Cellularity of bovine adipose tissues: developmental changes from 15 to 65 percent mature weight

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Abstract Changes in the cellularity of various adipose tissues in growing cattle were analyzed. Fifty male cattle were slaughtered between 15 and 65% of mature weight. The whole adipose mass, separated by dissection, was divided into three parts: subcutaneous, intermuscular, and internal adipose tissues. Lipid content, cell size and distribution, as well as cell number of these three parts were determined. Adipose cells became 15 times greater from 15 to 65% mature weight, whereas total adipose cell number increased only 1.8-fold. However, a significant hyperplasia occurred near 45% mature weight. These results suggested a cell size regulation by hyperplasia. Over the whole period studied (15–65% mature weight), hyperplasia was far higher in subcutaneous adipose tissue than in other tissues. This is discussed as related to the higher relative growth of this tissue. In each fatty tissue, two identical development periods were observed. Each of them began by an increase in small-sized cells (hyperplasia) followed by the filling of these cells (hypertrophy). These two periods were particularly clear in the case of subcutaneous tissue, in which the second hyperplasia occurred slightly later than in other fatty tissues. So, in all respects, subcutaneous fatty tissue appears to develop later than other tissues studied.—**Robelin, J.** Cellularity of bovine adipose tissues: developmental changes from 15 to 65 percent mature weight. *J. Lipid Res.* 1981. **22:** 452–457.

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One of the main goals in meat production research is to decrease lipid deposition and to increase protein deposition. This goal cannot be attained until adipose tissue development is better understood. In particular, it is interesting to know the changes in adipose cell size and number during growth, in relation to the deposition of fat in domestic animals. These changes have been studied extensively over the last ten years in rodents (1–4), pigs (5–7), and sheep (8, 9). In contrast, only little information has been obtained for cattle (10, 11), after the first results of Hood and Allen (12) concerning a short period of growth (8 to 14 months of age). Up to now, no detailed description of changes in adipose cell size and number in various fatty tissues of cattle involving a long life span has been published.

Over the last four years, we have pursued such a study from 15 to 65% mature weight. This work was part of a large research program dealing with the evolution during growth of daily liveweight gain, body composition, and feed efficiency of Charolais and Friesian bulls. This study analyzes the evolution within breed of the cellularity of the different fatty tissues in these growing animals.

MATERIALS AND METHODS

Animals

Fifty 4-month-old bulls, 25 Charolais and 25 Friesian, were randomly divided in each breed into one group of five, and five groups of four animals. These six groups of animals from each breed were slaughtered at 15, 25, 35, 45, and 65% of the estimated mature body weight (900 and 1,100 kg for Friesian and Charolais breeds, respectively).

After weaning, the Friesian bulls were fed ad libitum from 15 to 35% mature body weight a 60% concentrate, 40% hay diet. During the same period, Charolais bulls raised in a suckling herd were fed milk and green grass. After 35% mature body weight, the animals of both breeds were fed the same diet, 82% concentrate and 18% hay, ad libitum. The mean growth rate of animals was close to 1 kg/day, slightly higher between 15 and 45% mature weight, and slightly lower thereafter.

Friesian and Charolais bulls have a different propensity to fatten. At 15% mature weight, they have approximately the same amount of fatty tissues (approximately 5% body weight); at 65% mature weight

Friesian bulls are fatter than Charolais bulls (21 vs. 13% body weight). However, this study is only concerned with the mean evolution of cellularity with age (see section on statistical analysis).

Tissue sampling and body composition measurements

After slaughter, a sample of each of the subcutaneous, intermuscular, kidney, and peritoneal adipose tissues was taken to determine cell size. Subcutaneous fat samples were taken from the forelimb near the triceps brachii caput longum muscle, and at the same time from the hind limb near the biceps femoris muscle. They were dissected through the entire depth. Intermuscular fat sample was taken near the serratus thoracis cervicis muscle.

After careful dissection, using the method previously described (13), the total weight of subcutaneous, intermuscular, and internal fat was recorded. Internal fat is referred to as the sum of the pelvic and channel fat, kidney fat, peritoneal, mesenteric, and heart fat. The three groups of fatty tissues were minced separately and their lipid content was determined after chloroform—methanol extraction and purification (14).

Cell size measurements

Adipose cells were fixed by osmium tetroxide (15), and isolated in urea solution as proposed by Etherton, Thompson, and Allen (16). The fixed cells were trapped on a cellulose nitrate filter (pore size, 0.45 μm). A sample of this filter was then photographed through a microscope. The overall magnification of microscope and photograph was 80. One or two hundred cells per tissue sample were measured, separately by two operators, and divided into 19 groups according to their diameter, 2, 3, or 4 mm on the photograph, or, in fact, 25, 37.5, or 50 μm. The percentage of cells in each class was then recorded. For each fatty tissue (j), the mean volume of adipocytes (Vj) was computed from the volume (vi) of the cells of each class (i) and the percentage (pji) of cells in each class ($Vj = \sum_i pji\ vi$). There was no significant difference in the percentage distribution of cells and in the mean volume between peritoneal and kidney fat. So the mean values observed in these two deposits were recorded, and subsequently referred to as internal fatty tissue.

Determination of cell number

The number of adipocytes (Nj) in each deposit (j) (subcutaneous, intermuscular, and internal) was computed from the weight of lipids (lj) of each deposit, the mean volume of adipocytes (Vj) and the density (d) of lipids (Nj = lj/d Vj). As proposed by Lemonnier (17), 0.915 was considered to be the density of lipids in adipocytes. The number of cells (Nji) of each class (i) in each deposit (Nji = Nj pji), the total number of adipose cells per animal ($N = \sum_j Nj$), and the mean volume of adipocytes in each animal ($V = (\sum_j lj)/(dN)$) were computed.

TABLE 1. Correspondence between cell diameter and cell volume

Diameter	Volume	Diameter	Volume	Diameter	Volume
μm	$10^4\ \mu m^3$	μm	$10^4\ \mu m^3$	μm	$10^4\ \mu m^3$
20	0.4	70	18	120	90
30	1.4	80	27	130	115
40	3.3	90	38	140	144
50	6.5	100	52	150	177
60	11.3	110	70	160	214

Statistical analysis

The data were analyzed following a classical two-way (breed × slaughter group) variance analysis. Due to a highly significant interaction effect, the results have to be divided by 2 to clarify: 1) effect of slaughter weight; and 2) effect of breed and interaction with slaughter weight. The data presented here refer to the first part only. The results in the tables and figures are mean values per slaughter group and within-breed standard errors.

In the published results, both cell diameter and volume are used. In order to make the comparison with other results easier, the correspondence between cell diameter and volume has been tabulated (**Table 1**).

RESULTS AND DISCUSSION

Developmental changes in the whole adipose mass

The weight of all fatty tissues expressed as a percentage of empty body weight (full body weight − gut contents) increased from 5.4 to 17.4% from 15 to 65% mature body weight (**Table 2**).

A more careful analysis shows several periods in the development of adipose tissue (**Fig. 1**). Adipose cell size increased steadily and significantly ($P < 0.05$) from 15 to 45% mature weight. It was stabilized between 45 and 55% mature weight and seemed to increase again, but not significantly, later.

In contrast, the total number of cells increased slowly but not significantly between 15 and 25% mature weight; it remained nearly constant up to 45% mature weight, and increased significantly ($P < 0.05$) between 45 and 55% mature weight.

During the first period (15–25% mature weight), the number of medium-sized cells (diameter: 63–125

TABLE 2. Characteristics of fatty tissues in growing cattle between 15 and 65% mature weight: means and standard errors for eight animals per slaughter point

	Body Weight (% Mature Body Weight)					
	15	25	35	45	55	65
Number of animals	10	8	8	8	8	8
Age (days)	$123^a \pm 5$	$231^b \pm 5$	$306^c \pm 4$	$391^d \pm 5$	$488^e \pm 9$	$608^f \pm 17$
Empty body weight (kg)	$125^a \pm 4$	$220^b \pm 3$	$312^c \pm 3$	$400^d \pm 6$	$484^e \pm 11$	$579^f \pm 8$
Whole fatty tissues						
Fresh weight (kg)	$6.8^a \pm 0.5$	$19.1^b \pm 1.3$	$38.0^c \pm 2.6$	$55.6^d \pm 4.3$	$76.1^e \pm 4.3$	$100.6^f \pm 6.7$
Lipid weight (kg)	$2.3^a \pm 0.3$	$8.5^b \pm 0.8$	$20.7^c \pm 1.5$	$33.0^d \pm 3.1$	$49.3^e \pm 3.8$	$65.8^f \pm 6.6$
Cell volume (10^4 μm^3)	$4.7^a \pm 0.6$	$15.6^b \pm 2.7$	$34.4^c \pm 1.6$	$54.0^d \pm 5.7$	$55.6^d \pm 8.9$	$69.5^d \pm 7.6$
Cell number (10^{10})	$5.6^a \pm 0.4$	$6.6^a \pm 0.7$	$6.6^a \pm 0.4$	$7.1^a \pm 0.9$	$10.8^b \pm 1.5$	$10.2^b \pm 0.8$
Subcutaneous fatty tissues						
Fresh weight (kg)	$0.4^a \pm 0.1$	$1.6^b \pm 0.1$	$5.0^c \pm 0.5$	$7.7^d \pm 1.0$	$10.9^e \pm 1.0$	$16.5^f \pm 1.8$
Lipid weight (kg)	$0.1^a \pm 0.0$	$0.6^b \pm 0.0$	$2.5^c \pm 0.3$	$4.4^d \pm 0.6$	$6.7^e \pm 0.6$	$10.3^f \pm 1.4$
Cell volume (10^4 μm^3)	$3.1^a \pm 0.7$	$6.7^b \pm 1.3$	$18.1^c \pm 3.0$	$33.0^d \pm 3.0$	$32.5^d \pm 5.5$	$40.0^d \pm 5.2$
Cell number (10^{10})	$0.5^a \pm 0.1$	$1.1^b \pm 0.2$	$1.7^b \pm 0.3$	$1.5^b \pm 0.2$	$2.5^c \pm 0.2$	$2.8^c \pm 0.4$
Intermuscular fatty tissues						
Fresh weight (kg)	$4.3^a \pm 0.3$	$11.4^b \pm 0.7$	$20.7^c \pm 1.3$	$28.3^d \pm 1.9$	$38.6^e \pm 2.2$	$46.8^f \pm 2.3$
Lipid weight (kg)	$1.3^a \pm 0.1$	$4.2^b \pm 0.4$	$9.6^c \pm 0.6$	$14.0^d \pm 1.2$	$21.4^e \pm 1.5$	$26.0^f \pm 2.4$
Cell volume (10^4 μm^3)	$4.9^a \pm 0.7$	$18.2^b \pm 3.8$	$38.5^c \pm 3.2$	$48.4^d \pm 6.5$	$56.4^d \pm 10.3$	$67.2^d \pm 6.6$
Cell number (10^{10})	$3.2^a \pm 0.3$	$3.3^a \pm 0.6$	$2.9^a \pm 0.2$	$3.5^a \pm 0.6$	$4.9^a \pm 0.9$	$4.3^a \pm 0.4$
Internal fatty tissues						
Fresh weight (kg)	$2.1^a \pm 0.2$	$6.1^b \pm 0.5$	$12.2^c \pm 0.8$	$19.5^d \pm 1.7$	$26.6^e \pm 2.1$	$37.3^f \pm 3.1$
Lipid weight (kg)	$0.9^a \pm 0.1$	$3.7^b \pm 0.4$	$8.6^c \pm 0.7$	$14.6^d \pm 1.4$	$21.1^e \pm 2.1$	$29.6^f \pm 3.2$
Cell volume (10^4 μm^3)	$4.9^a \pm 0.6$	$18.4^b \pm 2.1$	$46.0^c \pm 2.5$	$80.5^d \pm 8.2$	$74.7^d \pm 11.9$	$103.0^d \pm 13.5$
Cell number (10^{10})	$1.9^a \pm 0.2$	$2.2^a \pm 0.1$	$2.1^a \pm 0.2$	$2.1^a \pm 0.2$	$3.4^b \pm 0.5$	$3.1^b \pm 0.2$

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

μm) increased significantly ($P < 0.05$), whereas the number of small-sized cells decreased slowly but not significantly (**Fig. 2**). During the second period (25–45% mature weight), the number of medium-sized cells and large-sized cells (diameter greater than 125 μm) increased significantly ($P < 0.05$), whereas the number of small-sized cells decreased significantly ($P < 0.05$). During these two periods, lipid storage is achieved by filling small-sized cells, without apparent hyperplasia. In contrast, in the third period, and mainly between 45 and 55% mature weight, the number of the three kinds of cells increased; even this increment was not significant for small-sized cells, and it seemed that a new generation of cells appeared.

Remarkably, the number of large-sized cells (diameter greater than 125 μm) increased very slowly

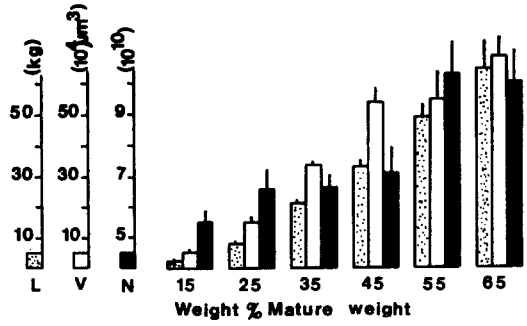

Fig. 1. Evolution of lipid weight of all fatty tissues (L,kg), mean volume of adipose cells (V, 10^4 μm^3), and total cell number (N, 10^{10}) in growing cattle between 15 and 65% of mature weight. Mean values and standard errors for eight animals per slaughter point.

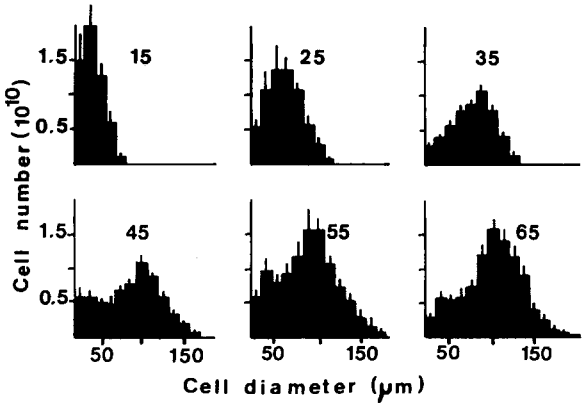

Fig. 2. Distribution of cells of all adipose tissues according to their diameter in growing cattle between 15 and 65% of mature weight. Mean values and standard errors for eight animals per slaughter point.

even in the last period; lipid storage apparently occurred selectively in medium-sized cells. Thus, these cells contained nearly 70% of total body lipids of the 65% mature weight animals, whereas large-sized cells contain only 24% of total body lipids.

The mean cell size observed at the latest stages of slaughter in our experiment (45–55% mature weight) is similar to that previously observed in young Friesian or Simmental bulls (10, 11), but it is far lower than the adipose cell size of Hereford × Angus steers of similar weight (12).

The larger size of Hereford × Angus adipose cells is probably related to the higher amount of total body fat, near 40% compared to only 13 and 20%, respectively, in Charolais and Friesian bulls of similar weight (18).

The apparent hyperplasia that occurs at 45% mature weight would disprove the hypothesis that adipose tissue growth was a result of hypertrophy only, following earlier growth stages. Such apparent late hyperplasia has already been observed in rabbits (9), rats, and mice (19). This apparent hyperplasia seems to be particularly high in rodents when the animals are fed high fat diets (17, 20, 21) and in fa/fa rats (22). Moreover, in these genetically obese animals, a bimodal distribution of cells appears at 10–20 weeks of age (22), as in our results at 45–55% mature weight (Fig. 2). The method of cell number determination used in the latter studies, similar to ours, does not indicate whether the apparent proliferation is actually hyperplasia or only a preadipocyte filling. However, the possibility of a true increase in the number of adipocytes at the latest stages of growth has now been clearly demonstrated in experiments on fatty tissue regeneration after lipectomy (23). This increase in the number of adipose cells may result either from a true cell multiplication, as shown by incorporation of labeled thymidine into DNA (2, 21) or from the development of undifferentiated cells into adipocytes (24, 25).

This apparent hyperplasia is accompanied in our results by a stabilization of cell hypertrophy; similar results have been observed in mice (17), rats (26), and rabbits (9); in these studies, the stabilization occurred when the mean cell size reached approximately 50 $\times 10^4$ μm^3. In other respects, we have also observed that the lipids were stored preferably in the medium-sized cells. Thus it seems that when a majority of cells reach a critical size, probably near 50 $\times 10^4$ μm^3 (diameter = 100 μm), a supplementary flow of energy, meant to be stored as lipids, induces hyperplasia. This induction may be related to the lipid-synthesizing capacity of adipocytes. Indeed, the lipid synthesis from acetate in lamb adipose tissue (27) decreases

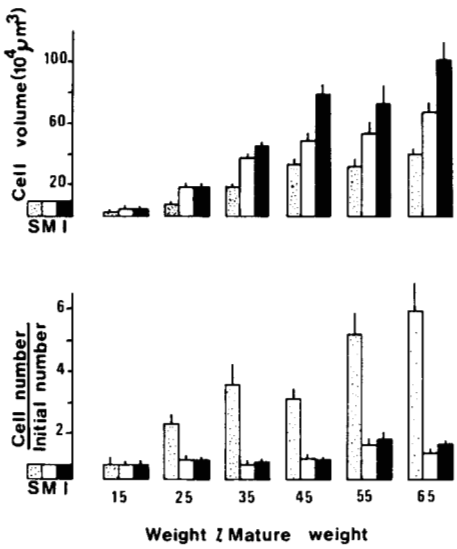

Fig. 3. Evolution of adipose cell volume and number in subcutaneous (S), intermuscular (M), and internal (I) fatty tissues in growing cattle between 15 and 65% of mature weight. Mean values and standard errors for eight animals per slaughter point. Cell number is expressed as a relative value in relation to the number observed at 15% of mature weight.

very rapidly when the mean adipose cell size exceeds 30 $\times 10^4$ μm^3 (diameter = 85 μm). Faust et al. (20) has already suggested such an hypothesis, but the proposed critical size was greater; the fact that hyperplasia was induced in adult rats instead of in growing animals, could possibly explain this difference in critical size. Finally, it should be noted that in growing animals cell proliferation could be related to puberty; it occurs immediately after this stage in young bulls (our results) as well as in man (28). The mechanisms of this hyperplasia induction are still not thoroughly known. The lack of induction in rat, when obesity is induced by a hypothalamic lesion (1), allowed us to think of a possible role for this organ.

Differences between fatty tissues

The various fatty tissues follow quite different growth patterns (Table 2, **Fig. 3**). From 15 to 65% mature weight, lipid weight increased 100-fold in subcutaneous fatty tissue, but only 20- and 33-fold in intermuscular and internal fatty tissues, respectively. Simultaneously, the adipose cell size increased 13-, 14-, and 21-fold in these three deposits whereas cell number increased 5.6-, 1.3-, and 1.6-fold. Thus the higher the hyperplasia, the higher the relative growth of tissues.

In the subcutaneous fatty tissue, an apparent cell proliferation occurred between 15 and 25% mature weight; the numbers of small-sized and of medium-sized cells increased significantly ($P < 0.05$; **Table 3**).

TABLE 3. Number of various cells in fatty tissues in growing cattle between 15 and 65% mature weight: means and standard errors for eight animals per slaughter point

	Body Weight (% Mature Body Weight)					
	15	25	35	45	55	65
	number of cells ($\times 10^7$)					
Whole fatty tissue						
Small[f]	5,456 ± 438[a]	4,560 ± 994[a]	2,116 ± 241[b]	2,161 ± 538[b]	3,426 ± 846[ab]	2,099 ± 287[b]
Medium[g]	108 ± 39[a]	2,067 ± 350[b]	4,442 ± 255[c]	4,622 ± 355[c]	6,749 ± 774[d]	7,114 ± 701[d]
Large[h]	0	0	49 ± 17[a]	304 ± 90[b]	600 ± 135[c]	983 ± 201[c]
Subcutaneous fatty tissue						
Small	469 ± 103[a]	1,068 ± 248[b]	1,055 ± 259[b]	525 ± 85[a]	1,077 ± 249[b]	837 ± 160[b]
Medium	3 ± 2[a]	62 ± 25[b]	625 ± 77[c]	956 ± 108[d]	1,379 ± 133[e]	1,959 ± 318[e]
Large	0	0	0	2 ± 1[a]	9 ± 5[a]	24 ± 15[a]
Intermuscular fatty tissue						
Small	3,115 ± 310[a]	2,260 ± 754[a]	731 ± 149[b]	1,157 ± 355[ab]	1,282 ± 504[ab]	684 ± 227[b]
Medium	56 ± 18[a]	1,008 ± 183[b]	2,119 ± 153[c]	2,322 ± 230[c]	3,500 ± 516[d]	3,484 ± 359[d]
Large	0	0	9 ± 3[a]	64 ± 22[b]	158 ± 62[bc]	193 ± 59[c]
Internal fatty tissue						
Small	1,872 ± 157[a]	1,231 ± 227[b]	329 ± 59[c]	479 ± 116[cd]	1,067 ± 317[bd]	578 ± 69[bcd]
Medium	48 ± 26[a]	998 ± 159[b]	1,698 ± 129[c]	1,344 ± 129[c]	1,870 ± 199[d]	1,720 ± 285[cd]
Large	0	0	40 ± 18[a]	238 ± 70[b]	433 ± 96[bc]	766 ± 159[c]

[a,b,c,d,e]: Means on the same line with different superscripts differ significantly ($P < 0.05$).
[f] Small cells, 25–63 μm diameter.
[g] Medium cells, 63–125 μm diameter.
[h] Large cells, >125 μm diameter.

From 25 to 45% mature weight, there was only a lipid-filling of cells without proliferation; the number of small-sized cells decreased significantly ($P < 0.05$), whereas the number of medium sized cells increased ($P < 0.05$). The increase, between 45 and 55% mature weight, of both small-sized and medium-sized cell numbers, indicates a new apparent cell proliferation.

In the other tissues, intermuscular and internal, there were only two phases: lipid-filling between 15 and 35% mature weight, and apparent proliferation between 35 and 45% mature weight. This apparent proliferation, which appeared later in subcutaneous fat (45–55% mature weight), occurred in each deposit when the mean cell size reached approximately $30–40 \times 10^4$ μm^3 (diameter = 80–90 μm) (Table 3).

Cellularity seems to explain clearly the well-known hierarchy involved in the relative growth of adipose tissue in cattle (13, 29). Subcutaneous tissues, known to be late developing, have the highest relative growth and appear to be the youngest on a cellularity basis: small-sized cells, high and late hyperplasia. The same relationship between relative growth and cellularity was already observed in rabbits and sheep (9).

In conclusion, these detailed results on the cellularity of bovine adipose tissues confirm earlier observations made on other species, and, mainly, the possibility of a late hyperplasia. The occurrence of this late hyperplasia at different stages of growth in the various fatty tissues had not been previously observed, and its relationship to mean cell size must be stressed. In this respect, subcutaneous fatty tissue seems to be a good model for further research.

This study will be followed by the analysis of the variations in cellularity between genotypes. Several other aspects of cellularity of adipose tissues in cattle need to be studied. First of all, the earlier stages of development of various fatty tissues during fetal growth have not yet been studied on a cellular basis; research in progress in this field will probably help to explain the differences between tissues observed in this study. In another respect, a reduction in energy supply in cattle is known to decrease the adiposity of the animal at slaughter; it would be interesting to know to what extent hyperplasia and/or hypertrophy are modified in various fatty tissues. Finally, it seems that total adipose tissue mass and mean cell size are fairly well related in growing cattle (Fig. 1); this relationship suggests that it could be possible to assess the adipose tissue mass in living animals by the adipose cell size measured on a probe of subcutaneous fat; this would be of very high practical importance in research experiments on cattle; in this respect, this relationship needs to be carefully analyzed in animals varying in adiposity for different reasons, age, breed, sex, or level of energy supply.

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REFERENCES

1. Hirsch, J., and P. W. Han. 1969. Cellularity of rat adipose tissue: effects of growth, starvation, and obesity. *J. Lipid. Res.* **10:** 77–82.
2. Greenwood, M. R. C., and J. Hirsch. 1974. Postnatal development of adipocyte cellularity in the normal rat. *J. Lipid Res.* **15:** 474–483.
3. Bertrand, H. A., E. J. Masoro, and B. P. Yu. 1978. Increasing adipocyte number as the basis for perirenal depot growth in adult rat. *Science*. **201:** 1234–1235.
4. Nougues, J. 1975. Adipocyte growth of four adipose deposits in rabbit. *Ann. Biol. Anim. Biochim. Biophys.* **15:** 541–546.
5. Anderson, D. B., and R. G. Kauffman. 1973. Cellular and enzymatic changes in porcine adipose tissue during growth. *J. Lipid. Res.* **14:** 160–168.
6. Mersmann, H. J., J. R. Goodman, and L. J. Brown. 1975. Development of swine adipose tissue: morphology and chemical composition. *J. Lipid. Res.* **16:** 269–279.
7. Enser, M. B., J. D. Wood, D. J. Restall, and H. J. H. Mac Fie. 1976. The cellularity of adipose tissue from pigs of different weights. *J. Agric. Sci.* **86:** 633–638.
8. Haugebak, C. D., H. B. Hedrick, and J. M. Asplund. 1974. Adipose tissue accumulation and cellularity in growing and fattening lambs. *J. Anim. Sci.* **39:** 1016–1025.
9. Nougues, J., and A. Vezihnet. 1977. Evolution pendant la croissance de la cellularité du tissu adipeux chez le lapin et l'agneau. *Ann. Biol. Anim. Biochim. Biophys.* **17:** 799–806.
10. Schön, I. 1978. The development of fat cells in different anatomical positions in carcass of young bulls, heifers, and cow-heifers. *In* Patterns of Growth and Development in Cattle. Proceedings E.E.C. Seminar Held in Ghent. Martinus Nijhof, editor. The Hague. 255–266.
11. Enser, M., and J. D. Wood. 1978. The development of adipose tissue in cattle. *In* Patterns of Growth and Development in Cattle. Proceedings of E.E.C. Seminar Held in Ghent. Martinus Nijhof, editor. The Hague. 243–254.
12. Hood, R. L., and C. E. Allen. 1973. Cellularity of bovine adipose tissue. *J. Lipid. Res.* **14:** 605–610.
13. Robelin, J., Y. Geay, and C. Beranger. 1977. Evolution de la composition corporelle des jeunes bovins mâles entiers de race Limousine entre 9 et 19 mois. I. Composition anatomique. *Ann. Zootech.* **26:** 533–546.
14. Robelin, J., Y. Geay, and C. Beranger. 1979. Evolution de la composition corporelle des jeunes bovins mâles entiers de race Limousine de 9 à 19 mois. II. Composition chimique et valeur calorifique. *Ann. Zootech.* **28:** 191–208.
15. Hirsch, J., and E. Gallian. 1968. Methods for the determination of adipose cell size in man and animals. *J. Lipid. Res.* **9:** 110–119.
16. Etherton, T. D., E. H. Thompson, and C. E. Allen. 1977. Improved techniques for studies of adipocyte cellularity and metabolism. *J. Lipid. Res.* **18:** 552–557.
17. Lemonnier, D. 1972. Effect of age, sex, and site on the cellularity of the adipose tissue in mice and rats rendered obese by a high fat diet. *J. Clin. Invest.* **51:** 2907–2915.
18. Robelin, J., and R. Daenicke. 1980. Variations of net requirements for cattle growth with liveweight, liveweight gain, breed and sex. *Ann. Zootech.* **29:** 99–118.
19. Lemonnier, D., and A. Alexiu. 1973. Nutritional genetic and hormonal aspects of adipose tissue cellularity. *In* The Regulation of Adipose Tissue Mass. J. Vague and J. Boyer, editors. Excerpta Medica, Amsterdam. 158–173.
20. Faust, I. M., P. R. Johnson, J. S. Stern, and J. Hirsch. 1978. Diet-induced adipocyte number increase in adult rats: a new model of obesity. *Am. J. Physiol.* **235:** E279–E286.
21. Klyde, B. J., and J. Hirsch. 1979. Increased cellular proliferation in adipose tissue of adult rats fed a high fat diet. *J. Lipid. Res.* **20:** 705–715.
22. Kaplan, M. L., J. R. Trout, and P. Smith, 1980. Adipocyte size distribution in fa/fa rats during development. *Metabolism.* **29:** 333–339.
23. Faust, J. M., P. R. Johnson, and J. Hirsch. 1979. Adipose tissue regeneration in adult rat. *Proc. Soc. Exp. Biol. Med.* **161:** 111–114.
24. Björntorp, P., M. Karlsson, H. Pertoft, P. Pettersson, L. Sjöström, and U. Smith. 1978. Isolation and characterization of cells from rat adipose tissue developing into adipocytes. *J. Lipid. Res.* **19:** 316–324.
25. Van, R. L. R., C. E. Bayliss, and D. A. K. Roncari. 1976. Cytological and enzymological characterization of adult human adipocyte precursors in culture. *J. Clin. Invest.* **58:** 699–704.
26. Knittle, J. L., and J. Hirsch. 1968. Effect of early nutrition on the development of rat epididymal fat pads: cellularity and metabolism. *J. Clin. Invest.* **47:** 2091–2098.
27. Vezihnet, A. 1976. Etude du tissu adipeux chez l'agneau et le lapin aprés la naissance: développement, lipolyse, lipogénèse, influence de l'hypophysectomie et de l'hormone de croissance. Thèse USTL-Montpellier. N° C.N.R.S.:A.O. 12437.
28. Knittle, J. L., J. Timmers, F. Ginsberg-Fellner, R. E. Bown, and D. P. Katz. 1979. The growth of adipose tissue in children and adolescents: cross sectional and longitudinal studies of adipose cell number and size. *J. Clin. Invest.* **63:** 239–246.
29. Robelin, J., Y. Geay, and C. Beranger. 1974. Croissance relative des différents tissus, organes et régions corporelles des taurillons Frisons, durant la phase d'engraissement de 9 à 15 mois. *Ann. Zootech.* **23:** 313–323.