Development of swine adipose tissue: morphology and chemical composition

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Abstract Differentiation and growth of swine subcutaneous adipose tissue was assessed by chemical analysis of tissue components, cell size measurements of isolated adipocytes, and light and electron microscopic observations. At birth all adipocytes were multilocular (contained multiple small lipid droplets), but by day 3 postpartum, many were already differentiated to the unilocular state (one major, central lipid droplet). Microscopic observations of fixed tissue, cell size determinations on isolated adipocytes, and chemical analysis of tissue composition indicated a marked increase in adipocyte size accompanied by an increase in the size of the central lipid droplet with age. Small cells were observed at all ages (in both fixed tissue and isolated cell preparations), yielding biphasic size distributions. Although the adipocyte stem cell was not discerned, an early stage in differentiation, designated an adipoblast, was observed.

Supplementary key words pigs · adipocytes · neonatal development · adipocyte size

Recent studies regarding the lipogenic capacity (both carbon flux and enzyme activities) of swine adipose tissue, the major site of fatty acid synthesis in this species (1), have included comparisons among breeds of swine (2–4), among swine of various ages (2, 3, 5–11), and among several anatomical locations (1, 3, 12, 13). In addition, the relationship between cell size and metabolic activity has been examined in several laboratories (2–6, 10–12). Lipolytic capacity of swine adipose tissue has also recently been investigated (14, 15).

Subcutaneous adipose tissue, the major depot in swine, grows and differentiates with extreme rapidity, as exemplified by a total carcass composition of 1–2% fat at birth, about 15% by 2 wk postpartum, and as much as 40% at approximately 6 months of age (5). Marked compositional changes in adipose tissue structural components as well as adipocyte size occur during this period (10); consequently, the tissue basis on which lipogenic activity (carbon flux or enzyme activity) is expressed has profound effects on the observed pattern of enzyme adaptation (6, 10, 11, 16). In order to further define the changes that occur in adipose tissue of growing swine, we have quantitated selected structural components as well as adipocyte size and have compared these with the light and electron microscopic morphology of swine subcutaneous adipose tissue during the neonatal and growing periods.

METHODS

Crossbred pigs (Chester White sow × Hampshire boar) were obtained from a local breeder. The animals were allowed access to solid feed (18% protein, 4% fat) before weaning at 28–30 days postpartum. The same diet was fed ad lib. to about day 70 postpartum, after which the ration was changed to 15% protein and 4% fat. Animals were slaughtered by a cephalic blow followed by exsanguination. Backfat was removed from a region in the middorsal area beginning at the scapular level and extending cephalad. Tissue for microscopy was removed from the middorsal region, and portions for isolation of cells and for tissue component analysis were taken from comparable bilateral areas. The animals were of the following ages: day 0 (<12 hr), day 3 (2–3 days), day 9 (8–10 days), day 23 (22–24 days), day 40 (40–45 days), day 60 (65–70 days), day 110 (110–115 days), and day 160 (155–165 days).

The portion of tissue for chemical analysis was frozen in liquid nitrogen and stored at −20°C until analyzed for triglyceride, nitrogen, hydroxyproline, and DNA as previously indicated (10). Adipocytes were isolated from a weighed amount of fresh tissue after incubation with collagenase, and the yield was determined by comparison of the triglyceride contents of the tissue and the isolated cells (10). Cell size was determined on 300 isolated adipocytes per sample. An ocular micrometer method was used with methylene blue to distinguish cells from lipid droplets. The average diameter, surface area, and volume were calculated directly (Table 2). The biphasic frequency distribution of cell di-

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TABLE 1. Adipose tissue components

Parameter	Age (days)							
	0	3	9	23	40	60	110	160
No. of samples[a]	2	4	4	4	4	4	3	3
Pig weight, kg	1.2 ± 0.1	1.5 ± 0.1	2.7 ± 0.2	4.8 ± 0.3	13.8 ± 0.1	23.5 ± 1.3	59.8 ± 2.1	84.9 ± 3.7
Hydroxyproline[b]	2.46 ± 0.07	2.96 ± 0.36	4.08 ± 0.08	5.50 ± 0.41	6.04 ± 0.10	4.96 ± 0.39	3.16 ± 0.35	3.19 ± 0.59
Collagen[b,c]	18.3	22.1	30.5	41.0	45.0	37.0	23.6	23.8
Nitrogen[b]	10.00 ± 0.97	12.54 ± 0.39	11.08 ± 0.68	9.94 ± 1.60	13.68 ± 1.02	11.58 ± 0.75	6.75 ± 0.76	4.67 ± 0.54
Protein[b,d]	62.5	78.4	69.1	62.1	85.5	72.2	42.2	29.2
Noncollagen protein[b,e]	44.2	56.3	38.6	21.1	40.5	35.2	18.6	5.4
DNA[f]	1.68 ± 0.19	1.72 ± 0.07	1.53 ± 0.20	1.16 ± 0.08	1.00 ± 0.07	0.84 ± 0.05	0.50 ± 0.03	0.32 ± 0.02
Triglyceride[b]	73 ± 3	139 ± 14	528 ± 60	659 ± 27	511 ± 22	625 ± 52	765 ± 26	900 ± 99

Values are means ± SE of the indicated number of samples except for pig weight, where individual animal weights were used at all ages. Duplicate analyses were obtained on each sample.

[a] Numbers represent pooled tissue samples obtained as follows: day 0, two litters of seven and six piglets each; day 3, four litters of five pigs each; day 9, four litters of three pigs each. Other ages represent tissue samples from individual pigs as indicated, and at least two different litters are represented.

[b] mg/g adipose tissue.

[c] Hydroxyproline × 7.46 (10).

[d] Nitrogen × 6.25.

[e] Protein minus collagen.

[f] μmoles DNA phosphorus/g adipose tissue.

ameter for each sample was decomposed by estimating the parameters of a mixture of two normal distributions as previously indicated (10). Calculation of subpopulation characteristics were made from these parameter estimates (Table 3).

Tissue for light microscopy was fixed in buffered formalin, sectioned at 10 μm, and routinely stained with hematoxylin and eosin, whereas the Verhouff-van Gieson procedure (17) was used for demonstration of connective tissue. Tissue for electron microscopy was dissected into cubes less than 1 mm on edge and fixed in cold 3% glutaraldehyde–1% formalin in 0.1 M cacodylate buffer, pH 7.3. The fixed tissue was stored several days in the cold, washed in cacodylate buffer, and then postfixed in 2% osmic acid–0.1 M cacodylate buffer, pH 7.3, for 1 hr at 4°C. The tissues were dehydrated in alcohol and propylene oxide, embedded in an Epon–Araldite mixture, sectioned, and stained with uranyl acetate and lead citrate. Electron micrographs were obtained only to day 60 because the thin cytoplasmic area surrounding the central lipid droplet in the extremely large adipocytes at older ages was excessively fragile.

RESULTS

Chemical composition

The adipose tissue hydroxyproline levels and the derived collagen values doubled between birth and 3 wk of age, remained at this plateau until day 60, and declined thereafter (**Table 1**). The nitrogen content and the derived protein levels were high from birth until day 60 and were decreased at day 110 and more so at day 160. The day 40 nitrogen value (postweaning) may have been the greatest, but due to the variability this was not clear. The extrapolated noncollagen protein value was quite variable (partially because it was a difference measurement) until days 110 and 160, when it decreased. The DNA content decreased from birth to 160 days of age, whereas the triglyceride content

TABLE 2. Cell parameters

Parameter	Age (days)						
	3	9	23	40	60	110	160
No. of samples[a]	4	4	4	4	4	3	3
D (μm)	24.1 ± 1.5	33.9 ± 2.4	35.7 ± 1.8	49.1 ± 2.2	48.6 ± 1.6	78.6 ± 1.4	70.1 ± 7.7
A ($\mu m^2 \times 10^{-2}$)	26.1 ± 4.8	50.4 ± 6.6	58.6 ± 5.0	96.7 ± 6.8	96.2 ± 5.4	243.0 ± 7.0	215.0 ± 33.4
V ($\mu m^3 \times 10^{-3}$)	20.3 ± 7.1	49.1 ± 10.0	65.2 ± 6.6	113.5 ± 10.6	118.2 ± 8.6	438.6 ± 22.0	390.8 ± 70.6
Yield (%)[b]	27 ± 1	18 ± 2	24 ± 5	30 ± 2	21 ± 5	8 ± 1	7 ± 2
No. of cells/g ($\times 10^{-6}$)[c]	7.5	11.8	11.1	4.9	5.8	1.9	2.5

Values are means ± SE of the directly calculated values from each sample (300 cells per sample). D, diameter; A, surface area; V, volume.

[a] See Table 1, footnote *a*, for an explanation of sample numbers.

[b] mg triglyceride per cells obtained from 1 g tissue/mg triglyceride/g tissue.

[c] mg triglyceride per g tissue/mean volume × density of triolein (i.e., 915 mg/cm^3).

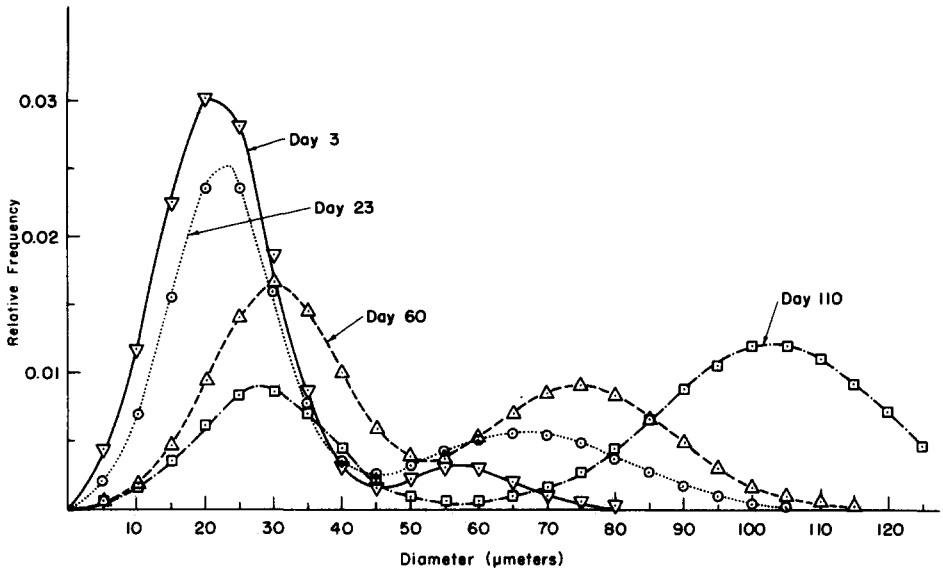


Fig. 1. Distribution of cell size. Relative frequency distributions at selected ages generated with the average parameters of Table 3.

rapidly increased to about 600 mg/g of tissue by day 23, decreased at day 40 to about 500 mg/g of tissue, and thereafter gradually increased.

Adipocyte size

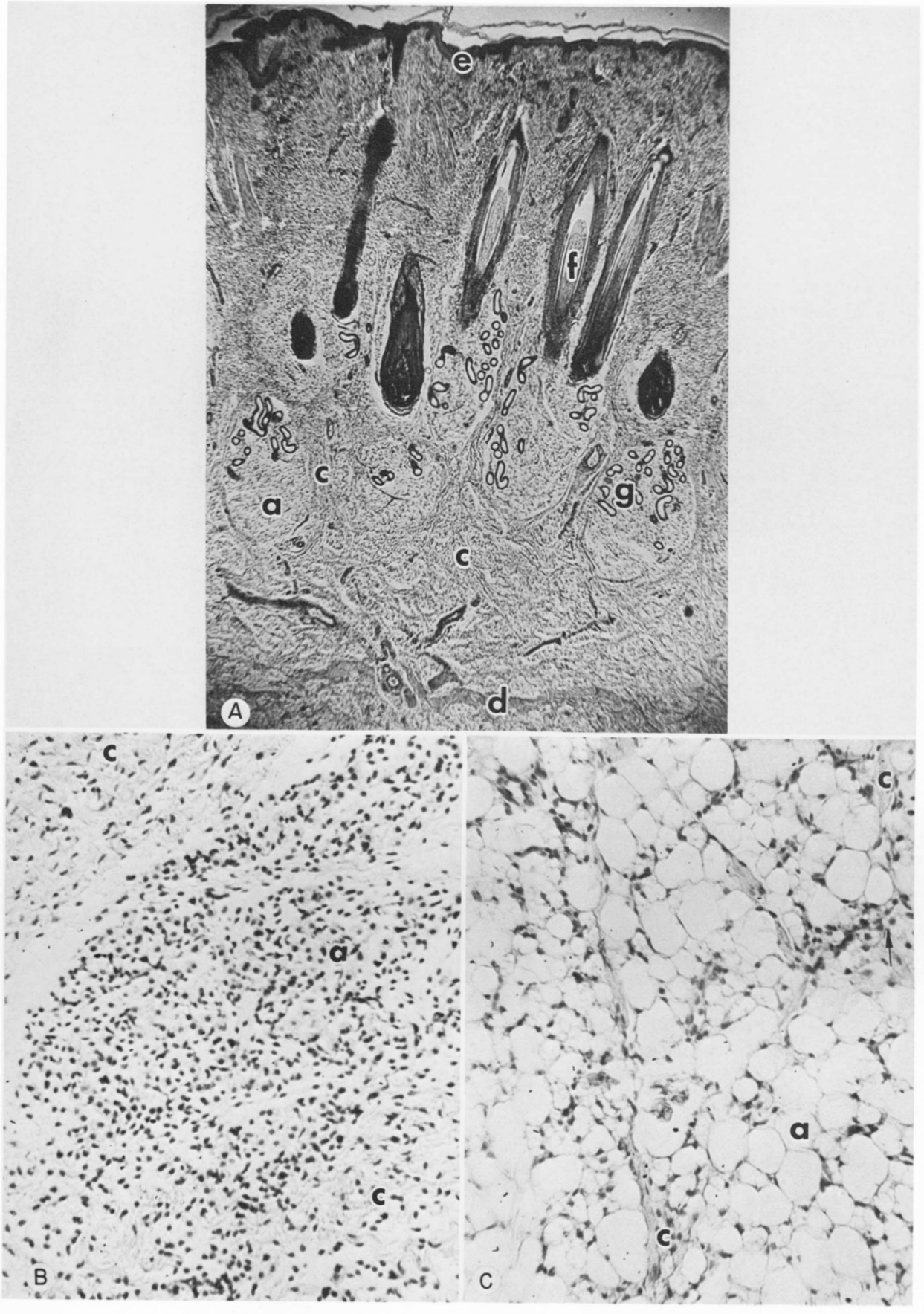
The mean diameter of isolated adipocytes generally increased with age, although there were several periods of only marginal increase (**Table 2**). The measurement of isolated adipocyte diameter did not yield the expected normal distribution, but was biphasic at all ages, as indicated in **Fig. 1**. Consequently, considerable errors were encountered when the average diameter, D (as well as surface area, A, or volume, V, calculated from it), was utilized to assess adipocyte size. The magnitude of the age-related increase in cell size was more definitively demonstrated by the surface area or volume values calculated from individual cell diameters rather than from the mean diameter (Table 2). The overwhelming contribution of the larger cells to the total surface area and particularly the volume is evident in **Table 3**, where cell diameter, surface area, and volume are expressed per population of adipocytes. The low yield obtained for cell isolation from 110- and 160-day animals results in questionable estimates of derived cell parameters at these ages. However, recent experiments with improved isolation techniques (yields > 50%) have also produced biphasic distributions at all ages.[3] It is not possible to discern any internal structure in the isolated cells, and therefore multilocular and unilocular small cells were not distinguished in these preparations. Also, no evidence was obtained in isolated cell preparations for the spindle-shaped cells seen in **Fig. 2**, *B*, possibly because they became spherical after isolation or because they did not float with the adipocytes during the preparative centrifugal washings.

TABLE 3. Mean cell diameter, surface area, and volume

Age	No. of Samples[a]	Parameter[b]	Population I[c] Mean	SD	%[d]	Population II Mean	SD	%
days								
3	4	D	21.6	11.9	91	57.1	12.3	9
		A	1,910		64	10,700		36
		V	10,100		47	111,000		52
9	4	D	24.4	13.6	75	63.5	15.6	25
		A	2,450		35	13,400		65
		V	14,700		22	158,000		78
23	4	D	22.5	11.2	69	66.4	22.3	31
		A	1,980		22	15,400		78
		V	10,400		10	205,000		90
40	4	D	21.8	10.4	38	65.7	17.7	62
		A	1,840		7	14,600		93
		V	9,160		3	181,000		97
60	4	D	30.2	13.6	56	74.6	19.8	44
		A	3,440		19	18,700		81
		V	24,100		10	262,000		90
110	3	D	28.3	14.1	31	102.9	22.9	69
		A	3,150		4	34,900		96
		V	20,800		1	655,000		99
160	3	D	18.3	10.3	32	98.3	28.3	68
		A	1,390		2	32,900		98
		V	6,260		0	621,000		100

[a] See Table 1, footnote *a*, for an explanation of sample numbers.
[b] D, mean diameter, μm; A, mean surface area, μm², derived from mean diameter and SD; V, mean volume, μm³, derived from mean diameter and SD.
[c] Populations I and II are separated according to age; this designation does not imply any necessary relationship within a given population at all ages.
[d] Percentage contribution to total.

[3] Mersmann, H. J., J. R. Goodman, and L. J. Brown. Unpublished data.



Morphological structure

The dorsal subcutaneous adipose tissue (Fig. 2, *A*) extended from the lower dermal layer of the skin to the muscle layers. (The dermal and epidermal structure of newborn [18] and adult [19] swine skin have been described.) The adipose tissue was divided into upper and lower layers by a connective tissue barrier observable in most animals on the day of birth. Near the bases of the hair shafts, connective tissue surrounded areas containing the tubular sweat glands and adipocytes. The remaining portion of the upper adipose layer as well as the lower layer was composed of less defined areas containing groups of adipocytes surrounded by loosened connective tissue fibers. In newborn and late fetal[3] animals, instead of adipocytes with large central lipid droplets (unilocular) these same areas contained small cells, most of which were spindle-shaped or fibroblastlike although they did not stain as connective tissue (Fig. 2, *B*). Careful examination of these cells (by continuous adjustment of the focal plane) indicated that many were more round in appearance and contained several lipid droplets (multilocular adipocytes). The general increase in adipocyte size was extremely rapid (Fig. 2, *C*) and continued to day 160.

Qualitative assessment of adipocyte diameter in the fixed sections indicated many adipocytes with diameters similar to the mean obtained with the isolated cell preparation from the same animal (Table 2). There was considerable variation among animals at all ages in regard to both the largest observed cell size and the proportion of larger cells. This variability was exhibited at day 0 by the relative proportion of spindle-shaped cells compared with multilocular adipocytes as well as the size of the latter cell type. Although unilocular cells were evident in all day 3 specimens examined, there was considerable variability among animals both in size and proportion of unilocular cells; by day 9, there were more unilocular cells. With increased age and concomitant enlargement of adipocytes, the supportive connective tissue was progressively loosened. Multilocular cells and small unilocular cells (<30 μm) were observed at all ages (up to and including 160 days) but were easily overlooked because of the overwhelming area occupied by the larger cells. (Note the percent of total volume occupied by the large cells in Table 3.)

Electron microscopic examination revealed that the multilocular adipocyte was generally round and contained a centrally located nucleus (**Fig. 3**). As the amount of lipid increased and the cell differentiated toward the unilocular state (**Fig. 4**), the nucleus achieved a peripheral location and conformed to the limited cytoplasmic space. The chromatin, although partially concentrated at the periphery, would be described as diffuse at all stages of development.

Cytoplasmic organelles (Golgi, mitochondria, smooth and rough endoplasmic reticula, and free ribosomes) were relatively abundant in early stages of differentiation (Fig. 3). As the amount of lipid increased and the cell differentiated toward the unilocular state, the cytoplasmic space became smaller and the organelles appeared to decrease in number.

Electron microscopy revealed only multilocular adipocytes on day 0, whereas by day 3 (Fig. 4) many cells had one to three large central lipid droplets with additional smaller peripheral droplets in the cytoplasmic space. The typical adipocyte or signet-ring cell, i.e., a large central lipid droplet surrounded by a thin peripheral cytoplasmic ring containing small lipid droplets and cell organelles, was evident by day 9. As the animals increased in age, larger unilocular adipocytes containing progressively larger central lipid droplets with concomitantly thinner peripheral cytoplasmic areas were observed.

The larger lipid droplets presumably accumulated and increased in size by coalescence of smaller droplets (**Fig. 5**), and evidences of this were common at all ages. Included in the lipid droplets of younger animals were crystalline-appearing clear areas (Figs. 3 and 4), which suggested a loss of dissolved material in dehydrating solvents (possibly the material was cholesterol). Another type of lipid droplet inclusion, more prevalent in older animals, was an electron-translucent area filled with varying amounts of a granular material that may be the result of cytoplasmic trapping upon coalescence of lipid droplets (data not included).

Collagen was abundant at all ages and existed as areas of massed fibers located throughout the adipose tissue layers (Fig. 4). The collagen structure and banding pattern did not appear to change with differentiation. At the periphery of individual adipocytes, there were numerous projections similar to collagen fibers that at higher magnification did not have the characteristic banding patterns evident in collagen and were smaller in diameter (Figs. 4 and 5). The nature of these fibers was not immediately apparent, although they may be elastin, a less mature form of collagen, or specialized structures.

Blood vessels of various types, i.e., arterioles, venules, and capillaries, were abundant; in many cases there was an intimate association between a capillary and adjacent adipocytes (Fig. 4).

Stem cells

Four similar cell types, easily observed at days 0 and 3, could be considered as the adipocyte stem cell. (*1*) The

Fig. 2. Light micrographs of adipose tissue fixed in buffered formalin sectioned at 10 μm and stained with hematoxylin and eosin. *A*, architecture of day 0 adipose layer, ×20; *B*, day 0 adipose tissue, ×128; *C*, day 9 adipose tissue, ×128. Indicated on the micrographs are adipocytes (*a*), connective tissue (*c*), connective tissue layer separating upper and lower adipose layers (*d*), epidermis (*e*), hair follicles (*f*), sweat glands (*g*), and, in *C*, multilocular adipocytes (*arrow*).

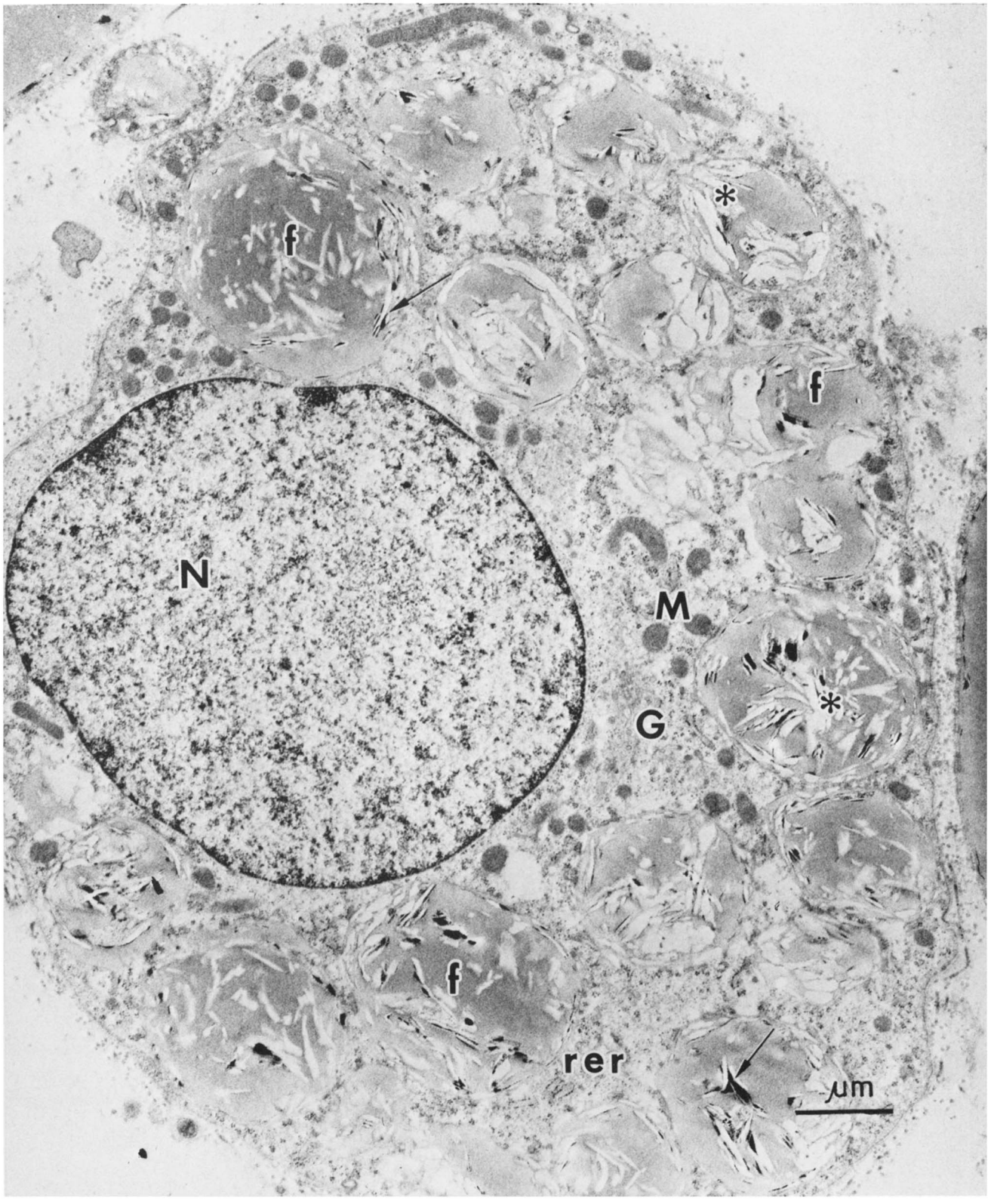


Fig. 3. Adipocyte, from a day 0 pig, with a rounded nucleus (*N*) containing dispersed chromatin. Numerous fat droplets (*f*) are evident with both thin strips of electron-dense material (*arrows*) and electron-translucent crystalloid shapes (*asterisks*). There are several mitochondria (*M*) and a scattering of rough endoplasmic reticulum (*rer*) and free ribosomes. There are a few Golgi vesicles (*G*) present. The scale is 1 μm.

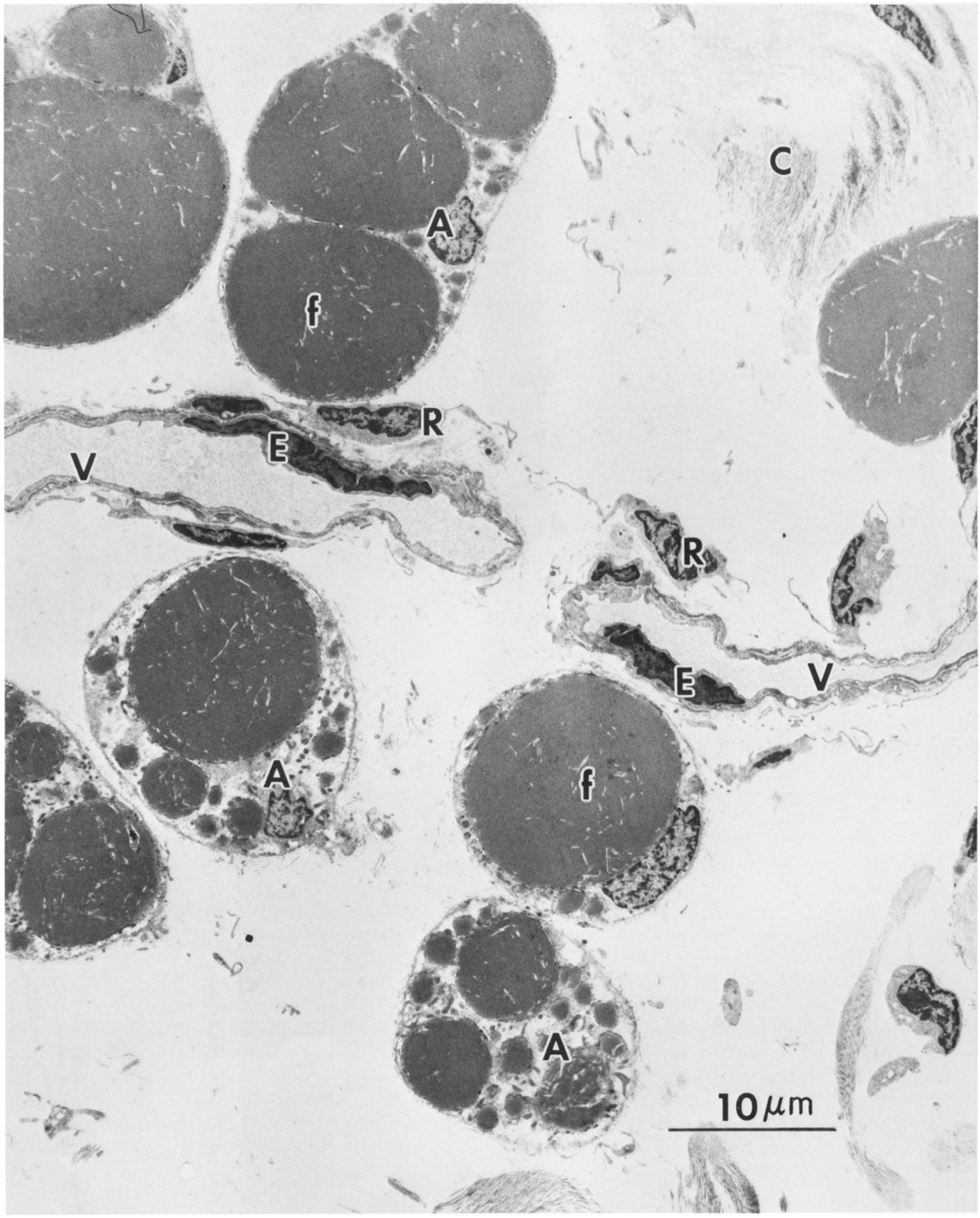


Fig. 4. This is a low magnification from a day 3 pig. There are several adipocytes (*A*) containing numerous locules of lipid (*f*). There are two blood vessels (*V*) with endothelial cells (*E*) and supporting reticular cells (*R*). Patches of collagen (*C*) are also present. The scale is 10 μm.

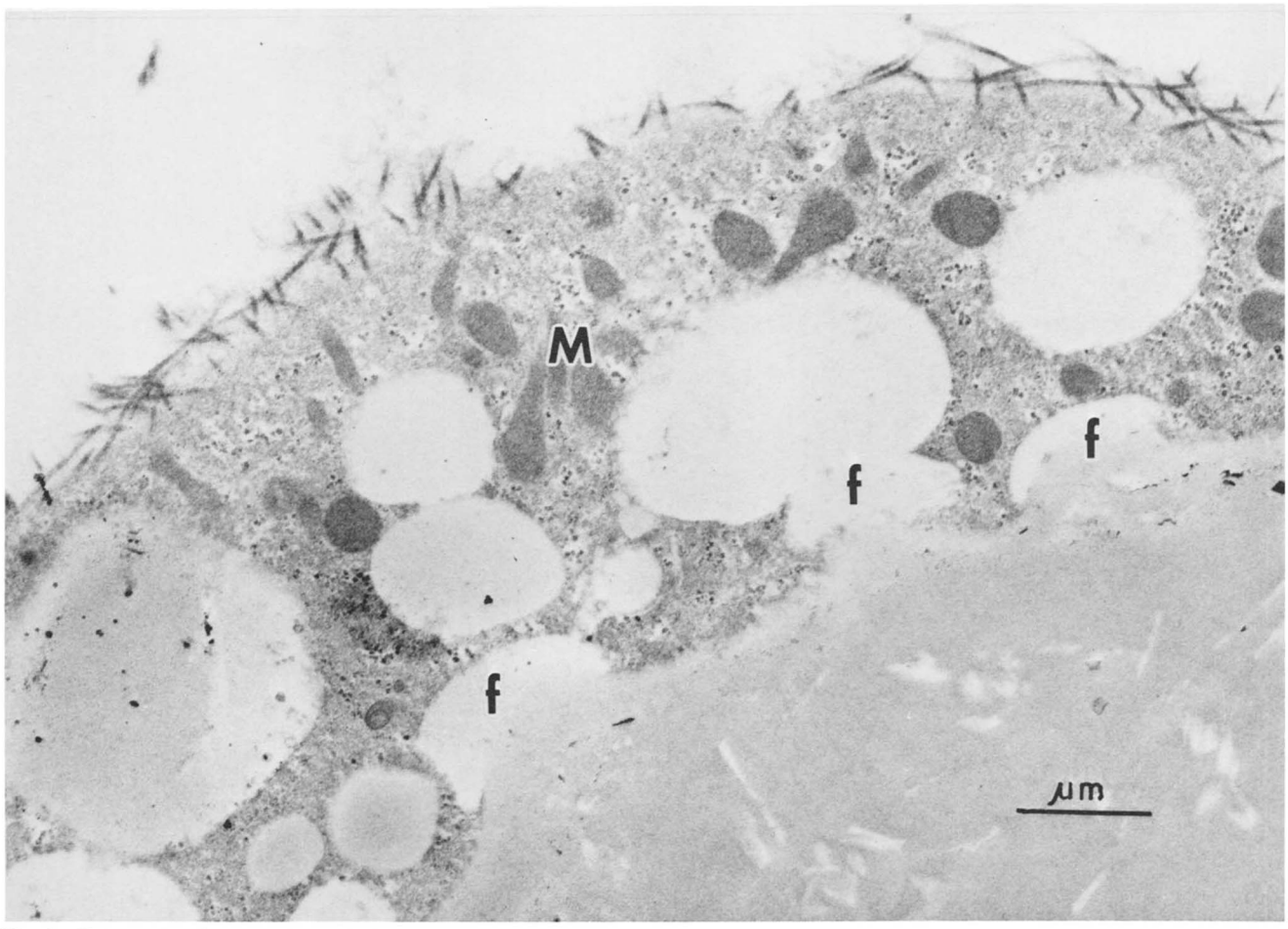


Fig. 5. This portion of the cytoplasm of a day 40 adipocyte demonstrates the coalescence of small lipid droplets (*f*) into the large central one. There are several mitochondria (*M*) and a scattering of ribosomes present. The surface of the cell has numerous short fibers that do not appear to be collagen. The scale is 1 μm.

elongate endothelial cell formed the lining of the capillaries (Fig. 4). (*2*) The reticular cell was contiguous to the endothelial cell and was distinguished by the accentuated spindle shape produced by long, thin, cytoplasmic tails (Fig. 4). A few of these cells contained a cytoplasmic lipid droplet. (*3*) An elongate, fibroblast-type cell with an elongated nucleus that occupied a considerable portion of the cell was observed in the collagen mass and was assumed to produce collagen (Fig. 4). (*4*) A somewhat elongated cell with a large ovoid-to-round nucleus, numerous mitochondria, abundant rough endoplasmic reticulum, many free ribosomes, and one or more cytoplasmic lipid droplets was designated an adipoblast (**Fig. 6**). A similar cell type without lipid droplets was also observed.

DISCUSSION

Tissue composition

The age-related fluctuations in tissue components observed in this study were similar to previously reported data (10). Consistent with an increase in adipocyte size during the animals' growth was the large increase in adipose tissue lipid, per unit weight, concomitant with the decrease in the concentration of DNA, protein, and collagen: components usually associated with cellularity. The derived noncollagen protein value, which may be an indicator of the number of adipocytes (10, 20), also decreased markedly at the oldest ages. Some of the minor differences between the current and the previous study (10) in both developmental patterns and concentrations of components may be the result of different weaning ages (28 and 21 days, respectively), of different breeds (Chester White × Hampshire and Duroc × Chester White, respectively), of dietary regime (solid feed plus sow's milk and sow's milk only, respectively), or of relatively small sample size in both studies.

Cell size

As indicated by size measurements on isolated adipocytes and confirmed by measurements on cells in fixed tissues as well as by the indirect evidence provided by chemical anal-

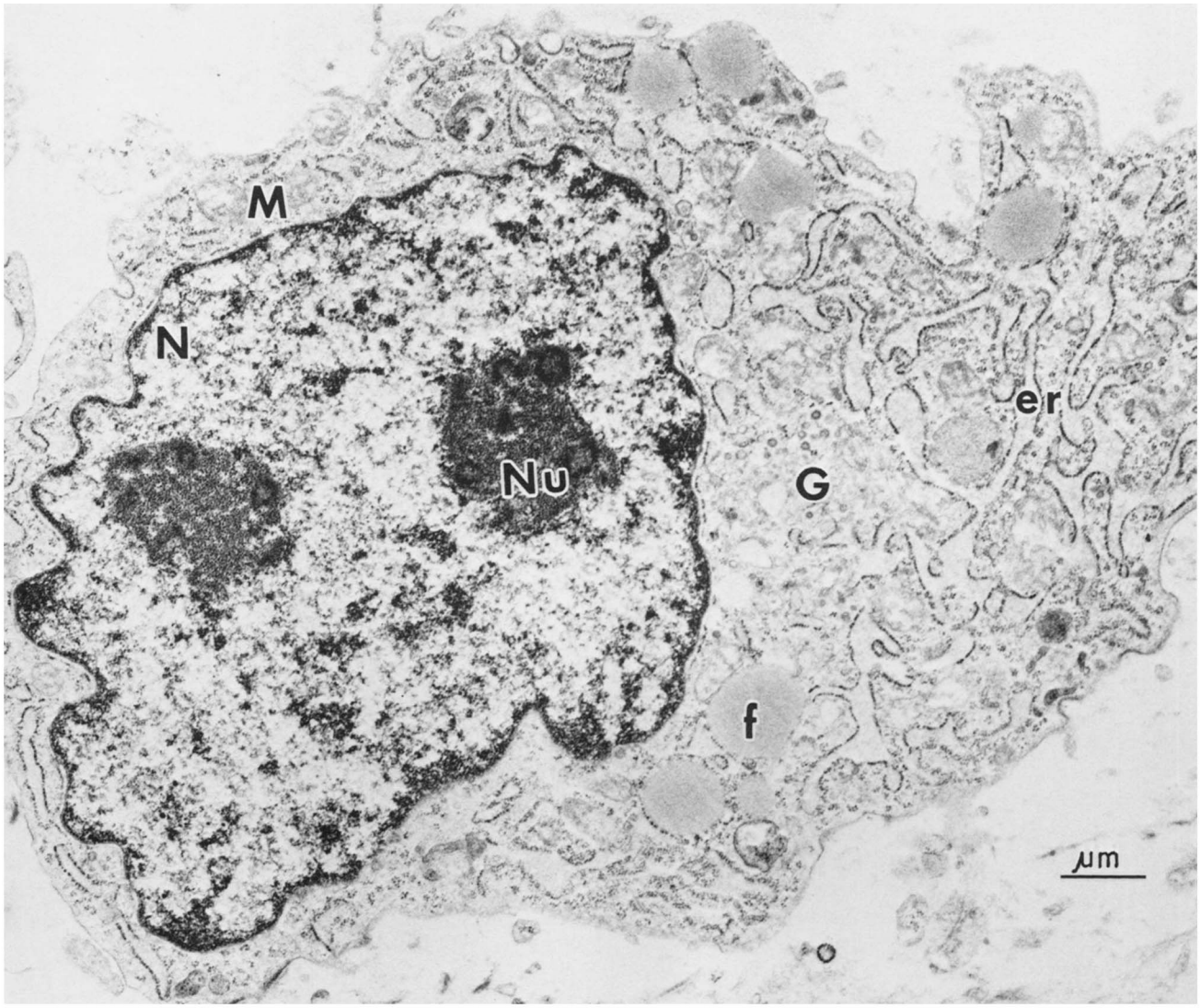


Fig. 6. An adipoblast with a reticular nucleus (*N*) containing two nucleoli (*Nu*). The cytoplasm has a few lipid droplets (*f*), extensive endoplasmic reticulum (*er*), mitochondria (*M*), and a Golgi (*G*). The scale is 1 μm.

ysis of the tissue, the average adipocyte size gradually increased with animal age and proliferation of the depots. Several groups previously observed the age-related increase in swine subcutaneous adipocyte size (2, 5, 6), although none reported the biphasic distribution of cell diameter measured on isolated adipocytes in these and previous studies from this laboratory (10). Numerous lines of evidence lead to confirmation of the presence of small cells in swine adipose tissue until 160 days postpartum. For example, light microscopic observations of the fixed, stained tissue indicated small cells at all ages although they were more difficult to visualize at older ages because of distortion of the paraffin sections during preparative solvent extraction of lipid. (The area occupied by large fat droplets yielding holes in the sections may be approximated by attention to the area calculations in Table 3.) The acuity of the observer was also lessened by the thick sections (10 μm) necessary to hamper complete disruption. However, at all ages, including day 160, careful observation coupled with continual manipulation of the focal plane indicated large numbers of small adipocytes. Observations on frozen sections stained with hematoxylin and eosin and on suspended isolated cells exposed to Sudan black and trypan blue as well as to methylene blue also indicated small cells. In the presence of the latter dyes, cells were distinguished from lipid droplets by their appearance. At times, the formation of lipid droplets was observed upon rupture of individual cells, providing immediate contrast between cells and lipid. We have recently separated a population of cells with lipolytic activity from animals 80 days of age by means of a nylon screen with a grid size of about 40 μm.[3] Further, this population as well as the total isolate can be fixed in glutaraldehyde, in which cells are easily distinguished by their rough surface compared with the smooth outline of lipid

droplets. Small cells are obvious in both preparations. Fixation in osmium did not as readily distinguish between cells and lipid droplets.

Our ocular method of cell size determination on free adipocytes does not discard the cells smaller than 25 μm as is the case in the usual methods employing osmium fixation followed by a filtration procedure. Disregarding probable cell shrinkage in the osmium procedures, the cells eliminated (<25 μm) constitute the major portion of population I (Fig. 1 and Table 3). Because cell number increased in rats (21, 22) only to about 3 months of age and in mice (23) to about 2 months of age, observations of small adipocytes would not be expected in most studies in these species even if methods to conserve the small cells were employed. However, Anderson and Kauffman (5) observed a continued increase in swine adipocyte number to 5 months of age and, consequently, observations of small adipocytes would be expected in swine adipose tissue at all of the ages in the current study.

The tissue parameter used as the base for expression of metabolic data obtained from growing swine adipose tissue influences not only the quantitative nature of the data but also may drastically alter the qualitative pattern (6, 16). Although there is general agreement that the most meaningful expression of swine adipose tissue metabolic data is on a cell basis, elimination of the small cells (<25 μm) from size and particularly from number considerations may be misleading. For example, in backfat of growing swine, positive correlations of malic enzyme activity (expressed on a cell basis) to the cell volume and to the soluble protein per cell have been indicated (2). On the other hand, our data (10) indicated a maximum in adipocyte lipogenic capacity (carbon flux from glucose into lipids) at 60–80 days postpartum with a subsequent decrease regardless of the tissue base used (wet weight, cell, unit surface area, or unit volume). It thus appears that the inclusion of the small cells in the calculation of cell number may greatly influence not only the mean cell size parameters but also the observed metabolic patterns. The measurement of cell size in fixed tissue slices as indicated by Sjöström, Björntorp, and Vrána (24) may be the method of choice for subcutaneous adipose tissue from growing swine because it avoids the loss of small cells as well as the potential loss of large adipocytes by rupture during collagenase isolation procedures. Although large human adipocytes isolated by collagenase procedures were not selectively ruptured (25), no definitive information is available regarding rupture of swine adipocytes.

Morphological structure

Human and mouse white adipose tissues (light microscopic observations) differentiated from highly vascularized regions within connective tissue (26, 27). Cells were observed that were indistinct from other mesenchymal cells except that many had small fat droplets. Wassermann (26) described the origin of the mesenchymallike adipocyte stem cell from the blood vessel wall, whereas Simon (27) described the differentiation of the adipocyte from a reticular cell. Similar observations were recently reported for the development of fetal swine adipose tissue in the orbit of the eye, around the thoracic duct, and in the prescapular lymph node (28). Preadipose lobules containing multilocular adipocytes were abundant during the 3rd month of gestation; unilocular adipocytes were observed by the terminal stages of pregnancy (4th month). In contrast, we have observed no unilocular adipocytes in swine subcutaneous adipose tissue in the newborn (confirmed by electron microscopy). The discrepancy between the two swine studies may be the result of different anatomical depots or possibly the use of only light microscopy in the former work (28).

Rat epididymal white adipose tissue differentiated from an elongate fibroblastlike cell containing abundant rough endoplasmic reticulum, few Golgi, an elongate nucleus, round-to-elongate mitochondria, and several small lipid droplets (29, 30). The gradual increase in lipid (only beginning by 9 days postpartum) was accompanied by a decrease in the rough endoplasmic reticulum and by coalescence of lipid droplets to yield the typical signet-ring shape. Our electron microscopic observations on developing swine adipose tissue were similar except that, in the newborn pigs, the partially differentiated state (no unilocular cells) was observable for less than 2 days after birth. In developing swine adipose tissue, we have not seen the extensive glycogen deposits noted by Napolitano (30) for rat adipose tissue, nor was any change in mitochondrial shape apparent during differentiation, nor were abundant pinocytotic vesicles present. These may be the result of species differences.

The stem cell from which the swine adipoblast (Fig. 6) has differentiated was not elucidated. Although the endothelial cell lining the capillary, the supportive reticular cell, and the collagen-producing fibroblast are all potential precursors, a cell type similar to the adipoblast but without lipid droplets was quite common in tissue from young animals and appeared to be a more likely precursor. Delineation of the adipoblast was possible only when lipid droplets were present in the cytoplasm (as in Fig. 6), although even this designation may be misleading, for reticular cells sometimes have a lipid droplet.

Investigations of the development of swine adipose tissue in regard to morphology, cell size, and chemical composition seem warranted in other adipose depots at various fetal ages and with several nutritional variables. Especially needed is a quantitative, morphometric analysis of the developing swine adipocyte.

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