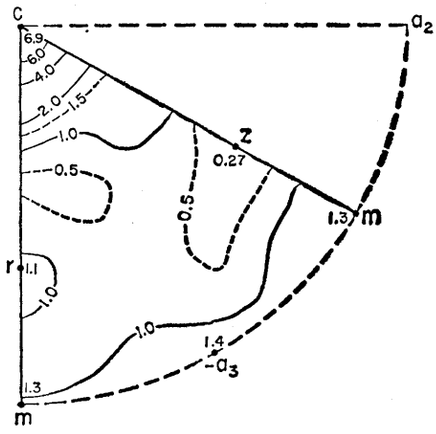

Fig. 4. Inverse pole-figure (equal area projection) of DT-459 showing the distribution of the compression axis with respect to crystal coordinates. Calculated from ten observed profiles with 16th order harmonics; (r is the strong, z the weak reflecting unit rhombodedron).

pole-figure were indirectly obtained by using Roe's method for spherical harmonic analysis of fabric data (*2*). The adaptation of this method to the trigonal symmetry of quartz and its application to the determination of quartz fabrics are described in detail elsewhere (*3*). The inverse pole-figure of DT-459 (Fig. 4) was deduced using 16th order Legendre polynomials, and several profiles have been calculated which compare satisfactorily with the observed data (Fig. 1). The main feature of the inverse pole-figure of DT-459 is a strong maximum of compression axes parallel to the c-axis (7 times uniform distribution). A secondary concentration of compression axes is in the zone of the prisms (1.5 times uniform distribution). Unlike specimens deformed in the α-field (*3*), in this specimen deformed in the β-field there is no significant tendency for other crystallographic directions to have additional preferred orientation. In DT-460 the high concentrations necessitated high-order spherical harmonics to represent the steep topography. With the limited data available, the serious termination errors in these high-order harmonic calculations were reduced using hexagonal symmetry, but still produced artificial secondary features. The c-axis maximum in the direction of compression was determined with 20th order harmonics to be 46 times uniform distribution.

Annealing thus increased the strength of the c-axis maximum six- to eightfold and obliterated the concentration of c-axes perpendicular to the compression. Green (*1*) has determined the concentration of c-axes in DT-460 optically (U-stage). His results are compared in Table 2 with the x-ray data obtained from different methods. Within the range of experimental errors there is agreement between the different data. The x-ray values for DT-460 have to be regarded as a lower limit because the beam-size is slightly larger than the highly oriented area and minute disalignments bring this area out of the focus. In the optical analysis only the larger grains (~0.03 mm) were measured, whereas by the x-ray technique the orientation of all grains is recorded. Green estimates that the small grains are less well oriented, and that their inclusion could reduce the concentration by as much as a factor of 2.

The fact that c-axis fabrics of quartz have been directly measured by x-rays offers new opportunities in structural petrology. It appears that presently available improved diffractometers are sufficiently better than ours to permit the direct measurement of c-axis fabrics in fine-grained quartz rocks with as much sensitivity as optical techniques.

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Brown and White Fats: Development in the Hamster

Abstract. *Sites occupied by multilocular brown fat in the adult hamster are occupied by unilocular cells in very young animals. Immature brown fat cells are laid down in the unilocular cell matrix at 3 to 5 days of age. White fat in the hamster does not develop from cells closely resembling mature brown fat.*

Recent demonstrations of the thermogenic function of brown fat (*1*) have led to renewed interest in this tissue. An unanswered question is its relationship to white fat. Mature brown-fat cells appear histologically similar to multilocular cells found in developing white fat in such animals as rabbits and mice. This similarity has led to the suggestion that brown fat may be identical, or closely related, to immature white fat (*2*). However, recent investigations of the two types of multilocular cells with the electron microscope have indicated that some differences exist (*3*). The differences are well discussed in a recent review (*4*). We present further evidence, based upon light-microscopic investigations, that brown fat is not identical to developing white fat in the hamster. Furthermore, unilocular cells are formed several days before the appearance of multilocular cells.

In the adult hamster the subscapular region is occupied by brown fat, while the inguinal region has white fat. We have studied the development of adipose tissue in these regions as examples of the development of brown fat and white fat, respectively, and have then compared the processes in the two sites.

The animals studied ranged from the 12-day fetus, through newborn to animals 30 days old. They were obtained from adults maintained on a 12-hour light cycle with unlimited access to food and water (*5*). Tissues from both sites were removed from animals of known age, fixed in 10 percent formalin or Bouin's fixative, and appropriately processed for staining in Mallory's triple stain, hematoxylin and eosin, Oil Red O, or Sudan III. Fetal animals were usually studied in whole-body serial sections.

No evidence of multilocularity has been seen in presumptive subscapular adipose cells in the fetal hamster, but occasional unilocular cells were observed. The cell type which will give rise to the unilocular cells of the subscapular deposits becomes visible at about 12 days of gestation. These precursor cells are numerous by 13 days and are seen as cellular deposits in the intermuscular connective tissue of the subscapular region. In the 14- and 15-day fetus the deposits increase in size, with little evidence of other specialization. Parturition occurs during the 15th day of gestation.

At 1 to 2 days of age, a light-colored tissue deposit is grossly visible in the subscapular region. Histological study reveals the same appearance as described for the 13- to 15-day fetus, except the cells are more

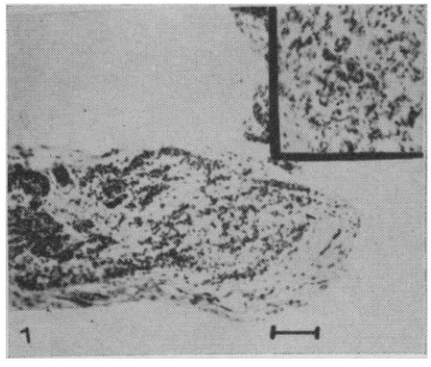
numerous in the older animal. Groups of densely packed cells are visible in a loose connective tissue matrix (Fig. 1). A few small (4 to 20 μ) unilocular cells with peripheral nuclei are present (Fig. 2). Such unilocular cells increase in number and size in subsequent days, and at 4 days of age they are the major cell type present (Fig. 3). Except for their smaller size and more distinct ring of cytoplasm, these unilocular cells closely resemble the "signet-ring" cells of mature white fat. Multilocular cells have not been observed in the process of development of the unilocular cells described here.

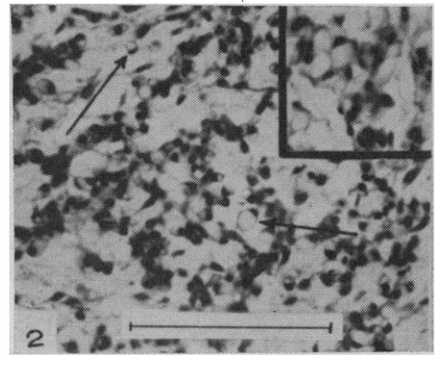
At 3 to 5 days of age, clusters of small cells scattered throughout the unilocular cells of the subscapular deposits become visible (Fig. 3). These clusters are closely associated with blood vessels. The cells within the clusters have a very thin (1 to 2 μ) cytoplasmic ring surrounding a fairly large (7 μ) nucleus, and are tightly packed together in the 5-day-old hamster. At later stages these small cells enlarge and differentiate into brown fat. They will, therefore, be referred to as immature brown-fat (IBF) cells. The IBF cells do not superficially resemble the immature adipose cells in the fetus which develop into the unilocular cells of the subscapular deposits.

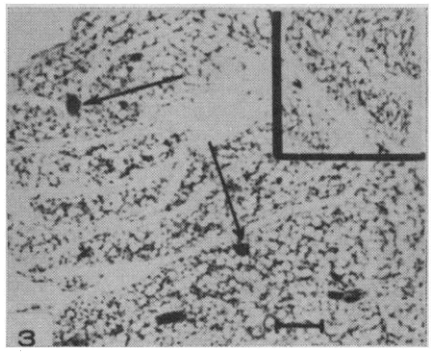
Addition of unilocular cells in the subscapular region continues for several days after the appearance of the IBF cells, so that for a time unilocular and IBF cells are added simultaneously. As new unilocular cells are added they store lipid, but lipid storage does not commence in the IBF cells until about 9 days of age, as judged by the appearance of a few multilocular cells at that time. At 9 to 10 days of age, the IBF cells make up approximately 50 percent of the tissue mass (Fig. 4), and at 14 to 15 days they are the predominant cellular type (Fig. 5), but multilocularity is not pronounced at this time. Lipid droplets become readily visible in the cytoplasm of most IBF cells by 16 to 18 days. Lipid storage is very rapid during this time, and the tissue stains intensely with fat-soluble dyes by 18 to 20 days. Lipid storage continues in the IBF cells, and the tissue is recognizable as multilocular brown fat before 30 days of age (Fig. 6).

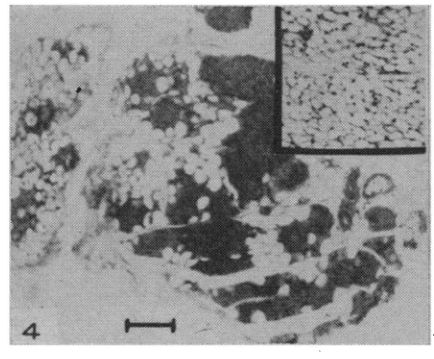
The developmental pattern described for subscapular brown fat is found in all brown-fat deposits in the scapular region. In all cases the appearance of multilocular cells is preceded by the appearance of unilocular cells. We have not studied brown fat in other sites of the body.

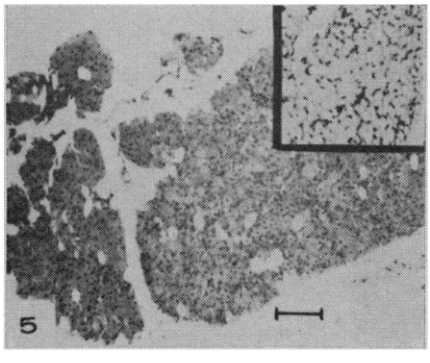
The developmental pattern of inguinal white fat is initially very similar to that described for subscapular fat. In the 13- to 14-day fetus, groups of small, densely packed cells become visible in the inguinal connective tissue. Their appearance is followed by the formation of a few small unilocular cells. Multilocular cells have not been observed before the appearance of the unilocular cells. Great similarity is seen between the development of subscapular and inguinal fats for the first 4 days (Figs. 1–3). At 4 days the unilocular cells in the inguinal fat are histologically indistinguishable from the unilocular cells of the subscapular tissue from the same or different animals of the same age (Fig. 3). The clusters of IBF cells do not appear in the inguinal region, however, and the tissue continues to develop into white fat (Figs. 3–6). No multilocular cells similar to those found in mature brown

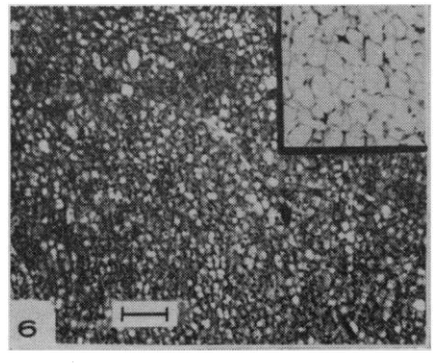












The lower portion of each figure is subscapular fat. Insets are inguinal white fat at the same age and magnification. Line on each figure, 150 μ. Fig. 1. Developing subscapular fat. Age, 2 days. Inset: 2-day inguinal fat. Fig. 2. Higher magnification of sections from Fig. 1. Arrows show unilocular cells of different sizes. Fig. 3. Subscapular fat. Age, 4 days. Note that cells are mainly unilocular. Arrows indicate clusters of immature brown fat (IBF) cells. Inset: inguinal fat. Fig. 4. Subscapular fat at 8 days of age. Note increase in mass of IBF cells over Fig. 3. Inset: inguinal fat. Fig. 5. Subscapular fat at 15 days of age. A few cells are multilocular. Inset: inguinal fat. Fig. 6. Subscapular fat at 30 days of age. Cells multiocular. Inset: inguinal fat.

fat have been observed in developing inguinal fat.

Epididymal and ovarian deposits of white fat show patterns of development identical to that described for the inguinal tissue, but development commences at about 6 days of age. Multilocular cells have not been observed in these tissues.

To our knowledge the early stages of adipose tissue development in the hamster have not been studied previously, although a study of later developmental processes has been presented (*6*). The developmental patterns we have described do not correlate well with previous investigations in other species. There is no doubt that brown-fat cells are laid down in a tissue consisting primarily of unilocular cells. These unilocular cells develop without passing through a multilocular stage, as observed with the light microscope. They closely resemble similar cells found in white-fat deposits. If the unilocular cell types of scapular and inguinal deposits are indeed identical, and if the cells which give rise to brown fat represent a different cell type, as it appears, then we must conclude that brown fat is not related to a precursor form of white fat, but is laid down in a preexisting matrix of white fat, at least in the hamster. If this is a general phenomenon, it might indicate that brown fat is a younger tissue in evolutionary terms than white fat is. Recent studies tend to support this view. Some mammalian forms do not contain brown fat, but the presence of white fat appears universal in mammals. Nor do marsupials contain brown fat (*7*). Our studies of the developing opossum confirm this and demonstrate well-developed deposits of white fat in the scapular region normally occupied by brown fat in many mammalian forms. A large amount of additional work is needed both phylogenetically and ontogenetically before the view that brown fat arose after white fat can be finally accepted. In particular, extensive studies of the relations between the precursors of unilocular cells in the scapular and inguinal deposits are needed in the hamster. The relationships of these precursors to the IBF cell must also be established. The source of the IBF cell is uncertain, but the closeness of its association with the blood vessels may indicate that it arises from the endothelium of blood vessels. This has been previously demonstrated in the cold-adapting rat (*8*).

The source of lipid accumulated in the IBF cells is not known. It probably does not come from the unilocular cells, since these virtually disappear several days before the major lipid accumulation in the IBF cells at about 17 days. We have demonstrated a major accumulation of glycogen in brown fat at 16 days (*9*). This has been demonstrated in other tissues prior to lipid synthesis (*10*). The IBF cells do not store lipid at a time of apparently active storage in adjacent unilocular cells. This can be taken as strong circumstantial evidence that the unilocular cells appearing in the scapular deposits differ, at least physiologically, from the IBF cells deposited later. In addition it may indicate that the brown and white fat cells are not as closely related metabolically as has been previously assumed.

It is our opinion that a great number of uncertainties currently associated with development of adipose tissue can be overcome by studies of the hamster. In this species development of unilocular and multilocular cells is separated in time, and metabolic studies can readily be correlated with morphological events.

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24 July 1967

Antibiotics in the Laboratory-Rearing of Cecropia Silkworms

Abstract. *A mixture of aureomycin and kanamycin prevents a fatal intestinal infection that usually occurs in all cecropia silkworms reared in the laboratory. Thus, for the first time, laboratory experimentation with these larvae and with those of other wild silkworms is practical.*

Studies of the growth and metamorphosis of the wild silkworm, *Hyalophora cecropia,* have been handicapped by the necessity of rearing the larval silkworms outdoors. When reared under laboratory conditions, the larvae invariably succumb to an intestinal disease characterized by diarrhea and the cessation of feeding; the disease routinely strikes in the fourth or early fifth instar. It then spreads rapidly throughout the entire stock despite one's maximal efforts to contain it. Staal (*1*) successfully reared cecropia by raising the temperature to 32°C. At Harvard, neither this nor any of numerous other regimens has met with any success.

Stress has been considered a promoter of intestinal infections in many insect larvae (*2*). The causative bacteria are normally present in small numbers in the gut. When the larvae are subjected to stress, changes in the microenvironment of the gut favor the growth and multiplication of these potential pathogens, resulting in an outbreak of a highly infectious disease. This theory, however, does not satisfactorily account for the rapid spread of the disease once it appears.

One thing is certain—stress is unavoidable in laboratory-rearing. Antibiotics have been used to prevent the outbreak of disease among some lepidopterans. Daily applications of streptomycin (*1*) and of streptomycin, aureomycin, terramycin, or tetracycline (*3*) have prevented disease in *Papilio polyxenes* and *Bombyx mori,* respectively. But, in our experience, neither streptomycin nor a mixture of penicillin, streptomycin, and aureomycin has been effective in protecting cecropia (*4*).

My attention was called to a previously untested antibiotic, kanamycin,