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FIRST OBSERVATIONS OF BROWN FAT IN BIRDS

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ABSTRACT.—Brown fat, the multilocular fat involved in non-shivering thermogenesis in many mammals, is generally believed to be absent in birds. Physiological data indicate that non-shivering thermogenesis does not occur in adult birds. Adipose tissues from a Ruffed Grouse (*Bonasa umbellus*) and two Black-capped Chickadees (*Parus atricapillus*) were found to show typical histological and ultrastructural features of mammalian brown fat. The cells of these tissues were polygonal with central nuclei, multiple lipid droplets and numerous well-developed mitochondria. An extensive capillary bed permeated the tissue. Although no direct demonstration of thermogenesis by this tissue was attempted, its remarkable similarity to mammalian brown fat suggests that non-shivering thermogenesis in birds should be reinvestigated.

Typical white fat of birds and mammals is white to yellow in color and comprised of large cells containing a single lipid droplet that occupies most of the cell volume. The nucleus is flattened against the cell periphery by the lipid. Extraction of the lipid during routine histological processing results in the typical "signet ring" appearance of these cells. Many mammals, especially those that hibernate, have a second distinct type of adipose tissue that is brown and appears almost glandular, the cells being smaller and more closely packed than in white fat (Smith and Horwitz 1969). Cells of this "brown fat" contain much more cytoplasm and the fat is distributed as numerous smaller lipid droplets associated with abundant large mitochondria. The nuclei are more or less spherical and are not displaced to the cell periphery. Brown fat also possesses a richer vascular supply and innervation than white fat (Napolitano 1965).

In mammals, brown fat has been implicated as the primary site of non-shivering thermogenesis during neonatal life (Dawkins and Hull 1965), during cold acclimation in adults (Foster and Frydman 1978), and during arousal from hibernation (Hayward and Lyman 1967). Adult birds are not known to exhibit non-shivering thermogenesis (West 1965, Chaffee and Roberts 1971). Freeman (1971) presented evidence of non-shivering thermogenesis in newly hatched domestic chickens (*Gallus gallus*, var. *domesticus*) but could not find brown fat in these birds. Histological examination of tissues from 11 species of adult and neonatal birds known to exhibit poor thermoregulation and/or torpor also disclosed no brown fat (Johnston 1971).

Multilocular or "plurivacuolar" fat cells in birds have been reported (Clara 1923, 1929; Luckenbill and Cohen 1966) and such fat from the cere of a pigeon was described and illustrated by Lucas and Stettenheim (1972:594). None of these authors suggested that these tissues were brown fat or presented other structural features compatible with their being brown fat. It is known that white fat can often take on multilocular appearance. Luckenbill and Cohen (1966) presented positive evidence that the "mulberry" cells they observed differed from mammalian brown fat in lacking numerous mitochondria.

During a routine examination of tissues from a Ruffed Grouse (*Bonasa umbellus*), I noticed tissue adjacent to the adrenal gland that had the histological appearance of brown fat. Subsequently I examined a Black-capped Chickadee (*Parus atricapillus*) and found that its fat was grossly and histologically similar to mammalian brown fat. A second chickadee was collected to confirm the nature of this tissue by electron microscopy. This paper describes the structure of the fat from these three birds.

METHODS

The Ruffed Grouse (adult female) was collected in November 1971 near Saskatoon, Saskatchewan. A variety of organs were removed in the field and placed in standard Bouin's fixative. These tissues were processed routinely and 7-μm sections were stained with hematoxylin and eosin.

The first chickadee was obtained in March 1980 (Ithaca, New York) and the second in March 1982 (Saskatoon). I made a complete gross dissection of both chickadees and examined their fat tissue under a dissecting microscope. Small representative pieces of fat were removed and placed in fixative (either 5% glutaraldehyde in 0.2 M S-collidine buffer or 2% osmium tetroxide in 1.25% sodium bicarbonate buffer at pH 7.4). The tissues were processed routinely and embedded in epon, except for some larger glutaraldehyde-fixed

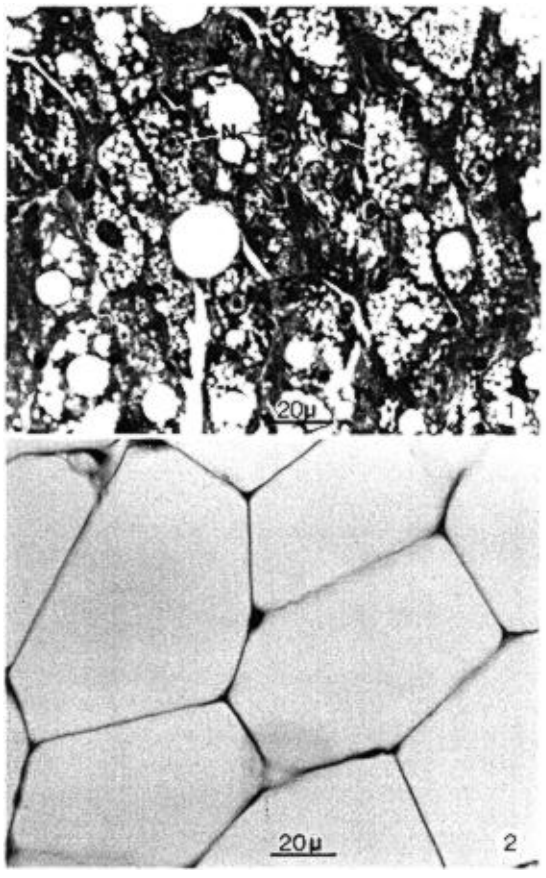


FIGURE 1. Periadrenal fat from a Ruffed Grouse. The spherical nuclei (N) are centrally placed and contain a large dense nucleolus. The cytoplasm surrounding the clear lipid droplets is filled with eosinophilic granules. Capillaries (C) surround the tightly packed cells. Paraffin section, light micrograph.

FIGURE 2. White fat from the ovarian mesentery of a Ruffed Grouse. Compare the large unilocular cells typical of white fat with the multilocular fat in Figure 1. Paraffin section, light micrograph.

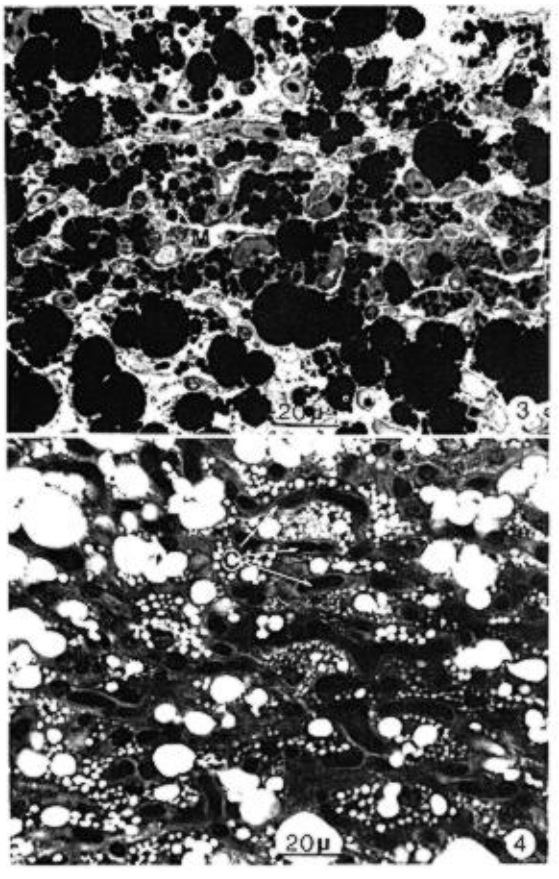


FIGURE 3. Fat from the neck of a Black-capped Chickadee. The abundant mitochondria (M; in center of figure) fill the cytoplasm between the more densely stained lipid droplets. One-micron epon section, light micrograph.

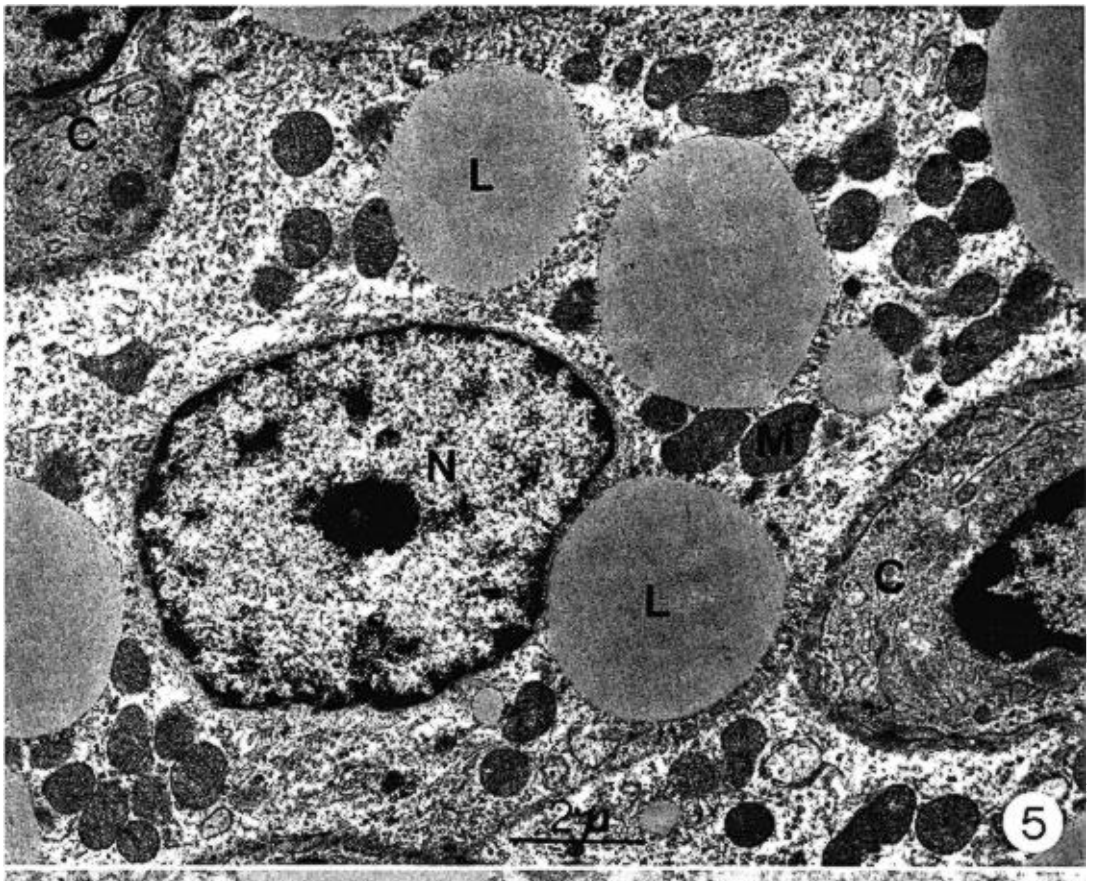
FIGURE 4. Thoracic fat from a chickadee. The extensive capillary beds (C) typical of brown fat are well shown. Two-micron methacrylate section, light micrograph.

pieces that were embedded in glycol methacrylate. One-μm epon sections were stained with 1% toluidine blue 0 and thin sections for electron microscopy were stained with uranyl acetate and lead citrate. Glycol methacrylate sections were cut at 2 μm and stained with hematoxylin and eosin.

OBSERVATIONS AND DISCUSSION

Sections from the adrenal gland (only one collected) of the Ruffed Grouse included a 3×4 mm lobular tissue mass adjacent to the adrenal consisting of tightly packed polygonal cells. A few small lobules consisted of unilocular fat cells but most of the lobules contained what appeared to be typical brown fat (Fig. 1). Adipose tissue associated with the kidney, spleen, ovary and caeca of this bird consisted entirely of larger, typical white fat cells (Fig. 2).

The nuclei of the multilocular cells were large, spherical and centrally located. The chromatin was primarily euchromatic and many cells had a single large nucleolus. The cytoplasm contained a variable number of lipid droplets. A few cells contained one or more large droplets, most cells contained many droplets the size of the nucleus or smaller, and some cells had only a few small droplets. The cytoplasm between the lipid droplets was filled with small eosinophilic granules 0.5–1 μm in diameter. These granules were most evident in cells containing small amounts of lipid and were presumed to be mitochondria. It has been my experience that mitochondria are often adequately preserved with Bouin's fixative and are seen as intensely stained eosinophilic granules. The cytological appearance of these cells was similar to that of brown fat from thirteen-lined ground squirrels (*Citellus tridecemlineatus*), hamsters, (*Mesocricetus auratus*), and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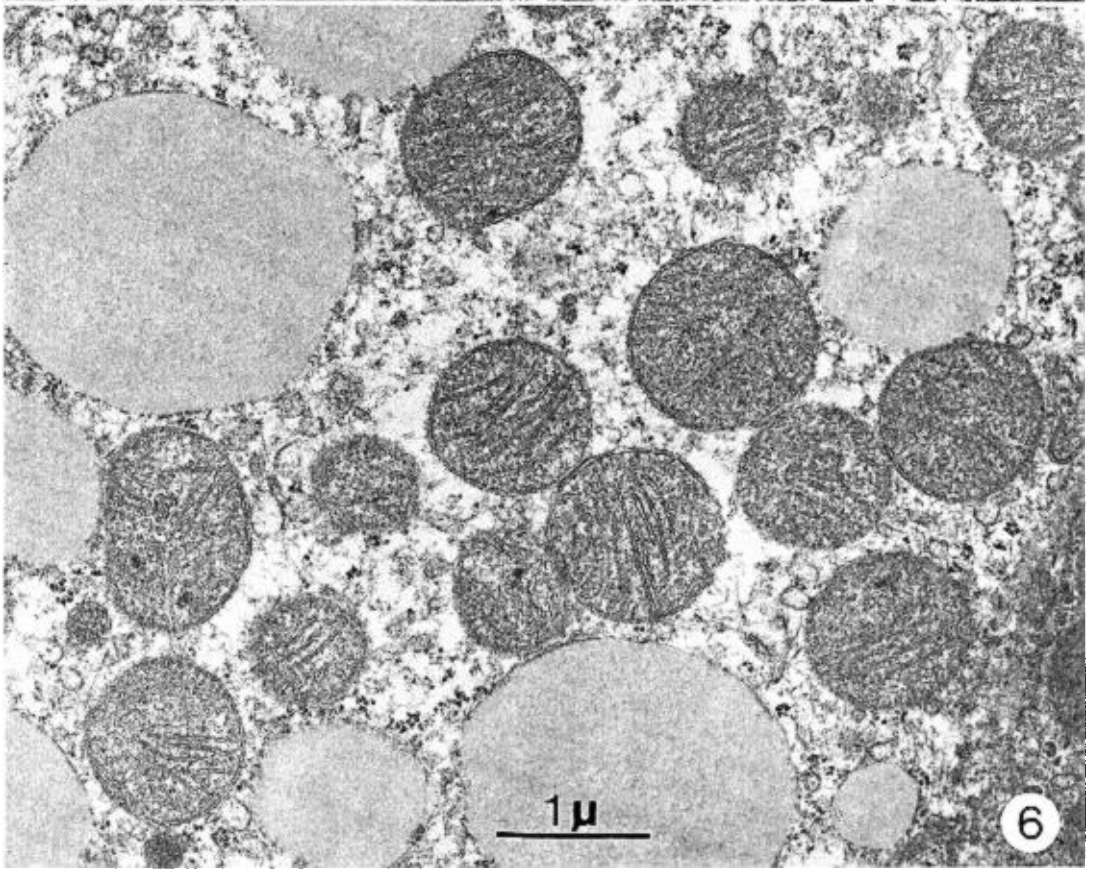
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cold-acclimated laboratory mice (*Mus musculus*) (Oliphant 1967).

Scattered among the fat cells were numerous smaller, more angular and darkly stained nuclei belonging primarily to endothelial cells of a dense capillary network. Larger blood vessels and small myelinated nerves were present in the loose connective tissue between the lobules.

The chickadees both possessed fat at the base of the neck, along the posterior edge of the thorax, in the intestinal mesentery and base of the heart. The color of this fat varied from pinkish to a light brown rather than the more typical bright yellow of most wild birds. Under the dissecting microscope the tissue appeared well vascularized. A small piece of fat that was dehydrated and cleared in xylene showed an extensive capillary network made visible by the red blood cells within the vessels.

The fat from all body areas of the chickadees was histologically similar to the multilocular fat of the Ruffed Grouse (Fig. 3). The cells were polygonal and tightly packed into an epithelial-like mass. Capillaries formed a dense network surrounding each cell (Figs. 4 and 5). Abundant, small densely-stained granules were present in the cytoplasm around the lipid droplets. These were more distinct than in the Ruffed Grouse tissue due to better fixation and thinner sections.

Electron micrographs showed that these cytoplasmic granules were indeed mitochondria (Figs. 5 and 6). They were large, spherical to ovoid, and contained abundant plate-like cristae. The relatively abundant cytoplasm contained cytoplasmic filaments, free ribosomes and a few smooth-surfaced membranes. The cell membranes of adjacent cells were closely opposed with very little intervening connective tissue. Typically two or more capillaries were in contact with each fat cell, separated by only the thin basal lamina surrounding the endothelial cells (Fig. 5). The morphology of the capillaries was unusual in that the lumenal side of the endothelial cells had abundant microvilli.

The periadrenal fat from the Ruffed Grouse and all the fat from the two chickadees showed all the recognized histological attributes of mammalian brown fat, i.e., high vascularity, small tightly packed polygonal cells, spherical central nuclei, multilocular lipid, and numerous large mitochondria (Napolitano 1965, Smith and Horwitz 1969).

The distribution of this multilocular fat throughout the body of the chickadees, to the apparent exclusion of typical white fat, is in distinct contrast to the situation in mammals where brown fat is restricted to certain localized body areas (Smith and Horwitz 1969). The apparent restriction of brown fat to the periadrenal area of the Ruffed Grouse is just as unusual. Although many mammals have brown fat beside the adrenals (Smith and Horwitz 1969; Oliphant, unpubl. observ.) they generally have brown fat in other body regions as well. Since I did not make a gross dissection of the grouse it is possible that other pads of multilocular fat were missed.

The apparent absence of brown fat in other birds examined previously (Johnston 1971) may be due to the types of birds chosen and the conditions under which they were collected. Johnston's choice of birds that exhibit poor thermoregulatory ability and/or torpor was logical, since hibernating mammals have well developed brown fat. His specimens were collected, however, in the southern United States from late March to September, a region and time in which little thermal stress might occur. Johnston pointed out the possible conversion of typical brown fat to a unilocular state which would be difficult or impossible to distinguish from white fat with routine histological methods. This could account for the apparent lack of brown fat observed in these birds, if indeed the species examined have such tissue.

Birds living in extremely cold climates are subject to considerable thermal stress. Winter temperatures around Saskatoon often fall to −40°C for several weeks at a time. From the standpoint of needing a source of heat, birds exposed to those conditions might be more logically expected to have brown fat than those previously examined. The turnover rate of fat in small passerines at low temperatures is known to be very high (Chaffee and Roberts 1971; J. Mosher, pers. comm.). The direct oxidation of lipid to produce heat in the fat cell, as opposed to its mobilization and use by skeletal muscle for heat production by shivering, would seem to be efficient from a physiological standpoint. The cellular machinery (mito-

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FIGURE 5. Electron micrograph of a multilocular fat cell of a chickadee. The nucleus (N) is central and mitochondria (M) and lipid droplets (L) are the most prominent cytoplasmic features. Portions of two capillaries (C) are closely apposed to the cell with little or no intervening connective tissue.

FIGURE 6. Electron micrograph of the mitochondria and lipid from a multilocular fat cell of a chickadee. Note their spherical shape and their extensive plate-like cristae.

chondria) necessary for direct oxidation is present in the multilocular fat examined in this study.

Although the fat described in this paper meets all the anatomical criteria for considering it brown adipose tissue, I have no physiological data to confirm a direct thermogenic role for it. The thesis that shivering is the primary source of additional body heat in birds exposed to cold has a firm physiological basis. West (1965) demonstrated a linear relationship between shivering and metabolic rate in four species of birds with no increase in metabolic rate before the onset of shivering. Norepinephrine, which mediates non-shivering thermogenesis in many mammals, is not effective in birds (Chaffee and Roberts 1971). It is possible that the multilocular fat described in this paper is highly modified for rapid *mobilization* of lipid rather than direct oxidation in the fat cells themselves. We know that the fat of many migratory birds has a remarkable capacity for rapid lipogenesis and lipolysis (Odum 1965).

It is reasonable, however, to expect differences in both the structure and function of adipose tissue in different species of birds and certainly between birds and mammals. The fact, for example, that norepinephrine fails to initiate non-shivering thermogenesis in birds may simply indicate that the mediating agents in birds differ from those of mammals. Other hormones such as thyroid hormone, corticosterone and glucagon have been found to be effective in stimulating metabolic rates in birds (Chaffee and Roberts 1971). Likewise it is unwise to assume that because non-shivering thermogenesis could not be demonstrated in the few avian species studied to date, that all birds are incapable of non-shivering thermogenesis.

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