A STUDY OF GRANULE FORMATION IN THE BAT PARAFOLLICULAR CELL

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SUMMARY
The fine structure of the bat thyroid parafollicular cell has been examined at monthly intervals throughout the hibernating period. During November and December parafollicular cells appear either partly or totally degranulated and intact dense secretory granules are relatively sparse. The degranulated cells exhibit an inconspicuous Golgi complex and relatively few lysosome-type bodies. Few degranulated parafollicular cells are present in thyroid glands from bats collected in January. When found they are characterized by the presence of whorls of cytoplasmic agranular membranes which enclose a central mass of cellular debris. January bat thyroids are characterized by the presence of three different types of parafollicular cell. One type contains no secretory granules. The cytoplasmic matrix of this type is rich in granular endoplasmic reticulum and free ribosomes and its small Golgi complex consists of several slightly dilated saccules. In close proximity to the Golgi complex are numerous small to medium-sized vesicles which often appear to merge with Golgi elements. Such vesicles are considered to represent the vehicle by which secretory product is transferred from the endoplasmic reticulum to the Golgi complex. The second type of parafollicular cell differs from the first in containing large numbers of intact dense secretory granules. It is also characterized by an extensive Golgi complex which appears to be forming new secretory granules, and by a less extensive granular endoplasmic reticulum. The third type of parafollicular cell shows a structure intermediate between the first two. The cytoplasm of all three types of January parafollicular cells contains many structures belonging to the lysosomal-vacuolar system, including autophagic vacuoles, vacuolated dense bodies and multivesicular bodies. By February and March only parafollicular cells of type 2 are observed. They contain few lysosome-like structures. It is concluded that during mid-hibernation (January), parafollicular cells undergo a series of intracellular changes during which new dense secretory granules are produced. Accompanying granule formation is an augmentation of lysosome-like structures which probably serve as a means of digesting debris from previous secretory cycles.

INTRODUCTION
The functional histological unit of the thyroid gland, the thyroid follicle, is made up of three principal components: the lining follicular cells, the luminal colloid, and the basal parafollicular cells. Follicular cells and the luminal colloid are involved in the formation, storage and release of the iodinated thyroid hormones, thyroxine and
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triiodothyronine (De Groot, 1965). The relatively sparse parafollicular cells, which comprise approximately 2% of the cell population of the thyroid gland of the normal rat (Stux, Thompson, Isler & Leblond, 1961), have until recently been considered by many to be either non-secretory (Saito, 1956) or a stage in the life-cycle of the follicular cell (Nonidez, 1932; Ehrenbrand, 1954; Gabe, 1959; Voitekevich, 1963). With the recent demonstration that calcitonin is of thyroid origin (Foster, Baghdiantz, Kumar, Slack, Soliman & MacIntyre, 1964; Dale, Roth & Garcia, 1965; Hirsch, Gauthier & Munson, 1963; Hirsch, Voelkel & Munson, 1964), several groups of investigators have been studying the possibility that the parafollicular cell is the site of calcitonin production (Foster, MacIntyre & Pearse, 1964; Bauer & Teitelbaum, 1966; Hargis, Williams, Tenenhouse & Arnaud, 1966). Although some of the findings have been contradictory (Hargis et al., 1966), the histochemical work by Pearse and his associates (Pearse, 1966; Bussolati & Pearse, 1967; Carvalheira & Pearse, 1967) indicates that the parafollicular cells may indeed be the source of calcitonin. Published micrographs of parafollicular cells, such as those of the hog (Pearse, 1966), rat (Wissig, 1962; Young & LeBlond, 1963) and dog (Tashiro, 1964) indicate that the main cytoplasmic constituent of parafollicular cells is their many small pale spherical vesicles, although in occasional cells variable numbers of small dense granules are present. Pearse has postulated that the pale vesicles may be the intracellular storage site of calcitonin (Pearse, 1966). On the other hand Matsuzawa & Kurosami (1967) and Capen & Young (1967) suggest that the small dense granules may be the hormone storage sites.

Another possibility, based on the fluorescence and autoradiographic studies of Gershon & Ross (1966), Larson, Owman & Sundler (1966), Ritzén, Hammarström & Ullberg (1965) and Falck et al. (1964), is that the parafollicular cells may be involved in the synthesis and storage of catechol- and/or indolamines which are found in relatively high concentrations in some mammalian thyroid glands (Erspamer, 1966). In this connexion, Larson et al. (1966) have speculated that the amines are stored in the dense granules of these cells.

We have previously studied the cycle of intracellular changes which occur in the parafollicular cell of the thyroid gland of the bat (Myotis and Nyctula) (Nunez et al. 1967). We found that the glands in active bats have large numbers of granule-containing parafollicular cells which undergo alterations related to seasonal changes in the physiological states of the animals. During spring and early summer the cytoplasmic matrix of the bat parafollicular cell is filled with dense secretory granules (Nunez et al. 1967), and similar granular parafollicular cells have also been observed in other species of bat (Azzali, 1967). Some of the parafollicular cells of bats caught in late summer contain large dense ‘secretory’ granules. Studies on late summer bat parafollicular cells suggest that the secretory material making up the small dense granules is packaged in the Golgi apparatus, while the larger granules, which are found enclosed within a ribosome-studded membrane, may grow by direct accretion of newly synthesized materials at their surface (Nunez, Gould & Holt, 1968). But parafollicular cells show further changes during early hibernation (late November). The large dense granules are now absent, while cells containing small granules are either partly of totally degranulated (Nunez et al. 1967).
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As electron-microscope studies have failed so far to reveal in parafollicular cells any cycle of events which indicates that they are typical secretory cells, we have now examined the fine structure of the bat thyroid parafollicular cell at monthly intervals throughout the entire hibernating period (November to April) in order to elucidate the mode of formation of the small dense cytoplasmic granules.

MATERIALS AND METHODS

Groups of 3-6 hibernating bats of both sexes of the species Eptesicus serotinus were collected in Hungary in their natural habitat late in November, December, January, February and late March. Animals were anaesthetized with chloral hydrate, the thyroid glands were removed in toto and immersed for 6 h in Karnovsky's cacodylate-buffered formalin/glutaraldehyde mixture (Karnovsky, 1965) or for 2 h in 6.25% glutaraldehyde in 0.067 M cacodylate buffer, pH 7.3. After fixation, the tissues were washed in ice-cold 0.25 M sucrose/0.067 M cacodylate buffer (2-4 h) and then post-fixed in 0.067 M cacodylate-buffered 1% osmium tetroxide (pH 7.3) for 1 h. Some thyroids were fixed in 1% osmium tetroxide in 0.067 M phosphate buffer, pH 7.3, for 1-2 h. Subsequently, all tissues were dehydrated through a graded series of alcohols and embedded in either Epon or Araldite. Thin sections were stained with a 4% aqueous uranyl acetate solution (Watson, 1958) and/or lead citrate (Reynolds, 1963). The sections were examined in a Philips EM 200 or an AEI EM 6 electron microscope. One-micron sections for light microscopy were stained with 1% toluidine blue and were used for orientation purposes.

RESULTS

Sections of thyroids from animals collected in either November or December (Fig. 1 and inset) show that parafollicular cells are either partly or totally degranulated, with the degree of degranulation varying considerably from cell to cell. Since the fine structure of degranulated bat parafollicular cells has already been described (Nunez et al. 1967), only a brief summary of the findings is given here. Many of the cells show a marked depletion of secretory material and are typical of all the bat thyroid glands examined during early hibernation (Fig. 1). Their cytoplasmic matrix is pale and contains relatively few intact granules, which vary greatly in electron density. In extensively degranulated cells, the cytoplasmic matrix contains many vesicles which are bounded by a smooth membrane, often broken in places, and which contain only a fine granular material (Fig. 1, inset). Short, rod-like profiles of the endoplasmic reticulum are occasionally distributed throughout the ground substance (Fig. 1). Round to elongate mitochondria with a moderately dense matrix are present and the Golgi complex consists of short to elongate flattened sacs, vacuoles and vesicles. Only a few dense, lysosome-like bodies are found in these cells. Parafollicular cells containing the large dense granules, several microns or more in diameter, seen in August bats were not observed in sections of thyroids from bats captured in November and December.

By January, degranulated parafollicular cells of the type just described are rarely observed. Instead, the degranulated parafollicular cells, which are now very sparse, are characterized by the presence of large whorls of agranular cytoplasmic membranes. These whorls enclose a centrally located mass of cytoplasmic matrix filled with granules, ribosomes and other cellular material (Fig. 3). Apart from the rarely observed degranulated cell, the thyroid glands of bats examined in mid-hibernation (January) con-
tain three types of parafollicular cells with differing fine structure (Fig. 4). The first type contains neither dense secretory granules nor depleted light vesicles (Figs. 4, 5). The cytoplasm of the second type of parafollicular cell contains large numbers of dense secretory granules (Fig. 7). The third type (Figs. 4, 11) has an intermediate structure.

In detail, the first type of parafollicular cell has a light to moderately dense cytoplasmic texture. Its most salient feature is the prominent rough-surfaced endoplasmic reticulum (Figs. 4, 5). Occasionally, this consists of irregularly shaped cisternae which vary in size, show slight to pronounced dilatations, and contain a material of moderate density (Figs. 4, 5). More commonly, the endoplasmic reticulum consists of numerous short, rounded units bounded by membranes which are both granular and agranular (Figs. 5, 6). The appearance and frequency of occurrence of the Golgi complex also varies. Although not obvious in the parafollicular cells characterized by pleomorphic ergastoplasmic profiles (Fig. 4), it is usually present in those cells which contain large numbers of short, round ergastoplasmic profiles (Figs. 5, 6), where, although not extensive, it consists of several closely packed, slightly dilated sacules and vacuoles. Close to the Golgi complex are numerous small to medium-sized vesicles containing material of similar density to that found in the ergastoplasm (Fig. 6). These vesicles are often seen in intimate association with elements of the Golgi apparatus, and occasionally appear to fuse with Golgi cisternae (Fig. 6, upper inset). Numerous free ribosomes, singly or in clusters, are distributed throughout the ground substance of these parafollicular cells (Fig. 6). Dense, round-to-elongate mitochondria are scattered throughout the cytoplasm (Figs. 5, 6). As a rule their cristae are transversely disposed but occasionally show a longitudinal orientation (Fig. 6, lower inset). The nuclei usually contain a single nucleolus (Fig. 4). Adjacent plasma membranes follow a relatively straight course with an occasional infolding (Fig. 5). Conspicuous components are the numerous dense bodies found throughout the cytoplasm. They vary greatly in electron opacity (Fig. 2), and their size ranges from about 0.1 μm (Fig. 2) to several μm in diameter (Fig. 5). They are usually round, although large spindle-shaped structures are occasionally encountered (Fig. 5, inset), and show considerable structural variation. Ferritin-like particles and long slender elements are frequently present (Fig. 2, inset). The dense bodies are bound by smooth membranes and, although they are encountered in all parts of the cytoplasm, tend to be more numerous in the basal area (Fig. 2).

The second type of parafollicular cell found in the thyroid glands of January bats differs from the first in that it has a greater cytoplasmic density and contains large numbers of small granules (Fig. 7). The cytoplasmic granules characteristic of this cell type possess a dense core which is often separated from the outer agranular limiting membrane by a narrow electron-transparent region (Figs. 7, 10). The granules are mostly round and have a mean diameter of about 0.3 μm. The rough-surfaced endoplasmic reticulum is less abundant and consists of individual, slender to slightly dilated profiles which are distributed throughout the cell (Figs. 7, 10). The Golgi complex is much more extensive than that of the first type of parafollicular cell and usually consists of 3–5 groups of elongated, flattened sacules which are arranged in a roughly circular or horseshoe shape (Figs. 7, 9). In many instances the Golgi cisternae
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appear to contain a dense material similar to that of the secretory granules (Fig. 9 and lower left inset). Terminal dilations of Golgi cisternae having similar dimensions to the opaque cytoplasmic granules also contain dark granular material (Fig. 9, lower right inset). Elements of the granular endoplasmic reticulum are often seen in juxtaposition to those of the Golgi complex (Fig. 7, inset). These ergastoplasmic elements have long, slender profiles and the surface membrane adjacent to the nearest Golgi saccule is free from ribosomes. The centre of the Golgi region contains large numbers of dense granules, vacuoles and smaller vesicles as well as variable numbers of coated hazy vesicles (Figs. 7, 7 inset, and 9). Large numbers of dense pleomorphic mitochondria are scattered throughout the cytoplasmic matrix (Figs. 7, 10).

Another feature of these granule-containing parafollicular cells is the appreciable number of large, complex dark bodies which are distributed throughout the cytoplasm; one type contains clearly recognizable sequestered cytoplasmic organelles such as mitochondria (Fig. 8). Other commonly observed structures are irregularly shaped dense bodies, composed of an electron-opaque, finely granular material which surrounds sharply defined vacuoles (Fig. 8, inset). These structures vary greatly in size and, as a rule, the larger they are the greater the size of the vacuolar compartment. Multivesicular bodies are also frequently found distributed throughout the cell (Fig. 10). They are often surrounded by vesicles which are similar to those lying within the multivesicular body. Groups of comparable vesicles are found in the cell matrix, often in close proximity to the Golgi complex (Fig. 10).

The third type of parafollicular cell is intermediate between the two just described and shows features of both. The nuclei of this intermediate cell type are round, ovoid or irregularly shaped, and usually eccentrically placed in the cell (Fig. 11). A prominent feature of some cells of this type is the arrangement of the endoplasmic reticulum into slender, slightly dilated units. A regular finding is that as the number of dense granules increases, there is an increase in the number of slender ergastoplasmic profiles and a decrease in the number of short, round profiles (Figs. 4, 11). Furthermore, the cells with a larger number of dense granules appear somewhat larger than the type 1 parafollicular cells. Numerous free ribosomes are found dispersed throughout the cytoplasmic ground substance. The Golgi complex of the intermediate forms of the parafollicular cell is larger in those cells having an increased number of dense granules. Dense material is often seen in these Golgi cisternae (Fig. 11). The thyroid glands from bats collected in February and March contain parafollicular cells which have a cytoplasmic matrix packed with small dense granules. These cells contain relatively few lysosome-type bodies and the granular endoplasmic reticulum consists of individual slender profiles.

DISCUSSION

During the early stages of hibernation there is a considerable decrease in the dark contents of the secretory granules which results in them appearing as more or less empty vesicles. Some of these contain short segments of membrane and myelin-like figures which may represent intermediate stages in the release of secretory material,
while the empty vesicles are perhaps residual membrane shells of the granules after their dense contents have been discharged. The degree of granule depletion and the long duration of this process is indicative of a direct response of the parafollicular cells to hibernation. Although these observations are consistent with the postulated secretory function for the parafollicular cell (Bensley, 1914; Nonidez, 1932; Sugiyama, 1954; Sato, 1959; Pearse, 1966), the function of these cells in the hibernating process will remain uncertain until the role of their secretory granules is precisely elucidated.

If, as has been suggested, the parafollicular cells secrete calcitonin (Pearse, 1966) and their dense granules are storage sites for the hormone (Matsuzawa & Kurosumi, 1967), it is likely that they are responding to, or perhaps controlling, the seasonal changes in the plasma calcium concentration reported by Riedesel (1957) and Azzali (1967). The recent demonstration that calcitonin normally acts on bone to prevent calcium release (Robinson, Martin, Mathews & MacIntyre, 1967) suggests that secretion of the hormone by bat parafollicular cells may slow down bone resorption during hibernation, when the animals are quiescent for long periods (Ransome, 1968) during which lack of stimulation by mechanical stress would be expected to lead to bone resorption and thence to an elevation of the serum calcium level (Deitrick, Whedon & Shorr, 1948).

During the mid-hibernation period (January) very few degranulated parafollicular cells were encountered in the bat thyroid preparations examined by electron microscopy. Instead, the glands were characterized by parafollicular cells, the fine structure of which showed considerable variation, ranging from those with an abundant, vesicular, rough-surfaced endoplasmic reticulum, but with no secretory granules, to those packed with dense granules. Towards the end of hibernation (February and March), however, only fully developed granule-rich parafollicular cells were seen. Since there were no signs of parafollicular cell death, or of autophagic activity during or immediately following the loss of its granular content, it seems most likely that the depleted parafollicular cell prepares itself for a new cycle of secretory granule production. Only morphological data are available so far, and based on these, it appears that there is an initial transitional phase during which a proliferation of rough-surfaced endoplasmic reticulum occurs, followed by a progressive development of the Golgi complex. These observations are consistent with the operation of the accepted cellular processes for the synthesis of protein (Siekevitz & Palade, 1960; Potter & Kuff, 1961), during which protein to be secreted is collected in the intracisternal spaces of the ergastoplasm and then channelled to the Golgi complex (Caro & Palade, 1964). Further observations on the present system do, in fact, show that in the early stages of Golgi development small to medium-sized vesicles containing a material morphologically similar to that within the ergastoplasmic profiles are seen in intimate association with the Golgi elements, and thus may be transporting newly synthesized material to them in the manner proposed by Palade (1961) and others (Zeigel & Dalton, 1962). The observed gradual reduction in the size and number of the ergastoplasmic profiles and the progressive expansion of the Golgi complex with the appearance of dense material in its cisternae is consistent with the termination of this phase of synthesis and transport. The occurrence of dense masses in those terminal dilations of Golgi cisternae that have the same order of diameter as that of the secretory
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Granules is further suggestive of their derivation by budding from these cisternae. To obtain more direct evidence for the synthetic and secretory pathways involved, it is planned to trace intracellular movements of injected tritiated amino acids using combined techniques of autoradiography and electron microscopy.

In parallel with the changes which follow degranulation is the appearance throughout the cytoplasmic matrix of large numbers of dense bodies of various sizes. Such bodies are almost entirely absent at other times of the year. Their morphology suggests that they belong to the lysosomal system which serves largely as a means of intracellular digestion, for they severally resemble the various components of this system, including autophagic vacuoles and residual bodies, as described in other cells (Miller & Palade, 1964; Smith & Farquhar, 1966; Ericsson, Trump & Weibel, 1965; De Duve, 1963; Novikoff & Essner, 1962). The more complex of these structures (vacuoles containing recognizable cell debris), and smaller bodies resembling the residual bodies of the liver parenchymal cell, were usually seen in those parafollicular cells in which dense secretory granules were re-forming, while other lysosome-like bodies were more numerous in granule-free parafollicular cells.

It has been demonstrated that lysosome formation is augmented in certain pathological processes (Hruban et al. 1963; Biavi, 1965), or as a result of various unusual physiological or pathological influences (Ashford & Porter, 1962; Trump & Ericsson, 1965). In such cases the increase in the lysosome population appears to be a specific cellular response to functional changes, whereby unwanted organelles are sequestered within a membrane and are digested by lysosomal acid hydrolases (Novikoff & Essner, 1962; Swift & Hruban, 1964). Thus, it seems reasonable to assume that during the transitional phase of granule formation in the bat parafollicular cell, a number of cytoplasmic residua from previous cell cycles—for example, the membranes remaining from the previous population of secretory granules—are removed by similar autophagic digestion. Studies with preparations cytochemically stained to show the presence and location of acid phosphatase are now in progress in the hope of obtaining more direct evidence for the operation of these lysosomal processes.

The role of multivesicular bodies during the period of granule restoration is far from clear. Although these bodies are occasionally seen at other times of the year, they appear in considerable numbers during the mid-hibernation period. It has been postulated that multivesicular bodies represent common digestive vacuoles for both exogenous as well as endogenous materials (Merker, 1965; Smith & Farquhar, 1966). However, the origin and function of multivesicular bodies is still poorly understood, although recent evidence presented by Friend & Farquhar (1967) in the case of cells of the vas deferens suggests that they may be assembled from the population of small, coated vesicles seen in the Golgi complex. Consistent with this view is the finding that numerous vesicles, many of which are of the coated type, and multivesicular bodies, are also prominent near the extensive Golgi complex present in parafollicular cells undergoing regranulation.

Many questions remain to be answered concerning the function of bat parafollicular cells. Among these is the nature of the stimuli which lead to the degranulation and granule re-formation which are observed at various stages during hibernation. In this
connexion, the known rapid release of calcitonin following the administration of high
doses of calcium (Matsuzawa & Kurosumi, 1967; Care, Duncan & Webster, 1967)
does not appear to be consistent with the long duration of degranulation observed in
the cells containing small granules. On the other hand, the fact that cells containing
large dense granules (Nunez et al. 1967) were not observed at any time during hiberna-
tion suggests that degranulation of these cells is a rapid process and that they must
also be considered as candidates for the source of the hormone. In such an event,
another function must perhaps be ascribed to the cells containing small granules. For
example, they may contain biogenic amines such as 5-hydroxytryptamine which has
already been reported to be present in parafollicular cells of sheep and goats (Falck et al.
1964; Falck & Owman, 1968).

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<table>
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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>aut</td>
<td>autophagic vacuole</td>
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<tr>
<td>c</td>
<td>colloid</td>
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<tr>
<td>cv</td>
<td>coated vesicle</td>
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<tr>
<td>d</td>
<td>small dense granule</td>
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<td>db</td>
<td>dense body</td>
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<td>er</td>
<td>endoplasmic reticulum</td>
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<td>fc</td>
<td>follicular cell</td>
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<tr>
<td>g</td>
<td>Golgi complex</td>
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<tr>
<td>m</td>
<td>mitochondria</td>
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<tr>
<td>mv</td>
<td>microvilli</td>
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<tr>
<td>mvb</td>
<td>multivesicular body</td>
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<td>n</td>
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<td>pc</td>
<td>parafollicular cell</td>
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<td>r</td>
<td>ribosomes</td>
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<tr>
<td>s</td>
<td>intercellular space</td>
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<td>v</td>
<td>vesicle</td>
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<td>vac</td>
<td>vacuole</td>
</tr>
<tr>
<td>vdb</td>
<td>vacuolated dense body</td>
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Figs. 1–11 are electron micrographs of thyroid parafollicular cells from bats collected at monthly intervals throughout hibernation (November–March). The series of micrographs depicts the intracellular events involved in the re-formation of the small dense granules.

Fig. 1. Electron micrograph showing portions of several partly degranulated parafollicular cells from the thyroid gland of a hibernating bat collected in November. The cytoplasmic matrix has a pale texture and contains dense bodies (db), granular endoplasmic reticulum (arrows), Golgi complex (g), mitochondria (m) and nucleus (n). × 18000. The inset shows several light vesicles (v) which characterize the cytoplasm of totally degranulated parafollicular cells; × 20000.

Fig. 2. Basal area of a type 1 parafollicular cell (January animal). Note large numbers of dense bodies (db) which range in size from approximately 0.1 (arrows) to 1 μm in diameter. Part of a nucleus (n), granular endoplasmic reticulum (er) and intercellular space (s) are also seen. × 30000. The inset shows several other types of dense bodies found in type 1 parafollicular cells; × 32000.
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Fig. 3. Degranulated parafollicular cell (pc) from a hibernating bat examined during January. The cell is characterized by the presence of agranular whorls of cytoplasmic membranes which enclose a mass of cytoplasmic material. Colloid (c), a follicular cell (fc), microvilli (mv) and the intercellular space (s) are also present. × 23 000.
Fig. 4. Basal region of a thyroid follicle from the thyroid gland of a January bat. The figure illustrates several different types of parafollicular cells. One type (pc-1) exhibits a cytoplasmic matrix free of secretory granules. The cytoplasmic matrix contains numerous free ribosomes (r) and many large, irregular or round profiles of the granular endoplasmic reticulum (er). A second type of parafollicular cell (pc-2) differs from the first in the following manner. Its cytoplasmic matrix contains many dense secretory granules and the granular endoplasmic reticulum consists of slender profiles (er). A third type of parafollicular cell (pc-3) is intermediate between the first two. Also present are colloid (c), follicular cells (fc), dense bodies (db), mitochondria (m), nuclei (n), multivesicular body (mvb) and the intercellular space (s). × 15 000.
Fig. 5. The most typically observed type 1 parafollicular cell (pc) is illustrated in this figure. They are characterized by a cytoplasmic matrix packed with medium-sized rounded profiles of the granular endoplasmic reticulum (er). Note the relatively small Golgi complex (g), large dense bodies (db) and absence of dense secretory granules, colloid (c), follicular cell (fc), nucleus (n) and intercellular space (i). × 16,000. The inset shows the detailed structure of the spindle-shaped dense body seen in Fig. 5; × 35,000.
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Fig. 6. This is a higher magnification (× 32 000) of the Golgi regions of the cell seen in Fig. 5. Note the large numbers of small to medium-sized vesicles (v) that are closely associated with the Golgi saccules (g). These vesicles contain a material similar to that in the granular endoplasmic reticulum (er). The upper left inset shows a vesicle (v) seemingly merging with a Golgi saccule, × 45 000. The inset at the lower right illustrates a mitochondrion with longitudinally oriented cristae; × 40 000.
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Fig. 7. A type 2 parafollicular cell (January bat), × 22,000. Such cells show a prominence of dense secretory granules and the extensive Golgi complex (g). Note the slender profiles of the endoplasmic reticulum (er) and the presence of a large vacuolated dense body (tub), colloid (c), part of a follicular cell (fc), mitochondria (m) and vacuoles (vac). Inset shows elements of the granular endoplasmic reticulum (er) in juxtaposition with those of the Golgi complex (g). Also seen in the Golgi region are coated vesicles (cv); vesicles (v) and vacuoles (vac); × 32,000.
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Fig. 8. Portion of a type 2 parafollicular cell (January bat), $\times 32\,000$. The figure shows several autophagic vacuoles ($aut$), one of which contains a mitochondrion ($m$). Inset shows a vacuolated dense body ($vdb$) and a multivesicular body ($mvb$) which are commonly observed in type 2 parafollicular cells; $\times 25\,000$.

Fig. 9. This electron micrograph illustrates the extensive Golgi complex typical of many of the type 2 parafollicular cells. Stacks of Golgi elements ($g$) are arranged in a roughly circular pattern. In the centre of the Golgi complex are numerous vesicles ($v$), coated vesicles ($cv$), dense granules and vacuoles ($vac$), $\times 24\,000$. The lower left inset shows dense material in Golgi elements; $\times 28\,000$. The lower right inset shows a dilation of a Golgi element containing dense material.
Fig. 10. This figure shows part of the cytoplasm of a type 2 parafollicular cell. Note that the multivesicular bodies (mVB) are surrounded by small vesicles (arrows). Groups of similar vesicles (v) are found in the cell matrix, the lower group being associated with the Golgi complex (g). Prominent mitochondria (m) and profiles of granular endoplasmic reticulum are also present (er). × 31000.
Fig. 11. A type 3 parafollicular cell (January bat). The cell contains a few dense secretory granules scattered throughout the cytoplasm. The endoplasmic reticulum consists of both round (short arrow) and slender (long arrow) profiles and the Golgi complex (g) is larger than in type 1 cells. Colloid (c), a follicular cell (fc), and the intercellular space (s) are also shown in this field. $\times 24,000$. 