Longitudinal study of adipose cell size in the dorsoscapular and perirenal depots of the growing rabbit

Y. Reyne, J. Teyssier, J. Nouguès, and Soraya Tébibel

I.N.R.A., Station de Physiologie Animale, E.N.S.A., Place Viala, 34060 Montpellier Cedex, France

Abstract The changes in fat cell size during normal growth of New Zealand rabbits were investigated longitudinally with serial dorsoscapular and perirenal fat biopsies. A remarkably complex pattern of changes appeared when individual evolutions were considered. About 50% of the rabbits were characterized by "significant drops" of mean diameter during fat tissue growth with shifting of distributions toward the smaller cells. These "drops" could not be attributed to regional variation observed within each depot or to growth or food intake disorders. Differences in behavior of perirenal and dorsoscapular depots were noted. The "drops" occurred earlier in perirenal than in dorsoscapular depots. The meaning of these "drops" remains unclear, but the results suggest that they may be due to a discontinuous recruitment of new observable cells. These results are discussed in relation to the hypothesis of a "critical size" of adipocytes. —**Reyne, Y., J. Teyssier, J. Nouguès, and S. Tébibel.** Longitudinal study of adipose cell size in the dorsoscapular and perirenal depots of the growing rabbit. *J. Lipid Res.* 1985. **26:** 1036–1046.

Supplementary key words adipocyte size distribution • fat depots • longitudinal study • rabbit

Knowledge of age-related changes in cellularity during normal growth allows a better understanding of adipose tissue development. Interpretation of the results, however, is limited by the fact that most of the studies are based on cross-sectional data. Significant variations in cell size and number between individuals are common and tend to obscure developmental trends that might be present in individual subjects. Longitudinal studies are preferable in this regard but they are not commonly performed, except in man, and are often interpreted by means of average parameters (1–3) which again tend to obscure individual trends. In only a few clinical experiments, changes in fat cell size are given for each individual after diet-induced weight variation (4, 5). The scarcity of such individual longitudinal data prompted us to perform the current study.

In contrast to the great number of studies on the rat, there are relatively few cross-sectional reports on adipose tissue cellularity in the rabbit (6–8). Late perirenal and dorsoscapular adipose tissue growth was only examined by Nouguès and Vézinhet (8). These authors reported that, until 6 months of age, growth is due to an increase in both the number and size of adipocytes. However, the increase in adipocyte number is relatively low until about 70 days for perirenal and 105 days for dorsoscapular depots, with a marked increase after these ages. Mean adipocyte size stabilizes at approximately 6 months of age and later growth is mainly due to increase in the number of adipocytes.

In order to analyze more carefully the changes in fat cell size and distribution of adipocyte sizes for each animal, a group of New Zealand rabbits has been investigated longitudinally with serial biopsies of dorsoscapular and perirenal fat depots from preweaning period to adulthood.

The study of the individual evolutions shows that regular and continuous evolution of mean cell size reported in previous cross-sectional studies may be misleading. Especially, beyond the early period of rapid hypertrophy, "significant drops" of mean diameter with shifting of distributions toward decreasing cell size can occur frequently. The meaning of these unexpected events remains unclear but the most likely hypothesis is the recruitment by pulses of new small cells into the countable adipocyte population.

MATERIALS AND METHODS

Animals and experimental procedure

All animals were male New Zealand rabbits and were studied from birth to 11 months of age. After weaning (28 days of age), they were housed in individual wire cages and allowed free access to water and a commercial pelleted diet (15% crude protein and 14% crude fiber in the dry matter), with a caloric concentration of 3.7 kcal/g.

The room temperature was 20 ± 1°C and the animals were subjected to a lighting schedule that consisted of 12 hr of light and 12 hr of darkness. In order to verify that serial biopsies did not influence growth and food consumption of each studied rabbit, body weights and food intakes were recorded weekly.

The biopsies were performed on 18 animals at 3, 5, 7, 11, 15, 19, 23, 38, and 47 weeks of age. Thus, results of nine biopsies were available for each rabbit. Immediately after the last biopsy, the rabbits were killed and their dorsoscapular and perirenal fat pads were removed and weighed. Samples of dorsoscapular and perirenal fat depots were obtained by surgical biopsies alternatively on the left and right side of the body. The biopsies on the same body side were performed along an antero-posterior line and were separated from each other by approximately 1 cm, so that each site was biopsied only once. Intervals between two consecutive biopsies from the same body side were, respectively, 4, 8, 8, and 24 weeks (left side), 6, 8, and 19 weeks (right side).

Animals were anesthetized by intravenous injection of Nesdonal (20 mg/kg body weight until 7 weeks of age and 12 mg/kg body weight after this age). The anesthesia was of short duration and the entire procedure required approximately 10 min for each rabbit.

Cell size measurements

Small fragments of adipose tissue (about 100 mg) were fixed for 48 hr in 2% buffered osmium tetroxide solution at pH 7.4 and 37°C as described by Hirsch and Gallian (9). The fixed cells were freed by washing with distilled water through a 300-μm nylon mesh filter and collected on a 20-μm nylon mesh filter.

With a projection microscope, 300 cells were sized by means of an image analysis system equipped with a classification program (model Kontron Mop AM03). This instrument provides directly a frequency distribution of cell diameters, with class intervals of 10 μm. Plotting of histograms was realized on a plotter Benson®.

Previous studies carried out in our laboratory with 6-month-old New Zealand male rabbits have shown that regional variations in mean adipocyte size within a depot were quite small. In these studies, when the rabbits were killed, the dorsoscapular and perirenal depots were visually divided in two or three equal parts, respectively. Fat samples for cellular determination were taken in the middle of each part; 14 and 8 rabbits, respectively, were studied for the dorsoscapular and perirenal fat pads. Results of Student's paired t test were never significant. The greatest site-to-site differences in mean cell diameter were 11 and 17 μm, respectively, in the dorsoscapular and perirenal fat depots. Expressed as percentage of the mean cell diameter, the greatest site-to-site differences were, respectively, 8 and 11% (unpublished data).

The total numbers of fat cells in the dorsoscapular and perirenal depots at the time of killing were estimated by dividing the total lipid weight of each depot by the mean lipid weight of adipocytes. Fat cell weight was obtained by assuming that the density of fat cells is that of triolein (0.915 g/ml) as proposed by Lemonnier (10).

The microscopic method utilized here for measuring and counting adipocytes gives quantitatively reliable data for fat cells down to the size of about 20 μm diameter.

RESULTS

Changes with age in mean adipocyte size

Individual data were pooled and plotted against age for dorsoscapular and perirenal fat depots (**Fig. 1**). Mean fat cell size increased rapidly until about 7 weeks of age in the perirenal (biopsy 3) and 11 weeks in the dorsoscapular (biopsy 4). Thereafter, a less rapid increase in diameter was observed in both cases. The greatest values were found at 15 weeks of age in the perirenal (biopsy 5) and 23 weeks in the dorsoscapular depot (biopsy 7). After these ages a slight decrease in mean diameter occurred.

For the two studied depots, the individual evolutions of mean fat cell diameter with age are given in **Table 1** and **Table 2.** Some rabbits were characterized by unexpected and important "drops" of mean diameter between two consecutive biopsies. In order to determine whether regional variations within a depot could account for these "drops," we compared the magnitude of each "drop" to a value calculated from results of the previous study reported above (see Materials and Methods). These results provided an estimation of maximal site-to-site variation, which was about 8 and 11% of the mean cell diameter, respectively, in dorsoscapular and perirenal depots. According to these findings, a "significant drop," which cannot be attributed to regional variation observed within each depot, is defined by the following equations:

$$D_n - D_{n+1} > 0.08\ (D_n + D_{n+1}) \text{ for dorsoscapular depot}$$

$$D_n - D_{n+1} > 0.11\ (D_n + D_{n+1}) \text{ for perirenal depot}$$

where $D_n - D_{n+1}$ is the difference between mean cell diameter from two consecutive biopsies when $D_{n+1} < D_n$. "Significant drops" are shown in Tables 1 and 2.

The use of the regional variation in mean adipocyte size observed for the 6-month-old rabbits may not be applicable to other ages. For this reason, regional variation was investigated for the ages at which each depot showed the greatest occurrence of "significant drops" in mean adipocyte size, i.e., for dorsoscapular depot at 38 weeks and for perirenal depot at 11 and 15 weeks. The procedure used was the same as that described for the

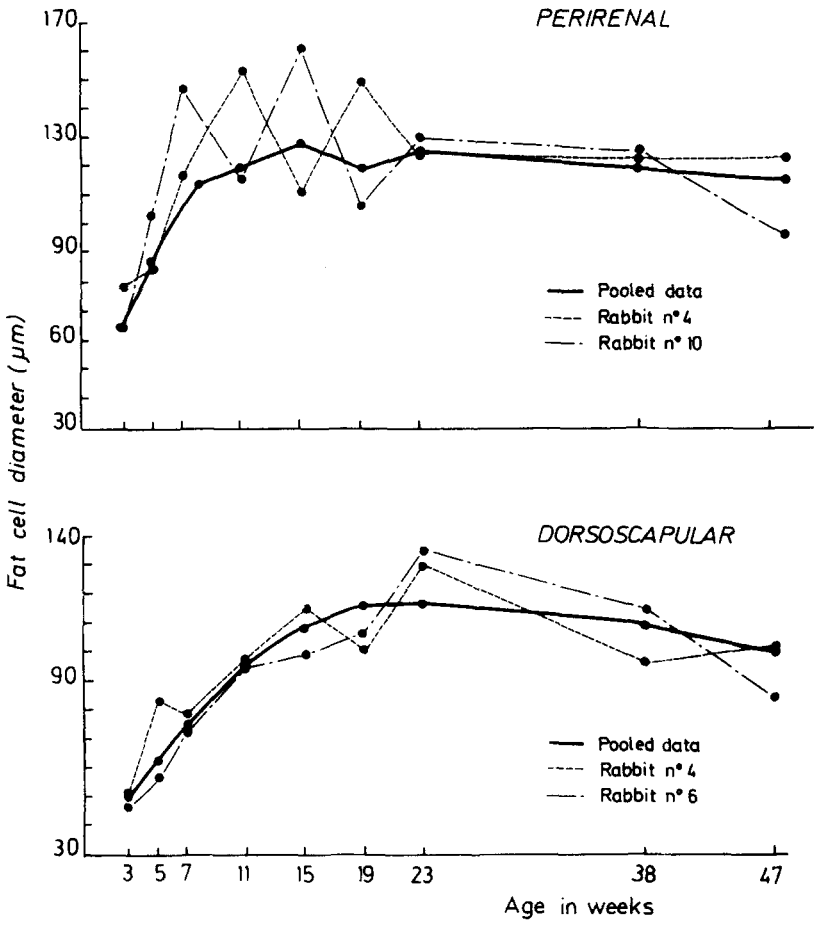

Fig. 1. Fat cell diameter in relation to age for perirenal and dorsoscapular depots. Solid lines represent the evolution of mean fat cell diameter when all animals are pooled. Dotted lines are examples of individual rabbits.

6-month-old rabbits. Mean adipocyte diameters from two sites in dorsoscapular depot and three sites in perirenal depot were compared. Nine rabbits were studied at 38 weeks of age (dorsoscapular), 11 at 11 weeks of age, and 11 at 15 weeks of age (perirenal). The maximal site-to-site variations, expressed as percentage of the mean cell diameter, were similar to those observed for the 6-month-old rabbits, i.e., the regional variation in mean adipocyte size within the depots did not seem to change during adipose tissue growth in the rabbit. The equations used for defining "significant drops" remain valid whatever the ages studied. The number of "significant drops" presented in Tables 1 and 2 are thus not altered.

The earlier occurrence of these "drops" in perirenal depot compared with dorsoscapular depot is quite evident. They are found after biopsy 5 for perirenal and biopsy 7 for dorsoscapular; i.e., approximately the end of the period of hypertrophy in both cases. For a given rabbit, the "drops" were frequently found after the greatest value observed during evolution of mean cell diameter. In both depots, the earlier "drops" were generally followed by an increase in mean cell diameter, while the latter were often followed by a relative stability or even by a new "drop," particularly in the dorsoscapular depot. Examples of individual evolutions characterized by "significant drops" are plotted in Fig. 1.

There was no significant difference in mean body weight gain and mean cumulative food intake between animals with "significant drops" and animals without "significant drops" of mean fat cell diameter (**Table 3**). Body weight gains and food intake between the two biopsies corresponding to each "significant drop" were also investigated and they were always within the range of values observed during the same period for animals without "significant drops." The "significant drops" noted in Tables 1 and 2 are thus apparently not related to perturbations in growth or feeding behavior; they are associated in most cases with substantial increases in body weight and never associated with a decrease.

Serial correlations between individual mean cell di-

TABLE 1. Perirenal fat cell diameter (μm) in relation to age for each rabbit

Rabbit No	Biopsy 1 3 Weeks	Biopsy 2 5 Weeks	Biopsy 3 7 Weeks	Biopsy 4 11 Weeks	Biopsy 5 15 Weeks	Biopsy 6 19 Weeks	Biopsy 7 23 Weeks	Biopsy 8 38 Weeks	Sacrifice 47 Weeks
1	90.2	97.9	118.9	138.9	171.3	114.4	128.6	114.0	106.2
2	81.6	72.7	67.4	108.2	105.0	116.5	132.3	103.5	108.7
3	108.3	74.7	112.2	161.9	122.4	107.6	113.1	130.5	—
4	78.7	85.0	117.1	153.1	110.3	149.3	124.2	122.5	121.8
5	55.9	56.1	99.2	109.6	142.2	110.6	118.0	116.1	107.2
6	61.8	65.5	98.8	118.8	113.6	116.1	126.3	142.9	117.3
7	61.0	68.9	124.3	122.0	110.2	145.8	112.7	109.5	112.1
8	59.0	106.1	120.3	115.9	122.0	122.1	134.3	112.6	125.1
9	56.7	93.6	121.0	118.7	138.1	120.1	124.0	114.2	97.7
10	65.9	103.2	146.3	115.0	159.4	105.3	129.0	126.8	95.1
11	72.6	79.5	94.3	113.5	124.5	114.9	116.3	104.7	92.6
12	74.6	95.1	108.1	105.8	123.5	109.3	130.6	104.5	115.0
13	60.6	80.9	95.8	95.4	121.4	109.7	123.0	123.7	136.8
14	49.5	106.6	151.6	138.6	137.7	145.8	132.4	118.6	119.6
15	58.4	107.1	131.5	120.9	150.7	108.5	109.2	124.3	151.2
16	62.2	91.7	112.0	99.0	147.3	132.1	128.2	133.9	124.1
17	49.6	76.5	103.1	112.0	106.4	125.9	110.9	130.9	118.7
18	47.7	74.4	105.5	116.3	100.1	102.9	109.8	110.0	106.9
Mean ± SEM	66.4 ± 3.4	88.2 ± 3.8	114.7 ± 4.4	119.4 ± 3.8	128.8 ± 4.6	119.2 ± 3.3	124.8 ± 2.5	118.8 ± 2.7	114.8 ± 3.6

Solid lines above two consecutive values indicate the presence of "significant drops" as described in the text and defined by $D_n - D_{n+1} > 0.11 (D_n + D_{n+1})$, where $D_n - D_{n+1}$ is the difference between mean cell diameter from two consecutive biopsies when $D_{n+1} < D_n$.

ameters of two consecutive biopsies were calculated and are given in **Table 4.** There is a great difference between the two depots. Significant correlation was noted only between mean cell diameter at biopsy 2 and mean cell diameter at biopsy 3 for perirenal depot. In the case of dorsoscapular depot, significant correlations were observed over a longer period of time, from biopsy 2 to biopsy 7. It is obvious that the presence of significant cor-

TABLE 2. Dorsoscapular fat cell diameter (μm) in relation to age for each rabbit

Rabbit No	Biopsy 1 3 Weeks	Biopsy 2 5 Weeks	Biopsy 3 7 Weeks	Biopsy 4 11 Weeks	Biopsy 5 15 Weeks	Biopsy 6 19 Weeks	Biopsy 7 23 Weeks	Biopsy 8 38 Weeks	Sacrifice 47 Weeks
1	56.7	52	76.6	126.7	120.9	129.9	122.0	103.2	104.8
2	50.6	53.9	58.3	79.0	88.6	115.6	106.4	101.0	90.8
3	58.1	53.2	71.4	131.5	111.0	85.3	106.3	124.5	—
4	51.5	83.2	78.3	97.4	114.2	99.8	129.9	96.2	101.8
5	48.9	47.1	77.4	104.4	95.9	125.5	123.9	105.2	89.1
6	45.5	56.7	72.6	94.3	98.1	116.6	134.2	113.9	84.9
7	45.6	56.1	70.5	111.6	105.5	109.7	127.9	109.0	103.8
8	37.6	55.6	68.6	74.0	80.4	113.3	99.4	126.5	117.0
9	43.0	55.9	72.7	72.4	92.7	112.7	115.2	102.3	106.8
10	49.9	87.8	81.9	112.2	139.0	142.7	125.0	119.5	76.6
11	49.4	61.4	64.0	76.7	95.2	104.9	103.2	108.9	82.5
12	48.8	58.9	77.3	85.5	112.2	97.9	99.8	101.9	98.9
13	41.4	56.0	59.3	79.6	97.1	102.6	114.6	104.2	122.8
14	68.7	67.9	73.1	110.4	146.6	144.3	91.8	121.7	111.2
15	50.1	78.4	84.3	120.1	125.8	133.9	151.5	110.2	115.3
16	54.3	61.0	66.9	108.4	115.5	132.3	142.4	118.3	109.1
17	51.3	54.3	69.6	74.1	99.2	102.6	105.7	114.4	109.4
18	44.6	58.6	59.2	72.0	87.0	76.4	80.6	97.6	90.6
Mean ± SEM	49.8 ± 1.5	62.0 ± 2.5	73.8 ± 2.5	97.4 ± 4.4	108.3 ± 4.0	115.0 ± 4.1	117.1 ± 4.0	109.9 ± 2.5	100.9 ± 3.2

Solid lines above two consecutive values indicate the presence of "significant drops" as described in the text and defined by $D_n - D_{n+1} > 0.08 (D_n + D_{n+1})$, where $D_n - D_{n+1}$ is the difference between mean cell diameter from two consecutive biopsies when $D_{n+1} < D_n$.

TABLE 3. Body weight gain and total food intake between the first biopsy and the time of killing for animals with "significant drops" (A) and animals without "significant drops" (B) of mean fat cell diameter

	A	B	
Perirenal			
Body weight gain (g)	3357 ± 297	3703 ± 285	NS
Food intake (kg)	44.4 ± 1.9 (8)	42.9 ± 4.1 (9)	NS
Dorsoscapular			
Body weight gain (g)	3613 ± 360	3658 ± 211	NS
Food intake (kg)	44.9 ± 3.0 (9)	42.1 ± 3.2 (8)	NS

Values are mean ± SD; number of animals in parentheses; NS, non-significant (two-tailed Student's t test).

relations corresponds to the lack of "significant drops" and reveals the period where hypertrophy takes place in most animals.

Changes with age in adipocyte size distribution

Histograms showing the average distribution of adipocyte diameters of the dorsoscapular and perirenal depots at the various ages studied are depicted in **Fig. 2.**

With increasing age the population of fat cells shifted toward increasing cell size until 7 weeks of age (biopsy 3) for the perirenal depot and 19 weeks of age (biopsy 6) for the dorsoscapular depot. Then, the average distribution stabilized before a slight shifting in the opposite direction between 38 and 47 weeks of age.

The dispersion of cell diameters increased with age but occurred sooner and was greater for perirenal until about 19 weeks of age (biopsy 6). Later in development the scatter of distribution was very similar. In both depots, small cells were always present.

The study of changes in distribution of adipocyte diameters for each rabbit often showed a more complex pattern of evolution. The general trends described for the average distribution were also found for individual evolutions. They revealed the presence of some bimodal distributions, particularly for the perirenal depot at 5 weeks of age (biopsy 2); the shifting of distributions toward the smaller cells between two consecutive biopsies, corresponding to the "drops" in mean adipocyte size previously described; and a considerable variation, at a given age, in the shape of adipocyte distributions, indicating that the average distribution is very misleading.

To illustrate these findings, histograms of the perirenal and dorsoscapular depots at the various ages are presented in **Fig. 3** and **Fig. 4.** In each case, only two rabbits are selected as examples, one with a "significant drop" and the other with a more regular evolution. It is interesting to examine more carefully the histograms of biopsies 4 and 5 (Fig. 3, rabbit number 4) and biopsies 7 and 8 (Fig. 4, rabbit number 15); a "significant drop" in mean diameter occurred between these two consecutive biopsies. In these two examples, there was apparently a recruitment of small cells, and cells less than 60–80 μm in diameter were not observed before the "drops."

These examples are representative of most of the "drops" noted for the perirenal depot. The generalization of this statement is less obvious for the dorsoscapular depot, in which the "drops" occurred later in development.

Some histograms are also presented for perirenal depot at 5 and 15 weeks of age (biopsies 2 and 5), as examples of the great variation between individuals at a fixed age (**Fig. 5**). We note that skewness and peakedness of diameter distributions often showed considerable departure from normality.

Presence of "drops" and adipocyte number at the time of killing

In **Table 5,** information is provided at the time of killing on the number of adipocytes for animals with "significant drops" during the evolution of mean adipocyte diameter, and animals without "significant drops." In the two depots, mean adipocyte number was larger for animals with "drops" than for animals without "drops," but the differences were never statistically significant (one-tailed Student's t test).

DISCUSSION

When data from all rabbits are pooled, the age-related changes in mean diameter are in agreement with the results reported by Nouguès (7) in a cross-sectional study carried out on the same rabbit species. The values of mean adipocyte diameter observed at the various ages are very similar to those noted by this author and confirm that serial biopsies had little or no influence on fat cell size. The increase in mean fat cell diameter occurs until about the age of 15 weeks in the perirenal and 23 weeks in the dorsoscapular depot. The slight decrease in mean diameter noted for the last biopsies could be related to the observations of Stiles, Francendese, and Masoro (11) and De Martinis and Francendese (12). These authors re-

TABLE 4. Correlation coefficients between mean cell diameters of two consecutive biopsies for perirenal and dorsoscapular depots

Biopsies	Perirenal	Dorsoscapular
B_1–B_2	−0.01	0.18
B_2–B_3	0.68[a]	0.50[b]
B_3–B_4	0.17	0.59[b]
B_4–B_5	0.05	0.71[a]
B_5–B_6	−0.18	0.61[a]
B_6–B_7	0.17	0.49[b]
B_7–B_8	−0.10	0.01
B_8–Sacrifice	0.38	0.17

[a] $P < 0.01$.
[b] $P < 0.05$.

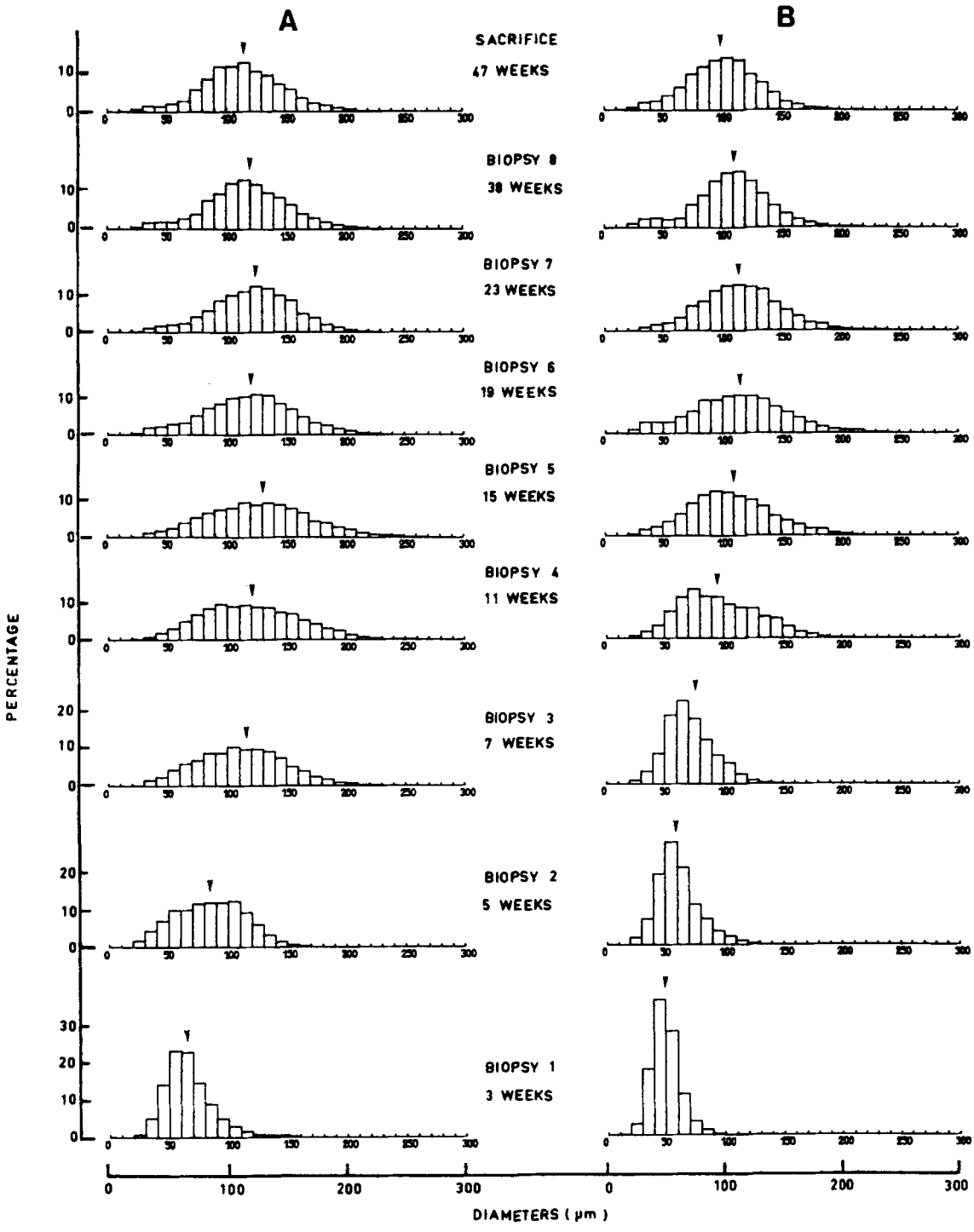

Fig. 2. Histograms of the average distribution of adipocyte diameters from the perirenal (A) and dorsoscapular (B) depots as a function of age. Arrows point out the mean cell size for each histogram.

ported that fat cell size decreases in the epididymal depot of the rat after the age of 52 weeks, as a result of an increase in cell number with very advanced age. In the present study, the decrease in mean diameter is also probably due to an increase in adipocyte number, since the cross-sectional study of Nouguès and Vézinhet (8) shows that late perirenal and dorsoscapular growth results from an increase in the number of adipocytes until approximately 10 months of age. The evolution of the average distribution of adipocyte diameters in the peri-

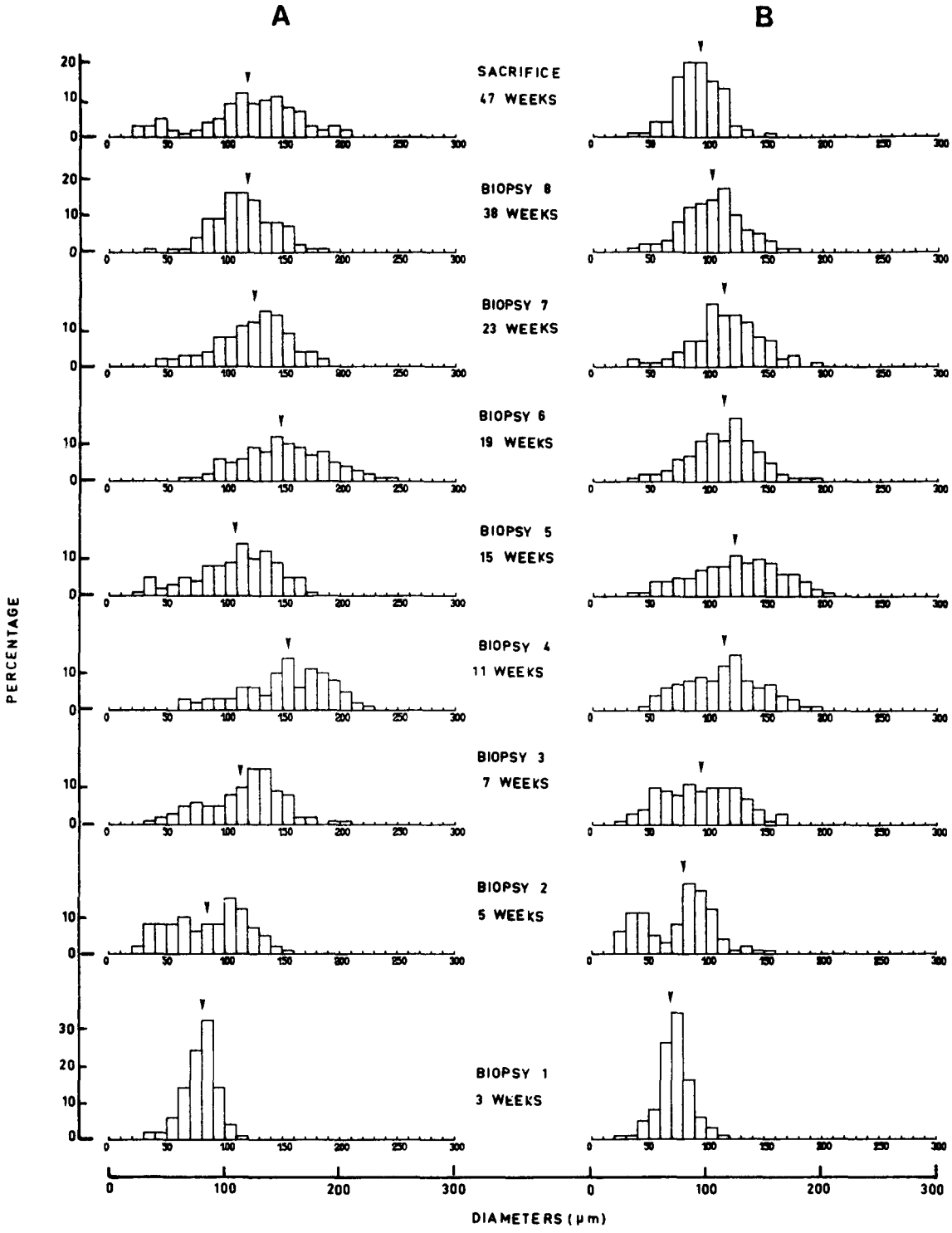

Fig. 3. Histograms of distributions of adipocyte diameters for the perirenal depot of two rabbits as a function of age. A, Example of a rabbit characterized by a "significant drop" of mean diameter: rabbit no. 4, where the "significant drop" occurs between biopsy 4 and biopsy 5. B, Example of a rabbit characterized by a more regular evolution: rabbit no. 11. Arrows point out the mean cell size for each histogram.

renal depot is also in agreement with the description of Nouguès (7).

When the individual evolutions of mean diameter or diameter distributions of adipocytes are considered, a remarkably complex pattern of changes becomes evident that would have been completely undetected if we had

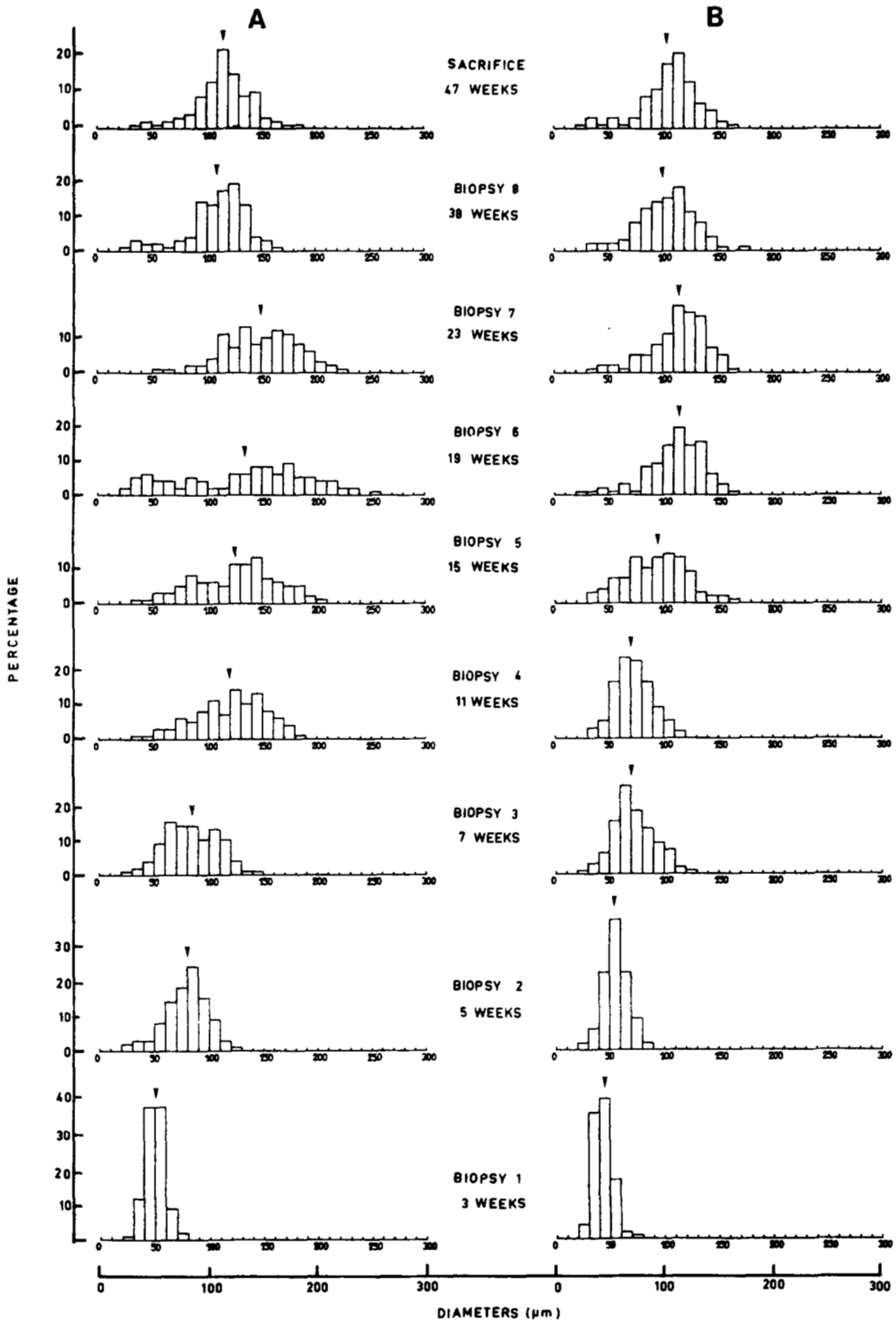

Fig. 4. Histograms of distributions of adipocyte diameters for the dorsoscapular depot of two rabbits as a function of age. A, Example of a rabbit characterized by a "significant drop" of mean diameter: rabbit no. 15, where the "significant drop" occurs between biopsy 7 and biopsy 8. B, Example of a rabbit characterized by a more regular evolution: rabbit no. 9. Arrows point out the mean cell size for each histogram.

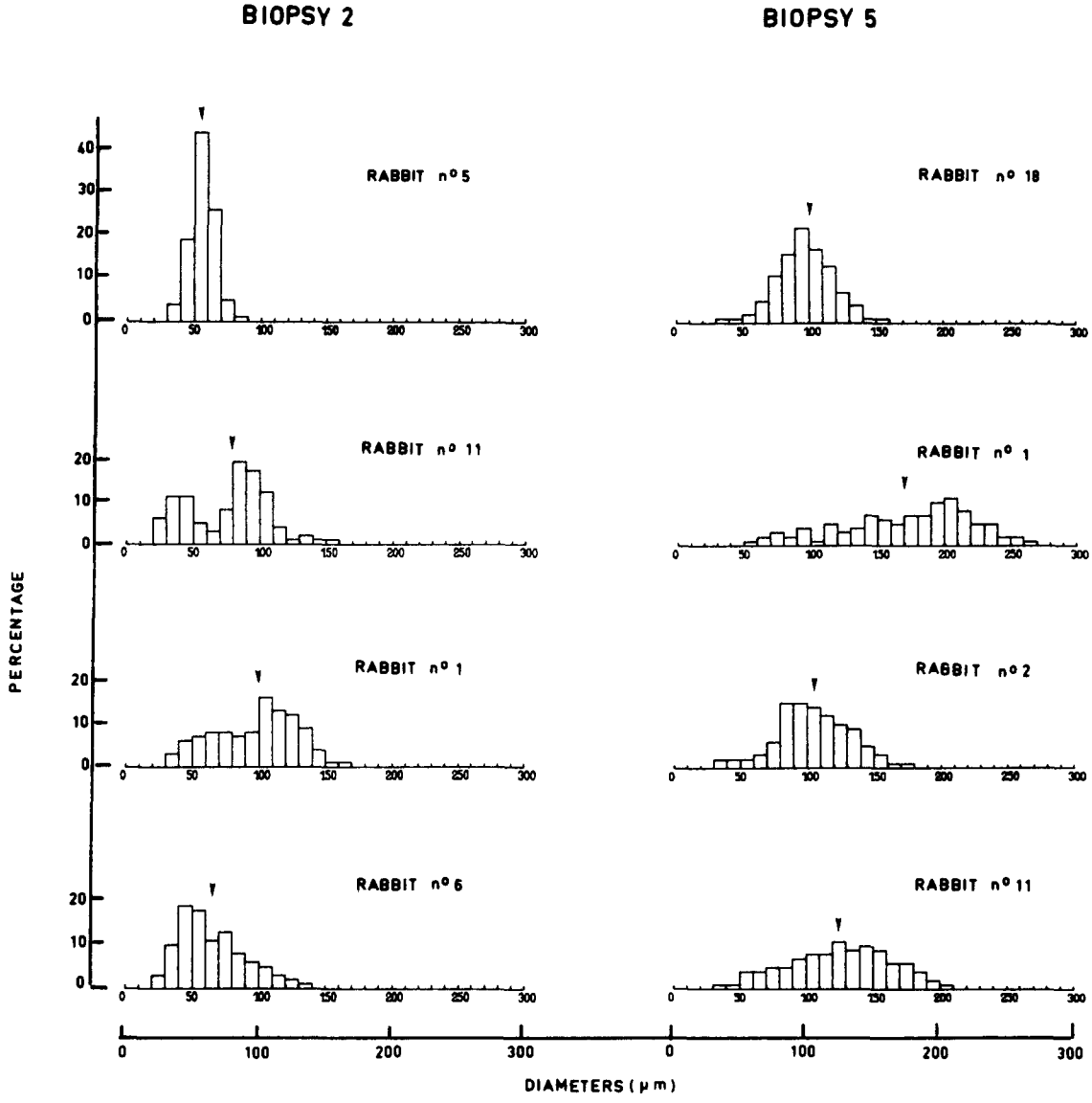

Fig. 5. Example of some individual histograms from perirenal depot at 5 and 15 weeks of age (biopsies 2 and 5). Arrows point out the mean cell size for each histogram.

examined only the average cell size or the average distribution. Some rabbits are characterized by "significant drops" of mean diameter between two consecutive biopsies. These "drops" cannot be attributed to regional variation observed within each depot or to growth or food intake disorders. They might reflect some redistribution of the fat cell sizes by delipidation. This hypothesis is, however, unlikely because it implies that delipidation is possible without detectable impairment of growth, while the "drops" are associated in most cases with substantial increases in body weight and are never associated with a decrease. Moreover, even if a transient delipidation caused by stress following surgical biopsy occurred, it is unlikely that this delipidation would remain visible at the end of the interval between two consecutive biopsies. Lastly, this hypothesis of delipidation does not explain why the "drops" occur at different ages in the dorsoscapular and perirenal depots. As our study took place

TABLE 5. Adipocyte number at time of killing (47 weeks of age) for animals with "significant drops" (A) and animals without "significant drops" (B) of mean fat cell diameter

	A	B
	cell number × 10^6	
Perirenal	283 ± 97 (8)	248 ± 124 (9)
Dorsoscapular	65 ± 19 (9)	47 ± 31 (8)

Values are mean ± SEM; number of animals in parentheses.

during the period of rapid fat tissue growth, the observation of "drops" may be more consistent with the mean cell size decrease associated with weight gain, noted by Ashwell and Garrow (4) during experimental overfeeding of volunteers. Their results of a paradoxical decrease in mean cell size with weight gain were due to the recruitment of small cells. In our case there is also, apparently, a recruitment of new fat cells into the measurable pool of mature adipocytes. Cells less than 60–80 μm in diameter are not observed before the "drops," but are present afterwards in most cases for the perirenal depot. This is less obvious for the dorsoscapular depot in which the "drops" occur later in development, but we must remember that intervals between biopsies are larger during this period, making the interpretation of latter "drops" more difficult.

Furthermore, for the two depots the presence of "drops" corresponds to the periods of marked increase in adipocyte number described by Nouguès and Vézinhet (8) in a cross-sectional study.

All of these results strengthen the idea that the "drops" in mean adipocyte size are due to the introduction of new small cells into the countable depot population.

The "significant drops" are frequently found after the greatest value observed during individual evolution of mean cell diameter. One possible explanation is that the "drops" occur when adipocyte hypertrophy stops, whereas there is a continuous and rapid increase in the observed number of fat cells. Another speculation is that there may be an important interaction between fat-cell size and cell number. When a certain large adipocyte size, characteristic of each rabbit, is attained, a stimulus for new cell production or differentiation may be produced.

The careful examination of individual evolutions of mean fat-cell diameter and of distributions of fat-cell diameter is more in favor of the second hypothesis and suggests that the recruitment of new small cells may be discontinuous, i.e., may occur by pulses, as a result of either a reinitiation of hyperplasia or multiphasic periods of differentiation from preadipocytes and subsequent lipid filling. Since our study was performed by measurement of the size of mature fat cells by fixing these cells with osmium tetroxide followed by sizing, the frequency distribution data of adipocyte diameters do not permit us to differentiate between hyperplasia or differentiation of preexistent cells.

The discontinuous recruitment of new observable cells, suggested by our results, is in agreement with the hypothesis of a "critical size" of adipocytes expressed recently by numerous authors (3, 13–15). In that case, the critical size would be reached at different times in perirenal and dorsoscapular regions. However, the presence of bimodal adipocyte diameter distributions in the perirenal depot at 5 weeks of age suggests that, in the early growth period, new small cells may be recruited by pulses, without relation to the size of available adipocytes. It is possible that different regulatory systems operate during early or late growth periods. In the rat, the experiments of Greenwood and Hirsch (16) using in vivo injections of ^{3}H thymidine have shown that the rate of proliferation and the time spent in the differentiating compartment are variable with age.

The fact that only about 50% of the animals present "significant drops" may be due either to different patterns of evolution of fat cell populations or to the methodological procedures. Biopsies are only pictures of a complex evolution, i.e., "drops" are not always examined at the same stage and some may be undetected. At the time of killing the rabbits, the mean adipocyte numbers of perirenal and dorsoscapular depots are not significantly different between animals with "drops" and animals without "drops." Consequently one must be cautious in the interpretation of such variability between animals, and we cannot distinguish two groups of animals on this basis.

The present investigations also provide evidence that bimodal distributions of adipocyte diameters can be observed in isolated cell preparations of individual normal growing animals. Other studies involving very small fat cells (cells 8–35 μm in diameter) led De Martinis and Francendese (12) to conclude that bimodality may be a usual component of normal adipose tissue. All of these results refute the impression gained from earlier studies that bimodality is most likely to be found in adipose depots of farm animals noted for excessive weight gain or in genetically obese animals (17–20).

The differences in behavior of perirenal and dorsoscapular depots described in our study must be related to differences previously observed in our laboratory between these two depots. During the postnatal period in the rabbit, the perirenal fat shows an increasing allometry, while the subcutaneous fat shows a slightly decreasing allometry (21). Surgical removal of dorsoscapular fat did not lead to regeneration in the rabbit, whereas regeneration of the perirenal fat was substantial regardless of the age of the animals at the time of surgery (22). These data, while derived from a variety of experimental approaches, might indicate the existence of different fat-cell populations.

Lastly, our results show that longitudinal studies are of great interest for the description of changes in adipocyte size that occur during adipose tissue growth. The wide diameter range, the skewness of adipocyte distributions, the presence of bimodal distributions, and the occurrence of "drops" of mean adipocyte diameters during growth demonstrate, in any case, that the examination of only average cell size or average distribution evolutions may be misleading.

Although interpretation of the results remains difficult, they lead to some interesting questions. We are aware of the fact that all our arguments supporting the hypothesis of discontinuous recruitment of new fat cells are associative and only theoretical, but there is no conclusive argument that would seem to prevent this interpretation.

We wish to thank Mrs. Odette Frecerro, Thérèse Chery, Josette Soriano, and Mr. E. Bouthier for skilled technical assistance.

Manuscript received 4 April 1984.

REFERENCES

1. Häger, A., L. Sjöström, B. Arvidson, P. Björntorp, and U. Smith. 1977. Body fat and adipose tissue cellularity in infants: a longitudinal study. *Metabolism.* **26:** 607–614.
2. Knittle, J. L., K. Timmers, F. Ginsberg-Fellner, R. E. Brown, and D. P. Katz. 1979. The growth of adipose tissue in children and adolescents. *J. Clin. Invest.* **63:** 239–246.
3. Sjöström, L., and T. William-Olsson. 1981. Prospective studies on adipose tissue development in man. *Int. J. Obes.* **5:** 597–604.
4. Ashwell, M., and J. S. Garrow. 1973. Full and empty fat cells. *Lancet.* **2:** 1036–1037.
5. Ginsberg-Fellner, F., and J. L. Knittle. 1981. Weight reduction in young obese children. I. Effects on adipose tissue cellularity and metabolism. *Pediatr. Res.* **15:** 1381–1389.
6. DiGirolamo, M., L. Thurman, and J. Cullen. 1974. Observations on adipose tissue cellularity and development in rats and rabbits fed ad libitum. *In* The Regulation of Adipose Tissue Mass. J. Vague and J. Boyer, editors. Excerpta Medica, Amsterdam. 175–180.
7. Nouguès, J. 1975. Adipocyte growth of four adipose deposits in rabbit. *Ann. Biol. Anim. Biochim. Biophys.* **15:** 541–546.
8. Nouguès, J., and A. Vézinhet. 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.
9. Hirsch, J., and E. Gallian. 1968. Methods for the determination of adipose cell size in man and animals. *J. Lipid Res.* **9:** 110–119.
10. 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.
11. Stiles, J. W., A. Francendese, and E. J. Masoro. 1975. Influence of age on the size and number of fat cells in the epididymal depot. *Am. J. Physiol.* **229:** 1561–1568.
12. DeMartinis, F. D., and A. Francendese. 1982. Very small fat cell populations: mammalian occurrence and effect of age. *J. Lipid Res.* **23:** 1107–1120.
13. Faust, I. M., P. R. Johnson, J. Stern, and J. Hirsch. 1978. Diet-induced adipocyte number increase in adult rats: a new model of obesity. *Am. J. Physiol.* **235:** E279–E286.
14. 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.
15. Björntorp, P., M. Karlsson, and P. Pettersson. 1982. Expansion of adipose tissue storage capacity at different ages in rats. *Metabolism.* **31:** 366–373.
16. Greenwood, M. R. C., and J. Hirsch. 1974. Postnatal development of adipocyte cellularity in the normal rat. *J. Lipid Res.* **15:** 474–483.
17. 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.
18. Allen, C. E. 1976. Cellularity of adipose tissue in meat animals. *Federation Proc.* **35:** 2302–2307.
19. Kaplan, M. L., J. R. Trout, and G. A. Leveille. 1976. Adipocyte size distribution in ob/ob mice during preobese and obese phases of development. *Proc. Soc. Exp. Biol. Med.* **153:** 476–482.
20. Kaplan, M. L., J. R. Trout, and P. Smith. 1980. Adipocyte size distribution in fa/fa rats during development. *Metabolism.* **29:** 333–339.
21. Vézinhet, A., and M. Prud'hon. 1975. Evolution of various adipose deposits in growing rabbits and sheep. *Anim. Prod.* **20:** 363–370.
22. Reyne, Y., J. Nouguès, and A. Vézinhet. 1983. Adipose tissue regeneration in 6-month-old and adult rabbits following lipectomy. *Proc. Soc. Exp. Biol. Med.* **174:** 258–264.