A simple method to determine fat cell size and number in four mammalian species

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Among the methods by which the size and number of fat cells in adipose tissue can be determined are those based on direct microscopic determination of the diameter of fat cells isolated by collagenase incubation and stained (3, 6, 11, 15, 17, 26). These methods, relatively simple and economic, take advantage of the spherical shape assumed by isolated fat cells floating on the surface of an aqueous medium.

In this report, we shall analyze one such method and the assumptions underlying the principles involved. We shall determine the degree of precision and reproducibility of the method and discuss the advantages and limitations. We shall then report on the application of this methodology to the determination of fat cell size and number in adipose tissue of over 200 animals from four mammalian species, and in four separate locations of adipose tissue in the 2-month-old male rat.

MATERIALS AND METHODS

Animals

a) Male Wistar rats, from age 5 weeks to 16 months, fed Purina laboratory chow ad libitum; b) male golden hamsters, from age 5 weeks to 12 months, fed Purina laboratory chow ad libitum; c) male albino guinea pigs, from age 6 weeks to 13 months, fed Purina guinea pig chow ad libitum and green food once weekly; d) female mongrel dogs of unknown age. The dogs were fed Purina dog chow for a minimum of 1 week before sacrifice. The dietary composition and details of sacrifice of the animals were the same as previously described (7, 9).

Adipose tissue was obtained from the epididymal fat pads of rats, hamsters, and guinea pigs and from the perirenal, mesenteric, and subcutaneous areas of rats. In the dog, samples were obtained from the right subcutaneous inguinal region.

Experimental Procedures

Immediately after sacrifice, the two epididymal fat pads were excised, weighed without prior blotting, and sections of tissue (weighing 100–300 mg) were cut along the longitudinal axis, weighed, and either placed in chloroform:methanol 2:1 for lipid extraction or placed in warm medium with collagenase for morphological studies.

Morphological studies. Tissue fragments were incubated
in 3 ml of Krebs-Ringer bicarbonate medium (CaCl₂ concentration 1.22%) containing, per ml: 3 μmoles glucose, 40 mg defatted bovine serum albumin (Pentex Corp.), and collagenase from Clostridium histolyticum (Worthington Corp.) at a concentration of 5-10 mg/g adipose tissue. The pH of the medium, adjusted to 7.4, did not vary more than ±0.1 unit during the incubation. The osmolality of the medium was found to be 287-290 mOsm/liter, the SG 1.016-1.018. The incubation was carried out in a Dubnoff shaker at 37°C for 1 hr, at 60-80 strokes/min. The glassware was siliconized with Dri-tote (Fisher Scientific Co.); polyethylene vials, test tubes, and tubing were used in the handling of fat cells. The isolation, washing (in medium lacking collagenase), collection, and dispensing of fat cells were done following the procedure described by Rodbell (18).

**Determination of fat cell diameter.** A 1-ml aliquot of the isolated fat cell suspension was added to 4 ml of warm medium in a Packard plastic scintillation vial to which 1 mg of methylene blue was added. After staining for 2-5 min, successive 0.2- to 0.4-ml aliquots of the stirred suspension of stained cells were placed on a siliconized glass slide and examined with a Zeiss microscope equipped with a

![Image](https://via.placeholder.com/150)

**FIG. 1.** This figure illustrates basic microscopic determination of fat cell diameter. Stained fat cells, floating on surface of medium, are aligned on caliper scale, brought into focus, and transverse diameter is recorded in units. Distance between 0 and 1, or 1 and 2 is divided in 10 units of 14 μm interval or 20 units of 7-μm interval. Cells are grouped in classes with midpoints of successive 7-μm multiples. Actual magnification 220X. Free fat cells seen in this photograph are derived from epididymal fat pads of a 620-g rat and range from 92 to 152 μ in diameter.

Polaroid camera attachment. The insertion of a micrometer disc in a focusing eyepiece placed in the phototube of the camera attachment produced a projected caliper scale (Fig. 1). At a magnification of 190X, the caliper scale was calibrated so that the unit marks had a constant interval of 7 μ. The free fat cells, floating on the surface of the medium, were recognized by the spherical shape, the stained nucleus with one or two nucleoli, and the stained cytoplasm; the latter features readily distinguished the fat cells from occasional droplets of floating lipid. Three hundred to 600 fat cells from the same population were brought in the caliper field with systematic motion of the stage control knobs. The cells were aligned on the caliper scale, the equatorial plane of the cell was brought into focus, and the fat cell diameter was determined with an accuracy of ±3.5 μ. In this fashion, cell diameters between 7 and 154 μ were recorded in classes with midpoints of successive 7-μ multiples to provide a frequency distribution of diameters in 22 categories of size. The sizing and grouping of 300-400 fat cells were performed by one observer in approximately 15-20 min. From this, the mean diameter and the standard deviation about the mean could be rapidly calculated by the usual formulas (5). Figure 2 shows a representative histogram of fat cell diameters in the rat. Polaroid photographs were taken in representative samples of each fat cell population studied. Repeated determinations in fat cells with diameters 20-130 μ showed a ratio of cell membrane width to total cell diameter consistently lower than 1%. Thus, in this study the terms cell diameter and diameter of lipid droplet are used interchangeably.

**Calculation of mean surface area and volume of a fat cell population.** From the data on diameters (D) of 300-600 fat cells in suspension, the frequency distributions could also be plotted for the fat cell surface area (SA = πD²) and cell volume (V = πD³/6). However, taking into consideration the nonlinear transformation involved in the calculation of surface area and volume from the mean diameter of each class of fat cells, all the cells falling within the class intervals were assigned a surface area or volume equal to the average of the smallest and largest surface area (or volume, respectively) within that class interval (see Fig. 3). This resulted in a closer approximation to a “true” surface area for
Whatman paper no. 12, and the solvent again evaporated to yield the adipose lipid as an oil. Three 0.1-ml volumetric pipettes were recalibrated by filling to the mark with mercury at 20 C, expelling the mercury into a tared vessel, and weighing it. The weight of the oil at 20–25 C over the volume of the calibrated 0.1 ml pipette, in triplicate, provided a measure of the lipid density.

**Number of fat cells in adipose tissue.** The estimate of the number of fat cells in a fragment of adipose tissue can be obtained by dividing the total lipid content of the tissue by the average lipid content of the fat cells. The lipid content of the average fat cell is derived by the mean cell volume X density of lipid. The series of operations involved in the determination of fat cell number in a sample of tissue are depicted in Figure 4. This method is based on two assumptions: that all or most of the adipose tissue lipid is intracellular and that the lipid droplet assumes spherical configuration in isolated fat cells so that the determination of the transverse diameter of the cell essentially represents the diameter of the lipid droplet of the cell.

The three key operations required to determine the total number of fat cells (n) in a sample of adipose tissue are: determination of lipid content of the sample (A); determination of mean cell volume (B); and determination of lipid density (D).

**Statistical evaluation of data.** Means, standard deviation, and standard error of the mean were estimated in the usual way. A measure of the relative variation in the analytical techniques employed and in the operations necessary to determine fat cell volume and number was obtained by the determination of the coefficient of variation (CV = standard deviation/mean) for 5–15 repeated determinations of the same samples. The CV for the various operations, expressed as a percentage, is shown in the results. The significance of differences between group means was estimated by the Student t test. Values for P of <.05 were taken to indicate significance.

**RESULTS.**

The results of this study are divided in two major sections: 1) evaluation of the methodology and validation of it, 2) application of the method to the determination of adipose tissue characteristics in four animal species.

![Diagram](https://via.placeholder.com/150)

**FIG. 4.** Schematic representation of operations necessary to estimate total number of fat cells in a given weight of adipose tissue. See text for details.
ADIPOSE CELL SIZE AND NUMBER

Evaluation of Methodology and Validation

The operations listed under experimental procedures in MATERIALS AND METHODS were subjected to an estimate of relative variation in separate and preliminary experiments.

The diameter of individual fat cells (ranging from 20 to 100 μ) was measured 15-20 times by successively realigning the cell with the caliper scale and refocusing on it. Almost always, the cell diameter fell within the limits of the same class so that little error resulted from this operation.

The calculations to estimate mean surface area and mean volume of a fat cell population were subjected to independent evaluation. The histograms of the surface areas and volumes obtained from the histograms of 400 fat cell diameters, derived from five isolated fat cell populations, were individually explored by the application of Simpson’s rule for irregular areas (23). The application of Simpson’s formula, although elaborate and time consuming, provided a rule for irregular areas (23). The application of Simpson’s formula, although elaborate and time consuming, provided the values for surface area and volume in close agreement with the values obtained as indicated above. In addition, the values obtained for mean cell volume with the method described were compared to values obtained by calculating the volume from the mean and the variance of the diameters according to Goldrick (12). The mean volumes of 20 populations of fat cells (ranging from mean diameter 20 to 100 μ) were calculated with both methods. The volume calculated according to Goldrick exceeded the estimate with our method by less than 1 % for fat cell populations with mean diameters between 50 and 100 μ, and by 2-4 % for fat cell populations with mean diameter below 50 μ.

The relative variation of the techniques to determine lipid content (both gravimetric and colorimetric) and to determine lipid density was evaluated by calculating the coefficient of variation, CV, of 5-15 replicate determinations from the same lipid samples. CV was found to be 0.73 % for the gravimetric and 1.51 % for the colorimetric determination of lipid and 0.27 % for the lipid density determination. CV for determination of tissue lipid content was 0.9 %.

The reliability of the sizing procedure was evaluated by repeating the determination of diameter in six replicated 100 fat cell samples from the same cell population obtained from epididymal adipose tissue of a 115-g rat and a 628-g rat. The comparison of the six means of 100 fat cell diameters (Table 1) revealed a mean of 30.99 μ with a range of 30.0-32.0 μ for rat A and a mean of 87.48 μ with a range of 86.1-89.9 μ for rat B. The coefficient of variation for the sizing operation was 2.2 and 1.6 %, respectively, for the cell diameters from rats A and B, indicating a good degree of reproducibility for this operation.

The next study was done to ascertain whether the isolated fat cells were representative of the population of cells present in the intact epididymal pad. Measurements of fat cell diameters were done on 400 consecutive fat cell samples obtained during the collagenase incubation of the two epididymal pads from a 202-g rat, at 15-min intervals during the 1-hr collagenase treatment and from cells that had incubated in a collagenase-free medium for additional 2 hr. The proportion of fat cells released by the tissue at 1 hr, the proportion of fat cells that were present at 1 hr of incubation, does not affect preferentially cells with diameters at the extremes of the bell-shaped curve.

We then proceeded in determining how many fat cells have to be sized in order to determine with an acceptable degree of precision the average fat cell diameter, surface area, and volume. An inspection of Figs. 1, 5, and 6 and examination of the data summarized in Table 1 reveal that cell populations present varying degrees of heterogeneity in size. Characterization of the sample of fat cells by the cell measurements would include a “methodological” variation (due to the technique employed in the determination of cell diameters and to chance in the selection of the sample cells) and a “biological variation” reflecting a true natural dispersion in fat cell size.

An estimate of the methodological variation can be obtained by calculating the coefficient of variation of six replicate determinations of the mean diameter of 100 cell samples from the same fat cell population. As already indicated, CVs were 2.2 % in the case of rat A of Table 1 and 1.6 % for rat B. On the other side, an estimate of the biological variation in size of the cells present in each population

| TABLE 1. Adipose cell measurements in two populations of rat cells isolated from epidymal depot of a lean rat (rat A) and a large rat (rat B) |
|-----------------|-----------------|-----------------|
|                | Cell Diam, μ    | Cell Area, μm²  | Cell Vol, μl |
| Rat A           | Mean ± s.d.     | Mean ± s.d.     | Mean ± s.d.  |
| Mean CV        | Mean CV         | Mean CV         |
| Rat B           | 87.48 ± 1.41    | 7.21 ± 0.29     | 17.50 ± 0.46 |
| Mean CV        | 0.050           | 0.016           | 0.050        |

Rat A, age 5 weeks, weight 115 g; Rat B, age 15 months, weight 628 g. For each of the two fat cell populations, six replicated samples of 100 cells were taken and all measurements obtained as described in the text. Mean and s.d. represent mean and standard deviation for measurements of the total 600 fat cells. CV1 = coefficient of variation = s.d. / mean. CV1 and s.d. are the mean and s.d. of the six mean values of cell measurements repeated on six aliquots of 100 cells from the same cell population. Therefore, Mean1 = Mean1 ± s.d., CV2 = s.d. of six mean values / mean.
can be obtained by calculating the coefficient of variation of cell diameter determinations of 300–600 fat cells \( (CV_1 = \text{SD/mean}) \) and then subtracting from this the \( CV_2 \) value due to the methodological variation. In Table 1, rat \( A \) presents a biological variation expressed by a CV of 27–22%, that is, approximately 25%; rat \( B \) presents a CV of 15–16%, that is, approximately 13%.

The precision of the determination of mean cell measurements will increase as the number of cells is increased. In rat \( A \) of Table 1, if 100 cells are employed, CV will be 2.7, 5.4, and 8.5%, respectively, for mean cell diameter, surface area, and volume (as derived by \( CV/\sqrt{n} \)); for 300 cells it will be 1.6, 3.1, 4.9%, respectively; for 500 cells it will be 1.2, 2.4, 3.8%. In the larger rat \( B \), CV for 300 cells is 0.9, 1.7, and 2.6%, respectively, for the three cell measurements. For this reason, sizing of at least 300 fat cells is recommended in order to keep the estimate of mean fat cell volume correct within 5%, a limit considered acceptable in many biological measurements.

The method proposed to determine the number of fat cells in adipose tissue was evaluated. Since the determination of fat cell number in a tissue sample requires several operations (Fig. 4), the method proposed was evaluated as follows: 1) evaluation of CV for the number of cells \( (n) \) by application of the formula for the propagation of error:

\[
CV_n = \sqrt{CV_A^2 + CV_B^2 + CV_D^2},
\]

where \( CV_A \), \( CV_B \), and \( CV_D \) are variances of CV for operations \( A \) (lipid content of tissue), \( B \) (mean cell volume), and \( D \) (lipid density), respectively; 2) by comparing in the epididymal pads of 11 rats (Table 2) the total numbers of cells obtained by the method proposed to those obtained by methods 2 and 3 described below.

In the method proposed, method \( I \), \( CV_A \) was found to be 0.9%, \( CV_B \) 4.9%, and \( CV_D \) 0.27%. The calculated \( CV_n \) was 5.0%. Thus, the 95% confidence limits for the number of fat cells in adipose tissue, estimated by the proposed method, are \( 2 \times CV \) or \( \pm 10\% \). This appears to be within limits considered acceptable in the determination of biological variables. If one determines the mean cell volume on more than 300 cells, or if one is measuring cells with diameter larger than 60 \( \mu \) (as seen in Table 1), then the accuracy of the estimation for both cell size and number improves so that the 95% confidence limits become \( \pm 5-7\% \).

Method 2 is based on the following principle: the total number of fat cells in a sample is equal to the lipid content of the sample divided by the mean lipid content of a counted number of suspended free fat cells. This method requires the count of fat cells in an aliquot of the cell suspension. This was done according to the techniques described by Gliemann (11) and Turtle and Kipnis (24). The coefficient of variation for the operation of counting 48 consecutive 0.1-\( \mu \) aliquots of a suspension of fat cells was 52%, for 12 consecutive 0.4-\( \mu \) aliquots was 12.3%, and for six consecutive 0.8-\( \mu \) aliquots was 11.1%. Thus, this operation shows a poor degree of reproducibility. When \( CV_n \) was estimated for this method with a formula for the propagation of error analogous to the one employed for method \( I \), it was found to be 11.2% so that the 95% confidence limits were \( \pm 22\% \).

Method 3 is based on the determination of the “lipocrit” measurement was obtained by filling an hemocrit capillary tube with an aliquot of the fat cell suspension and, after centrifugation, by measuring the ratio of the volume occupied by the cell suspension to the total volume containing a known number of fat cells, as determined by the count on a similar aliquot. The lipocrit determination had a CV of 8.8%. The total number of fat cells could then be estimated by dividing the lipid content of the adipose tissue sample by the mean lipid weight of the cells (cell volume obtained by the lipocrit estimate \( \times \) lipid density).

The coefficient of variation for the total number of fat cells \( (CV_n) \), estimated with the application of the formula for the propagation of error to this method, was found to be 14.9%; the 95% confidence limits were \( \pm 28\% \).

The comparison of the estimates of the number of fat cells contained in adipose tissue samples from the same rats, shown in Table 2, shows general agreement in the results obtained with the three methods. Method \( I \), however, was considered superior to the other two in simplicity of operation and in the confidence limits.

**Application of Methodology**

This second section of the results shows the application of the method proposed to observations in four mammalian species.

**Fat cell size in four mammalian species.** The epididymal adipose tissue from 136 rats, 28 hamsters, and 28 guinea pigs during growth and ad libitum feeding from age 5 weeks to 14-16 months was studied. In addition, the subcutaneous adipose tissue from 20 female dogs with varying degrees of adiposity was also studied. The morphological observations in the dogs have formed the basis of another report (9).

In all four species, the application of the methodology described to isolate fat cells by collagenase incubation and to determine the mean fat cell diameter gave satisfactory results. When the cell diameters were greater than 14 \( \mu \), the suspensions of fat cells revealed that the isolated fat cells assume rapidly and uniformly a spherical conformation. In general, fat cells with larger diameter were found in fatter animals at an advanced stage of development and following ad libitum feeding for longer periods of time. The diameter of the fat cells in the four species ranged from 11
to 150 $\mu m$ for the rat, from 20 to 120 $\mu m$ for the hamster, from 30 to 70 $\mu m$ for the guinea pig, and from 14 to 120 $\mu m$ for the dog. In fat cell populations derived from adipose tissue of younger, leaner animals, the smallest recognizable floating fat cells had a diameter of 11–15 $\mu m$. This indicates that a cell volume of at least 1–2 pl, corresponding to approximately 0.9–1.8 mg of triglyceride, is necessary to make a fat cell float.

In all the animals, the mean fat cell diameter and the standard deviation were then calculated for 300 free fat cell samples from the adipose tissues as described. The degree of heterogeneity in diameter for each fat cell population was indicated by the coefficient of variation of diameter. It was found (Fig. 5) that, in each species, the enlargement of the fat cells in adipose tissue leads to fat cell populations that are more homogenous in size, that is, less dispersed about the mean diameter. The consistency of these observations in the four species studied suggests that the increased homogeneity in fat cell diameter observed in all populations of cells, when the mean diameter increases from 40 to 70–120 $\mu m$, is a biological variable common to adipose tissue from all species.

Calculations of mean fat cell volume were also obtained from the histograms of the diameter for the 300 fat cell samples from rats, hamsters, and guinea pigs. The volume estimates ranged from as low as 12 pl to as large as 1,200 pl. From the histograms of the diameter for the 300 fat cell samples from rats, hamsters, and guinea pigs were obtained from epididymal fat depots, cells of dogs from subcutaneous adipose tissue.

Determination of lipid density in adipose tissue in animals from four mammalian species in relation to fat cell size. In previous reports (4, 15) the density used to estimate the weight of the lipid in fat cells has been that of triolein = 0.915 or of triglyceride, 0.92. In view of the possible changes in fatty acid composition of the adipose triglyceride from different animals at different stages of growth and nutrition, the lipid density in several animal groups was determined in the four species studied. The results are shown in Table 3. The lipid density did not appreciably change in the dog and hamster adipose tissue with enlargement of the fat cells, while in the rat and guinea pig the adipose tissue containing larger fat cells showed a lower lipid density. These findings indicate that appropriate determinations of the lipid density are necessary to obtain an accurate estimate of the mean lipid weight of the cells from the mean cell volume.

Diameter, volume, and number of fat cells in adipose tissue from four different locations in the rat. The convenient location of the epididymal fat pad in the rat and in other species, together with facility of recognition of its boundaries, has made this pad the most frequently used fragment of the adipose tissue for metabolic studies. However, some reports (22) indicate that adipose depots other than epididymal differ in metabolic performance, when expressed per unit of wet weight, suggesting interdepot compositional differences. In this series of experiments, detailed morphological and compositional analyses were conducted in four different locations of adipose tissue in the same animal. The results are summarized in Table 4. In six male rats, 7–9 weeks old, pieces of adipose tissue, 200–400 mg in size, were removed from the following areas: epididymal, perirenal, mesenteric, and subcutaneous (this latter was removed from the left groin region). The tissue samples were processed as described in the METHODS section.

The results of these experiments can be summarized as follows: 1) the four adipose depots of the 7–9-week-old rat differed markedly in the proportion of tissue constituents (lipid and DDR) and in fat cell size from one location to the other; 2) the percent lipid content of the tissue was highest (75.7 %) in the epididymal and lowest (41.2 %) in the mesenteric location in the following order: epididymal > perirenal > subcutaneous > mesenteric; 3) the percent defatted dry residue followed the reverse order, being lowest (3.1 %) in the mesenteric and highest (7.8 %) in the epididymal location; 4) the mean diameter and volume of the fat cells were largest for the epididymal and subcutaneous locations, smallest for the mesenteric location, and intermediate for the perirenal depots; 5) the number

![Graph](image-url)
of fat cells contained in 1 g of adipose tissue from the mesenteric region was consistently and significantly greater than the number of cells contained in 1 g of tissue from the three other locations.

TABLE 3. Determination of lipid density in lipid extracted from adipose tissue of four mammalian species at different stages of animal development

<table>
<thead>
<tr>
<th>Animals</th>
<th>n</th>
<th>Body Wt Range, g</th>
<th>Fat Cell Vol Range, pl</th>
<th>Lipid Density</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>6</td>
<td>150-170</td>
<td>20-30</td>
<td>0.915 ± 0.002</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>550-650</td>
<td>200-300</td>
<td>0.881 ± 0.004</td>
</tr>
<tr>
<td>Hamster</td>
<td>4</td>
<td>40-50</td>
<td>50-100</td>
<td>0.917 ± 0.002</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>158-174</td>
<td>250-600</td>
<td>0.992 ± 0.004</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>3</td>
<td>250-200</td>
<td>50-100</td>
<td>0.951 ± 0.006</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>900-1000</td>
<td>70-200</td>
<td>0.873 ± 0.010</td>
</tr>
<tr>
<td>Dog</td>
<td>12</td>
<td>18-28</td>
<td>12-400</td>
<td>0.909 ± 0.001</td>
</tr>
<tr>
<td>Triolein</td>
<td></td>
<td></td>
<td></td>
<td>0.915</td>
</tr>
</tbody>
</table>

Lipid density was determined in triplicate samples from n of animals for each group, as described in the text. The mean of determinations ± se is shown. In the rat, hamster, and guinea pig the epididymal adipose tissue was employed; in the dog the subcutaneous adipose tissue was used.

TABLE 4. Studies on adipose tissue composition and on isolated fat cells from four adipose depots in six rats

<table>
<thead>
<tr>
<th>Adipose Tissue</th>
<th>Total Lipids, mg/100 mg</th>
<th>DDR, mg/100 mg</th>
<th>Mean FC Diam, µm</th>
<th>SD, µm</th>
<th>Mean FC Vol, pl</th>
<th>No. of FC in 1 g tissue, X10³</th>
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<tbody>
<tr>
<td>Epididymal depot</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>75.7</td>
<td>3.1</td>
<td>43.5</td>
<td>9.4</td>
<td>50.4</td>
<td>17.0</td>
</tr>
<tr>
<td>Range</td>
<td>(71.2-80.0)</td>
<td>(2.8-4.0)</td>
<td>(39.6-49.8)</td>
<td>(8.5-15.8)</td>
<td>(37.8-60.0)</td>
<td>(13.0-25.0)</td>
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<tr>
<td>SE</td>
<td>79.1</td>
<td>3.7</td>
<td>47.7</td>
<td>10.9</td>
<td>66.6</td>
<td>22.4</td>
</tr>
<tr>
<td>Range</td>
<td>(73.2-86.2)</td>
<td>(3.3-5.3)</td>
<td>(42.0-53.0)</td>
<td>(9.7-14.6)</td>
<td>(60.6-81.4)</td>
<td>(17.6-30.0)</td>
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<td>Subcutaneous depot</td>
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<td></td>
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<tr>
<td>Mean</td>
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<td>7.0</td>
<td>29.4</td>
<td>6.5</td>
<td>16.0</td>
<td>29.4</td>
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<td>Range</td>
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<td>(5.9-8.8)</td>
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<td>(26.0-58.4)</td>
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<td>3.4</td>
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<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Perirenal depot</td>
<td></td>
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<tr>
<td>Mean</td>
<td>56.8</td>
<td>6.9</td>
<td>43.3</td>
<td>9.7</td>
<td>51.7</td>
<td>12.8</td>
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<tr>
<td>Range</td>
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<td>(5.9-8.5)</td>
<td>(37.3-51.4)</td>
<td>(6.3-10.9)</td>
<td>(39.4-81.7)</td>
<td>(18.6-56.8)</td>
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<tr>
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<td>4.0</td>
<td>0.1</td>
<td>2.1</td>
<td>1.4</td>
<td>7.1</td>
<td>1.6</td>
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<tr>
<td>P value</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Perirenal depot</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>66.6</td>
<td>4.6</td>
<td>37.2</td>
<td>10.9</td>
<td>37.0</td>
<td>22.6</td>
</tr>
<tr>
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<td>(27.9-43.4)</td>
<td>(8.9-14.3)</td>
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<td>(14.1-42.3)</td>
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<tr>
<td>SE</td>
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<td>0.5</td>
<td>6.1</td>
<td>4.1</td>
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<td>P value</td>
<td>&lt;.005</td>
<td>&lt;.05</td>
<td>&lt;.05</td>
<td>NS</td>
<td>NS</td>
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</tr>
</tbody>
</table>

The six rats were 7-9 weeks old, fed ad libitum. Average body weight was 171.2 ± 3.5 (sd) g, range 155-178 g. Total lipids and defatted dry residue (DDR) expressed as mg/100 mg wet wt. Mean of diameters of 400 fat cells (FC) in each rat depot. SD = standard deviation of diameter. Mean of volumes of 400 fat cells in each rat depot. This mean and related sd represent the average ± se for the six rats of the various determinations in each of the four adipose depots. The P value represents the level of significance of the differences between various mean determinations from mesenteric, subcutaneous, and perirenal adipose depots and the corresponding epididymal adipose depot values. NS means nonsignificant.

These results, illustrating one of the possible uses of the methodology described, show that, in the growing rat, significant interdepot variations exist in the composition and morphology of the adipose organ.

DISCUSSION

One of the original objectives of this study was the measurement of two variables: the size of the fat cells (expressed as mean diameter or volume) in a given sample of adipose tissue and the number of these cells in the tissue sample. It soon became evident that in any sample of the tissue there is considerable heterogeneity with regard to fat cell size so that the actual problem with regard to size became the estimation of: a) mean size and b) the degree of dispersion within the population about this mean. With regard to adipose cell number c), this variable represents a single value in any tissue sample, which the investigator attempts to measure as accurately as possible.

The method described, based on the direct microscopic measurement of fat cell diameter of a nonfixed free-fat cell suspension, permitted the characterization of a fat cell population in terms of parameters a, b, and c above. This method is similar in principle to methods that have been reported previously from other laboratories (3, 15, 17, 26). The present study evaluates the reliability of such a method by investigating the index of precision of the operations involved in the determination of mean fat cell size and number and by determining the relative error found in the determination of fat cell number in fragments of adipose tissue. Two related methods, described as methods 2 and 3, appear to be less accurate and more complex than the method 1 chosen and are therefore only reported for purposes of comparison.

In recent years, important methodological contributions to the study of morphology and cellularity of adipose tissue have come from the laboratories of Bjurulf (2), Zingg et al. (27), Wassermann et al. (25), Goldrick (12), and Hirsch and Gallian (15) with a variety of different techniques. In spite of the remarkable progress in the methodological approaches to these problems, it is important to realize that the techniques available, including the one here presented, encompass possible sources of systematic error that cannot be completely eliminated. Sampling artifacts, due to intra-depot variations in mean cell diameters, and imprecision of diameter determination for cells exposed by sections in noncoronal planes afflict the techniques based on histological preparations. Problems of incomplete adipose cell recovery are encountered in methods where separation of stromal from adipose cells is achieved by passage through screens of pore size that might permit loss of adipocytes with diameter less than 25 µ (15). Similar problems of incomplete adipose cell recovery are present in techniques based on collagenase incubation, such as the one presented here, where a proportion, however small, of cells liberated by collagenase might be lost during floatation and washing because of having a density greater than that of the medium.

It is not known to what degree these possible sources of systematic error affect calculation of cell size and cell number of adipose tissue, with the available methods. Although subject to the limitations of certain assumptions and technical procedures, the technique proposed and...
utilized here shows certain valuable points: a) an estimate of total fat cell number with 95% confidence limits of ±5 to 10%; b) valid representative sampling of the original cell population in the tissue, deriving from sampling of large numbers of free fat cells, thoroughly mixed; c) the possibility of quantitating the degree of dispersion in cell size in a given fat cell population; heterogeneity in cell size appears to be an important natural attribute of a cell population; d) simplified and low-cost operations that make this method easily available to students of adipose tissue physiology; e) the possibility of quantitating accurately the surface area of a cell population, therefore providing an important unit of measure for studies of hormone-tissue interaction and cell membrane permeability.

The study of the morphology of adipose tissue in various adipose depots from four mammalian species, by this method, has indicated that the method is generally applicable to fragments of adipose tissue containing fat cells varying markedly in size. Of considerable interest is the observation that fat cell populations derived from different tissue samples can vary markedly with regard to the heterogeneity in size of the individual cells. A definite pattern has emerged which appears to be common to all four species. As shown in Fig. 5, within each species, populations of fat cells with smaller mean diameter have significantly greater relative dispersion in size (greater coefficient of variation, CV) than populations of cells with larger mean diameter from older, fatter animals. This finding suggests the following hypothesis. Enlarging fat cells may have a limit for cell expansion dictated by intracellular or interstitial controlling mechanisms; during enlargement the cells would get closer to the maximal size and therefore the relative dispersion in size of the cell population would decrease. The maximal cell size attained by the fat cells in a given species may also be characteristic for that species and be an important regulator of cell metabolic activities.

The findings summarized in Table 3 show that the lipid density in adipose tissue varies, in at least two species, when the fat cells enlarge. In addition, the lipid density of the adipose tissue from 250- to 280-g guinea pigs appears to be abnormally high. This suggests that compositional differences might be present with regard to the triglyceride fatty acids or to the relative proportion of mono- and diglycerides present. A systematic study of the relationship between fat cell size, lipid density, and lipid composition would be needed to further clarify these points.

The observations on four different adipose depots from the 2-month-old rat (Table 4) reveal that, in an early stage of growth of the animal, certain locations of the adipose organ differ significantly from each other with regard to proportions of lipid and defatted dry residue in the tissue, to mean cell diameter and volume and to the number of fat cells per gram of tissue. Goldrick (12) has recently reported that in older rats, in which the mean fat cell diameter was greater than 90 μ, little difference in mean cell size was found when the fat cells from three depots, namely epididymal, retroperitoneal, and subcutaneous, were compared. Thus, it is possible that the process of fat cell enlargement which results from age and ad libitum nutrition might lead to a progressive accretion of intracellular triglyceride which by necessity finds a limit to further increase in a limited capacity of the fat cells to enlarge beyond a diameter of 140–160 μ. Adipose cells approaching a maximal size in the different adipose depots of older, fatter animals would then become more homogeneous in size, not only within one depot (as is suggested by the observations on CV seen in Fig. 3), but also within the different sites of the adipose organ.

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