Some Observations on the Fine Structure of the Adrenal Cortical Cell of Domestic Fowl.

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Since the electron microscopic works of LEVER (1955), and BELT and PEASE (1956), the mitochondrial crests of the adrenal cortical cell in most mammals have been believed to be not-laminar but villous or tubular. Though this structure in the adrenal cortical cell of the domestic fowl is also villous or tubular as FUJITA (1961) reported, the characteristic crests were found to be described in the present paper.

Concerning the new formation of mitochondria, various observations have been published for several organs. FAWCETT (1955), FREY-WYSSLINC (1960), LAFONTAINE (1960), SHERIDAN (1960), and DAVID (1962) reported the dividing of the mitochondria in several organs, and GANSLER and ROUILLER (1956), ROUILLER and BERNHARD (1956), OBERLING, ROUILLER and DONTCHEFF (1956), OBERLING (1959), and ROUILLER (1960) found the mitochondrial formation from the microbody. Then, BRANDT and PAPPAS (1959), and HOFFMAN and GRIGG (1958) suggested that the mitochondria are originated from the nuclear membrane, and ROBERTSON (1959) proposed a hypothesis that mitochondria arise from the cell membrane. In the adrenal cortical cell of the embryos and young chicks, we noticed the mitochondrial dividing in a fairly large number.

The production mechanism of the secretory substance in this organ has also been discussed by many investigators. LEVER (1955) and BELT (1958) concluded that some of the mitochondria transform into the liposomal or fat droplets, while ROSS, PAPPAS, LANMAN and LIND (1958) suggested the relationship between the agranular endoplasmic reticulum and steroid hormone, and FUJITA (1961) has believed that the lipid implicated in cortical hormone might be in the cytoplasmic vacuole. Concerning the production mechanism of the secretory material, several facts were found to be added to the previous paper.

I. Materials and Methods.

About twenty White-Leghorn domestic fowls, ranging in age from 16 days of incubation to 10 days after hatching were used in this study. The adrenal gland was removed, cut into several pieces and fixed in the 1% osmium-tetroxide solution buffered at pH 7.35 by PALADE’s solution. After 1 hour fixation, the materials were dehydrated with alcohol and embedded in epoxy-resin by LUPT’s method.
sections made with a PORTER-BLUM ultramicrotome and stained by saturated uranyl-acetate water solution, were examined with a HU-11 A type electron microscope.

II. Observations.

In the domestic fowl, it is impossible to differentiate three zones in the adrenal

Fig. 1. Part of a normal adrenal cortical cell of a 2 day-old chick. Several mitochondria with tubular crests, and cytoplasmic vacuoles are seen. ×52,000
cortex, because the cortical tissue is distributed in the form of irregularly arranged columns throughout the gland. As FUJITA (1961) reported, in the adrenal cortical cell, a large proportion of the cytoplasm is made up by numerous mitochondria and cytoplasmic vacuoles.

Though mitochondria are oval and have irregularly arranged, villous or tubular crests projecting from the inner limiting membrane as reported for several animals, some of the crests are parallel with one another in a few mitochondria (Figs. 1 and 2), and rarely the crest reaches the inner limiting membrane of the opposite side forming a bridge-like structure (Fig. 3). However, it is not clear whether this bridge-like crest is laminar or tubular. A most striking feature in the mitochondrion is an occurrence

Fig. 2. Part of a normal adrenal cortical cell of a 2 day-old chick. In a few mitochondria some crests are parallel with one another. Several smooth-surfaced cytoplasmic vacuoles are seen.

× 52,000
of the circinate tubular crest (Fig. 4). This is found in a few mitochondria of cortical cells in some normal chicks. One of the longest tubular crests which whirls for 5 times in the mitochondrion is about 10 μ in total length. This crest starts from the inner mitochondrial membrane and ends at the center of the whirlpool as a blind tube. In this mitochondrion, except for this long crest, there are also many short villous crests as usual (Fig. 4). DE ROBERTIS and SABATINI (1958) who described the several kinds of mitochondrial change in the cortical cell of the normal hamster, did not find such a long crest as this.

The diameter of the tubular crests, including limiting membranes is about 20~25 m. There are some vesicles, about 20~35 m in diameter, in the mitochondrion. Comparing the diameters of them, the vesicles are considered to be transverse or oblique sections of tubular crests. A few mitochondria have one or two large vacuoles, round, oval or irregular in shape, with low internal density in the center or periphery of this organoid (Fig. 5.) The vacuole is about 0.1~0.4 μ in diameter and might be originated from the enlarged tubular crest.

In the cortical cells of embryos and young chicks, the figures suggesting the mitochondrial dividing were found (Figs. 6 and 7). The dividing process is as follows: First, a transverse bridge-like crest appears in a mitochondrion, and secondly, furrows of the outer limiting membrane of the mitochondrion appear at both terminal points of this bridge-like crest. Then the furrows become deeper and deeper to make the mitochondrion divide into two. The size of both daughter mitochondria is not always equal.

A number of cytoplasmic vacuoles which FUJITA (1961) described as endoplasmic reticulum, with a distinct limiting membrane, are noticed in the cortical cell (Figs. 1 and 2). These vacuoles are usually in contact with mitochondria. Though most vacuoles look smooth-surfaced, ribosomes are scattered in the cytoplasm not only among the cytoplasmic vacuoles and mitochondria, but also on the outer surface of a few cytoplasmic vacuoles. As FUJITA (1961) reported, in the cortical cells of embryos and

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Fig. 3. Part of a normal adrenal cortical cell of a 10 day-old chick. A bridge-like crest is noticed in a mitochondrion. ×33,000
chicks, there are no highly dense droplets which are usually seen in a fairly large number in the adult domestic fowl. Moderately dense homogeneous substances are found in a very few cytoplasmic vacuoles of young chicks (Fig. 8). This substance which tends to accumulate to the central portion of the vacuole in the osmium fixed and epon embedding section, might be a lipid related to steroid hormone. Sometimes the cyto-
plasmic vacuoles containing the osmiophilic substance are seen near the plasma membrane to be secreted into the pericapillary region (Fig. 9). Most of the cytoplasmic vacuoles, low in electron density, are considered to contain diluted lipid, because the Sudan black B reaction is positive throughout the cortical cell from 10 day-old embryos.

Concerning the perivascular structure, YAMORI, MATSUURA and SAKAMOTO (1961) described the subendothelial space without basement membranes between cortical cells and endothelial ones in the rat. However, we found two basement membranes and perivascular space as shown in the other endocrine organs (Fig. 9). In some cortical cells of embryos and young chicks, centrioles showing an usual structure are found near the nucleus (Fig. 10).
III. Discussion.

DAVID and KETTLER (1960) reported that, in the liver, the vacuole appears in the mitochondrion as a sign of degenerative change of this organoid. However, BARGMANN and KNOOP (1960) and YAMORI, MATSUURA and SAKAMOTO (1961) found vacuolated mitochondria in the secretory cells of the normal anterior pituitary and in the adrenal cortical cells of normal and stressed animals respectively.
Fig. 7. Mitochondrial dividing in a cortical cell of a 2 day-old chick. × 65,000
From our observations, all normal embryos and chicks have not always the mitochondrial vacuoles in the adrenal cortical cell, and some normal animals have vacuolated mitochondria in the cortex. This fact suggests that the appearance of the vacuole in the mitochondrion might be related to the functional state of this gland.

There have been published numerous theories, as cited in the preface, on the formation of mitochondria. We observed the mitochondrial dividing in the adrenal cortical cell of the embryos and young chicks. No other mechanisms for mitochondrial formation have not yet been found in this organ, but the mitochondria are sometimes situated near the nuclear or plasma membrane, and we could not deny the relationship between mitochondria and plasma membrane.

Concerning the lipid formation in the cortical cell, LEVER (1955), and YAMORI,
MATSUURA and SAKAMOTO (1961) published that the granules related to steroid hormone are originated from mitochondria, while ROSS, PAPPAS, LANMAN and LIND (1958) suggested the relationship between the agranular endoplasmic reticulum and steroid hormone. FUJITA (1961) also discussed on this problem in the previous paper, and concluded that the lipid related to steroid hormone is in the cytoplasmic vacuole (endoplasmic reticulum). This view seems to be supported by the evidence that the moderately dense substance was found in a few cytoplasmic vacuoles. The lipid implicated in cortical hormone might be contained not only in the moderately dense vacuoles but also in most cytoplasmic vacuoles of low electron density. The grade in osmium staining of vacuoles might depend on the concentration of the lipid which is considered to become denser with age. Previously, FUJITA (1961) suggested that the osmiophilic substances which appear in mitochondria or within cytoplasmic vacuoles in adult or old domestic fowls are related to aging. However, some of the highly dense droplets accumulated in the cytoplasmic vacuoles are considered to be a highly condensed lipid related to steroid hormone, and the dense droplets elaborated from mitochondria may be a substance related to aging. To conclude, we believe that the

Fig. 9. A large cytoplasmic vacuole containing the moderately dense substance is near the pericapillary region. ×55,000
Fig. 10. A centriole in a cortical cell of a 3 day-old chick. \( \times 50,000 \)

cortical lipid, which is not always osmiophilic in the diluted state in the embryos and young chicks, is produced in the cytoplasmic vacuole with the aid of mitochondria which are usually near them, and it becomes denser and more osmiophilic with age.

IV. Summary.

Normal adrenal cortical cells of embryos and young chicks of White-Leghorn domestic fowl were examined with the electron microscope.

1. Though most mitochondrial crests are not laminar but villous or tubular and are arranged irregularly in the cortical cell, some crests are parallel with one another in a few mitochondria, and long circinate tubular crests were rarely found. A few vacuolated mitochondria were found in the cortical cells of some normal embryos and chicks.

2. Mitochondrial dividings were noticed in a fairly large number in the cortical cell of the embryos and young chicks.

3. Moderately dense substances were observed in a few cytoplasmic vacuoles (endoplasmic reticulum) of the cortical cells in the young chick. Relationships among
secretory substances, cytoplasmic vacuoles and mitochondria were discussed. We assume that the lipid implicated in cortical hormone is produced in the cytoplasmic vacuole with the aid of mitochondria.

内 容 自 抄.

白色レグホン種家鶏の孵卵16日から孵化後10日までの若鶏の副腎被質を電子顕微鏡で観察して、Fujita (1961) が発表したものに補遺すべき2, 3 の知見を得た。鳥類の皮質は細胞質と混在し、哺乳類のような3層を形成せず、哺乳類のそれとはかなりおもむきを異にする。

家鶏の副腎皮質細胞の糸粒体の機能は一般に管状で不規則であるが、少数の糸粒体では数本の管が平行に並んでいる。細胞に長く浸漬した（時には5回）、10μ に達する管状の管が見出された。また空胞をもった糸粒体も時々存在する。胎児や若鶏の皮質細胞にはかなり多数の糸粒体の分裂像が見られる。

細胞質の大部分は糸粒体と、これに接する滑面（少数は粗面）空胞からなっているが、これらの間にレリボーム (ribosomes) が散在し、細胞中心小体が見られる。滑面空胞の中には細胞内密度の電子密度をもった、均質な物質が見られ、この空胞中にホルモンに関係深い物質の存することを思わせる。Fujita (1961) のいうように他の大部分の低電子密度の空胞中にも、稀薄な、これと同様の物質が含まれるものと推測される。これらの所見から皮質の分泌物は、滑面（または粗面）小胞体の中に、それに接する糸粒体の作用を受けて形成されるものと考えられる。

References.

Some Observations on the Fine Structure of the Adrenal Cortical Cell of Domestic Fowl. 89


