

# Observations on the Fine Structure of Propylthiouracil-Induced "Brown Degeneration" in the Zona Reticularis of Mouse Adrenal Cortex<sup>1</sup>

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**ABSTRACT** Propylthiouracil (6-propyl-2-thiouracil), an anti-thyroid agent, was fed to mice in a concentration equal to 0.1% of their diet for periods of 10 and 15 weeks. The cells of the inner zone of the adrenal cortex were examined with the electron microscope. In animals receiving propylthiouracil for ten weeks mitochondria were altered and the smooth endoplasmic reticulum (SER) showed a marked focal proliferation. In contrast to control animals rough endoplasmic reticulum was abundant and was frequently associated with the hyperplastic SER. After 15 weeks these alterations were no longer present but had been replaced by a spectrum of "brown degeneration." The less affected cells were characterized by increased numbers of liposomes and lysosomes and the more affected cells by liposomal and mitochondrial degeneration. These observations emphasize that "brown degeneration" is a true degenerative process and not a spontaneous proliferation of ceroid pigment. It is suggested that the changes described may be directly related to an alteration in cholesterol metabolism.

"Brown degeneration" is a lipid pigmentation process which occurs in the adrenal glands of mice under certain experimental conditions. This phenomenon has been described in mice following treatment with estrogen hormones (Cramer and Horning, '37; Schardein et al., '67), after gonadectomy (Frantz and Kirschbaum, '49), and after hypophysectomy of gonadectomized animals (Bern et al., '59). In an extensive study Schardein and associates ('67) confirmed the ceroid nature of the pigment and described the pigmentation process at the fine structural level. The present report is concerned with the occurrence of propylthiouracil-induced "brown degeneration" and with additional observations on the development of this phenomenon.

## MATERIALS AND METHODS

Twenty-eight adult female albino Swiss mice were divided into two experimental groups (10 animals each) and one control group (8 animals). The animals of the experimental groups were fed a diet containing 0.1% propylthiouracil (PTU) for

periods of ten weeks (group 1) and 15 weeks (group 2). Under sodium pentobarbital anesthesia all mice were prepared for electron microscopy by perfusion through the left ventricle. Infusion of the fixative was preceded by the administration of a cold normal saline prewash containing 0.5% procaine hydrochloride and 100 mg of sodium heparin per 100 cm<sup>3</sup> of solution in order to flush and vasodilate the animal's circulatory system. The fixative used was 3% glutaraldehyde in 0.1 M phosphate buffer at a pH of 7.4 and an osmolarity of 500–550 mosm. After the perfusion was completed, the adrenal glands were removed, quartered, and placed in cold fixative for an additional 2-hour period. The tissue was then washed in cold buffer and post-fixed in 1% osmium tetroxide in 0.1 M phosphate buffer. Subsequently, the tissue was dehydrated in a graded series of cold alcohols and em-

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bedded in Epon (Luft, '61). Care was taken to orient each quarter of gland such that each section cut would demonstrate the capsule, each cortical zone, and medulla. One micron thick sections were cut from each block and viewed for selection of the area to be examined. Thin sections were made with a diamond knife on an MT-2B Porter-Blum ultramicrotome and picked up on copper grids. The tissues were stained with lead citrate and uranyl acetate to enhance contrast and were viewed with an RCA-EMU-3G or a Philips 201 electron microscope.

## RESULTS

### *Control*

For this study only the innermost cells of the cortex were of interest, therefore, the description of control mouse adrenal cortex will be limited to the zona reticularis (fig. 1). Observations on the fine structure of normal mouse adrenal cortex described here are essentially as reported by Wetzstein ('57) and Zelander ('59, '64).

The reticular zone consisted of a layer 2-3 cells thick adjacent to the medulla. Numerous microvilli were present on the cell surface. Nuclei were large, irregularly spherical, and often lacked a distinct nucleolus. Small, oval mitochondria occupied much of the cell. Internal mitochondrial appearance varied greatly but was essentially found to consist of straight, parallel membranes (rectimembranous) or short tubules with dilated ends (tubulosaccular). Occasional concentric, parallel (cyclomembranous) mitochondrial membranes were seen. The cells contained sparse, although varied, numbers of liposomes and often no liposomes at all. Golgi complexes were usually small and compact. Numerous lamellar membrane structures were seen which, when cut in full section, appeared as closely packed parallel, circular membranes resembling a cut onion. The smooth surfaced endoplasmic reticulum was tubular and most obvious adjacent to mitochondria. Dense lysosomal structures which were uniform in size and appearance were scattered randomly throughout the cell. Small liposomes were seen often in relation to the Golgi complex. Small clusters

of free ribosomes appeared randomly in the cytoplasm.

### *Experimental*

The most prominent of the changes observed after ten weeks treatment with PTU (figs. 2, 3) was a marked proliferation of the endoplasmic reticulum. Hyperplastic ER often occupied large areas of the cell. While predominantly of the smooth variety, combinations of rough and smooth ER were common and in some cases appeared to be intimately related. Most ribosomes were associated with membranes in contrast to the free ribosomes seen in the controls. Numerous small liposomes were grouped throughout the cell and in many cells were associated with the proliferations of endoplasmic reticulum. Mitochondria were larger and more varied in shape, tending to be elongated. While the internal structure of control mitochondria was distinctive and clearly visible, the internal mitochondrial structure of treated zona reticularis cells was indistinctive and blurry. Cristae appeared as short tubular or tubulosaccular projections of the inner mitochondrial membrane. Lysosomes were an infrequent component of these cells.

After 15 weeks treatment with PTU the structure of reticular cells differed remarkably from the pattern seen at ten weeks. "Brown degeneration" was obviously present in the reticular zone; however, individual cells were affected to varying degrees with some cells containing no pigment and others containing large amounts. The complex membrane structures present at ten weeks were conspicuously absent (fig. 4).

In cells which contained no pigment the tubular smooth endoplasmic reticulum and Golgi complexes were well developed. Free ribosomes were numerous in the cytoplasm; however, only a small amount of granular endoplasmic reticulum was present. Mitochondria were larger than in controls and varied in shape from spherical to elongated. In other cells in which mitochondrial alterations were more striking, tubulosaccular elements were not as common and most mitochondria had straight parallel cristae or concentrically arranged parallel cristae (figs. 4, 5). Liposomes varied greatly in number from cell to cell but appeared to be significantly increased.

Large lysosomes were a common component and often appeared to encroach on neighboring liposomes (fig. 4).

In cells in which the pigmentation process had begun, lysosomes were abundant and usually associated with clusters of liposomes. In their earliest recognizable form small pigment bodies were consistently related to groups of liposomes and were of like size and electron density. Cells which possessed numerous small pigment bodies contained few liposomes; that is, the more pigment observed in a cell, the fewer the number of typical liposomes observed (fig. 5). Mitochondria which resembled a bull's-eye were common in these cells and frequently possessed numerous concentrically arranged, parallel membranes.

Cells which displayed advanced stages of "brown degeneration" contained clusters of mature pigment bodies and other structures which consisted of concentric, membranous lamellae (fig. 6). Additional parallel membranes surrounded individual pigment bodies and groups of pigment bodies. In different cells the pigment bodies and membranous structures combined in one or two clusters within the cell which was greatly enlarged (fig. 7). Recognizable organelles of the type usual to these cells were no longer present. The few mitochondria present were small, elongated and possessed a few cristal elements oriented across the short mitochondrial axis.

#### DISCUSSION

Propylthiouracil (PTU) and other thiourea derivatives are known to block the synthesis of thyroid hormones by preventing the uptake and utilization of iodine by the thyroid gland. Although adrenocortical size and function are significantly affected by alterations in thyroid function (Deane and Greep, '47; Money, '55), the effect of thiourea derivatives on the adrenal cortex appears to be independent of the thyroid and pituitary (Money, '55; Steinetz and Beach, '63). Recent ultrastructural studies have revealed that modifications which occur in the adrenal cortex of thyroidectomized and PTU treated rats are distinctly different (Callas, '71; Moore and Callas,

unpublished observations) and lend support to this hypothesis.

Although thiourea effects on the adrenal cortex are well documented (Baumann and Marine, '45; Deane and Greep, '47; Leblond and Hoff, '44; Money, '55), the present report appears to be the first indication that propylthiouracil can induce the pigmentation process referred to as "brown degeneration" and that the process can be preceded by a noticeable alteration in the cellular fine structure. The most striking alteration observed after ten weeks of treatment was the presence of vast amounts of rough and smooth endoplasmic reticulum. The proliferation of smooth endoplasmic reticulum resembles that reported in the rat by Volk and Scrapelli ('64) following the inhibition of cholesterol synthesis by triparanol treatment, by Marek et al. ('71) subsequent to administration of aminogluthethimide which blocks the conversion of cholesterol to pregnenolone, and by Nickerson et al. ('70) after the transplantation of an ACTH, STH, and prolactin secreting tumor. Christensen ('65) has correlated the presence of such proliferations of smooth endoplasmic reticulum with the ability of the cell to produce cholesterol from acetate, and Rhodin ('71) has theorized that the endoplasmic reticulum hypertrophies when stimulated to produce large amounts of hormone for an extended time. In addition to containing coenzyme A which is necessary for the formation of cholesterol from acetate (Olson, '65), the microsomal fraction of steroid producing cells also contains 3- $\beta$ -hydroxysteroid dehydrogenase which converts pregnenolone to progesterone (Hayano et al., '56) and 21-hydroxylase which converts progesterone to 11-deoxycorticosterone (Ryan and Engel, '57). The presence of increased numbers of liposomes largely related to the arrays of smooth ER is compatible with the evidence that lipid is produced in the ER and Golgi (Rhodin, '71) and that lipid droplets store cholesterol and cholesterol esters (Moses et al., '69; Sayers, '50). In view of the work of Dallner and associates ('66a, '67b) on the development of smooth ER in hepatocytes, it is probable that the large amounts of granular ER serve to synthesize the protein com-

ponent of membranes and enzymes of the agranular reticulum. Other investigators have suggested that this function can also be attributed to the granular ER of fetal adrenal (McNutt and Jones, '70) and fetal testis (Black and Christensen, '69).

After 15 weeks the reticularis cells are characterized by the absence of the proliferations of smooth endoplasmic reticulum, the absence of granular endoplasmic reticulum, and the presence of mitochondria which resemble those normally present. The fate of the massive amounts of ER is not known, although it appears that much of the lipid produced by it remains.

Our study is not intended to be an extensive description of the fine structure of "brown degeneration" since our observations for the most part agree with the impressive work of Schardein et al. ('67). Some reconsideration may be in order, however. These investigators described the pigment bodies as being derived by spontaneous proliferation of small pigment granules which in their earliest form possessed "a basic structure not unlike lysosomes" and as having an "intensely positive reaction" for acid phosphatase. Based on our present understanding of the lysosomes in the cell in general (De Duve, '63) and in the adrenocortical cells in particular (Idelman, '70), these membrane bound structures may in actuality be lysosomes rather than immature pigment bodies. Although we have not observed an actual union between these lysosomes and any other cellular organelle, the proximity of lysosomes and liposomes and the reduction in the number of each as the amount of pigment increases suggests that the pigment may be formed by a true lysis of the lipid droplets of the cell. Furthermore, the presence of recognizable mitochondria which contain concentric, parallel membranes leads to speculation that such mitochondria, if degenerative, may give rise to the membranous component of the large pigment bodies. This supposition is of interest inasmuch as Schardein et al. ('67) offer no conjecture as to the fate of the normal cellular organelles and simply allow that "no typical lysosomal remnants" of these organelles were observed. It seems reasonable then that these organelles may

be consumed as part of this process and that "brown degeneration" is true degeneration and not simply the spontaneous production of a ceroid pigment.

Obviously, this phenomenon is unique in that it does not comply to the typical pattern of cellular degeneration described in the inner zones of mammalian adrenal cortex (Idelman, '70), but it is not so unique when compared to cytoplasmic degradation described in other tissues (Hartcroft and Porta, '65; Howes et al., '64; Stenger, '66; Tan and Heptinstall, '69). The cytoplasmic bodies reported in these cases seem to represent a common response to cellular injury and appear to differ in quantity and quality because of the cell type involved and the nature of the treatment (Hruban et al., '63). The large amount of lipid present in the adrenal cells prior to the onset of brown degeneration may be a significant factor in the distinctive appearance of this process. Support for this possibility is found in the remarkable similarity between the supposed lipid component of "brown degeneration" and the degenerative process observed in the renal tubular epithelium of hypercholesterolemic rabbits (Wellman and Volk, '71). Yates et al. ('68) have further shown that the ultrastructure of cytoplasmic bodies present in the adrenal cortex of hamsters after cholesterol inhibition is indeed dependent on the type of inhibitor used.

The factor which initiates the process of "brown degeneration" and how propylthiouracil relates to this factor is unknown. Other investigators have considered the process to be the result of an endocrine dysfunction, a degenerative process related to aging (Chester-Jones, '48), or a degenerative change related to altered metabolism (Firminger, '52; Schardein et al., '67). In view of the present work consideration should be given to the possibility that this phenomenon is related specifically to an alteration in cholesterol metabolism. Whether it might represent a nonspecific effort of the cell to purge itself of a defective or overabundant product or whether the cell is simply so exhausted or otherwise insulted that it can no longer carry on normal function remains to be determined.

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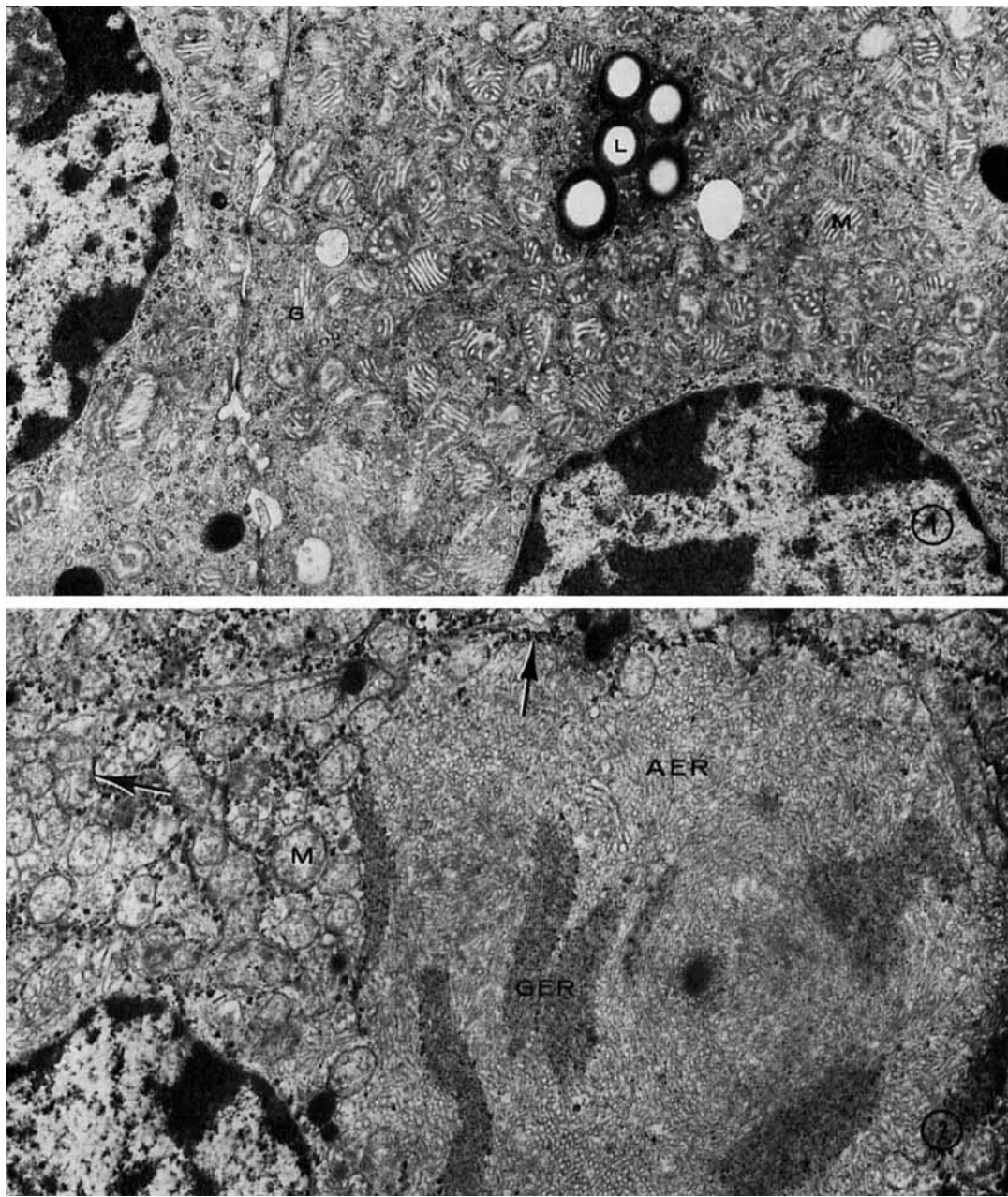
*Abbreviations*

AER, Agranular endoplasmic reticulum	L, Liposomes
G, Golgi apparatus	M, Mitochondrion
GER, Granular endoplasmic reticulum	P, Pigment bodies

PLATE 1

EXPLANATION OF FIGURES

- 1 A survey of cells of the zona reticularis of a control mouse. Numerous small, oval mitochondria (M) fill the cell. The internal mitochondrial structure varies from rectimembranous to tubulosaccular. The endoplasmic reticulum is moderately developed and the Golgi apparatus (G) is small. Few liposomes (L) occur in this zone. Free ribosomes are scattered throughout the cytoplasm.  $\times 14,600$ .
- 2 A reticular cell of a mouse after ten weeks treatment with propylthiouracil. Large proliferations of agranular endoplasmic reticulum (AER) occupy much of the cell. Combination of agranular and granular endoplasmic reticulum (GER) are common. Most ribosomes appear to be associated with membranes (arrows). Internal mitochondrial structure (M) differs greatly from controls.  $\times 14,600$ .

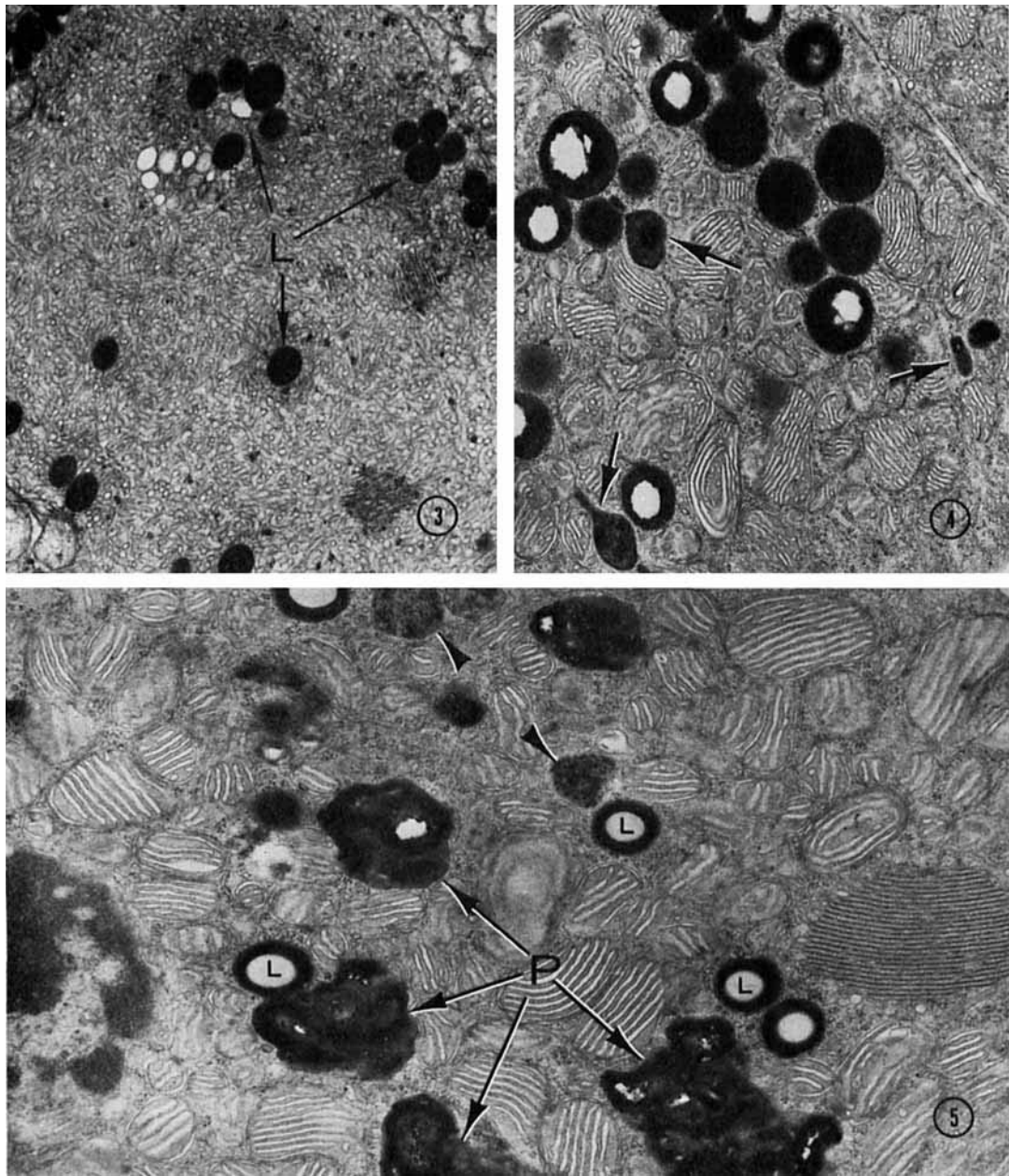


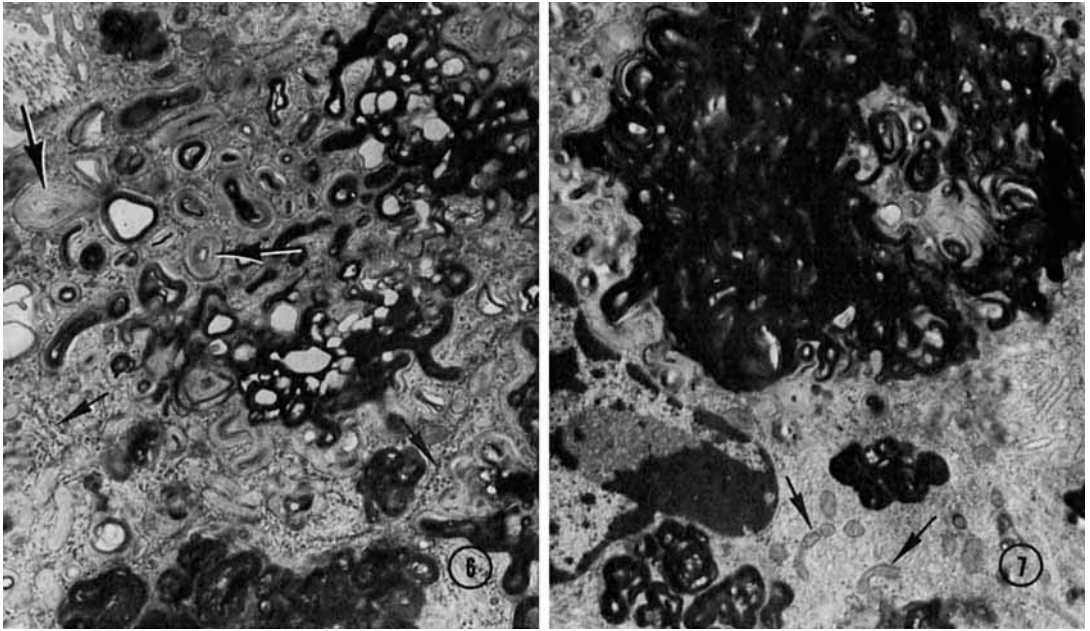
## PLATE 2

### EXPLANATION OF FIGURES

- 3 Details of a zona reticularis cell after ten weeks treatment with propylthiouracil. The numerous tubules of hypertrophic agranular endoplasmic reticulum fill the cell. Small patches of granular endoplasmic reticulum are also seen. Numerous small lipid droplets (L) are present in the cell, often in close association with the proliferations of endoplasmic reticulum.  $\times 14,600$ .
- 4 Portion of zona reticularis cell after 15 weeks treatment with propylthiouracil. These cells contain large numbers of liposomes and many lysosomes (arrows) which are usually closely related to the lipid bodies.  $\times 14,600$ .
- 5 A reticular cell of a propylthiouracil treated mouse after 15 weeks. Several mature pigment bodies (P) are present, and only a few liposomes (L) remain. Lysosomes (arrowheads) always occur in proximity to the groupings of pigment and liposomes. Mitochondria are larger and almost totally consist of straight and concentric membranes.  $\times 16,400$ .







EXPLANATION OF FIGURES

- 6 Other cells are drastically changed after 15 weeks treatment. Mitochondria and liposomes of the type seen in controls are not present. The dark masses are ceroid pigment which may result from a degeneration of the cellular lipid. The structures consisting of concentrically arranged parallel membranes (large arrows) may be mitochondrial in origin. Granular endoplasmic reticulum occurs throughout the cell (small arrows).  $\times 9,680$ .
- 7 This is the most advanced form of "brown degeneration" observed in the propylthiouracil treated mouse. The pigment bodies are clustered together within the cell. A mitochondrial form unlike the usual reticular mitochondrion appears with the cell (arrows).  $\times 9,680$ .