Microscopic fat cell size measurements on frozen-cut adipose tissue in comparison with automatic determinations of osmium-fixed fat cells

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ABSTRACT Diameters of fat cells in adipose tissue slices, floating in an isoosmolar solution, were measured under a microscope. The slices were obtained from percutaneous biopsies by freeze-cutting after brief formaldehyde fixation. All cells in a given part of the slice were measured, thus avoiding selection. A normal distribution of fat cell diameters could be demonstrated with this method, as has been found with previously described methods. The error of the method was 2.6% for diameter and 8.0% for weight determinations. Storage of adipose tissue at 4°C for 48 hr had no effects on cell size determinations.

Results with this microscopic method were compared with those obtained from a previously described method for automatic determinations of osmium-fixed fat cells. The latter method was slightly modified by using a viscous electrolyte, which prevented sedimentation of large fat cells, and by using sonication to complete cell separation. The methods agreed closely.

A method for calculating mean fat cell weight using osmium-fixed fat cells is described, which makes determinations of sample wet weight and ratio of lipid to wet weight unnecessary.

SUPPLEMENTARY KEY WORDS electronic determination · osmium tetroxide · sonication · glycerol electrolyte · Celloscope · Coulter Counter

INFORMATION on the size and number of adipose tissue fat cells has proved to be of considerable interest in clinical and metabolic studies, but the methods utilized so far have sometimes apparently not been adequate. Microscopic measurements on conventional thick (1–3) or thin (2–4) histological preparations have several drawbacks. These methods are time-consuming, and shrinking of the tissue (2) may give erroneous results. With thin preparations, mathematical corrections for underestimation of fat cell size must be included (2, 5, 6). When fat cells situated on the surface of fat lobuli are measured (7–9), the cell size is probably underestimated, because fat cells in a lobulus are smaller in the periphery than in the center (2).

Fat cells liberated by collagenase treatment (10) have been utilized for both microscopic (9, 11–15) and automatic (16) determination of size. Enlarged fat cells might be expected to break more easily than small cells, but in several studies it has been claimed that this is not the case (9, 11, 15).

The DNA content of adipose tissue correlates with the number of fat cells (3), but there is a large amount of DNA from other cells (10, 17), thus rendering this technique too inexact.

Recently, Hirsch and Gallian (16) described an adequate method for automatic counting or sizing of osmium tetroxide-fixed fat cells in a suspension.

In the present work a simple, rapid, and inexpensive microscopic method is described, which has a sufficiently small error to allow accurate determinations of fat cell size in frozen-cut slices of adipose tissue. This method correlates closely with the method described by Hirsch and Gallian (16) and has apparently none of the drawbacks described above.

METHODS

Measurements with the Microscope

Adipose tissue (2–15 mg), obtained by surgical techniques or by percutaneous needle biopsy (18) and kept in Ringer's solution at room temperature, was fixed in 35% formaldehyde for 7 min. Thereafter, the adipose tissue samples were frozen with carbon dioxide snow on the table of a microtome (Jung type 1205, Jung AG, Heidelberg, West Germany). A slice 200 μ in thickness from the central part of the tissue was, while still frozen, rapidly transferred to a glass plate with the aid of a chilled scalpel. A glass cylinder (diameter 7 mm, height 3 mm), ground at each end, was put around the adipose tissue slice and tightly held to the glass plate with the aid of vacuum grease. The glass cylinder was then filled with Ringer's solution and the slice of adipose tissue floated spontaneously or after a slight manipulation with a piece of wire. A covering glass was placed over the glass cylinder.

With a Zeiss photomicroscope 100 cell diameters were measured using an eyepiece micrometer at 256 × magnification (objective magnification 16 ×, ocular magnification 16 ×). The eyepiece was supplied with a scale graded in 100 parts. One scale unit corresponded to 2.44 μ. In order to circumvent errors caused by polarization of the fat cells, 50 cell diameters were measured in one direction and then 50 in another direction perpendicular to the first. The largest diameter of each cell was focused and measured. All cells through the whole thickness of the slice were included in the measurements if they fell within a surface delineated by the whole width of the scale when this was moved over the sample. The measured diameters were recorded on a tape recorder instead of writing down the results; by using a tape recorder operated by a pedal, both hands of the examiner are free for adjustments of the microscope.

Fig. 1 shows the equipment used for these measurements and Fig. 2 shows the quality of the preparations. Fig. 2 also demonstrates the necessity of keeping the slice of adipose tissue in isoosmolar solution during measurements. Without this protection the fat cells were rapidly deformed, apparently due to disappearance of extracellular water.

The calculation of the average fat cell size was performed by the following two procedures.

Method I:a. The average fat cell volume was determined from measured diameters, with the assumption that the measured cells were spheres, as the average of all separate cell volumes using an IBM 360/65 computer. Mean cell weight was calculated in the same way using the density of human fat cell triglycerides (0.915 g/ml) (19) to convert the individual volumes to weights.

Method I:b. With the aid of the average diameter ($\bar{d}$) of the fat cells and its standard deviation (s), the mean fat cell volume was obtained using an Olivetti table computer (Programma 101) according to the formula: $\frac{\pi(3s^2\bar{d} + \bar{d}^3)}{6}$ (Ref. 8). The computer also converted the mean fat cell volume to mean fat cell weight, utilizing the density of human fat cell triglycerides (19).

Osmium Fixation Methods

Method III of Hirsch and Gallian (16) was utilized with the following modifications: *1.* An electronic particle

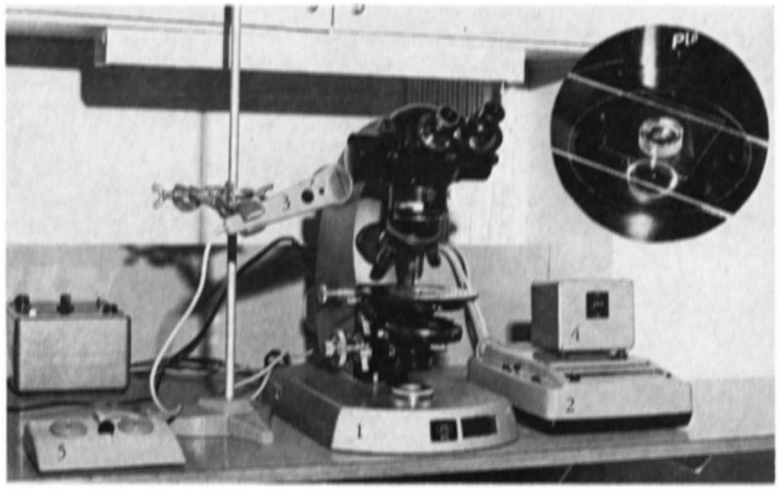


FIG. 1. The equipment for determination of fat cell size. *1*, microscope; *2*, tape recorder; *3*, microphone; *4*, counter; *5*, pedal for tape recorder. The insert demonstrates the frozen-cut adipose tissue slice floating in a closed chamber. For each observation to be dictated the tape recorder was started, which automatically resulted in a count on the counter. The records obtained were transferred to a table computer for calculations.

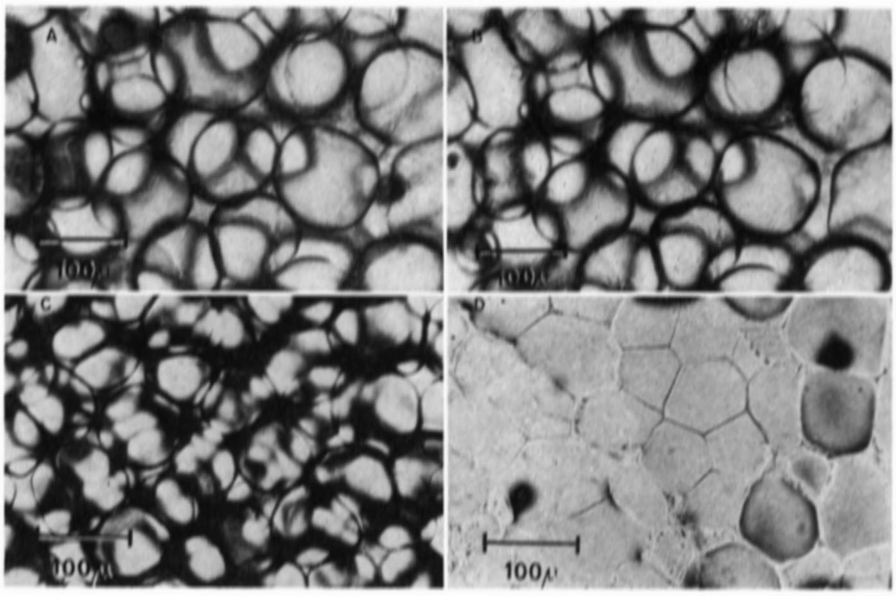


FIG. 2. The effect of storage on formaldehyde-fixed frozen-cut slices of adipose tissue. *A*, slice floating in Ringer's solution immediately after preparation; *B*, same slice as in *A* after 18 hr; *C*, slice on a glass plate without protection of isoosmotic fluid immediately after preparation; *D*, same slice as in *C*, 10 min later.

counter designated Celloscope 302 (AB Lars Ljungberg, Stockholm, Sweden) was usually used instead of a Coulter Counter model B. The orifice of the capillary tubing was 600 instead of 400 μ. Cell suspension aliquots of 5 ml instead of 2 ml were counted. *2.* In order to prevent sedimentation, the osmium-fixed cells were counted in a glycerol electrolyte instead of in normal saline. *3.* Adherence of fat cells to each other was avoided by sonication of the sample before counting. *4.* A 10-μ filter instead of a 25-μ filter was used when collecting the osmium-fixed cells.

Comments to Modification 1. In a preliminary series of determinations, results obtained by the microscopic method were compared with results obtained with a Coulter Counter model B. However, this counter was not available when the microscopic method was finally worked out and, instead, a Celloscope 302 was used. The grading of the settings for measurements of volumes with the Celloscope can be varied less than the Coulter Counter (factors 160 and 570, respectively). The 600-μ orifice proved to be more convenient under these circumstances and made possible measurements of particles with diameters between 14 μ and 161 μ. Larger cells could be counted but not measured. Blockage was practically never present with this large orifice.

Comments to Modification 2. It proved difficult to suspend the fat cells evenly in normal saline when the average cell weight was above 0.8 μg. Therefore, the cells were suspended in a viscous solution with the following composition: glycerol, 555 ml; water, 445 ml; and NaCl, 38.5 g. This solution was filtered through a 10-μ Millipore filter (LCWP 04700, Millipore Corp., Bedford, Mass.). The density of the solution was 1.148 g/ml, with an electrical resistance of 212 ohm/cm, resulting in a resistance of 27 kohm over the capillary tube, which corresponded to an optimal sensitivity of the Celloscope. Mixing of the suspension by propeller was either insufficient or, with increasing speed, caused high and irregular blanks due to air bubbles being drawn into the solution along the rotating axis. Therefore, a magnetic stirrer and a special pump (Hydrogator, AB Lars Ljungberg) were used together to suspend the cells evenly.

Comments to Modification 3. Adherence of the osmium-fixed fat cells to each other after passing them through the 250-μ Nitex screen (Tobler, Ernst & Traber, Inc., New York) was observed in the microscope. This was the case with suspensions in both normal saline and the glycerol solution. Fig. 3 shows that after sonication of the cell suspension for 3 min the number of particles was constant. The increase in counts per milliliter was between 20 and 45% in different experiments. The sonication corresponded to separation of cells in the microscope without any signs of cell rupture. Consequently, 3 min was chosen for the routine treatment of samples. The stability of counts after this sonication (measured up to 40 min) suggests that the original separation of cells by osmium fixation and filtering procedures (16) was insufficient, rather than the phenomenon being explainable by aggregation of liberated fat cells.

Comments to Modification 4. A 10-μ filter (LCWP 04700, Millipore) was used in order to trap as small cells as possible.

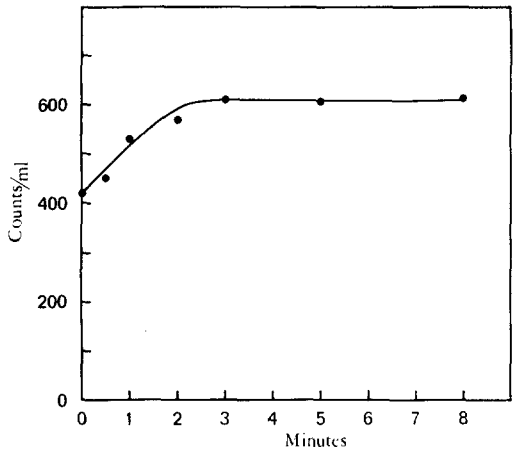


FIG. 3. The relationship between sonication time and number of counts per ml in a glycerol solution containing osmium-fixed fat cells. During careful stirring, aliquots of osmium-fixed fat cells suspended in glycerol solution were taken from a retort with a bottom stopcock. With all fat cells sedimented in a beaker, the aliquots were sonicated in an ultrasonic tank (22 × 17 cm) with one transducer and a middle effect of 80 w at 26 kc (Renggli 75 T, Renggli AG, Rotkreutz, Switzerland). Then, after stirring for 3 min, the aliquots were counted at settings corresponding to the mean diameter minus 1 SD as determined with the microscopic method.

Calibration

Corn pollen (AB Lars Ljungberg) was used for calibration of the Celloscope. These grains had a diameter in the glycerol solution of 89.5 ± 8.56 μ (mean ± SD, n = 400) measured with the microscope. The settings of the Celloscope at which 50.0% of the corn pollen in a suspension were counted was considered to correspond to 89.5 μ.

Correction for Coincidence Effect

Correction of the coincidence effect (20–24) was performed according to: $N = \frac{n}{1 - k \cdot n}$ (Ref. 22), where N is the corrected number of particles, n is the observed number, and k is a constant. The constant (k) for the 600-μ orifice was determined by dilution experiments. Fig. 4 shows the number of corn pollen grains per milliliter plotted against dilutions of a suspension. This curve is rectilinear up to 200 particles per ml, but after that it declines. With the aid of the regression equation for origin and the five lowest concentrations, N values were calculated for each observed dilution. The average of all k values from the formula was then calculated and found to be $1.60 \times 10^{-4} \pm 0.44 \times 10^{-4}$ (mean ± SD, n = 11).

Since calculated ($k = 1.60 \times 10^{-4}$) and observed n values were not significantly different (paired t test), the correction technique was considered adequate.

Counting with the Celloscope

The Celloscope 302 has nothing which corresponds to the Coulter Counter automatic particle size distribution analyzer (model J). Thus, when determining the total number of cells in a sample, there was no simple way to determine a setting corresponding to the smallest particle in the distribution. For each sample the number of cells was therefore determined at different size levels, and thereafter cumulative curves could be constructed for volume or diameter or both. Fig. 5 demonstrates the importance of determining the setting corresponding to the smallest cell diameter. With too low settings falsely high counts might be obtained due to a high background. To keep the coincidence at a low level the cell suspension was diluted when needed.

The calculation of the average fat cell size was performed by the following two procedures.

Method II:a. The wet weight of two adipose tissue samples from the same adipose tissue site was determined. One sample of about 200 mg was utilized for lipid determination by extraction according to the method of Dole (25) as modified by Trout, Estes, and Friedberg (26) and weighing of the residue after evaporation of a heptane aliquot. Except for modifications described above, alternative II:a was identical to method III of Hirsch and Gallian (16).

Method II:b. From cumulative determinations the number of particles were counted in narrow classes of arbitrary volumes over the whole range of fat cell sizes. The arbitrary class mean volume × number of fat cells in each of these classes was calculated, and the products obtained were added. This gave an arbitrary total volume of the osmium-fixed fat cells. This total volume was divided by the total number of fat cells calculated as described above; thereby an arbitrary average volume was obtained. From the calibration of the Celloscope, this arbitrary average volume could be converted to an absolute mean fat cell volume, and this to a mean weight using the density of human fat cell triglycerides.

Statistical Methods

Conventional Student's t test and linear regression analysis were utilized. The significance of a regression coefficient ($P[t_b]$) was determined by the t test method, using a standard error (27). Standard error of a single determination was calculated according to $\sqrt{\frac{d^2}{2n}}$, where d is the difference and n is the number of duplicates (28), and is expressed as a percentage of the mean of all determinations. When judging the quality of slides (Table 3), the nonparametric τ test was used (29).

RESULTS

Direct freeze-cutting of adipose tissue caused breakage and distortion of fat cells, and therefore fixation with

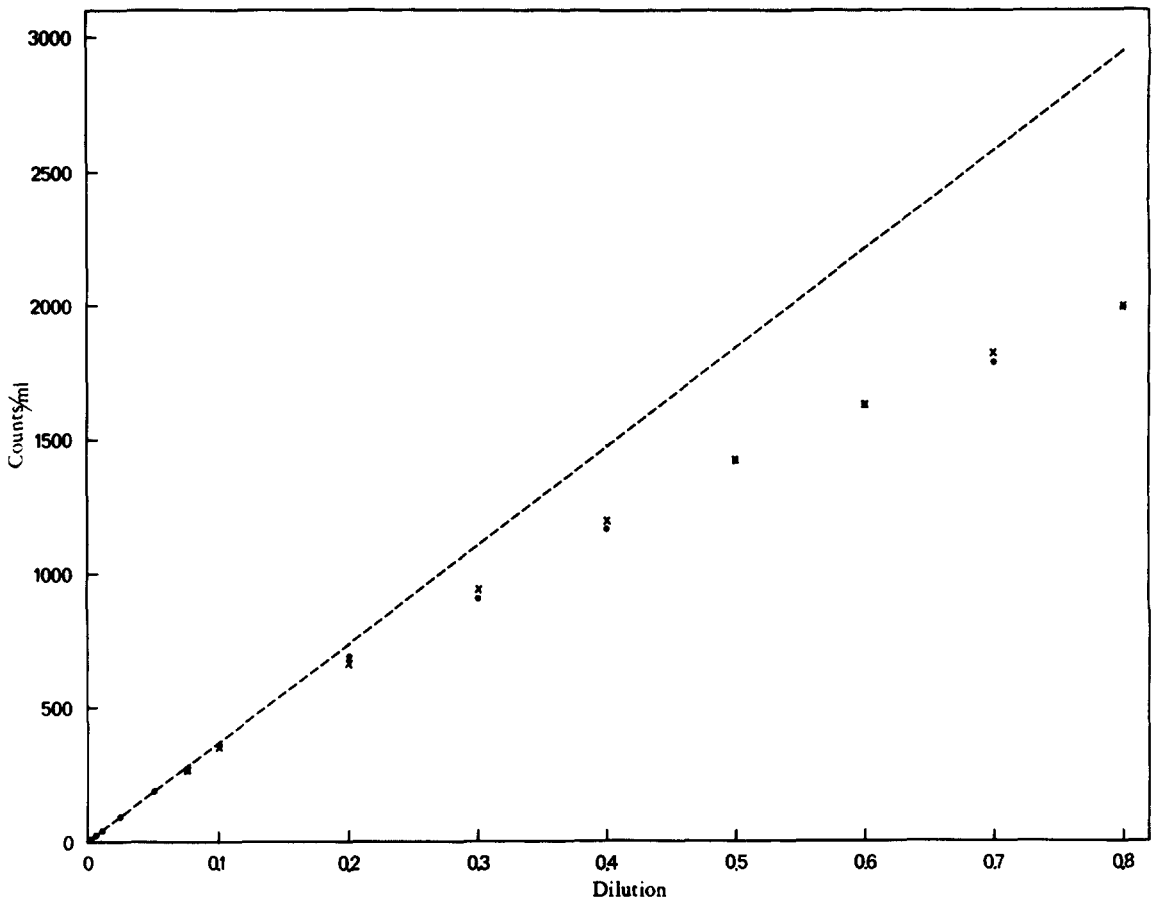


FIG. 4. The coincidence effect demonstrated by the relationship between dilution of a corn pollen suspension and observed number of counts in an automatic particle counter. From a suspension of corn pollen in a glycerol electrolyte, sonicated for 30 sec, dilutions were prepared as indicated, and after stirring for 3 min the number of counts per ml was determined (●) at settings corresponding to the mean diameters minus 1 SD. The regression equation for origin and the five lowest concentrations was $y = 0.368x + 1.87$ (dashed line). X indicates calculated n values obtained according to the formula $n = N/(1 + Nk)$, where k is $1.60 \cdot 10^{-4}$ (see text). Countings were performed with a Celloscope with a 600-μ aperture.

formaldehyde was necessary. With prolonged fixation, shrinking of fat cells was observed. Preliminary experiments with different formaldehyde concentrations and times of fixation showed that the chosen procedure was optimal.

When cutting adipose tissue, a number of fat cells will be divided. Principally, the largest diameter of a divided cell is situated within the slice of tissue under study if the diameter of that cell decreases from a maximal value when the focus is changed both upwards and downwards (2). With the freeze-cutting technique this was almost always the case, probably due to the fact that only intact cells, or cells cut leaving the maximal diameter in the preparation, were remaining; cell remnants of less than one-half the cell might have been rinsed away during preparation.

Mean fat cell weights obtained after calculations according to microscopic method I:a and I:b gave nearly identical values ($y = 0.993x - 0.000132$; $r = 0.999$, $n = 273$). Thus, in the following only calculations according to the less elaborate alternative I:b are given.

Photographs of cells in the flotation chamber at different times up to 18 hr after slicing revealed no changes of shape or size of the fat cells (Fig. 2).

The error of the method for diameter determinations was essentially the same in intraindividual and interindividual measurements on the same or different slices (Fig. 6, *A*, *C*, *E*). The same data transformed to weight will result in higher errors (Fig. 6, *B*, *D*, *F*).

Table 1 shows that the average error of method for diameter and weight determinations was 2.6% and 8.0%, respectively. The error of diameter determinations decreased with increasing diameter.

Fat cell weight determinations after different times of storage in Ringer's solution at 4°C were practically identical (Table 2). The error of the method also appeared to be constant. The quality of the slices was not decreased at the times indicated, as judged by inspection. These circumstances indicate that adipose tissue can be stored in this way for at least 48 hr before measurements.

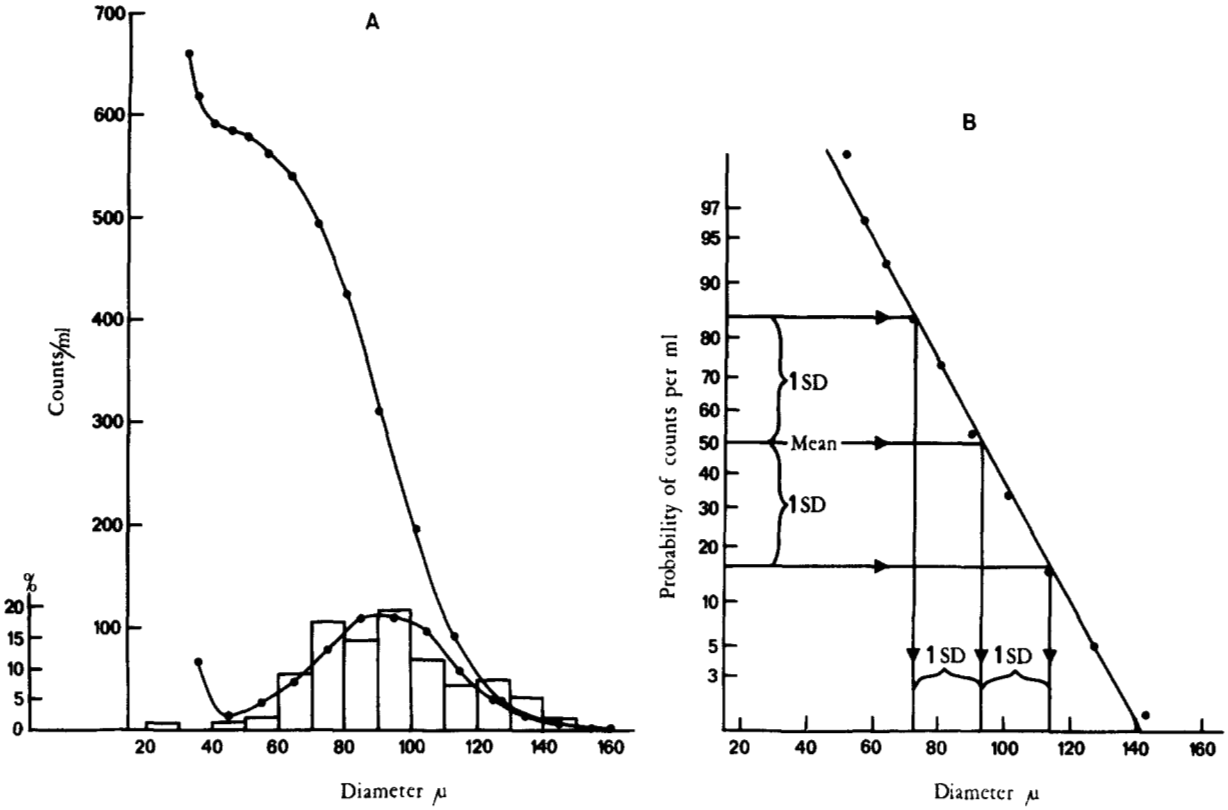


FIG. 5. Comparison between determinations of fat cell size with the microscope method and with osmium-fixed fat cells in an automatic particle counter (method II:b). *A*: The curves demonstrate Celloscope determinations. The cumulative curve is given in counts per ml and the normal distribution in counts per ml and in %. The bars represent the diameter distribution in % as obtained with the microscopic method. *B*: A cumulative plot of the Celloscope determinations on probability paper. A straight line is obtained, indicating normal distribution. The figure demonstrates how mean diameter ± SD is easily obtained from 50 ± 34.13 probit units.

Table 3 demonstrates the effect on fat cell weight determinations of storing the adipose tissue samples frozen in Ringer's solution before freeze-cutting. Some samples were frozen in Ringer's solution at −25°C directly after the biopsy, then thawed and formaldehyde-fixed immediately before freeze-cutting. Other samples from the same adipose tissue were first formaldehyde-fixed, then frozen in Ringer's solution. There was no significant difference between the cell weights obtained with fresh or frozen preparations. The correlation coefficients between freshly examined samples and samples stored frozen were, however, lower than in determinations of two samples not stored frozen (Fig. 6, *F*). This was probably a consequence of a decreased quality of the preparations, which subjectively was rather obvious although not possible to show statistically. After freeze-storage some preparations are thus technically unsatisfactory and the error of method is probably increased.

TABLE 1 RELATIONSHIP BETWEEN ERROR OF METHOD AND FAT CELL SIZE

Fat Cell Diameters	Error of Method: Diameter Determination	Error of Method: Weight Determination	Number of Samples
μ	%	%	
<85	3.7	14.3	28
85–115	2.3	6.6	47
>115	2.0	6.7	7
All	2.6	8.0	82

Data in Fig. 6 and 0- vs. 3-hr values of Table 2 are pooled.

The correlations between method II:a and II:b for the Celloscope 302 and for the Coulter Counter model B are seen in Fig. 7. The correlation coefficient was close to unity, and there was no significant difference between these results with either the Celloscope or the Coulter Counter.

Figs. 8 and 9 show the close agreement between the osmium tetroxide methods and the microscopic method.

DISCUSSION

When the average fat cell diameter is to be determined, selection of fat cells for measurements must be avoided. Therefore, the widest diameter of all cells was measured

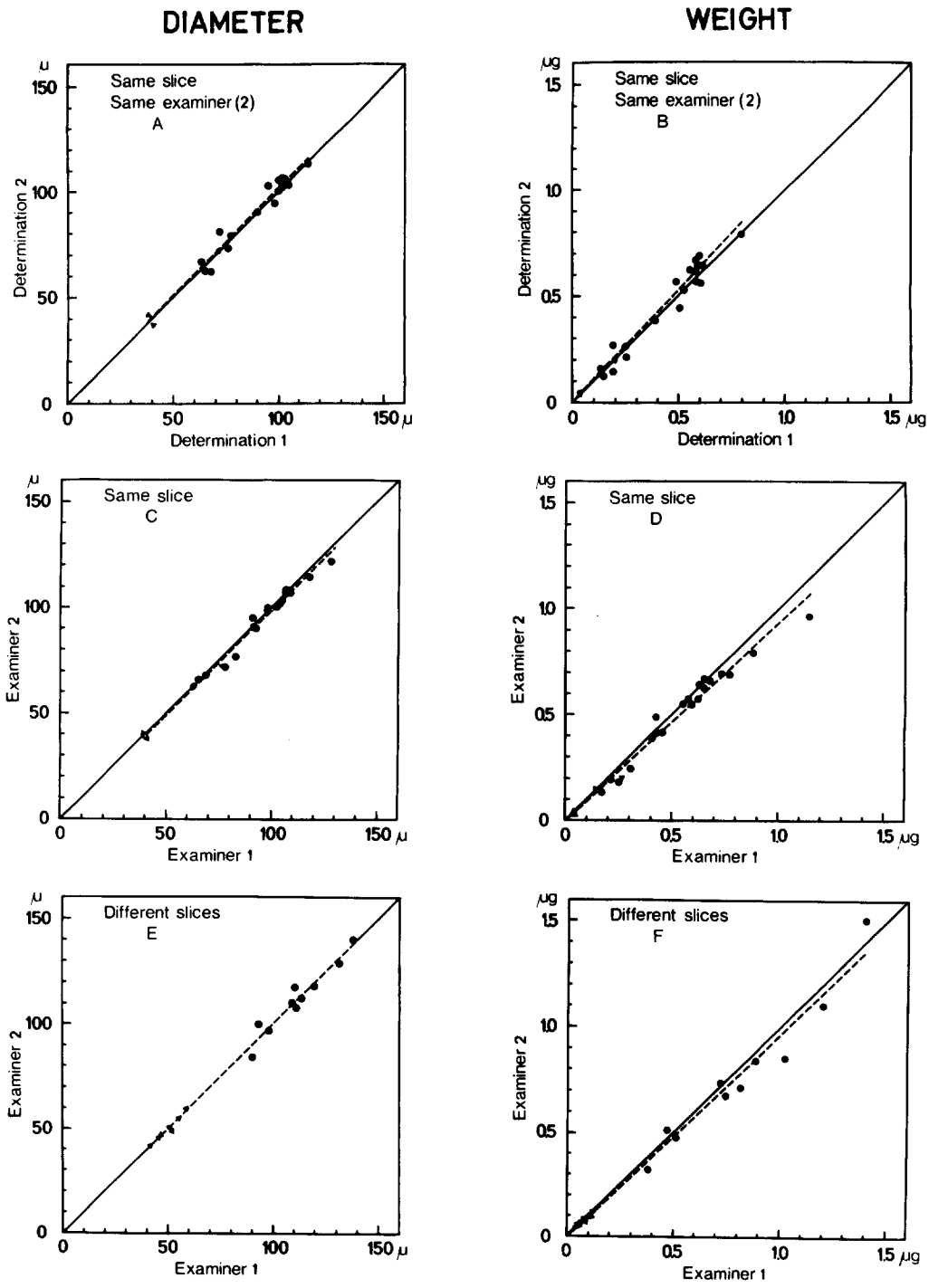


FIG. 6. Error of method in inter- and intraindividual determinations of fat cell diameters and weights. Solid lines, identity lines; dashed lines, regression lines; circles, human adipose tissue; triangles, rat adipose tissue. In *A*, one examiner made two subsequent determinations of all preparations studied. $y = 1.025x - 0.771$; $r = 0.985$; error = 3.4%. In *C*, measurements were made on the same preparations by two examiners. $y = 0.989x - 0.340$; $r = 0.980$; error = 2.4%. In *E*, adipose tissue samples were determined in different preparations by two examiners. $y = 1.002x - 0.297$; $r = 0.995$; error = 2.6%. Different samples were used in *A*, *C*, and *E*. From the diameter determinations, the corresponding weights were calculated and are given in *B*, *D*, and *F*. For *B*, $y = 1.057x - 0.004$; $r = 0.981$; error = 8.5%. For *D*, $y = 0.917x - 0.011$; $r = 0.986$; error = 8.2%. For *F*, $y = 0.969x - 0.009$; $r = 0.990$; error = 10.3%. In all six cases $P(t_b)$ was <0.001.

within a column of adipose tissue with a base surface of about 250 × 200 μ (width of measuring scale × thickness of slice). The size of this base surface was selected because fat cells above 200 μ are only rarely seen (compare Figs. 6 and 9), and because in thicker slices of adipose tissue the translucence decreases, thus rendering microscopy difficult. Measurements of cells on photographs (8, 16) are principally unsatisfactory

TABLE 2 STABILITY OF FAT CELL WEIGHT DETERMINATION AFTER STORAGE OF ADIPOSE TISSUE SAMPLES IN RINGER'S SOLUTION AT 4°C FOR DIFFERENT TIMES BEFORE FREEZE-CUTTING

	Hours				
	0	3	6	24	48
μg (mean ± SD)*	0.49 ± 0.25	0.50 ± 0.25	0.50 ± 0.27	0.49 ± 0.26	0.50 ± 0.25
Correlation vs. 0 hr (r)		0.991	0.982	0.978	0.962
$P(t_b)$ vs. 0 hr		<0.001	<0.001	<0.001	<0.001
n	19	19	18	18	14
Error of method, %, calculated on differences as indicated	5.8	8.3	6.8	7.5	

* Determined on samples examined at all indicated times (0, 3, 6, 24, 48 hr) (n = 13).

TABLE 3 EFFECT OF FREEZE-STORAGE ON FAT CELL WEIGHT DETERMINATIONS

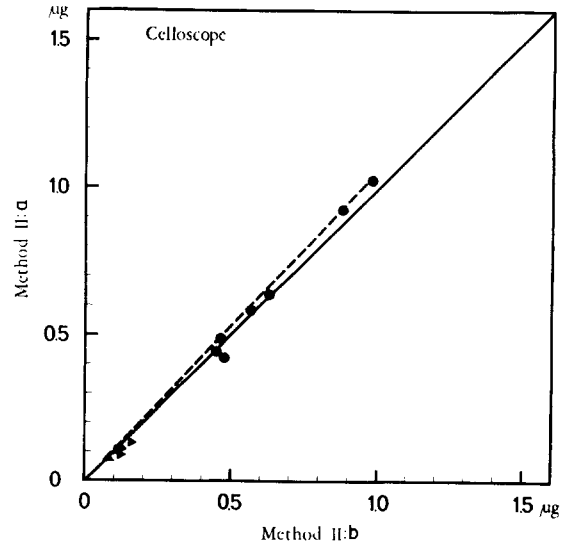
	No Freeze-storage	Formaldehyde Fixation before Freeze-storage	Formaldehyde Fixation after Freeze-storage
μg (means ± SD)	0.44 ± 0.27	0.46 ± 0.28	0.46 ± 0.29
n	17	17	17
Correlation vs. no freeze-storage (r)		0.948	0.956
$P(t_b)$ vs. no freeze-storage		<0.001	<0.001
Quality of slices, ranking means*	1.750	2.042	2.208

* n = 12. The quality of slices was estimated in an arbitrary manner from 0 to 3 (0 = completely unusable; 3 = perfect) by two examiners, and the mean values obtained were ranked. No significant differences between groups could then be found when analyzed by τ test.

because it is difficult to tell which cells were focused at maximal diameter.

To obtain correct results with the microscopic method, fat cells should be spherical. Particularly large fat cells are, however, packed and form polyhedrons with approximately 14 sides (30, 31). It has been suggested that the error of calculations caused by this is limited (7, 8). Osmium fixation probably fixes fat cells in the form they have *in situ*. Distribution curves for diameters of spheres, equivalent to the volumes obtained with the osmium fixation method II:b, coincide well with those obtained with the microscopic method (Figs. 5 and 9). This demonstrates that the error is insignificant when assuming that the measured fat cells are spheres.

With the osmium fixation method II:b, a large number of fat cells were counted in each sample, and the diameters of these cells were normally distributed resulting in straight lines on probability paper (see Fig. 5). The formula utilized in the microscopic method for calculation of fat cell volume from diameter and standard deviation (8) is valid only when the diameters are normally distributed. That this was the case after measurements of 100 cells is shown by the very strong correlation between method I:a and I:b.



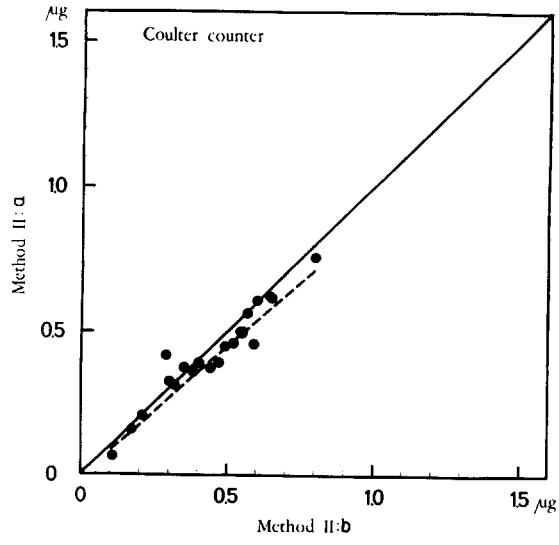


FIG. 7. The relationship between method II:a and method II:b in determinations with the Celloscope 302 and Coulter Counter model B (see text). Symbols as in Fig. 6. For the Celloscope, $y = 1.080x - 0.030$; $r = 0.998$. For the Coulter Counter, $y = 0.877x + 0.037$; $r = 0.954$. For both, $P(t_b)$ was <0.001.

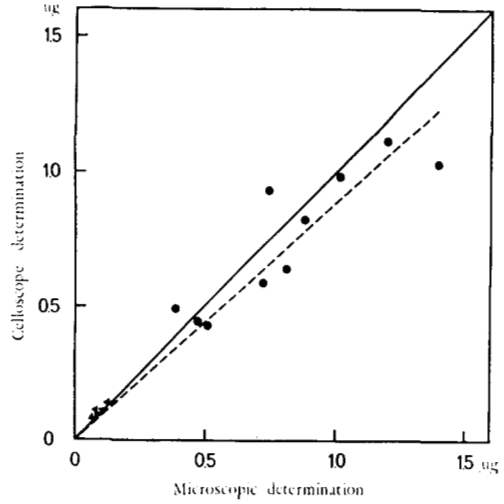


FIG. 8. The relationship between determinations of cell weights with the Celloscope (method II:a) and the microscopic method. Symbols as in Fig. 6. $y = 0.8429x + 0.0565$; $r = 0.960$; $P(t_b) < 0.001$.

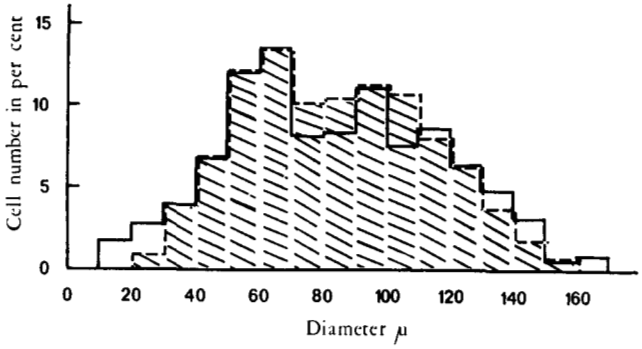


FIG. 9. Comparison of the distribution of diameters determined automatically with osmium-fixed fat cells and with the microscopic method. Fractions given in % of total number of cells. Broken line and striped bars indicate method II:b, and continuous line method I:b (see text). The mean difference between methods in diameter classes was 0.92%. Fat cell diameters >160 µ were not possible to size in the Celloscope (see Methods). All results obtained with methods I:b and II:b were pooled, except that samples containing a significant number of cells >160 µ were excluded from comparisons.

When sizing osmium-fixed fat cells electronically with the Coulter counter, Hirsch and Gallian (16) obtained higher values than with any other method tested; this was interpreted to be due to swelling of cells during osmium fixation. There was no indication of such swelling in the present study, as judged by measurements in either the Coulter Counter or the Celloscope (Fig. 7).

The error of the method for diameter determination was essentially the same in different preparations as in intraindividual measurements on the same slice (Fig. 6, *E* and *A*). This indicates a limited variation in average fat cell size within one region of human subcutaneous adipose tissue.

By utilizing arbitrary units, a mean cell volume could be calculated from the calibration of the particle counter (method II:b). This renders weight and triglyceride measurements of samples unnecessary; this is a considerable advantage when working with small amounts of tissue such as needle biopsy specimens. With the Coulter Counter model B, supplied with an automatic size distribution analyzer (model J), this appears to be a most useful procedure.

Principally, automatic cell counting methods are superior to manual methods. Automatic methods are objective, and a large number of fat cells are counted. These methods give volume as primary data and this probably reduces the error of method for fat cell weight determinations. However, determinations of very small fat cells by the osmium method is apparently difficult, presumably because they contain too small amounts of lipid to be properly fixed (32, 33). Another disadvantage when counting small cells could be interference by non-fat cell particles. Fig. 9 illustrates the possibility of measuring small cells (10–30 µ) with the microscopic method which are apparently incompletely registered with the osmium fixation methods. Thus, for small cells, the microscopic method described in the present work is probably preferable. The error of the method for determination of small fat cells can be brought down by increased magnification. The microscopic method also offers the advantage of requiring a smaller amount of tissue than the osmium fixation method (2–5 mg vs. 25–200 mg), and it is considerably simpler, less expensive, and requires no preparations the days before measurements. Time consumption per sample is approximately 30 min, including biopsy, preparation, measurements, and calculation. It should be observed, however, that a training period is required before the examiner obtains reliable values.

The described method for determination of fat cell size has proved useful in clinical and metabolic studies (34–41), and it is hoped that it might provide additional possibilities for such studies.

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REFERENCES

1. Hellman, B., I.-B. Täljedal, and S. Westman. 1962. Morphological characteristics of the epididymal adipose tissue in normal and obese-hyperglycemic mice. *Acta Morphol. Neer.-Scand.* **5:** 182–189.
2. Bjurulf, P. 1959. Atherosclerosis and body-build with special reference to size and number of subcutaneous fat cells. *Acta Med. Scand. Suppl.* **166:** 7–99.
3. Björntorp, P., and A. Martinsson. 1966. The composition of human subcutaneous adipose tissue in relation to its morphology. *Acta Med. Scand.* **179:** 475–481.
4. Zingg, W., A. Angel, and M. D. Steinberg. 1962. Studies on the number and volume of fat cells in adipose tissue. *Can. J. Biochem. Physiol.* **40:** 437–442.
5. Wicksell, S. D. 1925. The corpuscle problem. A mathematical study of a biometric problem. *Biometrika.* **17:** 84–99.
6. Hellman, T. 1926. Die Altersanatomie der menschlichen Milz. Studien besonders über die Ausbildung des lymphoiden Gewebes und der Sekundärknötchen in verschiedenen Altern. *Z. Angew. Anat. Konstitutionslehre.* **12:** 270–415.
7. Reh, H. 1953. Die Fettzellgrösse beim Menschen und ihre Abhängigkeit vom Ernährungszustand. *Virchows Arch. Pathol. Anat. Physiol. Klin. Med.* **324:** 234–242.
8. Goldrick, R. B. 1967. Morphological changes in the adipocyte during fat deposition and mobilization. *Amer. J. Physiol.* **212:** 777–782.
9. Smith, U. 1971. Morphological studies of human subcutaneous adipose tissue in vitro. *Anat. Res.* **169:** 97–104.
10. Rodbell, M. 1964. Localization of lipoprotein lipase in fat cells of rat adipose tissue. *J. Biol. Chem.* **239:** 753–755.
11. Martinsson, A. 1968. Methods of isolation and characterization of human subcutaneous fat cells. *Acta Morph. Neer.-Scand.* **7:** 41–50.
12. Zinder, O., R. Arad, and B. Shapiro. 1967. Effect of cell size on the metabolism of isolated fat cells. *Israel J. Med. Sci.* **3:** 787–791.
13. Bray, G. A. 1969. Studies on the composition of adipose tissue from the genetically obese rats. *Proc. Soc. Exp. Biol. Med.* **131:** 1111–1114.
14. Bray, G. A. 1970. Measurements of subcutaneous fat cells from obese patients. *Ann. Intern. Med.* **73:** 565–569.
15. Sailer, S., F. Sandhofer, H.-J. Lisch, and H. Braunsteiner. 1969. Grösse der Fettzellen und relatives Körpergewicht in verschiedenen Regionen des Menschen. *Wien. Z. Inn. Med. Grenzgeb.* **50:** 374–381.
16. Hirsch, J., and E. Gallian. 1968. Methods for the determination of adipose cell size in man and animals. *J. Lipid Res.* **9:** 110–119.
17. Hollenberg, C. H., and A. Vost. 1968. Regulation of DNA synthesis in fat cells and stromal elements from rat adipose tissue. *J. Clin. Invest.* **47:** 2485–2498.
18. Hirsch, J., and R. B. Goldrick. 1964. Serial studies on the metabolism of human adipose tissue. I. Lipogenesis and free fatty acid uptake and release in small aspirated samples of subcutaneous fat. *J. Clin. Invest.* **43:** 1776–1792.
19. Keys, A., and J. Brozĕk. 1953. Body fat in adult man. *Physiol. Rev.* **33:** 245–325.
20. Mattern, C. F. T., F. S. Brackett, and B. J. Olson. 1957. Determination of number and size of particles by electronical gating: blood cells. *J. Appl. Physiol.* **10:** 56–70.
21. Mercer, W. B. 1960. Calibration of Coulter counters for particles ~1 μ in diameter. *Rev. Sci. Instrum.* **31:** 1515–1520.
22. Wales, M., and J. N. Wilson. 1961. Theory of coincidence in Coulter particle counters. *Rev. Sci. Instrum.* **32:** 1132–1136.
23. Berg. R. H. 1965. Sensing zone methods in fine particle size analysis. *Mater. Res. Stand.* **5:** 119–125.
24. Stracke, J. 1966. Technical note. Coincidence loss in blood-counters. *Med. Biol. Eng.* **4:** 97–99.
25. Dole, V. P. 1956. A relation between non-esterified fatty acids in plasma and metabolism of glucose. *J. Clin. Invest.* **35:** 150–154.
26. Trout, D. L., E. H. Estes, Jr., and S. J. Friedberg. 1960. Titration of free fatty acids of plasma: a study of current methods and a new modification. *J. Lipid Res.* **1:** 199–202.
27. Woolf, C. M. 1968. Principles of Biometry: Statistics for Biologists. Van Nostrand-Reinhold Co., Inc., Princeton. 193–198.
28. Dahlberg, G. 1948. Statistical methods for medical and biological students. George Allen & Unwin, London. 122–126.
29. Kendall, M. G. 1948. Rank Correlation Methods. Griffin, London.
30. Lewis, F. T. 1925. A further study of the polyhedral shapes of cells. I. The stellate cells of *Juncus effusus*; II. Cells of human adipose tissue; III. Stratified cells of human oral epithelium. *Proc. Amer. Acad. Arts. Sci.* **61:** 1–34.
31. Bjurulf, P. 1960. Fettcellernas inbördes läge och form hos människa. *Commun. Dept. Anat. Univ. Lund, Sweden.* **No. 5.**
32. Hirsch, J., and P. W. Han. 1969. Cellularity of rat adipose tissue: effects of growth, starvation, and obesity. *J. Lipid. Res.* **10:** 77–82.
33. Hirsch, J., and J. L. Knittle. 1970. Cellularity of obese and nonobese human adipose tissue. *Federation Proc.* **29:** 1516–1521.
34. Björntorp, P., and L. Sjöström. 1971. The number and size of adipose tissue fat cells in relation to metabolism in human obesity. *Metab. Clin. Exp.* In press.
35. Björntorp, P., Kristina de Jounge, L. Sjöström, and L. Sullivan. 1970. The effect of physical training on insulin production in obesity. *Metab. Clin. Exp.* **19:** 631–638.
36. Björntorp, P., and J. Östman. 1971. Human adipose tissue. Dynamics and regulation. *Advan. Metab. Disord.* **5:** 277–327.
37. Björntorp, P. 1971. Disturbances in the regulation of food intake. *Advan. Psychosom. Med.* In press.
38. Björntorp, P., A. Gustafson, and G. Tibblin. 1970. Relationships between adipose tissue cellularity and carbohydrate and lipid metabolism in a randomly selected population. *Atheroscler. Proc. Sec. Int. Symp.* 374–378.
39. Björntorp, P., P. Berchtold, and G. Tibblin. 1971. Insulin secretion in relation to adipose tissue in men. *Diabetes.* **20:** 65–70.
40. Björntorp, P., A. Gustafson, and B. Persson. 1971. Adipose tissue fat cell size and number in relation to metabolism in endogenous hypertriglyceridemia. *Acta Med. Scand.* In press.
41. Björntorp, P., A. Jonsson, and P. Berchtold. 1971. Adipose tissue cellularity in maturity onset diabetes mellitus. *Acta Med. Scand.* In press.