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Received February 19, 1953. P.S.E.B.M., 1953, v82.

Age Changes in the Fine Structure of Anterior Pituitary of the Mouse.* (20147)

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Morphologic changes which have been described in the cells of aging animals include loss of regularity of nuclear and cellular outline(1-3): increased occurrence of cells with giant nuclei(4) and multiple nuclei(5,6); increased amounts of cytoplasmic pigments (1,7,8), and vacuolization of the cytoplasm (3). Recently Payne(9-11) has made extensive observations on cellular changes in the anterior pituitary, adrenal, and thyroid glands of the aging cock. His most striking findings involved swelling and vacuolization of the mitochondria, in some cases so extensive that they destroyed the cell. Jayne(8) has corroborated this work in the adrenal and pituitary of the rat.

To amplify the studies of Payne and others on the morphological changes in cell structure with age, a study of the anterior pituitary gland of the Swiss Albino mouse has been made, using the greater resolving power now available with the electron microscope. In addition to swelling and disruption of the mitochondria, irregularity of the nuclear outline, a disruption of the fine structure of the endoplasmic reticulum, and loss of the highly organized appearance of the young cell have been observed.

Materials and methods. Five Swiss albino mice 1-2 months of age were compared to 6 old individuals ranging in age from 12 to 14 months. The animals were killed by beheading and bleeding, and the pituitaries were dissected out within 5 minutes of the time of death. The procedures used for fixation and embedding closely followed those which had been outlined by Palade(12). The gland was fixed for 4 hours in 1% osmic acid in Ringer's solution buffered with acetate veronal to a pH of 7.5. The material was then washed 3 times in distilled water and dehydrated by successive passage through 50%, 70%, 95%, and absolute ethanol. The tissue was transferred from absolute ethanol to a half and half mixture of absolute ethanol and methacrylate, where it remained for an hour. The methacrylate solution was made up of three parts butyl methacrylate to one part methyl methacrylate. After three washings in 100% methacrylate solution, the tissue was trans-

* This investigation was supported by a research grant from the National Heart Institute, of the National Institutes of Health, Public Health Service.

† Damon Runyon Memorial Fund Fellow.

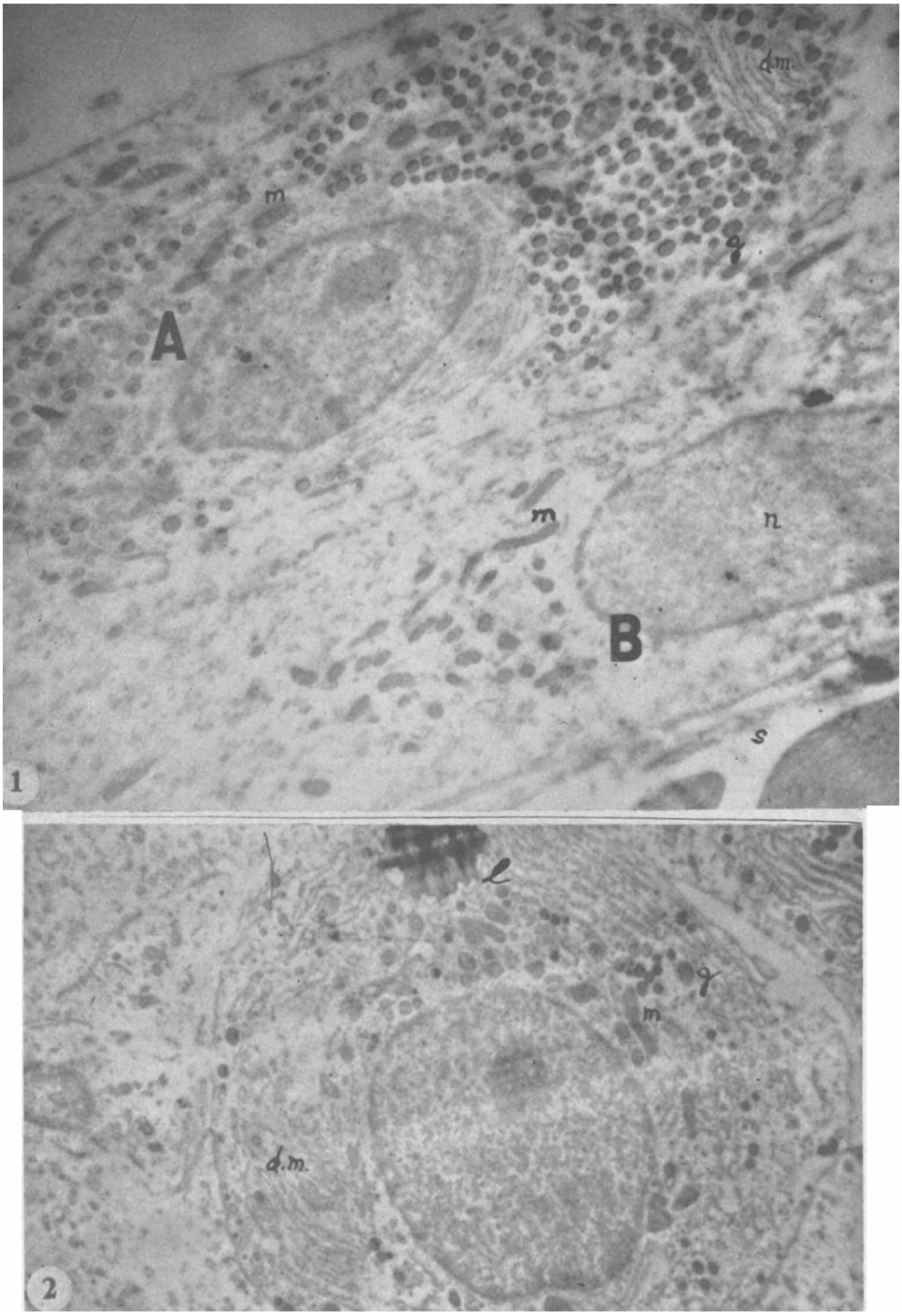

FIG. 1. Electron micrograph of acidophil (A) and chromophobe (B) from the anterior pituitary of a one-month-old mouse. Magnification approximately 6000 ×. l = lipid inclusion, m = mitochondrion, g = granule, d.m. = double-membraned system, n = nucleus.

FIG. 2. Electron micrograph of basophil from anterior pituitary of a one-month-old mouse. Magnification approximately 6000 ×.

ferred to methacrylate to which the catalyst, benzoyl benzoate, was added. Polymerization was begun at room temperature for 12 hours, followed by incubation at 47°C for 24 hours. This slow polymerization was found to decrease the incidence of tissue explosion. Sections were obtained by using the Minot microtome, modified as described by Dempsey and Lansing(13), and equipped with a glass knife. Sections were examined without removing the plastic in an RCA model EMU 2a electron microscope with a 40 μ aperture in the objective lens and a centerable condenser aperture. The electron micrographs were taken at an original magnification of from one-to five-thousand times, and were enlarged photographically thereafter as desired.

Observations. Individual anterior pituitary cells as seen with the electron microscope contain a centrally placed ovoid nucleus. The nuclei are bounded by a membrane which in many sections appears to be doubled. The nucleoplasm is granular. Clumps of randomly scattered dense material give the impression of chromatin. A circumscribed dense nucleolus is sometimes seen. Embedded within the finely granular cytoplasm are three classes of formed elements: granules, mitochondria, and a double-membraned system, which Porter(15) and Palade(12) have referred to as the endoplasmic reticulum. The granules are circular in cross-section, homogeneous, and vary considerably in density. They vary in diameter from 0.1 μ to approximately 0.5 μ. The distribution of granules throughout the cytoplasm in random, although there is a suggestion in some of the micrographs that in the neighborhood of capillaries or sinusoids the population of granules is greatest on that side of the cell nearest the circulation.

In the young cells the mitochondria are much as described by Palade(14), being bounded by an outer membrane from which fine double-membraned septa extend inward (Fig. 3). The matrix is homogeneous and of varying density. The mitochondria range in diameter from approximately 0.2 μ to 0.5 μ, and often reach 2 μ to 3 μ in length. They appear in sections as round, ovoid or elongated bodies (Fig. 1 and 2).

The third formed element within the cytoplasm is the double-membraned system which has been described by Porter(15), Palade (12), and Bernhard(16). This system, in section, consists of a double membrane of varying length usually connected at one end and sometimes at both (Fig. 3). It occurs at either an extreme pole of the cell or as a perinuclear cap or demilune (Fig. 1 and 2). Its long axis is usually parallel to the surface of the nucleus. The distance between the 2 components of the double-membraned system varies considerably along its length. At some points the membranes appear to be in contact whereas at other points they may be separated by as much as 0.4 μ.

In both young and old anterior pituitary glands there seem to be 3 general classes of cells. The first group contains cells the cytoplasm of which is almost devoid of the previously described granules and double-membraned system. These we consider to be the chromophobes (Fig. 1). In the second group are cells whose cytoplasm is packed with granules; the double-membraned system, although present, is not a conspicuous element. On the basis of the high content of relatively large and uniform granules we assume that these cells are the acidophiles (Fig. 1). Finally, there are cells which typically contain few granules and large amounts of the double-membraned system. Because of the sparsity of granules and the large amount of endoplasmic reticulum it appears that these cells are the basophiles (Fig. 2). The granules, where present in all 3 groups, do not differ in appearance, nor does the double-membraned system differ from cell to cell. The number of mitochondria is about the same in cells of each class.

The organelles of the cell which show morphologic changes with advanced age are the nucleus, the mitochondria and the double-membraned system (Fig. 4). The changes were present in all cell types. The nucleus becomes irregular in outline, and the nuclear cortex is made conspicuous by a ring of electron dense material. In some specimens the nuclear membrane is recognizable as a fine double membrane, similar to that seen in the young cell.

The most striking age change is found in

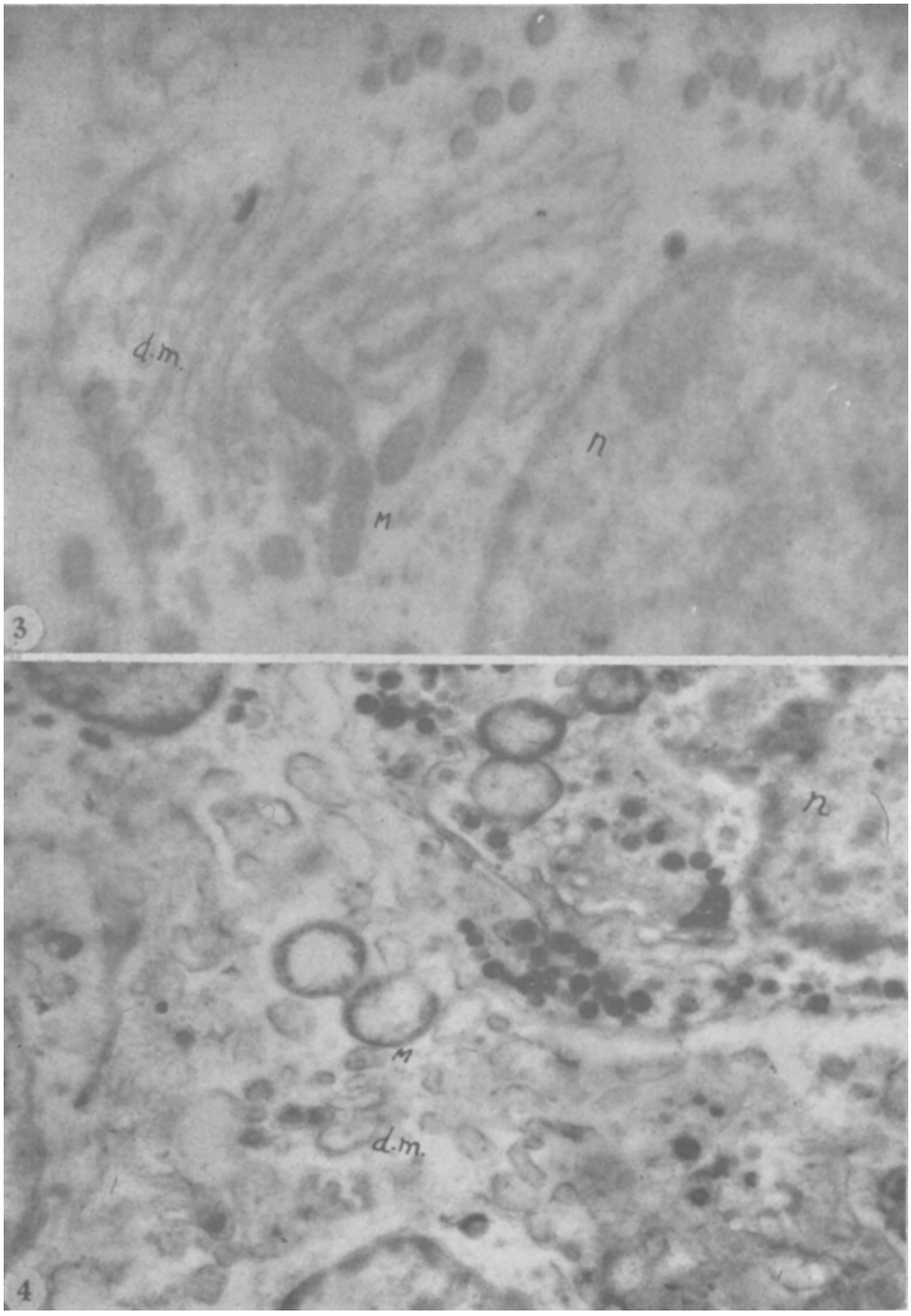

FIG. 3. Electron micrograph at approximately 16000 × of one-month-old mouse anterior pituitary illustrating the architecture of the double-membraned system, mitochondria, and nucleus.

FIG. 4. One-year-old mouse anterior pituitary at approximately 8000× illustrating swelling of mitochondria, disruption of the double-membraned system, and increased density of the nuclear cortex.

the mitochondria. Most of the mitochondria in old cells have a diameter from 2 to 5 times greater than those of young cells. They appear to be vacuolated, in that the mitochondrial matrix is very much reduced in amount or changed in kind as evidenced by low electron density. The double-membraned septa, which in youth extend into the medulla of the mitochondrion are reduced to small, inward-directed, stumps. The peripheral membrane is still finely delineated, and at times appears double. Occasional normal mitochondria can be found, as well as numerous intergrades to the most swollen form.

In young cells without exception the double-membraned system is highly organized as a perinuclear cap or as a condensation at one pole of the cell. This orderly localization is completely lacking in old cells, in which the double-membraned system may be found scattered throughout the cytoplasm in a more or less fragmented form. The membranes that make up the system are not well defined and are difficult to resolve.

The effect of the irregularity of the nuclear outline, the swelling of the mitochondria, and the disruption of the double-membraned system is to produce cells which lack the orderly cytoplasmic architecture of the young cell.

Discussion. Payne's cytological analysis of the age changes in the mitochondria of the anterior pituitary, adrenal, and thyroid cells of the cock indicate that there is a progressive breakdown in the structure of mitochondria, involving a vacuolization and accumulation of pigment in these structures. Definite cytological identification of mitochondria depends upon the *in vivo* uptake of janus green by these organelles. Tinctorial reactions such as the Altman(17) and the Bensley-Cowdry(18) procedures, although effective stains for mitochondria, lack specificity. Under such circumstances one might question the identity of structures presumed to be mitochondria which have undergone extensive structural changes. Indeed, in depending upon tinctorial reactions one might fail to recognize mitochondria which either do not stain in the conventional manner or else are too small to be readily visualized in the light microscope.

The description by Palade(14) of a definitive internal structure in mitochondria makes possible recognition of these structures through the use of the electron microscope. We have observed that the mitochondria in the anterior lobe of the mouse pituitary gland conform closely to the description of this organelle by Palade. In addition we found a sizable variation in the diameter of mitochondria including some that were as small as one-tenth of a micron in cross-sectional diameter. Structures of this size probably would not be recognizable in the light microscope.

In the cells of the anterior pituitary gland of old mice the mitochondria are very much enlarged and appear vacuolated. The mitochondrial matrix material is reduced and the double membraned internal septa are compressed against the outer mitochondrial membrane. Despite these marked changes, enough of the internal structure remains in these mitochondria to permit their identification. Although Payne's description of age changes in the mitochondria of the cock anterior pituitary gland emphasized their occurrence in the basophiles, our electron micrographs indicate that these changes appear in all of the 3 cell types.

While structural observations with the electron microscope do not justify functional interpretation, it is reasonable to assume that mitochondria, profoundly altered as they are in aging pituitaries, do not function in the same manner or as effectively as do young mitochondria. There is abundant evidence from many sources that respiratory enzymes (19-21), enzymes associated with the Krebs' cycle(22,23), and enzymes associated with the oxidation of fatty acids(24,25) are at least in part contained within the mitochondria (see also the reviews of A. I. Dounce(26), and Blaschko and Jacobson(27).) There is also evidence that these enzyme systems are dependent upon proper spatial orientation for their function. In their recent review Blaschko and Jacobson state that the enzyme's "spatial arrangement in the cell must clearly be of great importance when the catalytic system is complex." They state that "attempts to separate the components of the dehydrogenase-cytochrome system (apart from the soluble component c) have been unsuccessful. The activity of the preparation

from heart muscle seems to depend on the way in which the single components are built into the framework of the insoluble tissue proteins."

It is quite possible that the architectural disruption of mitochondria in old pituitaries may result in impairment of their enzymatic activities and, in particular, in the respiratory activities of the cells involved. In this connection it should be recalled that various investigators have noted alteration in cellular respiration in certain aged organs(28-30). It would be particularly desirable to determine simultaneously the anatomical and enzymatic changes with age in a single organ.

We have noted that there is a consistent increase with age in the electron density of the cortex of the nucleus. It has not been feasible to determine the source of this added density; it may be that there is a shift in the distribution of chromatin towards the periphery of the nucleus, or that there is an accumulation of insoluble materials in this region. In this connection it may be recalled that calcium increases with age in both the cortex of the nucleus and of the cytoplasm(31). There does not seem to be a significant age change in the fine structure of either the nuclear membrane or cell membrane.

In agreement with the observations of others we have found that the double-membraned system of normal cells is a highly oriented structural complex. According to Bernhard *et al.*(16) the system as seen in the electron microscope is identical with the basophilic component of cytoplasm or the chromidial substance. The presumption is, then, that ribonucleoprotein is concentrated in the double-membraned system. We have recently determined (to be published separately) that exposure of pancreas to ribonuclease results in digestion of this system, a fact which confirms the expectation that ribonucleic acid is a significant component of this organelle.

In the aged pituitaries the double-membraned system is no longer geometrically oriented, and indeed is markedly fragmented. One might expect that in such cells protein synthetic processes may not be carried out as effectively as in normal young cells.

Summary. Electron micrographs of the anterior pituitary gland of the Swiss albino mouse reveal that the cytoplasm of chromophobes, acidophiles and basophiles contain granules, a double-membraned system and mitochondria in varying amounts. The nuclear membrane appears to be double. With aging the nuclear membrane becomes irregular in outline, the nuclear cortex becomes dense, the mitochondria become enlarged and vacuolated, and the double-membraned system fragments. The implications of these observations are discussed.

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Received February 19, 1953. P.S.E.B.M., 1953, v82.

Influence of Pregnancy upon Hypertension Induced in Rats by Sodium Chloride and Desoxycorticosterone.* (20148)

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Pregnancy in rats invariably causes a fall of blood pressure when hypertension has been previously induced by a partial constriction of the renal artery(1-3). This reduction of hypertension is manifest in the second half of pregnancy and is maximal just prior to delivery, following which the hypertension returns to the prepregnancy level. The same phenomenon has been observed in dogs(4-6) and in rabbits(2,7). We are not aware of any reports regarding the influence which pregnancy might have upon other forms of experimental hypertension in the rat. Masson, Lewis, Corcoran, and Page(8), however, have stated that the administration of desoxycorticosterone acetate (DCA) to pregnant rabbits results in paralysis or convulsions and hepatic necrosis with subcapsular hemorrhages. Inasmuch as several authors have implicated adrenal cortical-like hormones and sodium chloride in the etiology of human eclampsia (reviewed by Mastboom(9)), the interaction of pregnancy and sodium chloride-DCA hypertension in the rat was studied. Although experimental results in rats are not directly applicable to women, especially because of the recognized differences in placental anatomy and function and the absence of spontaneous toxemia in lower mammals, it was hoped that this study might determine whether there are differences between renal hypertension and sodium hypertension, or whether a syndrome resembling eclampsia might develop in "sodium sensitized" animals.

Methods. Adult female rats of the Long-Evans strain were unilaterally nephrectomized, and 60 or 70 mg of DCA in the form of discs or cylinders were implanted subcutaneously, in the manner described by Masson, Corcoran, and Page(10).† The animals were placed on a diet consisting of 3 parts of Purina fox chow and one part of casein supplemented with the known essential vitamins. One per cent sodium chloride solution was supplied as drinking water with special traps to catch any spill. Systolic blood pressures were determined 2 or 3 times a week, using the microphonic method described by Friedman and Freed(11). Fluid intake and urinary output, quantitative proteinuria and body weights were determined frequently. Since the estrus cycles became quite irregular under this regime, males were placed with the females overnight at arbitrary intervals rather than selecting the time on the basis of estrus. Examinations for sperm

* This investigation was supported by research grants from the National Heart Institute of the National Institutes of Health, Public Health Services; and the Edwards Fund. We wish to acknowledge the valuable assistance of Jane O'Connell and Leonard Olson.

† We are indebted to Dr. Ernst Oppenheimer of Ciba, Inc. for a liberal supply of DCA cylinders, 15 mg each, and for DCA powder, from which Dr. G. M. C. Masson kindly prepared discs of 35 mg each.