Cellularity of bovine adipose tissue

R. L. Hood[1] **and C. E. Allen**

Department of Animal Science, Meat Science Laboratory,
University of Minnesota, St. Paul, Minnesota 55101

Abstract Subcutaneous and perirenal adipose tissue from bovine animals that had different fat deposition patterns were characterized in terms of the weight of the adipose tissue organ and adipose cell number and mean cell size as determined by electronic counting of osmium-fixed adipose cells. Similar parameters were also measured in the interfascicular adipose tissue dissected from four muscles. Adipose tissue from animals of the leaner Holstein breed contained smaller cells than the respective tissues from the fatter Hereford × Angus animals. The small subcutaneous deposit in the Holstein animals was due to a small number of adipose cells that were small in size. During growth of the bovine animal, an increase in adipose tissue mass was accompanied by cellular hypertrophy and hyperplasia. However, by 14 months of life hyperplasia was complete in all but the interfascicular adipose tissue. In the 14-month-old Hereford × Angus steers, interfascicular adipose tissue had an appreciable number of small cells and a bimodal distribution for cell diameter. The results of this study suggest that interfascicular adipose tissue is a late developing depot and that hyperplasia is still an active process in this depot at 14 months of life, whereas hyperplasia appears to be nearly complete in the subcutaneous and perirenal depots of bovine animals by about 8 months of life or shortly thereafter. Correlation coefficients indicated that intramuscular lipid content was positively related to the number of interfascicular adipose cells per 100 g of muscle in four different muscles. However, average cell diameter and volume were significantly correlated to intramuscular lipid content in only one of the four muscles studied.

Supplementary key words adipose cell · cell size · cell number · subcutaneous fat · perirenal fat · interfascicular fat · breed and tissue differences

Studies of cellularity of adipose tissue have been reported for man (1, 2), rats (3–5), mice (6), and swine[2] (7). Limited and less extensive information is available for the bovine species where fat deposition has economic as well as scientific interest. Bell (8) reported on the increase in fat cell diameter of bovine muscle during growth. Moody and Cassens (9) found that bovine adipose cells varied in size according to depot site (subcutaneous, intermuscular, interfascicular). Furthermore, they found that the largest average adipose cell diameter in the interfascicular depot was associated with the largest adipose cell mass within a particular muscle.

Allen (10) reviewed some of the technical and economic aspects of fat deposition in meat animals. It was evident that interfascicular adipose tissue, the visible portion of which is known as marbling, makes a positive contribution to the palatability characteristics of meat and generally increases the economic value of the beef carcass due to a higher quality grade. However, it was also noted that excessive deposition of subcutaneous, intermuscular, and perirenal fat does not contribute to meat quality, but results in excessive fat trim and economic losses. Review (10) of 1968 United States Dept. of Agriculture estimates indicated that retailers in the United States trimmed greater than two billion pounds of fat from fed beef cattle. Due to differences in cost of production and value received, this amounted to greater than a billion dollar loss. Thus, it is evident that the site and quantity of fat deposited in beef cattle are of major importance to meat quality and efficiency of production.

In the present study, the technique of Hirsch and Gallian (11) was employed to study the cellularity of bovine subcutaneous and perirenal adipose tissue during growth and the differences between animals with different propensities to deposit fat. In addition, the cellularity of interfascicular adipose tissue was studied in muscles with known differences for accumulating lipid.

MATERIALS AND METHODS

Animals

Six 14-month-old steers from each of the Holstein and H×A breeding groups and six 14-month-old and six 8-

Paper no. 8026, Scientific Journal Series, Minnesota Agricultural Experiment Station, St. Paul.

Abbreviations: H×A, Hereford × Angus.

[1] Present address: C.S.I.R.O., Division of Food Research, North Ryde, New South Wales, Australia.

[2] Hood, R. L., and C. E. Allen. Unpublished data.

TABLE 1. Bovine carcass and adipose tissue characteristics

Trait	Animal Groups			
	Holstein	Hereford × Angus	Hereford	Hereford
Age (mo)	14	14	14	8
Carcass wt (kg)	$277.9^{a} \pm 2.0$	$273.0^{a} \pm 12.8$	$168.4^{b} \pm 4.2$	$111.3^{c} \pm 2.9$
Perirenal adipose tissue				
% of carcass	$3.4^{a} \pm 0.5$	$3.9^{a} \pm 0.5$		$0.3^{b} \pm 0.01$
Cell diameter (μm)	$119.5^{a} \pm 3.9$	$138.2^{b} \pm 4.4$	$75.0^{c} \pm 3.1$	$55.5^{d} \pm 3.5$
Cell volume (μm^3) (10^4)	$115.8^{a} \pm 11.7$	$167.8^{b} \pm 20.3$	$25.8^{c} \pm 2.8$	$10.4^{d} \pm 2.7$
No. cells/perirenal depot (10^9)	$9.65^{a} \pm 1.14$	$7.44^{a} \pm 0.58$		$3.15^{b} \pm 0.29$
Subcutaneous adipose tissue				
Thickness (cm)	$0.36^{a} \pm 0.03$	$1.22^{b} \pm 0.10$	$0.23^{c} \pm 0.02$	$0.25^{ac} \pm 0.08$
Cell diameter (μm)	$106.9^{a} \pm 6.1$	$133.3^{b} \pm 3.9$	$67.4^{c} \pm 4.0$	$60.1^{c} \pm 4.1$
Cell volume (μm^3) (10^4)	$92.0^{a} \pm 10.2$	$150.3^{b} \pm 11.9$	$20.6^{c} \pm 3.1$	$14.6^{c} \pm 5.1$
Standardized rib				
Weight (kg)	$4.67^{a} \pm 0.10$	$5.43^{b} \pm 0.29$		
Weight of dissectible subcutaneous fat (g)	$296.8^{a} \pm 18.9$	$735.3^{b} \pm 56.1$		
Weight of fat-free tissue (kg)	$4.37^{a} \pm 0.11$	$4.69^{a} \pm 0.25$		
No. cells/standardized rib (10^8)	$3.75^{a} \pm 0.62$	$5.73^{b} \pm 0.38$		

^{a,b,c} Means on the same line with different superscripts differ significantly ($P < 0.05$).

month-old Hereford steers were studied at average live weights of 475, 470, 320, and 215 kg, respectively. The Holstein and H×A steers were fed a high energy corn diet (12). The Hereford steers were fed a low energy roughage diet. All of the animals had growth rates typical of steers fed these diets and in no case was there a period of weight loss or abnormally rapid weight gain.

Tissue sampling and analyses

Subcutaneous adipose tissue samples were taken dorsal to the 13th rib and 13 cm lateral to the midline. Perirenal adipose tissue was sampled adjacent to the left kidney. Interfascicular adipose tissue was dissected from the longissimus dorsi, semimembranosus, trapezius, and pectoralis profundus muscles from the H×A steers.

Samples of all adipose tissues were fixed in osmium tetroxide and processed according to the methods outlined previously (11) for determining fat cell diameter, volume, and number.

A standard 9th, 10th, and 11th rib, which is commonly used by meat scientists to predict carcass composition (13), was used to estimate the relative amount of subcutaneous adipose tissue in the Holstein and H×A carcasses. The rib was cut caudal and adjacent to the 8th and 11th ribs and on a line parallel to the spinal cord and midway between the sternum and the point of attachment of the rib to the thoracic vertebrae. All subcutaneous adipose tissue on this standardized rib was dissected and weighed. The weight of dissectible fat and the number of adipose cells per gram of tissue were used to calculate the number of adipose cells per standardized rib.

Statistical analyses

Statistical analyses of the data were conducted according to procedures described by Steel and Torrie (14). Differences between treatment means were determined by comparing each treatment with each other treatment using a simple F test.

RESULTS AND DISCUSSION

Growth and fat distribution

Mature animals of Holstein and H×A breeding had different patterns of fat deposition (Table 1). The quantity of perirenal fat was similar in both groups, whereas steers of the beef breeding group (H×A) deposited significantly ($P < 0.05$) more fat in the subcutaneous adipose depot. This difference was reflected in the subcutaneous fat thickness and the weight of fat dissected from a standard rib (Table 1). The 8-month-old (215 kg) Hereford steers deposited only small quantities of fat in both the subcutaneous and perirenal adipose depots. No increase in fat thickness was observed in the Hereford steers during growth from 215 kg to 320 kg live weight (Table 1). However, no increase in fat accumulation was expected because these animals were fed a low energy, roughage ration during this period of growth. The younger Hereford steers had a similar, although slightly lower, subcutaneous fat thickness than the Holstein steers, but significantly ($P < 0.05$) less subcutaneous fat than the H×A steers.

Steers of the Holstein breed contained significantly ($P < 0.05$) less dissectable subcutaneous fat from a standard 9-10-11th rib than H×A steers of equivalent age and live weight (Table 1). Standard ribs from the H×A steers were heavier ($P < 0.05$) than the corresponding Holstein ribs; however, no significant difference ($P > 0.05$) was observed when the rib weights were compared after removing the subcutaneous fat (Table 1).

Cellularity during growth and development

Fig. 1 depicts the volume frequency distributions of adipose cells isolated from bovine subcutaneous and perirenal adipose tissue. Each bar of the histogram represents the contribution made by adipose cells within a specified diameter range to the total volume of a unit weight of adipose tissue. Both adipose tissue sites in the 8- and 14-month-old Hereford steers had similar volume frequency distributions. However, in the 14-month-old H×A steers the volume frequency distribution was located in the larger diameter ranges, indicating that the adipose cells had undergone cellular hypertrophy. Evidence of cellular hypertrophy was also apparent from comparing data obtained for H×A steers with that for Hereford steers (Table 1). In these two breeding groups, which had a similar growth rate and fat deposition pattern, adipose cell diameter and volume were approximately 2.2 and 10 times greater, respectively, in the subcutaneous adipose tissue of the 14-month-old H×A steers than in the 8-month-old Hereford steers. Perirenal adipose cell volume was observed to increase 16-fold during this growth period. It is possible that part of the difference between the 14-month-old H×A steers and either the 8- or 14-month-old Hereford steers was genetic. However, other data (15) indicate that the Hereford and Angus breeds have similar quantities of subcutaneous fat. Therefore, one would predict that these differences are developmental and not due to major differences in genotype. Smaller differences (perirenal significant $P < 0.05$) in both adipose cell volume and diameter were observed in either subcutaneous or perirenal adipose tissue between the 8- and 14-month-old Hereford steers fed a low-energy diet (Table 1). In the 14-month-old animals, the perirenal adipose tissue contained adipose cells with a larger mean volume than the subcutaneous adipose cells (Table 1).

As with the young porcine animal,[2] cellular hypertrophy was not solely responsible for the increase in perirenal adipose tissue observed between 8- and 14-month-old Hereford and H×A steers, respectively. In comparing these two groups, the number of adipose cells per perirenal depot increased from 3.15×10^9 to 7.44×10^9 (Table 1). Therefore, in the young bovine animal, an increase in adipose tissue mass was achieved by both cellular hypertrophy and hyperplasia. Porcine[2] (7) and rat (3, 4) adipose tissue have been shown to grow in a similar manner during early development. However, beyond a certain stage, adipose tissue growth in these species is due solely to cellular hypertrophy. Although not clearly demonstrated, a similar situation presumably exists in the bovine animal; that is, at some point in time adipose tissue growth is due exclusively to cellular hypertrophy.

The absence of small cells (< 70 μm) in the H×A perirenal and subcutaneous tissues (Figs. 2 and 3) indicates that cellular hyperplasia was complete in the steer

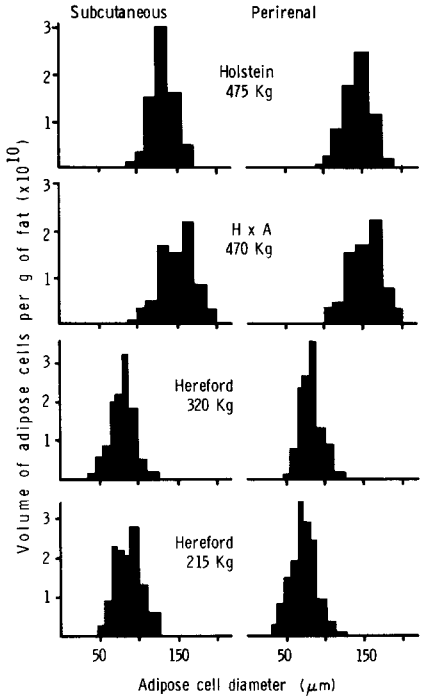

Fig. 1. Volume frequency distribution of adipose cells from bovine adipose tissue (H×A, Hereford × Angus animal). The values (ordinate) are to be multiplied by 10^{10}.

before 14 months of life. This was also true for the 14-month-old Holstein steers (Figs. 1 and 2). In addition, very few small cells (25–35 μm) were observed in the subcutaneous and perirenal adipose tissue of the 8-month-old Hereford steers (Figs. 2 and 3), which indicates that hyperplasia was nearly complete in the steer by 8 months or shortly thereafter. The final adipose cell number per animal is difficult to ascertain due to the variation in cell size between depots and consequently the number of cells per gram of fat. However, the value can be estimated to fall between 70 and 120 × 10^9 cells per carcass, assuming 34% carcass fat (16).

Cellularity differences due to breeding

Perirenal and subcutaneous adipose tissue in the H×A steers contained larger ($P < 0.05$) cells than the respective tissues from Holstein steers of similar age and live weight (Table 1). This relationship is also indicated in Fig. 1, where the volume distribution for the H×A steers was located in the larger cell diameter ranges than for the same tissues of the Holstein steers.

The cellular basis for the distinctly different patterns of subcutaneous fat deposition was of particular interest. This was studied by measuring the size and number of adipose cells in the subcutaneous fat dissected from a standardized rib section. Subcutaneous adipose cell diameter and volume were significantly ($P < 0.05$) smaller in ribs from Holstein steers than from H×A steers (Table 1 and Fig. 2). In addition, the number of adipose cells in

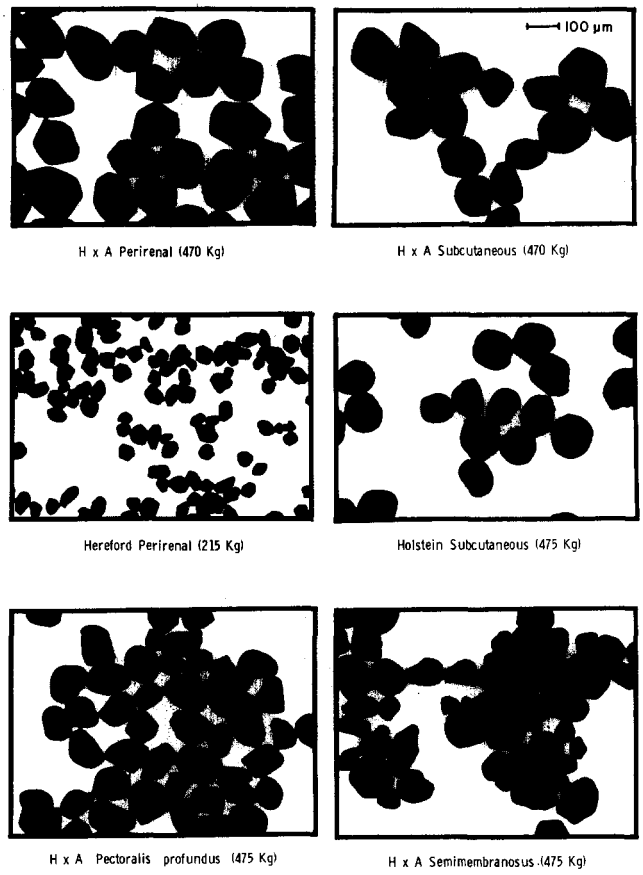

Fig. 2. Photomicrographs of osmium-fixed adipose cells from bovine adipose tissue (H×A, Hereford × Angus animals).

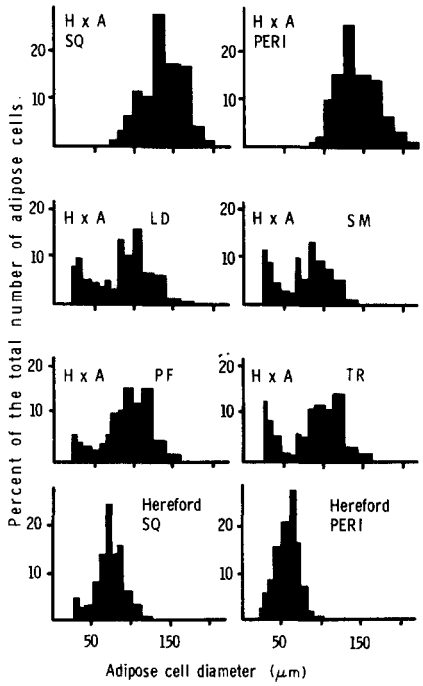

Fig. 3. Number frequency distribution of adipose cells from bovine adipose tissue. H×A, Hereford × Angus animals, 14 months old and 470 kg live weight; Hereford animals, 8 months old and 215 kg live weight; LD, SM, PF, and TR refer to adipose cells from longissimus dorsi, semimembranosus, pectoralis profundus, and trapezius muscles, respectively. SQ and PERI refer to subcutaneous and perirenal adipose tissue, respectively.

the subcutaneous fat dissected from the standardized rib of the Holstein steers was significantly ($P < 0.05$) less (Table 1). Therefore, the Holstein steers had less subcutaneous fat than H×A steers due to both a smaller number of adipose cells and cells of smaller size.

There were no significant correlations between cell size (diameter or volume) and size of the adipose depots (subcutaneous or perirenal) in either the H×A or Holstein steers. However, correlations of 0.75–0.80 suggest that cell number was highly related to depot size in the Holstein steers, whereas correlations of 0.51–0.75 suggest that in the H×A steers both cell size and number were related to depot size.

Interfascicular adipose tissue cellularity

Differences in the percentage intramuscular lipid were observed between the longissimus dorsi, trapezius, semimembranosus, and pectoralis profundus muscles; the pectoralis profundus muscle contained significantly ($P < 0.05$) more intramuscular lipid than the other muscles (Table 2). The higher lipid content of the pectoralis profundus muscle can be attributed to both a larger cell size (diameter and volume) and a larger number of cells per 100 g of muscle (Table 2). In general, the muscles with less lipid (longissimus dorsi, semimembranosus, and trapezius) tended to have a smaller adipose cell size and fewer cells per 100 g of muscle. Adipose tissue is richly supplied with a vascular system, and it has been clearly demonstrated (9, 17) that the main interfascicular deposits are close to or within a heavy vascular network.

Fig. 3 compares the adipose cell size distribution from interfascicular tissue of four bovine muscles and the number distributions found in subcutaneous and perirenal tissue from the same animals. It is apparent that interfascicular adipose tissue contained cells that were smaller than those in subcutaneous and perirenal adipose tissue (Figs. 2 and 3) from the same animals. The actual cell diameters and volumes are recorded in Tables 1 and 2. In the porcine animal, cell volume and diameter of interfascicular adipose cells were about 40–70% of those in subcutaneous fat (18). Moody and Cassens (9), using an ocular grid method for measuring fat cell diameter, reported that bovine longissimus dorsi interfascicular adipose tissue contained smaller cells than either intermuscular or subcutaneous adipose tissue. Noticeably absent from the subcutaneous and perirenal adipose cell distributions was the presence of cells smaller than 70 µm (Figs. 2 and 3). However, smaller cells were abundant in the later-developing interfascicular adipose tissue, indicating that interfascicular adipose tissue from these steers was actively

TABLE 2. Contribution of adipose cell size and number to lipid content of four bovine muscles

Muscle	Intramuscular Lipid	Avg Cell Diameter	Avg Cell Volume	No. of Cells/ 100 g Muscle
	%	μm	$(\mu m^3)(10^4)$	(10^6)
Longissimus dorsi	$6.3^a \pm 1.8$	$90.9^b \pm 5.9$	$61.9^a \pm 10.2$	$11.5^a \pm 1.8$
Semimembranosus	$4.2^a \pm 1.1$	$74.2^a \pm 5.1$	$36.6^b \pm 4.8$	14.2 ± 3.8
Trapezius	$7.1^a \pm 1.5$	83.5 ± 2.4	$50.4^a \pm 4.1$	16.2 ± 2.9
Pectoralis profundus	$13.2^b \pm 2.5$	$95.0^b \pm 5.5$	$63.1^a \pm 9.4$	$29.8^b \pm 7.5$

[a,b] Means in the same column with different superscripts differ significantly ($P < 0.05$). No superscript indicates lack of significance ($P > 0.05$).

growing by both hyperplasia and hypertrophy. The lack of small cells in the subcutaneous and perirenal tissues suggests that hyperplasia is complete in these two depots (Fig. 3). The differences in mean adipose cell size observed among the four muscles can be attributed to the proportion of small (< 50 μm) cells present in the cell population (Fig. 3). This relationship was also apparent when the photomicrographs of the pectoralis profundus and semimembranosus adipose cells were compared (Fig. 2). In all muscles, the majority of the cells were between 70 and 130 μm, the average cell diameter being influenced by the number of cells in the smaller diameter ranges (e.g., interfascicular adipose tissue of the pectoralis profundus has the largest average cell size and the lowest number of small cells). The counting technique used in these studies has some limitations because adipose cells smaller than 25 μm in diameter were not counted. Thus, in certain depots such as the interfascicular, not all the adipose cells will be recovered and counted.

Differences in lipid content among muscles were due to alterations in both cell size and number (Table 2). The relationships between these variables are reflected by the correlation coefficients listed in Table 3. With the exception of the longissimus dorsi muscle, which contained the fewest number of cells per 100 g of muscle, correlations between average cell size (diameter and volume) and percentage intramuscular lipid were low and nonsignificant. The number of cells per 100 g of muscle was significantly correlated to the percentage intramuscular lipid both within and across muscles, suggesting that cell number was the main factor that influenced the quantity of interfascicular adipose tissue in the H×A steers. The presence of small cells in the interfascicular adipose tissue suggests that the final cell number had not been reached in this tissue of 14-month-old steers.

TABLE 3. Correlation of intramuscular lipid with number and size of interfascicular adipose cells from 470-kg Hereford × Angus steers[a]

	Intramuscular Lipid (percent) vs.		
	Avg Cell Diameter	Avg Cell Volume	No. of Cells/ 100 g Muscle
Longissimus dorsi	0.79	0.75	0.81
Trapezius	−0.36	−0.37	0.94
Semimembranosus	−0.33	0.08	0.91
Pectoralis profundus	−0.28	−0.29	0.89
All muscles	0.32	0.35	0.87

[a] Correlation coefficients are significant at levels of $P < 0.01$ (if $r > 0.92$) and $P < 0.05$ (if $r > 0.81$) within muscles and $P < 0.01$ (if $r > 0.55$) and $P < 0.05$ (if $r > 0.43$) across muscles.

Adipose tissue from the 8-month-old (215 kg) Hereford steers would also be a growing tissue (Fig. 3). However, subcutaneous and perirenal tissue from these animals and the H×A steers had an apparent development pattern for cell diameter quite different from the growing interfascicular adipose tissue (Figs. 2 and 3) of the H×A steers. In the interfascicular adipose tissue (particularly semimembranosus and trapezius muscles) of the H×A steers, a bimodal distribution was evident, whereas in the subcutaneous and perirenal tissue of the 8- and 14-month-old animals a unimodal distribution was observed. Therefore, it appears that interfascicular adipose tissue has a development pattern distinctly different from the subcutaneous and perirenal adipose tissues. This difference suggests that hyperplasia is still an important process for growth of the late-developing interfascicular adipose tissue of the bovine animal.

Additional studies[2] on the H×A steers have shown that on a cellular basis in vitro lipogenesis was three to five times greater in adipocytes from the subcutaneous depot than in either the perirenal or interfascicular adipocytes. In addition, it has been reported that fasting (19), mild exercise (20), and norepinephrine infusion (21) result in increased levels of intramuscular lipid in other species. Therriault et al. (20) speculated that conditions, such as mild exercise, which cause the free fatty acid level of muscle to exceed the energy demand, result in deposition of intramuscular triglyceride. Thus, it may be that the interfascicular adipocytes have extended hyperplasia and reduced rates of lipogenesis because they are functionally serving as storage depots in response to the high-energy ration fed to fattening cattle. However, it is not unusual to find bovine animals with very large subcutaneous or perirenal adipose depots and a near absence of interfascicular adipose tissue. Thus, the exact reason for the apparently different developmental pattern of interfascicular adipocytes and subcutaneous or perirenal adipocytes is not apparent and provides an interesting area for continuing research.

This research was supported in part by a grant from the American Meat Institute Foundation.

Manuscript received 11 July 1972 and in revised form 15 June 1973; accepted 18 June 1973.

REFERENCES

1. Hirsch, J., and J. L. Knittle. 1970. Cellularity of obese and nonobese human adipose tissue. *Federation Proc.* **29:** 1516–1521.
2. Salans, L. B., E. S. Horton, and E. A. H. Sims. 1971. Experimental obesity in man: cellular character of the adipose tissue. *J. Clin. Invest.* **50:** 1005–1011.
3. Hirsch, J., and P. W. Han. 1969. Cellularity of rat adipose tissue: effects of growth, starvation, and obesity. *J. Lipid Res.* **10:** 77–82.
4. Hubbard, R. W., and W. T. Matthew. 1971. Growth and lipolysis of rat adipose tissue: effect of age, body weight, and food intake. *J. Lipid Res.* **12:** 286–293.
5. Johnson, P. R., L. M. Zucker, J. A. F. Cruce, and J. Hirsch. 1971. Cellularity of adipose depots in the genetically obese Zucker rat. *J. Lipid Res.* **12:** 706–714.
6. Johnson, P. R., and J. Hirsch. 1972. Cellularity of adipose depots in six strains of genetically obese mice. *J. Lipid Res.* **13:** 2–11.
7. Anderson, D. B., and R. G. Kauffman. 1973. Cellular and enzymatic changes in porcine adipose tissue during growth. *J. Lipid Res.* **14:** 160–168.
8. Bell, E. T. 1909. II. On the histogenesis of the adipose tissue of the ox. *Amer. J. Anat.* **9:** 401–438.
9. Moody, W. G., and R. G. Cassens. 1968. A quantitative and morphological study of bovine longissimus fat cells. *J. Food Sci.* **33:** 47–52.
10. Allen, E. 1969. Importance of adipose tissue to the meat industry. *Proc. Meat Ind. Res. Conf.* **21:** 1–8.
11. Hirsch, J., and E. Gallian. 1968. Methods for the determination of adipose cell size in man and animals. *J. Lipid Res.* **9:** 110–119.
12. Hood, R. L., E. H. Thompson, and C. E. Allen. 1972. The role of acetate, propionate and glucose as substrates for lipogenesis in bovine tissues. *Int. J. Biochem.* **3:** 598–606.
13. Hankins, O. G., and P. E. Howe. 1936. Estimation of the composition of beef carcasses and cuts. USDA Technical Bulletin no. 926.
14. Steel, R. G. D., and J. H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill, New York.
15. Gaines, J. A., G. V. Richardson, W. H. McClure, D. W. Vogt, and R. C. Carter. 1967. Heterosis from crosses among British breeds of cattle: carcass characteristics. *J. Anim. Sci.* **26:** 1217–1225.
16. Garrett, W. N., and N. Hinman. 1971. Fat content of trimmed beef muscles as influenced by quality grade, yield grade, marbling score and sex. *J. Anim. Sci.* **33:** 948–957.
17. Blumer, T. N., H. B. Craig, E. A. Pierce, W. W. G. Smart, Jr., and M. B. Wise. 1962. Nature and variability of marbling in longissimus dorsi muscle of beef carcasses. *J. Anim. Sci.* **21:** 935–942.
18. Lee, Y. B., and R. G. Kauffman. 1971. Lipogenic enzyme activities in intramuscular adipose tissue of the pig. *J. Anim. Sci.* **33:** 1144. (Abstr.)
19. Masoro, E. J., L. B. Rowell, and R. M. McDonald. 1966. Intracellular muscle lipids as energy sources during muscular exercise and fasting. *Federation Proc.* **25:** 1421–1424.
20. Therriault, D. G., G. A. Beller, J. A. Smoake, and L. H. Hartley. 1973. Intramuscular energy sources in dogs during physical work. *J. Lipid Res.* **14:** 54–60.
21. Carlson, L. A., S-O. Liljedahl, and C. Wirsen. 1965. Blood and tissue changes in the dog during and after excessive free fatty acid mobilization. *Acta Med. Scand.* **178:** 81–102.