The Fine Structure of Brown Adipose Tissue in the Newborn Mouse and Rat

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ABSTRACT

Interscapular fat from newborn rats and mice was fixed in buffered 1 per cent osmium tetroxide and thin sections of the methacrylate-embedded tissue were studied with the electron microscope. The findings have reaffirmed the epithelioid character of brown adipose tissue, and have provided additional information on the relation of its cells to each other and to the rich capillary bed. For the most part, the earlier description of the fine structure of brown adipose cells by Lever, has been confirmed, but our observations on the mitochondria and their relation to fat droplets have led us to different conclusions concerning the role of these organelles in lipogenesis. Mitochondria were often found to be very closely associated with lipid inclusions, but no actual communication between the two was observed and no evidence was found to support the hypothesis that mitochondria are transformed into lipid droplets. Large dense bodies which showed a highly ordered fine structure suggesting a crystalline protein were seen in the matrix of some mitochondria. The cytoplasm of the adipose cells contained fine granules that seemed to be of two kinds: particles of uniform size (~150 Å) and appreciable density that are believed to be ribonucleoprotein, and granules of lower density and more variable size that are tentatively interpreted as a form of glycogen. The Golgi complex of the adipose cells was small and the endoplasmic reticulum almost entirely absent. The significance of the poor development of these organelles is discussed in relation to current concepts of their function.

The interscapular brown fat of rodents was formerly thought to have a function related to winter dormancy and it was therefore called the hibernating gland, but it is now generally believed to be a special form of adipose tissue. Although it has been a subject of renewed interest to histologists, biochemists, and virologists in the past few years, its physiological significance remains largely unknown. For a review of the early literature on this tissue, a description of its histology, and a tabulation of its occurrence in various mammalian species, the reader is referred to the classical paper by Rasmussen (17) in 1923. Later work on the microscopic structure, histochemistry, and metabolism of brown adipose tissue, and its relation to the endocrine glands is reviewed in the recent paper by Remillard (18). The interscapular brown fat of adult rats has previously been investigated with the electron microscope by Lever (12, 13), and a cervical lobule of this tissue in the mouse has recently been described and illustrated by Ekholm (4), who mistook it for the parathyroid gland. This unfortunate error, since corrected (5), is not the first instance of misidentification of this tissue. It recalls the paper by Pende in 1914 (16), who found small islands of multilocular adipose cells in the neck of a young boy and, being struck by their resemblance to the cells of the adrenal cortex, gave them the name glandula insularis cervicalis in the belief that he had discovered a diffuse endocrine gland. These incidents serve to emphasize the gland-like appearance which this type of adipose tissue may have under certain conditions.
The high lipide content of brown fat in adult rodents creates problems of fixation and microtomy that make it unusually difficult to obtain electron micrographs of good quality. The study of interscapular fat bodies of newborn animals which contain relatively little lipide obviates some of these technical problems and, at the same time, affords an opportunity to examine the cells in a period of active lipogenesis.

Materials and Methods

The observations are based upon the study of the interscapular pad of fat in newborn mice and rats. For electron microscopy, small blocks of tissue (1 x 1 x 2 mm) were cut from the excised fat pad and fixed by immersion for 2 hours in 1 per cent osmium tetroxide adjusted to pH 7.4-7.6 with veronal-acetate buffer. After a brief washing in distilled water the tissue was dehydrated in increasing concentrations of ethanol (60, 80, 95, and 100 per cent) and infiltrated for 3 hours in 3 changes of n-butyl methacrylate containing 2 per cent laurocapram as catalyst. Polymerization of the plastic was carried out in an oven at 48°-60°C. Sections, showing yellow interference colors, were cut on a Porter-Blum microtome and examined directly without removal of the plastic. Micrographs were made on an RCA electron microscope, model EMU-3B, at original magnifications of 2,000 to 6,000 and enlarged photographically to the desired final magnification.

For light microscopy, blocks of tissue were fixed in cold Rossman’s fluid (4°C.). Sections were stained for glycogen by the periodic acid-Schiff reaction. Control slides were similarly stained after preliminary treatment with saliva. Other blocks were fixed in Zenker-formol and stained for mitochondria by Mallory’s phosphotungstic acid hematoxylin method.

Observations

The interscapular brown adipose tissue of the newborn consists of richly vascularized lobules of polygonal cells containing relatively little lipide (Fig. 1). Almost every fat cell is in contact with one or more capillaries. Both the fat cells and capillaries are invested by a thin layer of moderately dense extracellular material which appears amorphous at the resolutions attained in the present study. This layer resembles the extraneous coating on the sarcolemma of muscle, or on the Schwann cells of nerves, and apparently corresponds to the “basement membrane” of epithelia as this structure is now defined by electron microscopists. Connective tissue is very sparse around the capillaries and is usually represented by widely spaced bundles of three to thirty loosely associated unit fibrils of collagen that are seldom more than 300 A in diameter (Fig. 3). No appreciable perivascular space is observed around the capillaries. The adipose cells, although epithelioid in appearance, are not in intimate contact with other fat cells over their entire surface, but leave angular interstitial spaces, particularly at their rounded corners (Fig. 1). Small unmyelinated nerves are occasionally found adjacent to the capillaries or lodged in the interstices between the fat cells (Fig. 4). Some of the axons are naked while others are sheathed by Schwann cells. Electron micrographs of interscapular fat from adult animals reveal that these nerves remain unmyelinated in the mature animal, and their small size suggests that they terminate among the fat cells. In addition to small dense mitochondria their axoplasm sometimes contains minute vesicles in varying number. Whether or not the latter are to be identified with the so called “synaptic vesicles” of De Robertis and Bennett (3) must await further study. Experimental evidence, however, suggests that the mobilization of lipide from brown adipose tissue is, at least to some extent, under nervous control (10, 19), and therefore it would not be surprising to find in the nerves among the fat cells the same vesicular component that occurs at synapses in the central nervous system and at sites of termination of nerves upon their effectors.

The adipose cells are about 18 to 20 μ in diameter and usually have a single, centrally located nucleus. Binucleate cells are observed occasionally (Fig. 4). The nuclei generally possess two dense, compactly organized nucleoli and show numerous irregular masses of intermediate density that have a distribution corresponding to the chromatin pattern seen in stained preparations examined with the light microscope. The cytoplasm has a fine granular character due to the presence of abundant 150 to 250 A particles. In some instances, these fine particles are of rather uniform appearance throughout the cytoplasm (Fig. 2), but in other cells two types of granules can be distinguished on the basis of their differing densities (Fig. 14). The sharply defined, dense particles that are present in relatively small numbers appear to correspond to the nucleic acid-rich granular component of the cytoplasm, first described by Palade. The identity of the less dense particles, that are present in much greater number is in doubt, but it is possible that they are the same as the granules in other tissues that have been interpreted as a particulate form of glycogen (7, 8). This interpretation is supported by the observation that, in adult animals, this type of
granulation is particularly conspicuous after insulin administration or refeeding after a period of fasting, experimental conditions known to favor the accumulation of glycogen in adipose cells. Work now in progress on the centrifugal isolation and biochemical characterization of these particles may clarify this point.

An unusual feature of the cytoplasm is the near absence of cytoplasmic membranes. Only rarely does one encounter elongated profiles that can be definitely identified as elements of the endoplasmic reticulum by the presence of dense granules adhering to their membranes. A limited number of empty appearing, smooth surfaced vesicles of varying size are scattered through the cytoplasm, but it is not clear whether these are agranular components of the reticulum, or small vesicles arising at the cell surface in pinocytosis, or possibly vesicular components of the Golgi apparatus. The Golgi complex is unusually small and is often situated at the periphery of the cell (Fig. 12).

In cytological preparations viewed with the light microscope, the mitochondria are numerous and appear to vary in shape from ovoid to rod-like. In electron micrographs, they are found to be generally more elongate and more pleomorphic than described by workers using the light microscope (6). Short rods and longer filamentous forms seem to predominate but, owing to their random orientation with respect to the plane of section, the mitochondrial profiles in electron micrographs vary from less than 0.5 μ to more than 6 μ in length. They frequently possess bud-like lateral branches (Fig. 6) which give them a T or V shape (Fig. 3 and Fig. 11) and occasional ring forms are encountered. As a rule, the mitochondria of brown adipose tissue have a remarkably complex internal structure consisting of numerous, closely packed, parallel cristae that extend across the organelle, forming more or less complete septa (Fig. 5, M1, Figs. 7, 10, and 13). One finds many mitochondrial profiles, however, which show no internal membranes but simply consist of a pair of limiting membranes enclosing a rather dense, homogeneous matrix (Fig. 5, M2). A detailed consideration of the significance of this lack of visible internal structure will be deferred until the discussion. Let it suffice here to state that the empty appearance of most of these mitochondrial profiles seems to be an artifact of thin sectioning and they are not to be regarded as immature forms or intermediate stages in the transformation of mitochondria into lipide inclusions.

Elongated mitochondrial profiles are also observed which possess closely spaced cristae at one end but are devoid of transverse membranes at the other end (Fig. 5, M3). Absence of internal structure confined to the ends of elongated mitochondrial profiles does not seem to be artifactual but probably reflects a real absence of cristae. The localization of these structureless areas at the tips of mitochondria suggests the possibility that these are incompletely differentiated regions of mitochondria that were in the process of elongating.

Dense bodies of varying size are found in the matrix of some of the mitochondria. These are larger and more variable in size than the dense granules described in the mitochondrial matrix of other tissues (17). They are generally elongate in form, and when the plane of section coincides with their long axis, they show an orderly internal structure consisting of thin dense lines closely spaced in parallel array suggesting a lamellar organization (Fig. 9, Cr1 and Fig. 10, Cr2). When cut transversely, however, they present a porous, grid-like aspect made up of tightly packed circles or contiguous polygons about 150 A in diameter (Fig. 9, Cr2). In other instances, they show a rectangular lattice (Figs. 8 and 15). The internal organization of these apparently crystalline bodies cannot be described more precisely from the micrographs now at hand. The question as to whether they are composed of dense walled tubular elements or a square packing of parallel filaments must await further analysis in electron micrographs of higher resolution.

Lipide droplets of varying size are found free in the cytoplasmic matrix. These do not have a limiting membrane, but they may appear to have one because the lipide often retracts from the original oil-water interface during dehydration, leaving a thin layer of osmiophilic material adhering to the cytoplasm (Fig. 5, Lp). The lipide droplets present varied appearances in electron micrographs. They are sometimes osmiophilic throughout but show alternating bands of greater and lesser density (Fig. 1, Lp1 and Lp2), an appearance which is a common sectioning artifact seen in tissue components that are considerably hardened by the fixative. Not infrequently the droplets show a dense outer zone surrounding a central cavity (Fig. 1, Lp3; Fig. 5, Lp). In still other examples, they have a dense center with a clear zone around the periphery (Fig. 4, Lp3). These differing appearances are probably artifacts.
attributable to incomplete penetration of the fixative and to shrinkage during specimen preparation, but one cannot exclude the possibility that there are, within the droplet, zones of lipide of differing degrees of saturation that might react differently with osmium.

Mitochondria are often closely applied to the surface of the larger lipide droplets but the two structures are always distinct. We have rarely, if ever, seen a genuine example of lipide within the mitochondrial matrix, but it is not uncommon for large mitochondria to be wrapped around small lipide droplets in such a way that if sectioned in the appropriate plane a spurious appearance of intramitochondrial lipide might result (see the interrupted line in Fig. 4).

**DISCUSSION**

The observations reported here on the fine structure of brown adipose tissue are in substantial agreement with those of Lever (13), but our interpretation differs with respect to the structure of the mitochondrial matrix and particularly their relationship to the lipide droplets. It was noted in the present study that the appearance of the mitochondria in electron micrographs of brown adipose cells is not uniform. Many mitochondrial profiles show numerous parallel cristae, while others appear to be devoid of transverse membranes. Mitochondria of these two different appearances occur side by side and often in nearly equal numbers (Figs. 2 and 5). Such a variation in the appearance of mitochondria within the same cell is rarely seen in electron micrographs of other tissues, and this might lead one to conclude that the possession of two types of mitochondria is a characteristic feature of brown adipose tissue. However, for reasons that will become apparent in a later discussion of the geometry of mitochondrial structure, two main categories of profiles are to be expected in randomly oriented sections of any cell type whose mitochondria have highly ordered parallel cristae and a rather dense matrix. This expectation is borne out when micrographs of human heart muscle are compared (Figs. 17 to 19) with those of adipose cells. The mitochondria of cardiac muscle (Fig. 17) have an internal structure quite similar to those of brown adipose cells and in this tissue also, two kinds of mitochondrial profiles are observed (Figs. 17 and 19). The explanation for this may not be immediately apparent to readers who do not have occasion to study electron micrographs of thin sections. It may be well, therefore, to go into this matter in some detail, because a clear comprehension of this point is essential for an understanding of the basis of our disagreement with previous interpretations of the relation of mitochondria to the formation of lipide droplets.

In micrographs of thin sections, membranes only appear as clearly defined dense lines when the plane of section is nearly perpendicular to that of the membrane. The lower the angle of incidence of the section with the membrane, the less dense is the resulting linear contour in the micrographs and the more indistinct are its limits. For example, if the mitochondrion depicted in Fig. 18 were to be cut with the microtome along the line \( A-B \) in a plane normal to the page, the section would pass nearly perpendicular to the internal membranes but would intersect the limiting membranes of the organelle at \( A \) and \( B \) at an angle of 135° or more. In an electron micrograph of this hypothetical section one would expect a profile such as that of \( M_1 \) or \( M_6 \) in Fig. 17 where the cristae, cut normally, are clearly defined whereas the limiting membranes, cut obliquely, are represented only by an ill defined gradient of density between that of the mitochondrial matrix and that of the cytoplasmic matrix. Also in Fig. 17, mitochondrion \( M_2 \) illustrates the point that, where a membrane is surrounded by a matrix of appreciable density, the orientation of the membrane with respect to the plane of section need depart only very little from perpendicular in order for it to become invisible. The arrows at \( a \) point to the closed free edges of several clearly defined cristae. These evidently undergo a slight torsion from their apex to their points of attachment and, as a result of this change in their orientation, the attached ends of the same cristae (in the area indicated by the arrow at \( b \)) are completely invisible. Presumably they would still be visible, albeit indistinct, if the mitochondrial matrix were of lower density. It is apparent from these considerations that it is not necessary for the plane of section to coincide with a crista or to fall between successive cristae to give a mitochondrial profile that appears to be devoid of internal structure. Referring again to Fig. 18, a section along the path of \( C-D \) would reverse the conditions described for \( A-B \) in that it would intersect the limiting membrane of the mitochondrion at nearly a right angle while the cristae, being parallel, would all be cut at the same low angle. The density of the obliquely sectioned cristae would be no greater than that of the matrix, and the mitochondrion would there-
In the present study of brown adipose tissue in a plane oblique to the limiting membrane of the mitochondrion, their contents develop their internal structure. The faint amorphous matrix instead of forming by plication apparently acquires some of the features of an intercristal plane. The cycle of events which develop their internal structure is the same as that observed for these two planes of section. In other cell types containing mitochondria with relatively few irregularly oriented cristae, any plane of section will pass perpendicular to some of the cristae and consequently all mitochondrial profiles in such cells will show internal membranes. Obviously the more numerous the cristae and the closer their spacing, the more nearly parallel must they be. In turn, the more perfect the parallelism of the cristae, the greater will be the frequency, in electron micrographs, of mitochondria which "appear" to be devoid of internal structure.

It has already been pointed out by previous authors (14) that the cristae and limiting membranes of mitochondria can be clearly seen only when the plane of section is nearly normal to that of the membranes. The above detailed rediscussion of the problems of interpreting mitochondrial structure from electron micrographs may therefore seem an unnecessary statement of the obvious, but it is apparent from interpretations already in the literature that these matters are not universally understood. For example, round or oval profiles limited by a double membrane and having a homogeneous content have been interpreted as transitional stages in the transformation of mitochondria into lipide droplets (11, 12). The present work indicates that, in brown adipose tissue, these so-called "intermediate bodies" are explicable simply on the basis of the geometry of mitochondrial structure and have no bearing upon the origin of lipide. There have been repeated references in the literature to "open mitochondria" (1, 2, 11), and micrographs have been published which purport to show mitochondria "freely open to lipide droplets" (12). From the foregoing discussion and the examples given in Figs. 17 and 19, it seems quite clear that if a mitochondrion is situated adjacent to a fat droplet and if the two are cut in a plane oblique to the limiting membrane of the mitochondrion, a specious appearance of continuity will result. Instances of this kind can easily be misinterpreted as evidence that mitochondria contribute their contents to the enlarging fat droplets. In the present study of brown adipose tissue in a phase of active lipogenesis we have seen no real evidence of continuity between the mitochondrial matrix and the cytoplasmic matrix or of open communication between the interior of a mitochondrion and a lipide droplet, and we consider previous reports of this kind to be based upon misinterpretation of images resulting from oblique planes of section, or breaks in membranes caused by expansion during polymerization.

Although it is likely that the great majority of the mitochondrial profiles which lack visible internal structure can be accounted for on the basis of the plane of section, as explained above, this explanation does not seem to apply to those occasional long profiles which have numerous cristae in the body of the mitochondrion, but show no transverse membranes at one or both ends (Figs. 5, 6, and 11). It could be argued that a sudden bend in a long mitochondrion might result in an abrupt change from distinct cristae in one part, to no visible membranes in another part of the same profile. However, if the organelle were curving out of the plane of the section, its limiting membrane would usually be cut obliquely at the bend and hence this part of the profile should be indistinct or invisible in the electron micrograph. Actually this configuration is seldom found. We suggest, therefore, that the absence of internal membranes at the ends of these long profiles may reflect the true condition, and a small fraction of the empty appearing round or oval mitochondrial profiles observed, may be transverse sections through such regions. Mitochondria which exhibit complex shapes including one or more side branches and even closed loops are a familiar sight to those who observe tissue cultures with phase contrast microscopy. In lapsed-time cinematographs of cells in culture, the mitochondria can be seen to elongate suddenly or to thrust out lateral branches (9, 21). These changes in form take place with surprising rapidity, even if allowance be made for the acceleration of events that results from lapsed-time exposure. Mindful of the remarkable activity and plasticity of mitochondria in the living state, it is tempting to speculate that the end segments of mitochondria which lack transverse membranes are newly formed growing tips that have not yet developed their internal structure. The faint transverse markings sometimes seen in these areas suggest that the cristae may arise de novo from the amorphous matrix instead of forming by plication of the inner mitochondrial membrane, followed by elongation of the resulting fold. Much further work...
must be done before such a hypothetical process of mitochondrial elongation followed by internal differentiation, can be accepted. The results of the present investigation indicate, however, that brown fat of newborn animals may be a favorable tissue in which to study the intriguing problem of the morphogenesis of mitochondria.

It has been the experience of electron microscopists studying a variety of tissues, that there is, in general, a fairly good correlation between the energy requirements of a particular cell type and the extent of the membrane surface of its mitochondria. In brown fat the presence of many mitochondria with elaborate internal structure thus constitutes additional indirect support for the thesis that this is a tissue of high metabolic activity. Of the many cell types that have been studied to date, relatively few (e.g. insect flight muscle, mammalian skeletal and cardiac muscle) have mitochondria with as large a number of closely spaced parallel cristae as those of brown adipose tissue. The large number of cristae per unit volume in mitochondria of muscle is believed to be related to the important role of mitochondrial enzymes in providing energy for contraction. Obviously brown adipose tissue does not carry out conversion of chemical energy into mechanical work, but it is known to have an unusual capacity for synthesis of lipide from carbohydrate (22), and the complexity of its mitochondria may well be related to the very high energy requirement for this transformation.

The marked tendency for the mitochondria to cluster around developing fat droplets no doubt also has significance in relation to this synthetic activity. Palade and Schidlovsky (15) have recently described mitochondria closely apposed to the surface of lipide droplets in the liver and pancreas of animals fasted or treated with cortisone. It was noted that the outer layer of the mitochondrial membrane could not always be discerned, but these investigators found no continuity of the mitochondrial matrix with the lipide drop. It was suggested that, under the experimental conditions described, the cells had shifted from utilization of carbohydrate to oxidation of lipide as the principal source of cellular energy, and that the apposition of mitochondria to lipide droplets brought the fatty-acid oxidases, residing in these organelles, into closer association with their substrate. At the same time, it was pointed out that these reactions are reversible and that under other conditions, such as cortisone administration, a functional association of mitochondria and lipide inclusions might be indicative of active lipide synthesis. This latter alternative is more likely to be the correct interpretation of the juxtaposition of mitochondria and lipide droplets in the brown adipose tissue of newborn animals.

Crystalline inclusions in the mitochondrial matrix such as those described here do not seem to have been reported before, although smaller “dense granules” or “opaque areas” in the matrix have been described in the mitochondria of several epithelial tissues (20). It was noticed in certain of these earlier studies that the granules had a “complicated internal structure.” A more searching examination of the mitochondrial granules in some of these tissues may reveal that they, too, have a precisely ordered fine structure. No suggestion can be made as to the chemical nature or functional significance of the crystalline bodies in the mitochondria of brown fat, but further study of their variations under different experimental conditions may provide some clues.

Of particular interest in relation to the general cytological problem of the function of the several cell organelles, is the observation that the Golgi complex of the brown adipose cell is poorly developed and the endoplasmic reticulum is almost entirely lacking. Both of these organelles are well developed in cell types that are engaged in synthesis of protein-rich secretory products. Although the adipose cells described here are metabolically as active as glandular cells, their activity is largely concerned with synthesis of carbohydrate (glycogen) and lipide (triglycerides). The finding of a small Golgi complex and a rudimentary endoplasmic reticulum in such cells is consistent with the current belief that these organelles are cooperatively involved in the elaboration of protein cell products and suggests that they have little to do with carbohydrate metabolism or liponeogenesis.

A relatively unexplored aspect of the histophysiology of adipose tissue is the functional relation of the peripheral nerves to the fat cells. Experimental investigations involving unilateral denervation of the paired interscapular fat bodies have demonstrated a transient deposition of glycogen on the denervated side followed by a relative increase in its lipide content that persists for many weeks (10, 19). Although these results suggest that the fat cells themselves are innervated, it is difficult to exclude the possibility that the changes observed are secondary to local changes in circulation after denervation. Silver-impregnated histological sections show what
appear to be nerves intimately related to the
fat cells, but some workers have contended that
these are merely passing through the fat to end in
the overlying skin. Others, aware of the capricious-
ness of silver stains, have questioned whether the
fibers observed are actually nerves or whether they
are argyrophilic components of the connective
tissue stroma. Under the electron microscope, the
identification of nerves depends upon clear cut
morphological criteria, and all possibility of con-
fusing bundles of collagen fibers with axons is
eliminated. The finding of small unmyelinated
axons between the cells in electron micrographs of
brown adipose tissue permits us to reaffirm earlier
statements concerning the probable innervation
of brown fat cells (19).

Previous light microscope studies have drawn
attention to the lobular, gland-like organization of
brown adipose tissue and to its rich vascularity
(6). The observations reported here extend our
knowledge of the structure of this tissue to the sub-
microscopic level, demonstrating anew the epithe-
lioid character of its cells and their intimate re-
lation to capillaries and nerves. The distinguishing
cytological features of the adipose cells have been
shown to be a finely granular cytoplasmic matrix,
a small Golgi apparatus, a paucity of elements of
the endoplasmic reticulum, and a great abundance
of mitochondria with closely packed parallel
 cristae. The exact role of brown adipose tissue in
the bodily economy is still poorly understood, but
an impressive body of evidence indicating that it is a
highly active tissue and one that is probably
histogenetically and morphologically, and meta-
bolically distinct from ordinary depot fat.

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### EXPLANATION OF PLATES

**Abbreviations used in the Figures**

- **AdC**, adipose cell.
- **Ax**, axon.
- **BM**, basement membrane.
- **Cap**, capillary.
- **Chr**, chromatin.
- **Cr**, crystal-like body.
- **EnC**, endothelial cell.
- **ER**, endoplasmic reticulum.
- **G**, granules.
- **GC**, Golgi complex.
- **Lp**, lipide.
- **M**, mitochondrion.
- **Nd**, nucleus.
- **Nls**, nucleolus.
- **Vs**, vesicle.

#### PLATE 340

**Fig. 1.** A low-power electron micrograph of brown adipose tissue from a newborn mouse. It consists of polygonal epithelioid cells intimately associated with capillaries (Cap). Between the cells are small interstices occupied by a sparse, collagenous reticulum and occasional unmyelinated nerves. The adipose cells have numerous, dense mitochondria (M) randomly distributed in a finely granular cytoplasm. Occasional lipide droplets (Lp to Lp₃) are found in the cytoplasm. Mitochondria are often, but not always, closely clustered around the lipide droplets. The round nucleus (Nd) is usually compressed in sectioning to oval shape. It generally contains two nucleoli and shows a pattern of density variations in the karyoplasm corresponding to the distribution of chromatin in stained preparations viewed with the light microscope. X 10,000.
Fig. 2. A portion of a typical cell of brown adipose tissue. At the upper right is the nucleus (Nuc), showing two dark nucleoli (Nls) and clumps of chromatin (Chr) which appear in micrographs as irregular masses of fine granules with a density intermediate between that of the nucleoli and the background karyoplasm. The cytoplasm has an extremely uniform granularity and shows no profiles of the endoplasmic reticulum. The mitochondria vary in their shape and in the complexity of their internal structure. Some have numerous cristae; others appear to have none. At the upper left, mitochondria are closely applied to a fat droplet (Lp). The latter has retracted from the cytoplasm during specimen preparation, leaving a clear space around the osmiophilic lipide. X 14,000.

Fig. 3. A micrograph illustrating the intimate relationship of the fat cells to the capillary bed. An area of the peripheral cytoplasm of an adipose cell is seen at the left, and a portion of a capillary (Cap), at the upper right. The endothelial cell (EnC) is separated from the adipose cell (AdC) by 400 to 600 A except where the cell surfaces diverge slightly to pass around a small bundle of collagen fibrils (Cl). The interval between the cells is occupied by a thin layer of basement membrane material (BM). The cytoplasm of the fat cell contains small granules of variable density. The dense ones appear to be the same as those in the endothelial cell and are presumed to be ribonucleoprotein. The nature of the less dense granules is uncertain. Small vesicles (Vs) scattered through the cytoplasm of the fat cell may possibly represent rudiments of the endoplasmic reticulum. The mitochondria are quite pleomorphic. Branching forms (M) are not uncommon. The mitochondria (M) are often in close apposition to lipide droplets (Lp), but there is no continuity between the two. The arrow indicates a place where the mitochondrial limiting membrane appears to be absent owing to obliquity of the plane of section. X 24,000.
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FIG. 4. A portion of a binucleate adipose cell showing the usual abundance of mitochondria and several small droplets of lipide. Some of these droplets are free in the cytoplasm (Lp1), while others are in close contact with mitochondria (Lp2 and Lp3). For an explanation of the interrupted line, see text. X 14,000.

Fig. 5. An area of perinuclear cytoplasm from a typical adipose cell illustrating the extreme variation in the appearance of the mitochondria. M1 shows very numerous, closely spaced, transverse membranes, while M2 shows no internal membrane structure and M3 has clearly defined cristae at one end and none throughout the rest of the organelle. A possible explanation for this variation is offered in the text. One of the rare profiles definitely recognizable as endoplasmic reticulum is seen at the upper right (E.R.). X 24,000.
Fig. 6. A mitochondrion with a lateral bud (a) showing the abrupt change in direction of the cristae. The end of this mitochondrion (b) and of the one at the upper left shows no internal membranes. It is suggested that these are newly formed ends of elongating mitochondria that have not yet developed cristae. X 40,000.

Fig. 7. A group of typical mitochondria of brown fat. The short rod-shaped mitochondrion cut longitudinally at the upper right has many cristae that seem to extend completely across the organelle. This mitochondrion and the one in the upper half of Fig. 10 show an abundance and regularity in arrangement of cristae rivalled only by active tissues with high energy requirements, such as muscle. X 40,000.

Fig. 8. A mitochondrion containing a sizeable dense body (Cr) that shows a highly ordered repeating structure, an appearance suggesting the lattice of a crystal. X 46,000.

Fig. 9. A cross-section of a mitochondrion showing a disorderly arrangement of cristae around two dense, apparently crystalline, inclusions. The one (Cr₁) is cut in its long axis and in the original micrograph shows a closely spaced longitudinal striation. The other (Cr₂) is cut transversely and appears as if made up of closely packed circles or contiguous polygons. X 58,000.

Fig. 10. Portions of two mitochondria. The one at the lower half of the figure contains two apparently crystalline bodies (Cr) which, in longitudinal section, have a closely spaced lamellar appearance. X 57,000.
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FIG. 11. A mitochondrion of unusual form. The ends a, b, and c lack cristae. It is not unreasonable to believe that these may be regions of active growth and that the development of cristae lags behind the elongation of the limiting membrane and the formation of new matrix. X 31,000.

FIG. 12. The Golgi complex (G.C.) of the brown fat cell is small and tends to be located at the periphery instead of near the nucleus. Notice at x the branching crista and at y, one that forms a loop. X 34,000.

FIG. 13. This mitochondrion illustrates a crista that extends nearly to the opposite side, then makes a hairpin turn (at arrow) and returns to the side of origin. The mitochondrion on the right contains a mass of dense material showing crystalline (Cr) order in some areas. X 34,000.

FIG. 14. An area of cytoplasm shown at higher magnification, illustrating fine particles of similar size, but different densities. The dense granules (G1) appear to be nucleoprotein. It is considered likely that the lighter ones (G2) are a particulate form of glycogen. X 60,000.

FIG. 15. A mitochondrion with an inclusion that shows a flat crystal face (at the arrow) and a particularly regular grid-like pattern in its interior. X 60,000.

FIG. 16. A triangular interstice between adipose cells occupied by a small unmyelinated nerve. X 15,000.
Fig. 17. An area of sarcoplasm from human heart muscle presented here to clarify certain points in the interpretation of mitochondrial structure in electron micrographs. The mitochondrial profile $M_1$ results from a plane of section such as $A-B$ in Fig. 18 which is perpendicular to the cristae but oblique to the limiting membrane. The cristae are therefore clearly defined, but no limiting membrane is discernible. The mitochondrial profile $M_5$ exemplifies the opposite situation (corresponding to a section through $C-D$ in Fig. 18) where the section is normal to the limiting membrane, but oblique to all of the cristae. Thus the mitochondrial membrane is clearly defined in the micrograph, but no membrane structure is visible in the interior. In profiles $M_4$ and $M_6$, the plane of section is perpendicular to both the internal and external membranes. Mitochondrion $M_4$ demonstrates that in the presence of a rather dense matrix the internal membranes become invisible if they depart only slightly from perpendicular. The arrows at $a$ point to the free ends of several cristae. These evidently twist slightly and, as a result, the attached ends of the same cristae in the area indicated by arrows $b$ are invisible. The significance of these interpretations is discussed in the text. × 50,000.

Fig. 18. A mitochondrion from brown adipose tissue serving only to indicate the orientation of hypothetical planes of sections $A-B, C-D, E-F, G-H$ referred to in the text in a discussion of the relation of the parallel orientation of the cristae to the frequent occurrence of mitochondrial profiles which appear to lack transverse membranes. × 57,000.

Fig. 19. A group of mitochondria from human heart muscle demonstrating that “empty appearing” mitochondrial profiles ($M_2$ and $M_3$) are not confined to brown adipose tissue, but are to be expected in any tissue where the mitochondrial cristae are numerous and parallel in their arrangement ($M_1$). × 50,000.