Methods for the determination of adipose cell size in man and animals

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ABSTRACT Four methods for the sizing of adipose cells in small samples of human or animal adipose tissue are compared. These methods depend on the preparation of cell suspensions by incubation of the tissue with collagenase or by prolonged fixation with osmium tetroxide and separation of the fixed cells. A Coulter electronic counter was used to count and size the suspended cells and a Zeiss particle size analyzer for the sizing of cells in photomicrographs. The use of the Coulter counter to count cells in a suspension derived from a known amount of tissue and subjected to osmium tetroxide fixation is recommended for accuracy and general applicability to adipose cells of all sizes in man and animals.

KEY WORDS adipose tissue · adipose cell · cell size · cell number · cell suspensions · electronic counting · Coulter counter · cell fixation · osmium tetroxide · collagenase

THE IMPORTANT METABOLIC ROLE of adipose tissue is well-recognized. It is also known that genetic and nutritional influences as well as various experimental manipulations can cause major changes in the size of the adipose depot. In order to determine to what extent such changes in adipose tissue mass come about because of changes in adipose cell size or because of changes in cell number, and also to explore the effect of such changes in cell size and number on the metabolism of this tissue, we have examined various methods for determining adipose tissue cellularity.

In the past, such measurements have been made microscopically on appropriate histologic preparations (1–5) or by the determination of DNA (4–6), which provides a nuclear, and hence cellular, tally. The former method is tedious, since it involves the measurement and counting of large numbers of cells, which have the appearance of irregular polygons, in various orientations to the cut plane. The measurement of DNA in the intact tissue is an inexact estimate of adipose cellularity, since blood vessels, fibrous tissue, mast cells, macrophages, etc., contribute unknown amounts of DNA to the total. It has been estimated that the DNA derived from these other cells can exceed the DNA content of the adipocytes (7). It is, of course, possible to determine the DNA content of adipose cell suspensions prepared by digestion of the tissue with collagenase. In the case of human tissue, or of animal tissue containing large cells, collagenase treatment usually causes a great deal of cell breakage, and the final suspension may not be a representative or valid sample of original cell sizes.

The present communication describes other techniques for the sizing and counting of adipose cells. These newer methods are evaluated, and one technique, method III, is recommended because of simplicity, accuracy, and general applicability to rat, mouse, and human adipose tissue. Methods I, II, and IV are tedious both to describe and to perform. Details are included here in order to provide some basis for evaluating the recommended method, as well as to illustrate the advantages and disadvantages of several approaches to this problem.

MATERIALS AND METHODS

Adipose tissue was obtained from the epididymal fat pads and from the retroperitoneal and subcutaneous areas of rats and mice. In most instances, human samples were obtained from the subcutaneous fat depot by needle aspiration (8); but some samples, from both deep and subcutaneous sites, were also obtained at surgery. Cellularity was determined on adipose cell suspensions obtained by one or both of the following methods.

A preliminary account of this investigation was presented at the 1966 Meeting of the Federated Societies for Experimental Biology.

TCA–Glutaraldehyde-Fixed Cells

Tissue shreds, each 5–100 mg in weight (total less than 500 mg), were washed free from adherent fat in warm isotonic saline and incubated for 1 hr at 37°C in a 30 ml plastic vial containing collagenase (Worthington Biochemical Corporation, Freehold, N. J.), glucose, and albumin in 3 ml of Krebs-Ringer bicarbonate buffer, as described by Rodbell (9). At the end of the hour, the vial was shaken in an Eberbach shaker at 37°C at a rate of 160 cycles/min for 1–3 min, so as to liberate most of the cells from the tissue. The vial and its contents were then cooled for 1 hr in a cold-room at 4°C. About 10 ml of cold fixative (0.25% trichloroacetic acid and 6.25% glutaraldehyde in water) was added, and the reaction was allowed to proceed for an additional hour.

The fixed, free cells were separated from tissue and debris by passage through a nylon screen (pore size, 250 μ) marketed as "Nitex" and obtained from Tobler, Ernst, and Traber, Inc., 71 Murray Street, New York. The filtrate was passed through a smaller screen (pore size, 25 μ) and the trapped cells were quickly washed from the filter into a plastic beaker with cold, isotonic saline. The entire procedure was performed in a cold-room at 4°C. The suspension of cells obtained by this method and referred to as the TCA–glutaraldehyde-fixed cell suspension was then processed for cell counting and sizing by the methods described below.

B. Osmium-Fixed Cells (Fig. 1)

Tissue shreds not exceeding 200 mg in total wet weight were first thoroughly washed with warm isotonic saline or buffer on tared circular discs of nylon screen (pore size, 250 μ). The discs were about 5 cm in diameter and had been washed in chloroform–methanol 2:1 and dried in air before being weighed. The disc was suspended in a Nalgene filter unit made without the usual fine membrane and available in this form from The Nalge Co. Inc., Rochester, N. Y. A large hole was cut in the bottom of the receptacle to allow easy filtration. The tissues were washed many times with small quantities of warm saline or buffer, pH 7.4, in order to free them from oil droplets. The filter disc was then removed and rapidly blotted on the undersurface with absorbent paper, and the wet weight of the tissue was obtained.

The filter and tissue were then immediately plunged into a plastic counting vial containing 30 ml of 2% osmium tetroxide in 0.05 M collidine–HCl buffer at pH 7.4 and 37°C. In the preparation of this buffer, 25 ml of 0.2 M collidine and 22.5 ml of 0.1 N HCl are customarily mixed, and the volume is made to 100 ml with water; in these experiments, the mixture was made to volume with 0.15 M saline instead of water so that the final

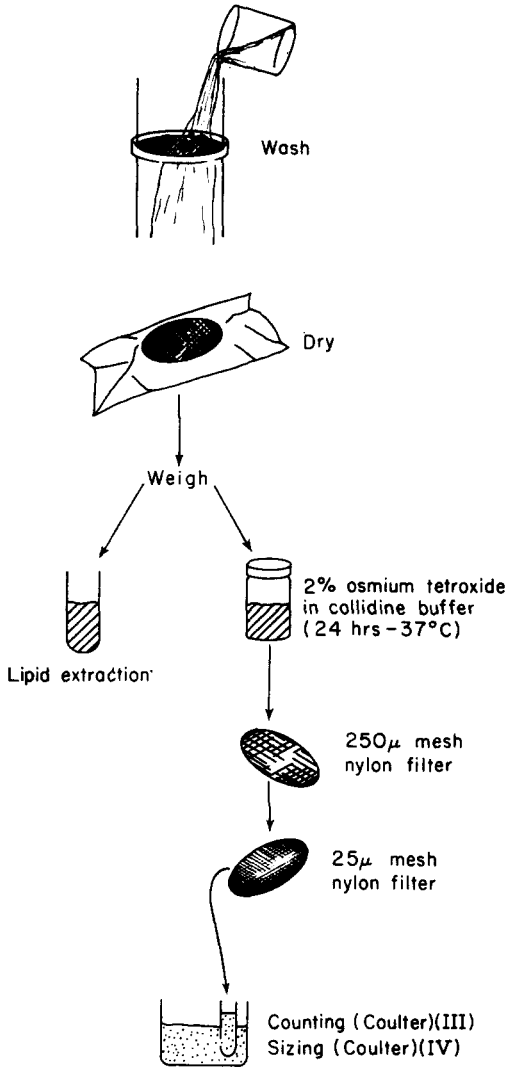

FIG. 1. A schema for the preparation of a suspension of osmium tetroxide-fixed cells. III and IV are techniques for sizing and counting described in the test. Method III depends on the simultaneous determination of the ratio of lipid to wet weight in adipose tissue samples. This is done by extracting a separate sample of known wet weight in chloroform–methanol, separating the phases with the addition of water, and then determining the lipid content of the chloroform phase.

preparation of 2% osmium tetroxide in buffer would be close to isosmolar. Fixation at 37°C was allowed to continue for at least 24 hr and for 48–72 hr with tissue samples weighing more than 100 mg. Osmium tetroxide is quite volatile at room temperature, and hence all procedures with this toxic substance should be performed with adequate ventilation.

After fixation, the contents of the plastic container were thoroughly washed through a nylon screen (250 μ) with distilled water. The Nalgene filter unit, as prepared above, was useful for this washing procedure. The filtrate contains most of the fixed free cells, but fibrous tissue

and some intact shreds of fixed adipose tissue remain on the filter. The tissue shreds were gently rubbed by hand on the filter while the washing was continued. This procedure completely separated the tissue into free cells and allowed total recovery of cells in the filtrate, which was then washed with distilled water onto a finer screen (25 μ). The trapped cells were washed with copious amounts of water and then rinsed, with isotonic saline, into a 400 ml tared, glass beaker. The weight of the cell suspension was determined. The density of the suspension was approximately the same as the density of isotonic saline; hence the density of saline at room temperature was used to determine the volume of the cell suspension (volume = weight/density). The total volume of suspension was useful in further calculations as described below.

Counting and Sizing Devices

A. Zeiss Particle Size Analyzer

Photographs of TCA–glutaraldehyde-fixed cells, which have the photomicrographic appearance of almost perfect spheres, were sized by means of a Zeiss particle size analyzer (model TGZ-3) (Carl Zeiss, Inc., New York, N. Y.). With this instrument, photomicrographs containing images between 1.2 and 27.7 mm in diameter can be rapidly sized to provide a frequency distribution of image diameters in 48 categories of size.

B. Coulter Counter

A Coulter counter (model B) and Coulter automatic particle size distribution analyzer (model J) (Coulter Electronics, Hialeah, Fla.) were used. The passage of suspended particles through a small aperture in the counter interferes with the electrical conductivity of the aperture in such a way as to provide counts of particles passed per unit volume of suspension, and, with the analyzer, a frequency distribution of the various volumes of particles passing through the aperture is obtained. For cells of the usual size (diameter greater than 55 μ or lipid content greater than 0.08 μg/cell), a 400 μ aperture was

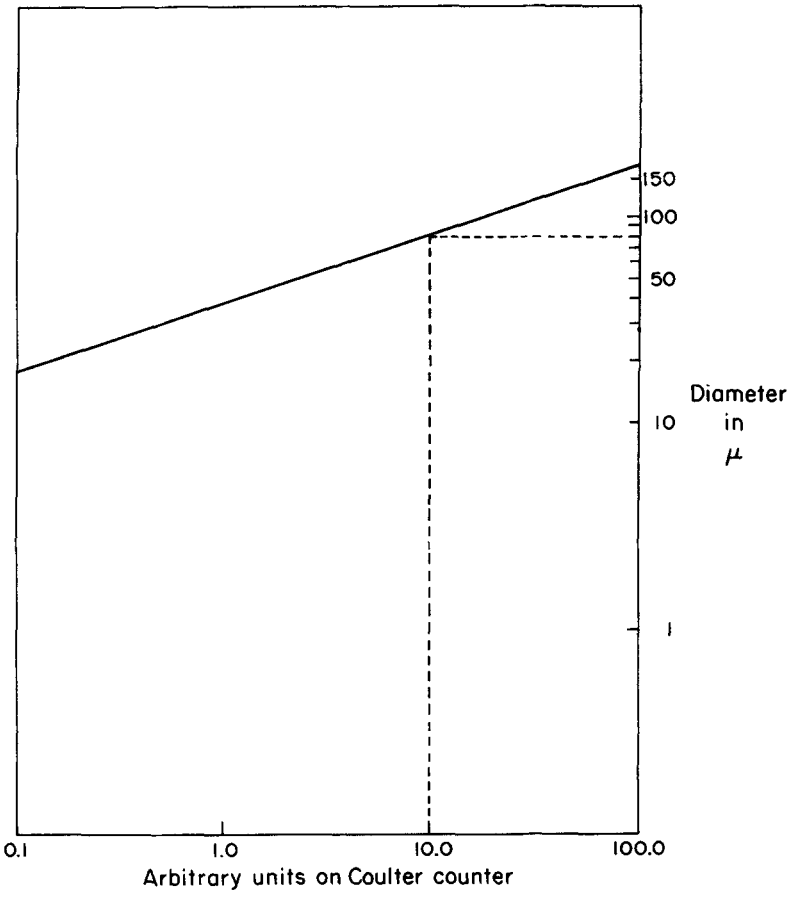

Fig. 2. This nomogram was constructed from two points of gate setting (arbitrary units on Coulter counter) obtained with corn and ragweed pollen particles and equated with diameters of 85.9μ and 19.6μ (the independently measured mean diameter of these particles). It should be noted that the sloping line from which projections are made to Coulter units and diameter represents a specific "gain" (aperture current and amplification) of the counter. For other "gains," appropriate lines can be drawn parallel to the sloping line shown in this figure. Thus, 10 Coulter units is equal to 80 μ of diameter at this "gain"; but at twice this gain, for example, 10.0 Coulter units would equal only 40 μ.

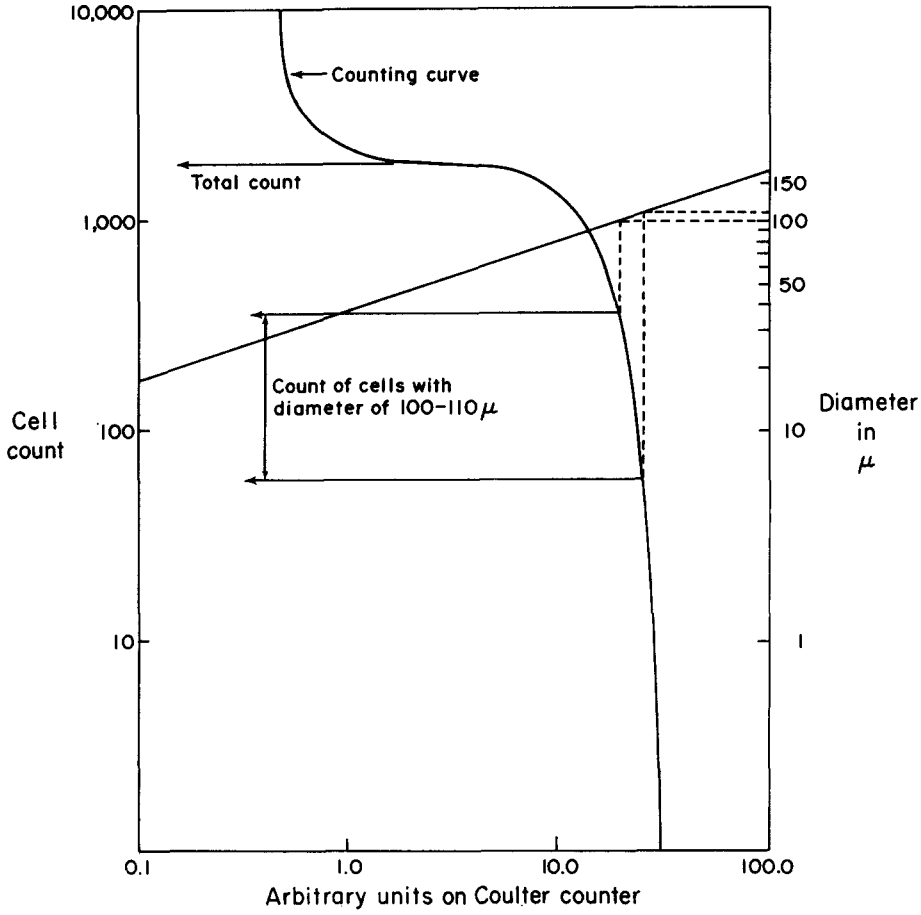

FIG. 3. A counting curve obtained by counting a cell suspension with various settings of the lower "gate" is shown. Such a curve can be obtained automatically by the Coulter analyzer. From this curve and the nomogram (see Fig. 2) one can obtain a frequency distribution of particles as to intervals of diameter. The use of the nomogram for obtaining the numbers of particles in the 100–110 μ interval is shown. Note that the logarithmic scales accentuate the flatness of the plateau.

used and for smaller cells, a 280 μ aperture.

Pollen particles from corn and ragweed were obtained from the Hugh Graham Laboratories, Dallas, Tex., for the calibration of the Coulter counter. Suspensions of the pollens wetted with ethanol and diluted with isotonic saline were photographed and sized with the Zeiss particle size analyzer. Corn pollen was found[1] to be 85.9 ± 3.4 μ (mean ± SD) in mean diameter, and ragweed pollen, 19.6 ± 1.6 μ. Suspensions of the pollens were counted in the Coulter counter, which can be "gated" to exclude or include particles of different size in the total count, and the settings at which exactly 50% of the corn or ragweed pollen particles were excluded was considered to be the setting precisely equal to 85.9 or 19.6 μ and therefore equivalent to particle volumes of $\pi/6(85.9)^3$ μ^3 (332 × 10^3 μ^3) and $\pi/6$ $(19.6)^3$ μ^3 (3.94 × 10^3 μ^3) respectively. The gates of the Coulter counter are controlled by dials numbered from 0 to 100. Thus, for a given aperture current and amplification, precise dial settings were obtained for the volumes or diameters of two different particles suspensions. This served to calibrate the device.

From these data a nomogram could be constructed, as shown in Fig. 2. Any diameter as shown on the ordinate could be related to a given setting indicated along the abscissa as "arbitrary units on Coulter counter." Thus, at the particular settings of aperture current and amplification for which this nomogram was constructed, a dial setting of 10.0 was equal to a particle diameter of 80 μ.

This nomogram was used with a counting curve obtained as follows: counts or numbers of suspended adipose cells per 2 ml were obtained at various settings of the lower gate, beginning at low settings (designated by the abscissa) and thus excluding no cells, then at gradually higher settings until a counting curve could be plotted as shown in Fig. 3. The upper gate was set at infinity; thus at each setting all cells larger than the setting of the

[1] At least 500 cells were sized to provide these data. Mean and standard deviation were calculated by the technique shown in Fig. 5 and as discussed below, under Method I.

lower gate were included in the count. The curve shows a plateau from 1.0 to 10.0 Coulter units (gate settings). The inflection point along this plateau represents the count of all cells per 2 ml. To the left of this point, electrical background noise and small particles of dust or other extraneous material in the counting solution give extremely high and erroneous counts. Some of these unwanted effects can be reduced if carefully filtered isotonic saline is used. The saline used in these studies was Abbo-Liter (Abbott Laboratories, North Chicago, Ill.). To the right of the plateau, the counts at first slowly and then more rapidly decrease as more and more cells of increasing size are "gated" out of the total count. Thus, it is possible by use of the ordinate on the right of the nomogram to make projections from various diameters to the counting curve and find the reduction in counts attributable to cells with diameters falling between these values. The reduction in counts is determined from the ordinate on the left of the nomogram (Fig. 3). In this way, both a frequency distribution of cell size, as well as the total cell count, could be determined. For both of these determinations, the Coulter automatic particle size distribution analyzer (model J) has been found useful. This device automatically plots a counting curve similar to that shown in Fig. 3.

An additional calculation is required when total counts are determined. Any counting technique has the usual inherent counting errors which can be statistically determined, but by the Coulter technique of counting there is an additional source of error which may give falsely low results. This is a coincidence error which occurs when two or more particles enter the aperture simultaneously and are counted as though they were a single larger particle. The probability of this occurrence increases as the concentration of particles increases and also with increases in aperture size. It is not, however, related to particle or cell size. The probability that n cells will be counted simultaneously can be described by Poisson statistics and is equal to $(V^n e^{-v})/n!$ where V = the average number of cells per "critical" volume. The critical volume is that volume of suspension in and surrounding the aperture within which a cell will be counted. It is approximately three times as great as the cylindrical volume enclosed by the aperture (10). With this information, a correction curve can be made; however, such correction curves can also be made by directly observing the effects of progressive dilution on a solution of particles as shown in Fig. 4.

In this figure, the reduction in machine counts is shown as a suspension of corn pollen particles was progressively diluted from a given concentration, K. With low concentrations, i.e. when there are fewer than 1500 particles per 2 ml, the error due to simultaneous counting of more than one particle is negligible, thus the extrapolation of

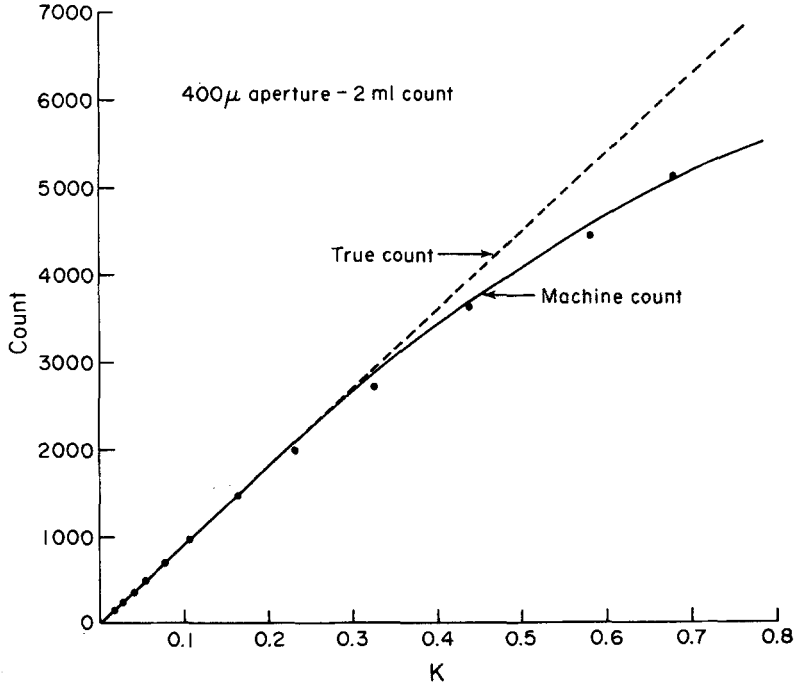

FIG. 4. A method for determining the counting error due to coincidence of particles in the aperture of the Coulter counter. A suspension of corn pollen of concentration K is progressively diluted to various dilutions relative to K as shown on the abscissa. The ordinate gives counts obtained at each dilution. The dotted line is a constructed line tangential to the curve of counts close to the origin. The difference between these curves is the coincidence "error" as explained in the text.

this portion of the curve to higher counts is the "true count." The difference between these two curves is the counting error. A determination of counting error must be made for each aperture size. In the data to be presented, counting errors of less than 25% were accepted and corrected accordingly. More concentrated solutions were diluted appropriately before counting. In practice, a simple computer program was used with the Olivetti Programma 101 computer to obtain the means of several counts and also to correct this mean for the counting error.

RESULTS

Four independent methods of cell sizing, utilizing the two types of cell suspension and the counting and sizing techniques described above, were examined. The methods and their results are described in detail below.

Methods I and II are performed on suspensions of TCA–glutaraldehyde-fixed cells and methods III and IV on osmium-fixed cells. Method III is the simplest and most generally applicable of these methods and is a direct measure of the average cell lipid content, which will be expressed in μg of lipid/cell. This is done by counting the total number of osmium-fixed cells obtained from a known weight of tissue lipid. Method II also directly measures lipid content per cell, but methods I and IV measure the diameters and volumes of the adipose cells, respectively. These data are transformed into μg of lipid/cell by assumptions and mathematical formulas described below. Thus all methods give final data in μg of lipid/cell, and the results of the various methods can be compared.

TCA–Glutaraldehyde-Fixed Cells

Method I

The suspension of fixed cells made from the residue of the 25 μ filter as described above was allowed to stand for several minutes and form a creamy layer, and a few drops of this thick cell suspension were removed and placed on a chilled plastic slide. A plastic coverslip was placed over the droplets and photomicrographs made. At least 500 circular cell shadows of adipose cells were then sized with the Zeiss particle size analyzer. The Analyzer was set to give a cumulative frequency distribution and the results were plotted on probability (probit) paper.

Fig. 5 shows a typical plot of cell diameters obtained by this method. As can be seen, the cumulative frequencies for the various intervals of diameter fall on a straight line on probit paper, which indicates that the diameters of the cells are normally distributed. The mean cell diameter is obtained from the cell diameter corresponding to 50% on the ordinate, and σ, or standard deviation, is obtained by the change in diameter between 15.9 and 84.1 probit units (a fixed property of the σ of any normal distribution). Assuming that each cell is a perfect sphere composed of material with the density of triolein (0.915), then a sphere of any given diameter, d (in microns), would weigh $0.915/10^6 \times \pi/6\ d^3$, or $(0.4791/10^6)\ d^3$ μg.

It is important to recognize that the mean lipid weight per cell is here defined as total lipid weight of a number of cells divided by the number of cells. If one defines mean weight as the weight of the cell with the mean diameter, a quite different answer is obtained. Thus, the transformation $[3\sigma^2 \cdot \text{mean} + \text{mean}^3]$ is used in place of $(\text{mean})^3$ in the calculation of mean cell weight, and the formula for mean lipid weight/cell is $0.4791/10^6\ [3\sigma^2 \cdot \text{mean} + (\text{mean})^3]$. This type of statistical transformation is described in most advanced texts on statistics. The precise derivation can be obtained from Dr. Bruce A. Barron of The Rockefeller University upon request. This transformation takes into account the fact that the distribution of the cubes of a normally distributed variable is skewed to the right. To illustrate the effect of such skewness in an extreme case, assume one is dealing with five cells of diameters 1, 2, 3, 4, and 5 μ. The average weight as defined above equals the total weight of lipid divided by 5, or 21.6×10^{-6} μg; however, the weight of an average cell (3 μ in diameter) equals only 12.9×10^{-6} μg. If the transformation described above is used, a more satisfactory estimate of 23.7×10^{-6} μg is obtained. The discrepancy between 21.6×10^{-6} and 23.7×10^{-6} is a residual error of the statistical assumptions made, but the transformation described is a sufficiently accurate technique for correction in most instances.

Method II

The suspension of TCA–glutaraldehyde-fixed cells was placed in a 250 ml plastic beaker in an ice bath. The entire mixture was taken to the Coulter counter where, under gentle agitation to maintain an even suspension, counts per 2 ml were rapidly determined. The automatic plotter was found useful in obtaining appropriate settings for an accurate count (see Fig. 3 and related description). Simultaneously, multiple 25-ml aliquots were withdrawn into a siliconized glass pipette. The pipette was emptied into a small glass funnel containing a loose filter bed of Celite particles (previously washed in chloroform and methanol) over a plug of glass wool. The pipette was repeatedly washed with cold saline and the washings were delivered to the filter bed. The bulk of the contents of the filter was emptied into a glass-stoppered tube and the remainder was rinsed into the tube with methanol. Sufficient chloroform was added to make the final composition of the mixture of chloroform and methanol

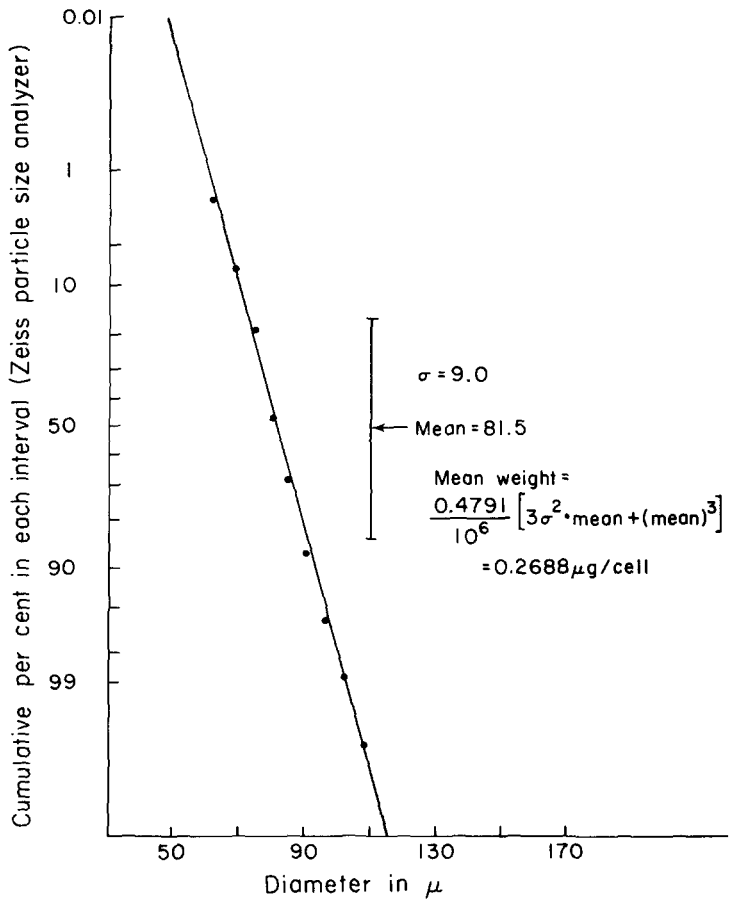

FIG. 5. Adipose cells from the epididymal pads were sized with the Zeiss particle size analyzer (Method I). The cumulative frequency distribution on probit paper is a straight line from which mean, standard deviation (σ), and mean weight of cells is derived as shown in this figure and explained further in the text.

2:1. After the addition of 0.2 volume of distilled water and separation of the phases, an aliquot was removed from the chloroform layer for lipid determination by measurement of carboxyl ester bonds. After appropriate calculation, the lipid content per 2 ml of cell suspension was divided by the corrected number of particles per 2 ml to give average lipid content per cell.

OSMIUM-FIXED CELLS

The cell suspension was prepared as already described and as shown in Fig. 1.

Method III

The suspension of osmium-fixed cells was washed with isotonic saline into a tared 400 ml beaker. The weight of cell suspension (approximately 350 g) was corrected for the density of saline to give the total volume of suspension. After an automatic plot had been obtained by means of the Coulter automatic particle size distribution analyzer, the setting of the lower gate corresponding to the point of inflection on the curve (see Fig. 3) was selected for 4 counts of 2 ml each. The corrected mean of these counts was then divided by 2 to give the number of particles per ml and multiplied by the volume of saline present in the beaker to give the total number of cells obtained from that tissue sample. Since the total wet weight of the sample had been determined, as well as the lipid to wet weight ratio in another sample (see Fig. 1), the average cell size was calculated as follows:

$$\text{average cell lipid content in } \mu g/\text{cell} = \frac{(\text{wet weight of sample in } \mu g) \times (\text{ratio of lipid to wet weight})}{\text{total number of cells in sample}}$$

Method IV

A suspension of osmium-fixed cells was sized in the Coulter counter by means of a curve such as that shown in Fig. 3. From such a curve and the nomogram of Fig. 3, the number of cells falling into each interval of diameter could be ascertained. The data were then plotted as a cumulative frequency distribution on probit paper. From the mean and standard deviation and the appropriate transformation discussed under method I, the average lipid content per cell was determined.

Intactness of Cells in Suspension

All of the methods described depend for accuracy on the assumption that the suspended bodies, in the suspensions fixed both with TCA and glutaraldehyde and with osmium, are single intact cells rather than oil droplets liberated by cell rupture. Some evidence for cellular integrity can be obtained by microscopic examination of the suspensions.

Cells Fixed with TCA–Glutaraldehyde. As shown in Fig. 6, the cells are of quite uniform size and the suspension does not contain the sprays of small droplets and the large floating droplets of oil that were found when unfixed cells at room temperature were subjected to the counting procedure. Furthermore, under higher magnification, in most cells there can be seen a small protrusion along the cell border, probably an intact nucleus.

Osmium-Fixed Cells. Fig. 7 shows that the cells, although similar in size, have irregular shapes resembling those of intact cells examined in the usual histologic sections of adipose tissue. The perfectly round shapes of osmium-fixed oil droplets are not seen. With higher magnification, particularly under phase contrast, many of the cells can be seen to have retained thin cytoplasmic coverings and even to exhibit structures resembling a nucleus and other subcellular organelles.

Comparisons of Methods

Methods I and II, which utilize suspensions of cells fixed with TCA–glutaraldehyde, have marked limitations

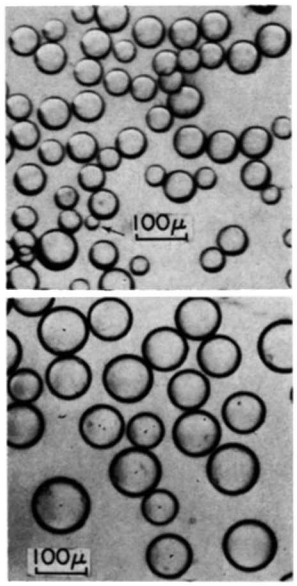

Fig. 6. TCA–glutaraldehyde-fixed cells, in suspension, from the epididymal pads of a small rat (above, 188 g) and from a large rat (below, 650 g). The cells are fairly uniform in size and in most instances are cells rather than oil droplets. In a few instances, small droplets are issuing from the cells (see arrow), which probably indicates the beginning of cell dissolution.

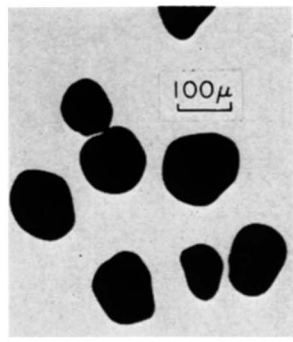

Fig. 7. Osmium tetroxide-fixed cells from human subcutaneous adipose tissue. The irregular shapes, similar to those seen in histologic sections, is taken as partial evidence that these shadows are intact cells, rather than osmium-fixed oil droplets.

when large cells occur, as in most human adipose tissue and in that of old or obese rats and mice. In these instances, the collagenase treatment and fixation lead to a great deal of cell breakage. For this reason, the method using osmium tetroxide fixation of tissue shreds was developed.

In order to compare the four methods, we applied them to epididymal pads from eight rats of small to average size. Smaller animals were chosen to minimize cell breakage with collagenase treatment and thus to assure better and more representative recoveries in methods I and II. As can be seen in Table 1, the cell sizes range from 0.06 to 0.26 μg of lipid per cell. To make an a priori judgment as to which method should be most accurate is difficult. It is clear from Table 1 that the osmium-fixed cells sized by the Coulter counter (method IV) always gave a higher estimate of cell size than the other three methods. This may be due to the fact that osmium tetroxide fixation is accompanied by cell swelling. The results of the other three methods were, however, quite similar.

TABLE 1 Determination of Adipose Cell Size in Eight Epididymal Fat Pads by Four Methods

Optical Sizing (Zeiss) I (TCA–Glut. Fixed)	Counting (Coulter) II (TCA–Glut. Fixed)	Counting (Coulter) III (Os. Fixed)	Sizing (Coulter) IV (Os. Fixed)	Mean of I, II, and III
		μg of lipid/cell		
0.2020	0.2540	0.2457	0.3095	0.2337
0.1799	0.1937	0.1942	0.2794	0.1892
0.2464	0.2992	0.2418	0.3937	0.2624
0.2639	0.3695	0.2852	0.4291	0.3062
0.0730	0.0780	0.0956	0.1445	0.0819
0.0640	0.0703	0.0946	0.1446	0.0763
0.2126	0.2835	0.2375	0.3261	0.2445
0.2126	0.2610	0.2363	0.3360	0.2366

Epididymal pads from eight rats, small to average in size, were used. Methods are denoted by the Roman numerals used in the text. TCA–Glut., trichloroacetic acid–glutaraldehyde; Os., osmium tetroxide.

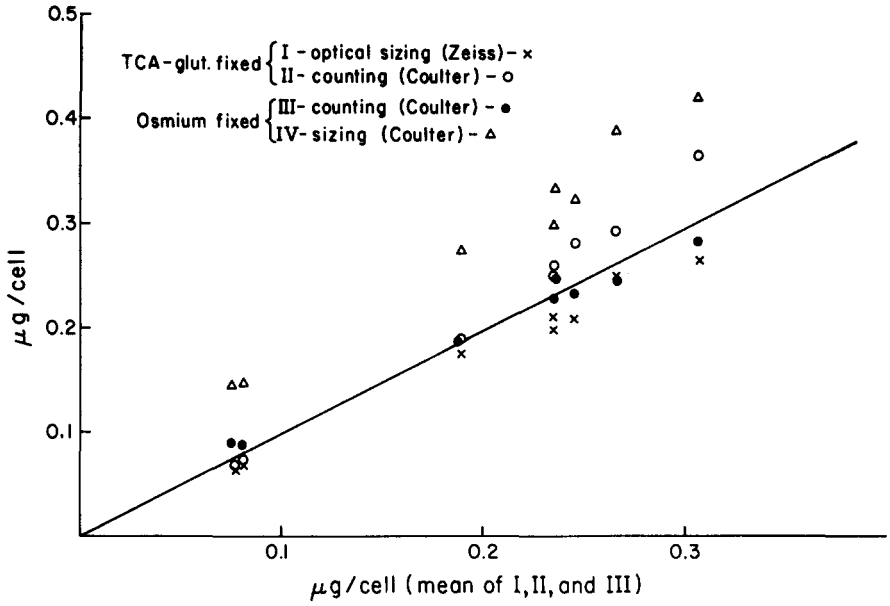

FIG. 8. A comparison of results with the four methods. For reasons discussed in the text, each of the four methods (results on ordinate) is compared with an arbitrary "true" result, namely the mean of methods I, II, and III plotted on the abscissa.

In a wholly arbitrary way, the mean of methods I, II, and III was used to obtain a best estimate of the "true" cell size. The four methods are compared with this arbitrary "true" cell size in Fig. 8. The result obtained using each method is plotted on the ordinate vs. the mean, or "true" cell size, on the abscissa. It can be seen that method III gives results which are as close or closer to this mean than any other method. For this reason and, perhaps more importantly, because it is generally applicable to cells of all sizes, we recommend method III as the most useful of these methods.

Variability

In order to test the variability of method III, it was applied to the left and right epididymal pads of four Sprague-Dawley rats, all close to 200 g in weight. The results, as shown in Table 2, indicate that the standard error of the mean for cell number and cell size is roughly 5–20% of the mean. Cell number was calculated by dividing epididymal pad lipid content by average cell lipid content.

TABLE 2 VARIABILITY OF CELL SIZE AND NUMBER IN RAT EPIDIDYMAL PADS, DETERMINED BY METHOD III

Four animals: wt = 200.7 ± 3.1 g

	No. of cells/pad ($\times 10^6$)	Size
		μg of lipid/cell
Right pads	3.52 ± 0.33	0.088 ± 0.018
Left pads	3.41 ± 0.46	0.080 ± 0.003

Values are mean ± SEM, n = 4.

DISCUSSION

Four related methods for the counting and sizing of adipose cells have been described. The method in which cells fixed with osmium tetroxide are counted with the Coulter counter, referred to as method III, has been found most generally useful. In the past 2 yr, nearly 1000 tissue samples have been processed by this method. These samples were from various adipose tissue sites of rats and mice, as well as from human subjects. One drawback is the expensiveness of the osmium tetroxide. However, it may be possible to recover much of the fixative by oxidizing the fixed cells and unused fixative and thereby reconstituting the osmium tetroxide. If this proves practical, the expense will be greatly reduced.

It is hoped that there will be many potential uses for such a method. To date, the method has been used for studies of adipose cellularity in human obesity and effects of early variations in nutrition on the development of adipose tissue in the rat. Preliminary reports of these studies have appeared (11–13).

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