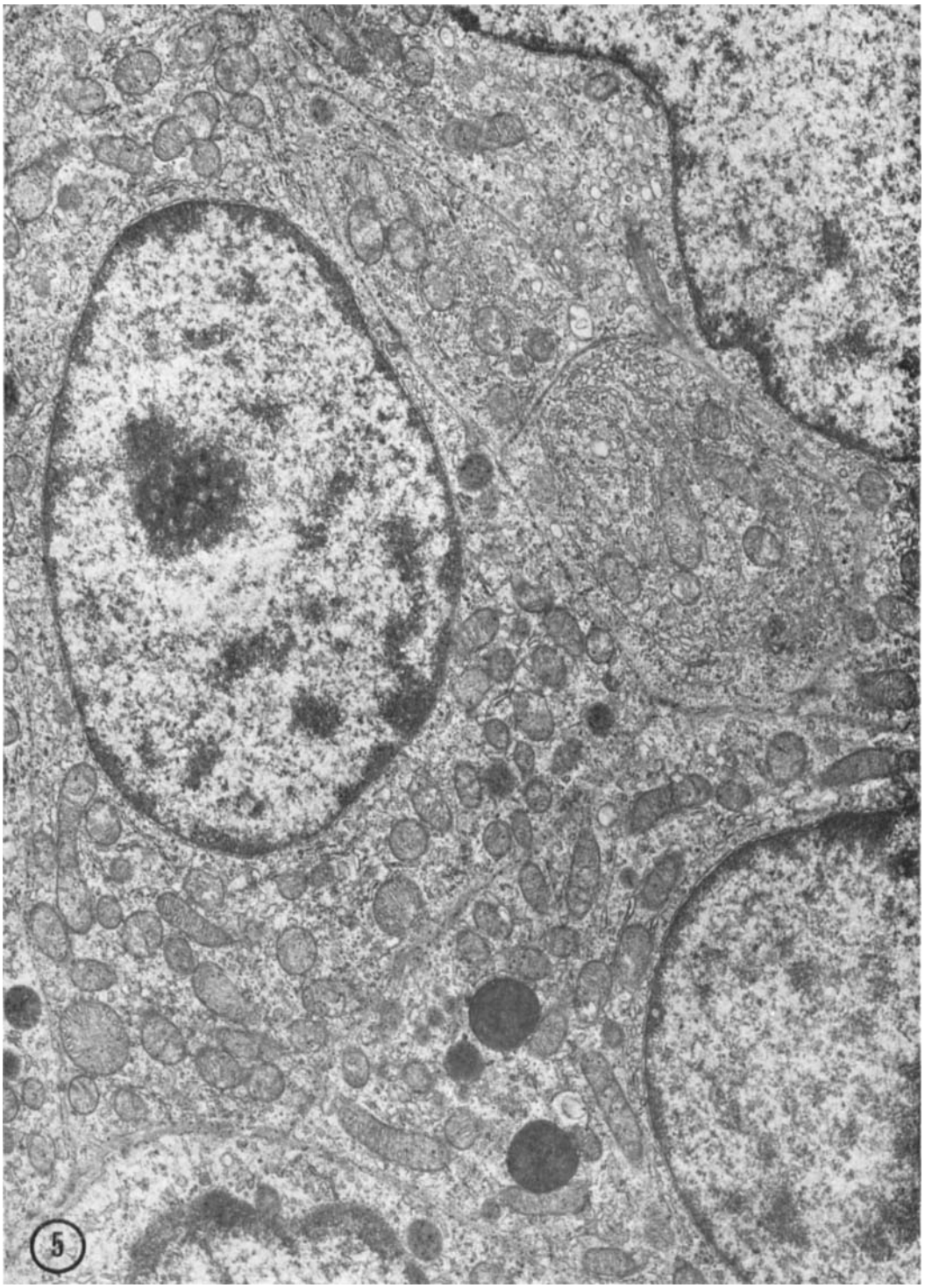
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PLATE 4

EXPLANATION OF FIGURES

6–7 Mitochondria in cortical cells in tissue culture of fetal rat adrenals treated with 0.3 μ mole/ml of chloramphenicol as described in figure 5. Note the numerous small granules in the mitochondrial matrix. They have a similar ultrastructure as cytoplasmic ribosomes but are smaller. $\times$ 49,500 and 99,000.

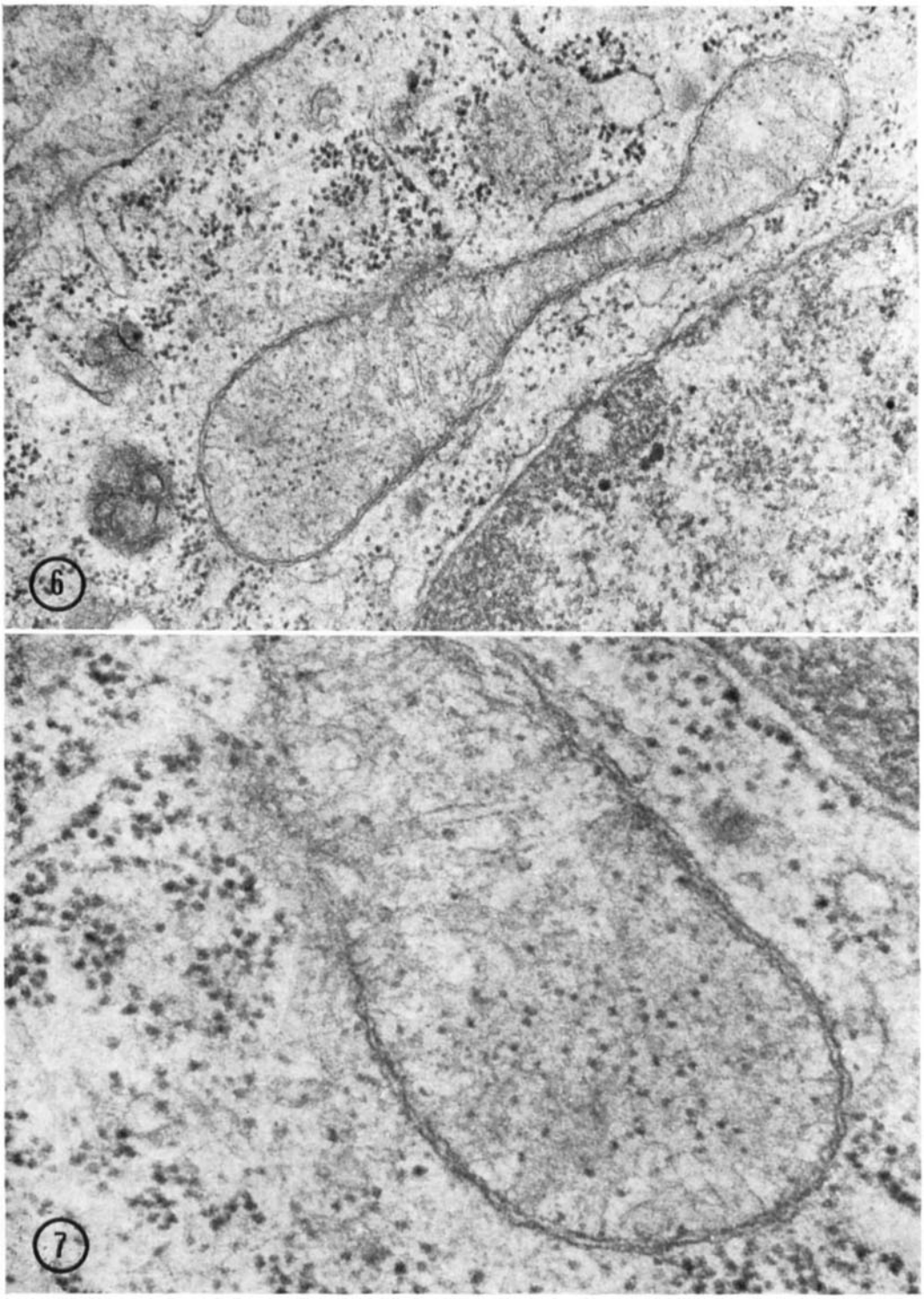
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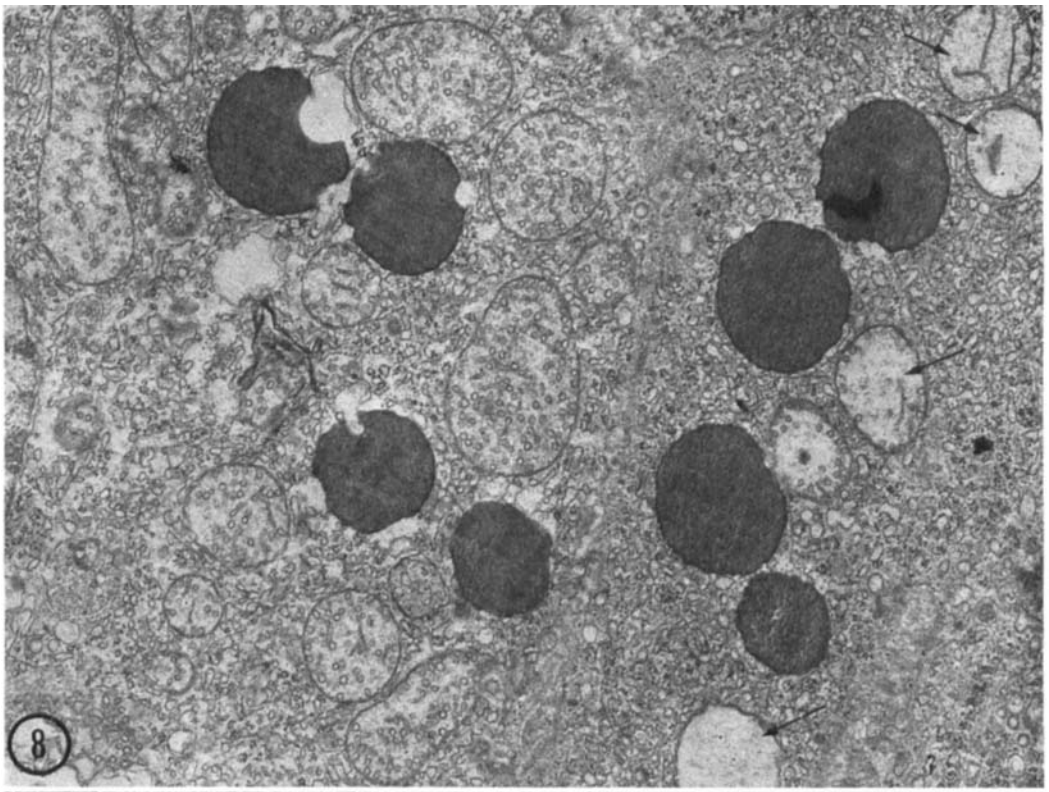
PLATE 5

EXPLANATION OF FIGURES

8 Cortical cells in monolayer colony in tissue culture of fetal rat adrenals treated with 0.03 μ mole/ml of chloramphenicol and 0.1 I. U./ml of ACTH for six days from the nineteenth day of cultivation up to and including the twenty-fourth day. Incomplete inhibitory effects of chloramphenicol on the ultrastructural organization of inner membranes of mitochondria are visible. In some mitochondria the organization of inner membranes is completely inhibited (arrows), but in other cells the mitochondria contain moderate amounts of well developed 600 Å vesicles. Other cellular organelles show no signs of chloramphenicol inhibition. × 15,900.

9 A cortical cell which had undergone mitosis during treatment with 0.03 μ mole/ml of chloramphenicol and 0.1 I. U./ml of ACTH. Note the incomplete inner limiting membrane in some mitochondria and complete lack of one limiting membrane in another and a few 600 Å vesicles in mitochondria with only a limiting membrane and an incomplete inner limiting membrane. × 34,800.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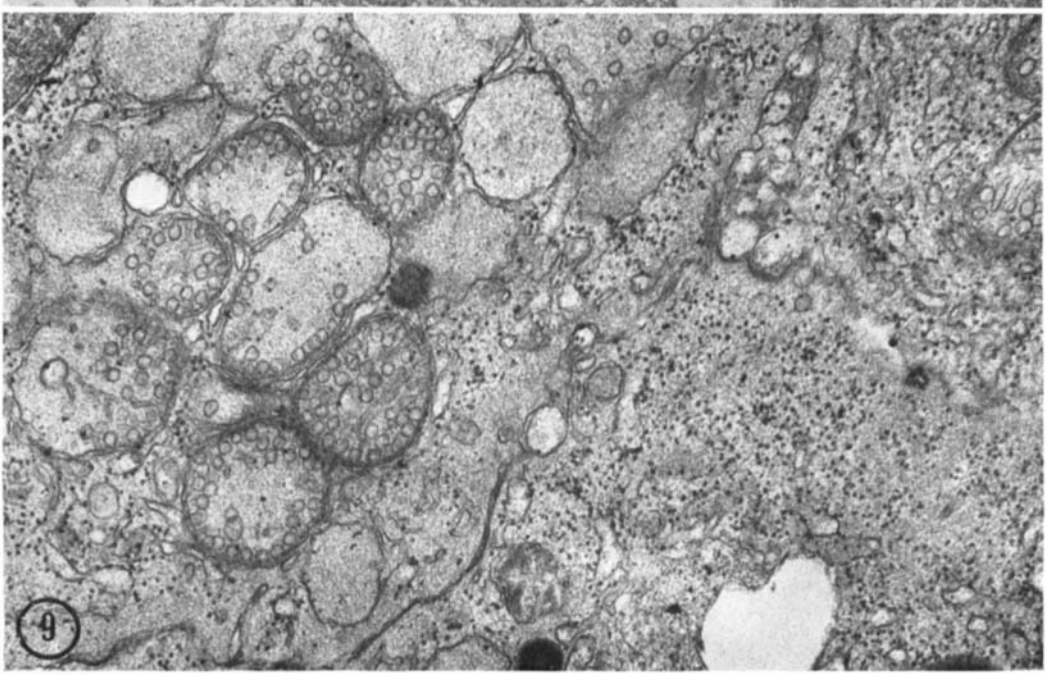
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PLATE 6

EXPLANATION OF FIGURE

10 Mitochondria in the cortical cells in tissue culture of fetal rat adrenals cultivated 25 days and treated with 0.3 μ mole/ml of chloramphenicol and 0.1 I. U./ml of ACTH for six days. Chloramphenicol has completely inhibited this ACTH-induced modification of mitochondrial inner membranes. Note that some mitochondria completely lack cristae and have a matrix of very low electron density. Note the well developed smooth surfaced endoplasmic reticulum membranes and a few sequestra in the cytoplasm. $\times$ 20,000.

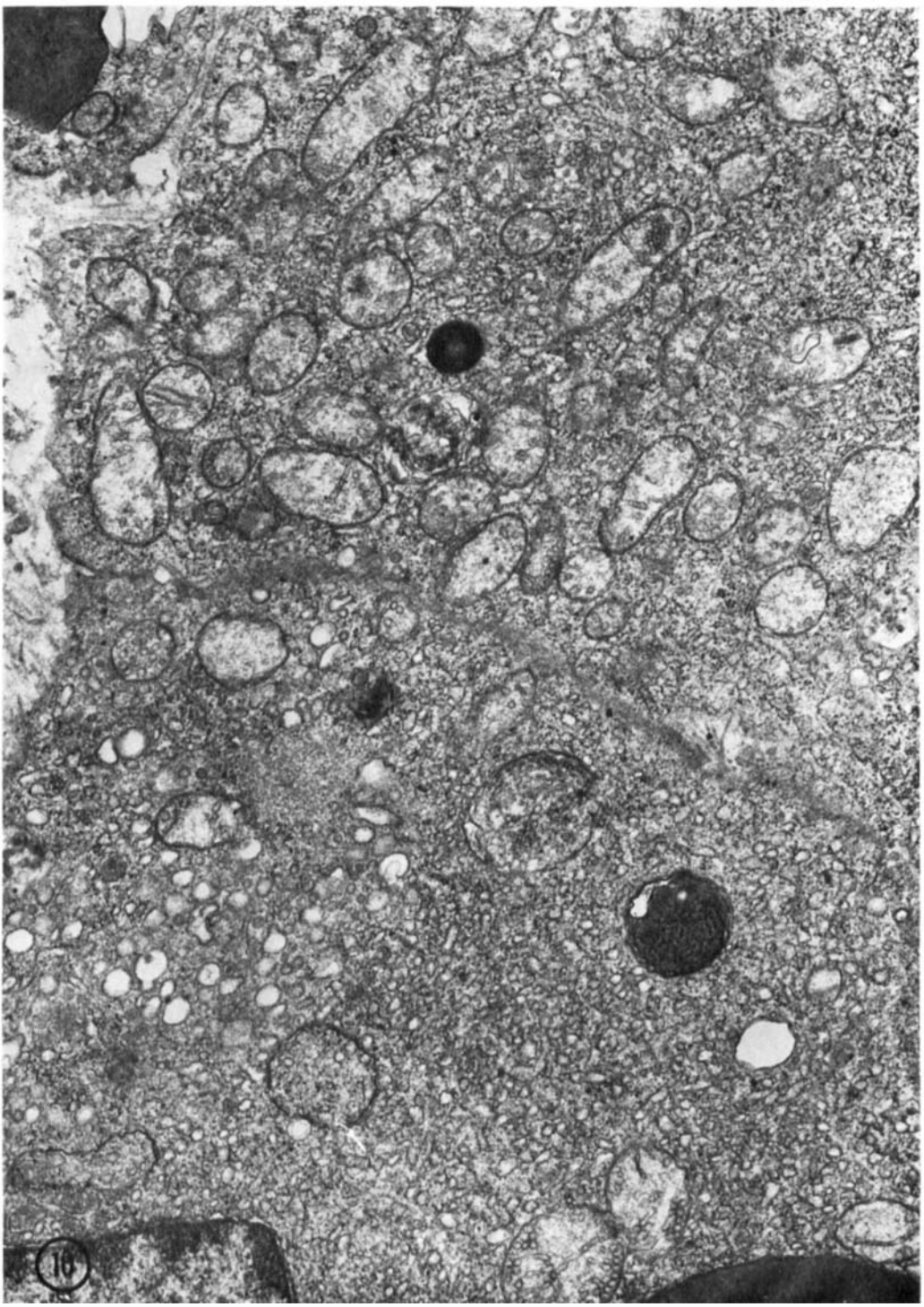
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PLATE 7

EXPLANATION OF FIGURE

11 Cortical cells in monolayer colony in tissue culture of fetal rat adrenals treated with 0.6 μ mole/ml of chloramphenicol and 0.1 I. U./ml of ACTH for six days. A complete inhibition of formation of mitochondrial cristae (600 Å vesicles) has occurred. Chloramphenicol has induced no inhibition of development of other cytoplasmic organelles. Cultivated 25 days. $\times$ 13,600.

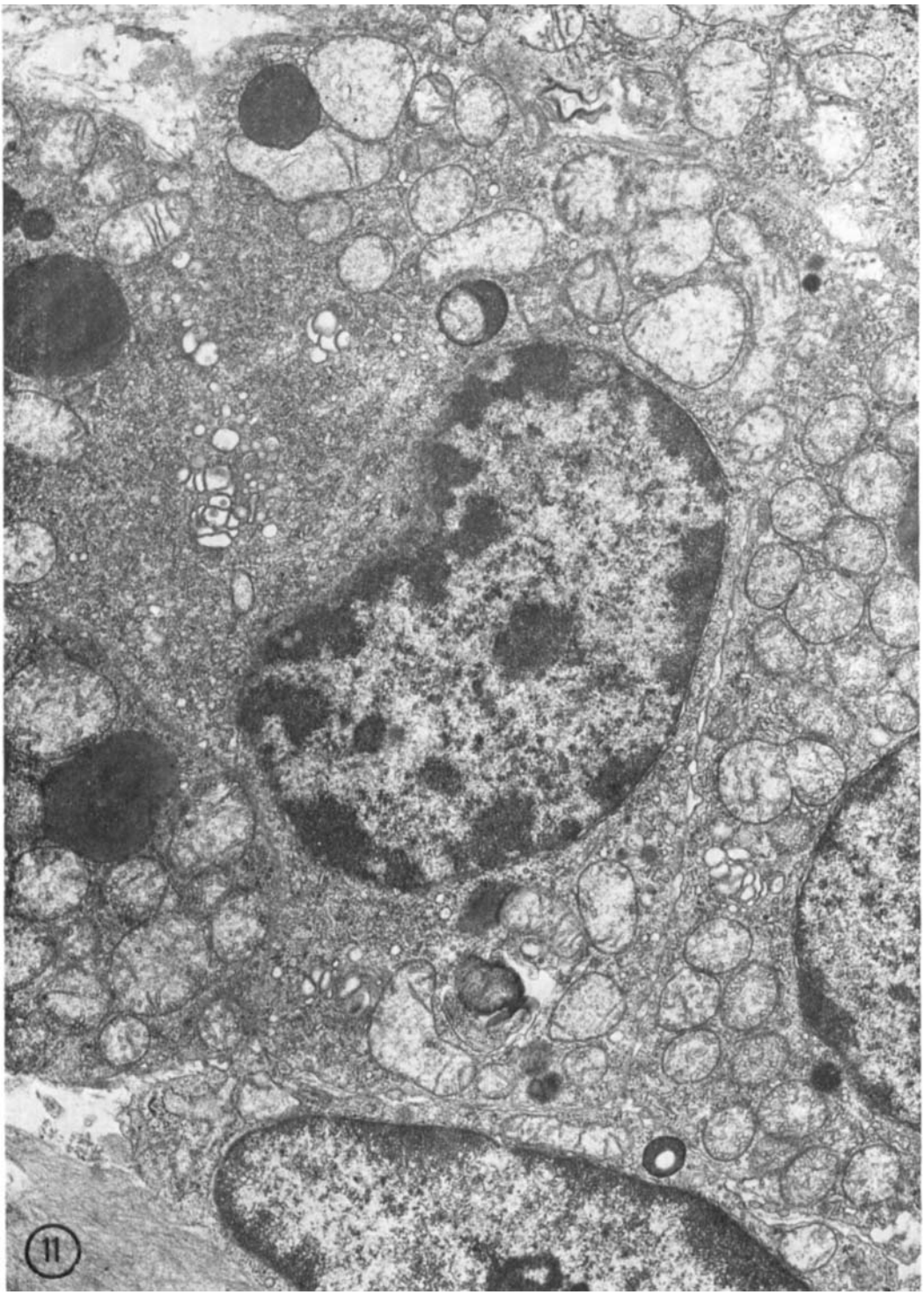
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PLATE 8

EXPLANATION OF FIGURES

12–13 Mitochondria in cortical cells treated with 0.6 μ mole/ml of chloramphenicol alone (fig. 12) and together with 0.1 I. U./ml of ACTH (fig. 13) for six days. Chloramphenicol has induced complete inhibition of formation of inner mitochondrial membranes (cristae) but has not inhibited the ACTH-induced growth of both limiting membranes of the mitochondria. Cultivated 24 days. $\times$ 34,800.

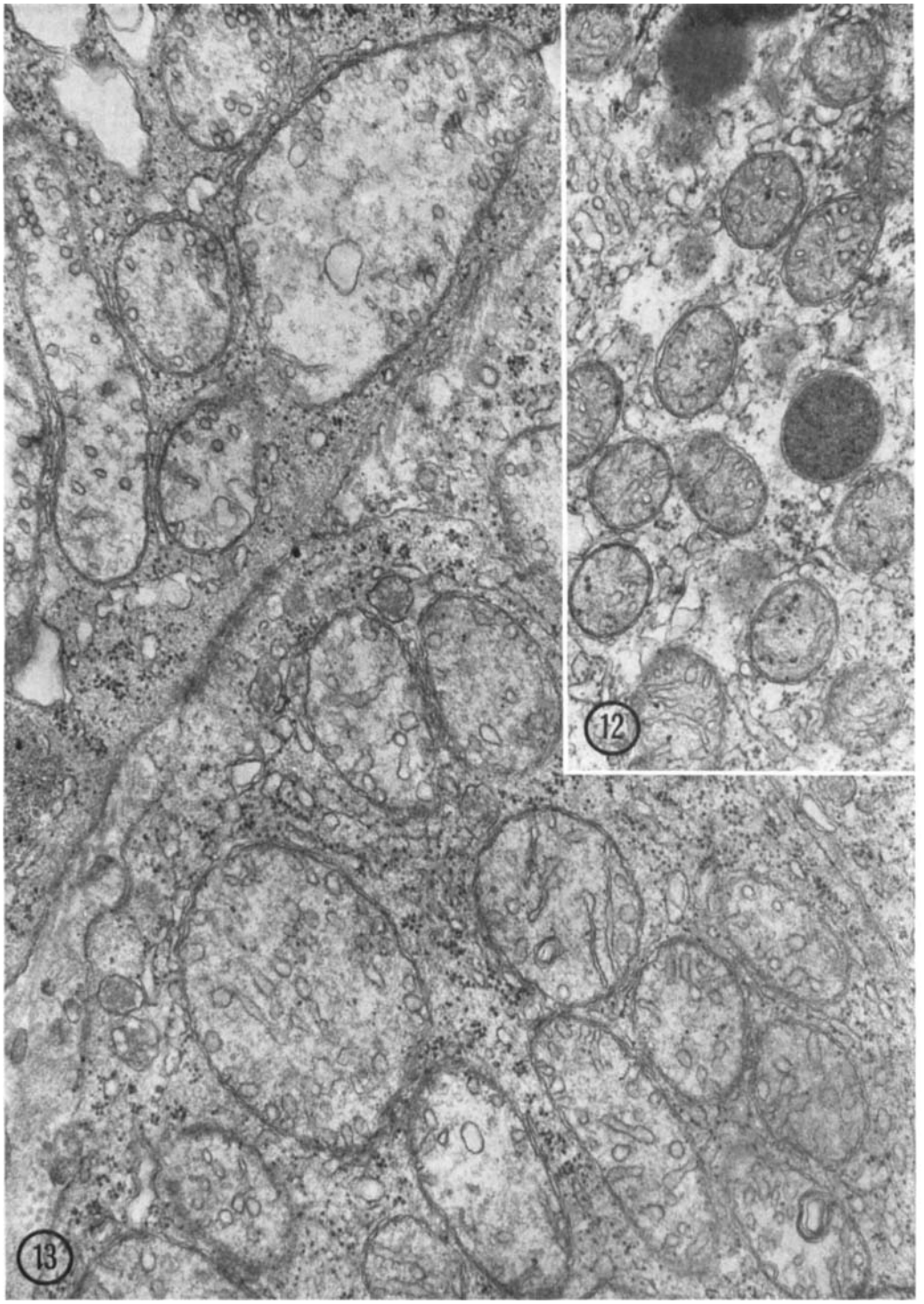
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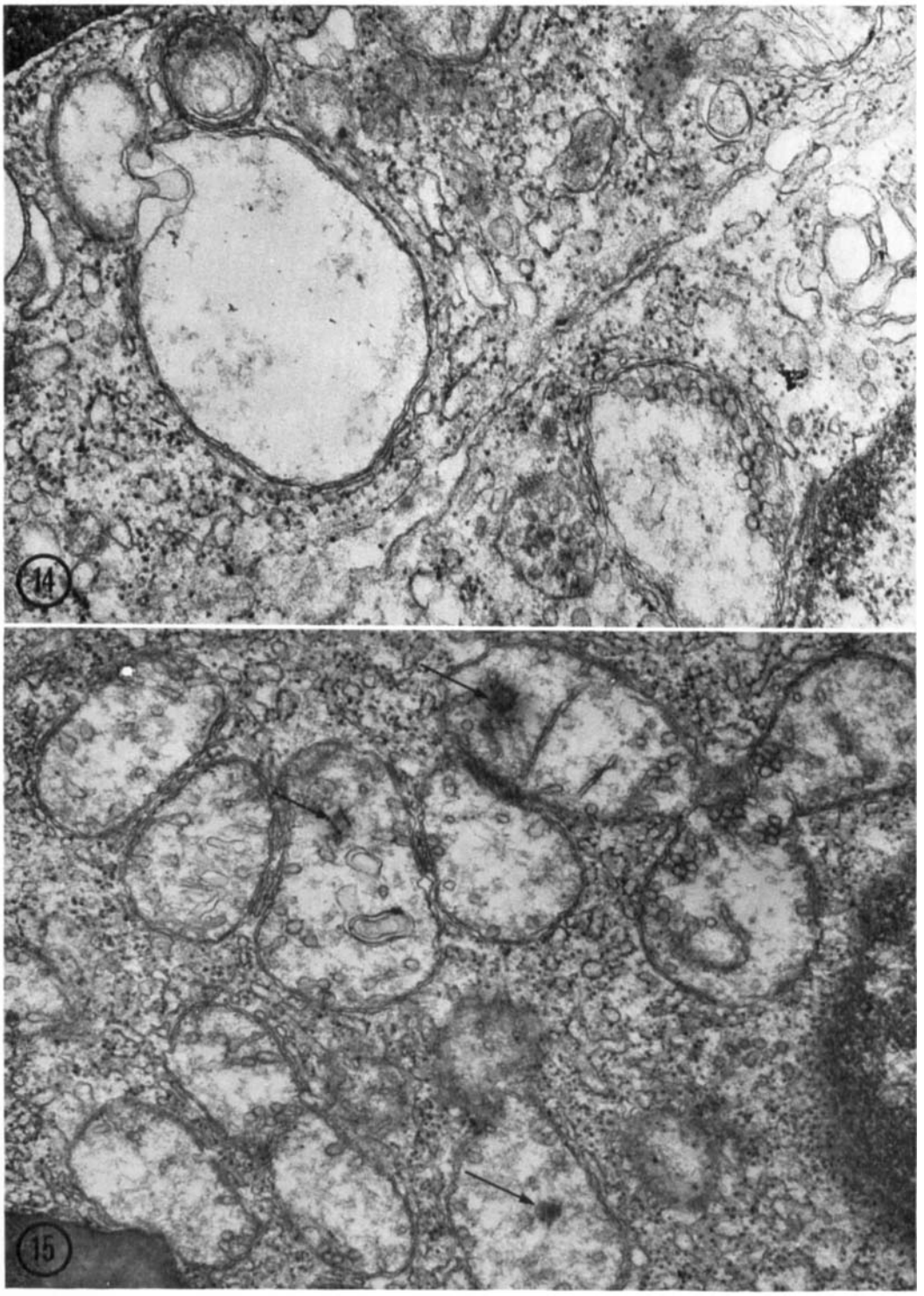
PLATE 9

EXPLANATION OF FIGURES

14 Mitochondria in cortical cell treated with 0.6 μ mole/ml of chloramphenicol and 0.1 I. U./ml of ACTH for six days. There is a complete lack of mitochondrial cristae after chloramphenicol inhibition. Cultivated 25 days. $\times$ 41,200.

15 Mitochondria in cortical cells treated with 0.6 μ mole/ml of chloramphenicol and 0.1 I. U./ml of ACTH for six days. Only a few regular 600 Å vesicles are visible in the matrix, which has a very low electron density and contains some irregular tubular or lamellar cristae and very frequently one or more electron dense bodies (arrows). Cultivated 25 days. $\times$ 33,000.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