Protein content and osmotic behavior of isolated fat cells

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SUMMARY Fat cells isolated by a modification of the method of Rodbell were washed in protein-free buffers, which allowed determination of their protein content. Osmotic behavior and response to norepinephrine and insulin were unimpaired.

KEY WORDS isolated fat cells · protein determination · osmotic properties · response · norepinephrine · insulin

IN HIS STUDIES of isolated fat cells, Rodbell (1) related his data to the triglyceride content of the fat cells. Since the ratios of both triglyceride and wet weight to nitrogen vary greatly with the age of the animal and the source of the adipose tissue (2), it would be preferable to relate lipolytic activity to the protein content of the cells. However, because of the high protein content of the medium in which the fat cells are isolated, protein estimation in the cell suspensions has not been feasible. In order to circumvent these difficulties, we isolated fat cells and washed them in an albumin-free buffer.

It has been suggested (3) that fat cells isolated according to Rodbell's procedure have lost their semipermeable properties. We therefore investigated the osmotic properties of the fat cells in our preparation. Because the removal of albumin from the buffer used for the isolation and washing procedures might cause a disruption in cell metabolism, the lipolytic response to norepinephrine and the effect of insulin on glucose incorporation into lipid were studied.

Cell Isolation. Epididymal adipose tissue, 2.5 g, was removed from 200-g male Holtzman rats which had been fasted for 18 hr. The tissue was rinsed with 0.9% NaCl and placed in 9 ml of Krebs-Ringer phosphate buffer (ph 7.4) containing 25 mg of purified collagenase (Nutritional Biochemicals Corp., Cleveland, Ohio). The mixture was incubated at 37°C for 90 min in a siliconized glass vessel. After incubation the cells were freed by gentle agitation, collected by slow centrifugation at 500 × *g*, and washed four times with warm (37°C) albumin-free Krebs-Ringer phosphate buffer. After each washing the suspension was centrifuged at 500 × *g* and the cells were transferred to fresh buffer by aspiration. The cells were finally diluted to a convenient volume with buffer and mixed thoroughly before the transfer of aliquots to siliconized incubation vessels for subsequent studies. The cells were always washed in cellulose tubes and transferred with a polyethylene pipette.

Protein Determination. An aliquot of the final cell suspension in buffer was digested for 1 hr at 100°C in N NaOH. A second portion of the suspension was centrifuged at 1400 × *g*, the cells were removed by aspiration, and a portion of the suspending medium was treated as above. Both digests were assayed for protein by the method of Lowry, Rosebrough, Farr, and Randall, (4) with crystalline bovine serum albumin (Nutritional Biochemicals Corp., Cleveland, Ohio) as a standard.

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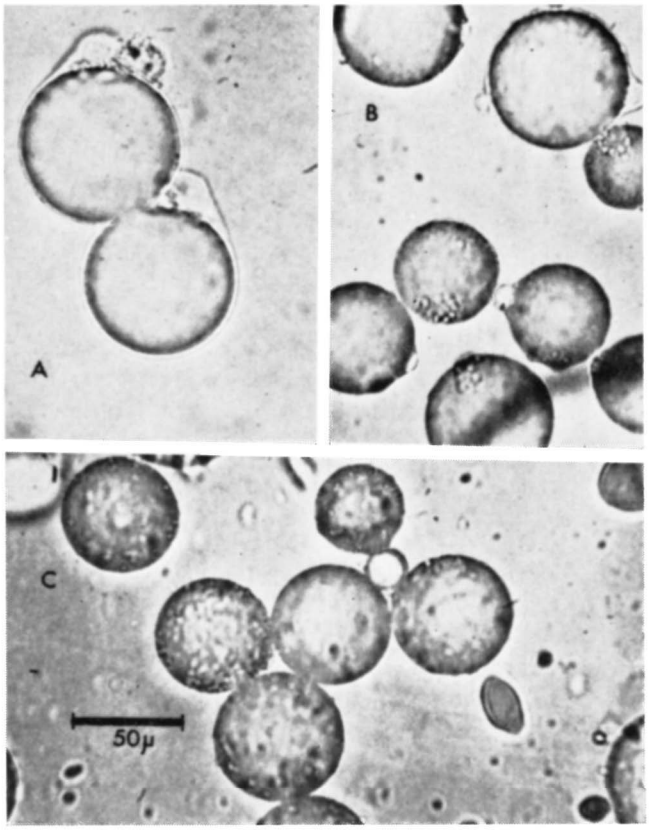

FIG. 1. Effect of hypotonic (A, B) and hypertonic (C) buffers on swelling of isolated fat cells. (Krebs-Ringer phosphate buffer pH 7.4.) Low power, × 340.

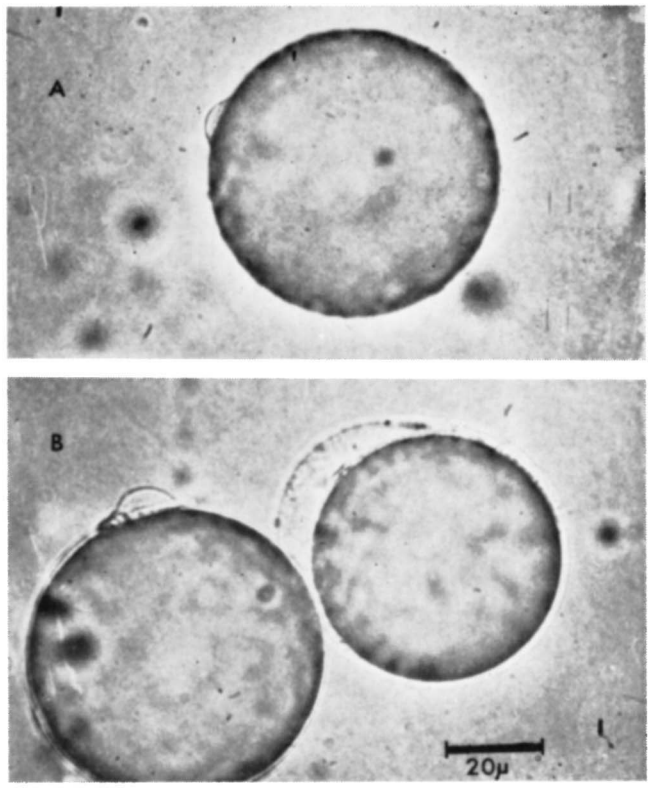

FIG. 2. **Effect of hypertonic** (A) and hypotonic (B) buffers on swelling **of isolated fat cells.** (Krebs-Ringer phosphate buffer pH 7.4.) High power, × 750.

The difference between the protein content of the cell suspension and that of the suspending medium was taken as an estimate of cell protein.

Microscopic Examination. Fat cells were isolated and washed as described above in Krebs-Ringer phosphate buffer with and without 5% bovine serum albumin, and in Krebs-Ringer bicarbonate buffer with and without 5% bovine serum albumin (Armour Pharmaceutical Co., Kankakee, Ill.), all at pH 7.4. In the experiments in which the osmotic effects were studied, the suspensions were diluted 1:1 either with warm (37°C) distilled water or with warm 2.7% NaCl. After 5 min of incubation at 37°C, portions of the cell suspensions were mounted, wet, between siliconized glass slides and cover slips. Cell counts were made and photomicrographs were taken under these conditions.

Metabolic Studies. For the norepinephrine studies, cells suspended in warm (37°C) Krebs-Ringer phosphate buffer (pH 7.4) were transferred to siliconized incubation vessels containing warm 5% bovine serum albumin in Krebs-Ringer phosphate buffer (pH 7.4) and appropriate concentrations of *l*-norepinephrine bitartrate (Winthrop Laboratories, New York, N.Y.). Net release of free fatty acid was measured by the difference between a zero time sample taken immediately after addition of cells and a second sample removed from the same beaker after 1 hr of incubation at 37°C. Free fatty acids released under hormonal stimulation were determined by the method of Dole and Meinertz (5).

When the response of isolated fat cells to insulin was to be studied, the fat cells from fed, 200-g rats were isolated and washed in Krebs-Ringer bicarbonate buffer (pH 7.4) containing 1 μmole/ml of glucose. Aliquots of the cell suspension were transferred to siliconized vessels containing warm Krebs-Ringer bicarbonate buffer (pH 7.4) containing 5% bovine serum albumin and 1 μmole/ml of glucose, 1 mU/ml of glucagon-free insulin (Eli Lilly & Co., Indianapolis, Ind.), when used, and sufficient glucose-U-^{14}C (New England Nuclear Corp., Boston, Mass.) to give a final specific activity of 0.1 μc/μmole of glucose. Incubation was carried out at 37°C for 1, 2, and 3 hr, after which N H_2SO_4 was added to stop the reaction. The incubation mixtures were then extracted twice with redistilled chloroform and the chloroform fractions were washed with N H_2SO_4. Aliquots of the washed chloroform fractions were transferred to a scintillation fluid consisting of 0.4% 2,5-diphenyloxazole

TABLE 1 EFFECT OF TONICITY ON ISOLATED FAT CELLS IN VARIOUS BUFFERS

Buffer	Tonicity	Fraction Swollen
KRP	hypertonic	7/164
	hypotonic	97/119
KRP + 5% albumin	hypertonic	3/120
	hypotonic	143/152
KRB	hypertonic	4/127
	hypotonic	117/143
KRB + 5% albumin	hypertonic	4/119
	hypotonic	106/123

KRP, Krebs-Ringer phosphate buffer, pH 7.4; KRB, Krebs-Ringer bicarbonate buffer, pH 7.4

TABLE 2 EFFECT OF INSULIN ON THE INCORPORATION OF GLUCOSE-U-^{14}C INTO THE CHLOROFORM-EXTRACTABLE LIPIDS OF ISOLATED FAT CELLS

Incubation Time	Radioactivity	
	No Addition	Insulin, 1 mμ/ml
hr	*cpm/mg adipose cell protein*	
1	26,393	44,159
2	46,497	68,706
3	59,388	88,418

and 0.05% 1,4-bis[2-(5-phenyloxazolyl)]-benzene in toluene, and ^{14}C was determined in a Packard Model 3003 liquid scintillation counter.

Results. Six protein determinations on 1 ml aliquots of the same fat cell suspension gave a protein content of 762 ± 25 (SEM) μg/ml. As this example illustrates, the determination is very reproducible. Since we have been using this method the protein content of the suspending medium alone has rarely exceeded one-third that of the entire cell digest.

Because triglyceride represents most of the cell volume, changes in tonicity of the bathing medium would not be expected to make as gross changes as are seen, for example, in the red blood cell. If the microscope is carefully focused and the light suitably adjusted, however, any cytoplasmic bulge in the nuclear area can be seen when this part of the cell is in profile. In a hypotonic medium the nuclear area can be seen to be quite swollen in most of the cells, and in many instances the cytoplasm can be seen along the periphery of the central lipid inclusion (Figs. 1A, 1B, 2B). Under hypertonic conditions, very few, if any, cells swelled, although occasionally in the nuclear region a slightly expanded area was observed (Figs. 1C, 2A). Swollen and unswollen cells were counted under hypertonic and hypotonic conditions. Only those cells in which the nuclear area was seen in profile were

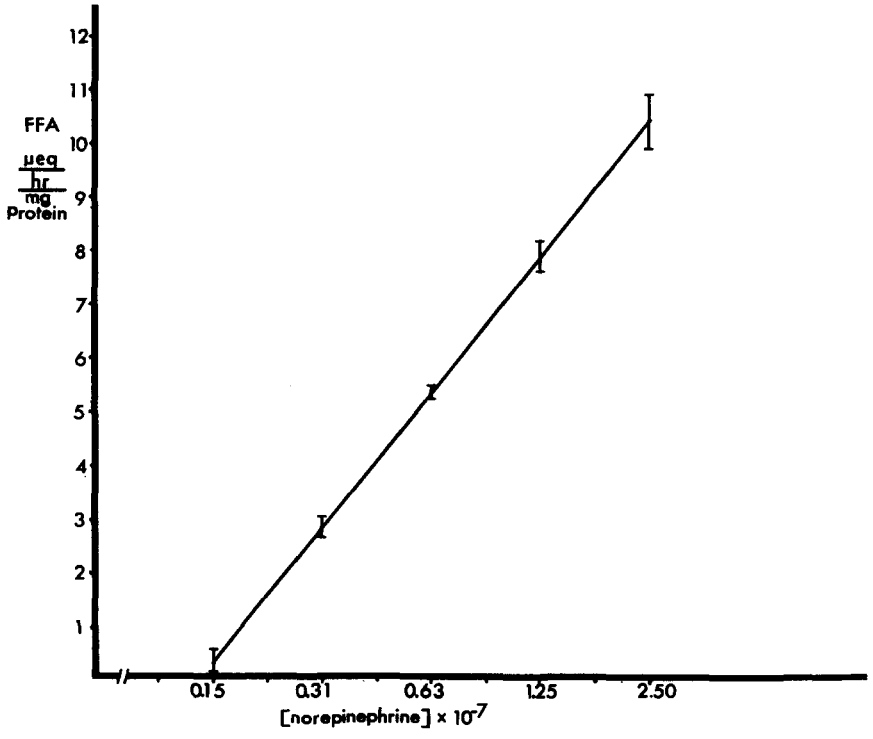

FIG. 3. Release of free fatty acids from isolated fat cells by norepinephrine. Ordinate: increase in free fatty acid release in response to norepinephrine. Each point represents the mean of three experiments ± 1 SEM.

counted. It is apparent (Table 1) that under hypotonic conditions a much larger proportion of cells are swollen than under hypertonic conditions.

Fat cells isolated under the described conditions respond in a dose-related fashion to added norepinephrine (Fig. 3). The free fatty acid response in Fig. 3 is the increased amount released under the stimulus of the neurohormone and is expressed as microequivalents per milligram of adipose cell protein per hour. Regression lines were calculated for each of three experiments and the responses for each dose of drug determined from the lines. The curve is a plot through the means calculated for each point. The data in Table 2 indicate that fat cells isolated in the absence of albumin respond to insulin similarly to the intact fat pad and to cells isolated according to the procedure of Rodbell. Thin-layer chromatography of the washed chloroform extract of the incubated fat cells showed that 94% of the radioactivity in the extract was in the fatty acid and triglyceride fractions.

Routine microscopic examinations of isolated fat cells had indicated to us the possibility that they responded to changes in the tonicity of the bathing medium. It was with some surprise that we noted the findings of Autor and Lynn (3), which indicated that the isolated cells did not respond to tonicity changes in their system. Our photographs show that changes in tonicity do affect the cells, although the conditions of viewing must be carefully selected. This finding is of considerable importance since it is an indication of the retention of intact physical characteristics of the cells after the isolation procedure. Further evidence for metabolic intactness is furnished by the characteristic response to added norepinephrine and insulin.

The technique described therefore allows preparations of viable fat cells to be obtained in which protein content can be measured.

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