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A histocytological study of age changes in the canine adrenal gland studied by light and electron microscopy

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IN THE CANINE ADRENAL GLAND STUDIED
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A HISTOCYTOLOGICAL STUDY OF AGE CHANGES

IN THE CANINE ADRENAL GLAND

STUDIED BY LIGHT AND ELECTRON MICROSCOPY

by

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INTRODUCTION

The persistent existential question concerning the meaning of aging and decline of the individual has surely tormented and haunted some men throughout the span of contemplative and reflective life on this planet. To others who have accepted the challenge of this question their efforts to find meaning in this apparent decline have led them, often rather serendipitously, to a real appreciation of the meaning for their life. Although man senses that this aging and period of decline is dramatized only in his community of individuals, it does appear that growth, development, maturation and decline are universal phenomena; all of us and our environments are all participants in this process.

From the first evidence of man's burying his dead, to the works of the alchemists in his efforts to produce the elixir of life, to the efforts of today's molecular biologist, one may conclude that man is very interested in and concerned about the period of decline in his life.

Notwithstanding that, as Comfort (1964b) suggests, in much of aging research "...scientists have discovered more about the patterns of irrational thinking in the human mind than aging," our society is currently directing no small portion of its resources to the study of aging. The goal of this contemporary aging research is surely not to prevent the ultimate decline of the organism, but by the increased understanding gained concerning the basic aging process, to be able to exercise some measure of control over the length of productive and creative life.

The aging of living organisms is being explored by specialists ranging from the physicist and biochemist at the molecular level to the clinician who applies the results of aging research in treating the geriatric patient. But between these two poles of the investigation effort are the morphologists, physiologists, endocrinologists and a host of others who engage in both basic and applied research and serve to fuse the gap between discovery and application.

The morphologist must provide information and participate in the formation of questions regarding what is the so-called "normal" morphology of the cells, tissues and organs of living organisms as they age. For although structure and function are ultimately reflections of one another, it is imperative that a non-ambiguous concept of basic structure be initially elicited. Thus, a primary objective of this thesis is to present evidence as to the pattern of tissue and cellular morphological changes with age in the dog adrenal gland.

Initial superficial examination of the dog adrenal led this author to formulate questions related to these observations. What is the "normal" pattern of adrenal cortical zoning and how does this vary with age? How are the parenchymal cells of the adrenal cortex replaced in a species with such a well-developed zona glomerulosa and is this mechanism altered with age? What is the origin of the cortical nodules and their relation to age? What is the electron microscopic structure of the adrenal tissues and cells? What are the functional states of these adrenal cells with advancing age and can any of these alterations in structure and function be related to more primary age changes such as

1) increased amounts of connective tissue stroma, 2) increased intracellular pigment or 3) alterations in the blood vascular system leading to decreased nutrition of the cells?

The hypothesis to be researched herein is that there are important and basic morphological alterations in the tissues and cells of the canine adrenal related to advancing age. The hypothesis was tested in a group of 128 dogs which were evaluated at various ages for adrenal cortical morphology.

The observations and results offered in support of this hypothesis along with the conclusions of the author are included as a part of this dissertation.

LITERATURE REVIEW

The object of this review of the literature is to present an overview of a portion of the extensive literature on general adrenal morphology and an in depth review of selected works which are representative of the significant amount of research on the adrenal glands and their role in the aging process. Since much of our knowledge of structure and function comes from comparative studies, emphasis has been placed upon reviewing research covering the adrenal structure and changes with age of many species as well as the dog. To properly correlate and evaluate the changes observed in this study, it has been necessary to use macroscopic and mesoscopic as well as light and electron microscopic evaluation. Therefore, the initial portion of this review is of the general morphology and arrangement of the adrenal tissues. After next examining the relevant light microscopy of adrenal investigations, the most significant studies with electron microscopy will be enumerated followed by findings in the area of aging research pertinent to the research of this dissertation.

General Adrenal Morphology

The morphology of the adrenal glands of numerous species has been actively investigated from the year 1563 when Eustachius first detailed his observations of these fascinating glandular structures. During the 405 years of investigation following Eustachius' report, the adrenals of many of the domesticated animals, the common laboratory animal species, numerous other vertebrates as well as invertebrates, the avian species and the primates have been subjected to scientific inquire regarding their general and specific morphology. The scientific literature records a large compilation of observations, hypotheses, experimentations and theories regarding the macroscopic, mesoscopic and microscopic structure and function of these organs.

The excellent treatises covering many areas of current adrenal research edited by Eisenstein (1967) and by Moon (1961) present reviews of the past adrenal research relevant to contemporary research problems.

Review articles and texts dealing specifically with adrenal morphology include those of Luse (1967 and 1961), Zelander (1964), Bourne (1961), Greep (1961), Bachmann (1954) and Bourne (1949). Bachmann presented an exhaustive review of the literature prior to the early 1950's and Luse reviewed the literature available on the adrenal cortical fine structure.

The texts by Goldzieher (1944), Hartman and Brownell (1949), Chester Jones (1957) and Soffer, Dorfman, and Gabrilove (1961) contain excellent discussions of adrenal morphology with some comparative information and extensive reviews of the literature.

Dog (Canine familiaris) Adrenal Morphology

The macroscopic anatomy of the dog adrenal including the general morphology, embryology, blood supply, venous drainage and innervation has been reviewed previously (Flint, 1900; Baker, 1937; Stockard, 1941;

Randolph, 1950; Bachmann,1954; St. Clair, 1957; Smithcors, 1964; Haensly and Getty, 1965; Hullinger, 1966).

Cortical and Medullar Arrangement

As is characteristic of the mammalian species, the dog has an adrenal composed of two glandular structures, the cortex and the medulla (Bourne, 1949). Two different germ cell types contribute to the formation of the adrenal gland; mesoderm differentiates to form the adrenal cortex and neuro-ectoderm differentiates into the adrenal medulla. Bourne (1961) indicated that some of the migrating neuro-ectodermal cells may become incorporated into the capsular stroma. Thus, with a few notable exceptions, in the fully developed adrenal gland a medulla of neuro-ectoderm is surrounded in nearly all areas by a cortical layer of mesodermal origin. ((Cortex (corticis = Latin for bark), the outer portion of an organ; medulla (= Latin for marrow), the center of a part (Stedman, 1961).))

A further subdivision of the adrenal, this dealing with the cortex, was, according to Yoffey (1953), first proposed by Arnold (1866). Arnold's description of the human adrenal cortex was based on morphological criteria, chiefly the blood vessel and connective tissue arrangement. From the capsule inward to the medullary border he described a zona glomerulosa, a zona fasciculata, and a zona reticularis. Nicander (1952) suggested that Gottschau (1883) followed Arnold's basic trilaminar classification, but that he was the first to submit that the three morphologically distinct zones represented functional phases in the life of the cortical parenchymal cells. The subsequent work with the adrenal cortex was based on these classifications established by Arnold (1866) and Gottschau (1883).

Hoerr (1931), working with the guinea pig adrenal, used Arnold's three basic categories of cortical subdivision and added to these two modifications; a differentiation between the inner and outer portions of the zona fasciculata and a fourth zone, the juxtamedullary zone. He related that he was unable to establish distinct boundary margins between the zona fasciculata and zona reticularis or between the zona reticularis and the juxtamedullary zone. Zwemer, Wotton and Norkus (1938) working with monkey, cat, frog and human cortices described the three classical zones with the exception of, like Hoerr (1931), adopting the further subdivision of the zona fasciculata into inner and outer regions.

A further attempt to be functional in classification rather than strictly morphological was made by Bennett (1940). His histochemical work with the feline adrenal led him to classify the zona glomerulosa as the presecretory zone, the outer zona fasciculata as the secretory zone, the inner zona fasciculata as the postsecretory zone, the zona reticularis as the senescent zone and a juxtamedullary portion of the senescent zone where there were greater concentrations of senescent cells. Bennett emphasized the impossibility of drawing sharp zonary boundaries between these regions in the cortex.

Deane and Greep (1946) concluded that the rat adrenal cortex was composed of a zona glomerulosa, zona fasciculata and a juxtamedullary zone. Other authors have characterized the adrenals of several mammalian species, including man and the dog, using only the three basic zones reported by Arnold (Gruenwald and Konikov, 1944; Gruenwald, 1946).

Nicander (1952) submitted that it was because of physiological experimentation conducted in the early 1940s that many began to modify the classification of the cortical zones. Swann (1940) and Sarason (1943), out of their work with the hypophysectomized rat, advanced the theory that the zona glomerulosa was responsible for the production of mineral regulating hemostatic substances contrasted with a carbohydrate metabolizing substance produced by the zona fasciculata. Nicander evaluated the cortical zoning in an extensive study of the adrenal cortex in eleven species of domesticated and laboratory animals. He discussed species differences based on the following six possible cortical regions.

1) zona glomerulosa
2) intermediary zone
3) outer zona fasciculata
4) inner zona fasciculata
5) zona reticularis
6) juxtamedullary zone

The juxtamedullary zone was observed by Nicander only in the horse, rabbit, rat and the non-pregnant female mouse. Thus, he failed to confirm Hoerr's (1931) observation of a juxtamedullary zone in the guinea pig and Bennett's (1940) observation of this zone in the cat.

Very interesting, indeed, was Nicander's demonstration of an intermediary zone in all eleven species he evaluated. He appears to be the first to have so definitively characterized this zone, but Nicander suggests that Mulon in 1903 first observed this zone and called it the "zone de transition." Nicander believed it to be the same zone observed by Engstrom (1936), Hall and Korenchevsky (1937), Mitchell (1948) and Greep and Deane (1949) in the rat and referred to by each as the boundary layer, the demarcation zone, the zone of compression and the transitional zone, respectively.

Bennett (1940) reported that in the cat there was a "narrow inner portion of the presecretory zone" and an "inner or columnar portion" of the zona glomerulosa. Deane and Greep (1946) specified a cortical area variously as a "lipid-free zone" between the zona glomerulosa and zona fasciculata, a "very narrow subglomerulosal layer," and a "transitional zone of compact cells" which they believed to be a subdivision of the zona fasciculata.

Some authors working with the dog adrenal have distinguished only between inner and outer cortical zones (Hill, 1930; Gruenwald and Konikov, 1944). Hill used the terms superficial and deep to indicate the glomerulosa and fasciculata respectively.

Elias (1948) and Elias and Pauly (1966) emphasized the importance of accurate descriptive morphology when naming the subdivisions of the adrenal cortex. Flint (1900) used Arnold's classification but indicated that there was "great variation in the cortex of different animals."

The cortex and medulla have been determined, in most reports, to be structurally separate in the mammalia (Bourne, 1949). However, Hill (1930), Randolph (1950) and Nicander (1952) showed that in the early postnatal dog the separation between the cortex and medulla may not be distinct. Flint (1900) portrayed a generally distinct separation between the two elements but concluded that "it is not uncommon to find projections of either of the constituent portions into the other."

Parenchymal Cell Replacement in the Adrenal Cortex

Gottschau (1883), in proposing the first functionally based classification of the adrenal cortex, hypothesized that these differences in function were brought about by an inward migration of cortical parenchymal cells from a zona glomerulosa storage area into the active zona fasciculata and eventually into the zona reticularis where aging and degeneration of the cells occurred (Nicander, 1952). His work became the basis for a theory of cortical cell replacement based upon a centripetal flow of cells from a cell division region at the periphery to a degeneration region in the center of the gland. Hoerr (1931) believed that Gottschau did not distinguish between the zona reticularis and the medulla, calling them both the zona consumptiva, a mistake Hoerr attributes to poor fixation of the medulla which Gottschau probably encountered.

Although modified in some way by nearly all subsequent workers, Gottschau's theory has served as the catalyst for a protracted debate regarding the origin and functional significance of the adrenal cortical cell types. The literature concerning this moot question has been reviewed by Bachmann (1954), Chester Jones (1957), Skelton (1959) and Brenner (1963).

Gruenwald (1942) stressed the point that the question of centripetal migration assumes new significance when one is dealing with species such as the dog and horse which have a well-developed zona glomerulosa. He stated that for him it was "difficult to imagine how such new-formation (of cortical cells) should take place in the many species with a more distinctly differentiated zona glomerulosa."

According to Hoerr (1931), Canalis (1887) was the first to detect mitotic figures in the adrenal cortex and to experimentally increase their number. Elias (1948) and Hoerr (1931) commented on the observations of germinative areas in the zona fasciculata by Mulon (1903). Hoerr made reference to the mitotic figures found by Mulon in the guinea pig cortex. Mulon determined that they were greatest in number "four, five or six rows of cells in from the capsule," this being the point of junction between the zona glomerulosa and zona fasciculata. Graham (1916) working also with the guinea pig, studied the toxic effects of chloroform on the cortex and observed the mitotic index during subsequent regeneration periods. He discovered that repair regeneration was from parenchymal cells and stromal cells and that in the adult the "growth center" was in the zona glomerulosa and outer zona fasciculata. Graham proposed that cells from this "growth center" migrated centripetally to replace degenerated cells in the more central zones.

Kolmer (1918), according to Hoerr (1931), studied many mammalian species of various ages and found in most a process of reproduction and destruction of cortical parenchyma at the same time. In nearly all cases he observed mitosis and concluded that there was a constant regenerative process in the cortex.

Hoerr (1931) indicated that da Costa (1913) did not support the hypothesis that the zona glomerulosa was the germinative area for the cortex. He detected nearly all mitotic figures in the zona fasciculata and suggested that this zone was the germinative zone from which cells moved into the zona reticularis and outward to the zona glomerulosa.

In his researches with the guinea pig adrenal cortex Hoerr (1931) noted mitosis in the zona glomerulosa only rarely and then it was confined to the younger animals. He also detected mitotic figures in the zona reticularis of the young animal. When the guinea pigs were stressed, Hoerr obtained greater numbers of mitotic figures (approximately 75 per cent of the total) in the outer zona fasciculata. He ascribed considerable significance to the reticular fibers lateral to the parenchymal cells and suggested that they "marshall" the movement of parenchymal cells inward as they are pushed by cells proliferating nearer to the capsule. Hoerr concluded that cells move centripetally from a cell division area which is concentrated mainly at the zona glomerulosa-zona fasciculata border.

Zwemer, Wotton and Norkus (1938) investigated the adrenals of the cat, monkey, frog and human and detected capsular extensions into the zona glomerulosa. These extensions contained cells which were more rounded than the zona glomerulosa cells, with vesicular nuclei and they contained some lipoid droplets. These investigators designated the glomerular and arcade forms in the zona glomerulosa to be sectional artifacts caused by oblique or transverse sectioning of glomerular zone cords arising from germinal areas in the capsule. They submitted that in the adult, cortical cell replacements are derived from undifferentiated cells in the capsule by an uninterrupted centripetal movement and maturation of these cells. To support their theory they cited evidence of cortical cell foci which appeared when capsular sites for cell multiplication were experimentally activated. They proposed that, when sectioned in the proper plane or serial sectioned, these islands of cells surrounded by connective tissue could be demonstrated to be connected to the cortex or to extend superficially to form accessory cortical nodules. These authors considered the zona glomerulosa to be a "transition zone between the capsule and the mature fascicular cell." This work was followed by further experimental evaluations by Salmon and Zwemer (1941) and Wotton and Zwemer (1943) who drew the same conclusions.

Bachmann (1937), according to Bennett (1940), supported centripetal migration on the basis of reticular fiber morphology in the cortical zones and in 1939 he described, in the human, a "germinal zone" interposed between the zona glomerulosa and the capsule. The same "zone" was observed by Gruenwald (1946) in a young child and Gruenwald (1942) suggested that perhaps Zwemer, Wotton and Norkus (1938) had considered this same area part of the capsule.

Bennett (1940) used the cat adrenal in his extensive studies, drew from the works of Lux, Higgins and Mann (1937), Higgins and Ingle (1938) and Turner (1939) with transplants and concluded that the capsule was the germinative zone of the cortex. But Bennett in his same report qualified this conclusion and specified that at least the germinative zone was in the outer zones of the cortex. He used centripetal migration to explain his findings and presented evidence of mitotic figures only in "the inner or columnar portion" of the presecretory zone.

Deane and Greep (1946) and later Greep and Deane (1949), working with the hypophysectomized rat, observed postsurgically that the zona glomerulosa continued to enlarge as did the "transition zone between the glomerulosa and fasciculata." They theorized that the zona glomerulosa and zona fasciculata were of different "structure, origin and function." They interpreted their findings by what they termed the "escalator theory" and, thus, supported the centripetal migration theory. But they made an important exception; the migration of cells was not from the zona glomerulosa inward. These authors noted that few cells of the glomerulosa migrated into the fasciculata and, in fact, cells were observed to degenerate in the zona glomerulosa. The highest mitotic index was at the outer fascicular margin, the transitional zone. When normal rats were stressed, greater numbers of mitotic figures were observed in the outer fasciculata leading to hypertrophy of the fasciculata which confirmed Hoerr's (1931) report. Mitotic figures were observed throughout the cortex in rats subjected to stress (Baxter, 1946; and Mitchell, 1948). Blumenthal (1940) in the guinea pig observed mitotic figures only at a site confined to the inner zona glomerulosa and outer fasciculata.

Deane and Greep (1946) concluded that the transitional zone was the germinative zone for the fasciculata and agreed with Gruenwald and Konikov (1944) when they specified that the glomerulosa arose from the capsule. Gruenwald and Konikov (1944) indicated that many dog adrenals showed no evidence of cortical cell formation in the capsule but in others there were often several groups of cortical cells in the capsule. They interpreted this evidence as suggesting that nodules of glomerulosa cells in the capsule replaced zona glomerulosa cells by "moving into their new position as a compact layer." They also stressed that each species might manifest a different mechanism of cortical cell replacement. Gruenwald and Konikov suggested that moderate requirements for new cortical cells could be met by mitosis at the outer zona fasciculata border. Further demand might be met by zona glomerulosal cells migrating into the fasciculata and, in cases of severe stress, by regeneration of cells from the capsule. ((The second response mechanism reflected Gruenwald's conclusion that the zona glomerulosa was "a reserve store of cortical cells." (Gruenwald, 1942).))

Nicander (1952) observed mitotic figures in the outer layers of the cortex and concluded from his study of eleven species that in the normal adult cortex no migration of cells occurred. Yoffey (1953) was similarly opposed to the centripetal migration theories and he pointed to the glomerulosa of the horse as an obvious obstacle for proponents of this theory. He believed local mitosis to be the mechanism of cellular replacement in all zones.

The adrenal cortices of the horse, cattle, sheep and goat were studied by Elias (1948). He stressed the differences between fetal and early postnatal development and growth versus the pattern in the adult adrenal. He commented that in some species there are "fluent transition" areas between the capsule and cortex for the exchange of new cortical cells. Elias also considered the nodules formed in the capsule to be of blastemal origin and eventually to be incorporated into the cortex.

Tonutti (1942) proposed that the adrenal cortex was composed of two "transformation fields"; the outer and inner transformation regions. He suggested that the capsule and zona glomerulosa constituted the outer field and the zona reticularis and parts of the zona fasciculata constituted the inner field. By either progressive or regressive transformation in these two fields these cells can become functional fasciculata cells or functional cells of their respective zones. He considered both the zona glomerulosa and zona reticularis to be reserve zones.

Chester Jones (1957) presented his theory of cortical cell genesis based upon a cord of cells which undergoes mitosis at a selected portion of the cord and the new cells resulting push away from this focus. The morphology of the distal ends of the cord, the zona glomerulosa and zona reticularis is determined by the stromal network which serves to limit and divert the migrating cells. He suggested that the zona intermedia represented the products of this focal cellular division.

Parenchymal cell replacement in the dog adrenal cortex has been evaluated briefly by the following workers. Dempster (1955) was unable to obtain cortical regeneration from enucleated dog adrenal glands and he concluded, on the basis of his negative findings, that no evidence of centripetal migration was seen in the dog adrenal. The capsule was believed by Randolph (1950) to be the germinative zone in the dog adrenal from birth to two years of age. Hullinger (1966) characterized the zona intermedia in the dog and suggested that this zone played a role as a germinative zone in the older animal.

With the development of tritiated thymidine and radio-autography in the late 1950s a new method of analysis became available for application to the problem of adrenal cortical cell origin. Diderholm and Hellman (1960) used labeled thymidine in young rats and found the uptake to be greatest in the zona fasciculata and zona glomerulosa. This pointed to a high mitotic index in these areas. Messier and Leblond (1960) applied this technique to evaluate cell migration in the mouse and rat but were unable to confirm the centripetal migration process. Walker and Runnels (1961) performed similar work using female mice and reported a high uptake rate in the zona fasciculata and zona glomerulosa, but recorded no evidence of cell migration.

Brenner (1963) obtained a high level of uptake of the thymidine label in mice and went on to suggest that other workers had failed to allow enough time for migration to occur. He traced the distribution of the label for up to 24 weeks in his specimens. Initial uptake was in the glomerulosa-fasciculata region and after six weeks the label was found in the zona reticularis where many degenerative cells were detected. Significant amounts of the label, however, remained in the outer cortex. Large numbers of labeled fibroblasts and cells of the endothelium were discovered in the connective tissue deep within the cortex. It is interesting to note that Hoerr (1931) observed mitotic figures in the capillary endothelium of the inner cortex of rats subjected to stress. Ford and Young (1963) conducted experiments quite similar to those of Brenner (1963) and joined him in favoring the centripetal migration theory.

Sabatini and De Robertis (1961) studied the ultrastructural zonation in the rat adrenal cortex and, on the basis of morphological alterations in the mitochondria, supported the theory of centripetal migration of cells. These same authors (1962) discussed the fine structure of the mitochondria in the zona intermedia as it related to the same theory.

Light Microscopy of the Canine Adrenal Gland

Capsule

Hill (1930) made reference to the relatively large adrenal capsule in the newborn pup. Also, Vincent (1922) emphasized the thickness of the adrenal capsule in the adult. Hullinger (1966) quantified the capsular thickness and observed a progressive increase in thickness up to six months of age but no further significant change through 13.6 years.

Flint (1900) referred to the capsule as the "fibrous envelope" of the adrenal gland which was composed of "connective tissue cells, smooth muscle fibres, and some nerves and ganglion cells." His results with digestion studies indicated that the capsule was composed of two layers; an outer dense layer of white fibrous tissue and an inner layer of reticulum. Bachmann (1954) indicated that the elastic fibers and smooth muscle cells which he and others had observed were not indicative of a "static organ" concept. He attributed a dynamic role to the capsule in addition to its role as a stromal support.

Randolph (1950) studied development from birth to two years of age and stressed a distinct adrenal capsule which possessed a highly cellular inner portion and an outer fibrous layer. From the capsule there were frequent "cellular indippings" which extended into the cortex and some extended as fibrous trabeculae deep into the cortex. Between the capsular layers Randolph reported undifferentiated cells which he suggested might develop into either cortical or medullary cells. Lane and deBoda (1952) described a thin capsule which was relatively cellular and contained delicate collagenous fibers.

Hullinger (1966) demonstrated that the ratio between cellular and fibrous components of the capsule was dependent upon age. At birth the capsule was predominantly cellular and few fibrous septa extended into the cortex. However, with advancing age this picture was altered until at 13.6 years the capsule was heavily laden with fibrous connective tissue, the cellular areas being confined to some of the great numbers of septae which extended into the cortex.

Zona glomerulosa

Flint (1900) described the stroma of the zona glomerulosa as being composed of white fibers and reticular fibers which passed as septae into the regions of the cortex. The larger septa went deeper into the regions of the cortex while the smaller septae divided the glomerulosa laminae into oblong and ovoid spaces. These spaces were further subdivided by fine reticular fibers. Flint (1900) and Chester Jones (1957) emphasized the well-defined nature of the dog's zona glomerulosa versus that of many of the other species.

According to Flint (1900) the spaces in the stroma of this zone were "occupied by coiled columns of more or less irregular cylindrical or columnar cells." Depending somewhat upon the plane of section, the coiled column commonly appeared "curved in horseshoe or U shapes with the convexity directed towards the capsule and the open end to the medulla." However, variations in this pattern included shapes "like an omega or letter S." He also noted that the zona glomerulosa followed the large fibrous septae into the deeper cortical regions.

Flint (1900) continued by describing the glomerular cells as being "spindle, wedge, or irregularly columnar in shape" and extending at each end to the reticular fiber laminae. The reticulum was not observed to separate individual cells. Gruenwald and Konikov (1944) wrote that the "zona glomerulosa in the dog's adrenal is very conspicuous" and is composed of "large epithelial arches with their convexity toward the capsule." Nicander (1952) described laminae of glomerulosa cells which were "folded in the vertical plane" and in sections appeared "often broader beneath the capsule." "Thin sheats of collagenous fibers surrounded the cell complexes" and the typical cellular morphology was "long, slender, often slightly bent and columnar." The "highly vacuolated cytoplasm" was "weakly eosinophilic" and contained a large, elongated nucleus often with "flattened ends" and no pigment granules.

Kolmer (1918) referred to a varying morphology in the zona glomerulosa ranging from those having "short columns of flatly compressed cells" to others having "columns wound about, giving an arcuate appearance." He observed no pigment in these cells. Hill (1930), in early postnatal pups, spoke of a "well-formed zona glomerulosa" composed of "flask-shaped masses of tall columnar cells arranged side by side in a single layer" which showed a definite lipoid reaction."

Randolph (1950) stressed the role of "trabecular indipping" in the formation of the zona glomerulosa arches. He noted that septa "surrounded the cell columns" of "pyramidal cells" arranged in "rounded or arched groups." According to Dempster (1955) the dog possesses a well-developed zona glomerulosa consisting of cell groups arranged in "large double arches with their convexities outward." Lane and deBoda (1952) referred to the zona glomerulosa as an "outer shell or mantle" composed of "radially placed" columnar cells arranged such that their long axis lies parallel to the capsule; "radially placed stacks of disc-shaped units." The cells were lipid positive and were separated by delicate collagenous strands.

Hullinger (1966) described the glomerular parenchymal cell as a lipid and cholesterol rich columnar cell which extended to the sinusoidal space at each base. At birth the cells were cuboidal and randomly arranged but with advancing age the cells became columnar and arranged in cords. These became progressively more well-defined as vertical arcades separated by prominent collagenous septa.

Zona intermedia

Nicander (1952) reported that as early as 1903 Mulon described the "zone de transition" in the dog. Nicander characterized this zone and advanced the term intermediary zone. He described this zone as a narrow band of "small, low columnar cells" which were arranged in generally "verticle rows, which formed the continuation of the cords of the zona glomerulosa." They contained an eosinophilic cytoplasm and some cells were "cuboidal with spherical nuclei."

Hullinger (1966) followed Nicander's classification and described a well-defined zona intermedia in the dog at one month of age. This zone appeared between the zona glomerulosa and zona fasciculata, increased with advancing age and, in the mature gland, appeared to be 4 to 8 cells in thickness. These low columnar cells were lipid and cholesterol negative. There were larger amounts of collagenous fibers in this zone than in the adjoining glomerular or fascicular zones.

Flint (1900) did not make any reference to cells of this zone but in his description of the reticulum he did note an interlacing of fibrils at the inner layer of the zona glomerulosa. Lane and deBoda (1952) described a "narrow subglomerular transitional area." Randolph (1950) stressed the envolopment of glomerular cells in collagenous connective tissue but made no reference to a zona intermedia-like region or cells of that morphology.

Zona fasciculata

Flint (1900) described a stroma for this zone composed of some large septae arising directly from the capsule and many smaller septa from the inner margin of the zona glomerulosa. The course of these fibers served to keep the parenchymal cell columns at right angles to the capsule and radiating from the medulla. From these larger fibers between the cords came fine reticular fibers which supported the individual cells. Flint noted that this zone was "composed of polyhedral cells arranged in anastomosing columns, the long axis of which run at right angles to the capsule." The columnar arrangement of cells resulted from what he believed to be "the capillaries which separated the cells." He observed that usually there were two cell cords between two capillaries but the number varied from one to four. These cells contained a light staining, vesicular nucleus which contained two to three nucleoli. Hill (1930) referred to "large ovoid cells arranged roughly in short columns radiating from the center of the gland" as composing that portion of the cortex not including the zona glomerulosa.

Nicander (1952) divided the zona fasciculata into an inner and outer portion. The outer zone was composed of "moderately large cells with highly vacuolated cytoplasm" and more eosinophilic versus zona glomerulosa cells. The nuclei were "smaller and darker than those of the zona glomerulosa, and the nuclear membrane was slightly uneven." He observed some pigment granules in these cells.

The inner fasciculata was characterized by Nicander as being composed of cells "somewhat smaller, with more eosinophilic and less vacuolated cytoplasm than in the outer fasciculata." The nuclei were larger and somewhat elongated and pigment granules were more prevalent. This morphological description of the inner and outer fascicular zones was confirmed by Randolph (1950) and Hullinger (1966).

Vincent (1922) emphasized that the dog possessed a well-defined zona fasciculata which merged into the zona reticularis.

Zona reticularis

The stroma of the zona reticularis was described by Flint (1900) and Hullinger (1966). Flint indicated that as the fibrils from the zona fasciculata reached the reticularis, "they branch and anastomose to form a dense meshwork of reticulum." He pointed out that nearly every cell had a space in the reticulum. He detailed areas of random arrangement but also pointed out areas where cords of cells tended to be deflected at right angles and go parallel to the medulla. He suggested that the parenchymal cells of this zone differed from those of the zona fasciculata in that they were a "trifle smaller and usually do not contain the fatty globules." These cells occurred in small groups and were surrounded by capillaries.

Nicander (1952) considered this zone to be "distinct and well demarcated." His description supported that of Flint (1900). However, he described the entire zone as occasionally appearing as "a 'medullary capsule' with scattered 'intracapsular' cell groups."

Dempster (1955) emphasized the well-defined nature of the zona reticularis and reported that he found no evidence of degeneration in this region. Randolph (1950) generally supported the findings of Flint (1900).

Hullinger (1966) discussed the development of the zona reticularis and noted that this cortical region first appeared at about two months of age. At this age its classification as a cortical zone was based on tissue and not cellular characteristics. The cells were "observed to deviate laterally and run parallel to the cortical-medullary border." After one year the cells of this zone showed evidence of fatty metaplasia and some degenerative change.

Cortico-medullary border

The boundary between the cortex and medulla has been described by many authors as being clearly demarcated (Flint, 1900; Kolmer, 1918; Hill, 1930; Randolph, 1950; Hullinger, 1966). Kolmer (1918) indicated that in the older dogs a definite band of fibrous tissue occurred at the cortico-medullary border. According to Hill (1930), in the young pup the cortico-medullary boundary was not a definitive line as it was in the adult and he demonstrated cortical cell islands in the medulla. Flint (1900) observed that the cortex and medulla projected alternately into the substance of the other at some foci but he spoke of a dense network of fibers at these points of "invagination" and believed that the network still separated the cortex and medulla.

Hullinger (1966) described invagination of reticular zone cells through the collagenous band of the boundary and observed that these cells appeared as isolated cell masses within the medulla. Flint (1900) referred to these as "aberrant islands of cortical tissue." Hill (1930) did not observe any cortical nodules within the medulla in the adult.

Medulla

Flint (1900) described the canine adrenal medullary parenchymal cells as

> ".....generally polyhedral in shape, although at the periphery they may have an irregular columnar form. The cells have round vesicular nuclei with considerable chromatin substance and they contain several nucleoli...and they do not possess a membrane, nor is there any demonstrable ecto- and endoplasmic differentiation of the cytoplasm."

The cells occurred as irregular groupings of cells surrounded by capillaries and the nuclei of these cells were at the base of the cell away from the capillary surface. The supporting reticulum of the medulla, according to Flint, arose from the zona reticularis and it was described by him as being similar to the zona glomerulosa reticulum, but more delicate.

Vincent (1922) described the medulla as being composed of columns or bundles of cells having a small amount of connective tissue with "medium-sized spaces" surrounding them. Hill (1930), in young dogs, observed cortical nests in the medulla. Hullinger (1966) observed those islands of cortical tissue in the medulla in nearly all age groups and prolapses of cortical tissue into the medulla in older dogs.

In the young pup Flint (1900), Randolph (1950), and Hullinger (1966) observed medullary cell islands in the various layers of the cortex and the capsule.

Capsular nodules

Morphology and distribution The occurrence of accessory cortical tissue in the form of nodular isolates has been described by many authors in many different species. Flint (1900) observed them frequently in the dog within the capsule and explained their development as follows:

> "As the ingrowing cell groups leave their embryonic position beneath the capsule, they often draw portions of the capsule after them, and these processes either partially or entirely occlude parts of the cortex."

Accessory nodules or cellular proliferations of other descriptions have been observed in man (Flint, 1900; Cooper, 1925; Gruenwald and Konikov, 1944; Goldzieher, 1944; Gruenwald, 1946; Bourne, 1960 and 1961; Andrew, 1966), in the horse (Gruenwald and Konikov, 1944; Elias, 1948; Nicander, 1952), the cat (Bennett, 1940; Wotton and Zwemer, 1943; Gruenwald and Konikov, 1944; Nicander, 1952), in the pig (Flint, 1900; Gruenwald and Konikov, 1944; Nicander, 1952), the guinea pig (Zwemer, Wotton and Norkus, 1938; Gruenwald and Konikov, 1944), monkey (Gruenwald and Konikov, 1944), in the sheep (Elias, 1948; Nicander, 1952), in the goat (Elias, 1948), in the ox (Elias, 1948; Nicander, 1952), in the rat (Yeakel, 1946), in the rabbit (Gruenwald and Konikov, 1944; Nicander, 1952) and in the dog by Flint (1900), Goodpasture (1918), Randolph (1950), Nicander (1952), Dempster (1955), Dämmrich (1960) and Hullinger (1966).

In the dog these nodules have been reported in assorted forms. Randolph (1950) spoke of cells in the capsule which developed into nodules or nodule-like structures. Flint (1900) indicated that there were isolated cortical cell groups within the capsule. Gruenwald and Konikov (1944) noted that some dogs showed no nodule formation in the capsule while others showed "cortical cell groups of varying number and size." They spoke of small groups of cells possessing no connection to the cortex and larger ones showing cell cords which were "no longer separated from the glomerulosa."

Goodpasture (1918) evaluated the adrenals of senescent dogs and found that "the adrenal surface is made irregular by the presence of smooth, more or less spherical, protuberances varying in size from one millimeter to two centimeters in diameter." These were multiple and bilateral and when sectioned they projected from the cortex through the capsule.

Dämmrich (1960) observed 100 acute and chronically diseased dogs ranging in age from about three months to fifteen years. In all but 29 there were cortical nodules either within or upon the capsule. He determined that the nodules grew in size with advancing age until they appeared macroscopically. He believed these nodules were formed from rows of cells lying under the capsule which begin to assume a rounded form and frequently extended through the capsule. With further nodular development the connection to the underlying zona glomerulosa was lost and the nodule was independent on the surface of the capsule covered only by peritoneum. The cell morphology in the nodule was dependent on the nodule size; as the nodules increased in size, so did their constituent cells and columnar cells became oriented much like the zona glomerulosa. He observed that these cells contained a sudanophilic substance.

Dempster (1955) observed small glomerular cell isolates in the capsule in 70 per cent of the normal adrenals he examined in the dog. Furthermore, in autotransplanted adrenals he observed that in the thickened capsule there was a striking increase in the cellularity and nodules of the glomerulosa cell type arose from cells in the capsule. These nodules were usually intimately related to large blood-vessels. They contained cholesterol and there did seem to be a tendency for these cells to grow "laterally within the capsule." Contrary to Gruenwald and Konikov (1944) in the dog and Elias (1948) in ungulates, who suggested that these nests of cells are moving inwards, Dempster suggested these cells "may be moving outwards."

Hullinger (1966) stressed the progressive occurrence of these "extracortical parenchymal nodules" from 14 days to 13.6 years; they increased in size and number with age. These cholesterol and neutral lipid-rich nodules could be seen both within and upon the capsule and many of them demonstrated connections to the underlying glomerulosa. He observed a zonation within the larger nodules typical of the parent cortex.

<u>Role of the nodules</u> Randolph (1950) believed that these nodules developed within the capsule and "similar to medullary development, pushed the inner capsular layer ahead and became incorporated into the glomerulosa zone of the cortex." Flint (1900) believed that they were merely isolated parenchymal nodules. Gruenwald and Konikov (1944) used

nodule formation in the dog as further support for their theory of a germinative center in the adult capsule which provides new cells for the cortex. They suggested that as the nodules proliferate "toward" the cortex, their structure "resembles more and more that of the zona glomerulosa."

Goodpasture (1918) stated that "such areas not infrequently are found penetrating the capsule and forming small excrescences upon the gland." Dämmrich (1960) interpreted the occurrence of these nodules as a sign of increased functional activity due to the adaptation syndrome of Selye (1946). Dempster (1955) submitted that "the cells may be merely a herniation through a weak area of the capsule and of no further significance." Hullinger (1966) concluded that the nodules were differentiated cortical cells which were evaginating through the capsule, rather than moving inward to the cortex. He advanced the hypothesis that these nodules were compensitory changes to meet an increased demand for functional parenchyma.

Electron Microscopy of Canine Adrenal Gland

There are very few descriptions of the fine structure of the dog's adrenal gland in the literature. Luse (1967) and Kaminsky, Luse and Hartroft (1962) expressed a concern stemming from the need for the electron microscopy of the normal dog adrenal stressing the physiological similarities between the dog and human.

Luse (1967) presented a very short description of her electron microscopic findings in the dog adrenal. She wrote that the zona glomerulosa cells were smooth-surfaced and outlined by a distinct basement membrane. She described abundant lipid droplets, variable endoplasmic reticulum and numerous free ribosomes. The elongated mitochondria of the glomerular cells possessed laminar or leaf-like cristae. The zona fasciculata cells were contrasted with the glomerulosa cells as having ovoid, relatively small mitochondria which possessed vesicular cristae. Vesicles were abundant in these cells. The compact reticularis zone cells contained many smooth surfaced membranous structures of variable shape and a prominent Golgi apparatus. The mitochondria varied in shape from round to elongated and contained cristae of laminar or vesicular types.

Definitive electron microscopy of the normal adrenal cortex has been reported in the human (Carr, 1961), the rat (Lever, 1955; Sabatini and De Robertis (1961), the mouse (Zelander, 1959), the guinea pig (Sheridan and Belt, 1964), in the hamster (deRobertis and Sabatini, 1958), in the opossum (Long and Jones, 1967) and the Rhesus monkey (Brenner, 1966).

Changes in the Adrenal with Age

Korenchevsky (1961) and Jayne (1953) have commented that the adrenals have not been widely viewed as playing a primary role in the aging process. Comfort (1964b) similarly stated "...the supposedly primary and 'senile' histological appearances in endocrine glands require serious investigation before they are made the basis for statements that senescence is a consequence of the deterioration of a particular gland."

Bourne and Jayne (1961) discussed aging in the human adrenal and the adrenals of some other mammalia. Along with a review of the literature, they suggested that the primary changes with age in the adrenal included: 1) accumulation of pigment, 2) an increase in connective tissue and 3) a loss of active lipids and an accumulation of fats. They suggested that vascular dilation in both the cortex and medulla are seen in some species. They also included cytological alterations such as fragmentation and vesiculation of mitochondria, the formation of vesicles leading to pyknosis of the nuclei with an infiltration of leucocytes as changes often associated with advanging age.

Korenchevsky (1961) suggested that pathological change occurs only in senescence in the adrenal. He described atrophic and degenerative changes which accompanied a general increase in the connective tissue stroma. Jayne (1953) believed that the displacement of parenchyma by stroma in the rat adrenal was of relatively less significance when compared to the atrophy and degenerative changes occurring in the parenchymal cells.

Korenchevsky (1961) summarized the works of Delmare (1903) and Puech (1953) and noted they both concluded that in man degenerative cellular changes are due primarily to eschemia brought about by various degrees of arteriosclerosis of the adrenal vasculature. Korenchevsky and Paris (1950) and Korenchevsky, Paris and Benjamin (1953) discussed the cellular changes in senescent rat adrenals which included 1) cellular atrophy and nuclear pycnosis of the glomerulosa cells; 2) cells of the inner zones coalesced to form syncytia followed by nuclear changes; 3) fragmented mitochondria which were important in the formation of pigment granules. These focal changes, according to these authors, were nearly always compensated for by hypertrophy of parenchymal cells in the unaffected areas of the cortex. They considered nodular hyperplasis to be a progressive change with advancing age.

Cooper (1925) described changes in the human adrenal from birth to old age. She observed a progressive increase in capsule thickness and glomerulosa septa coupled with an increased predominance of the zona reticularis and sinusoidal spaces in the zona fasciculata. The cortical medullary border was also increased in prominance. These same primary connective tissue changes in the capsule and reticularis were reported in the hamster (Meyers and Charipper, 1956) the guinea pig (Blumenthal, 1945) and the rat (Dribben and Wolfe, 1947).

Other specific cellular changes in the parenchyma have been reported in the guinea pig and cat. Hoerr (1931) considered the light and dark cortical cells of the guinea pig cortex to indicate early stages of degenerating and senescent cells. Bennett (1940), in the cat, considered the zona reticularis to be the senescent zone of the cortex and noted variations in width directly proportional to age.

Aging in the dog adrenal has been characterized by only a few authors in some detail and other authors have mentioned only a few observations in their work. The fibrous capsule between the cortex and medulla is lacking in the newborn and young pup but appears with advancing age (Kolmer, 1918; Hullinger, 1966). Goodpasture (1918), as discussed previously, believed the nodule formation to be a constant and progressive finding with advancing age, a finding also supported by

Dämmrich (1960) and Hullinger (1966).

Bloom (1962) suggested that the changes observed in older dog adrenals could be grouped into 1) senile regressive changes, 2) "myelomatoid" nodules, 3) nodular hyperplasia, and 4) tumors. He considered senile regressive changes to include a thickening of the capsule and trabeculae, fatty infiltration into fascicular cells, and hyalinization of thickened arterioles and small arteries. Bloom considered "myelomatoid" nodules to be circumscribed areas of myeloid cells in the cortex of mostly older dogs. Nodular hyperplasia involving all areas of the cortex were described, especially in dogs over 10 years of age. He observed adenomas in approximately 10 per cent of the dogs over 10 years of age.

Hullinger (1966) stressed the possible significance of the zona intermedia, extracortical nodules and fatty metaplasia in the senile canine adrenal gland.

MATERIALS AND SURVEY OF METHODS

General

The material used in this research was obtained from the Beagle dog colony of the Department of Veterinary Anatomy, Iowa State University and from the Gaines Dog Research Laboratories, Kankakee, Illinois. The 128 dogs involved in this study included 109 Beagles, 5 Springer Spaniels, 4 Fox Terriers, 3 Golden Retrievers, 2 Irish Sitters, 2 Laboradoг Retrievers, 1 Basengi, 1 English Cocker, and 1 Corgi. These included 53 males and 74 females ranging in age from 8 hours to 13.6 years of age. Table 1 provides a description of these animals. This study was limited to animals from these two sources in an attempt to standardize the environments and eliminate possible wide variation due to widely differing environmental conditions which are otherwise quite difficult to control.

Carlson (1942) emphasized the imperative necessity for controlled studies in aging research. Recognizing that great numbers of factors are not amenable to control, he suggested that care be taken to insure 1) the standardization of nutrition, overall health status, heredity and 2) a thorough evaluation of the total endocrine status and the condition of the circulatory system. Cammermeyer (1963) expressed a similar concern for an accurate control of these variables.

Design of Program

A review of the general design of the gerontological research program involving the dog which is being conducted in the department of Veterinary Anatomy, Iowa State University, Ames, Iowa, was presented in an earlier dissertation (Hullinger, 1966). This program was instigated by Dr. R. Getty in 1952 when a mongrel dog colony was established for the purpose of supplying specimens for the gerontological research being carried on at that time. The colony grew in size and the pattern of management evolved until in 1955 a purebred Beagle colony was initiated and plans were made to gradually phase out the mongrel colony as the purebred colony increased. Since 1958 the colony has been composed entirely of purebred Beagles and a population of approximately fifty animals is currently maintained.

The animals provided for this research from the Gaines Dog Research Laboratories have been a part of their long-established colonies and have been involved in the feed consumption and palatibility studies being conducted there.

The post-weaning diet for all those animals maintained within the colony at Iowa State University is composed entirely of dry commercial dog feed.[1] This self-fed diet is provided free choice by means of sheltered, hopper-type self-feeders. Feed analysis showed the dry diet to be composed of 25 per cent protein, 40 per cent carbohydrates, 7 per cent fat, essential amino acids, vitamins and minerals with one pound of feed supplying 1500-1600 calories.[2]

Free access to a clean fresh water supply is provided by an automated

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[1]Provided by Gains Dog Food Division, General Foods Company, Kankakee, Illinois.

[2]Feed analysis provided by Gaines Dog Research Laboratories, Kankakee, Illinois.

watering system at all times.

The following procedures are employed routinely to access the general health status of the individual animals. General physical evaluations are made at regular, predetermined intervals of three months. The physical examination is supplemented by routine fecal examinations to determine the gastro-intestinal parasite level. The objective is to maintain this at a low level by the periodic administration of a commercial anthelmintic. As a further control measure the dog kennels are hosed clean with steaming-hot water as a daily routine.

The entire colony is involved in a rigid program of infectious disease prophalaxis. This includes routine vaccination for canine distemper and infectious canine hepatitis. As a further precautionary measure, an eight foot, solid wooden fence bounds the periphery of the colony and at all points is located at a minimum of five feet from the colony fences. This serves to prevent direct contact of colony dogs with stray animals. Very importantly also it serves to minimize possible distraction and excitement from outside the colony.

A necropsy evaluation is conducted at the time of death and specimen collection. In the case of this specific research the author was interested in these overall or specific appearances which might suggest an abnormally active or inactive adrenal gland.

Each animal was observed for any external symptoms of endocrine malfunction as outlined by Bloom (1962). Special attention was given to the condition of the hair coat noting any alopecia in frictional regions and/or rough, dry areas where the hair was easily pulled out. In addition to this external examination the blood cholesterol levels of some animals were monitored routinely at predetermined quarterly intervals as an indirect measure of cortical activity. A record of the results of that evaluation was maintained for each. The results of these evaluations combined with the negative pathology findings at necropsy indicated that all animals were in generally good physical health.

The colony could be properly termed "closed" with reference to the introduction of new individuals. The program of breeding is designed to provide that only a very limited number of new parent stock be occasionally introduced. Thus, an attempt is being made to maintain a certain degree of homogeneity to the parent stock and offspring among the Beagle animals.

The morphology of the circulatory system and other endocrines and their integrity has been reported previously (Getty, 1962, 1963, 1965; Haensly and Getty, 1965a) or is currently being conducted within this department.

Collection Procedure

Each animal was sacrificed by electrocution and exsanguination was accomplished by severing the axillary vessels. The animal was dissected as rapidly as possible after death and the adrenal was generally the first organ to be removed. These tissues were in the fixatives for light and electron microscopy 3 to 5 minutes after respiratory and cardiac arrest.

Histological Methods Employed for Light Microscopy

The fixing solutions used in this study were mercury formal-saline and standard calcium-cadmium procedures. Transverse slices were made through the capsule to facilitate the penetration of the fixative and to shorten fixation time. The right adrenal was generally fixed in mercury formal-saline and the left in calcium-cadmium. Fixation and storage were carried out at 4 degrees centigrade.

An attempt was made to remove identical cross sectional segments of mercury formal fixed tissue from a point nearly midway along the long axis, at such a point that nearly 0.5 cm. of tissue would be taken which would include a portion of the greatest diameter of the gland. In addition to this portion taken from all specimens, further segments were selected from some to provide longitudinal, oblique and tangential gland profiles and selected views of regions of nodular proliferation. These segments were embedded in Paraplast[1], serially sectioned at 6-8 microns and mounted for staining.

A similar procedure was used to select the segments of adrenals fixed in calcium-cadmium which were used for frozen sectioning. Sections were made at 5, 10, 15, and 20 microns with a Histo-Freeze apparatus equipped with a sliding surface microtome.[2] These sections were mounted on glass slides with the aid of egg albumin and allowed to air dry prior to staining cholesterol, but for the lipid staining they were stained

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[1]Scientific Products, Evanston, Illinois.

[2]Scientific Products, Evanston, Illinois.

before mounting.

Staining procedures used included the following:

1. Routine Hematoxylin and Eosin (Armed Forces Institute of Pathology, 1960)

2. Mallory's triple connective tissue stain (as modified by Crossman, 1937)

3. Heidenhain-Van Giesen-Weigert (as adapted by Getty, 1949)

4. Gomori's reticulum stain (Mallory, 1938)

5. Gomori's aldehyde fuchsin stain (Gomori, 1950)

6. Weigert's resorcin-fuchsin elastic stain (Mallory, 1944)

7. Oil-Red-O (as modified by Bell, 1959)

8. Schultz cholesterol (as modified by Weber, Phillips and Bell, 1956)

9. Sudan Black B Stain for fat (Chiffelle and Putt, 1951)

Photographic macro reproductions were made with a Leitz Aristophote II with Macro-Dia equipment and a 4 x 5 inch billows camera. Fine grained black and white panchromatic roll film, size 120, was used without filters.

Photographic micro reproductions were made with a Leitz Ortholux research microscope equipped with a 6V 30W lamp attachment, a FAS phototube, plano objectives and orthomat microscope camera. The color reproductions were produced on Kodachrome II professional film and were commercially processed. The black and white reproductions were produced on fine grain panchromatic, 35 mm. size, black and white roll film.

Histological Methods Employed for Electron Microscopy

Tissues from 17 dogs were processed for electron microscopy. From a point mid-way along both the left and right adrenals an approximately

15 millimeter transverse slice was removed and immediately immersed in 3% gluteraldehyde buffered with 0.1M sodium cacodylate buffer to pH 7.4 (Sabatini, Bensch and Barnnett, 1963).

The fixation was carried out at 4 degrees centigrade for 12 hours and then stored in the cacodylate buffer for further processing.

The thin slice of adrenal tissue was placed under a binocular dissecting microscope in a small watch glass containing the buffer. By varying the magnification the various cortical regions could be resolved (see Figure 1). From each cortical zone, the capsule and medulla at different points around the gland, at least two different samples were taken for further processing. These samples were gently dissected from the larger slice with the aid of a pointed scalpel blade and a wooden probe. The samples were approximately 0.5 mm cubes. The remaining adrenal slice was processed for routine light microscopy. By then evaluating these slices with the light microscope it could more accurately be determined from what region the sample had been taken and often what cell types might be included.

The glutaraldehyde-fixed samples were then post-fixed in 1% osmium tetroxide buffered with cacodylate. Following rapid dehydration through a graded series of alcohols, the samples were embedded in a mixture of Dow epoxy resins, D.E.R. 732 and 332 (Lockwood and Langston, 1964) and heat polymerization was used.

Glass knives in a Porter-Blum MT-2 microtome were used to produce sections exhibiting interference colors in the silver range. The thin sections were mounted on uncoated 300 and 400 mesh grids and were stained with 2% uranyl acetate (Huxley and Zubay, 1961) for 30 minutes followed by a 3 minute exposure to lead citrate, (Venable and Coggeshall, 1965) and examined in an Hitachi HU-11A electron microscope.

In addition a 0.5 micron and a 1 micron section was removed after the thin sections from the tissue blocks and mounted on a glass slide. These were stained with azure blue-methylene blue (Richardson, Jarett, and Finke, 1960) and examined with a light microscope to further identify the tissue being examined.

The electron photomicrographs were generally 8,500 times primary magnification and photographic enlargement was in the range of 3 to 4 times.

OBSERVATIONS AND RESULTS

Because of the relatively large size of the population being studied and to facilitate comparing of observations, the observations and results will be discussed according to groupings with respect to chronological age. Recognizing that these are rather arbitrary selections and that in the realm of biology one is often confronted with observations which refuse to be neatly categorized, nevertheless, these results are detailed according to the following systematization.

Group A -- Birth
Group B -- 4-63 days; 0.01 - 0.2 years
  Subgroup B1 - 4 and 9 days
  Subgroup B2 - 14 days
  Subgroup B3 - 28-36 days
  Subgroup B4 - 57 and 63 days
Group C -- 90-206 days; 0.3-0.6 years
  Subgroup C1 -- 90-100 days
  Subgroup C2 -- 178-206 days
Group D -- 238-373 days; 0.6-1.0 years
Group E -- 406-1520 days; 1.1-4.2 years
Group F -- 2192-3402 days; 6.0-9.3 years
Group G -- 3614-4980 days; 9.9-13.6 years

Group A: Birth (3 Specimens)

Parenchymal relationships

The adrenal glands at birth were composed of two different cell types indicative of their cortical and medullary analogues. However, the demarcation between the cortex and medulla portions was indistinct. Although medullary cell islets appeared in all areas of the cortex, in most regions these islets were seen as collections of centrally located medullary cells. A prominent central vein in the medulla appeared as the central axis around which were the satillite collections of medullary cells.

Capsule

A very delicate connective tissue capsule, measuring approximately 6 u in thickness, enclosed the parenchyma. The capsule was divisible into an outer and inner portion which shall be termed the fibrous and cellular portions of the capsule respectively. The outer fibrous portion was composed of 3-4 layers of fibroblasts and their intercellular ground substance which at the periphery blended very subtly with the surrounding adventitia. These fibroblasts contained a large, elongated, lightly stained nucleus which evidenced very little heterochromatin dispersed in the nuclear ground substance. The cytoplasmic limits of these spindle-shaped cells could not be resolved, but the cell was embraced on the long axis by delicate bands of collagenous fibers. These fibers are arranged such that they run in a circumferential direction on the outer capsule.

At this stage in development many small trabeculae were identifiable and appeared to be manifestations of the inner cellular portion of the capsule. The cells were predominate in these regions and few collagenous fibers were present. These cells seemed to be of the primitive mesenchymal type with the characteristically large, ovid nuclei containing very few grains of heterochromatin, and small amounts of cytoplasm extending into the relatively distinct fusiform cell processes. Cells of this description composed roughly the inner one half of the capsule. This inner capsular laminae was further modified as follows:

At regular intervals along its border with the underlying cortex, this cellular laminae was increased in thickness and gave rise to a funnel-shaped trabeculae which penetrated for a short distance into the cortex. In addition to a high concentration of the mesenchymal cell types, the larger of these trabeculae contained some collagenous fibers but the smaller ones did not. These extended into the cortex to blend imperceptively with the cortical parenchyma.

Still other patterns of capsule and cortex relationships were noted. At some foci there were direct communications between the inner capsular layer and the parenchyma. In still other regions there was a differentiation of cellular elements to the extent that sinusoidal spaces separated the capsule and the underlying parenchyma.

At those points where the developing capsule was covered by a layer of simple squamous epithelium, the peritoneum, there was an advanced progression of collagenous fiber development and a corresponding decrease in the size of cellular layer of the capsule.

Of considerable interest also were the islets of mesenchymal cells in the periadrenal adventicia. These discrete nodules of tissue contained cells typical of fat cells with a large central vacuole and a nucleus high in heterochromatin content displaced to the periphery of the cell. However, a large proportion of cells in these periadrenal fatty tissues displayed a nuclear and cytoplasmic morphology which in nearly all respects was similar to the parenchymal cells of the post-natal cortex, the spongiocytes.

Cortex

The corticies of these early postnatal dogs resembled in many respects those descriptions of the rat adrenal cortex. The cortex was in some areas of the zona glomerulosa very intimately associated with the capsule. The zona glomerulosa was narrow and blended subtly with the underlying cortex. A further sharp, definitive demarcation of the zonary boundaries within the cortex was not possible in this age group.

Zona glomerulosa This outer cortical zone was composed of large columnar cells whose cytaplasmic outlines were not well defined. The constituent cells were related to each other in their long axis and arranged juxtapositioned in an orderly register. Both free ends of these cells extend to border upon a sinusoid. While maintaining this relationship to each other and to the sinusoids, cords of these cells were in some areas seen to be oriented in such a manner as to form in a coil or ball or glomerulous. Thus, at the periphery and within the central portion of these formations there were sinusoidal spaces.

A centrally located, oblong nucleus with a well developed nucleolus and some scattered heterochromatin was typical of these zona glomerulosa cells. Each cell was characterized by a moderately acidophilic cytoplasm which with routine light microscopy appeared finely granular due to the great numbers of lipid vacuoles.

The intimate relationship of this zone to the capsule was very striking. The small trabeculae became partitions between those cell groups arranged as glomeruli and seemed to branch at the inner margin of the zona glomerulosa as "roots" to "contribute" mesenchymal cells to the zona glomerulosa, for at this point no longer could a distinction be made between the zona glomerulosal parenchyma and these trabeculae. At the inner margin of this zone there were some few circumferentially running collagenous fibers and sinusoids which, when coupled with the rather abrupt change from columnar to cuboidal parenchymal cell types, served as the inner "boundary" of the zona glomerulosa.

Zona intermedia No well-portrayed zona intermedia was evident in this age group. But the above mentioned circumferentially arranged collagenous fibers were indicative of this zone. These were formed from the lateral extension and anastomosis of the smaller trabeculae from the capsule.

Zona fasciculata This zone was the most prominent of the cortex in this age group. No distinction could be made which would have divided this zone into an inner and outer portion. The parenchymal cells were generally randomly distributed in large groupings which in selected regions were separated by lineal segments of fibroblast-like cells. These fusiform cells appeared either as delicate stromal elements with a fine collagenous component or as endothelial cells of the developing sinusoids. In some sections large trabeculae in this zone, having originated from the capsule, were detected. Within the collagenous matrix of these trabeculae were cells not of the fibroblast cell type, but much like the primitive mesenchymal cells of the inner capsular layer. These were found along the entire length of these larger trabeculae deep within the zona fasciculata and even extending to the cortico-medullar border. Two contrasting parenchymal cell types were apparent in this zone. The predominant cell type was a large cuboidal cell measuring up to 20u in diameter. A centrally located, large nucleus with numerous particles of heterochromatin dispersed in the nuclear ground substance was characteristic of these cells. The one, and often, two nucleoli seen in these nuclei were also conspicuous features of these cells. The cytoplasm contained large empty vacuoles and the filaments of cytoplasm forming the outline of these vacuoles were finely granular and faintly acidophilic.

The other prominent but less prevalent cell type was a smaller, more darkly staining cuboidal cell. The nuclei of these cells were smaller and contained a greater proportion of heterochromatin granules and strands than did the other zona fasciculata cell type. The nucleoli were still detectable in this darkly staining nucleus. The rather homogenous cytoplasm exhibiting a fine granularity and distinct acidophilia was notably lacking in vacuoles. These cells seen, in most cases, to be distributed randomly in all regions of this zone.

Zona reticularis The tissues in this juxtamedullary region were in general arrangement and specific cellular composition and morphology no different from that of the zona fasciculata. On the basis of these criteria no zona reticularis was present in this age group. However, near the cortical medullary border there were greater numbers of the darker staining smaller cells described above for the zona fasciculata. This cell type was confined to selected loci along the inner perimeter of the cortex and in other areas the cells were typical of the zona fasciculata. Therefore, with this one exception, the zona fasciculata was in all areas seen to extend to the medulla.

Cortico-medullary border.

The boundary between the cortex and medulla escaped quick detection with the light microscope. But when evaluated more closely at the point where cortical and medullary cells approximated, there were clearly distinct separations between the cortex and medulla. It was here that greater numbers of fibroblasts could be seen now to be arranged in a position around the circumference of the medulla and separating the cortex from the medulla. Trichrome staining revealed delicate collagenous fibers in greater concentrations here than in any other zona fasciculata region and very similar to the arrangement in the zona intermedia. Similar to the phenomena observed there, the larger trabeculae which had penetrated the cortex from the capsule were seen to terminate by branching and sending constituent fibers in a lateral direction to embrace the circumference of the medulla. The predominant cell type was the fibroblast, however, some primitive mesenchymal cells were evident.

Medulla

There was an abrupt change in the parenchyma-stromal ratio and relationship in the medulla versus the cortex. In the two dimensional field 8-10 medullary cells were arranged in a conglobate fashion surrounded by a well defined fibroblast web. These nests of parenchymal cells were uniformly distributed in the well-defined reticulum in all areas of the medulla save juxta to the central collecting veins. Here the parenchymal cells were arranged so as to "outline" these venous channels. One further exception was the regular occurrence of cortical cells as groups of two or three cells in the medullary parenchyma and along the large medullary veins.

The medullary cell was a large rounded cell with a proportionately large nucleus and scanty amounts of cytoplasm. The large vesicular nucleus contained usually a single nucleolus and scanty amounts of heterochromatin. With hematoxylin and eosin staining, the cytoplasm appeared less eosinophilic than the cortical cells but not basophilic. Most cells presented a uniformaly granular, dense cytoplasm but others were sparsely granular.

Contrasted to the poorly developed sinusoids of the cortex, the medullary sinusoids were well developed and prominent. They pervaded the regions between the conglobates of medullary cells and were distended with blood.

Cortical nodules

There were large, descrete sections of periadrenal and perirenal fat the cells of which, as mentioned above, bore a striking similarity to the zona fasciculata parenchymal cells. The capsule of the glands in this age group were very cellular especially the innermost portion of the capsule. There were in a few cases on the outer portions of the capsule pericapsular nodules of cortical cells. More prevalent, however, were areas of local hyperplasia of cells within the fibrous portion of the capsule which were termed intracapsular nodules.

In one specimen (B8) there were satellite nodules of tissue which were independent of the capsule of the adrenal. They were contained within a fibrous capsule and possessed a cellular morphology similar in all respects to that in the medulla. These appeared similar but, nevertheless, different from the paraadrenal sympathetic ganglia. They were supplied richly with blood and were considered as possibly being isolates of medullary cells.

Group B: 4-63 days (10 Specimens)

Subgroup B1: 4 days and 9 days (2 specimens)

Capsule A cellular capsule was prominent in this age group. This cellularity was confined to the inner portion of the capsule and was contrasted against a quite well-developed fibrous outer portion. Trichrome staining revealed large bands of collagen in this outer portion. The interstices between these fibers were occupied by the still active fibroblasts, as evidenced by its large oblong, vesicular nucleus.

The mesenchymal cells with their large vescicular nuclei composed the larger portion of the inner capsule. Occupying the same area however, were a few fibroblasts as evidenced by contrasting nuclear morphology and the presence of collagenous fibers. The further development of the underlying zona glomerulosa into prominent glomeruli and arching loops directly affected the morphology of this cellular capsule layer. Over these glomeruli and arcades of the zona glomerulosa were corresponding indentations into this cellular layer; the effect being to produce arches within this layer also. At the peak of these arches in the inner capsule layer the cellularity was decreased, while at the margins and supports (trabeculae) the cellularity was much increased.

These cellular projections continued into the small trabeculae which supported and subdivided the underlying zona glomerulosa. Also prominent at this stage of development were sinusoids between the glomerulosa cell arcades and the arches in the capsule. The net effect being to prevent the continuity of the cellular capsule with the underlying cortex in these regions. The only area of communication between the capsule and cortex were the numerous highly cellular trabeculae.

<u>Cortex</u> The corticies of this age group had differentiated cytologically to such a point that one could distinguish on the basis of cytological criteria the three primary cortical zones. However, the tissue arrangement and subtle blending of one zone into the next made easy recognition of the zonary boundaries impossible.

<u>Zona glomerulosa</u> The columnar cells of this zone were arranged in lateral register and each column of such cells had coiled upon itself so as to form many varying shapes which had a sinusoidal space in the center and at the periphery of the cord. The arrangements of these cords into glomeruli was the pattern most often seen, but, on occasion, profiles of S, M,Ω-shaped arrangements could be detected.

The vacuolated and faintly eosinophilic cytoplasm contained a large, spherical, vesicular nucleus with a prominent nucleolus. Each cell radiated from the center of the coil, where its base rested on the central sinusoid, to a similar position for the other base resting upon the peripheral sinusoid. In some regions of this zone the cells were low columnar and contained a relatively darkly staining nucleus. Often these cells were found at the inner margin of this zone. The cords and coils were limited by the smaller trabeculae which appeared to limit and direct the nature of the coil formed. At the inner margin of the zona glomerulosa the columnar cells blended very subtly with the mesenchymal elements of these trabeculae. Also within the central sinusoid of the glomerulosa coils these same mesenchymal cells could be seen. The change in cellular morphology and the appearance of intersellular collagenous fibers established the inner limits of the zona glomerulosa for this age group.

Zona intermedia At the inner margin of the zona glomerulosa where the coils seemed to originate, there were sinusoidal spaces and accompanying endothelial cell and collagenous fiber elements which ran parallel to the capsule. These elements appeared to arise from the divergency of the cells and fibers of the small capsular trabeculae. Along the course of this very narrow undulating region were many elongated, dark staining nuclei which betrayed resting stages of fibroblast cells. But at the inner margin of the zona glomerulosa where pyramidal shaped interstices between the glomeruli occurred, there were many primitive mesenchymal cells. These cells were also scattered along the course of this fine collagenous band between the zona glomerulosa and the zona fasciculata, the zona intermedia.

Zona fasciculata The cuboidal cells of this largest cortical zone were aligned in plates and anastomosing cords of parenchymal cells which radiated from the medulla toward the capsule in a "sun-burst" fashion. This morphology seemed to be directed by the many sinusoids now well-developed in this zone. These were lined by endothelial cells or littoral cells and, while traversing this zone from their origin at the zona intermedia sinusoids to the zona reticularis, they follow a strict radiating couse much like the spokes of a wheel.

The parenchymal cells nestled between these sinusoids were on the basis of cellular morphology subdivided into an inner and outer zona fasciculata, each composing about one half of the zone.

In the outer zone the cells were large and cuboidal with a large spherical nucleus with only limited amounts of heterochromatin. Their cytoplasm was highly vacuolated and possessed only a thin network of faintly acidophilic cytoplasm.

A similar nuclear morphology was manifest by the cells of the inner zona fasciculata but the cytoplasm was lesser in amount. The cytoplasm was replete with vacuoles but they were smaller than those of the outer zone and thus more intervenient cytoplasm was seen as a weakly acidophilic network. Herein also were detected cells of a second but less prevalent type. These were of the same size as the other parenchymal cells in this region, but were characterized by a finely granular cytoplasm and a dark staining basophilic nucleus.

Zona reticularis As the parallel cords and plates of the zona fasciculata approach the medulla, they reach a region where they rather abruptly lost this orderly arrangement and deviated in many varying patterns. These parenchymal cells possessed the same nuclear and cytoplasmic proportions as did the cells in the fasciculata regions. The cells occurred in groups of 10 to 15 in islands surrounded by large sinusoidal spaces or in cords and plates of cells parallel to the large sinusoids.

The parenchymal cells were of two general types. The most prevalent cell was a large cuboidal cell with a vacuolated but strongly eosinophilic cytoplasm. Their large rounded to ovoid nucleus contained a prominent nucleolus and 3-4 false nucleoli with several fine granules of heterochromatin in a large amount karyoplasm

A second type of cell occurred in significantly high numbers. They were characterized by smaller amounts of non-vacuolated, eosinophilic cytoplasm and a strongly basophilic nucleus with larger amounts of heterochromatin and an irregular contour of the nuclear envelope. These cells were not randomly distributed in this zone but rather occurred in groups of 4-5 near the cortico-medullary border.

A system of large diameter sinusoids permeated the zona reticularis. The endothelial lining cells were supported by delicate collagenous fibers as detected by trichrome staining.

Cortico-medullary border   The now rather sharp but undulating border between the cortex and medulla was accentuated by a narrow band of mature fibroblast and their collagenous fibers. This band seemed to arise from the confluence of the sinusoidal collagenous bundles and the trabeculae which had penetrated directly through the cortex from the capsule.

Medulla   The medulla had in large part achieved its central location but there were still isolates of medullary cells in the cortex. In one specimen (B 10) the medulla extended to the glomerulosa and some elements of this extension pervaded on through the zona glomerulosa into the capsule to form a periadrenal nodule of medullary cells. Medullary cell groups were surrounded by a reticulum of fibroblasts and numerous sinusoids permeated the entire tissue. The parenchymal cells were of two basic types. Both possessed a large vesicular nucleus with scanty heterochromatin but one was best characterized as having a large amount of "washed out" cytoplasm and the other as having a similar amount of acidophilic granular cytoplasm.

Frequently throughout the medulla, but especially near the central vein, islets of cells characteristic of the larger cells in the zona reticularis were encountered.

Cortical nodules A large intracapsular nodule of medullary (not cortical) tissue was observed in the 9-day old specimen. In other areas of the adrenals in this age group a few small descrete hyperplastic nodules of cortical cell types were seen within the cellular layers of the capsule.

Subgroup B2: 14 days (2 specimens)

At two weeks the adrenals were fairly well differentiated according to tissue morphology into four major cortical zones and the medulla. However, large portions of the medullary parenchyma still remained within the cortex and some was outside the capsule with an attachment to the underlying adrenal proper.

Capsule The thick capsule of this age group was divided into rather distinct laminae. The outer composed approximately one third of the capsular width and was the fibrous layer of the capsule. The elongated nuclei of actively secreting fibroblasts were sharply contrasted between the numerous large collagenous bundles. The outer surface of the fibrous layer was continuous with the adventitia which became a tunica serosa in areas bounded by the peritoneal cavity. This fibrous layer was found in most areas to be intimately related to the inner capsular layer, but in regions where intracapsular or pericapsular cortical nodules occurred, this layer was observed to bifurcate and pass in a plane which would enclose these nodules in a delicate capsule.

In the inner two thirds of the capsule the proportion of collagenous fibers to cells was low. With routine staining most of these cells lacked clearly defined cytoplasmic boundaries, but their nuclei were large and circular to ovoid. Their karyoplasm contained finely granular heterochromatin. These nuclei were seen in great concentrations in this layer and served as an index to the great numbers of cells in this region. At the summits of the underlying glomerular arches no continuity was observed between the capsule and cortex, but at the spaces between these arches the capsule sent small trabeculae deep into the zona glomerulosa. These were highly cellular and contained a cell which tested negative for lipid storage and possessed a morphology suggestive of a primitive mesenchymal cell. These appeared to be the avenues of communication between the cellular capsule and cortex.

Zona glomerulosa The columnar cells of this zone appeared more elongated than in the previous age group. The large nuclei were oblate-spheroid in shape and were located in the central portion of the cell. Each cell extended in its length from one sinusoid to the next adjacent one.

Cholesterol and lipid staining combined with the appearance of vacuoles in paraffin sections portrayed a distribution of cholesterol and lipid positive materials at the poles of these cells with the center of the cell being occupied by the large nucleus.

The columns of parenchyma cells were contorted into arches and glomeruli.

Zona intermedia Along the inner margin of these glomerular bundles a sinusoid ran parallel to the capsule. Around this sinusoid was a delicate collagenous support and mature fibroblasts and along its course were large, vescicular nuclei betraying the presence of the primitive mesenchymal cells. It was also at this boundary between the zona glomerulosa and zona fasciculata that the numerous small trabeculae from the capsule terminated and contributed their cellular contents to the zona intermedia.

Outer zona fasciculata The cells comprising this zone were generally arranged in radiating plates and cords with narrow sinusoids approximating at least one of the cell surfaces of each cell. These cells appeared juxta to the zona intermedia and were large and cuboidal in shape with a highly vacuolated cytoplasm. Frozen sections of this area stained highly positive for neutral lipids and these cells were the most strongly cholesterol positive of all the cortical cells, this due in part to the greater amount of cholesterol stored.

Inner zona fasciculata This zone was an inward continuation of the plates of cells from the outer fasciculata. The difference being a rather subtle change to a more acidophilic cell. The only differences between the cells of the two zones being a somewhat darker staining nucleus, great numbers of small vaculoles within the cytoplasm and less affinity for the cholesterol stain of the inner zone's parenchymal cells.

Zona reticularis The regular arrangement observed in the zona fasciculata was altered to produce a random association of parenchyma surrounded by large sinusoids and greater amounts of stroma in the zona reticularis. Also there was a rather abrupt cellular change to an accentuated acidophilia in all cells of this zone. Some cells contained a homogenous granular cytoplasm while others contained varying degrees of conspicuous vacuoles. The nuclei were similar in all respects to those of the zona fasciculata cells and the cytoplasm of the cells in this zone was strongly cholesterol positive.

Cortico-medullary border A large sinus at the periphery of the medulla was bounded by supportive mesenchymal tissue elements. Varying amounts of collagenous fibers, fibroblasts and primitive mesenchymal cells were observed along the course of this delicate band of connective tissue which separates the cortex and medulla.

Medualla The medullary tissue and cells differed in no significantaspects from the descriptions of earlier age groups. Similarly, large amounts of medullary parenchyma were still isolated within the cortex and in some areas large masses of medullar tissue were seen to be out of the parent adrenal gland and connected to the gland by a stock of tissue which penetrated the capsule.

Cortical nodules Several foci of intracapsular hyperplasia were detected in this age group. The smaller nodules were imbedded in the capsule but the larger ones measuring up to .5 mm in diameter, although they were within the capsule, had enlarged above the capsular profile and depressed the underlying cortex in their development. A substantial capsule surrounded each of these nodules, the majority of which were composed of cells which were similar in all respects to the underlying zona glomerulosa cells. These cells were Oil-Red-O and cholesterol positive and were, in the larger nodules, coiled into glomeruli and arches which were richly supplied with sinusoids.

Subgroup B3: 28-36 days (4 specimens)

Capsule In this age group the composition of the capsule had altered to a fibrous portion composing the outer two thirds and a cellular portion composing the inner one third. There was, therefore, a considerable increase in collagenous fibers over the previous age group. Many of the larger vesicular nuclei described in the preceding age groups now were replaced by nuclei which indicated a further differentiated cell type by being smaller, oval to elongated and more basophilic. However, cells in the inner cellular portion retained the properties ascribed to an undifferentiated cell.

Concurrent with the further development of the underlying zona glomerulosa the communications between cellular capsule and cortex were confined to the trabeculae. The larger trabeculae contained many of the undifferentiated cells, but the smaller ones contained very few. These smaller trabeculae contained a few prominent fibroblast cell nuclei and associated collagenous fibers and penetrated the zona glomerulosa to the zona intermedia.

Zona glomerulosa The columns of columnar cells had become more contorted and there was a thickening of this zone due to an elevation of these glomeruli and arches in a plane perpendicular to the capsule. In those columns of cells thrown into erect arches, the bases of the arch would be composed of columnar cells 3-4 times as tall as their diameter measure, and as one examined cells progressively to the peak of the arch, the cells increased in height to a cell which was nearly 9 times its diameter measurement. Only a faint reticulum of cytoplasm remained to highlight the perimeter of medium sized vacuoles which packed each pole of the cell from the basal ends to the nucleus. These cells were strongly cholesterol positive. The nucleus was elongated to conform to the shape of the cell; most were oblong, but some were cylindrical with blunt ends. Generally, two nucleoli were seen among scant amounts of heterochromatin in the karyoplasm.

Prominent sinusoids bordered these arches and coils of glomerular cells and thus effectively prevented the transfer of cells directly from the capsule or cellular trabeculae to the zona glomerulosa.

Zona intermedia   The cellular trabeculae from the capsule, after having traversed the zona glomerulosa, deposited their cellular contents at the junction of the zona glomerulosa and fasciculata. These cells seemed to fan out like the roots of a young plant and assume a horizontal position in this zona intermedia. In the areas between these trabeculae bases there were sinusoids, fibroblasts and delicate collagenous fibers separating the two larger cortical zones on either side. At the points of cellularity in the zona intermedia, the zona glomerulosa and zone fasciculata appeared to be continuous with this very narrow band of small cuboidal to low columnar cells. In two specimens (B 28 and B 29) the zona intermedia was more developed than in the others. The sections of these gland evaluated for cholesterol and lipid showed a narrow, descrete cholesterol and lipid deficient band at the region of the zona intermedia in an otherwise very cholesterol positive adrenal cortex.

Outer zona fasciculata This tightly packed zone just inside the zona intermedia was chacterized by a large cuboidal parenchymal cell containing a rounded vesibular nucleus in the center of a highly vacuolated cytoplasm. In these two dimensional profiles it appeared that as few as 8-10 of these very large vacuoles filled the equitorial profile of the cell.

Scattered at random in the outer fasciculata zone were smaller cells with acidophilic cytoplasm and a very basophilic nucleus.

Inner zona fasciculata The parenchyma of this region of the cortex was composed of cells which were a degree smaller than those in the outer zone. The nuclei were more basophilic but in all other respects seemed similar to those of the outer zone. The difference in size of the component cells was due to a lesser amount of cytoplasm which contained smaller sized vacuoles. Thus, the cytoplasmic hyaloplasm remaining gave a faint acidophilia to the cytoplasm. Scattered about in this zone were the darker staining cells described above for the outer zone.

Zona reticularis Rather abruptly there was a transition to strongly acidophilic cells randomly arranged in groups between sinusoids next to the medulla. These cells varied from those possessing a rather homogenous granular, nonvacuolated cytoplasm with a dense basophilic nucleus to those with varying numbers of vacuoles and often times binucleate cells. Zona reticularis-like cells were seen as isolets in many areas of the medulla.

Cortico-medullary border The separation between cortex and medulla was marked by more prominent strands of collagenous fibers in this age group. In all other respects this area was similar to those previously described for subgroup B2.

Medulla In this age group no evidence of extramedullary parenchyma was seen. All of the medullary cells appeared to be included within the medulla proper. The medullary cell was characterized by a large cuboidal cell pale staining cytoplasm and a large, rounded basophilic nucleus. A few cells contained a more homogenous granular cytoplasmic ground substance. These cells were grouped into conglobate nests of 8-10 which were contained by a prominent stromal network.

Cortical nodules A few descrete intracapsular nodules and some flattened and elongated pericapsular nodules closely applied to the fibrous capsule were noted in this age group. All were composed of cells similar to the zona glomerulosa parenchyma.

Subgroup B4: 57 and 63 days (2 specimens)

Capsule The undifferentiated cellular regions of the capsule, blastemal regions, were limited almost entirely to those areas where the capsule sent large and medium-sized trabeculae into the zona glomerulosa. The remainder of the capsule contained active fibroblasts and accompanying large collagenous fiber bands. At no point on the inner capsular surface was there evidence to suggest a continuation of capsule into the zona glomeruolsa. This separation was further augmented by the presence of an extensive subcapsular sinusoidal plexus.

Some trabeculae were quite large and could be followed in one plane from the capsule to corticomedullary border, suggesting that these were possibly small septae-like structures wedged into the cortical parenchyma. These stromal elements carried large blood vessels and nerves as well as primitive mesenchymal cells.

Zona glomerulosa   The plates and columns of cords present a more elongated inverted U shape than in previous age groups. In some areas profiles of laterally recumbent S shapes were evident as well as many other vermiform arrangements. The overall appearance was one of less randomly arranged coils and glomeruli and a trend toward more uniform arcades in this region. The trabeculae between the arcades and other cell groups of the zona glomerulosa contained large numbers of seemingly undifferentiated cells in a delicate collagenous fiber support. These cells were lipid and cholesterol negative. These extensions of the cellular layer of the capsule continued to the base of the zona glomerulosa where they spread and effectively "surrounded" or "capped" the inner bases of many of the glomerulosa columns.

Zona intermedia   This zone was formed in large part by the anastomosing of the diverging trabecular ends below the zona glomerulosa. The effect being to partially isolate the zona glomerulosa from the zona fasciculata. Along with the sinusoidal space, fibroblasts and collagenous fibers, and primitive mesenchymal cells this zone contained a few small low columnar cells. These cells were arranged in the plane of the zona intermedia and were stacked in groups of three or four. They contained a dark nucleus and a small amount of highly vacuolated cytoplasm and were only slightly positive for neutral lipids and cholesterol.

<u>Zona fasciculata</u> The zona glomerulosa arcades in a few areas were seen not to be bounded by the zona intermedia but to continue on into the zona fasciculata as low columnar cells and then gradually merge as cuboidal cells into the deeper regions of the zona fasciculata. However, in most cases the zona fasciculata was observed to abut directly upon the inner margin of the zona intermedia where this region appeared as the beginning of the outer zona fasciculata. The large cuboidal cells contained large droplets of lipid and cholesterol positive material. They were similar to those cells described in subgroup B3.

The inner two thirds of the zona fasciculata was composed of columns and plates of cells whose cytoplasm was not so distended with large droplets. This in effect produced a narrower cord diameter and was followed by an increase in the sinusoidal space size in this zone. The individual cells were filled with the smaller, fatty, Oil-Red-O positive droplets. The cytoplasmic and nuclear morphology of these cells was similar to that given in subgroup B3.

<u>Zona reticularis</u> The radiating columns of the inner zona fasciculata approached the medulla and quite abruptly lost their ordered array. These plates and columns had become the predominately cord and bundle-like arrangements of the zona reticularis. Large sinusoidal channels infiltrated between these cell groups. The cells were very lipid positive and some isolated cells displayed a selected affinity for the Oil-Red-O dye. The zone was moderately cholesterol positive and a significant proportion of the cells contained nuclei with considerable amounts of heterochromatin.

Cortico-medullary border The large trabeculae penetrating directly from the capsule to the medulla contributed cellular and fibrillar elements to this band of delicate connective tissue as in the previous age groups.

Medulla The medulla was not significantly different from that description given under subgroup B3. Islets of zona reticularis-like cells were within the parenchyma and were especially concentrated at the central vein.

Cortical nodules A few spherical to elongated intracapsular nodules of the zona glomerulosa-like cell type were noted. At one focus in the capsule the apex of the 3 or more arcades were prolapsed through nearly the entire capsule and projected for a short distance into the adventitia.

Group C: 90-206 Days (18 Specimens)

Subgroup C1: 90-100 days (5 specimens)

The general relationship between cortical and medullary elements, the zonary development and arrangement and the cellular morphology and histochemistry of this age group appeared to be that of a well-differentiated, "classical," "normal" adrenal gland.

Capsule The capsule was still divisible into a fibrous and cellular portion. The outer fibrous portion was composed of densely packed, coarse collagenous fibers. The interfibrillar spaces were very narrow and the fibroblast nuclei could be seen in these spaces as an elongated rod-shaped structure with much heterochromatin. These nuclei were characteristic of a resting fibroblast or fibrocyte. The inner layer was still composed of active fibroblasts and associated collagenous fibers. At regular intervals this layer gave off trabeculae at right angles to the capsule proper which penetrated deeply into the zona glomerulosa. At the point of origin beneath the capsule these rather broad based trabeculae were very cellular. These trabeculae then rapidly narrowed as they were compressed between the developing zona glomerulosa arcades only to expand into a divergent highly cellular base at the inner margin of the zona glomerulosa. Thus the inner and outer morphologies, the trabecular origins and ends, were quite similar. The cells at the respective extremities of the trabeculae were of the primitive mesenchymal cell type with a faintly acidophilic cytoplasm and a large vesicular nucleus. High magnification examination of thin sections of these cells revealed no lipid vacuoles and they were also shown to be Oil-Red-O and cholesterol negative. At the narrowed middle point these trabeculae contained only a sinusoid and its collagenous fiber support and reticular fiber staining revealed that delicate reticular fibers are predominant in these trabeculae.

Zona glomerulosa The arrangement of the columnar cell cords was predominantly in the form of an elongated inverted U with the convexity of the arch beneath the capsule and the ends extending inward and approaching the zona intermedia. The more elongated and larger diameter columnar cell was usually seen at the peak of the arches and those cells in the lateral extensions of the arches were progressively decreased in size toward the low columnar cell juxta to the zona intermedia.

Trichrome and reticular fiber staining failed to demonstrate any fibrillar material between these columnar cells. However, all of the cells did extend to the sinusoid at each end where an extensive reticular support surrounded these sinusoids.

The elongated columnar cells characteristic of this zone were often seen to be laterally compressed to such an extent that only a narrow rim of cytoplasm was present between the nucleus and the lateral cell membranes. The nucleus was elongated and cylindrical with blunted ends. Scattered granules of heterochromatin were evident in the karyoplasm along with a prominent nucleolus and several large false nucleoli.

The elongated nucleus occupied approximately the central 1/5th of these columnar cells and the remaining 2/5ths of the cytoplasm at each pole was filled with lipid droplets which contained large amounts of cholesterol positive material. The intervening hyaloplasm was faintly acidophilic.

Zona intermedia   At the inner margin of the zona glomerulosa there was a rather sharp line of demarcation between this zone and the zona fasciculata. With trichrome and reticular stains a prominent collection of collagenous and reticular fibers could be demonstrated to run parallel to the capsule at the base of the zona glomerulosa. This was the narrow zona intermedia.

A sinusoid was seen to follow the course of this narrow band of tissue and, at points corresponding to the inner portions of the capsular trabeculae, a group of cells resembling undifferentiated mesenchymal cells could be detected. These cells in some places exhibited a few cytoplasmic vacuoles and some degree of uptake of the cholesterol stains indicating that intermediate stages between these and the functional cortical cells were also present.

Outer zona fasculata From the lamellae of the zona intermedia collagenous fibers and delicate reticular fiber elements originated and penetrated into the zona fasciculata. These fibers were oriented parallel to each other and perpendicular to the capsule and they were located between the cell columns and plates and gave support to the sinusoids. From each of these, delicate reticular fibers extended laterally to surround and support small groups of cells and in some cases individual cells.

The large cuboidal parenchymal cell of this region contained a large, ovoid, vesicular nucleus with a prominent nucleolus. These cells were rich in neutral lipids and cholesterol. In this age group the cells appeared very uniform in size and staining characteristics, however scattered throughout the zona were a very few singly occurring cells which were small, very acidophilic and possessed a condensed, darkly staining nucleus.

Inner zona fasciculata The stroma of this region was similar to that of the outer zona fasciculata except that there were large reticular fibers around smaller nests of cells and each cell received an extensive reticulum network. The parenchyma was composed of branching cords and plates of cuboidal cells with narrow intervening sinusoids. Each of the moderately eosinophilic cells approximated a sinusoid on at least one cell surface. The centrally located nucleus was surrounded by a cytoplasm containing many cholesterol positive lipid droplets. These did not fill and distend the cytoplasm as in the outer fasciculata but they occupied the larger portion of the cytoplasm. Approximately 10 per cent of the cells in this zone displayed a pronounced eosinophilia, a condensed amount of vacuolated cytoplasm and a strongly basophilic nucleus.

Zona reticularis Continuing toward the innermost portion of the cortex, the stroma became increased in amount and individual groups of three or four cells were seen to be surrounded by prominent reticular and collagenous fibers. These gave rise to a delicate reticulum of fibers which embraced each parenchymal cell.

The cells of the zona reticularis were smaller than the other cortical cells. They were polyhedral with a spherical nucleus containing a single nucleolus and some considerable amounts of heterochromatin. Most of the cells displayed a cytoplasm with a limited number of cholesterol and lipid positive droplets, but there were others which seemed to be lacking these droplets. All of these cells were strongly eosinophilic, but those small cells without lipid droplets showed a greater affinity for the acid dyes.

Cortico-medullary border A condensation of collagenous and reticular fibers at the cortico-medullary border, when coupled with the prominent sinusoid in this location, produced a small but descrete band separating the two organs, the cortex and medulla.

Medulla Cortical parenchymal cells similar to the cells of the zona reticularis were observed isolated within the medulla. The medullary cells islands were contained by well-developed connective tissue at the perimeter. There were 10-12 cell profiles appearing in a cross section and they were of two general cell types. However, within an islet the cells were usually of the same type.

The more prevalent cell type was a large polyhedral cell possessing a large vesicular nucleus with a very prominent nucleolus and a finely granular cytoplasm which stained moderately blue with methylene blue-analine blue staining. The second cell type was a smaller cell which was of different shapes and outlines. However, the nucleus was of the same size and general morphology of the above mentioned cell type. The primary difference was the very fine, darkly staining cytoplasmic granules in these cells and the occurrence of a few small vacuoles.

With hematoxylin and eosin these cells differed only in that the more granular cell stained more eosinophilically. With light microscopy the cell membranes were not well delineated for either cell type.

Cortical nodules Several nodules of zona glomerulosa-like cells were seen within the capsule. These descrete intracapsular nodules varied from spherical to oblong in shape and were composed of mature functional parenchyma as evidenced by cholesterol and neutral lipid positive tests. These nodules were sharply delineated and separate from the capsular cells surrounding the nodule. No intermediate stages of development were noted among the nodule cells.

One large pericapsular nodule was seen whose diameter was approximately 1 mm. The zona glomerulosa cells had differentiated into coiled columns and cells of the zona fasciculata occurred in the center of this nodule.

Subgroup C2: 178-206 days (13 specimens)

The adrenal arrangement and morphology of this age group did not differ in any conspicuous mode from that of the subgroup C1. Only those few notable differences will be detailed.

Capsule The division of this limiting structure into an inner cellular and outer fibrous zone was no longer easily accomplished. Large, prominent trabeculae were observed to penetrate deeply into the cortical parenchyma and some extended to the medulla. These trabeculae contained large numbers of undifferentiated cell types which were neither cholesterol or neutral lipid positive.

Zona glomerulosa The arcades were the predominant mode of coiling for the cell columns of this zone and they were progressively more laterally compressed and elongated with their long axis perpendicular to the capsule. The glomerulosa was developed on all sides of the large trabeculae which penetrated the cortex.

Zona intermedia There was an increase in the amount of stromal fibers in this zone which, when viewed with a concurrent increase in the collagenous content of the small capsular trabeculae, which subdivided the zona glomerulosa, gave the appearance of compartmentalizing small areas of the zona glomerulosa. Thus, some arcades appeared to be bounded by the capsular trabeculae laterally, on their apex by the capsule and at their base by the zona intermedia.

There were greater numbers of the small, darkly, staining, partially differentiated cells in this zone than in previous age groups. There were sufficient numbers of these cells to render this narrow zone easily recognizable with low power magnification. Selected histochemistry procedures (cholesterol and lipid analysis) rendered evidence which suggested 1) this zone to be lipid and cholesterol deficient and 2) this zone to be wider than was morphologically apparent.

Zona fasciculata and zona reticularis   Distributed at random throughout these zones were individual cortical cells which appeared strikingly similar to the signet-ring cell of common fat cells. Oil-Red-O positive material occupied a single vacuole in the center of the cell and the condensed, dark staining nucleus was displaced to the periphery of the cell. These were considered to be cells which had undergone fatty metaplasia.

Cortico-medullary border   The contribution of the large capsular trabeculae to the formation of this structure was clearly evident. At the inner portion of these trabeculae near the medulla they broke up into large bundles of collagenous fibers which embraced the cords and nests of the zona reticularis parenchyma. There was a prominent precipitation of fibers between the cortex and medulla.

Cortical nodules   A limited number of small subcapsular, intracapsular and pericapsular nodules of cortical cells were detected. Some nodules of medullary cells were also within the capsule.

Group D: 238-373 Days (28 Specimens)

In this interval of nearly 4.5 months the adrenal gland morphology was altered only slightly but significantly from that description given for the previous group.

Capsule

A capsule was not altered in thickness but the inner cellular layer had been replaced by collagenous fibers to such a degree that the capsule was nearly all a dense fibrous connective tissue stroma. The trabeculae given off at right angles to the capsule and extending far into the cortex contained prominent collagenous fibers and only a few relatively undifferentiated cell types.

Cortex

Zona glomerulosa   The elongated inverted U shaped pattern of columnar cell columns in this zone was duplicated in the space between the trabeculae around nearly all of the area included in the zone. Different profiles of columns were often present but they were variants of this basic morphology. The elongated columnar cell with a centrally located nucleus, which took the same shape as the cell, extended to touch two sinusoidal spaces. The cytoplasm was filled on each side of the nucleus with great numbers of medium sized cholesterol and lipid positive droplets.

However, in a limited number of arcade regions the cellular morphology and arrangement was quite different. In these areas, limited by the trabeculae, small columnar cells with dark staining nuclei and strongly acidophilic cytoplasm were arranged in random whirls. These appeared much like smaller sized cords of cells which were coiled several times over. The cellular morphology was strikingly similar to that of the zona intermedia parencyma. In some cases these cells appeared to be outward extensions of the zona intermedia.

In as many as four specimens the zona glomerulosa was narrowed in width in some small areas such that the zona fasciculata approached the capsule. In these areas the zona intermedia was not evident and only a horizontally extending cord of zona glomerulosa cells separated the zona fasciculata and the capsule. Serial section evaluation revealed that in two of these specimens, in very limited areas, the zona fasciculata and capsule were not separated by the zona glomerulosa. At such points of contact the capsule was quite cellular and the evidence suggested a continuity between the capsule and the zona fasciculata. The cells in the capsule at these foci appeared to be well differentiated zona fasciculata cells wedged into the base of a still very fibrous capsule.

Zona intermedia This zone was increased in width due to a greater content of collagenous fibers and greater numbers of lateral compressed small columnar cells. The zone blended imperceptively with both the parenchyma of the zona fasciculata and zona glomerulosa.

Zona fasciculata and zona reticularis These zones were cholesterol, Oil-Red-O and Sudan Black B positive. All zones (inner and outer fasciculata and zona reticularis) were present in each specimen, but the amount of each zone varied, considerably within the group; ranging from a large to a very small component in the cortex. Some corticies contained scattered single cells which had undergone fatty metaplasia to form signet ring cells. In one specimen (C8) a few degenerative foci involving cells of the inner zona fasciculata were observed. In these areas 2-3 or as many as 10 cell profiles were seen and the cells were undergoing cytolysis. The cytoplasm was filled with facuoles and eosinophilic cell debris. The nucleus was small but not pyknotic.

Cortico-medullary border

At the inner portion of the zona reticularis the thick collagenous fibers were separated only by rows of parenchymal cells from this zone. These fibers followed the contour of the cortico-medullary boundary and gave the appearance of a medullary capsule containing parenchymal cells of the zona reticularis.

Medulla

The same basic arrangement of parenchyma cell islands surrounded by a prominent stroma and large sinusoids is persistent in this age group. With trichrome staining a brilliant red granulated cytoplasm is evident in some cell groups while others show a relatively homogenous pink cytoplasm. These cells with methylene blue-analine blue staining revealed dark blue granules filling the cytoplasm of one cell type and a homogenous light blue hyaloplasm in the other.

At the hilus of the adrenal the capsule was continuous with the adventitia of the central vein in the medulla. Cortical cells were seen to continue along the vein well into the medullary parenchyma. In several regions of many specimens the zona reticularis cells could be seen to have invaded the medullary parenchyma by perforating the cortico-medullary border.

Cortical nodules

Intracapsular, pericapsular and satellite nodules of medullary tissue were observed in this age group. The occurrence of cortical cell nodules was variable. Some specimens appeared to only have a moderate number of small intracapsular nodules, while others (C41, B56, B62, C17, A23) also exhibited several larger subcapsular, intracapsular and pericapsular nodules. In the case of B56 very large intracapsular nodules were present. These nodules contained large amounts of functional cortical tissue. Well formed zona glomerulosa and fasciculata cells were present along with cells typical of the zona intermedia. These zona intermedia-like cells were found in greatest concentrations at the points where it appeared the original prolapse of cortical tissue into the capsule had occurred. These cells were also observed to the continuous with the underlying cells of the zona intermedia.

Group E: 1.1-4.2 Years (40 Specimens)

The overall morphology and arrangement of adrenal cortical and medullary tissues in this age group was generally similar to the picture developed in group D. However, variations were commonly encountered throughout the age group. Those deviations and modifications of the "normal" pattern will be presented.

Capsule and glandular stroma

The fibrous capsule sent fibrous trabeculae into the parenchyma of the cortex. The stroma of the entire cortex seemed to be related in some direct or secondary mode to the capsule. The thickened trabeculae arose directly from the capsule and separated the arcades of zona glomerulosa cell cords. The individual cells of this zone did not receive any collagenous or reticular fiber support; their only relationship to the stroma being that each end of the cell extended to rest on a sinusoid. At the inner margin of the zona glomerulosa branches of these trabeculae extended laterally to join with similar extensions from other trabeculae. The thickness of this band of collagenous and reticular fibers varied within the group. In those specimens having a prominant fibrous band at the zona intermedia, the cell content was decreased. However, in those specimens showing an extensive number of cells in this zone, the fibrous content was proportionately decreased.

From the zona intermedia stroma smaller collagenous trabeculae again at right angles to the capsule, penetrated into the zona fasciculata. The cords and plates of parenchymal cells were positioned between these stromal elements and the individual cuboidal cells received fine reticular support fibers from these trabeculae.

As the fibers approached the region of the zona reticularis they became thicker and deviated from their regular pattern. Each parenchymal cell in the zona reticularis received a large number of reticular fibers and some were surrounded by collagenous fibers. A condensation of fibers at the cortico-medullary border seemed to provide a dense stroma as a thin capsule for the medulla.

Within the medullary parenchyma the large cell groups were separated by prominent collagenous bundles. It appeared that some small reticular fibers formed a network for a few individual cells, but most cells had none.

<u>Zona glomerulosa</u> In many foci of many different specimens within this group there were cords of functional zona glomerulosa

prolapsed or partially prolapsed through the fibrous capsule. These varied greatly in size but usually they involved only one or two arcade cords which seemed to have extended through the capsule.

In other regions of several specimens yet another modification of the arcades was noted. Rather than the space between the trabeculae being filled with the columns of mature columnar cells, the same area was filled with a bizarre arrangement of small, dark staining, relatively undifferentiated, low columnar cells. These whirls or "flowing" bundles of cells could be seen to have invaded the capsule and to be intimately related to the developing nodules. (See later description of nodules.)

Several specimens demonstrated limited areas characterized by a narrowing of the zona glomerulosa and a resultant closer positioning of the zona fasciculata and capsule. In a few instances the zona glomerulosa tapered to a narrow horizontal cord of cells and then for a short distance was not detectable. In such areas the capsule covered the cords of the zona fasciculata.

Zona intermedia   This zone was very extensively developed in some specimens. Small darkly staining columnar cells, which appeared to be extensions of the inward base of the capsular trabeculae, composed this zone. These cells appeared to be increased in number in those specimens which demonstrated a moderate degree of fatty metaplasia and cytolysis in the inner cortical layers.

There was a direct continuation of these cells with those similar cells of the whirls found in the zona glomerulosa, suggesting that possibly these were the same cells, having originated from a hypoplasia of the zona intermedia.

In the glands having an extensively developed zona intermedia the cells of that zone were generally cholesterol, Sudan Black B and Oil-Red-O negative. In those glands with only a narrow band the cells showed varying degrees of positive reactions for cholesterol and neutral lipids.

Zona fasciculata The division of this zone into an inner and outer portion was readily accomplished. The lipid and cholesterol histochemical results and the routine light microscopy suggested an irregularity of zonary widths. In some cases the zona fasciculata would extend nearly to the medulla but immediately adjacent to this the zona reticularis would extend far out into the outer zona fasciculata region. These areas were often delineated by the large septae. This suggested to this observer a type of functional lobulation or pyramid as seen in the kidney.

Some adrenals showed evidence of considerable numbers of cells which had undergone fatty metaplasia while other cells appeared to be disintegrating. These were characterized by a small condensed, pyknotic nucleus and a granular eosinophilic cytoplasmic debritis.

Zona reticularis As indicated above, this zone varied in width within the entire group and within a given adrenal. Many areas of apparent prolapse of zona reticularis cells into the medullary parenchyma could be detected in some specimens. Degenerative cell groups and a negative lipid and cholesterol reaction were in some cases coupled with a proliferation of the zona intermedia. However, in most cases this zone was positive for lipids and cholesterol.

Cortical nodules These areas of "ectopic" cortical cells were increased in numbers and size over the previous age group. With a few exceptions where the zona fasciculata parenchyma, not covered by a layer of zona glomerulosa, was "swollen" beneath the capsule, most nodules included some zona glomerulosa cells. In the smaller nodules this was the only cell type present.

In most areas the zona glomerulosa was extending into or through the capsule, but in others the zona intermedia appeared to play a role in the further development of the nodules. Most of the intracapsular nodules were composed of mature columnar cells. However, in the larger intracapsular and most pericapsular nodules there were a few undifferentiated cells resembling the zona intermedia cell type at the margin and/or base of the nodule. In many of these pericapsular nodules the zona intermedia seemed to be directly involved in the nodule formation.

Group F: 6.0-9.3 Years (13 Specimens)

The general morphology and arrangement of tissues and cells paralleled that of the previous age group. However, the alterations from the "normal" pattern described in group E were amplified in this age group.

Capsule

The capsule was composed of a thick layer of dense fibrous connective tissue. An increase in the amount of collagenous fibers throughout the stroma of the cortex was clearly evident.

Cortex

Zona glomerulosa In many areas small segments of zona glomerulosa cell columns invaded the capsule. There were areas of narrowed zona glomerulosa and at selected intervals the zona fasciculata was juxta to the capsule. Many intertrabecular spaces in this zone were occupied by the whirls of small, darkly staining cells of the zona intermedia. But within the same zone and juxta to these arcades, there were the characteristic columns of parenchymal cells composing the great portion of the zona and seemingly unaffected by the cellular and tissue alterations elsewhere.

Zona intermedia This zone was greatly enlarged in some specimens. In one specimen (M54) cells typical of this zone occupied a greater width of the cortex than did the outer zona fasciculata. These broad zona intermedia laminae were cholesterol and neutral lipid negative. These cells were observed to be closely related to the areas of cortical nodules and prolapses, either within the ectopic tissue or at the base of the nodule or prolapsed segment.

Zona fasciculata and zona reticularis The distinction between inner and outer fascicular zones and between these and the zona reticularis was readily accomplished along individual radiating planes, but the boundaries between zones were markedly serrated. Along a group of columns the zona fasciculata might be the major cortical zone, while in the neighboring radiating cords the zona reticularis would be the more greatly developed. In addition to the serrated border between the zones of the cortex, this variation gave a general striated mesoscopic picture to the cross sectional cortical profile. As a result, in sections other than those producing transverse sections of the cortical parenchyma, there appeared inner zona fasciculata cells surrounded by zona reticularis cells and the reverse was also observed.

Large numbers of foci in the zona fasciculata and the zona reticularis had undergone fatty metaplasia to signet ring cells. A smaller number of cells and cell groups appeared to be degenerating and in various stages of cytolysis.

Some specimens demonstrated extensive areas of prolapsed zona reticularis into the medulla through the cortico-medullary border.

Cortical nodules

Within the age group there was considerable variation as to the numbers and sizes of extra cortical nodules. However, this group, relative to those preceding, showed a steady increase in the quantity and size of these nodules. All specimens contained to some degree, small intracapsular nodules of functional cortical cells. Other intracapsular nodules were observed which were very large and possessed tissue zonations and cell types just like the parent cortex.

Those structures which were markedly increased in number, however, were the pericapsular nodules. Large masses of cortical parenchymal tissue generally encapsulated, but sometimes covered by only a delicate advential layer, protruded above the capsular surface. These nodules contained all cortical cell types including the zona intermedia cells. Some nodules possessed the same organization as the parent cortex while others were not organized by cell types into zones. Functional cell types as well as undifferentiated cells were present as indicated by lipid droplets and selective histochemistry. Within some of the larger organized nodules degenerative and fatty metaplasia foci were detected.

Group G: 10.0-13.6 Years (16 Specimens)

Capsule

The intercellular collagenous fiber was the predominate component of the thick fibrous capsule. The collagen fibers were cut in several profiles which indicated that they were disposed in several planes. The fibrocytes were dispersed in the interstices between the fibers but their cytoplasmic boundaries were not definable with light microscopy, and only their rod-shaped, dense basophilic nucleus could be detected.

At its peripheral margin the capsule was bounded by periadrenal fat, adventitia and the peritoneal serosa. The inner margin was sharply delineated and there was a prominent sinusoid just below the capsule separating the capsule and the arcades of the glomerulosa. Many arteries, arterioles and nerves were contained within the capsule. These structures sent their branches into an extensive lattice of trabeculae, which passed at right angles the capsule, into the cortex for varying distances. A few undifferentiated mesenchymal cells were scattered along these dense fibrous trabeculae but they were much less numerous than in previous age groups. These trabeculae passed between the arcades of the zona glomerulosa and became incorporated into the zona intermedia as mentioned previously.

Cortex

Zona glomerulosa The parenchymal cell of this zone was typically an elongated columnar cell with a moderately basophilic nucleus and a prominent nucleolus. The poles of the cells contained a finely granular eosinophilic cytoplasm with considerable numbers of medium sized vacuoles. There was, however, relatively far fewer of these vacuoles than in cells of the corresponding type in the younger age groups. These cells were arranged in the characteristic cords, but the cords were not typically arranged. The elongated arcades were present, but evident also were true glomeruli and bizzare coiled arrangements of the cords.

In some regions the zone was moderately wide, while in other areas it was very narrow and in a few areas the fasciculata or zona intermedia extend to the capsule. Although the cords generally tapped somewhat at their inward ends, they appeared to quite obviously blend with the cells of the zona intermedia. In other areas of some glands the collagenous core of the zona intermedia was so thick that it appeared to "isolate" arcades of zona glomerulosa. In some arcades the parenchyma showed evidence of fatty metaplasia.

Zona intermedia This zone was obvious in all specimens of this age group and in some it was greatly hyperplastic. In this age group a prominent collagenous band at the border between the zona glomerulosa and the zona fasciculata served as an earmark for the origin of this zone. There was a sinusoid contained therein and a few undifferentiated mesenchymal cells were distributed along its course.

In some glands there was a hyperplasia of cellular elements of the zona intermedia on the zona glomerulosa side of the collagenous band in the zona intermedia. These cells were much smaller than those of the zona glomerulosa, but were also spherical to elongated unlike the cuboidal cells of the zona fasciculata. A few vacuoles were distributed in their otherwise homogenous, strongly acidophilic cytoplasm. In some specimens this increase in cell numbers was accompanied by an increase in the numbers of this cell type in the overlying zona glomerulosa arcades. In others this cell type was incorporated into prolapsed glomerular columns as they extended through the capsule. In addition some specimens had large pericapsular nodules which contained large numbers of this cell type in a whirled arrangement. In still other corticies where the glomerulosa was not seen to penetrate the capsule, this proliferation of zona intermedia cells resulted in a separation of the collagenous core of this zone from the trabeculae of the zona intermedia and a movement of this band in toto deeper into the cortex much as a wave front. In such cases this band of collagenous tissue remained intact and cells of the outer zona fasciculata were located immediately below. A further manifestation of the proliferation of zona intermedia cells was the extension of a wedge-like projections of these cells from the zona intermedia into the zona fasciculata. These cells were, relative to other cortical cells, cholesterol and lipid negative.

Outer zona fasciculata The large cuboidal or polygonal cells of this zone stored large amounts of cholesterol and neutral lipids in large cytoplasmic vacuoles which distended the cell membrane. The cells were arranged in radiating cords and plates and some cells of these plates exhibited cytoplasmic changes indicative of fatty metaplasia.

Inner zona fasciculata These cells of similar size to those of the outer zone contained fewer lipid vacuoles and demonstrated a correspondingly greater cytoplasmic staining.

A significantly large number of these cells were altered in morphology. Some cells and cell groups showed fatty metaplasia in varying degrees. Still other cells and cell groups appeared to have undergone cytolysis and necrosis with a loss of cellular components as observed in some foci.

Zona reticularis Large amounts of connective tissue fibers separate groups of cells in this zone and each cell has an extensive reticulum for support. Prominent, circumscribed areas of cytolysis of parenchymal cells were commonly encountered. Often such changes involved large groups of cells which were positioned juxta to other quite normal appearing cells. Changes varying from pyknosis and acidophilia of the cytoplasm to karyolysis and cytoplasmolysis with loss of the cells were seen in this age group.

Often extensive masses of zona reticularis parenchyma were seen evaginated into the medulla. Those cells involved appeared much more active as functional cortical cells than those remaining within the zona reticularis. Some possessed a morphology typical of the outer zona fasciculata cells.

Cortico-medullary border

In this age group the dense collagenous tissue at the interphase between the cortex and medulla could aptly be called the medullary capsule. Much like the cortical capsule, fenestrations were present in this capsule which contained the prolapsed cortical tissue.

Medulla

The only further medullary change evident with the light microscope was an increase in the connective tissue stroma.

Cortical nodules

The number and extent of nodular hyperplasias in this age group was another distinguishing feature. The types of nodular hyperplasias encountered could be broadly grouped as intracortical, subcapsular, intracapsular and pericapsular.

Unique to this age group was the extensive intracortical hyperplasia. These appeared as circumscribed areas of hyperplasia within the zona intermedia, the zona fascicularis and the zona reticularis. The surrounding cortical parenchyma was displaced and compressed. The medullary tissue seemed to be the tissue ultimately violated and the usually large network of sinusoids and venous channels within the medulla were compressed to narrow slits. Subsequent to the occurrence of the intracortical nodules the "normal" arrangement and relationships of the cortical zones appeared distorted in direct proportion to the magnitude of the focal hyperplasia.

The subcapsular nodule formation generally involved only the parenchymal cells of the zona glomerulosa and zona intermedia. These nodules were generally encapsulated, but in some regions, as described above, (1) the collagenous band of the zona intermedia was observed to limit the nodule or (2) the nodule would be unencapsulated.

By definition the size of the intracapsular nodules was limited. However, great numbers of small to medium sized (up to 0.5 millimeters) nodules were observed to occupy a position within the capsule. Serial sectioning revealed that nearly all of these nodules were connected by a "stock" to the underlying cortex. These nodules were composed of zona glomerulosa and zona intermedia parenchyma and by virtue of their location were contained by a conspicuous capsule. The hyperplasia of these cells produced a bulging in this parent capsule.

Perhaps the most striking feature of the nodular hyperplasias were the numerous pericapsular nodules. These nodules were a constant finding in the adrenals of this age group. The pattern of development, histological and cytological appearance of these cortical proturberances was quite variable.

In some a large aperature in the capsule provided an avenue for an "outpouring" of cortical parenchyma. After passing through the fenestration in the capsule, the cortical tissue expanded so as to appear like a mushroom attached by a stock to the parent cortex. In other areas serial sectioning suggested that these large excresences were connected to the underlying cortex by only very narrow stocks through the capsule.

Some nodules were bulging proturberances surrounded by a thick capsule, while others were lateral extensions over a great surface area of the capsule, but were elevated very little above the surface. Many were heavily encapsulated while others were limited only by delicate adventitia.

In those nodules connected to the cortex by large stocks of cortical tissue there appeared to be no change in the cellular character, but only an alteration in relationships of the parenchymal tissues. However, in the nodules connected to the cortex by a small stock, the cells in this stock were often of the undifferentiated zona intermedia type which seemed to be percolating through this narrow slit in the capsule.

Within these mounds of cortical tissue there were present all of the cortical cell types. In some this was a random arrangement but in others the zones and zonal relationships were present as though these were a small adrenal corticies. The same patterns of cellular fatty changes and degeneration could also be detected.

Blood vascular morphology

To evaluate the possible relationships between the extensive and varied patterns of nodular hyperplasia and changes in the blood vascular system accompanying age, the arteries of this age group were examined for any such changes.

The cholesterol and neutral fat sections revealed no evidence of cholesterol clefts or "foam cells" in the intima layer of the capsular, cortical or medullary vessels. Von Kossa's calcium staining procedure produced no evidence of calcium deposition in the intima or medial vascular layers. Evaluation of the vessels on a morphological basis indicated that all the tissues and cells of the blood vessels examined would fit a normal pattern for these older dogs. In these older dogs as compared to those in the younger age groups, that portion of the vessel wall occupied by the tunica media was progressively increased while the tunica adventitia was progressively decreased without encroachment upon the vessel lumen.

Fine Structure as Revealed by Electron Microscopy

Capsule

The most prominent tissue component of the capsule was the fibroblast and its accompanying intercellular fibers. These elongated and flattened cells were enveloped by massive collagenous bundles. A large oval to elongated nucleus surrounded by a narrow rim of cytoplasm produced the widest portion of the fusiform shaped cell. The karyoplasm contained a nucleolus and numerous smaller, electron dense heterochromatin bodies which were generally uniform in distribution except for a peripheral condensation at the nuclear envelope. The cytoplasm was extended into narrow, elongated cell processes which continued between the bundles of collagen. Within the cytoplasm there were observed small, spherical and short rod-like mitochondria which were characterized by thin tubular cristae mitochondriales projecting into the matrix. Abundant granular endoplasmic reticulum permeated the cytoplasm and in some areas this organelle was seen to have dilated cisternae. The cytoplasm contained many clusters of free ribosomes and a Golgi apparatus. The plasmalemma was uniform in outline and did not appear to be related by desmosomes to other cells. Along the inner edge plasmalemma, however, there were numerous small vesicles which suggested that these cells were actively exchanging material with their environment.

In a significant number of capsule cells there were pigment granules of various sizes and constituencies surrounded by a single membrane. The cells containing these pigment granules differed in no recognizable morphological manner from the surrounding fibroblasts, and would appear to be functioning as fixed macrophages.

The extracellular fibrous material was observed to be sectioned in many different profiles which suggested that the fibroblasts and collagenous bundles run in many different planes within the capsule.

The relatively undifferentiated mesenchymal cells in the inner regions of the cortex differed from the fibroblasts and macrophages only in that they possessed a more vesicular nucleus and a less well-developed, granular endoplasmic reticulum.

Zona glomerulosa

At those points of where the parenchymal cells of this zone were juxta positioned, these elongated columnar cells of the presented a smooth plasmalemma without apparent desmosomes or a basal lamina. At such positions there was only a very narrow slit-like intracellular space which contained no fibrillar elements. However, at the poles of the cell where they approached a perisinusoid space or elements of fibrous connective tissue, the cells had elaborated a prominent basal lamina.

A large, elongated, cylindrical nucleus occupied the central portion of these cells. Fine granules of heterochromatin along with a prominent nucleus were scattered in the karyoplasm. On the inner surface of the inner membrane of the nuclear envelope the heterochromatin was condensed along the intervals between nuclear pores; only karyolymph was near the inner surface of the pores. Often centrioles were seen within the cytoplasm juxta to the nuclear envelope.

The cytoplasm contained oblong to elongated rod-shaped mitochondria. These parallel lamellae, cristae mitochondrioles, penetrated at random from the inner mitochondrial membrane from all sides into an electron dense finely granular matrix. Within these mitochdonria large portions of the matrix showed no evidence of cristae. In other regions some of the cristae formed complete septae across the mitochondrial matrix.

These major cytoplasmic components occurred in several different shapes in these zona glomerulosa cells. Some were elongated and branched; others were elongated and narrowed along its length; still others were generally spherical in shape; some appeared as though they were budding. The mitochondria appeared throughout the cytoplasm but there appeared to be a greater concentration of these structures near the bases of the cell near the sinusoids. Often times they were seen clustered about a fat droplet.

A Golgi complex was prominent within these cells and it occurred at variable positions within the cytoplasm. Flat cisternae, vesicles and vacuoles constituted these structures.

Along with the mitochondria numerous droplets of lipid material within the cytoplasm stood out as prominent features of these cells. These large droplets were of generally uniform size and were not membrane limited. Most droplets had been removed by the alcohols or other steps in processing and a large lipid vacuole remained the only evidence of their widespread occurrence. However, in some cells and regions of cells the apparently unsaturated lipids contained within the droplet had been reduced and rendered insoluble by the action of the osmium tetroxide. In these areas the lipid droplets showed varying degrees of electron density.

A granular endoplasmic reticulum did occur randomly distributed within these cells. However, the agranular form of reticulum was a prominent feature of these cells. A vast system of this tubular organelle was portrayed by many transverse and longitudinal profiles of single membrane-limited tubules. There were aggregates of free ribosomes scattered diffusely between these tubular organelles throughout the cytoplasmic ground substance.

Primary lysosomes, which occurred as electron dense, singly membrane-lines spherical structures, were observed in many cells. In a large number of cells these appeared to have been incorporated into larger, more complex structures which were termed pigment granules or cytosegrosomes. These large singly membrane-lined entities contained a variety of structural components in addition to the lysosome. These included large electron dense lipid droplets, multivesiculate bodies, small granules, and membranous outlines.

The columnar parenchymal cells were extended at each extremity to an interstitial region which contained sinusoids lined with endothelium, perisinusoidal cells, and collagenous fiber bundles. The endothelium of the sinusoids was attenuated, except for that region containing the nucleus, into narrow cytoplasmic extensions which presented numerous areas of discontinuity. These pores or apertures were bridged by a diaphragm. The endothelial extensions approximated each other but there were no desmosomes or junctional complexes observed. A basal lamina was peripheral to the outer endothelial cell membrane and the sinusoid was embedded in a large number of collagenous fibers. A prominent fibroblast-like cell type also occupied this space between the endothelium and the parenchymal cells. Its internal fine structure was similar to that of the fibroblasts described in the capsule. These cells were often observed to contain great numbers of large pigment granules or cytosegrosomes.

The parenchymal cells extended a few cytoplasmic processes into this perisinusoidal space. Mitochondria were prevalent at the base of the cells and some extended into the cell processes. No evidence was present to suggest any lipid droplets in these processes, within the perisinusoidal space or within the endothelial cells of the sinusoids.

Outer zona fasciculata

The perisinusoidal space extended between the parenchymal cells of this zone such that the intercellular space was distended. The cuboidal, polyhedral parenchymal cells lacked well-defined desmosomes, but at random intervals the plasma membranes of these cells were adherent. These appeared to be foci of attachment rather than bands or rings. The parenchymal cells sent an extensive and varied pattern of microvilli into the perisinusoidal space and a few were extended into the intercellular space.

A large, generally spherical nucleus occupied the central portion of the cell. Large amounts of heterochromatin were scattered throughout the karyoplasm and a prominent nucleolus was evident. The nuclear envelope with nuclear pores was easily distinguishable and it often followed an undulating contour around the nucleus.

A very prominent Golgi complex, a primarily agranular endoplasmic reticulum and lysosomes were present, as seen in the zona glomerulosa. Centrioles and pigment granules were not commonly encountered. The primary alterations in cytoplasmic constituents involved the mitochondria, lipid droplets and cytoplasmic "density." Mitochondria were much larger in size than those of the zona glomerulosa. They exhibited many differing sectional profiles, but primarily were of rounded morphology. The cristae mitochdonrioles were branched tubular alveolar in morphology and they packed the internal structure of the mitochondria. The dense matrix outlined well these alveoli of the cristae.

Many mitochondria, especially the elongated forms embraced the edge of lipid vacuoles. These lipid vacuoles were very large, generally smooth-outlined vesicles which were generally void of any lipid. However, some contained lipid in varying degrees of electron density.

Some cells, while possessing the same component organelles and inclusions as those cells in other regions of this zone, seemed to be "condensed" in that there was much less development of the agranular endoplasmic reticulum and the lipid vacuoles, mitochondria and other cytoplasmic organelles seemed to be compactly arranged within a now relatively homogenous and electron dense cytoplasm. These appeared to be the "dark cells" contrasted with the "light cells" of classical light microscopy.

A similar relationship between the parenchymal cells and the sinusoidal region existed in this zone as in the zona glomerulosa. However, a few important differences were present. The perisinusoidal space extended between the cells and also carried collagenous fibers between the cells. The perisinusoidal cells sent cell processes between the cells in this space and the parenchymal cells extended microvilli of various sizes into the surrounding subendothelial or perisinusoidal space.

Inner zona fasciculata

The relationships between the parenchymal cells and the sinusoidal system was as that described for the outer portion of this zone. There were greater amounts of collagenous fibers between the cells and the perisinusoidal cells extended far into the intercellular spaces.

The distinction between light and dark cells could still be drawn. Within the parenchymal cells of this zone the primary differences from those of the other zones described above were the still larger and more bizarre shaped mitochondria. These varied from elongated dumbbell shapes to very large spherical forms. The cristae varied from tubular to alveolar with a combination of both being the most prevalent type. Some mitochondria possessed very few cristae, others were filled nearly completely.

These cells contained fewer numbers of lipid droplets and vacuoles than those cells in the outer portion of this zone. Also a greater proportion of these cells contained lipid droplets in various degrees of unsaturation. Unlike the cells in the outer zona fasciculata these cells possessed many lysomes and some pigment granules. The perisinusoidal cells reflected this increase also in that they often contained large numbers of pigment bodies or cytosegrosomes.

Zona reticularis

The relationships between the parenchymal cells of this zone and the perisinusoidal space was basically unaltered, the only variation being the larger sinusoidal space.

The same distinction between light and dark cells prevailed. The light cells contained large amounts of agranular reticulum and widely separated organelles. The dark cells appeared similar in composition but with a condensed, compact cytoplasm.

The mitochondria of these cells were predominantly spherical with a moderate number of tubuloalveolar cristae. Lesser numbers of lipid vacuoles and droplets occurred in this zone than in the other zones described above. Those droplets present were seen to be generally smaller and unsaturated in the light cells versus the dark cells where they were large and often extracted. Numerous electron dense membrane-lined lysosomes and pigment granules were present within the cells.

Medulla

An irregularily spherical nucleus occupied the central portion of a cuboidal cell type. The intercellular space was narrow and often the cells were related by desmosomes. At selected, discrete intervals along the intercellular space there were marked dilations of this space.

The nuclei contained moderate amounts of uniformly distributed heterochomatin except when it was closely applied to the inner nuclear membrane, where it occurred in large aggregates. Nuclear pores were prevalent and the outer layer of the nuclear envelope was studded with ribosomes. A relatively small number of mitochondria compared to cortical cells, were present in these cells. A few lamellar shaped cristae transversed the matrix of these mitochondria. A granular reticulum was prevalent in the cytoplasm as well as liberal amounts of free ribosomes, but an agranular reticulum was not apparent. A well-developed Golgi complex was present near the nucleus and centrioles were detected on occasion.

The most prominent cytoplasmic constituents were rod-shaped, electron dense, singly membrane-limited granules. These varied in electron density and in morphology within a given cell and in numbers between cells. Some medullary cells contained great numbers and others only a few. This correlated well with the observed highly granular and less granular cells seen with the light microscope.

DISCUSSION

An advantage inherent in a morphological study is that direct evidence can often be presented in support of a hypothesis and in many ways this evidence may be self explanatory. Although function does begin and end in structure, structure is not properly understood until one has and understanding of its functional dynamics. Thus, to have an appreciation for and understanding of the changes with age in the adrenal one must also recognize the function of the adrenal and how that function is altered with age.

The monumental work of Selye (1946) demonstrated that the adrenal gland played a most important role in the stress response. He recorded the response by the medulla as being a rapid and abbreviated one, whereas that of the cortex was slow but prolonged. This response and response ability of the medulla and cortex is reflected in and is also a reflection of the morphology of the gland.

The observations made herein suggest that there are alterations in the tissues and cells of the adrenal which accompany advancing age. It is difficult to distinguish and delineate the normal aging pattern in an individual, however, one can more nearly approximate this pattern by evaluating a larger population of animals maintained under a controlled environment. In addition to variations between individuals, the adrenal, being an endocrine organ, is subject to rapid and extensive change within the individual such that to determine what is physiological and what is pathological aging is made more difficult due to the great variability brought about by the dynamic changes within these endocrine structures.

Khelimskii (1963) and Hullinger (1966) have previously described the variations in zonary widths with advancing age in the dog.

With variations in age and environmental stress there are corresponding variations in the pattern of cellular aging. The adrenal medulla, both parenchyma and stroma, and the stroma of the adrenal cortex appear relative to the parenchyma of the cortex more fixed and stable with a longer period of generation life. The cortical parenchyma, however, is continually undergoing genesis and gerontomorphic changes. It is, therefore, a model for the study of cellular aging due to its continued turnover of cells.

General Adrenal Gland Morphology

Cortex and medulla

For all tissues and organs there is a "standard appearance" or a "classical" description of their morphology which is portrayed as the "normal" for that tissue or organ. The observations made in this thesis indicated that in the dog adrenal after three months of age and in some limited number of individual specimens from still younger animals, the encapsulated mass of glandular tissue located near the anterior medial ventral aspect of each kidney possessed this classical or "normal" morphology (Figures 3 and 41).

In all of the age groups studied the adrenal could be divided into two rather distinct glandular types. The first of these was a centrally located mass of randomly arranged parenchyma which was readily identified as the medulla. In the younger animals, groups A and B, islets of medullary cells were often seen as discrete foci embedded at varying levels within the cortical parenchyma as was previously noted by Hill (1930), Randolph (1950) and Nicander (1952). In a similar manner, especially in the younger animals, medullary parenchymal cells could be detected within the larger capsular trabeculae which were extending inward to the medulla. DeGroot and Fortier (1959) described subcapsular nests of medullary cells in the rat. In some specimens of this current study the medulla appeared to extne from its central location "out" along these trabeculae and to approach the capsule. In other cases, generally confined to those age groups under 6 years, there were often nodules of cells within and upon the capsule which were Oil-Red-O and cholesterol negative. This histochemical finding coupled with their cellular morphology lead this author to conclude that these were nodules of medullary tissue. These isolated accessory medullary tissues which were well supplied with sinusoids were especially prominent in the groups A and B. These observations indicated that possibly the neuroectodermal cells which were destined to become the medulla, while invading the cortical mesodermal mass, were in addition to being isolated within the cortex, often incorporated into the capsular stroma as Bourne (1961) suggested. Others of these neuroectodermal cells develop into the numerous paraganglia found in the periadrenal connective tissue. Those islets of medullary cells within the cortex eventually migrated into the central medullary mass, for in the older individuals these were not detected. Those neuroectodermal cells remaining in the capsule apparently retained their responsiveness to trophic stimuli and underwent hypertrophy to, in effect, increase the amount of functional medullary tissue.

The second of the glandular masses composing the adrenal was the rather orderly arrayed mass of parenchyma which encompassed the medullary cells. Throughout all of the age groups examined small numbers of cortical cells, generally occurring as individual cells, were seen within the medullary parenchyma. These cells, typical of the zona fasciculata cell were most often noted at a position surrounding or cuffing the larger medullary vessels. Perhaps these cells remained in this position from the early developmental stages and were not displaced by the invading medullary parenchyma. Positive cholesterol and lipid staining, coupled with the presence of large cytoplasmic vacuoles, labeled these cells as functional steriod synthesizing cells. The fact that these cells appeared even in the older specimens suggested that they may be maintained by mitotic divisions and nourished by being in close proximity to the medullary vessels (Figures 21 and 34).

The cortico-medullary border, although irregular in outline, was evident as a sharp division between these two glandular elements. The exceptions to this generality occurred in the very young where the migration of medullary cells centripetally was not yet completed and in the older animals where frequent prolapses of zona reticularis cells into the medulla were observed. In the cases where cortical and medullary cells were interspersed, it was interesting to note an apparent affinity for a similar type cell, for the elements were not seen to be randomly mixed but appeared as aggregates of similar cells (Figures 44E and 39F).

Thus, it was apparent that, although the adrenal gland was composed of two functionally different glandular tissues and that there were some cases of less complete separations, the dog adrenal is composed of two anatomically distinct galndular elements. According to Symington (1960) the dog adrenal would be classified as a lipid rich gland and the observations reported herein would support this classification.

Cortical arrangement

The zones of parenchymal cells which are belt-like structures around the sphere of medullary tissue divide the dog adrenal cortex into several subunits. These divisions can be defined in one or more of several ways. Most authors have followed Arnold (1866) in referring to morphological criteria but others describe zonal differences based on functional differences similar to those suggested by Bennett (1940). This present work supports Nicander's (1952) description of five cortical zones in the dog which is a modification of the work of Arnold (1866).

The zona glomerulosa is a descriptive name applied to the outermost cortical zone. It is a term which comes from the early works on the human adrenal tissue before comparative anatomical studies had been carried out in the dog or other domesticated animals. The tissue arrangement within this zone is generally described as coils of cells wound into a ball with a central and peripheral sinus; thus the term zona glomerulosa. However, Elias and Pauly (1956) have described as many as 8 structurally distinct types of tissue orientation in the human zona glomerulosa in healthy individuals. In the dogs studied in this work glomerular arrangements of tissues were present, but the most prevalent form in this zone was that involving the parenchyma cells thrown into bows or arches. Thus, the term arcuate would be more accurate as a descriptive term and the zone might more properly be labeled the zona arcuata. As a further modification it was of interest to observe that, within each animal the pattern of cellular arrangement recorded was variable. This finding supported Elias and Pauly (1960) who reported that each individual human adrenal cortex was a mosaic of structural types. It was of some further interest to note that these authors described a normal pattern of human zona glomerulosa development which included a typus arcuatus caninus and that many of the patterns described by these authors in the human were seen occasionally in this study. The term zona arcuata would be more descriptively accurate, but for this thesis this area of the cortex was referred to by the commonly used term zona glomerulosa. The regularity of arrangement, the large columnar cells and the prominent arcades all combined to make this a very prominent zone which coincides well with Flint (1900) and Chester Jones (1957) who consider this region to be more well developed in the dog than in any other species (Figures 40, 41 and 42).

The next inner major cortical layer was the zona fasciculata. This name has apparently arisen from the two dimentional images one can see with the light microscope which suggests that the cells in this zone are arranged as bundles or rods. But the morphology of the gland is also in the third dimension and an accurate descriptive name must include that morphology. When the profiles of cells in the two dimensional plane were examined closely, it was clear that these cells were arranged as plates or sheets and anastomosing columns of cells. This observation corresponds to those of Pauly (1957) in the rat and Zelander (1964) in the mouse who, with the aid of three dimensional reconstruction, illustrated the relationships between the cells and sinusoids of this zone. They indicated the plates of cells were arranged such that each secretory parenchymal cell was juxta positioned to a subendothelial space of a sinusoid on at least one surface.

The innermost major cortical zone was the zona reticularis. As the name of this zone correctly implies this zone was compsed of cells which formed a network of anastomosing cell columns with an areolar pattern of sinusoids between them. This region in the dogs studied was of variable width but conformed well to this descriptive definition.

In addition to these three basic zones which were quite easily detected, the cortex portrayed a further subdivision closely related to the zona fasciculata. The more apparent of these was a subdivision of the zona fasciculata into an inner and outer portion based upon the cell size and amount of lipid stored in the cytoplasm. The outer zona fasciculata was composed of larger cells containing vacuoles very rich in lipids and a somewhat smaller, less lipid-rich cell was characteristic of the inner zona fasciculata. This subdivision is in agreement with the works of Hill (1930), Gruenwald and Konikov (1944), Randolph (1950), Nicander (1952) and Hullinger (1966) (Figures 24 and 28).

A further modification of the cortex was seen as a narrow zone which appeared at the outer border of the zona fasciculata and inner border of the zona glomerulosa. This relatively narrow zone of small laterally compressed, low columnar cells was considered to be the intermediary zone described by Nicander (1952). The name is descriptively accurate for this zona intermedia was interposed between the two larger zones in this region of the cortex, the zona glomerulosa and zona fasciculata. This constant finding supported earlier descriptions by Nicander (1952) and Hullinger (1966) in the dog. Mulon (1903) termed this zone the "zone de transition" and this may have been the area of fibrillar interlacing which Flint (1900) described in the dog adrenal cortex. Lane and deboda (1952) may have been describing this zone when they spoke of a "narrow subglomerular transitional area." Randolph (1950) discussed zona glomerulosa cells which were envoloped by connective tissue, but, considering the evidence presented in this present thesis that the zona glomerulosa cells proper were not enveloped by connective tissue, the cells of this region Randolph described could have been cells of the zona intermedia. A personal evaluation of some of Randolph's original thesis material suggested that in dogs from birth to two years of age included in his study, there was evidence of a zona intermedia (Figures 10, 12, 20, 40E and 40F).

It is the opinion of this author that due to the well-developed zona glomerulosa, the dog has a correspondingly well-developed zona intermedia. This same zona may be present in the many other species investigated to date, but in many, to a much lesser degree. This author would support Nicander (1952) when he suggested that others had described this zone by another name; the boundary zone (Engstrom, 1936); the demarcation zone (Hall and Korenchevsky, 1937); the zone of compression; the transition zone (Mitchell, 1948 and Greep and Deane, 1949). Perhaps Bennett (1940) was referring to the zona intermedia when he spoke of a "narrow inner portion of the presecretory zone" and also an "inner or columnar portion" of the zona glomerulosa. Deane and Greep (1946) may have observed the zona intermedia when they described a "lipid-free zone" between the zona glomerulosa and zona fasciculata, a "very narrow subglomerulosal layer" and a "transitional zone of compact cells."

In summary the general "normal" adrenal tissue morphology was that of two glandular tissues intimately related anatomically and the cortex was further subdivided into a zona glomerulosa, zona intermedia, outer zona fasciculata, inner zona fasciculata and zona reticularis.

Cytology of Dog Adrenal Gland

The cellular morphology of the parenchyma of the adrenal cortex is that of an internally secreting or endocrine cell; it is designed to produce, store and deposit into the circulatory system cell secretory products as outlined by Palay (1958). The major functions of the zona glomerulosa, zona fasciculata and zona reticularis have been generally designated to be the formation of mineralocorticoids, glucocorticoids and androgens respectively.

The cells of all the cortical zones and the medulla are characterized by a close contact with the circulatory milieu. The cortical cells to varying degrees send microvilli into the perisinusoidal space ostensibly as a means for an increase in surface area exposed to the fluid enviroment so as to facilitate nutrient absorption and the secretion of wastes and secretory products. There was an extension of the perisinusoidal space between the cells of the zona fasciculata and reticularis and a basal lamina covered the surfaces of the cortical cells exposed to the perisinusoidal space but did not extend between the cortical cells.

This finding supported Long and Jones (1967) in the opossum, but does not agree with Luse (1967) in the dog and Zelander (1964) in the mouse who described the cortical cells as being surrounded by a basement membrane (Figures 54 and 62).

There appeared to be a gradient of mitochondrial concentration with the greatest numbers of these organelles occurring near the plasmalemma bordering on a perisinusoidal space. The mitochondria of the cortex were considerably different from zone to zone. This variability of internal structure may reflect, according to Fawcett (1966), a need for great energy requirements in the steroid synthesis. Also the migration of cortical cells within the cortex will be accompanied by a certain degree of differentiation and resultant changes in the cytoplasma organelles according to Fawcett (1959) (Figures 60 and 63).

These steroid producing cells were also characterized by large amounts of smooth surfaced endoplasmic reticulum and a prominent Golgi complex. Enders (1962) demonstrated the extensive smooth reticulum in the lutein cells of several laboratory animals and Christensen and Fawcett (1966) showed this well-developed smooth reticulum in the interstitial cells of the testis which is also a steroid producing cell. The intracellular secretory products were deposited within the cytoplasm and were easily seen with the aid of the light microscope. The electron microscope has shown these to be nonmembrane limited (Figures 61, 64 and 65).

The medullary cell produces a protein for export and, as is typical of this kind of cell, variable numbers of spherical mitochondria were dispersed between scanty amounts of rough surfaced endoplasmic reticulum and a prominent Golgi complex. The inclusion granules were membrane limited and were generally near the plasmalemma. The cells were closely applied to one another without apparent desmosomes. At frequent intervals along the intercellular space a large dilated space was encountered which may have played a role in the secretory process. Friend (1965) demonstrated similar dilated lumina between the cells of the Brunner's glands in the mouse and theorized that they played a role in the process of secretion. De Robertis and Sabatini, 1960, showed the mechanism of secretion of the medullary cell to be by the fusion of the secretory granule membrane with the plasmalemma (Figures 66 and 67).

Thus there was a great variety of extensively developed cytoplasmic organelles and inclusions within the cells of the cortex and medulla. De Robertis Nowinski and Saez (1965) suggested that a heterogenous cytoplasmic picture indicated great functional activity. This fine structural appearance combined with the strongly positive histochemical tests for cholesterol and neutral lipids betrayed these cells as highly active elements (Figures 50 and 51).

Within the zona fasciculata and zona reticularis the parenchymal cells were classified with the light microscope as light and dark cells, as described by Luse (1961) in the mouse and by Penney, Patt and Dixon (1963) in the rat. The lipid and cholesterol evaluations indicated a difference in quantity of stored secretory product among the cells. A similar classification was made with the use of the electron microscope. Also the cells of the adrenal medulla evidenced varying amounts of granularity within the cytoplasm which with the aid of the electron microscope were seen to be differences in the amount of accumulated secretory product. These observations were interpreted to be evidence that these cells exhibited different functional stages; the cytomorphology directly mirrored that difference in function.

Based upon the evidence that no cortical parenchymal cell within the zona glomerulosa, zona fasciculata, zona reticularis or medulla was without some amount of secretory product, the cellular secretion must be a rather continuous process with synthesis exceeding secretion to the extent that storage within the cytoplasm is nearly always evident. However, the observation of light and dark cells of the cortex and the medullary cells with differing amounts of granules did suggest evidence for cyclic activity and in some cases cellular exhaustion. Thus, the cortical and medullary cells were caught at the moment of fixation in differing stages of their secretory cycle.

The fine structural findings of this study agreed in part with Luse (1967). She described a smooth-surfaced zona glomerulosa cell which this author did observe at the point of intercellular space between these parenchymal cells. However, between the distal ends of the cell and the endothelium of the sinusoid, these cells sent great numbers of microvilli into the perisinusoidal space. This author was able to confirm her description of elongated mitochondria with leaf-like cristae in these cells.

The zona intermedia cells presented no unique fine structural features in this investigation. However, Kahri (1966) and Sabatini and De Robertis (1962) both in the rat, have demonstrated mitochondrial differences in these cells versus the adjoining cortical regions.

Contrary to Luse (1967), this author observed the mitochondria of the zona fasciculata to be much larger than those of the zona glomerulosa and to present many different shapes. The cristae of these mitochondria were described as vesicular by Luse (1967), but this author favors a branched tubulo-alveolar descriptive terminology for these cristae. The findings herein supported her descriptions of the zona reticularis cell type. Further investigations of this morphology is definitely indicated in light of the evidence of the role these cells may play in adrenocorticogenesis presented in this thesis (Figures 54, 55, 60 and 64).

The intercellular spaces in the deeper cortical regions contained fibrous elements and portions of cell processes, but no evidence of secretory material was observed. Belt, Sheridan, Knouff and Hartman(1965) were able to demonstrate lipid-like deposits within these intercellular spaces and their thesis was that reverse pinocytosis into this space was an important mechanism of secretaion.

The medullary cells were similar to those described by Coupland (1965) and Burgos (1959) in the rat and frog respectively and Elfvin (1965a and 1965b) in the rat.

Cell Replacement within the Dog Adrenal Cortex

Adrenocorticogenesis is still a moot question. Much of this long-lasting debate has surely been due, as Pauly (1957) so correctly indicates, to the species differences encountered in the definitive research on this problem. The position of the blastemal, primordial, or germinative layer of cells in the young, mature and senescent adrenal gland has been directly or indirectly debated in many different species and its very existence in the mature gland has been questioned by some.

Some of the evidence in support of several theories and the elements of those theories were presented in a earlier chapter of this dissertation.

As an outgrowth of this current investigation into age changes within the adrenal gland, this author would suggest a modification of the current theories of cortical cell replacement as they may be applied to the dog.

In the dog adrenal cortex at birth there was considerable evidence to suggest a continuation of the relatively undifferentiated capsular cells with the parenchyma of the underlying cortex. At such areas there was no morphological evidence of any barrier between a capsular blastema and the newly formed cortex. Developmentally it is of some considerable importance to stress that the capsule and cortex are both of mesodermal origin. Seen in this perspective, it is then apparent that the capsule is in fact a condensation at the periphery of a mass of differentiating celomic mesoderm. Thus, a continuity of the cellular elements of both the capsule and cortex in the young adrenal would not be unexpected.

With further differentiation of the early postnatal cortex, those cells just below the capsule were enlarged to a columnar morphology and had become associated with one another in such a manner as to form veriform columns and plates of cells. There was established at this point a differential development within the cortex. Parenchymal cells external to the region of the zona intermedia developed in a centrifugal direction, whereas those internal to the zona intermedia laminae, developed in a centripetal direction. The blastemal layer on the inner surface of the capsule appeared to have effectively produced, by mitotic divisions, new parenchyma in an outer as well as an inward direction. The result was that the zona glomerulosa and zona fasciculata arose from this now "displaced" blastermal layer, the zona intermedia.

As the pattern of zona glomerulosa development was monitored through the early age groups, two important changes were detected. First, the columns of columnar cells appeared as tentacles arising from the zona intermedia toward the capsule. When these columns contacted the capsule, they were diverted and followed a course parallel to the inner capsular surface until they contacted a trabeculum from the capsule or a neighboring cell column. At this point this column were again diverted back in an inward direction toward the zona intermedia. The result of this development was the formation of coils or glomeruli (Figure 40).

Secondly, the spaces between these developing coils of the zona glomerulosa were occupied by stromal tissues which were extensions of the capsule which permeated into this region. At most points of contact between the parenchyma of this zona glomerulosa and the supportive stroma there were prominent sinusoids. This was very significantly the case between the inner cellular layer of the capsule and the parenchyma. This same effective separation of the capsule and the zona glomerulosa was reported in the Rhesus monkey by Brenner (1966). The result of such a physical separation is that further direct communication between the capsule and the underlying zona glomerulosa is effectively prevented (Figure 40).

The capsular trabeculae, extensions of the capsular tissue into the zona glomerulosa, were observed to penetrate to the region of the zona intermedia and then very subtly blend into that zone. This resulted in the partitioning of the zona glomerulosa into compartments within which rather bizzare patterns of zona glomerulosa development occurred.

In addition to their role in directing and limiting the development of the zona glomerulosa these trabeculae provided an avenue of connection between the primitive reticular cells or undifferentiated mesenchymal cells of the capsule and the new blastema of the cortex, the zona intermedia.

In summarizing the results of this early development of the zona glomerulosa in the dog this author has emphasized that the primary blastemal layer located originally at the inner surfacr of the adrenal capsule has been displaced to the inner boundary of the zona glomerulosa. This narrow band of cells is termed the zona intermedia.

It is interesting to note that Baker and Baillif (1938) observed after enucleation of the rat adrenals that the regeneration was from a once again very cellular capsule and that the zones were formed in the order of zona fasciculata, then zona reticularis and the zona glomerulosa was the last to develop. Brenner, Patt and Wyman (1953) concluded that the zona glomerulosa not the capsule was the regeneration center in their work with rats.

Evidence that the cells of the zona intermedia would fit the criteria of a blastema have from this current morphological evaluation included the following items. 1) These small cells resembled in many aspects those cells detected within the very cellular trabeculae which were in turn related to cells of similar morphology in the capsule. 2) The cells possessed many features of an undifferentiated cell, a large vesicular nucleolus and a homogenous cytoplasm. Examination of thin sections of these cells with the light microscope revealed that they contained, if any only a few small lipid droplets. 3) The results of tests for neutral lipids and cholesterol indicated that these cells were negative in most specimens and only weakly positive in others. 4) In specimens with a well-developed zona intermedia, evaluation of cells at the margins of the zone revealed cells in transitional stages between the balstemal cells and the mature functional cells of the zona glomerulosa.

In a similar fashion, this zona intermedia cell type was hypothesized to be the blastemal cell type for the zona fasciculata and zona reticularis. Cells arising from this germinative region were extended as plates and columns of cells inward to the medulla. At the medulla, much as the zona glomerulosa columns were diverted by the capsule, these cell columns and plates were diverted and compressed. The result was a breakdown of the orderly array of the zona fasciculata columns into a rather random arrangement or network, the zona reticularis.

It is quite probable that under conditions of normal control and compensation that the adrenal cortex can be and is maintained by indigenous mitotic divisions of the respective cortical zones. But under conditions of chronic or subacute stress when a noncompensated status exists, these divisions are supplemented by the activity of the zona intermedia. The proliferation of cells here is accompanied by an increase in the absolute width of the zona intermedia.

In order to establish this zone as a germinative zone one would need to demonstrate mitotic figures in this region. However, in this entire study no more than ten mitotic figures were confirmed. They are quite rare and unless some experimental procedure is used to increase their number and also mark their occurrence, their index is difficult to monitor. At this point the work of others proved helpful. Mulon (1903) determined that the mitotic index was greatest at the juction between the zona glomerulosa and zona fasciculata in the dog. Graham (1916) suggested that in the adult guinea pig the "growth center" was in the zona glomerulosa and outer zona fasciculata. da Costa (1913) observed nearly all the mitotic figures to be confined to the zona fasciculata and that this was the germinative zone from whence cells moved in both an inward and outward direction. Hoerr (1931) also working with the guinea pig found nearly 75 per cent of all mitotic figures in the outer zona fasciculata. Bennett (1940) found mitotic figures only in "the inner columnar portion" of the presecretory zone in the cat. Deane and Greep (1946) and Greep and Deane (1949) observed that this "transition zone between the glomerulosa and fasciculata" enlarged after hypophysectomy in the rat where they also observed the highest mitotic index. Blumenthal (1940) recognized mitotic figures only at the inner zona glomerulosa and outer fasciculata and Chester Jones (1957) suggested that the zona intermedia represented the products of cellular division. Reiter and Pizzarello (1966) were unable to demonstrate any mitotic activity at the zona intermedia.

Labeled thymidine was used by several investigators as a tool to measure mitotic activity in the adrenal cortex. Diderholm and Hellman (1960), Walker and Runnels (1961), Brenner (1963) and Ford and Young (1963) all used this technique in mice or, as in the Diderholm and Hellman research, rats and observed the greatest mitotic activity to be in an area corresponding to the inner zona glomerulosa and outer zona fasciculata. Bloom and Fawcett (1962) stated that in the human there is a greater tendency for mitotic divisions to occur at the transition zone between the zona glomerulosa and zona fasciculata.

Further indirect supportive evidence for the role of the zona intermedia in adrenocorticogenesis was the distribution of pigment granules within the parenchymal cells and perisinusoidal cells were most numerous within the zona glomerulosa and zona reticularis. The capsule also contained large numbers of these lipofuschin-like bodies which may have resulted from the phagocytosis by fixed macrophages in the capsule of pigment extruded from glomerulosa cells. The inner zona fasciculata contained fewer of these granules and the outer zona fasciculata rarely evidenced these intercellular inclusions (Figures 58, 59 and 62).

These observations of pigment granule distribution have led this observer to thorize that there are older cortical cell elements at both the inner and outer margins of the cortex, the zona reticularis and the zona glomerulosa. These less differentiated cells were observed at the inner regions of the zona glomerulosa and throughout the outer zona fasciculata. Accordingly, the inner zona fasciculata with respect to cellular age is an intermediate region between the outer portion of the fasciculata and the zona reticularis. Thus, the newest cortical cells are those nearest the zona intermedia which serves as the blastemal layer for both the zona glomerulosa and the outer zona fasciculata. Bourne (1960) emphasized the marked accumulation of pigment within the adrenal cortex with advancing age as did Bourne and Jayne (1961). Samorajski and Ordy (1967) and Belt (1958) have classified and considered the origins of pigments which included types similar to those described in this work.

Changes with Age in Dog Adrenal

Within this gland one can observe tissue alterations with progressive age and a panoramic view of the entire life cycle of parenchymal cells with a continued renewal of the cell population. Recognizing that tissues are composed of cells and their products, this discussion will view aging of the adrenal gland as a cellular phenomena.

Connective tissue

An increase in connective tissue throughout the adrenal cortex and medulla with advancing age was apparent in this study. A change from a very cellular capsule to a fibrous capsule over the early period of development from birth to one year was a striking alteration. This alteration was accompanied by an increase in the number and prominence of capsular trabeculae. At the zona intermedia there was a progressive increase in the amount of connective tissue fibrous elements until in the older animals there was a prominent boundary between the zona glomerulosa and zona fasciculata. Collagenous fibers were never observed, however, to extend between the columnar cells of the zona glomerulosa. There was a progressive increase with aging in the amount of connective tissue in the perisinusoidal space and the intercellular space within the zona fasciculata and zona reticularis. In the older dogs the cells of the inner region of the zona reticularis were individually embedded within fibrous elements. The separation between the cortex and medulla was emphasized with advancing age by a condensation of fibrous elements at this junction. This progressively increased and resembled a medullary capsule in the specimens from older animals. This general increase in cortical connective tissue was reported by Bourne (1960) in the human and conforms with the review presented by Bourne and Jayne (1961) who listed connective tissue proliferation as a general aging pattern in the mammalian adrenal.

The medullary cells were provided with a generous amount of supporttive fibers at birth and this network slowly increased in amount with age. The result was a further spatial and mechanical separation between the cell and the sinusoid.

Adrenocorticogenesis

Due in part to at least two changes with age, 1) the development of the zona glomerulosa and 2) the increase in the fibrous portion of the capsule, there was observed an increase in prominence of the zona intermedia with advancing age. This was interpreted to suggest that an alteration in adrenocorticogenesis had also occurred. As discussed above there was, in effect, a new blastemal layer in the older adrenal cortex.

Cellular arrangement

Alterations in the cellular arrangement of two of the cortical zones were especially prominent, the zona glomerulosa and the zona reticularis.

After its initial development and assumption of an arcade pattern of organization at bout 90 days, the regularity of cellular arrangement was only on occasion slightly modified until approximately 4 years of age. At this age level variations in this orderly arcade pattern were encountered. In most areas this was manifested by different patterns of glomerulosa cell organization, but in some regions there was a change in the constituent cell ytpe which appeared very much like the zona intermedia cells. This alteration within this zone was interpreted to be the preliminary alterations in this tissue just prior to the formation of nodules which will be discussed below.

A similar alteration in the regularity of cellular arrangement in the zone reticularis was observed. In this region the packed, randomly arranged parenchyma of the young animal were, with advancing age, increasingly separated by progressively larger sinusoidal spaces.

Cellular changes

The manner in which the adrenal gland aged was in large part reflected in and determined by the pattern of individual cell aging. Heilbrunn (1943) suggested, "In the last analysis, according to any theory, senescence is due to protoplasmic changes, changes which occur in individual cells." However, the question of primary and secondary, of causative and effective, of stimulus and response becomes important. Andrew (1952) suggested that in the case of some cells "...it is not the effects of aging of the cells as such in which we are to be primarily interested but rather the effects of ageing of the organism upon the structure and functioning of the cells."

The cell populations were classified according to catagories suggested by De Robertis, Nowinski and Saez (1965). The cells of the zona glomerulosa, zona fasciculata and zona reticularis of the cortex and the cells of the medulla were considered to be typical expanding cell populations. These were fully differentiated cells which were within a relatively homogeneous tissue. For normal maintenance of numbers these cells can undergo mitotic divisions as has been demonstrated by the labeling studies of Diderholm and Hellman (1960), Walker and Runnels (1961), Ford and Young (1963) and Brenner (1963).

There is yet another classification which can be applied to the adrenal cortex under conditions of increased stress. Under such conditions cells in the deeper cortical regions and possibly in the zona glomerulosa are undergoing senescence, degeneration and death at such a high rate that a rapid renewal is needed which cannot be met by the mitosis of surrounding cells. Such a population of cells is considered a renewing cell population. The renewal region in this study was hypothesized to be the region of the zona intermedia.

In certain specimens, generally three years of age and over, the typical spongiocyte of the adrenal cortical parenchyma was altered to resemble a signet-ring fat cell. This process was termed fatty metaplasia after Selye and Stone (1950). This type of cellular alteration was most commonly encountered in the zona reticularis and inner zona fasciculata. This change was observed in both sexes and was apparently not linked directly with an increase in age, for some of the older specimens revealed no evidence of such a change. In specimens demonstrating considerable amounts of this fatty metaplasia, it was noted that the zona intermedia was well-developed. This author would support Selye and Stone (1950) in suggesting that these cells are indicative of a cortex which is in the resistant phase of the adoption syndrome and reflects the effect of increased corticotrophin secretion upon the cortex. Jayne (1953, 1957, 1963, and 1966) has made similar observations and drawn similar conclusions in alterations with age and stress in the rat and mouse adrenal cortex.

In still other specimens individual cells and sometimes large groups of cells showed evidence of cytolysis. The entire cell was altered with the cytoplasm having lost its staining characteristics and evident only as faintly eosinophilic cellular debris. The nuclei had undergone pyknosis and chromatolysis and in some cases had disappeared. Bourne and Jayne (1961) listed these cytological changes as being commonly observed in the aged adrenal. Selye and Stone (1949) considered this to be a non-specific reaction to an intense stimulation by the corticotrophic hormones (Figures 20, 21 and 43D).

In other areas of the cortex rounded, punched out areas were noted which contained no cellular debris. These rounded spaces were not limited by a membrane, but were of the same size of a parenchymal cell. Selye and Stone (1950) called these areas lumina and considered them to be the result of intense systemic stress. Jayne (1953) considered the degenerative changes in cortical cells to be of more importance than a proliferation of connective tissue in altering the functionability of the adrenal cortex (Figure 43A).

From the observations made in this study this author concluded that the degenerative changes in the cells of the adrenal cortex were the result of nonspecific stress on the total organism which then affected and were manifested in the cellular changes within the adrenal cortex. These focal losses of cellular constituents would then be replaced by local hyperplasia typical of an expanding cell population. However, in the case of extensive loss of parenchyma, which in itself indicated an intense stimulation of the adrenal cortex, this stress was met and the regeneration was accomplished by proliferation of the zona intermedia as is typical of a renewing cell population.

Korenchevesky (1961) reviewed the evidence presented by Delmare (1903) and Puech (1953) which incriminated changes in the arterioles which resulted in ischemia and the subsequent cellular deterioration. This investigation did not produce any evidence of intrinsic adrenal arterial vascular alterations from the normal. This was also suggested in the studies of Das and Zashjian (1952) and Duncan and Buck (1960) whose observations were confined to the larger vessels, and would support Selye and Stone (1949 and 1950) who suggested that nonspecific stresses were responsible for these focal degenerative changes.

Extracortical nodules

The formation of extracortical nodules of parenchymal tissue was along with the changes in the zona intermedia the most striking alteration in adrenal morphology with advancing age. These nodules were observed in some form in nearly all specimens from birth to 13.6 years of age without apparent regard to sex or diet. However, the number and size of these nodules did increase with age. They varied from small intracapsular nodules at birth to the large pericapsular nodules at 13.6 years which extended over vast areas of the capsular surface.

Nodules of cortical tissue have been described by many workers in many species as was reviewed in an earlier section. The findings herein support those observations in the dog reported earlier by Flint (1900), Goodpasture (1918), Randolph (1950), Nicander (1952), Dempster (1955), Dämmrich (1960), and Hullinger (1966).

Selye and Stone (1950) described capsular nodules in or just below the capsule as being quite common in man. Anderson (1961) described nodular hyperplasia in the human adrenal much as has been described for the dog in this discussion. Smith, Beamer, Vellios and Schulz (1959) reported that one-third of the human antopsies evaluated demonstrated nodular hyperplasia and Commons and Callaway (1948) noted that in 2.86 per cent of 7,437 autopsy specimens nodules greater than 3 mm. in diameter. Bourne (1960) suggested that in the aged human such nodule development was commonplace. It was Kokko, Brown and Berman (1967) who attempted to establish a link between adrenal adenoma and hypertension but concluded, as nearly all others have, that nodule formation results in no systemic pathology.

These nodules were catagorized on the basis of location as being subcapsular, intracapsular, pericapsular or satellite nodules. All of the small nodules and most medium sized nodules contained zona glomerulosa cells and some small numbers of zona intermedia cells. The glomerulosa cells were typical of the mature glomerulosa cell as measured by morpholohy and histo-chemistry. The larger nodules often contained all of the cortical cell types and they were arranged according to the parent cortex. Murthy and Russfield (1966) described a similarily arranged internal structure of nodules in hamsters. The medium-sized nodules varied in morphology between those with only zona glomerulosa cells and those which appeared as accessory adrenal cortices (Figures 46 and 47).

The nodules were in most all cases shown by serial sectioning to be connected to the underlying parent cortex. The exception to this general rule were the satellite nodules which were completely surrounded by a thick connective tissue capsule and some of the intracapsular nodules of the very young animals. Denber (1949) described nonencapsulated cortical tissue in the periadrenal fat. These satellite nodules were most probably accessory chromaffin tissue and will not be discussed as far as origin from the parent cortex.

The formation of these nodules appeared to occur in one or a combination of three different manners. The first appeared to be by the proliferation of cellular elements within the capsule. This was the method of nodule formation encountered in the younger specimens. These islets of parenchymal cells were shown to be functional cells but they did not appear to be connected to the underlying cortex. These nodules were probably formed by the activation of blastemal cells which were still present within the very cellular capsule (Figure 5).

A second possible method for formation of the subcapsular, intracapsular and pericapsular nodules involved the zona intermedia. A hyperplasia of the zona intermedia to form the zona glomerulosa may be continued when there is a further need for zona glomerulosa parenchyma. Goss (1964) indicates that this hyperplasia is typical of undifferentiated cells such as are in the adrenal cortex. Continued formation of glomerulosa cell types would result in a continued coiling until, by the limits of the stroma in the zona glomerulosa, these cells, which were not individually limited by a collagenous support, may move by ameboid-like processes combined with the pressure of new cells being formed to migrate into and through the capsule (Figures 50, 47, and 48D).

A third method is similar to the second. However, in this method the hyperplastic zona intermedia cells invade the zona glomerulosa and thence into the capsule to lead the prolapsing cortical tissue out of the parent cortex. Once through the capsule they continue to multiply and differentiate into functional cortical cells. In still other specimens their was, in addition to the prolapsing of cells through the capsule, a similar evagination of the zona reticularis parenchyma into the medulla. Such cells retained their relationships to each other and did not permeate the medullary parenchyma. These mature cells appeared not as the cells remaining in the zona reticularis but resembled much more the cells of the inner zona fasciculata. In that they resembled active spongiocytes and were very cholesterol postive. If these cells were indeed of zona reticularis origin as they did appear to be, then the influence of the new environment had definitely altered their cytological appearance. Perhaps the appearance of a medullary capsule in the older animals which served as a barrier between the cortex and medulla also served to limit the dynamic functional ability of the inner cortex, much as the capsle may have limited the zona glomerulosa, and the cortical cells expanded through this barrier (Figures 47, 48, 50 and 51).

This evidence of nodule formation pointed to a response on the part of the adrenal cortex to stimuli in other parts of the organism, suggesting that these changes were secondary to stress or other primary changes elsewhere.

Possible stress sources

With the semi-controlled environment as outlined earlier in this thesis some of the large stress factors such as the need for food, water, shelter and protection from disease have been greatly reduced if not eliminated. However, stress on an individual basis may very considerably depending on the dogs response to his or her environoment and he or she may not interact with the other dogs in a group. Their response to environmental temperature fluxuations or subacute immune responses or slight mechanical trama induced by the environment or surgically are but a few examples that could effect a rapid and extensive stress response in the inner cortical zones. Holzbauer (1964) measured a rapid alternation in aldosterone secretion during operative stress.

Liebisch (1954) considered cortical hyperplasia in the dog to be a response to nonspecific stresses as explained by Selye (1946). The hyperplasia of the zona glomerulosa in dogs suffering from heart worm infestations was demonstrated by Nichols and Hennigar (1964). The response of the human adrenal to the stress of various disease conditions has been shown by Elias and Pauly (1960) and Elias, Murthy and Elias (1966) to result in as many as 16 different structural types in the zona glomerulosa. Giacomelli, Wiener, and Spiro (1965) described cytological alterations in the zona glomerulosa accompanying stress. Miller (1965a and 1965b) reported stress induced changes in all cortical zones of rats.

The outer zone or the zona glomerulosa is responsible for the production of mineralocorticoids and any shift in the homeostatic mineral balance would result in a stimulus upon the zona glomerulosa.

The the dog one routinely encounters mild to moderate degrees of kidney nephrosis with advancing age. Such alterations could be very quickly be relayed by the juxtaglomerular cells via the renin-angiotensin system to effect changes in the zona glomerulosa.

Such relationships between the kidney and adrenals have been investigated by Davis (1967), Ganong and Boryczka (1967), Ganong, Boryczka and Shackelford (1967), Fisher (1966), Dunihue (1965) and Davis, et al. (1967). Lindt (1958 and 1962) conducted morphological evaluations of dogs suffering from chronic nephritis and observed that with increasing chronic nephritis the adrenals displayed increasing cortical and medullary nodule formation with a widening of the transitional or sudanophobic zone (the zona intermedia of this thesis).

In all of the specimens studied in this research no evidence was present to indicate that these nodules were later to become incorporated into the parent cortex and, in effect, serve as a means for replenishing cortical cell elements. Thus, this work would not support the interpretation given these nodules in other species by Zwemer, Watton and Norkus (1938) and Elias (1948). However, the species differences again must be considered of primary importance. Similarly this research failed to support Gruenwald and Konikov (1944) who after having evaluated the dog adrenal, also suggested these nodules were moving inward. This author would support the reports of Goodpasture (1918), Dempster (1955), Dämmrich (1960) and Hullinger (1966) who evaluated the occurrence of nodules in the dog adrenal and suggested that these cells were moving outward through the capsule.

The Role of the Adrenal Gland in Aging

Solomon and Shock (1950) reported that in aged humans there was no decrease in adrenal cortical ability to secrete the 11-17 oxysteroid group hormones when stimulated by ACTH and Carlson (1942) cited reports of hyperplastic adrenal in individuals above 90 years of age. Verzar

(1960) has observed that compensatory hypertrophy in rats after unilateral adrenalectomy was less in the middle-aged interval than in the young or old.

Korenchevsky (1961) suggested that the adrenals have rarely been mentioned as a causitive factor in the aging process. Hickler (1962) similarly stressed the lack of evidence which would suggest that an alteration in adrenal function was significant or primary as a causative factor in aging. Blumenthal (1955) suggested that the changes in the aged adrenals were compensatory phenomena designed to delay the development of endocrine deficiencies.

From the observations made in this research it would seem that, although there were deleterious changes such as connective tissue proliferation and degenerative cellular changes which accompanied an increase in age, there were compensatory changes, namely the nodule formation, which compensated for these losses in functional ability and any needs for increased functional ability. Thus, along with Bourne (1960), this author would suggest that it is difficult to incriminate adrenal failure as a primary cause of the aging phenomena.

SUMMARY

1. The adrenal glands from 128 purebred dogs ranging in age from birth to 13.6 years of age were evaluated for histocytological changes with age. A description of those observations made with both the light and electron microscopes is included in this dissertation.

2. The developmental pattern for the younger age groups was delineated in this study. The capsule, cortex and medulla were defined at birth and the remaining cortical zones and subdivisions were detected by the tenth day after birth. The classification of cortical zones used in this study from without to within included the zona glomerulosa, the zona intermedia, the outer zona fasciculata, the inner zona fasciculata and the zona reticularis. These zones became progressively more well delineated until at three months of age the dog adrenal cortex conformed with classical form or established "normal" morphology of the adrenal gland.

3. Islets of medullary cells were observed in the younger specimens within the cortex and capsule but these were not present in glands beyond one year of age. Likewise, cortical cell isolates were found distributed within the medulla.

4. The cells of the zona glomerulosa, zona fasciculata and zona reticularis were considered to be expanding cell populations. The zona intermedia was classified as a renewing cell population.

5. The light and electron microscopic evaluations of the cellular structure suggested that a very actively secreting endocrine cell composed

the parenchyma of the major cortical zones and the medulla.

6. Selected histochemical tests revealed that the adrenal cortex of all ages stored large amounts of neutral fats and cholesterol materials. All of the cortical zones exhibited a great affinity for these dyes with the exception of the zona intermedia which was, along with the medullary parenchyma, negative when tested for these components.

7. The adrenal parenchymal cells demonstrated extensive modifications for interaction with the circulatory system. These included in all the major zones an irregularity of the plasmalemma which formed microvilli projecting into an extensive perisinusoidal space. In the zona fasiculata, zona reticularis and medulla this space extended between the parenchymal cells.

8. Desmosomes were observed to relate the cell boundaries of adjacent medullary cells but they were not apparent in any of the cortical zones. Striking mitochondrial differences existed between the cortical zones. These included size and shape differences as well as internal structural differences. A prominent Golgi complex and an extensive smooth surfaced endoplasmic reticulum reflected the morphology of a steroid metabolizing cell.

9. The cellular inclusions of the cortex were generally large lipid secretory droplets which did not have a limiting membrane and demonstrated varying degrees of saturation. The medullary cells contained numerous membrane limited secretory granules. Variations in the amounts of the

secretory inclusions along with the appearance of light and dark cells in the inner cortical regions were interpreted as evidence for functional variations within the cells of both the cortex and the medulla.

10. Evidence was presented for a modification of the current cell replacement theories as they apply to the dog adrenal cortex and which might be applied to other species with a similarly well-developed zona glomerulosa. The blastemal function which was exercised by the capsule at birth was, with the development of the zona glomerulosa, theorized to be transferred to the zona intermedia and evidence was presented in support of this theory. Under conditions of general stress and demand for more functional cortical parenchyma, this zone appeared to have undergone hyperplasia and expansion.

11. Changes in the connective tissue with advancing age involved a general increase in the size and number of fibrous intercellular elements. The capsule, the capsular trabeculae, the zona intermedia, the cortico-medullary border and the reticulum supporting the parenchyma reflected this general increase in fibrous elements.

12. The cytomorphology of the cortical cells was also altered with aging. These changes included fatty metaplasia leading to signet-ring cell formation, cytolysis, and lumina formation all of which occurred primarily in the inner cortical zones. These were considered to be focal alterations resulting from non-specific stress on the cortex.

13. Pigment bodies of varying morphologies were observed in the capsule, zona glomerulosa and zona reticularis. Large numbers of these

were also contained within the perisinusoidal cells of the respective zones.

14. No morphological or histochemical evidence of blood vascular pathology or variations from the normal aging pattern was obtained when the intrinsic blood vessels were evaluated.

15. The arrangement of cortical cells was also altered with age. Cells of the zona intermedia were observed to invade the zona glomerulosa and displace the functional parenchyma. Extracortical parenchymal nodules were observed in many of the adrenals ranging in age from birth to 13.6 years. These were classified according to location as subcapsular, intracapsular or pericapsular nodules of hyperplasia.

16. In the younger specimens with a cellular capsule the extracortical nodules were considered to be focal proliferations of blastemal cells remaining in the capsule. In the older animals these were considered to be evaginations of the zona glomerulosa and/or the zona intermedia through the capsule. Possible methods for the formation of these nodules were presented and evaluated. In some specimens nodules of cortical tissue also had evaginated into the medulla.

17. This research with the dog adrenal lends support to the theory that the changes with age in the adrenal are primarily compensatory in nature and that the histocytological changes with age in the canine adrenal are secondary to more primary changes affecting this organ.

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APPENDIX A: TABLE

Table 1. Sex, breed, diet and age of animals studied

Group	Identification number		Sex	Breed	Diet	Age in days	Age in years
Group A	B7		M	Beagle	CP	8 hours	Birth
	B8		F	Beagle	CP	8 hours	Birth
	B9		F	Beagle	CP	8 hours	Birth
Group B1	B52	5FFA	M	Beagle	CP	4	0.01
	B10		M	Beagle	CP	9	0.02
Group B2	B34	1DA	M	Beagle	CP	14	0.04
	B35	1DE	F	Beagle	CP	14	0.04
Group B3	B45	1DAA	M	Beagle	CP	28	0.08
	B46	1DAC	F	Beagle	CP	28	0.08
	B28		M	Beagle	CP	36	0.1
	B29		F	Beagle	CP	36	0.1
Group B4	B47	1DAB	M	Beagle	CP	57	0.2
		E26	M	Beagle	MP	63	0.2
Group C1		E23	F	Beagle	MP	90	0.2
	B26	13A	M	Beagle	CP	97	0.3
		E07	M	Beagle	HP	99	0.3
		E05	F	Beagle	HP	99	0.3
	B27	13C	M	Beagle	CP	100	0.3
Group C2		E08	M	Beagle	HP	178	0.5
		E06	F	Beagle	HP	178	0.5
	B76	C38	M	Beagle	CP	180	0.5
		M55	F	Springer spaniel	MP	187	0.5
		C35	M	Beagle	MP	184	0.5
		M56	F	Springer spaniel	MP	187	0.5
		M57	F	Springer spaniel	MP	187	0.5

Table 1. (Continued)

Group	Identification number		Sex	Breed	Diet	Age in days	Age in years
Group C2 (cont.)		M58	M	Springer spaniel	MP	187	0.5
		M59	F	Springer spaniel	MP	187	0.5
	B66	034	F	Beagle	CP	198	0.5
	B59	1D3	F	Beagle	CP	205	0.6
	B60	1D1	M	Beagle	CP	205	0.6
	B61	1D4	F	Beagle	CP	206	0.6
Group D	B72	A37	M	Beagle	CP	238	0.6
	B48	361	F	Beagle	CP	239	0.7
	B70	A22	M	Beagle	CP	244	0.7
	B71	A23	M	Beagle	CP	245	0.7
	B49	354	F	Beagle	CP	247	0.7
	B21		M	Beagle	CP	252	0.7
		C34	M	Beagle	MP	262	0.7
		A46	F	Beagle	MP	273	0.8
	B74	A39	F	Beagle	CP	280	0.8
	B22		M	Beagle	CP	281	0.8
	B67	009	F	Beagle	CP	283	0.8
	B53	352	M	Beagle	CP	302	0.8
	B79	C12	F	Beagle	CP	304	0.8
	B86	C36	F	Beagle	CP	305	0.8
		C39	F	Beagle	LP	305	0.8
		C40	M	Beagle	LP	305	0.8
		C17	F	Beagle	MP	329	0.9
		C8	F	Beagle	MP	335	0.9
		C42	F	Beagle	LP	335	0.9
		C41	F	Beagle	LP	335	0.9
		C10	F	Beagle	MP	336	0.9
	B55	353	M	Beagle	CP	336	0.9
	B54	1DD	M	Beagle	CP	364	1.0
	B56	1DB	M	Beagle	CP	365	1.0
	B57	1DC	M	Beagle	CP	365	1.0
	B58	3B5	M	Beagle	CP	365	1.0
	B62	1D6	F	Beagle	CP	367	1.0
	B83	C13	F	Beagle	CP	373	1.0

Table 1. (Continued)

	Identification number		Sex	Breed	Diet	Age in days	Age in years
		800	F	Beagle	LP	406	1.1
		804	M	Beagle	HP	406	1.1
		803	M	Beagle	LP	415	1.1
		500	F	Beagle	LP	419	1.1
		743	M	Beagle	HP	422	1.1
	B37	13B	M	Beagle	CP	437	1.2
		499	F	Beagle	HP	439	1.2
		504	M	Beagle	HP	447	1.2
		710	M	Beagle	MP	458	1.2
		491	F	Beagle	LP	462	1.3
		494	M	Beagle	MP	462	1.3
	B90	690	F	Beagle	CP	488	1.3
		A50	M	Beagle	MP	546	1.5
	B77	A08	M	Beagle	CP	556	1.5
	B80	A09	M	Beagle	CP	614	1.7
	B41	13D	F	Beagle	MP	626	1.7
	B81	A10	M	Beagle	CP	627	1.7
	B12		F	Beagle	CP	648	1.8
	B13		F	Beagle	CP	699	1.9
	B11		F	Beagle	CP	709	1.9
	B24	7DD	F	Beagle	CP	826	2.3
Group E	B25	7CA	F	Beagle	CP	827	2.3
	B23		M	Beagle	MP	962	2.6
		053	F	Beagle	HP	986	2.7
	B91	441	F	Beagle	CP	1072	2.9
	B92	442	F	Beagle	CP	1072	2.9
	B93	445	F	Beagle	CP	1072	2.9
	B94	444	F	Beagle	CP	1072	2.9
		046	F	Beagle	MP	1094	3.0
	B87	032	F	Beagle	CP	1128	3.1
	B88	029	M	Beagle	CP	1128	3.1
	B75	1D5	F	Beagle	CP	1169	3.2
	B82	017	F	Beagle	CP	1194	3.3
	B16		M	Beagle	MP	1214	3.3
	B89	008	M	Beagle	CP	1214	3.3
	B20		M	Beagle	MP	1226	3.4
	B36	13B	M	Beagle	MP	1245	3.4
	B65	1D	F	Beagle	MP	1304	3.6
	B95	1D2	M	Beagle	CP	1460	4.0
	B51	13C	F	Beagle	MP	1520	4.2

Table 1. (Continued)

Group	Identification number		Sex	Breed	Diet	Age in days	Age in years
Group F	B18		F	Beagle	MP	2192	6.0
	B30		F	Beagle	MP	2739	7.5
		M52	M	Basengi	MP	2748	7.5
	B42	2	F	Beagle	MP	2769	7.6
	B44	7	F	Beagle	CP	2829	7.8
	B43	4	F	Beagle	MP	2830	7.8
		M47	F	Golden retriever	MP	3017	8.2
		M53	F	Golden retriever	MP	3286	9.0
		M54	F	Golden retriever	MP	3288	9.0
	B33	13	F	Beagle	CP	3317	9.1
	B63	10	M	Beagle	CP	3355	9.1
	B19		M	Beagle	CP	3366	9.2
		M51	F	Irish setter	MP	3402	9.3
Group G		M46	F	Irish setter	MP	3614	9.9
	B64	1	F	Beagle	CP	3667	10.0
		M49	M	Lab. retriever	MP	3753	10.3
		M50	F	Lab. retriever	MP	3755	10.3
	B15	220	F	Beagle	CP	3791	10.4
		M48	M	English cocker	MP	3841	10.5
		M41	F	Fox terrier	MP	4069	11.1
		M42	F	Fox terrier	MP	4077	11.2
		M43	F	Fox terrier	MP	4145	11.3
	B73	5	F	Beagle	CP	4335	11.9
		M44	F	Corgi	MP	4383	12.0
		M45	F	Fox terrier	MP	4383	12.0
	B31	206	F	Beagle	CP	4520	12.4
	B17		F	Beagle	CP	4701	12.9
	B32	207	M	Beagle	CP	4952	13.6
	B14	K219	F	Beagle	CP	4980	13.6

APPENDIX B: FIGURES

Figure 1. The canine adrenal gland in transverse section. Specimen taken from 1.0-year old female Beagle (B83). For the purpose of general orientation one can resolve the following: nerve ganglia in the periadrenal fatty connective tissue (n), the capsule (C), the zona glomerulosa (g), the zona intermedia (Z), the zona fasciculata (f), the zona reticularis (r) and the postcava (P). Gluteraldehyde fixed, no staining. Magnification 12x.

[redacted]

Figure 2. Cross section of an adrenal gland from a 4-day old male Beagle (B52). A very cellular capsule (Ca) communicates with the developing glomerulosa (G). The cortex (C) and medulla (M) can be distinguished. Stained with Mallory's triple. Magnification 27x.

Figure 3. Cross section of an adrenal gland from a 90-day old female Beagle (E23). The cortex narrows at the hilus (H) and the capsule (Ca) sends small (t) and large (black pointer) trabeculae into the cortex. The remaining cortical zones are well-defined except for the zona intermedia. Stained with Mallory's triple. Magnification 15x.


Ca


Ca
H
t
Ozf
R
G
Izf

Figure 4. Longitudinal section of an adrenal gland from a 0.5-year old male Beagle (C35). The medulla (M) is surrounded by a well-developed cortex with a zona intermedia (Z). The capsule is very prominent with large trabeculae. Three kinds of nodule formation can be seen at (N), subcapsular, intracapsular and pericapsular and (white N; see black pointer below). Stained with Mallory's triple. Magnification 14x.

Figure 5. Cross section of the above gland from a 0.5-year old male Beagle (C35). The black pointer indicates a region which could, if sectioned properly, produce a nodule like the nodule (white N) above. The subcapsular nodules (N) are well developed. The zona intermedia (Z), zona reticularis (R) and medulla (M) are prominent. Stained with Mallory's triple. Magnification 24x.

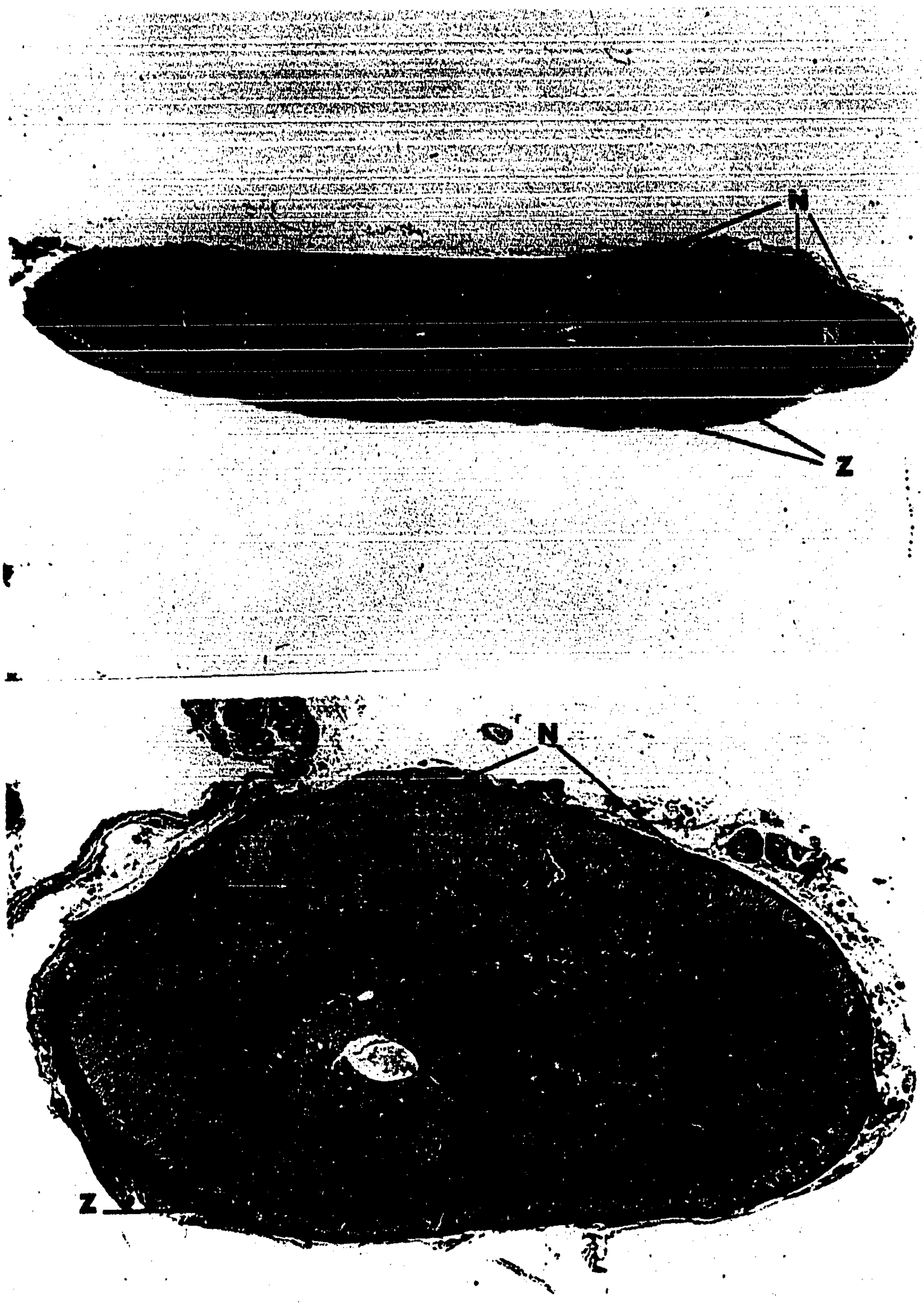

N
Z
N
Z

Figure 6. Cross section of an adrenal gland from a 0.6-year old female Beagle (B61). Capsule (Ca) outlines the well-developed zona glomerulosa. The zona intermedia (Z) and zona reticularis are distinguishable in this section. Medulla (M). Stained with Mallory's triple. Magnification 25x.

Figure 7. Tangential section of an adrenal gland from a 0.6-year old male Beagle (B72). A pericapsular nodule which contains both zona fasciculata (f) and zona glomerulosa (G) tissue as well as a capsule (ca) zona intermedia (Z) tissue can be detected in the parent cortex. Capsule (Ca). Stained with Mallory's triple. Magnification 15x.


Z
Ca

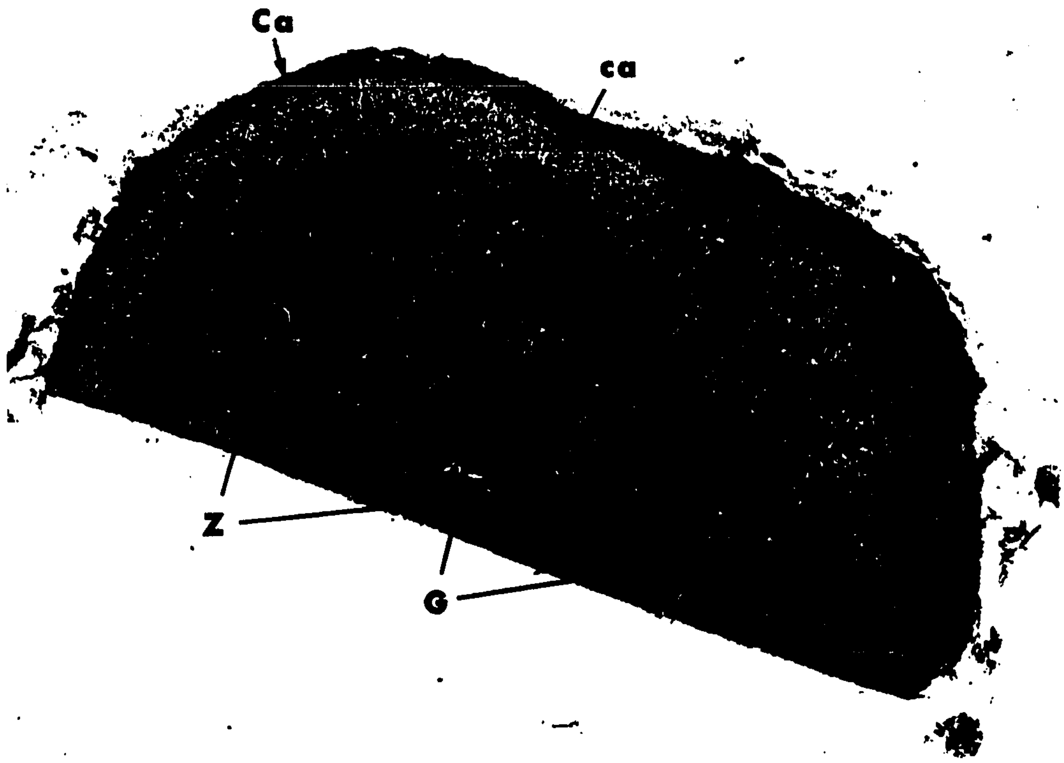
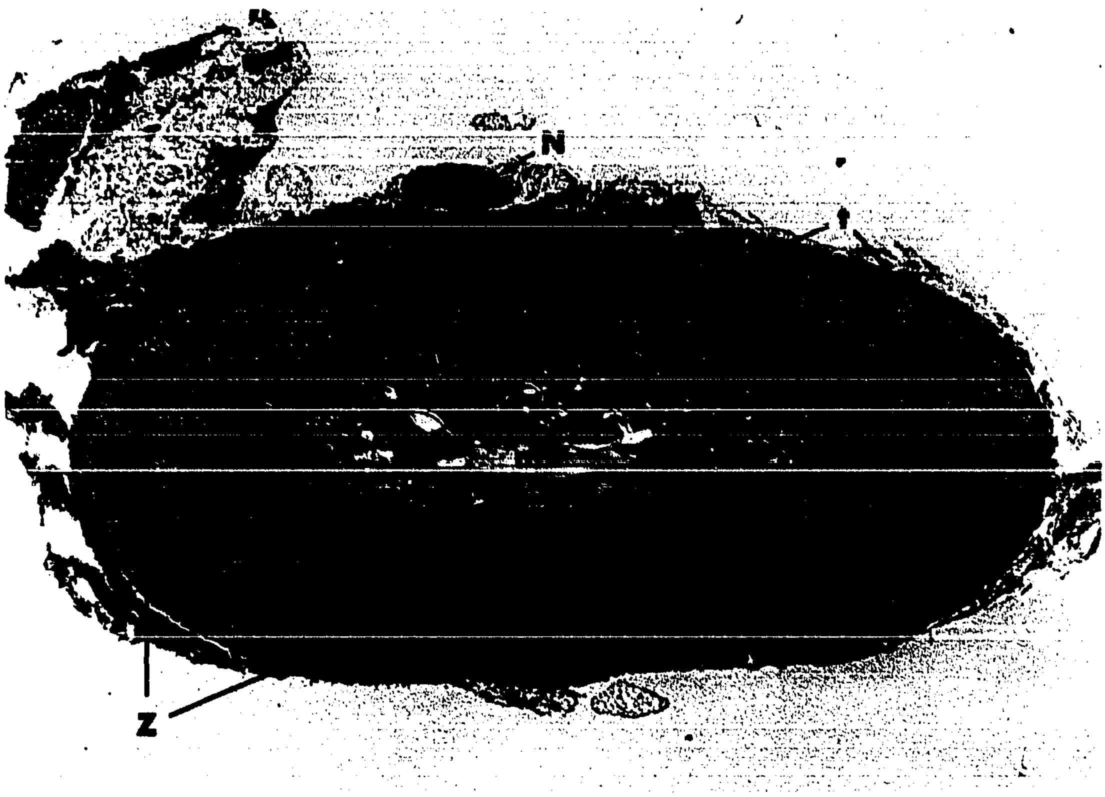

Ca
ca
Z
G

Figure 8. Cross section of an adrenal gland from a 0.7-year old male Beagle (B70). Extra cortical nodule of medullar tissue (N). The zona glomerulosa (G) is subdivided by trabeculae (t) and the zona intermedia (Z) is prominent. The inner cortex can be divided into outer (o), inner (i) fasciculata and reticularis (r) zones. The medulla (M) manifests a large medullary vein (mv). Stained with Mallory's triple. Magnification 25x.

Figure 9. Cross section of an adrenal gland from a 0.8-year old male Beagle (B22). The zona reticularis (R) is prominent but irregular in outline which suggests that there is a functional variation between the individual plates or fascicles. The zona intermedia (Z) is similarly well-developed. The inner cortex can be divided into outer (o), inner(1) fasciculata and reticularis (r). Medulla (m). Stained with Mallory's triple. Magnification 23x.


N
Z


R
Z

Figure 10. A tangential section of an adrenal from a 1.0-years old female Beagle (B62). Due to the sectional geometry of the zona reticularis (R) appears at many different levels in the cortex. An outer zona fasciculata (O) is well-developed as is the zona glomerulosa (G) and zona intermedia (Z). Inner zona fasciculata (i) and medulla (m) are also prominent. Nodules are indicated by the arrows. Stained with Mallory's triple. Magnification 20x.

Figure 11. A cross section of an adrenal from a 0.9-year old female Beagle (C17). This stain reveals the capsular trabeculae (t) and a very narrow zona reticularis (R). Stained with Mallory's triple. Magnification 14x.


R
Z


t
R

Figure 12. Longitudinal section of an adrenal gland from a 1.1-year old male Beagle (743). Nodules (N) and a prominent zona glomerulosa (G) and zona intermedia (Z). Fasciculata (F), reticularis (R) and medulla (M). Stained with Mallory's triple. Magnification 17x.

Figure 13. Cross section of an adrenal gland from a 1.1-year old male Beagle (743). The trabeculae (T) extend to the medulla to form the medullary capsule. The zona intermedia (Z) is prominent. Stained with Mallory's triple. Magnification 15x.


Z
N

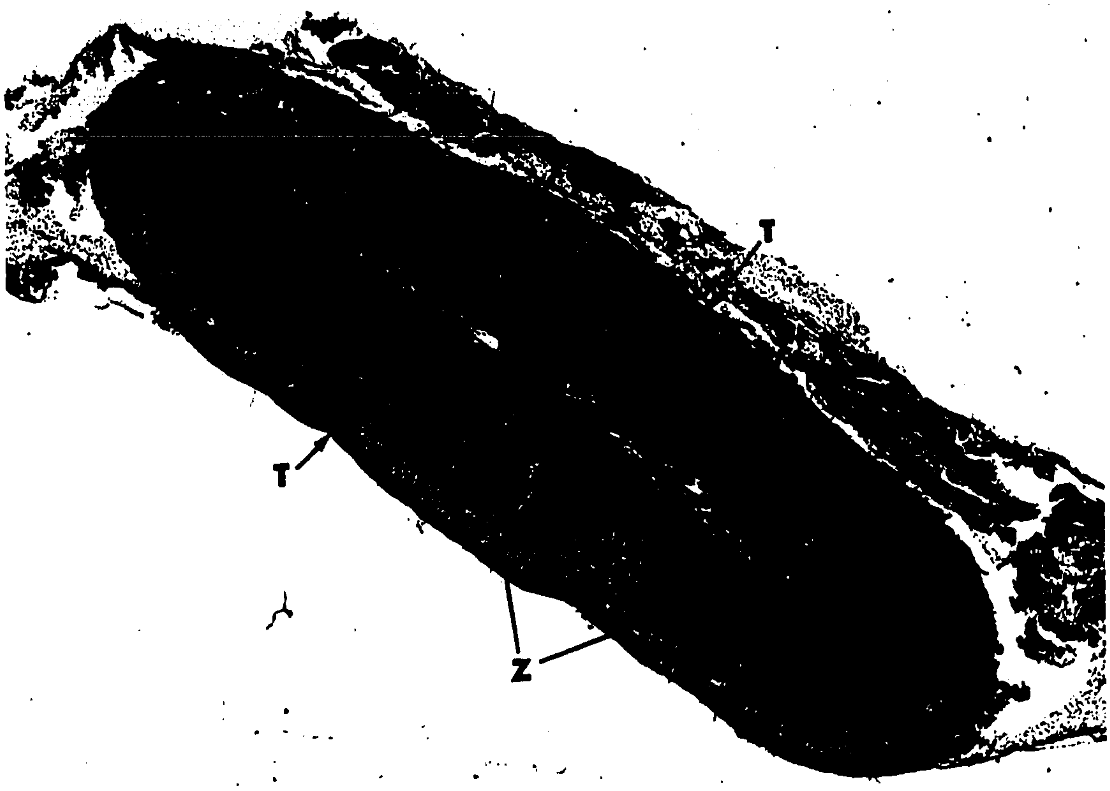

T
T
Z

Figure 14. Longitudinal section of an adrenal gland from a 1.2-year old male Beagle (504). This section shows a well-developed intracapsular nodule (N) and a prominent trabecular artery (Ta). The medulla (M) contains some zona reticularis (R) tissue due to the position of the sagital plane of section. Stained with Mallory's triple. Magnification 10x.

Figure 15. Cross section of the above adrenal gland from a 1.2-year old male Beagle (504). The large capsular trabeculae (T) direct the development of the underlying zona glomerulosa. The fasciculata (F) and reticularis (R) are quite prominent. The reticularis contains widely dilated sinusoids which seem to continue into the medulla (M). Stained with Mallory's triple. Magnification 16x.


R
Ta


T
R

Figure 16. Longitudinal section of an adrenal gland from a 1.3-year old female Beagle (491). Note the zona intermedia (z) and narrow zona reticularis (R). Stained with Malory's triple. Magnification 14x.

Figure 17. Cross section of an adrenal gland from a 1.3-year old female Beagle (491). The large pointers indicate areas of partial evagination of the zona glomerulosa through the capsule. Stained with Mallory's triple. Magnification 15x.


R
Z

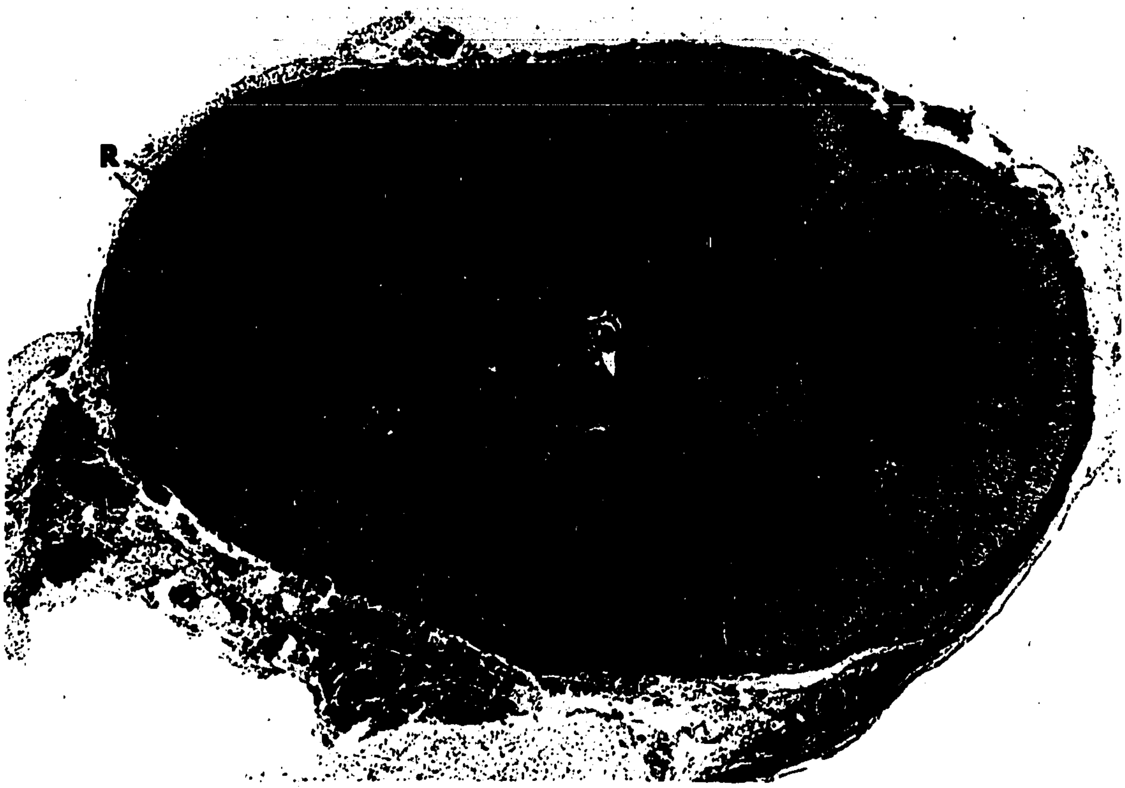

R

Figure 18. Cross section of an adrenal gland from a 2.7-year old female Beagle (053). The capsule (Ca) contains two small intracapsular nodules (small arrows). Note the fascicular zones, inner (i) and outer (o) as well as the reticularis (r) and the Medulla (M). Stained with Mallory's triple. Magnification 15x.

Figure 19. Cross section of an adrenal gland from a 2.9-year old female Beagle (444). The zona intermedia (Z) separates the zona glomerulosa and the zona fasciculata (F). Two trabecular arteries (Ta) penetrate in trabeculae to the medulla. Stained with Mallory's triple. Magnification 16x.

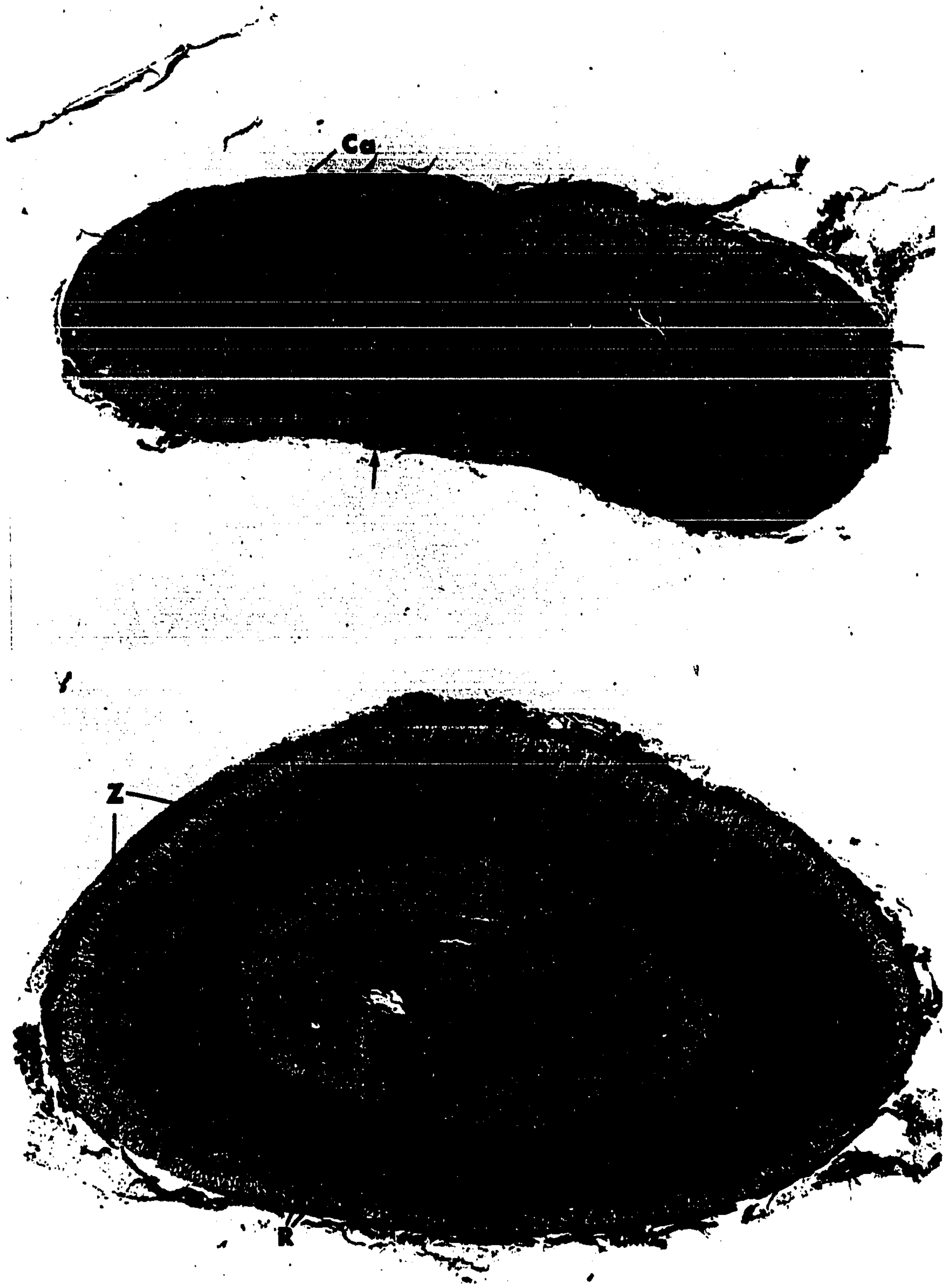
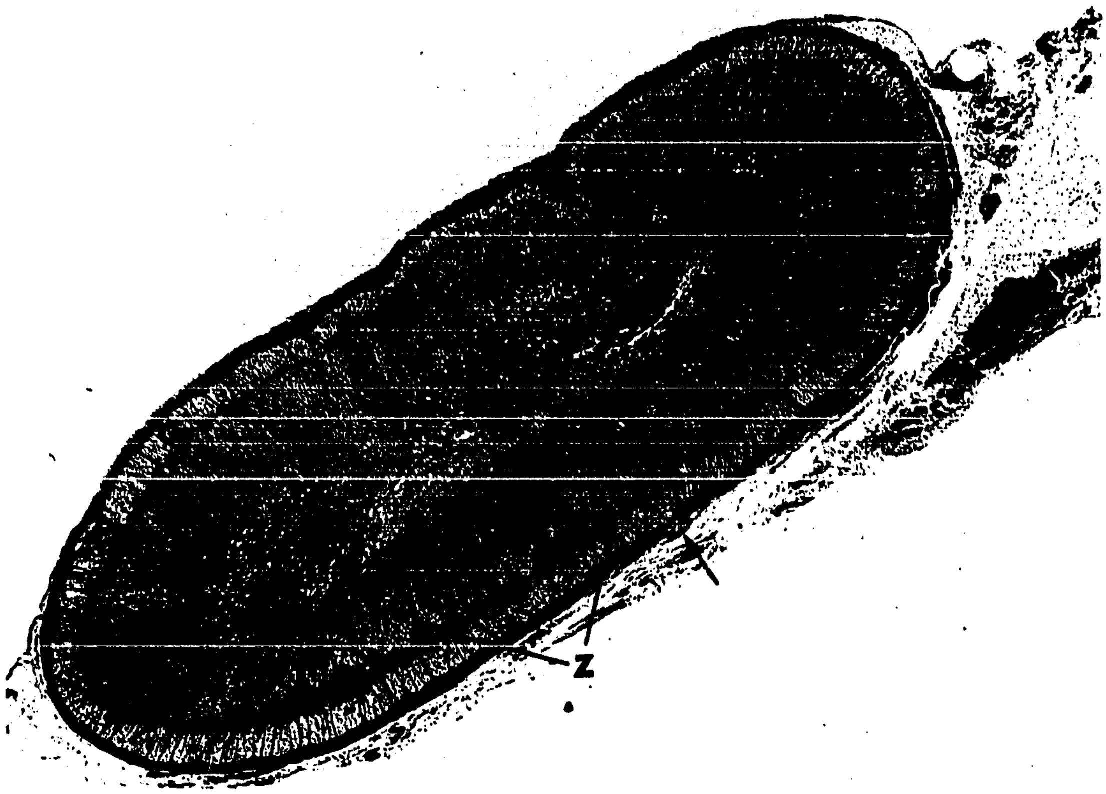

Ca
Z
R

Figure 20. Cross section of an adrenal gland from a 2.0-year old female Beagle (046). The reticularis (R) contains evidence of fatty metaplasia (at arrows). The zona intermedia (Z) and zona glomerulosa (G) outer (O) and inner (i) zona fasciculata are present. A small nodule is at end of arrow. Stained with Mallory's triple. Magnification 15x.

Figure 21. Cross section of an adrenal gland from a 3.6-year old female Beagle (B65). A thick capsule (Ca) is transversed by a nodule near a blood vessel (at arrow). The zona intermedia (Z) is evident as a dark line. An extensive zona reticularis (R) contains many areas of cellular change. Stained with Mallory's triple. Magnification 14x.


Z

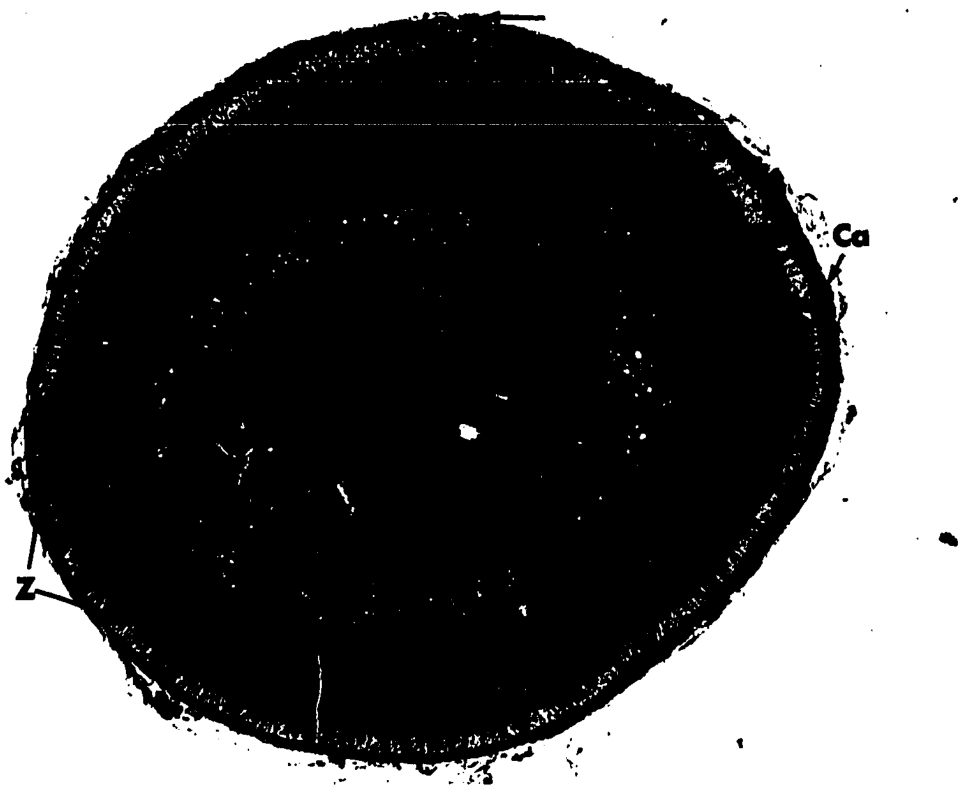

Ca
Z

Figure 22. Cross section of an adrenal gland from a 4.2-year old female Beagle (B51). The discrete zona glomerulosa (G), fasciculata (F), reticularis (R) and medulla (M) are the outstanding features of this gland. Small intracapsular nodules are present (small arrows). Stained with Mallory's triple. Magnification 17x.

Figure 23. Cross section of an adrenal gland from a 6.0-year old female Beagle (B18). The four larger cortical zones, glomerulosa (G), outer fasciculata (O), inner fasciculata (1), and reticularis (R) are very prominent but the zona intermedia is not easily resolved. The arrows indicate small nodules. Stained with Mallory's triple. Magnification 16x.

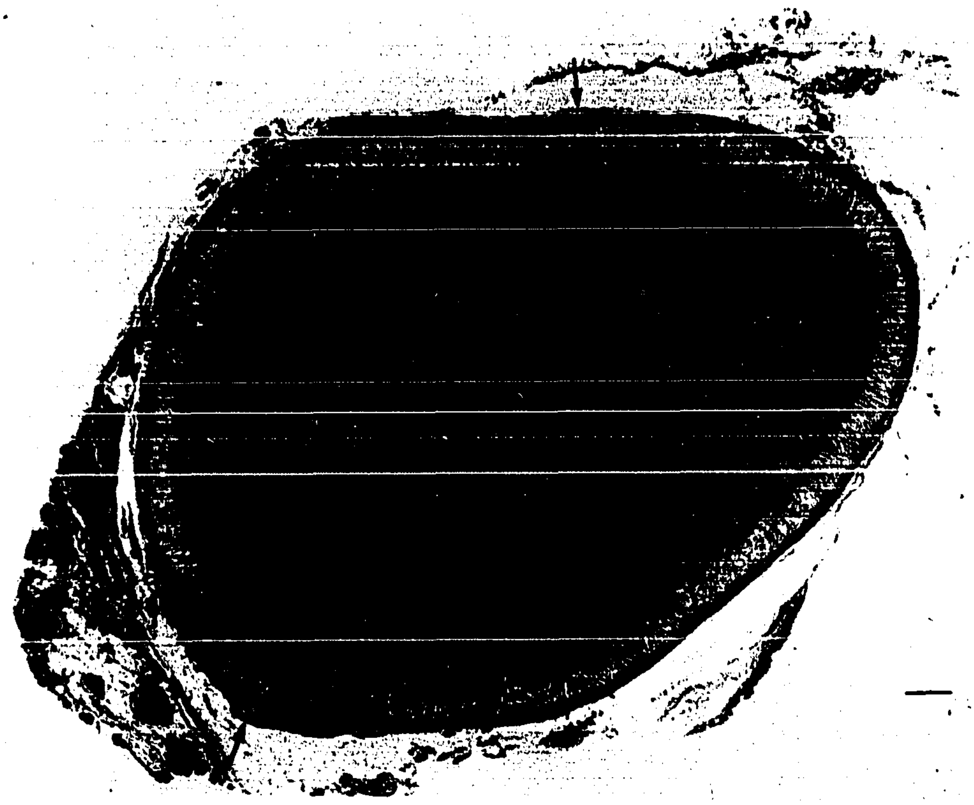


Figure 24. Cross section of an adrenal from a 9.0-year old female Golden Retriever (M54). Many nodules (arrows) are upon the capsule and the zona intermedia clearly extends into one of these nodules. Glomerulosa (G) and medulla (M). A large zona intermedia (Z) was more extensive than the outer zona fasciculata (O). The inner zona fasciculata (1) showed evidence of fatty metaplasia. The fasciculata has displaced the reticularis at some points. Stained with Mallory's triple. Magnification 14x.

Figure 25. Cross section of an adrenal from a 9.9-year old female Irish Setter (M46). Beneath the capsule at the black arrows the zona intermedia has invaded the zona glomerulosa. At the large black arrow the zona intermedia is invading the prolapsing nodule. An extensive reticularis (R) shows some evidence of fatty metaplasia. Stained with Mallory's triple. Magnification 16x.


G
Z
O
I
R

Figure 26. Cross section of an adrenal from a 9.9-year old female Irish Setter (M46). A very thick capsule is frequently perforated by the cortical cells (at small arrows). The medulla extends to the capsule (at large black arrow). The reticularis is prolapsed into the medulla (RP). Stained with Mallory's triple. Magnification 13x.

Figure 27. Cross section of an adrenal from a 10.0-year old female Beagle (B64). Note the trabecular artery (Ta) and nodules (at black arrows). Sectional geometry has resulted in the zona reticularis (R) being superimposed upon the zona fasciculata (F). The zona intermedia (Z), outer fasciculata (O) and inner fasciculata (1) are well-developed. Stained with Mallory's triple. Magnification 14x.

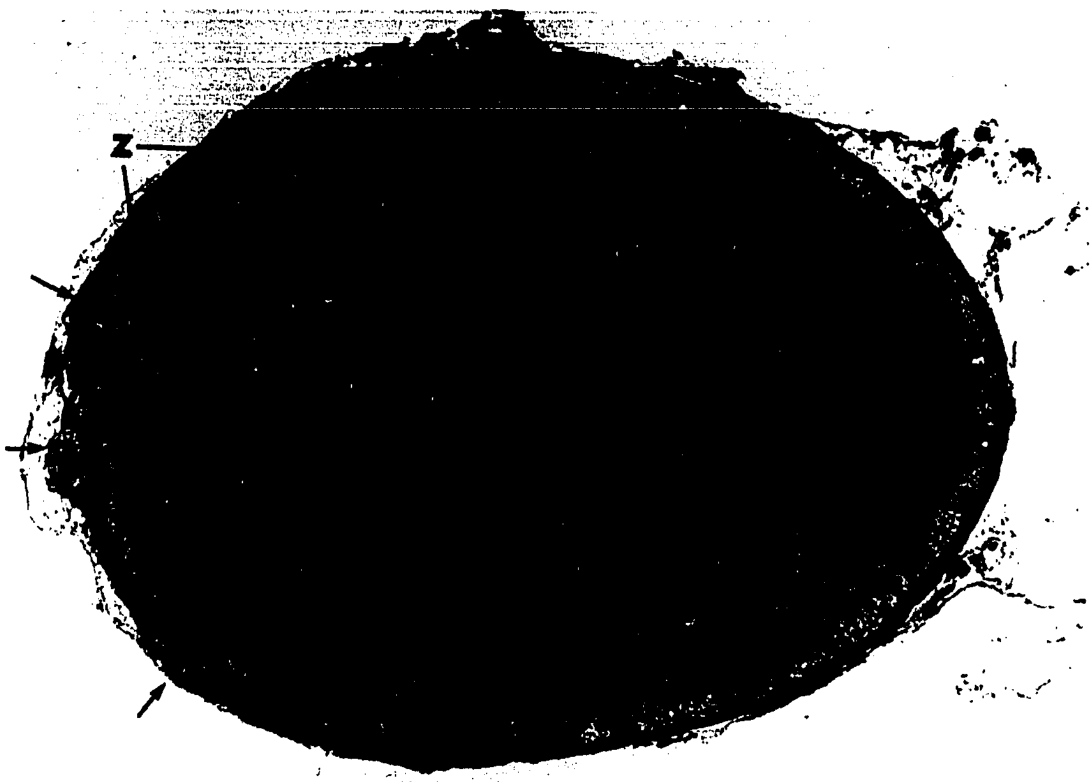
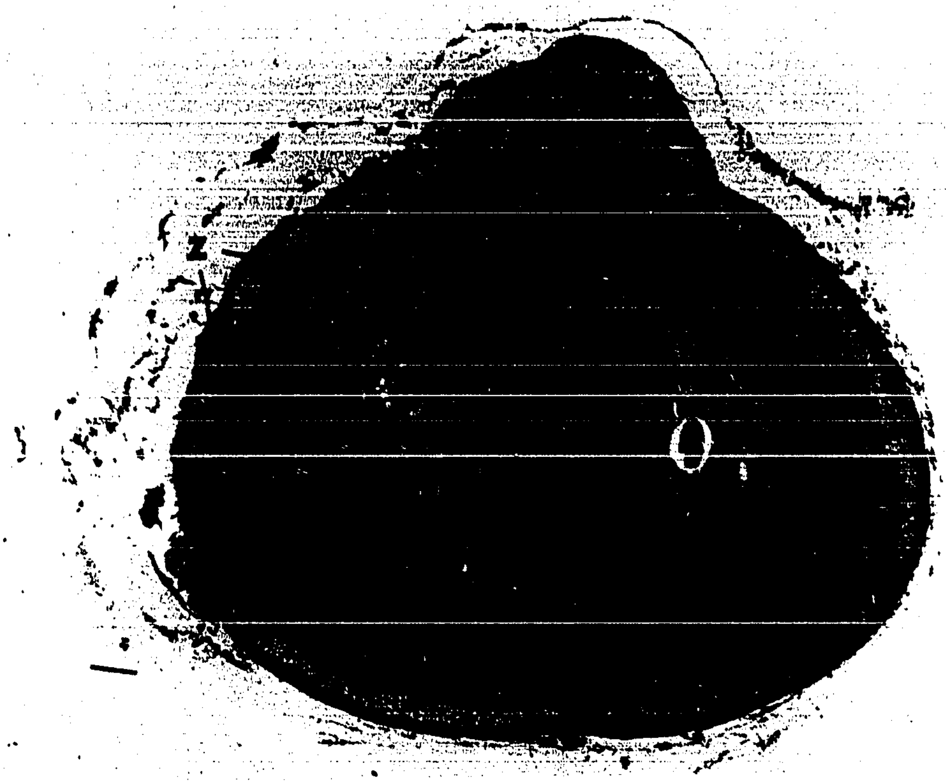

Z

Figure 28. Cross section of an adrenal gland from a 10.4-year old female Beagle (B15). The medulla (M) is seen in two regions due to sectional geometry. A trabecular artery (Ta) is passing deep into the cortex. The outer (O) and inner (i) zona fasciculata and the zona reticularis (R) are well-defined. The zona intermedia (Z) is a wide zone which can be seen at the black pointers to extend into the prolapsed cortical nodules. Stained with Mallory's triple. Magnification 15x.

Figure 29. Cross section of an adrenal gland from a 11.1-year old female Fox Terrier (M41). The medulla (M) is separated by a thick band of collogenous fibers (MC). Pericapsular nodules are numerous (small, black arrows). Stained with Mallory's triple. Magnification 18x.


Z

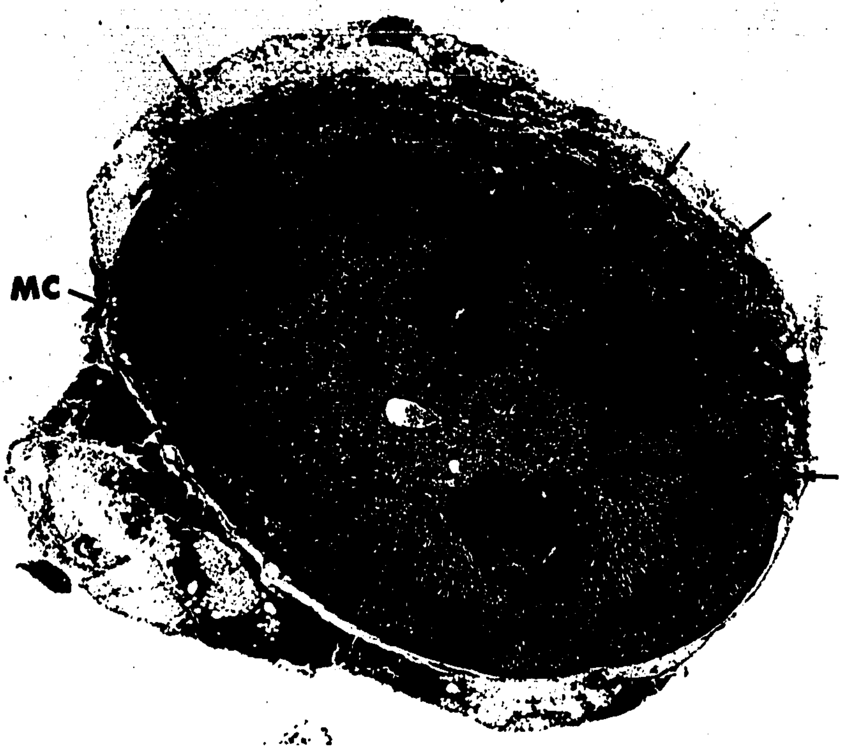
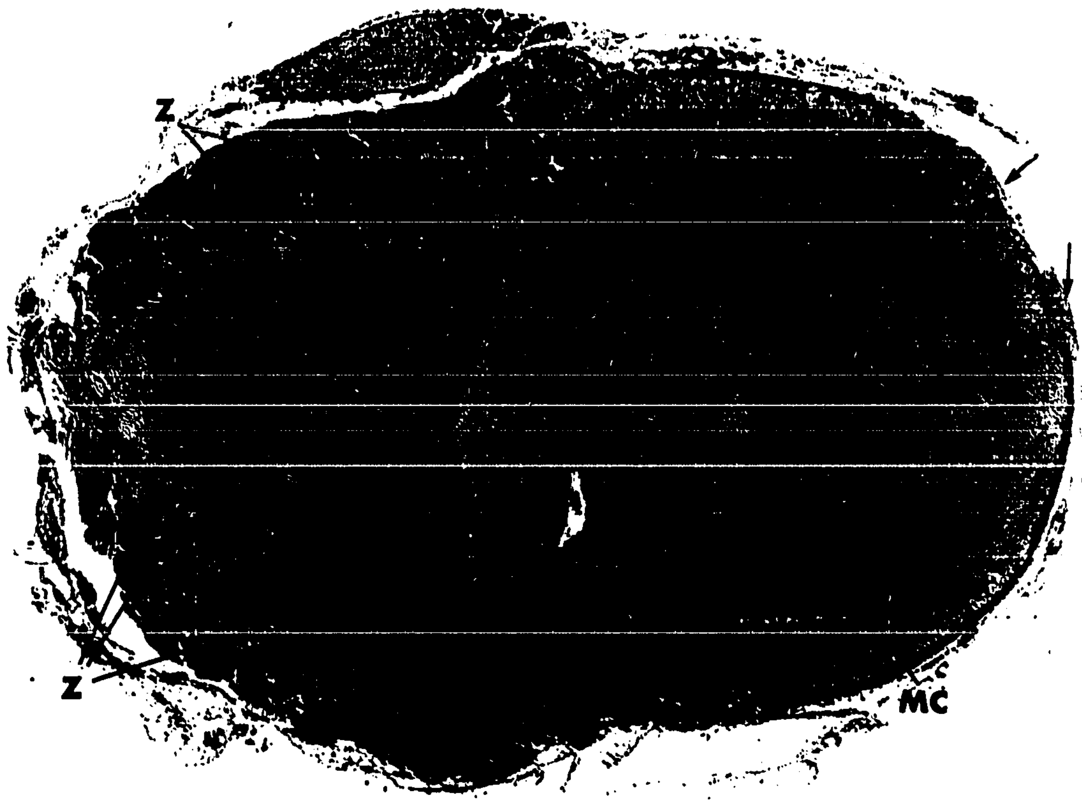

MC

Figure 30. Cross section of an adrenal gland from a 11.2-year old female Fox Terrier (M42). Extensive development of the zona intermedia has produced a great over-production of perecapsular nodules and a displacement of the zona glomerulosa (Z). The medulla (M) is surrounded by a "capsule" (MC). The reticularis (R) has prolapsed into the medulla. The small black arrows indicate the nodules of prolapsed tissue which has little or no connective tissue covering. Stained with Mallory's triple. Magnification 14x.

Figure 31. Cross section of an adrenal gland from a 11.3-year old female Fox Terrier (M43). The medulla (M) is violated by frequent zona reticularis evaginations. The zona intermedia (Z) is extensive. The outstanding feature in this section is the great development of the pericapsular nodules (small black arrows). Stained with Mallory's triple. Magnification 15x.


Z
Z
MC

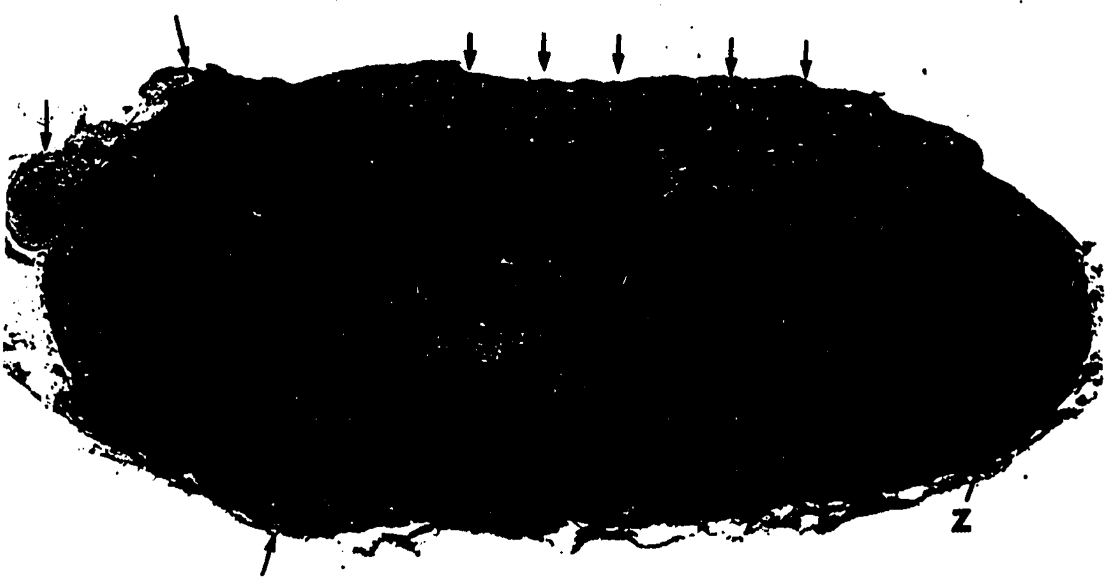
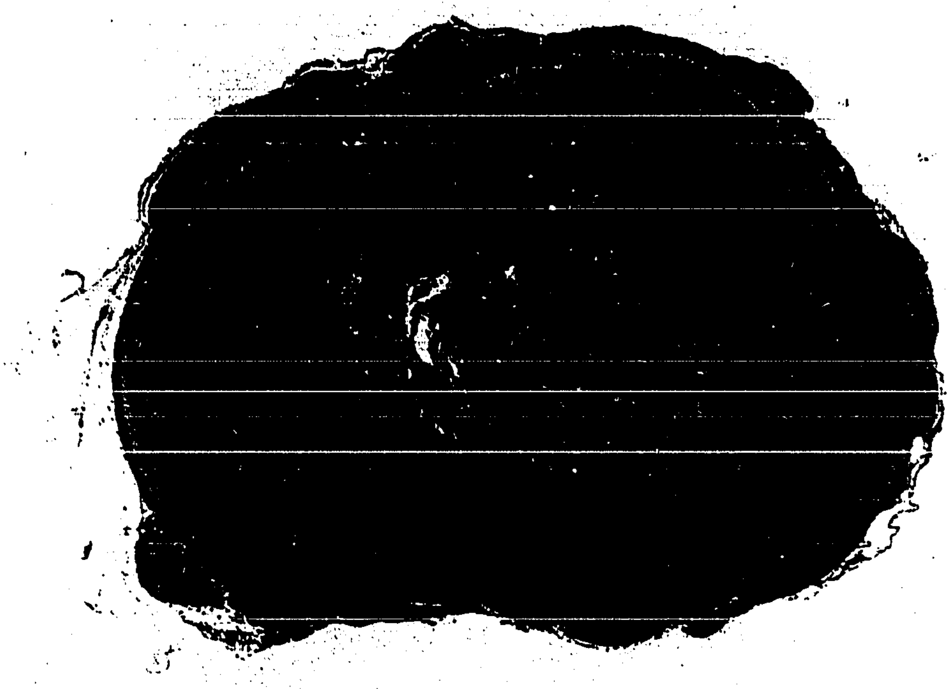

Z

Figure 32. Cross section of an adrenal gland from a 11.3-year old female Fox Terrier (M43). Nodule formation is extensive and metaplasia in the reticularis (R) is apparent (at black arrow). The large pointer indicates a medullary extension to the capsule. Stained with Mallory's triple. Magnification 16x.

Figure 33. Cross section of an adrenal gland from a 12.0-year old female Corgi (M44). Extensive nodule formation is accompanied by degenerative changes in the zona reticularis. Stained with Mallory's triple. Magnification 17x.



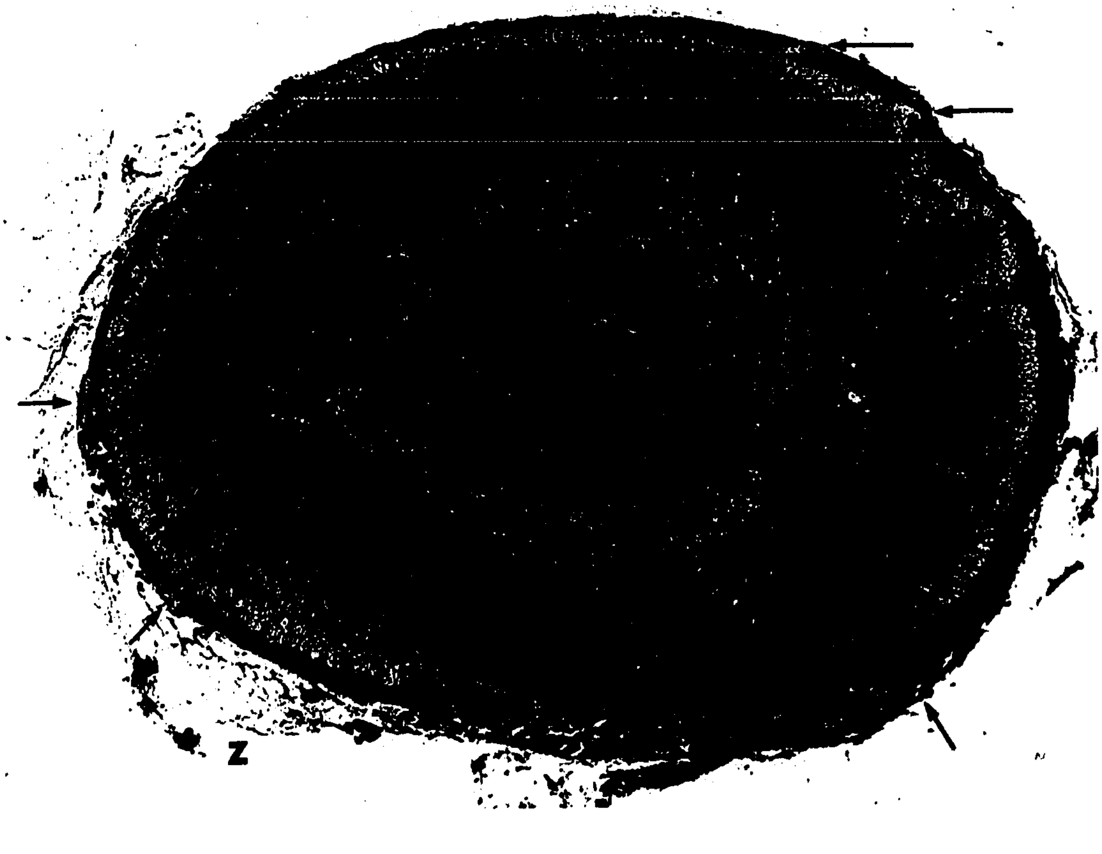
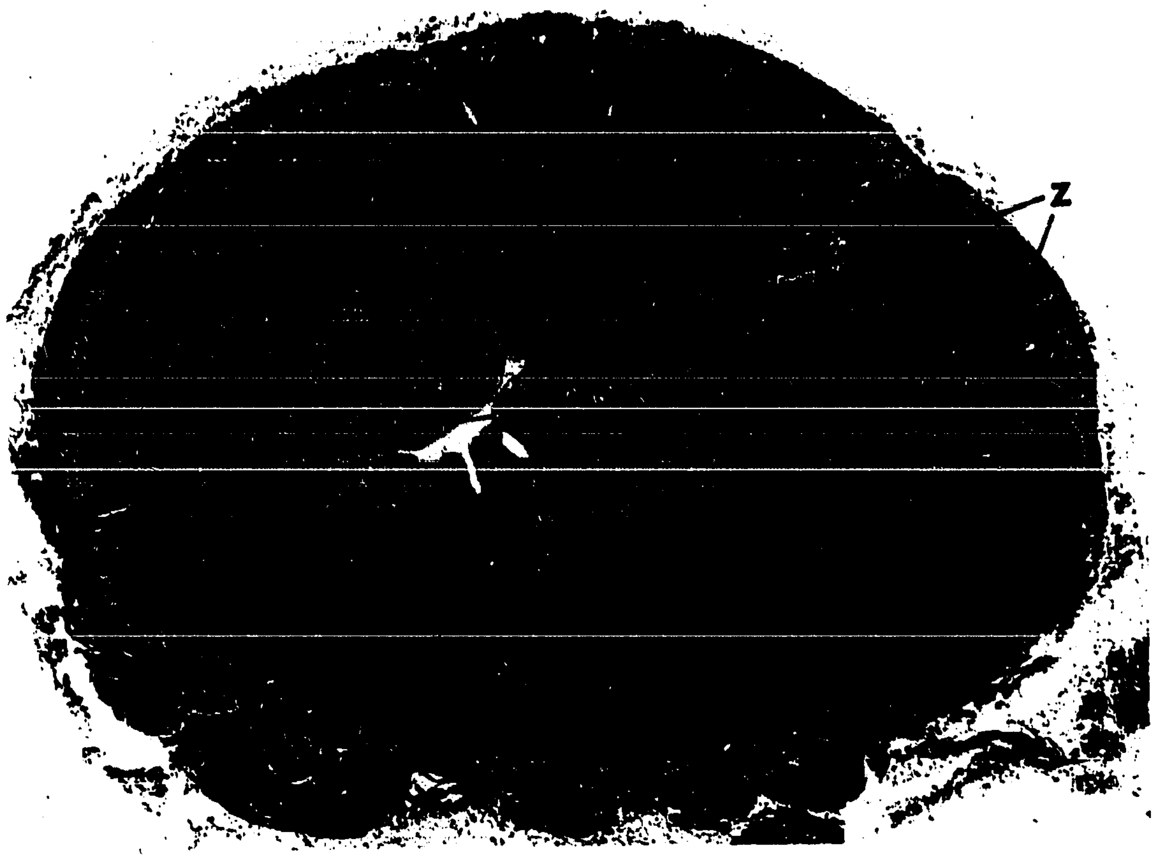

Z

Figure 34. Cross section of an adrenal gland from a 12.0-year old female Corgi (M44). Extensive development of the zona intermedia (Z) encroaching upon the zona fasciculata (F). Several areas of major evagination of the zona reticularis (R) into the medulla (M). Some reticularis cells occupy a position at the central vein in the medulla. Stained with Mallory's triple. Magnification 12x.

Figure 35. Cross section of an adrenal gland from a 12.0-year old female Fox Terrier (M45). Many subcapsular and intracortical proliferations of the zona fasciculata cells. The large zona intermediate (Z) and medulla (M). The fibrous band between medulla and cortex can be termed a medullary capsule. Stained with Mallory's triple. Magnification 13x.


Z
Z


MC
Z

Figure 36. Tangential section of an adrenal gland from a 13.6-year old Beagle (B14). Due to tangential cut the sectional geometry produces a mixing of the zonal arrangement. The zona reticularis (R), zona fasciculata (F) and zona intermedia (Z) are apparent. The large black pointers suggest areas of prolapse where the zona intermedia extends outwardly through the aperature. Contrast this gland with the youngest gland just below. Stained with Mallory's triple. Magnification 16x.

Figure 37. Cross section of an adrenal gland at birth. Beagle male (B7). All that can be resolved is the capsule (Ca), the cortex (C) and the medulla (M). Stained with Mallory's triple. Magnification 16x.


Z
R


Ca

Figure 38. Cortical and medullary regions of adrenal gland from a 1.0-year old male Beagle (B56)

A. Zona glomerulosa. The zona glomerulosa arcades (G) are displaced by the invading cells from the underlying zona intermedia. Stained with Mallory's triple. Magnification 500x.

B. Intracapsular nodule. Note the thick fibrous capsule around this nodule. The zona glomerulosa of the cortex (G) appeared to be continuous with the nodule (at black arrow) via cells similar to the zona intermedia. Within the nodule there has been a differentiation into zona glomerulosa (g) and zona fasciculata (f). Stained with Mallory's triple. Magnification 200x.

C. Outer zona fasciculata. The major portion of this tissue is composed of the spongiocytes typical of the outer zona fasciculata (O). A portion of the inner zona fasciculata is at the far right (l). Stained with Mallory's triple. Magnification 500x.

D. Inner zona fasciculata. The smaller spongiocytes are prominent in this area (l) and a portion of the zona reticularis (R) is at the far right. Stained with Mallory's triple. Magnification 500x.

E. Zona Reticularis. The orderly arrangement of cells into fascicles now seems to have given way to a random arrangement of parenchyma (R). These cells are more darkly staining and smaller than those in the outer fasciculata. Juxta positioned to the zona reticularis is the medulla (M). Stained with Mallory's triple. Magnification 500x.

F. Zona reticularis. Note that the zona reticularis (R) is permeated with large sinusoids (s). Stained with Mallory's triple. Magnification 500x.

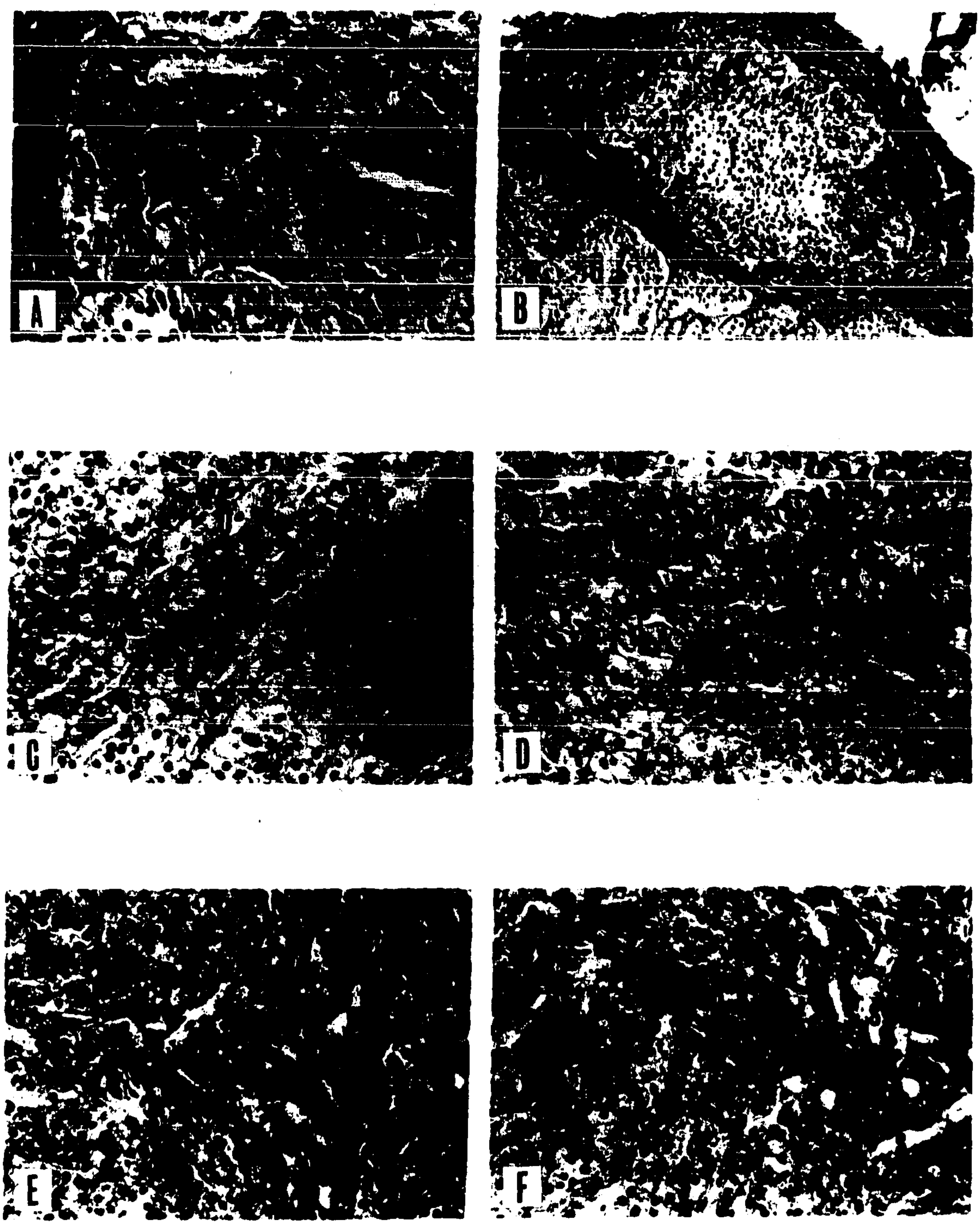

A
B
C
D
E
F

Figure 39. Cortical and medullary regions of adrenal gland from a 11.9-year old female Beagle (B73)

A. Zona glomerulosa. The fibrous capsule sends cellular trabeculae (large black arrow) into the zona glomerulosa. Stained with Mallory's triple. Magnification 500x.

B. Outer cortical regions. Note the zona glomerulosa (G) blends subtly with the zona intermedia (Z) as does the outer zona fasciculata (O). Stained with Mallory's triple. Magnification 500x.

C. Outer zona fasciculata. The large spongiocytes of this zone (O) in some areas show evidence of fatty metaplasia (black arrows). Stained with Mallory's triple. Magnification 500x.

D. Inner zona fasciculata and zona reticularis. The inner fasciculata (1) subtly is altered to conform to the morphology of the zona reticularis (R). Selected foci of cytolysis are observed within the zona reticularis (large black pointers). Stained with Mallory's triple. Magnification 500x.

E. Zona reticularis and medulla. Many foci of cytolysis (C) are present within the zona reticularis. Stained with Mallory's triple. Magnification 500x.

F. Cortico-medullary border. An aperature in the medullary capsule (MC) accommodates an evaginating mass of zona reticularis (dark pointers) which extends from the zona reticularis (R) into the medulla (M). Stained with Mallory's triple. Magnification 200x.

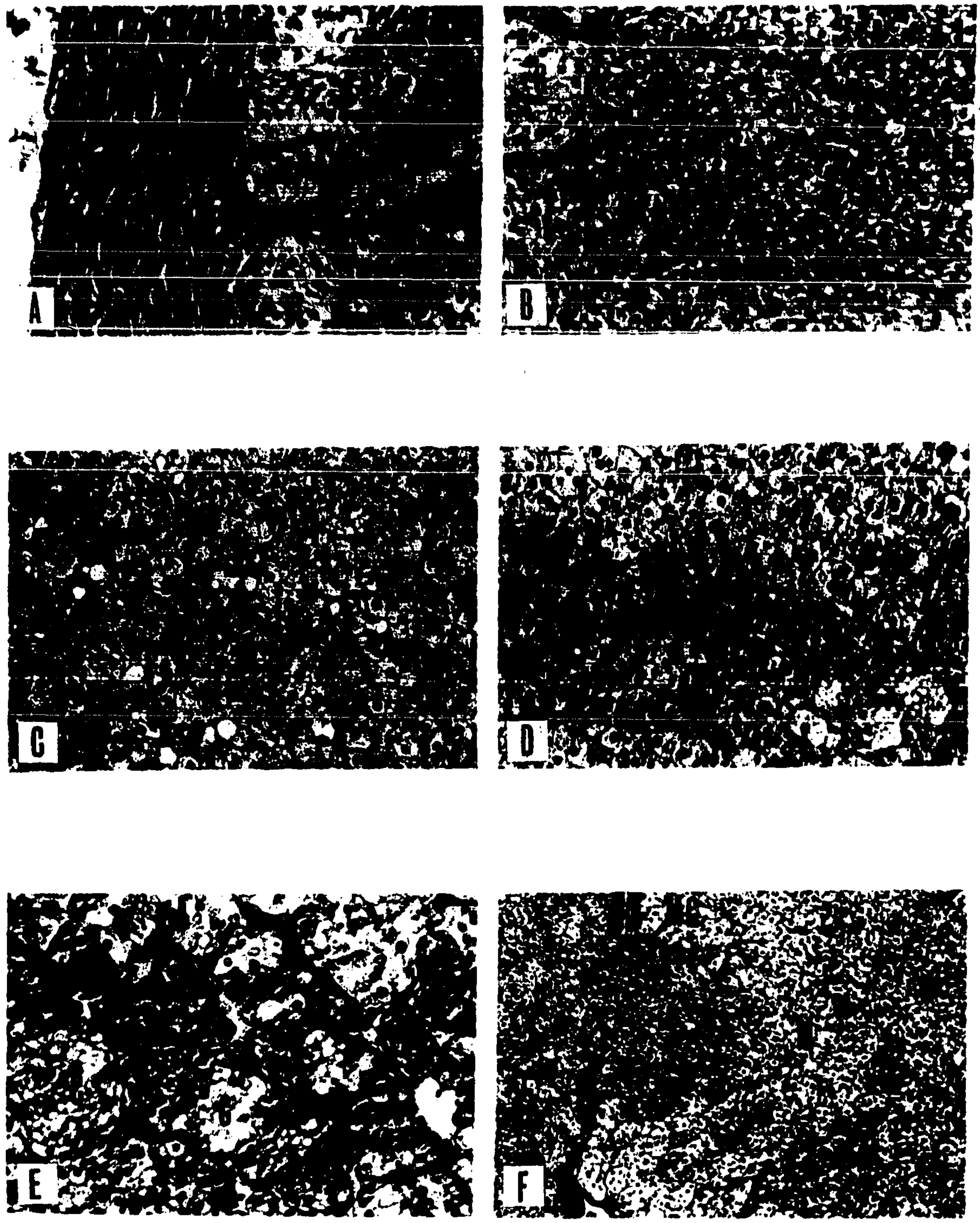

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Figure 40. The morphology of the early zona glomerulosa.

A. Zona glomerulosa. From a 9-day old female Beagle (B10). A very cellular capsule (C) sends equally cellular trabeculae into the zona glomerulosa (g). The glomeruli of cells (g) are separated from the capsule by sinusoids engourged with blood. Stained with Mallory's triple. Magnification 500x.

B. Zona glomerulosa. From a 9-day old female Beagle (B10). The sinusoid (s) between the glomerulosa (g) and the capsule would effectively prevent cellular continuity between the two. Stained with Mallory's triple. Magnification 500x.

C. Zona glomerosa. From a 9-day old female Beagle (B10). The sinusoid (s) is approaching the region of the zona intermedia. The glomerulosa (g) is developing at the expense of capsule and cortical communication. Stained with Mallory's triple. Magnification 500x.

D. Outer cortex. From a 0.3-year old male Beagle (B26). A very narrow zona glomerulosa (g) borders on to a very cellular capsule (C). The Outer zona fasciculata (O). Stained with Magnification 500x.

E. The outer cortex. From a 7.5-year old female Beagle (B30). A fibrous capsule (C) sends trabeculae between the arches of the zona glomerulosa (g). A well-developed zona intermedia (Z) is seen between the glomerulosa and the outer zona fasciculata. Stained with Heidenhain-Van Giesen - Weigert. Magnification 200x.

F. The zona intermedia. From a 7.5-year old female Beagle (B30). The (g) is the arcade extension from the zona intermedia. Similary the outer zona fasciculata was thought to have its origin from the inner surface of the zona intermedia. Stained with Heidenhain - Van Giesen - Weigert. Magnification 500x.

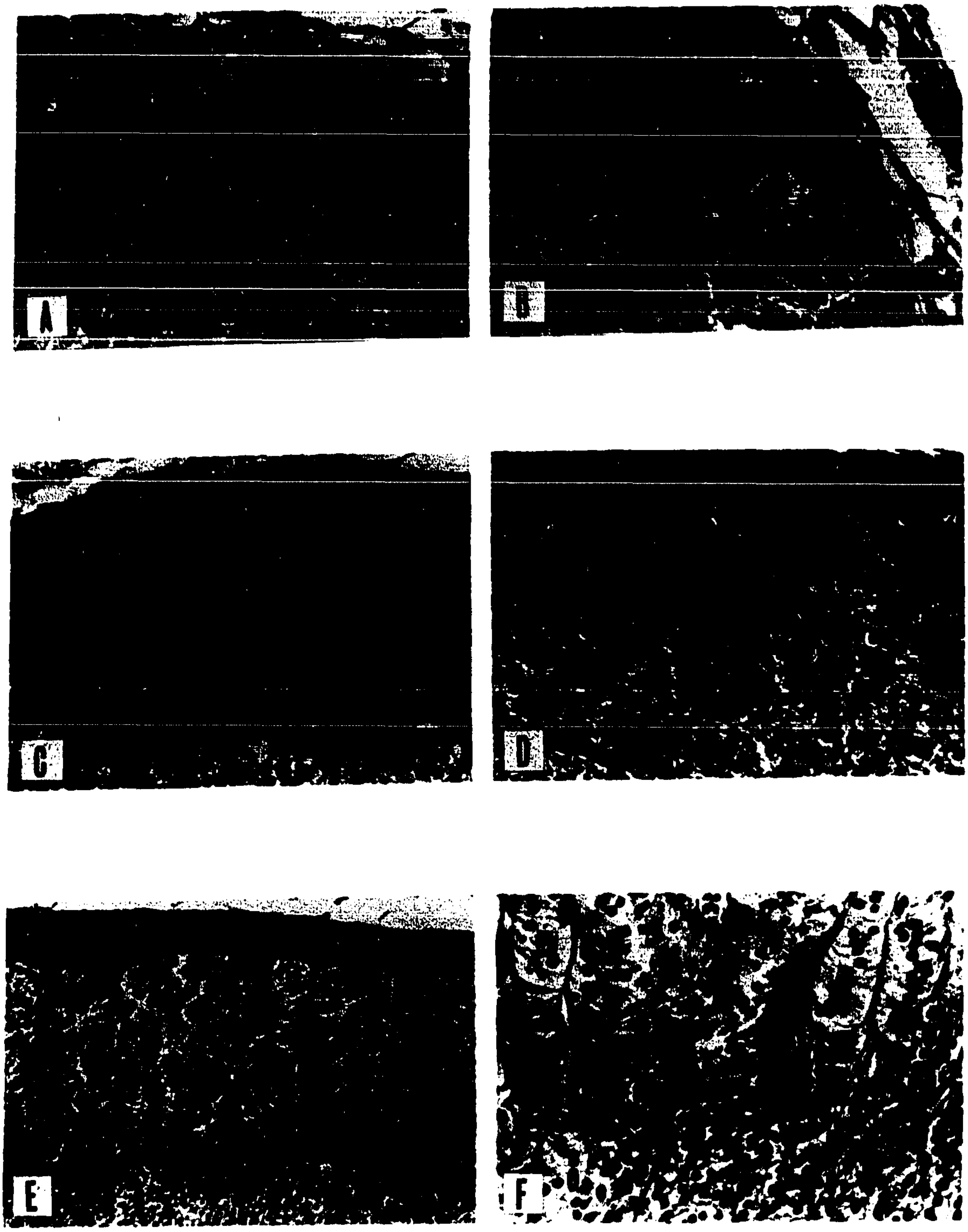
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Figure 41. The zona glomerulosa as seen by thin section evaluations

A. Zona glomerulosa. From a 0.3-year old female Beagle (E05). Note the trabeculum (t) and the sinusoid therein. The elonged columnar cell is very rich in lipid vacuoles. Stained with azure blue-methylene blue. Magnification 2000x.

B. Zona glomerulosa. From a 0.3-year old female Beagle (E05). Note their are smaller cells with small nuclei and a more homogenous cytoplasm with fewer lipid vacuoles. These are zona intermedia-like cells. Stained with azure blue-methylene blue. Magnification 2000x.

C. Zona glomerulosa. From a 0.3-year old male Beagle (E07). Between the arcades of the glomerulosa prominent sinusoids have a close contact to the ends not sides of the cells. Stained with azure blue-methylene blue. Magnification 2000x.

D. Zona glomerulosa. From a 0.3-year old female Beagle (E05). Some few cells appear, like zona intermedia cells. However the broken arrow points to a cell typical of those in the field. These are probably transverse sections through the nuclear region of a plate or column of glomerulosa cells. Thus the lipid vacuoles are few. Stained with azure blue-methylene blue. Magnification 2000x.

E. Zona intermedia. From a 0.3-year old female Beagle (E05). Cells in this field are separated by a sinusoid and its adventitia. Within this tissue are primitive reticular cells which may divide to produce new parenchyma. Note that the cells above sinusoid are low columnar in shape with some amount of lipid droplet storage (broken arrow). Those below the sinusoid are outer zona fasciculata cells. Stained with azure blue-methylene blue. Magnification 2000x.

F. Zona glomerulosa. From a 1.1-year old female Beagle (500). Tissue illustrates the tangential views of cells one encounters frequently. Stained with azure blue-methylene blue. Magnification 2000x.

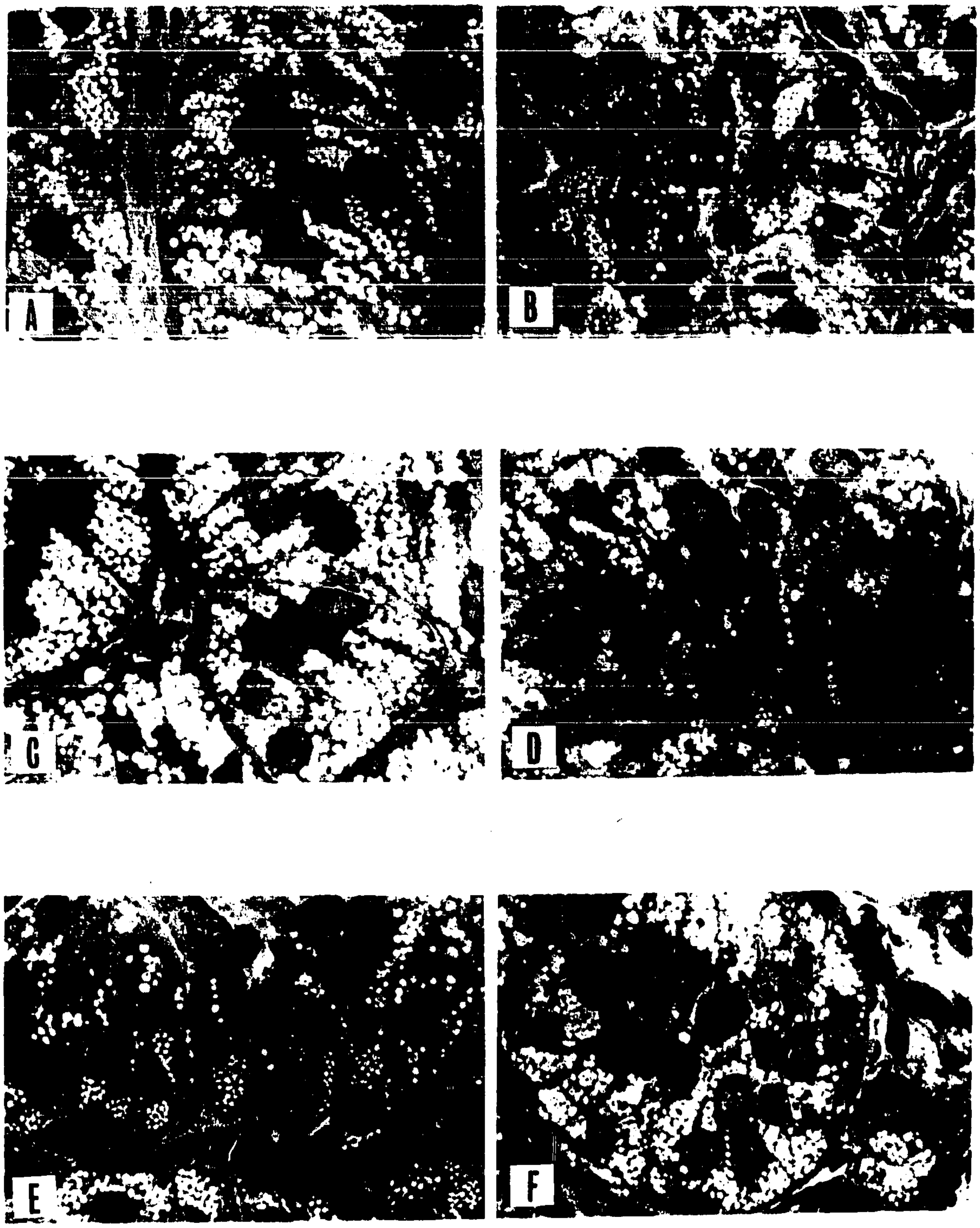
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Figure 42. The zona glomerulosa in the older adrenal gland.

A. Zona glomerulosa. From a 7.8-year old female Beagle (B44). The dark arrow indicates the connective tissue of the zona intermedia. Immediately above that the zona intermedia cells have proliferated and invaded the zona glomerulosa arcades. Stained with Mallory's triple. Magnification 200x.

B. Zona glomerulosa. From a 7.8-year old female Beagle (B44). A view of the zona glomerulosa which shows the variations in the arcade profiles when they are viewed in two cli-mentions. Stained with Mallory's triple. Magnification 500x.

C. Zona glomerulosa. From a 7.8-year old female Beagle (B44). This micrograph illustrates the proliferation of zona inter-media cells (black arrows) at the base of the zona glomerulosa. Stained with Mallory's triple. Magnification 500x.

D. Zona glomerulosa. From a 7.8-year old female Beagle (B44). The black arrows indicate the stroma of the zona intermedia. However, the zona intermedia cells are scattered within the zona glomerulosa. Stained with Mallory's triple. Magnification 500x.

E. Outer cortex. From a 10.0-year old female Beagle. The relationships between the fibrous capsule (C), cellular trabeculae, zona glomerulosa (G), zona intermedia (large arrow), and outer zona fasciculata (O) are well illustrated. Stained with Mallory's triple. Magnification 500x.

F. Outer cortex. From a 13.6-year old male Beagle. The arrows indicate the stroma of the zona intermedia and above it the zona intermedia parenchyma (Z) has undergone hyperplasia. The capsule (C) and zona glomerulosa (G). Stained with Mallory's triple. Magnification 200x.

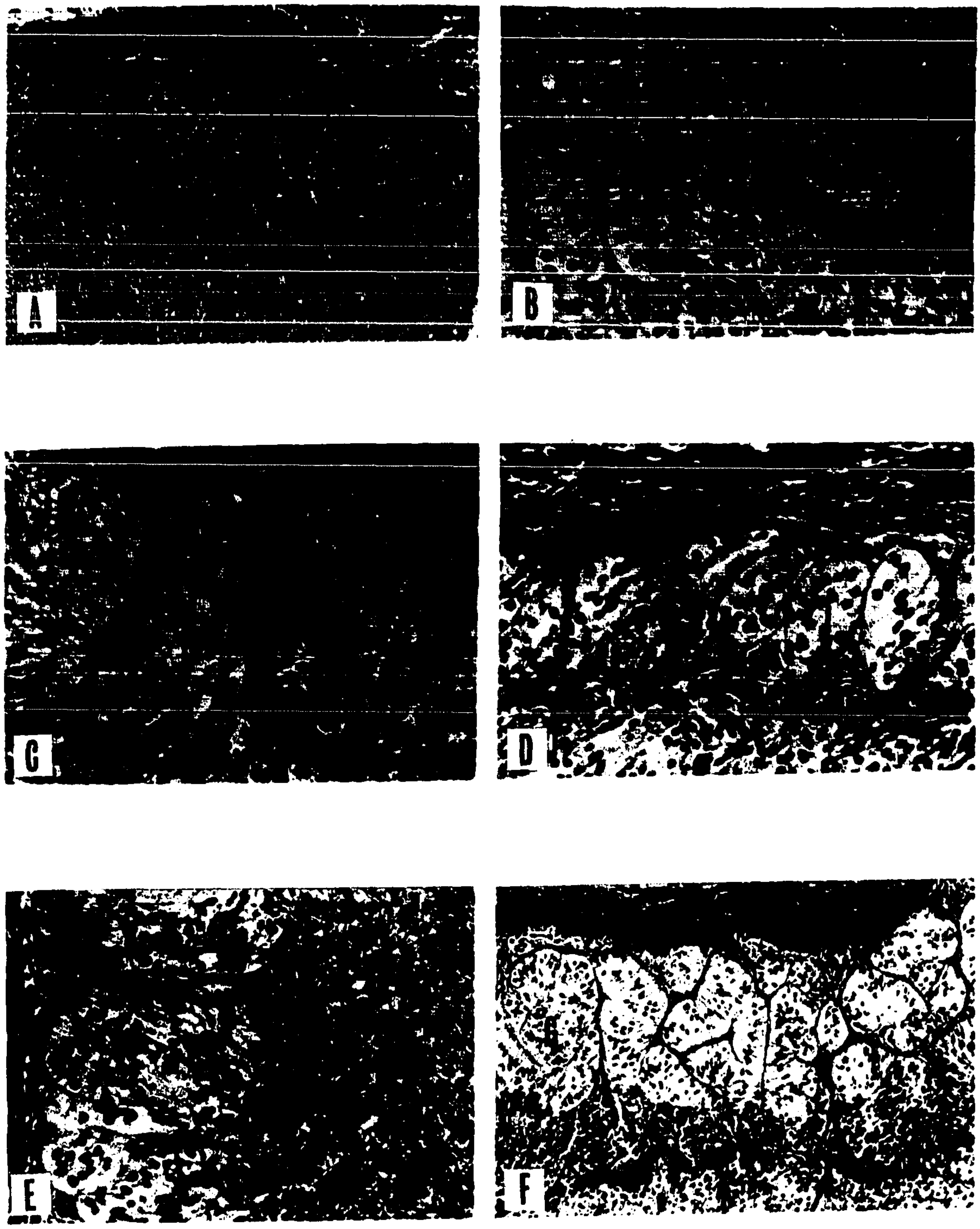
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Figure 43. Representations of the zona fasciculata

A. Outer zona fasciculata. From a 1.3-year old male Beagle (494). The dark pointers indicate areas of lumina formation within this cortical parenchyma. Stained with Mallory's triple. Magnification 500x.

B. Inner zona fasciculata. From a 9.3-year old female Irish Setter. The parenchymal cells were distended with lipid droplets and these varied in size between cells but were uniform in size within individual cells. At the right of the micrograph some cells have large lipid vacuoles. Stained with azure blue-methylene blue. Magnification 2000x.

C. Outer zona fasciculata. From a 10.0-year old female Beagle (B64). Stained with Mallory's triple. Magnification 500x.

D. Inner zona fasciculata. From a 9.3-year old female Irish Setter. The arrow indicates an area of fatty metaplasia. The adjoining areas of cellular change are filled with red blood cells. Stained with azure blue-methylene blue. Magnification 2000x.

E. Inner zona fasciculata. From a 10.0-year old female Beagle (B64). Stained with Mallory's triple. Magnification 500x.

F. Inner zona fasciculata. From a 10.3-year old female Lab. Retriever. This micrograph reveals a great number of varying sized lipid vacuoles. Stained with azure blue-methylene blue. Magnification 2000x.

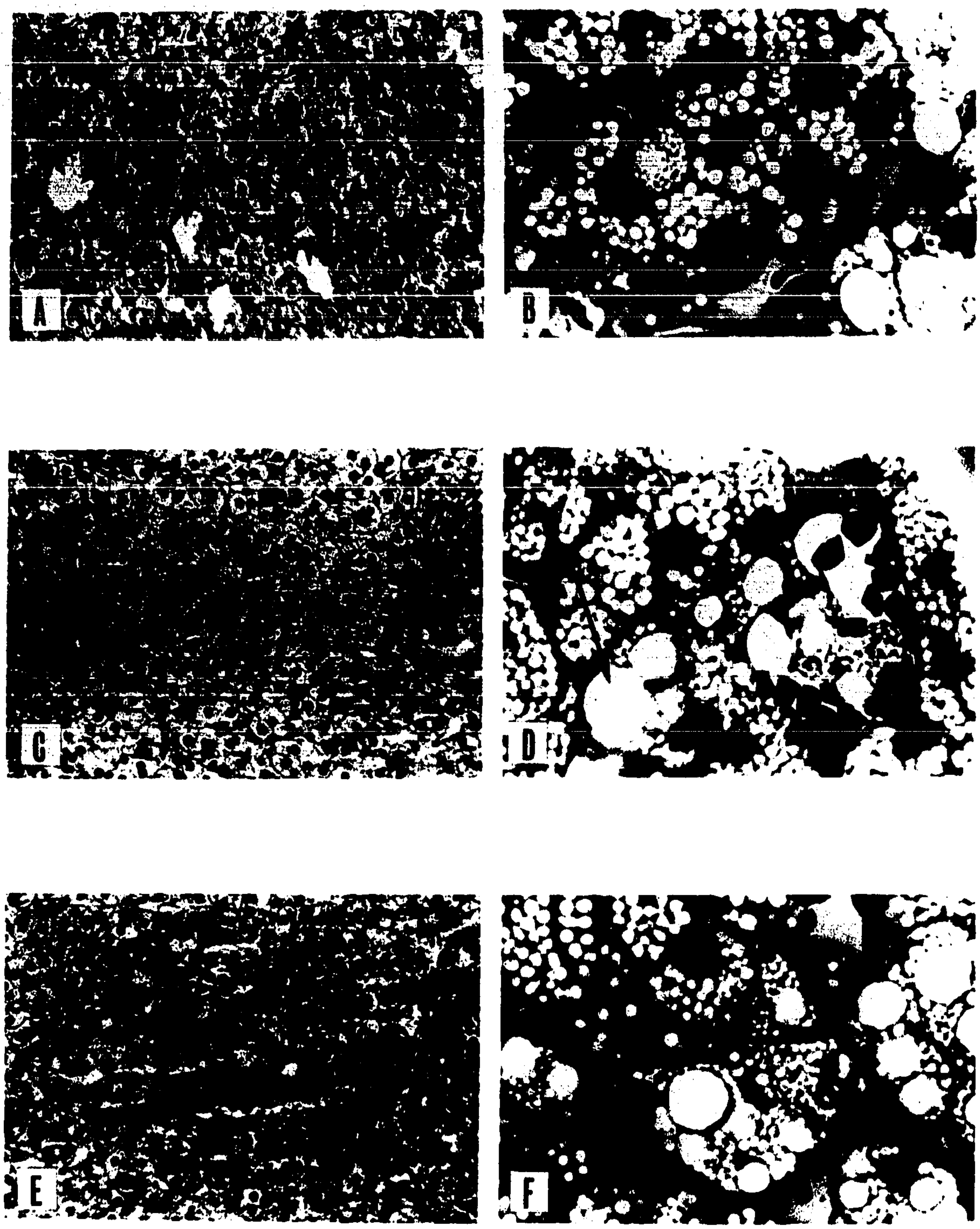
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Figure 44. Reprensentations of the zona reticularis

A. Zona reticularis. From a 1.3-year old male Beagle (494). There is a condensation of the stromal elements of the zona reticularis (R) at the border of the medulla (M). Stained with Mallory's triple. Magnification 500x.

B. Zona reticularis and medulla. From a 9.3-year old female Irish Setter (M51). The zona reticularis cells contain fewer lipid vacuoles and the sinusoids are quite prevalent. The medullary cells are finely granular and otherwise homogenous except for a very large nucleus. Stained by azure blue-methylene blue. Magnification 500x.

C. Zona reticularis. From a 10.0-year old female Beagle (B64). Stained with Mallory's triple. Magnification 500x.

D. Zona reticularis and medulla. From a 9.3-year old female Irish Setter (M51). The sinusoidal system (S) extends between many individual cells. Stained with azure blue-methylene blue. Magnification 2000x.

E. Zona reticularis and medulla. From a 13.6-year old male Beagle (B32). The arrow indicates an area of zona reticularis (R) evagination into the medulla (M). Stained with Mallory's triple. Magnification 200x.

F. Zona reticularis. From a 9.3-year old female Irish Setter (M51). The distribution of red blood cells indicates the extent of the sinusoidal development in this zone of the older specimens. Stained with Mallory's triple. Magnification 2000x.

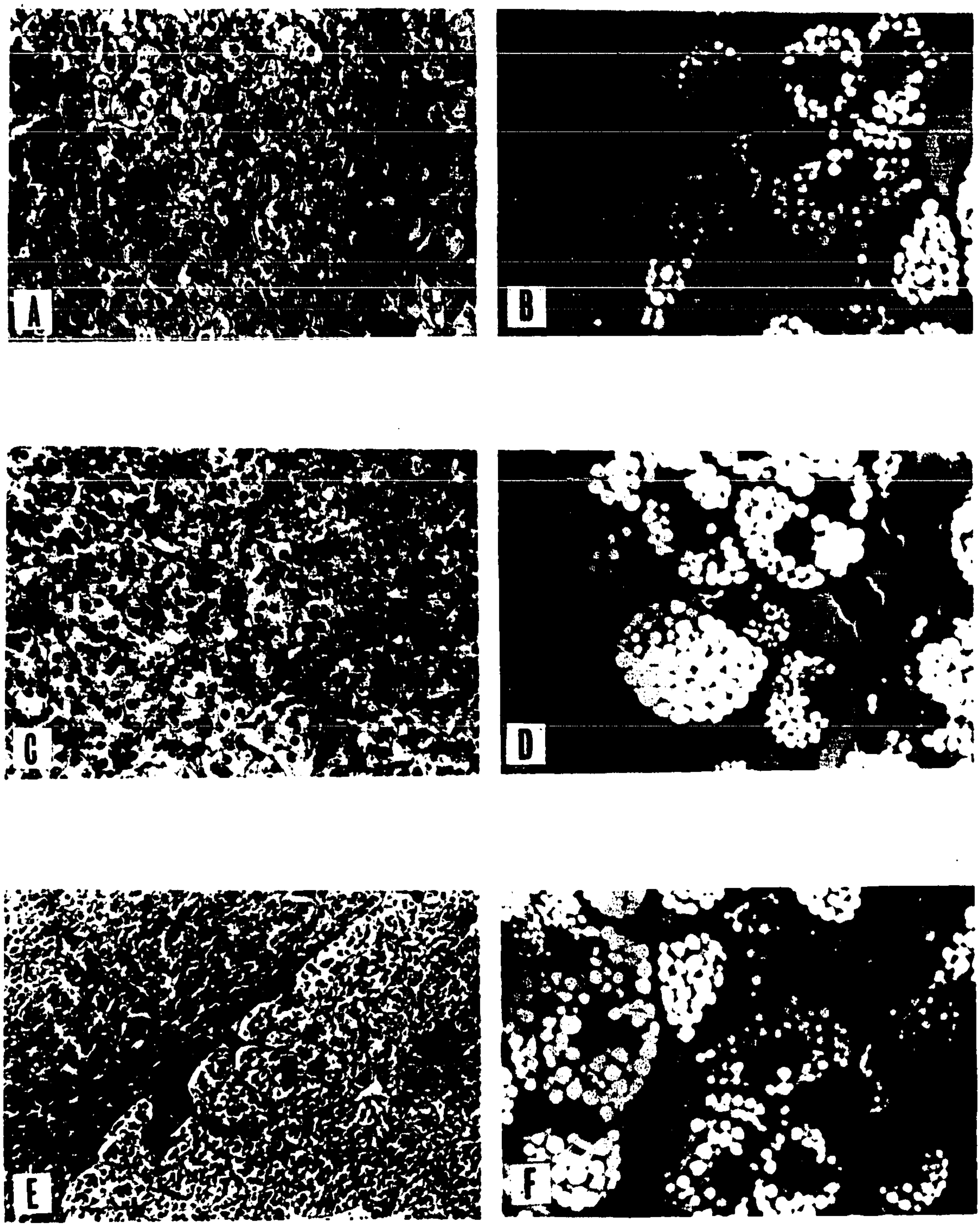
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Figure 45. Representations of medullary morphology

A. Medullary parenchyma. From a 0.9-year old female Beagle (C17). The very cell outlines is often delineated by a network (N) of fibroblasts. Stained with azure blue-methylene blue. Magnification 2000x.

B. Medullary parenchyma. From a 0.9-year old female Beagle (C17). Sinusoids penetrate the parenchyma along with the fibrous connective tissue. Stained with azure blue-methylene blue. Magnification 2000x.

C. Medulla. From a 1.3-year old male Beagle (494). The network (N) of supportative fibers are prevalent. Stained with Mallory's triple. Magnification 500x.

D. Medulla and reticularis. From a 10.0-year old female Beagle (B64). The medulla (M) and reticularis (R). Stained with Mallory's triple. Magnification 500x.

E. Medullary parenchyma. From a 13.6-year old male Beagle (B32). With older age the amount of stroma between the cells increases. Stained with Mallory's triple. Magnification 500x.

F. Medullary parenchyma. From a 13.6-year old male Beagle (B32). There is a great increase in the amount of connective tissue. Stained with Mallory's triple. Magnification 800x.

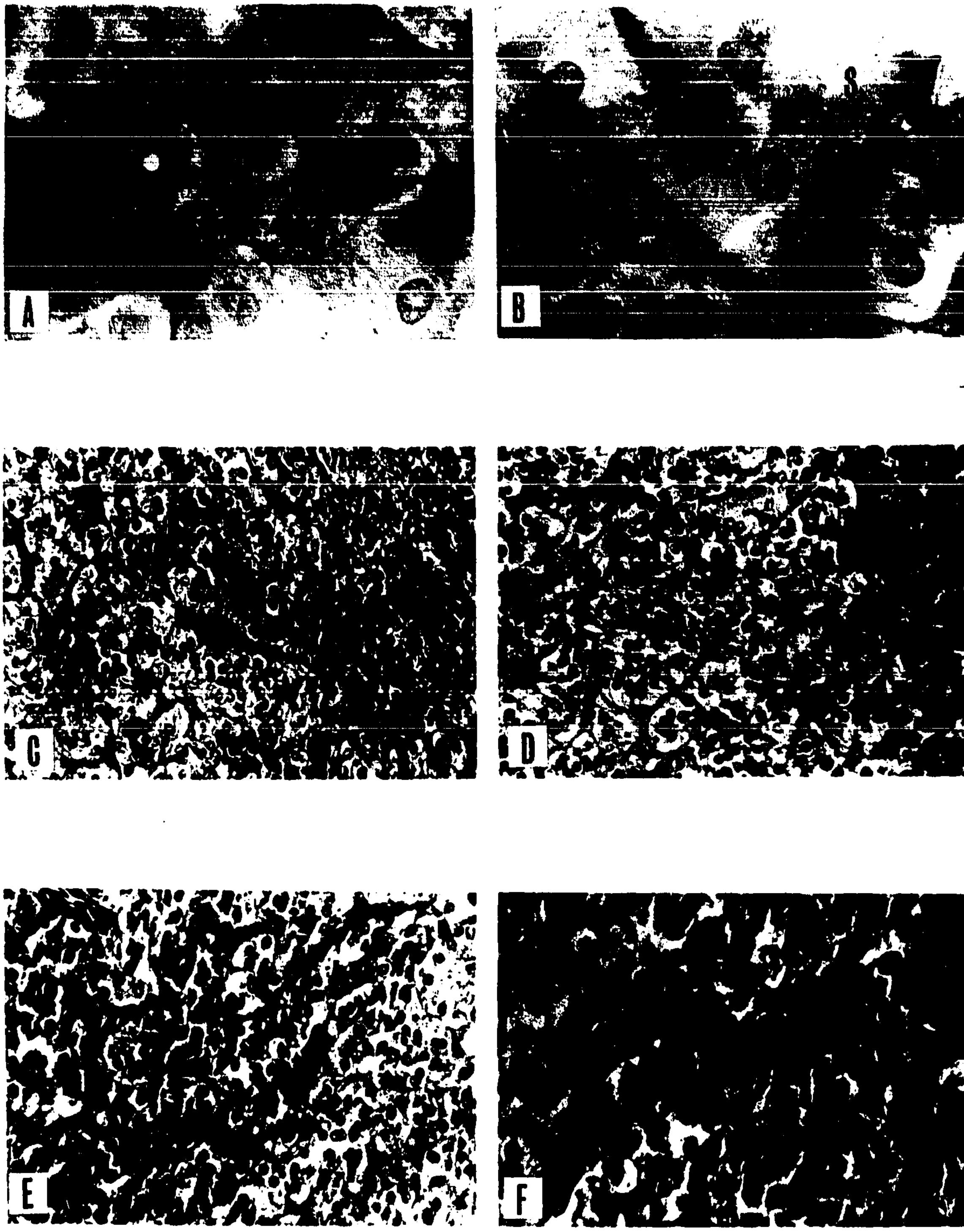
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Figure 46. Extracortical nodules I

A. Isolated extracortical nodules. From a 11.1-year old female Fox Terrier (M41). A well-differentiated nodule was observed in this tissue.(C and e indicate that further magnification was given in some of the following illustrations). Stained with Mallory's triple. Magnification 200x.

B. Intracapsular nodule developing. From a 12.0-year old female Corgi (M44). Note the sinusoids around the cells. These cells are mature and not generalted in the capsule. Stained with Mallory's triple. Magnification 500x.

C. Zona intermedia of the nodule in A). From a 11.1-year old female Fox Terrier (M41). Within this nodule the fasciculata (F) and zona intermedia (Z) are present. Stained with Mallory's triple. Magnification 800x.

D. Zona intermedia tissues. From a 12.0-year old female Fox Terrier (M44). The arrow indicates a zona intermedia bud. Stained with Mallory's triple. Magnification 500x.

E. Zona glomerulosa of the nodule in A). From a 11.1-year old female Fox Terrier (M41). Note the zona glomerulosa (G) is formed within the nodule also containing a zona fasciculata (F). Stained with Mallory's triple. Magnification 800x.

F. Zona intermedia tissues. From a 12.0-year old female Fox Terrier (M45). Cells in middle of an extracortical nodule are zona intermedia cells (Z). Stained with Mallory's triple. Magnification 500x.

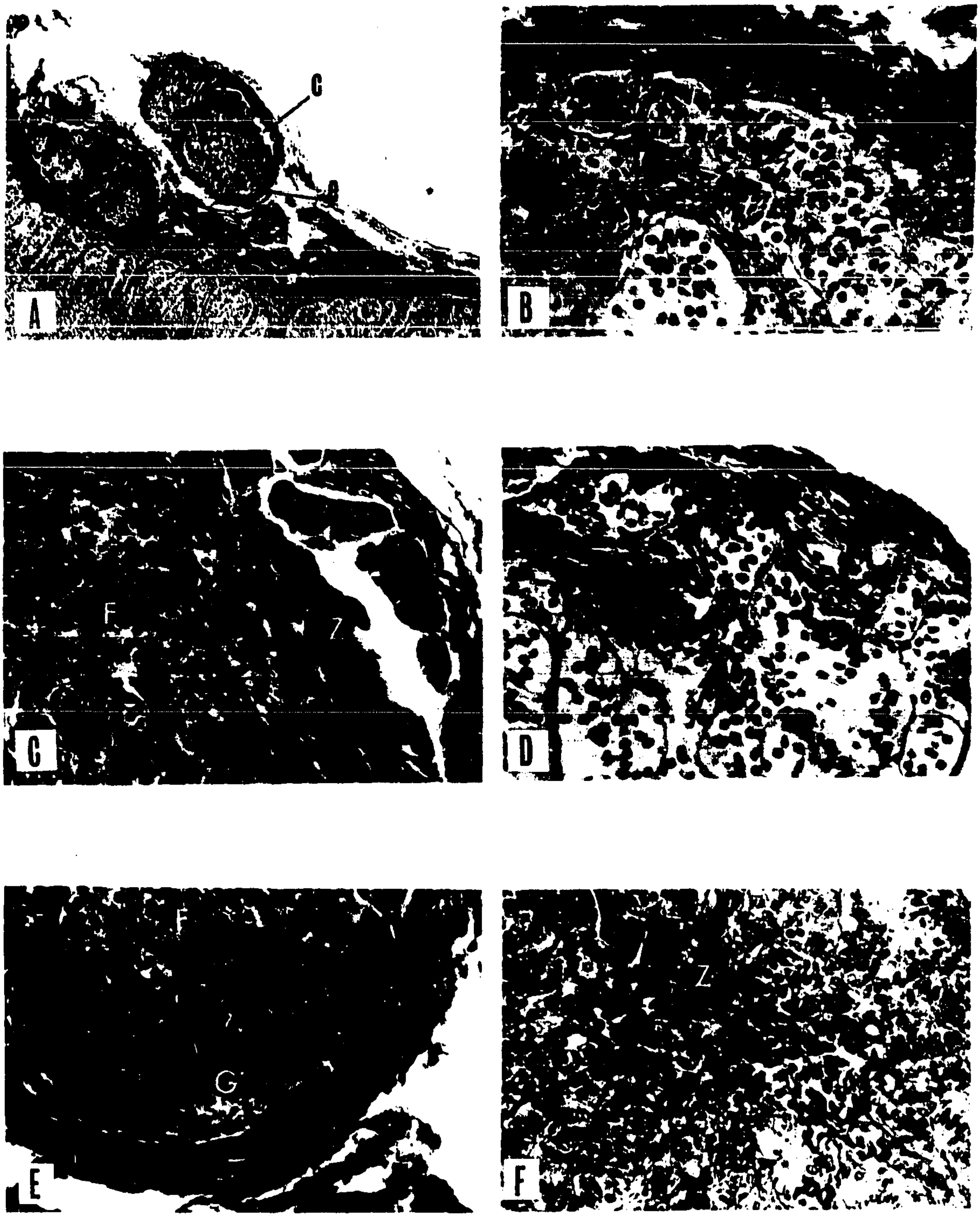
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Figure 47. Extracortical nodules II

A. Pericapsular nodule. From a 12.9-year old female Beagle (B17). The arrow signifies role zona intermedia may play in formation of the nodule. Stained with Mallory's triple. Magnification 200x.

B. Intracapsular nodule. From a 12.0-year old female Corgi (M44). An outgrowth of the zona glomerulosa is forming this nodule. Stained with Mallory's triple. Magnification 500x.

C. Pericapsular nodules. From a 13.6-year old male Beagle (B32).. Note extent of nodule formation and the role played by the zona intermedia. Stained with Mallory's triple. Magnification 80x.

D. Pericapsular nodule. From a 13.6-year old male Beagle (B32). Note the extent of development and that there is great disruption of the zona glomerulosa. Stained with Mallory's triple. Magnification 80x.

E. Pericapsular nodules. From a 13.6-year old male Beagle (B32). It appears that nodules form off of nodules in a very dynamic cycle of events envolving the zona intermedia. Stained with Mallory's triple. Magnification 80x.

F. Pericapsular nodules. From a 13.6-year old male Beagle (B32). Arrow suggests possible role of the zona intermedia in their formation. Stained with mallory's triple. Magnification 200x.

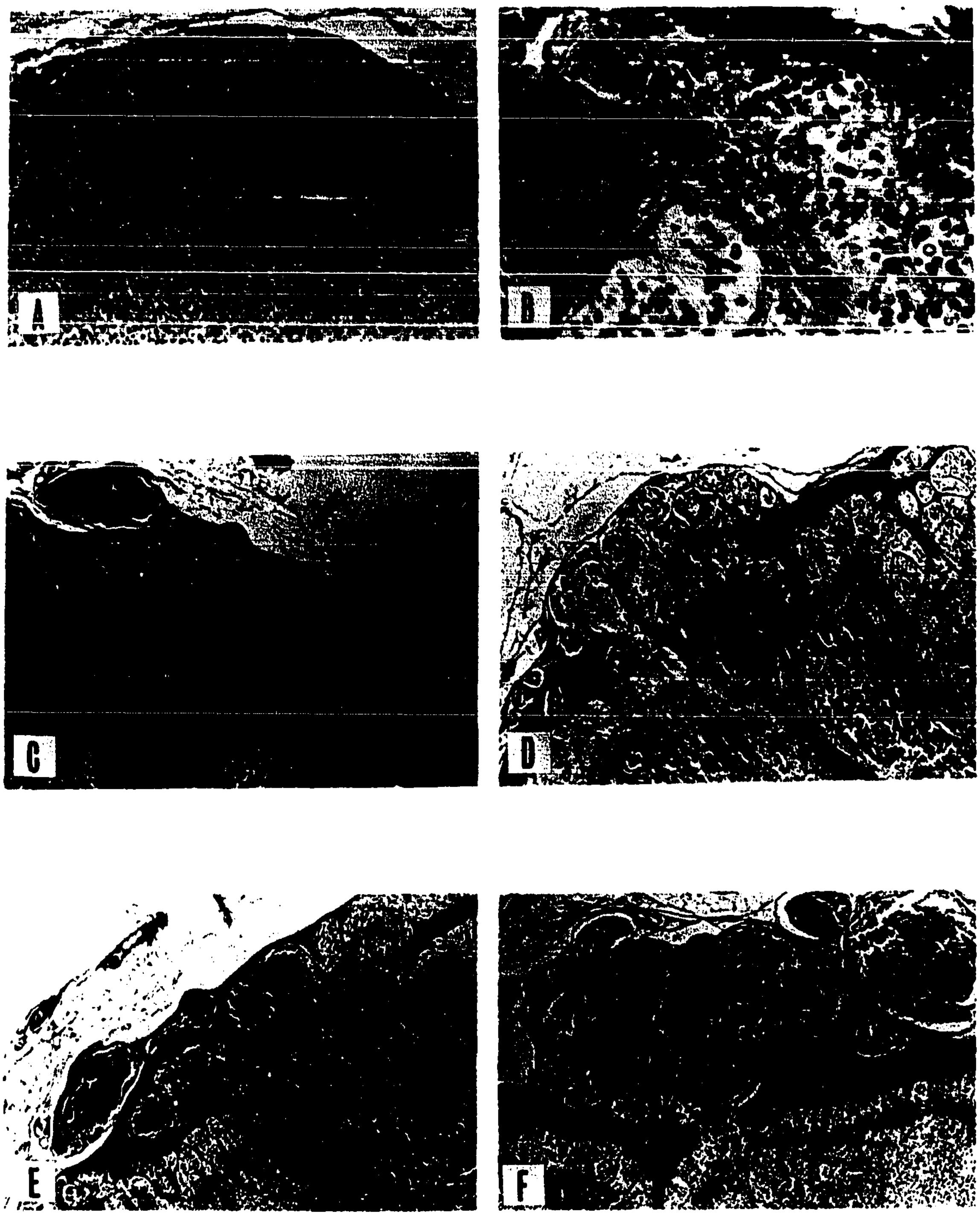
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Figure 48. Nodule formation

A. Arcade evagination. From a 0.2-year old male Beagle (E26). Note the proliferation and expansion of the glomerulosa tissue. The direction of the nodule movement (inward or outward) can be determined by the direction of flow of the capsular connective tissue. In this case the movement is outward. Stained with Mallory's triple. Magnification 500x.

B. Glomerulosa migration. From a 0.5-year old male Beagle (C35). Again as above the direction of pressure on the connective tissue is outward. Stained with Mallory's triple. Magnification 500x.

C. Proliferation due to activity of the zona intermedia. From a 0.6-year old female Beagle (B59). Some nodules seem to be formed from the activity of the zona intermedia as it appears in this micrograph at the arrow. Stained with Mallory's triple. Magnification 500x.

D. Proliferation due to activity of zona intermedia. From a 10.0-year old female Beagle (B64). Stained with Mallory's triple. Magnification 500x.

E. Extension of zona glomerulosa. From a 10.0-year old female Beagle (B64). Often times nodules result from a narrow extension of the zona glomerulosa. Then, when outside the capsule, there is a hypertrophy and hyperplasia of the cell elements. Stained by Mallory's triple. Magnification 200x.

F. The effects of sectioning. From a 10.0-year old female Beagle (B64). In this gland same as E) the same nodule appears as only a small intracapsular nodule. Stained with Mallory's triple. Magnification 200x.

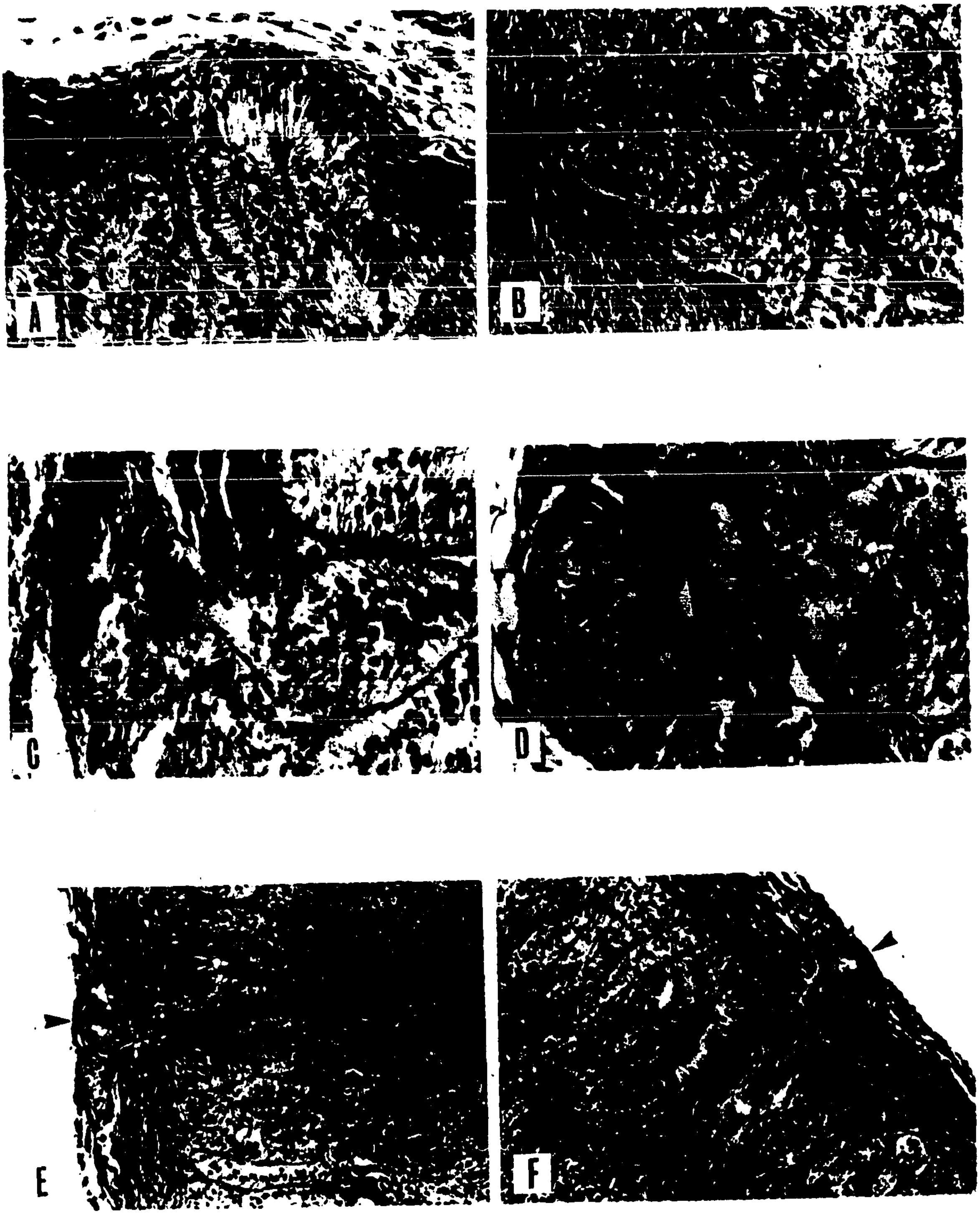
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Figure 49. Thin sections of the Cortical regions

A. Parenchyma of a nodule. From a 0.9-year old female Beagle (C17). As in most nodules the cellular morphology is generally that of the zona glomerulosa. The perisinusoidal space containes many primitive cells. Stained with azure blue-methylene blue. Magnification 2000x.

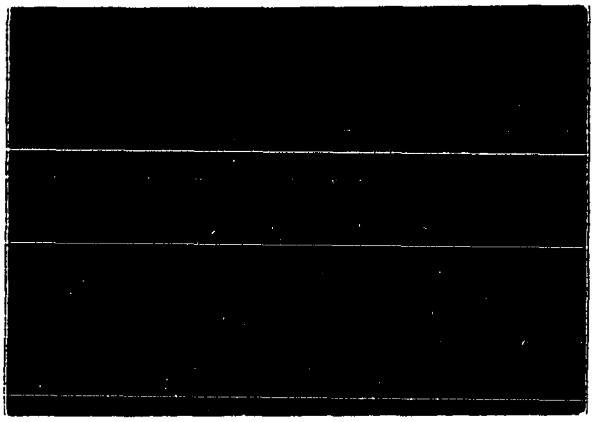
B Zona glomerulosa. Fromaa 0.3-year old male Beagle (E07). The mature secretory cells extend from one sinusoid to the other. The primitive reticular cells are numerous. Stained with azure blue-methylene blue. Magnification 2000x.

C. Zona intermedia. From a 10.3-year old female Lab. Retriever (M50). The upper portion is the zona glomerulosa, the central is the zona intermedia and the inner portion is the zona fasciculata. In the zona intermedia there are many primitive reticular cells and some show varying degrees of lipid droplet accumulation. Stained with azure blue-methylene blue. Magnification 2000x.

D. Outer zona fasciculata. From a 0.7-year old male Beagle (C34). Large polyhedral cells contained very large amounts of lipid droplets. Stained with azure blue-methylene blue. Magnification 2000x.

E. Inner zona fasciculata. From a 1.7-year old male Beagle (B80). The cells are smaller and more closely packed than in the outer zona fasciculata. These cells contain less amounts of lipid also. Stained with azure blue-methylene blue. Magnification 2000x.

F. Zona reticularis. From a 0.7-year old male Beagle (C34). In this zone there was a considerable increase in the amount of intercellular connective tissue. Staining azure blue-methylene blue. Magnification 2000x.



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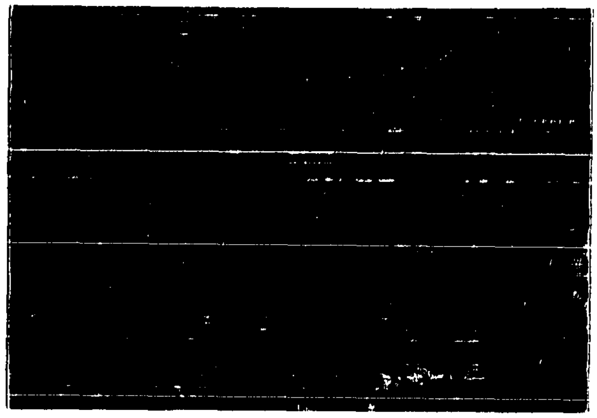

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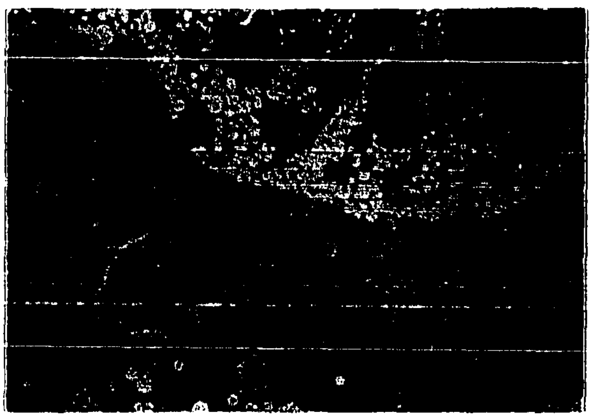

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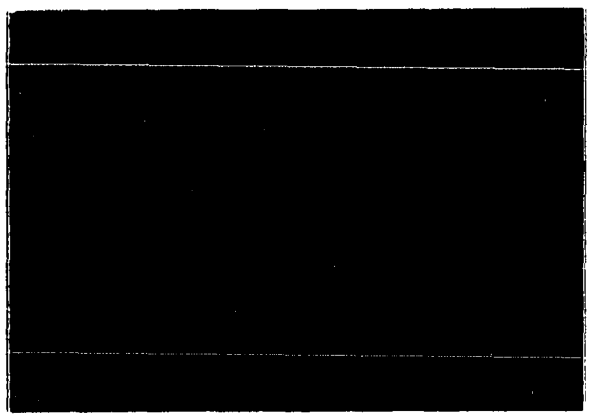

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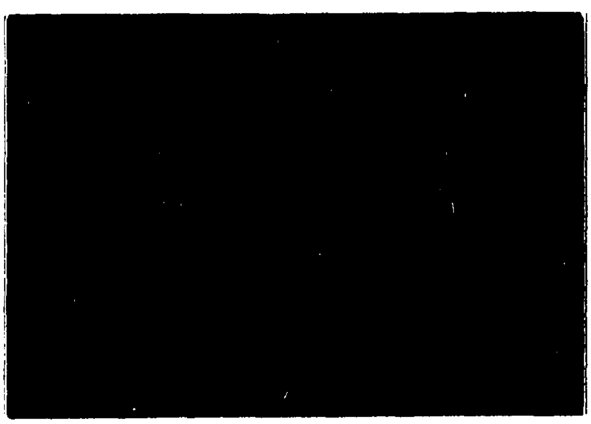

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Figure 50. Oil-Red-O distribution within the cortex

A. Zona glomerulosa. From a 7.8-year old female Beagle (B43). The zona glomerulosa and outer zona fasciculata are strongly positive. The zona intermedia is negative. Note that at the point of formation of the intra-capsular nodule envoles the zona intermedia. Stained with Oil-Red-O. Magnification 400x.

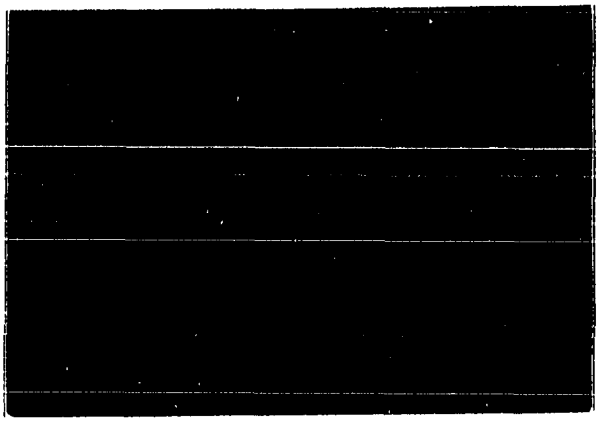
B. Zona fasciculata. From a 0.8-year old female Beagle (B67). The lipid is distributed within the cytoplasm as droplets. The nucleus is counter stained as a blue body. Stained with Oil-Red-O. Magnification 800x.

C. Zona reticularis. From a 10.0-year old female Beagle (B64). The morphology and arrangement of tissues is much as has been described before with the widely distended sinusoids. The cells vary in affinity for the dye, some are very positive and others contain relatively little. Stained with Oil-Red-O. Magnification 800x.

D. Capsular nodule. From a 9.1-year old male Beagle (B63). Fully mature cells are seen to be projecting through part of the capsule. There are not intermediate stages of cell development within the capsule. Stained with Oil-Red-O. Magnification 500x.

E. Pericapsular nodule. From a 7.8-year old female Beagle (B43). This large nodule is connected to the cortex by a narrow stock of zona intermedia cells. There is evidence in this photograph to suggest that the zona intermedia cells are at the base and periphery of the nodule. Stained with Oil-Red-O. Magnification 400x.

F. Prolapsing nodule. From a 9.3-year old female Irish Setter (M51). The zona intermedia is flowing into this nodule which projects into the capsule. The Oil-Red-O negative cells at the margins of the nodule may be medullary cells or zona intermedia cells. Stained with Oil-Red-O. Magnification 200x.



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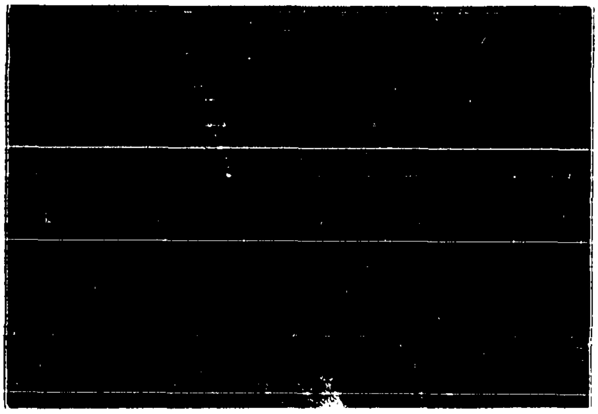

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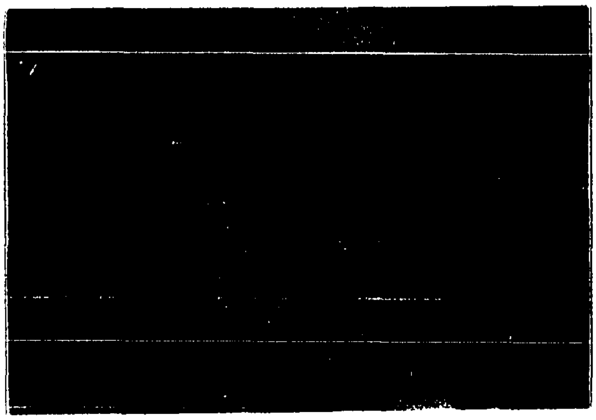

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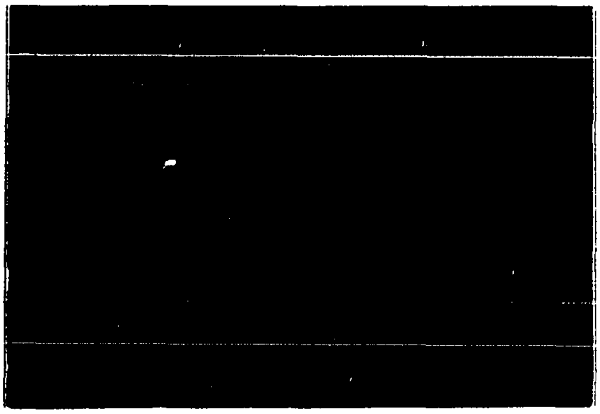

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Figure 51. Cholesterol distribution within the cortex.

A. Zona glomerulosa. From a 9.1-year old male Beagle (B63). Note the positive glomerulosa and fasciculata. The zona intermedia is distinctly void of cholesterol as measured by this test. (The dark ringed structures in the field are artifacts). Magnification 200x.

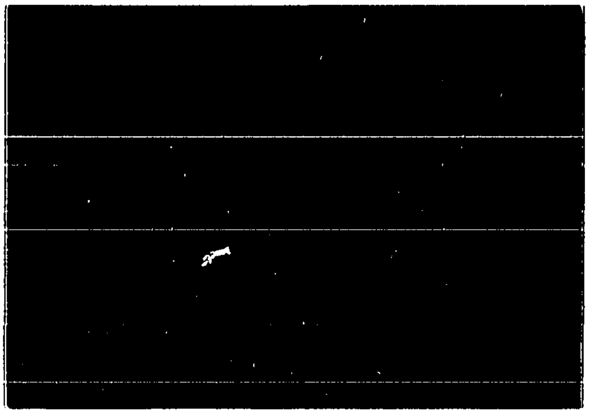
B. Zona intermedia. From a 9.1-year old male Beagle (B63). A portion of the glomerulosa and fasciculata are present as very positive components. The white to yellow foci are cell nuclei. Magnification 800x.

C. Outer zona fasciculata. From a 10.0-year old male Lab. Retriever (M49). This slide shows a very positive test and some of the cytoplasmic distribution can be resolved. Magnification 800x.

D. Inner zona fasciculata. From a 7.5-year old male Basenji (M52). The intracellular desposits of cholesterol positive material, the nuclei and intercellular space can be resolved. Magnification 800x.

E. Zona reticularis. From a 9.3-year old female Irish Setter (M51). The deposits of cholesterol are more selectively distributed, some are more positive than others. Magnification 800x.

F. Nodules. From a 10.0-year old female Beagle (B64). This nodule of cortical tissue demonstrates that the functional activity within the nodule varies considerably. Magnification 80x.



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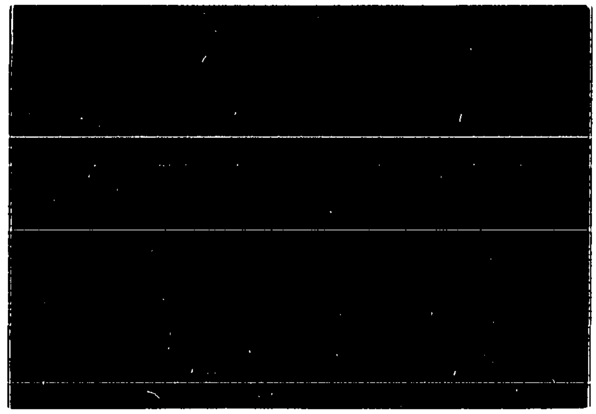

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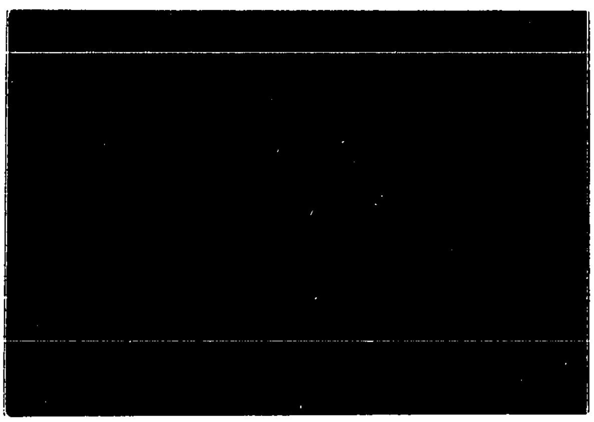

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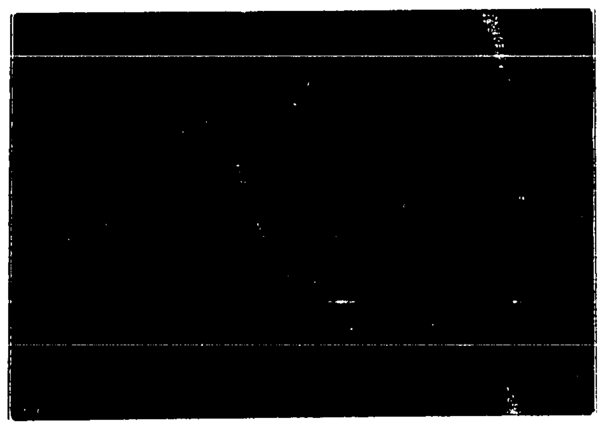

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Figure 52. Nodule formation

A. Zona intermedia. From a 0.7-year old male Beagle (B70). A prominent zona intermedia is present between the zona glomerulosa and outer zona vasciculata. Stained with Mallory's triple. Magnification 500x.

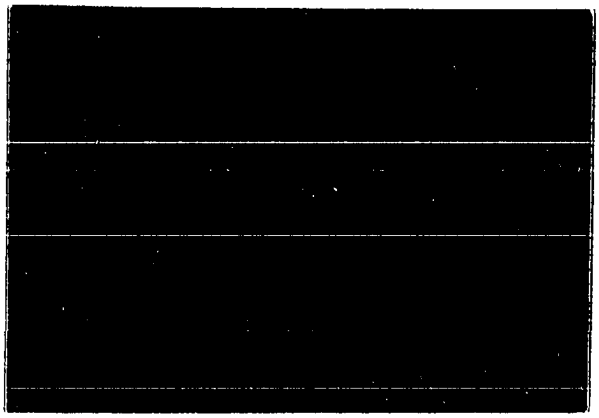
B. Zona glomerulosa. From a 1.7-year old male Beagle (B81). The prominent columns of the zona glomerulosa cells commonly lose their regularity of arrangement in regions in which nodule formation is taking place. Stained with Mallory's triple. Magnification 500x.

C. Zona intermedia in capsule. From a 3.2-year old female Beagle (B75). Regions similar to this appear quite dynamic in that the regularity of cellular and tissue morphology is lost and the cells are arranged at random. The cells constituting this mass resemble very closely those of the zona intermedia. Stained with Mallory's triple. Magnification 500x.

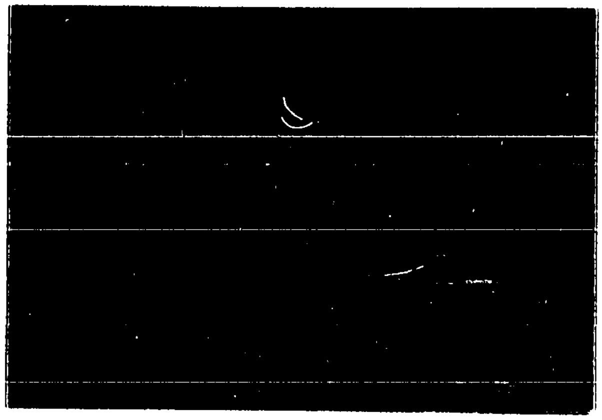
D. Intrcapsular nodule. From a 3.2-year old female Beagle (B75). Often times evaginating glomerulosa cells are seen at points close to capsular and pericapsular arteries. Stained with Mallory's triple. Magnification 500x.

E. Intracapsular nodule. From a 11.9-year old female Beagle (B73). In other instances the cellular arrangement is maintained and a column of glomerulosa cells appears to migrate *in toto* into the fibrous capsule. Stained with H and E. Magnification 500x.

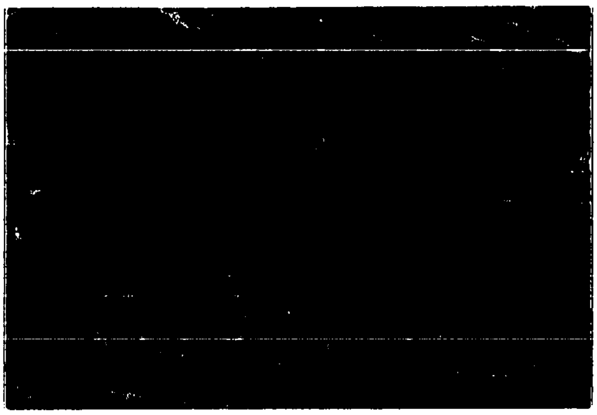
F. Pericapsular nodule. From a 11.9-year old female Beagle (B73). This nodule composed primarily of a glomerulosa cell type is connected to the underlying cortex by an unusually large stock. The zona intermedia can be seen to again play a role in the development of this pericapsular nodule. Stained with resorcin fuchsin. Magnification 200x.



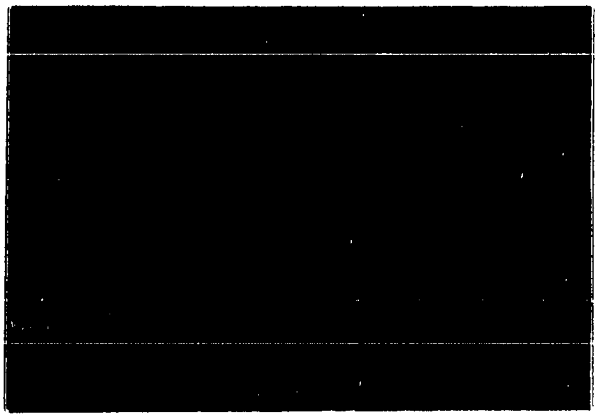
A



B



C



D

E

F

Figure 53. Adrenal capsule. Specimen is from a 7.5-year old male Basenji (M52). Portions of several fibrocytes are evident. These cells are embedded within a matrix of cell product, collagen (C). The narrow dark line points to interdigitation of cells. Typical mitochondria for this cell type are present (M) and an extensive rough surfaced endoplasmic reticulum (rm) fills the cytoplasm. A cytosegrosome (cy) may have been phagocytized after having been given off by the zona glomerulosa parenchyma. D.E.R. embedded, lead citrate stained. Magnification 36,000x.

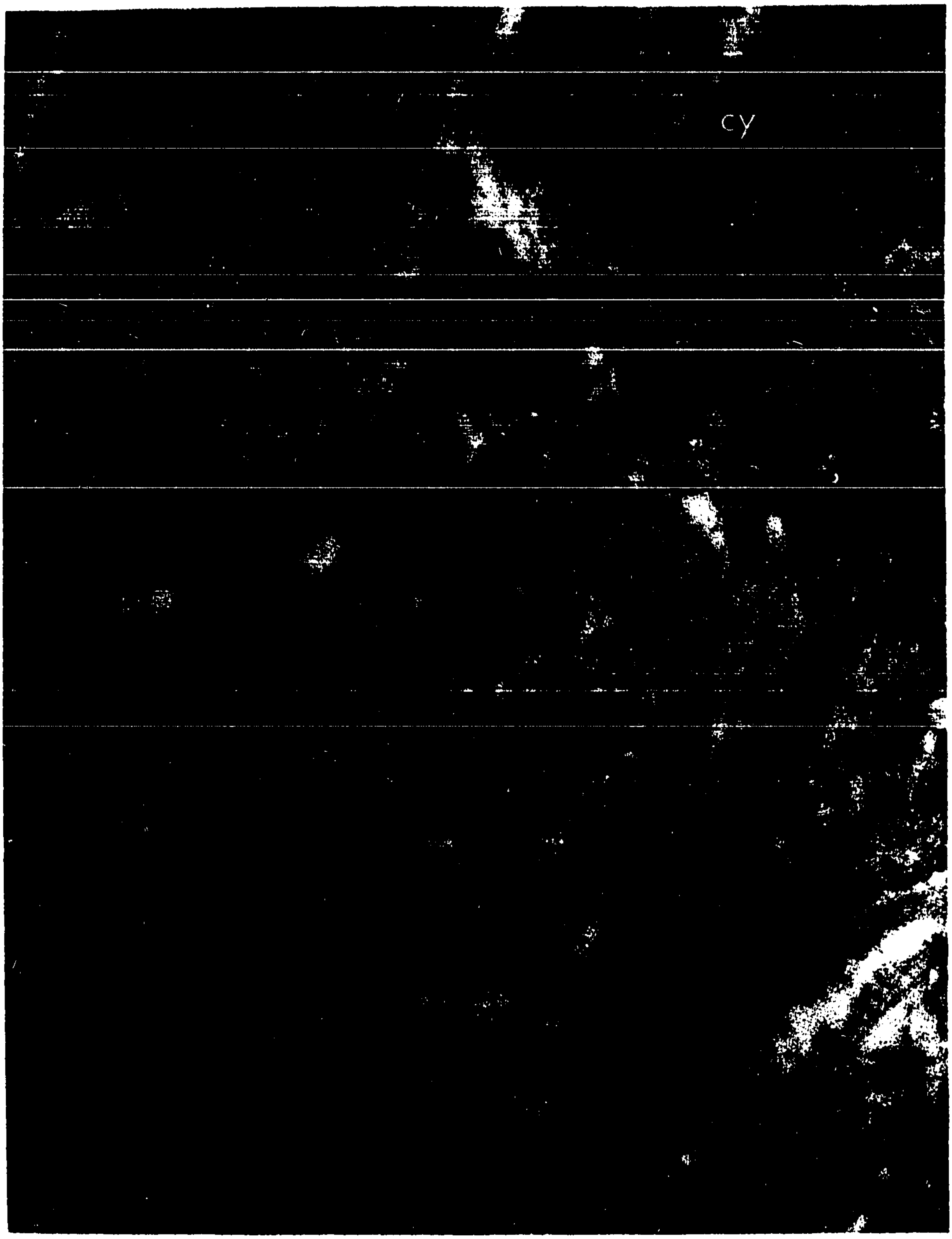

cy

Figure 54. Zona glomerulosa. Specimen is from a 0.3-year old male Beagle (E07). These elongated columnar cells possess nuclei (N) of similar morphology. The narrow intercellular space is smooth and without desmosomes (large black pointers). The small black arrows are points of plasmalemma invagination. The large lipid vacuoles (LV) are at the ends of the cells and the mitochondria are elongated and filamentous in shape with lamellar cristal. D.E.R. embedded, uranyl acetate and lead citrate stained. Magnification 20,000x.


LV

Figure 55. Outer zona fasciculta cells. Specimen is from a 1.7 year old male Beagle (B81). Portions of 4 parenchymal cells can be seen. Their cytoplasm is characterized by a large number of lipid droplets (L) which contain areas of varying electron density. These darker regions may represent less saturated lipids which bind more osmium. The lipid vacuoles (LV) show a margin of electron density at the interphase with the surrounding cytoplasm. This is the reminant of the lipid droplet after extraction. On close inspection portions of smooth endoplasmic reticulum can be seen eveloping some of the lipid droplets. The mitochondria (M) are quite large and variable in size and often are seen in close relation to the lipid droplets (large black arrow).

A sinusoid to the left of the micrograph contains a white blood cell with an irregularily shaped nucleus (N) and a well developed Golgi complex (G). The perisinusoidal space (P) is pervaced by many microvilli from the parenchymal cells and contains a bundle of collagenous fibers (C). D.E.R. embedded, lead citrate stained. Magnification 15,000x.

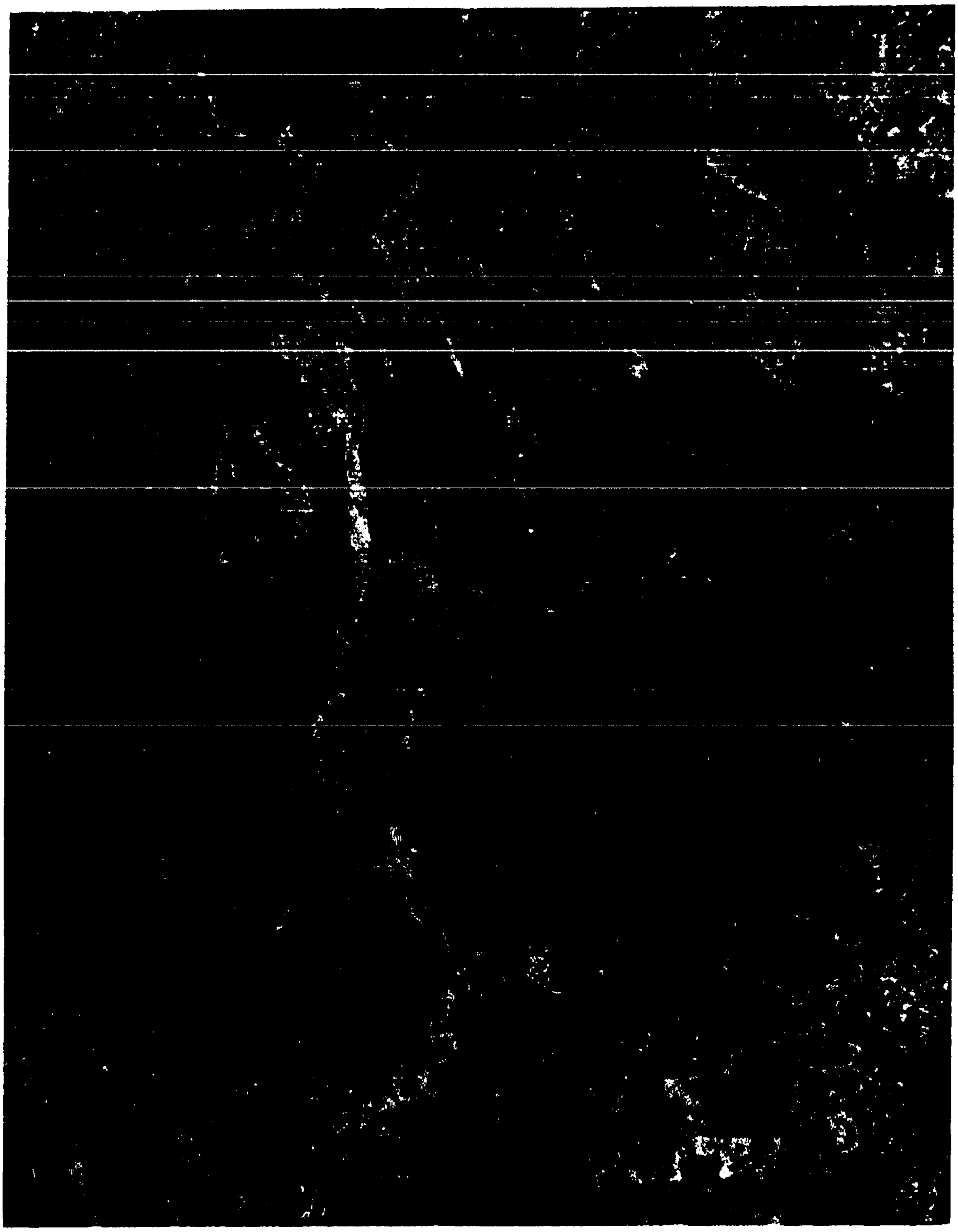


Figure 56. Inner zona fasciculata. Specimen is from a 0.3-year old male Beagle (E07). These parenchymal are often separated by a sparse network of collagenous fibers (C). This cell possesses a variety of mitochondrial shapes (M), large lipid vacuoles (LV), a Golgi complex (G) and a nucleus with considerable heterochromatin (HC). D.E.R. embedded, uranyl acetate and lead citrate stained. Magnification 26,000X.

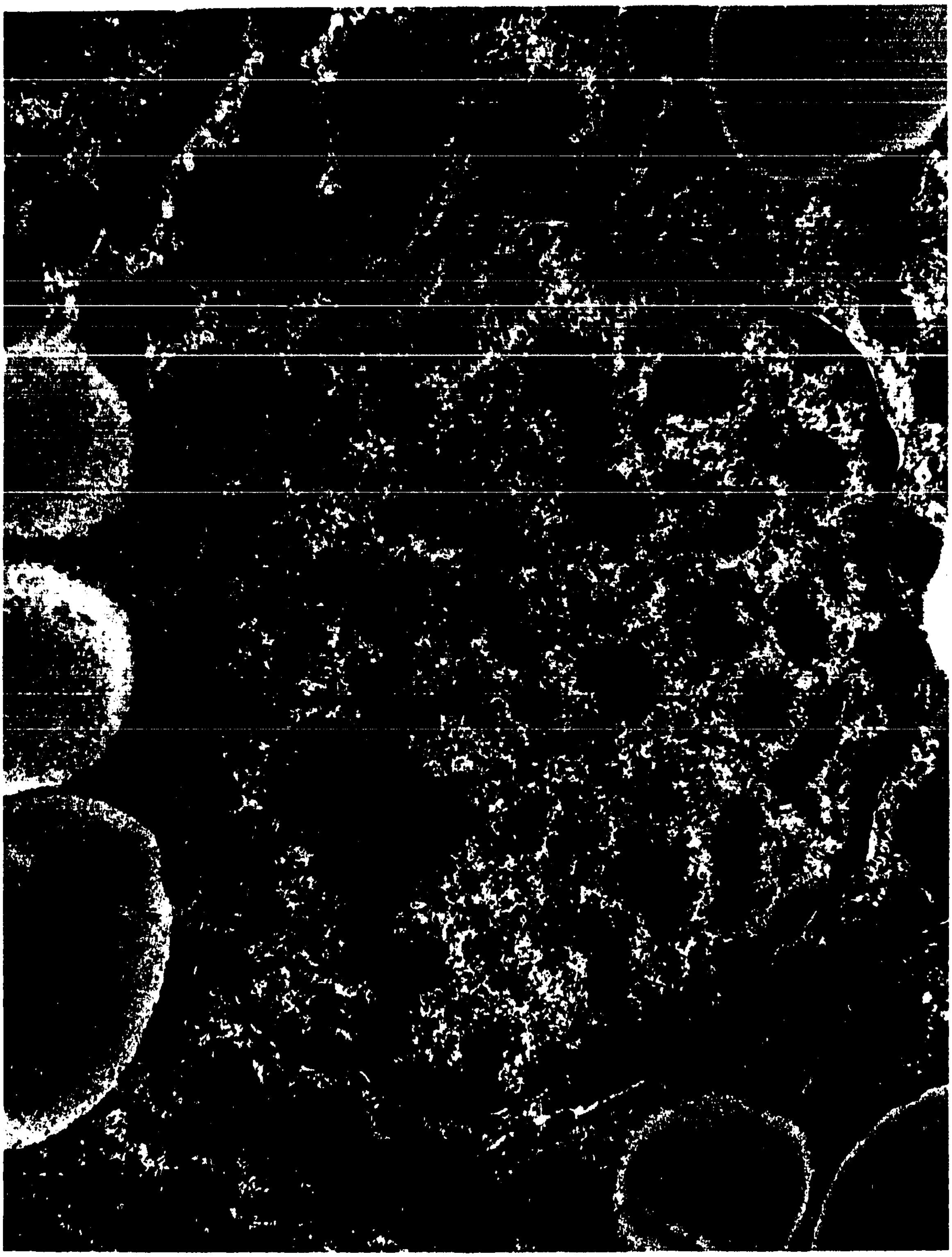


Figure 57. Inner zona fasciculata. Specimen is from a 0.3-year old male Beagle (E07). A light and a dark cell of this region are evident. The light cell shows a prominent Golgi complex (G) and both show similar mitochondria. The two cells are separated by collagenous fibers. D.E.R. embedded, uranyl acetate and lead citrate stained. Magnification 26,000x.

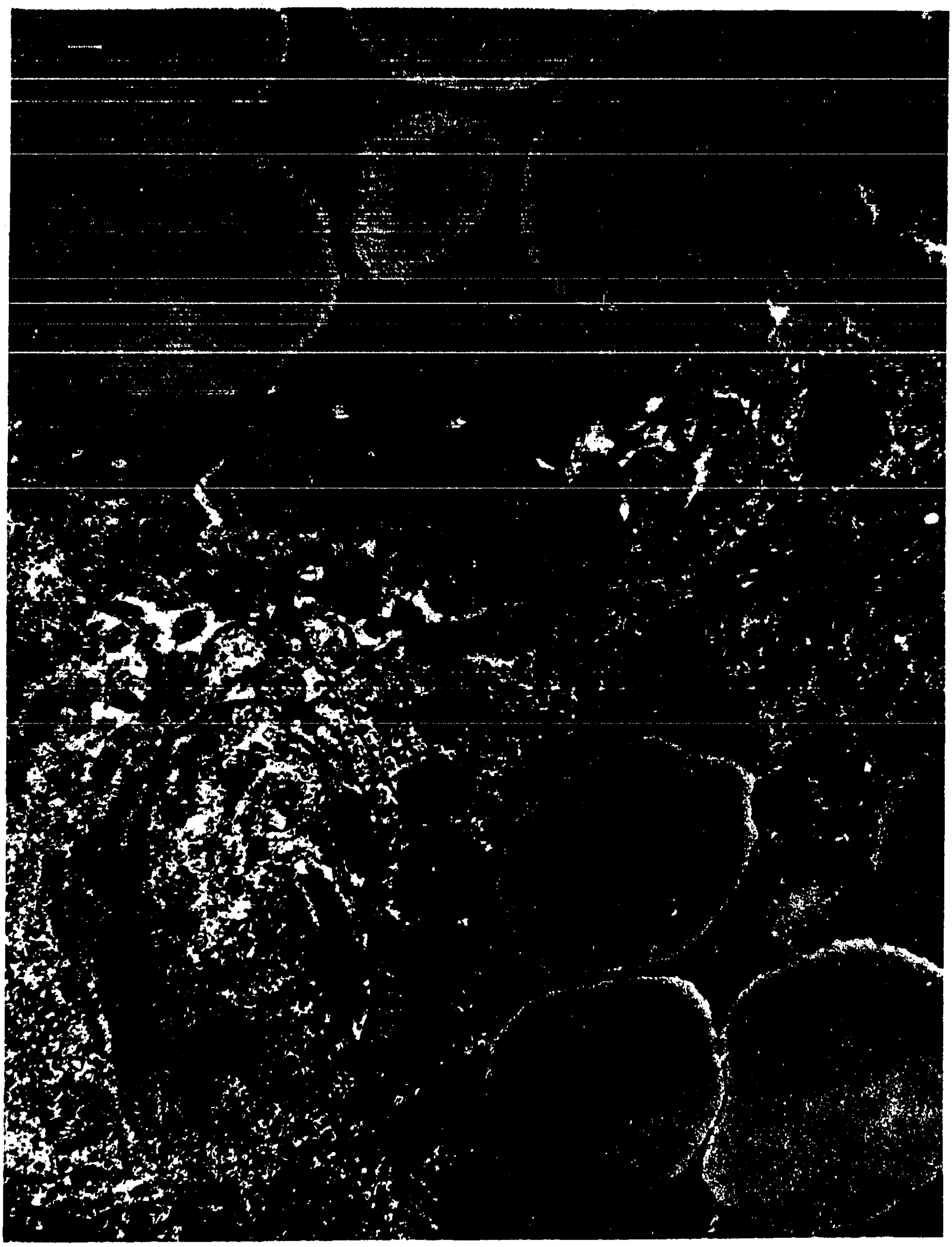


Figure 58. Inner zona fasciculata. Specimen is from a 1.7-year old male Beagle (B81). Seen here are portions of a parenchymal cell (left) and a perisinusoidal cell (right), the intercellular space separating them being indicated by the black pointers. By the mitochondrion morphology (white m) this cell can be classified as a non-steroid metabolizing cell. These perisinusoidal cells have phagocytic properties which is evidenced by the several membrane-limited vacuoles containing lipid droplets, dense bodies and granules. These are termed cytosegrosomes (CY).

The large dense body at the upper left has granules associated with it as well as smooth endoplasmic membranes (large dark arrow). At the lower left a large dark arrow indicates a cyclomembranous mitochondrion. The aggregates of free ribosomes (r) are interspersed between the extensive network of smooth surfaced endoplasmic reticulum (sm). D.E.R. embedded, lead citrate stained. Magnification 42,000X.

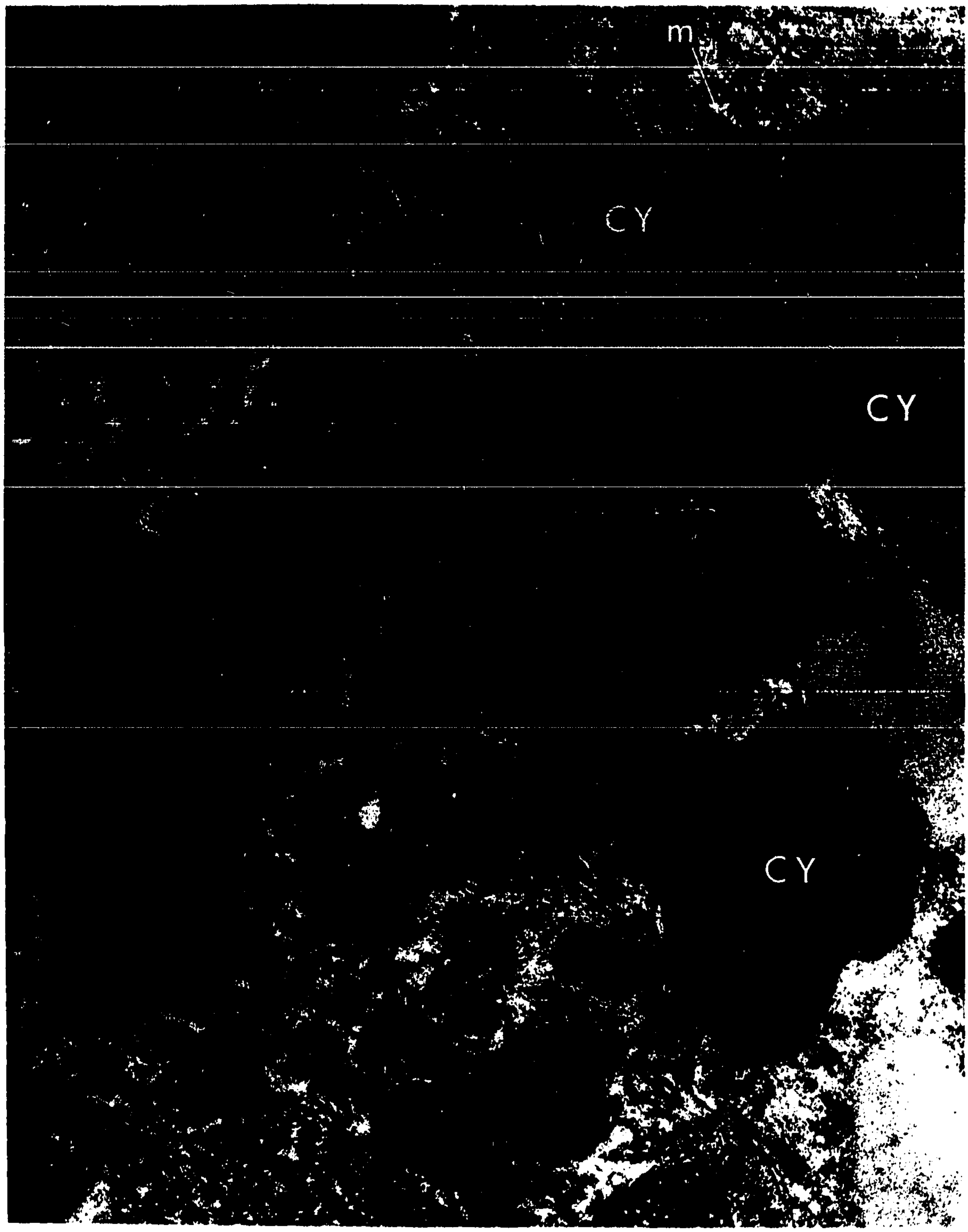

m
CY
CY
CY

Figure 59. Inner zona fasciculata. Specimen is from a 7.5-year old male Basengi (M52). This perisinusoidal phagocytic cell contains pigment granules (Pg), cytoplasmic dense bodies (cd), free ribosomes (r) and its typical mitochondria (M, upper right). The adjoining parenchymal cells contain lipid vacuoles (L) and large bizarre mitochondria (M, upper left). Collagen (C) fills the remaining intercellular space. D.E.R. embedded, uranyl acetate and lead citrate. Magnification 26,000x.

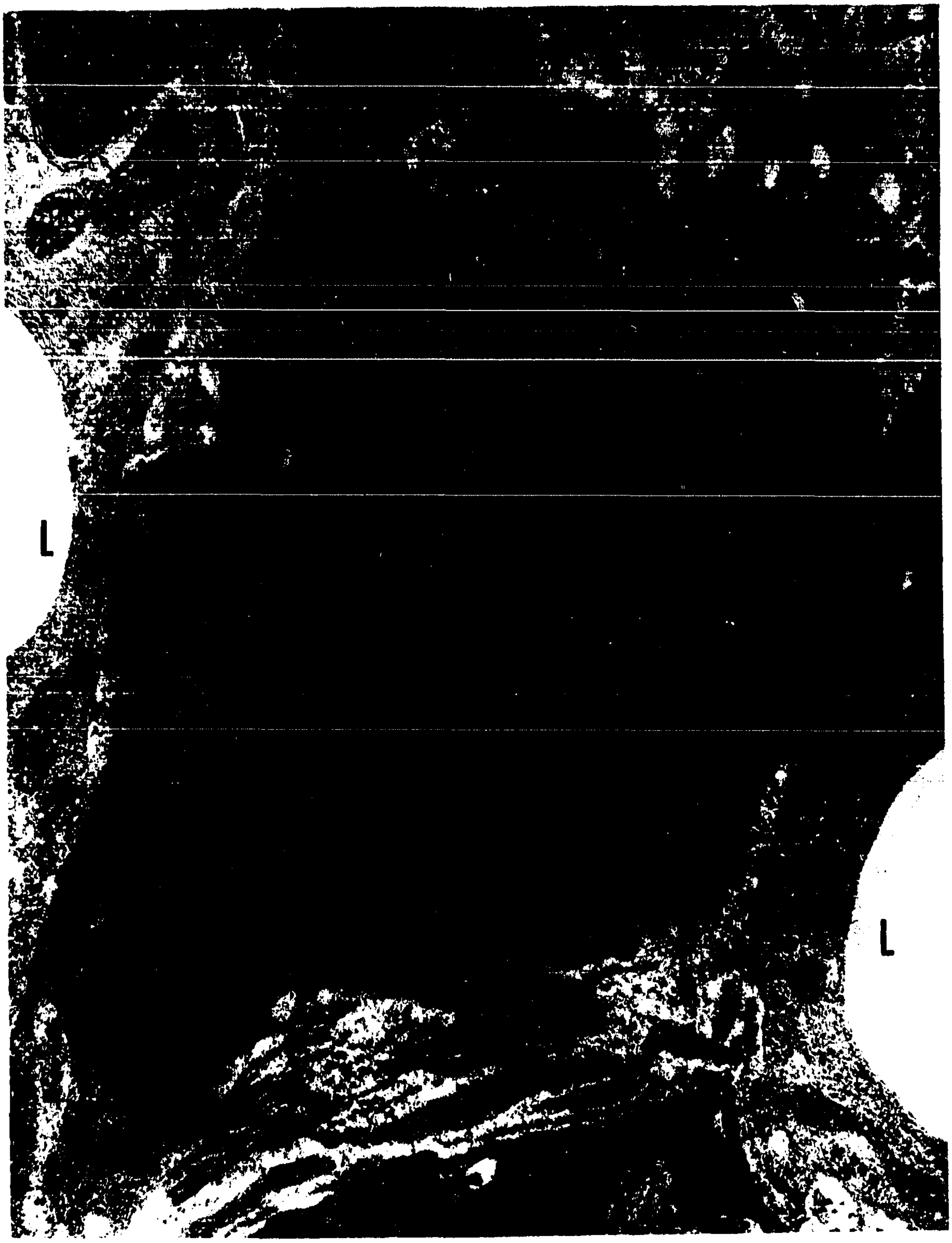

L
L

Figure 60. Inner zona fasciculata. Specimen is from a 7.5-year old male Basengi (M52). A perisinusoidal cell extends between the parenchymal cells (black pointers). The mitochondria are quite variable in morphology (M) and the lipid vacuoles (LV) are fewer in number. Nucleus of perisinusoidal cell (N). D.E.R. embedded, lead citrate stained. Magnification 26,000x.

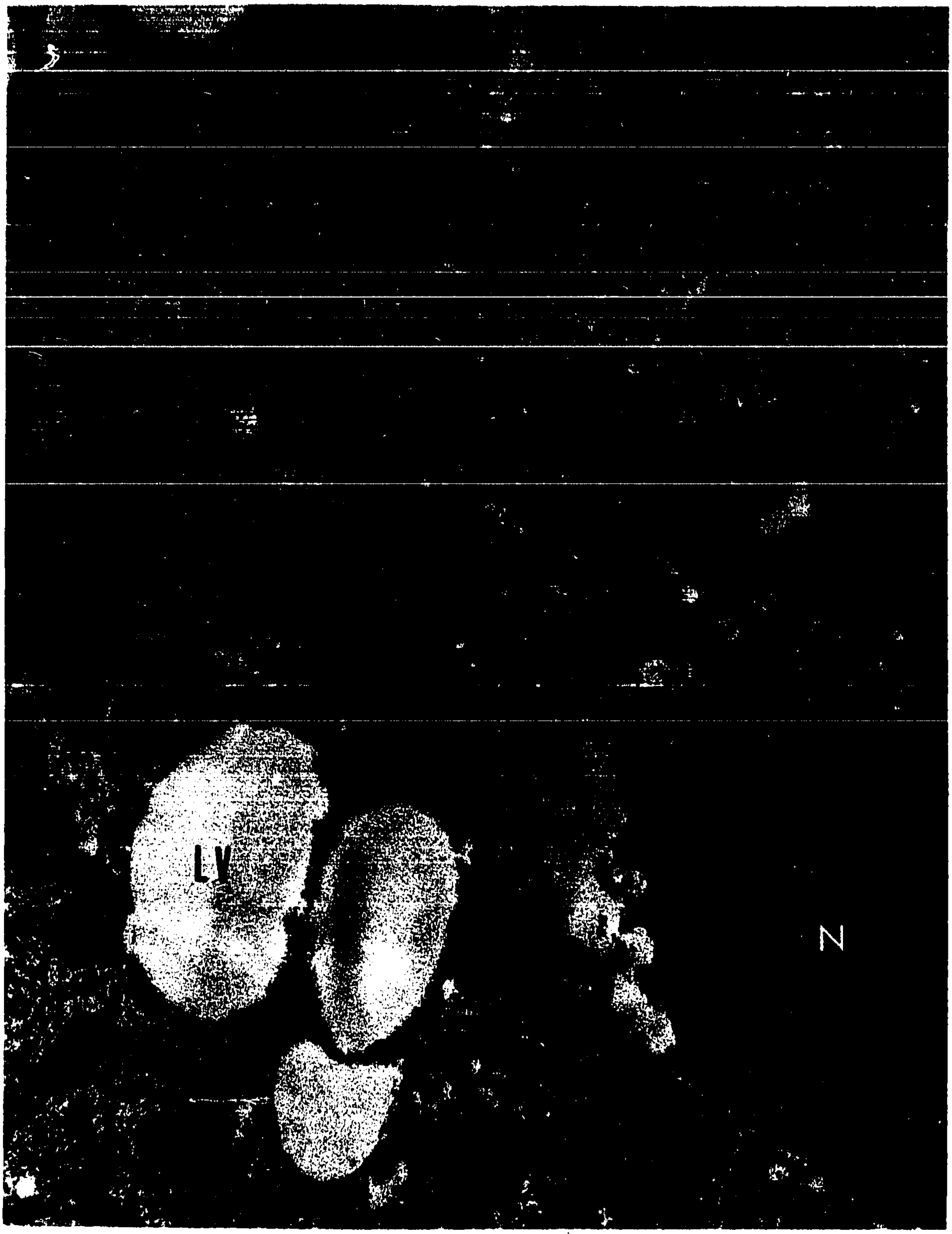

LV
L
N

Figure 61. Zona reticularis cells. Specimen is from a 0.3-year old male Beagle (E07). Portions of two cells separated by an intercellular space (L) are present. The plasmalemma of two cells (at black pointers) suggest a zonula adherens but no desmosomes are evident. A lipid vacuole (LV) and well-formed spherical mitochondria showing the alevoli, tubular and tubulo-alveolar profiles of cristae mitochondriales are demonstrated. The nucleus (N) contains nucleolus (nu) and the nuclear envelope showed nuclear pores (small black arrows). The smooth endoplasmic reticulum is very extensive (sm). D.E.R. embedded, uranyl acetate and lead citrate stained. Magnification 26,000x.

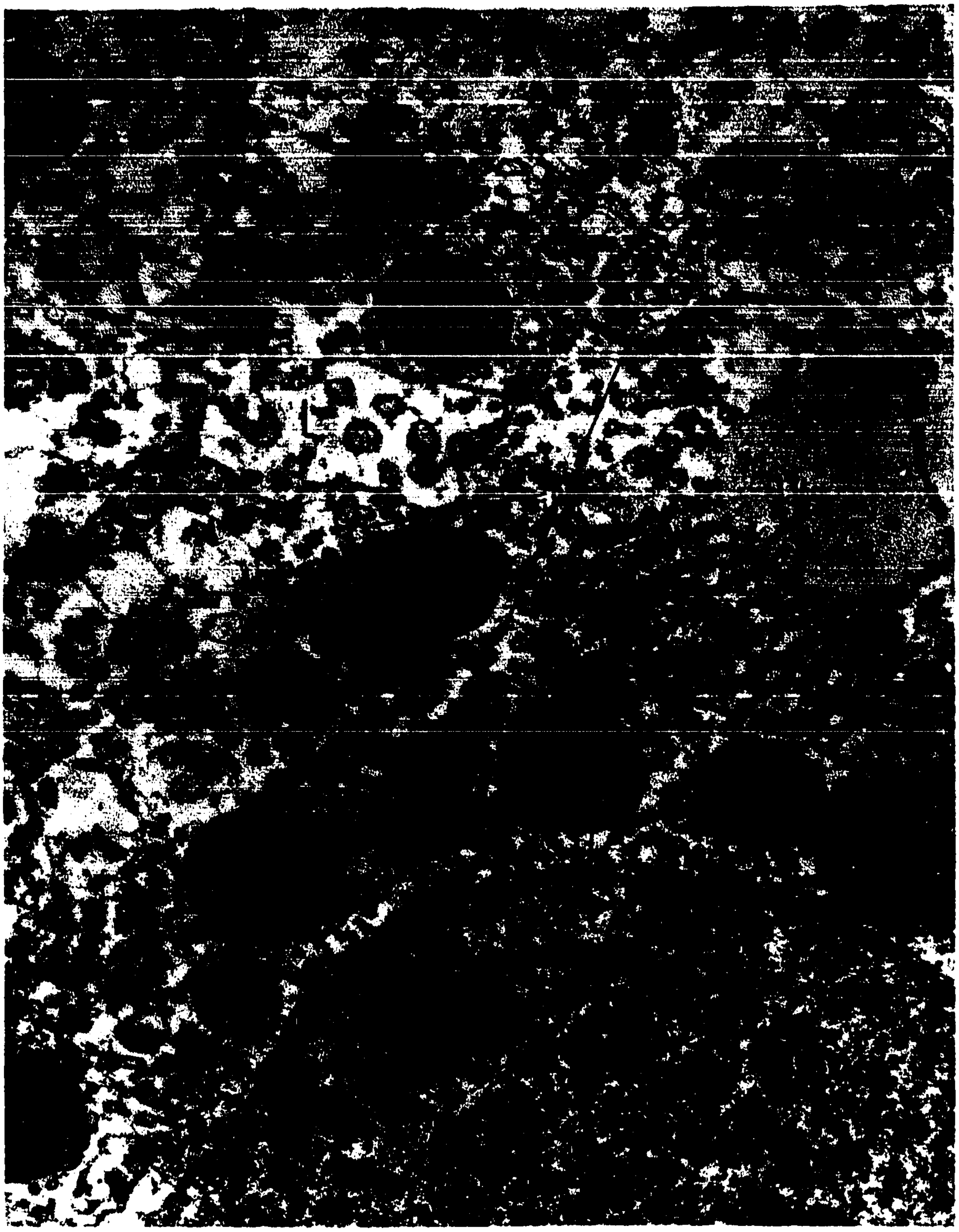


Figure 62. Zona reticularis cells. Specimen is from a 0.3-year old male Beagle (E07). Portions of three parenchymal cells and a perisinusoidal cell are evident. The perisinusoidal cell in the intercellular space demonstrates typical mitochondria (m), ribosomes (r), rough surfaced endoplasmic reticulum (small dark arrow) and a nucleus (N). The cytoplasm is attenuated in an extension between the cells and among the collagenous fibers (C).

The parenchymal cells are characterized by the large mitochondria (M), lipid vacuoles (LV) and numerous microvilli which project into the perisinusoidal space and due to sectional geometry produce large tubular profiles at the periphery of the cell cytoplasm (small black arrows). D.E.R. embedded, uranyl acetate and lead citrate stained. Magnification 32,000x.


E
S
cy
cd
cy

Figure 63. Zona reticularis cells. Specimen is from a 7.5-year old male Basengi (M52). The parenchymal cells are closely applied to the sinus (S) in this micrograph. The endothelial cell (E) is bounded by collagen (C) and a perisinusoidal cell process (P). A basal laminae has been formed juxta to the parenchymal cells. A nucleus (N), lipid vacuoles (LV), Golgi complex (G), lipid droplets (L), cytoplasmic dense bodies (cd), mitochondria (M), cytosegrosomes (cy) and smooth endoplasmic reticulum (sm) are characteristic of these cells. D.E.R. embedded, lead citrate stained. Magnification 26,000x.

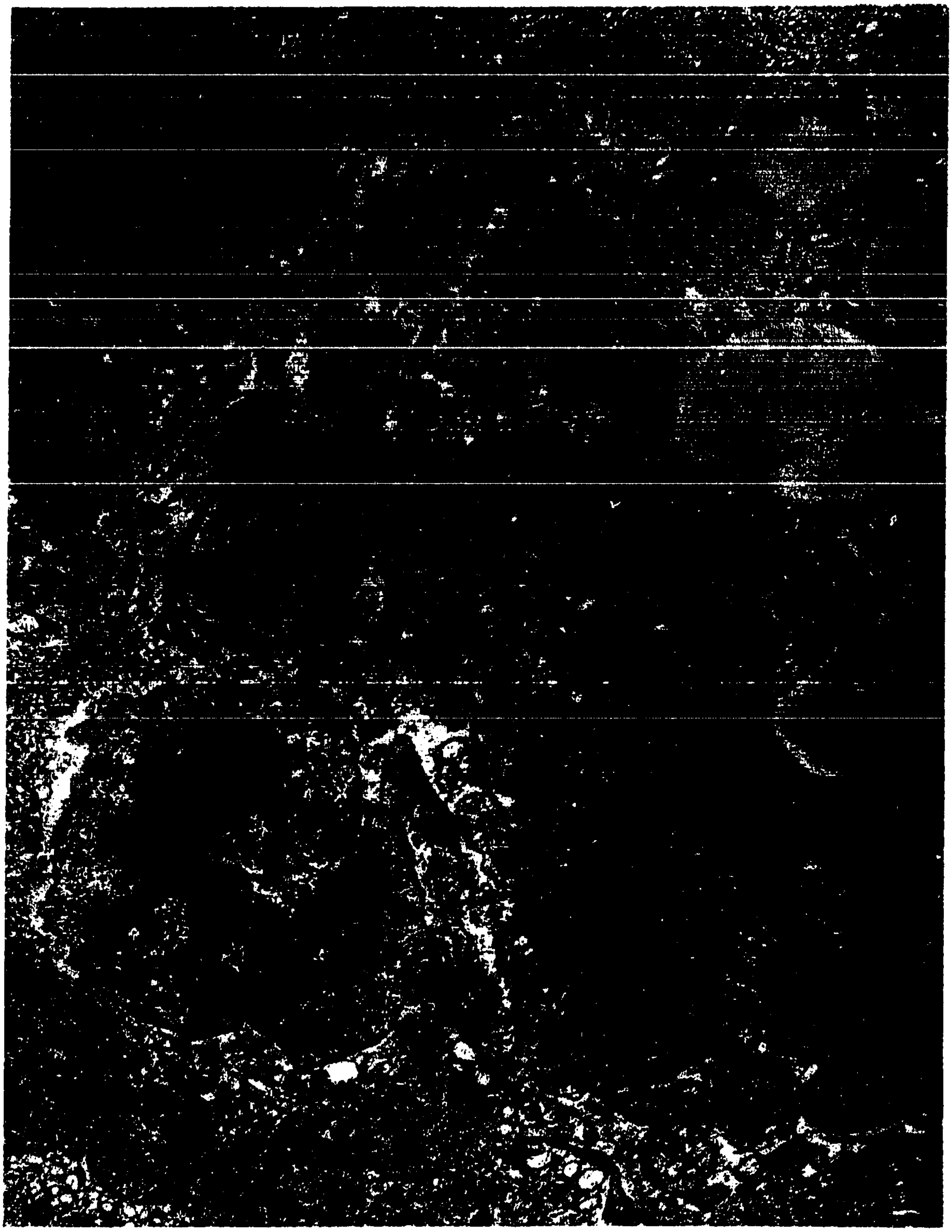


Figure 64. Zona reticularis cells. Specimen is from a 7.5-year old male Basengi (M52). Portions of a light and dark cell are present. The light cell demonstrates well the myriad of mitochondrial morphologies (M), extensive smooth surfaced endoplasmic reticulum (sm), free ribosomes (r), lysosomes (Ly) and lipid droplets (L). D.E.R. embedded; uranyl acetate and lead citrate stained. Magnification 30,000x.

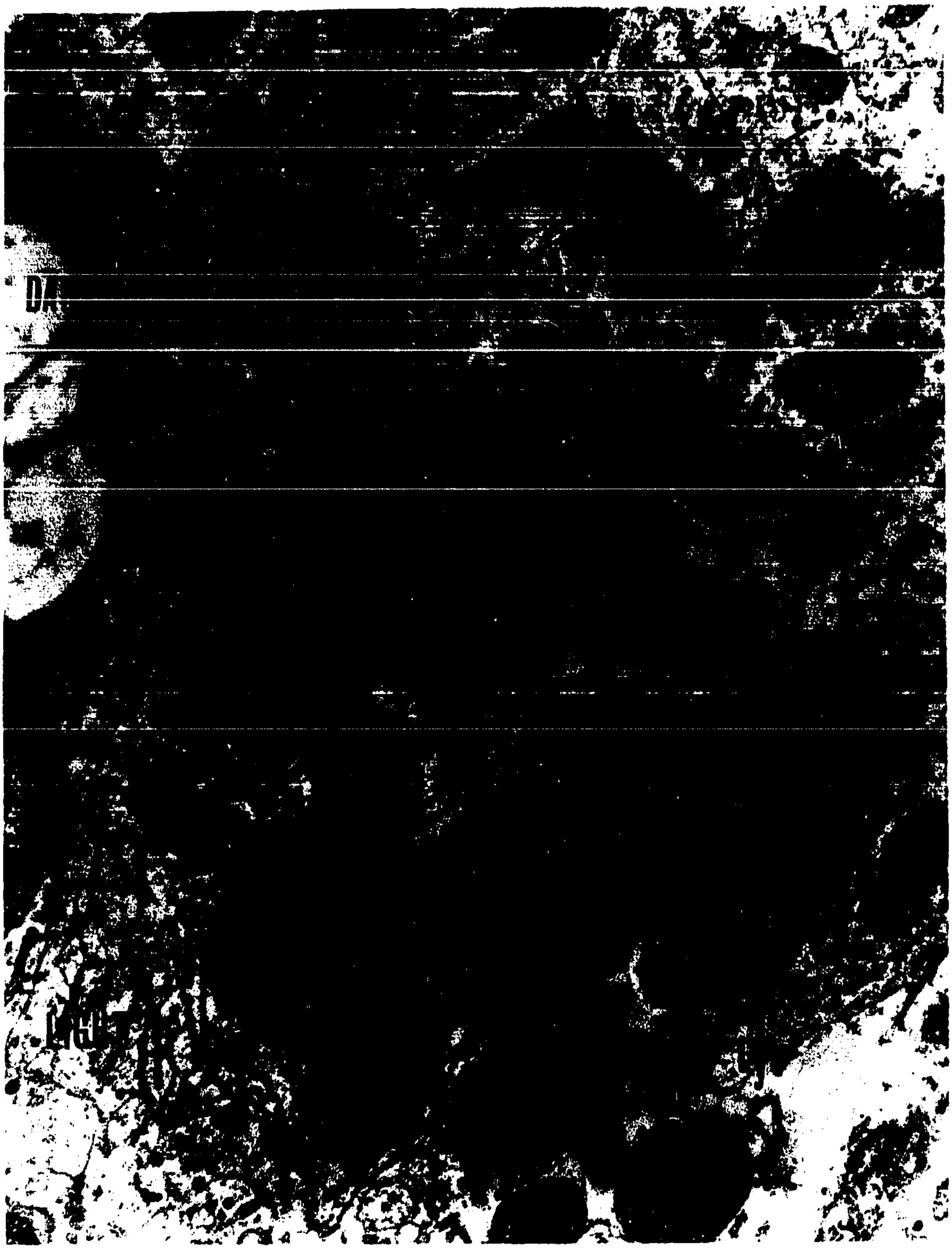


Figure 65. Zona reticularis cells. Specimen is from a 7.5-year old male Basengi (M52). Portions of three cells can be seen, two light cells and one dark (so labeled). A nucleus (N), Golgi complex (G), lipid vacuoles (L) and ribosomes (r) are present in both cell types. The extensive smooth endoplasmic reticulum (sm) and mitochondria (M) appear to differ only in their special arrangement. D.E.R. embedded, uranyl acetate and lead citrate staining. Magnification 26,000x.

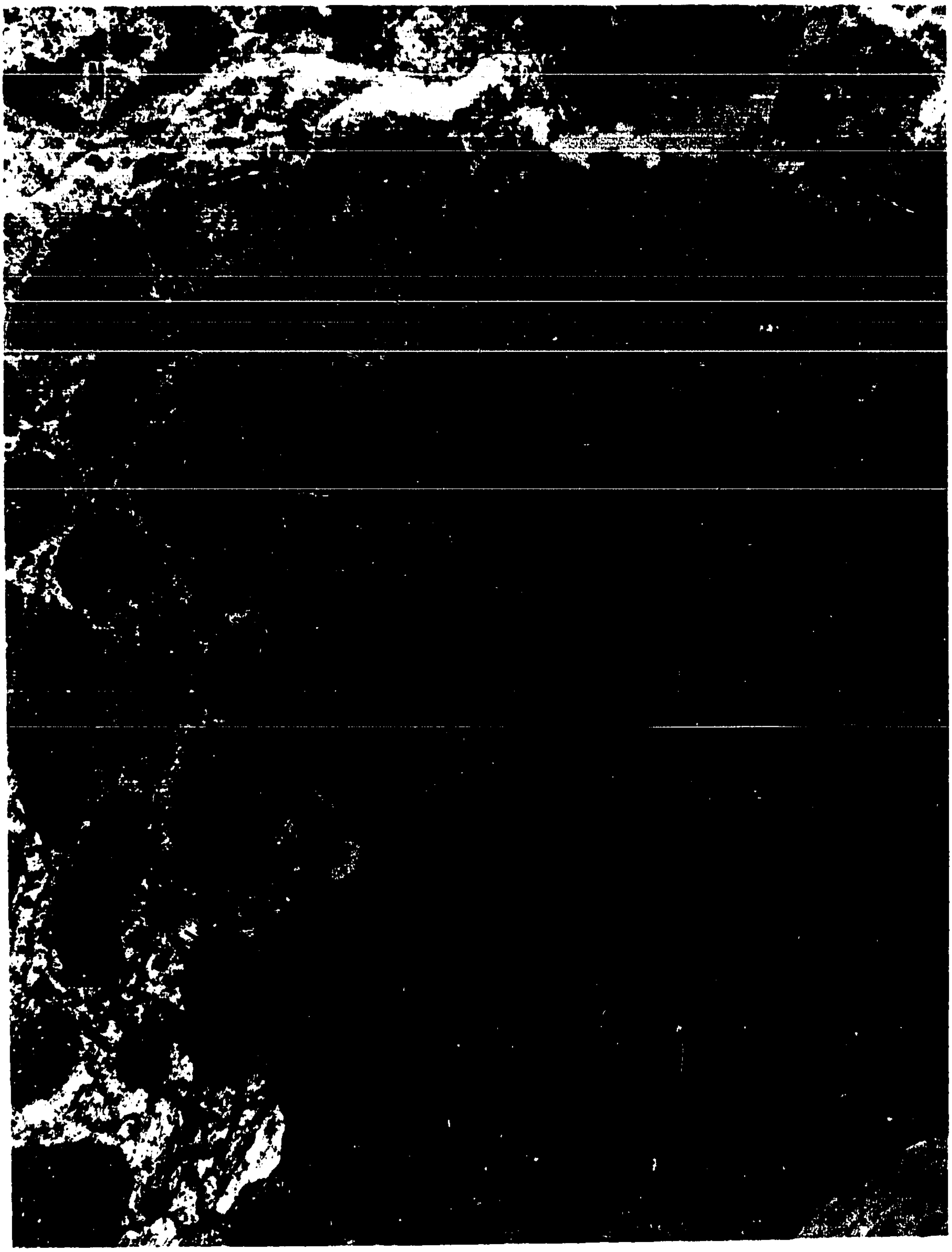


Figure 66. Adrenal medulla. Specimen is from a 7.5-year old male Basengi (M52). This large medullary cell contains numerous secretory granules (GR), elongated mitochondria (M), some rough surfaced endoplasmic reticulum (rm) and a nucleus (N). A Basal laminae is interspersed between the cell and the collagen in the intercellular space. D.E.R. embedded, uranyl acetate and lead citrate stained. Magnification 26,000x.

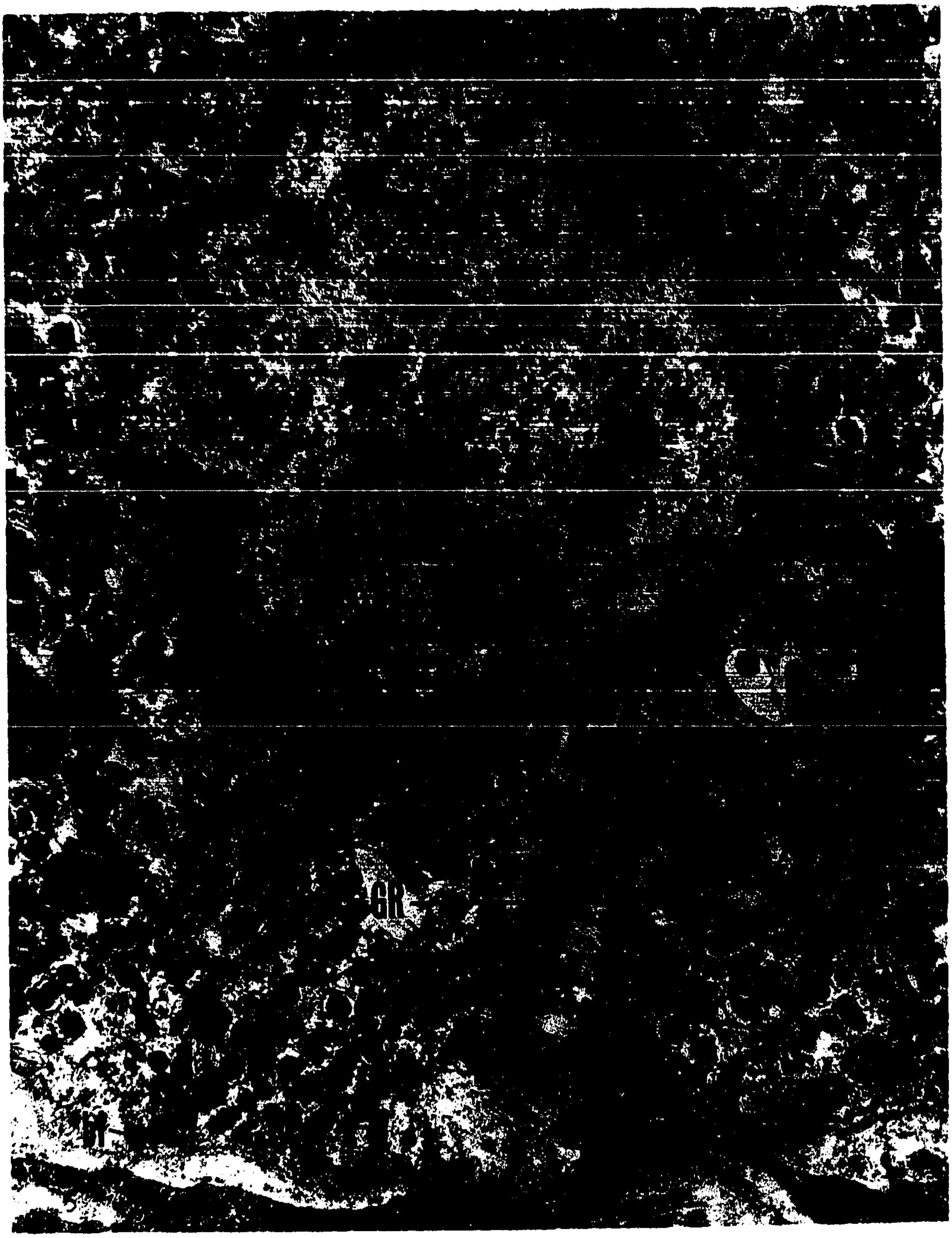
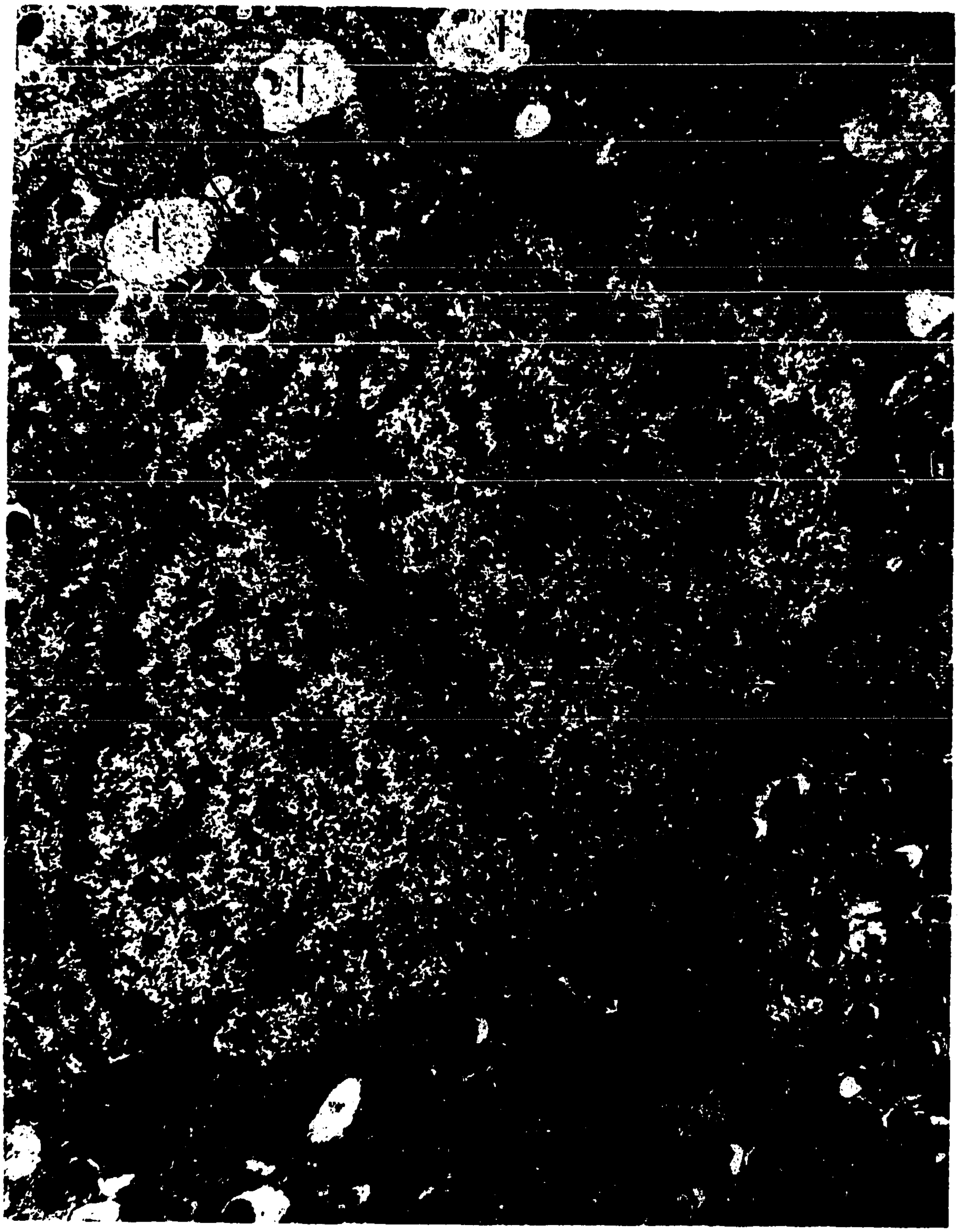

GR

Figure 67. Adrenal medulla. Specimen is from a 7.5-year old male Basengi (M52). This medullary cell presents an interesting nuclear morphology which is due to an invagination of the nuclear membrane (large black arrow). The cells contain mitochondria (M), secretory granules (GR), and rough surfaced endoplasmic reticulum (rm). Between the cells are interesting dilated spaces (1) which may serve to facilitate secretion. D.E.R. embedded, uranyl acetate and lead citrate stained. Magnification 26,000x.



AFTERWORD

Believing that contemporary man is acting on behalf of all that has gone before him and for all that is to come; that man is laying the corner-stones in the past and the foundations for the future, this author presents these creative expressions of three true brick layers.

The past _____

> "The living cell
> which has perpetuated itself through
> countless generations amid the great
> vicissitudes of the physical world,
> which has created flourishing communities
> of infinite variety, and which, through its
> highest composite, Intelligent Man, may still
> find its greatest adventures in the future."
> _____ W. D. Andrew, 1952. Cellular Changes with Age

The present _____

> "It was the best of times, it was the worst
> of times, it was the age of wisdom, it was
> the age of foolishness, it was the epoch of
> belief, it was the epoch of incredulity, it was
> the season of Light, it was the season of Darkness,
> it was the spring of hope, it was the winter of
> despair, we had everything before us, we had
> nothing before us, we were all going direct
> to Heaven, we were all going direct the other
> way _____"
> _____ Charles Dickens, 1859. A Tale of Two Cities

It is the year of our Lord one thousand nine hundred and sixty-eight.

The future _____

> "Far from being the pious injunction of a Utopian
> dreamer, the command to love one's enemy is an
> absolute necessity for our survival. Love even for
> enemies is the key to the solution of the problems
> of our world."
> _____ Martin Luther King, Jr. 1963. Strength to Love

So be it.