5. Cornwell, D. G., Kruger, F. A., *J. Lipid Res.*, 1961, v2, 110.

6. Kunkel, H. G., in *The Plasma Proteins*, V. 1, p294, 1960, Ed., F. W. Putnam, Academic Press, New York.

7. Mangold, H. K., *J. Am. Oil Chem. Soc.*, 1961, v38, 708.

8. Stoffel, W., Chu, F., Ahrens, E. H., Jr., *Anal. Chem.*, 1959, v31, 307.

9. Farquhar, J. W., Insull, W., Jr., Rosen, P., Stoffel, W., Ahrens, E. H., Jr., *Nutrition Rev.* (Suppl.) 1959, v17.

10. Hirsch, J., Farquhar, J. W., Ahrens, E. H., Jr., Peterson, M. L., Stoffel, W., *Am. J. Clin. Nutrition*, 1960, v8, 499.

11. Havel, R. J., Goldfien, A., *J. Lipid Res.*, 1961, v2, 389.

12. Dawson, A. M., Isselbacher, K. J., *J. Clin. Invest.*, 1960, v39, 150.

13. Dawson, A. M., Isselbacher, K. J., *ibid.*, 1960, v39, 730.

14. Borgström, B., Jordan, P., *Acta Soc. Med. Upsaliensis*, 1959, v64, 185.

15. Dustin, J.-P., Fredrickson, D. S., Laudet, P., Ono, K., *Fed. Proc.*, 1961, v20, 270.

16. Laurell, S., *Acta Physiol. Scand.*, 1959, v47, 218.

17. Stein, Y., Shapiro, B., *Am. J. Physiol.*, 1959, v196, 1238.

18. Kay, R. E., Entenman, C., *J. Biol. Chem.*, 1961, v236, 1006.

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Extractable Lipid, Water and Residue of Rat Epididymal Fat Bodies.* (27609)

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The epididymal fat bodies of male rats or mice constitute a convenient source of adipose tissue for the study of fat metabolism either *in vivo* or *in vitro*. Smith *et al.*(1) suggested that changes in lipid content of the epididymal fat bodies might provide a reasonable means for estimating the influence of hormones and other compounds on adipose tissue in general. Tracht *et al.*(2), Levy and Ramey(3,4) and White and Engel(5) have used the change in lipid content of the fat bodies before and after a period of fasting, as an index of lipid mobilization. In addition, concentration of lipid has been used to show changes in the character of epididymal fat as the result of various treatments(1). Consequently, the relationships between the weight of the fat bodies, their lipid content and lipid concentration are important, especially as these are altered by treatment effects. The present study shows the excellent correlation between lipid content and wet weight of epididymal adipose tisue. The relation of lipid, water and residue concentration of this fat depot are also reported.

Method. Male albino rats of the Sherman strain bred at the Lederle Laboratories Wyckoff Colony were housed individually and given free access to Purina Laboratory Chow Pellets or ground pellets and tap water. Rats weighing between 50 and 330 g were sacrificed and either one or both epididymal fat bodies were taken for analysis. Lipid extractions were performed with chloroform-methanol (2:1) according to a procedure similar to that of Folch *et al.*(6). The weight of extractable lipid obtained after evaporation of solvent and drying was not changed significantly by reextraction of the lipid with petroleum ether (6). The residue remaining after extraction was dried to constant weight. Water content of the tissues was calculated by difference. All percentages were calculated on the basis of fresh weight of the adipose tissue. The regression relations were calculated by the method of least squares.

For convenience the quantities are represented symbolically as follows:

*This work was conducted in the Metabolic Chemotherapy Dept.

W_T = weight of fresh adipose tissue
W_L = weight of extractable lipid
W_R = weight of residue
W_W = weight of tissue water, obtained by difference according to the relation:
$W_W = W_T - (W_R + W_L)$

Results. A positive linear relationship existed between the fresh weight of epididymal adipose tissue and extractable lipid content for tissue weights greater than 0.5 – 1.0 g as shown in Fig. 1. The regression relation for Fig. 1 was

$$W_L = -0.1193 + 0.871\ (W_T) \quad (1)$$

The coefficient of correlation was 0.9943. Extension of the regression line in the area below 500 mg fresh weight indicated that it must curve to intersect the origin. In view of the excellent correlation above 500 mg fresh weight, however, it was possible to estimate lipid content from fresh weight of the fat bodies quite accurately. This procedure may be especially useful for routine determination of epididymal fat lipid content.

The relation between concentration of extractable lipid and fresh weight was curvilinear when plotted against fresh weight on a linear scale (Fig. 2).

Division of equation (1) by W_T gave the equation describing the relation between proportion of lipid, W_L/W_T, and fresh weight:

$$W_L/W_T = 0.871 - 0.1193\ (1/W_T) \quad (2)$$

Fig. 3 shows the plot of the proportion of lipid (expressed as per cent) *vs* the reciprocal of fresh weight, $1/W_T$. Residue concentration (expressed as per cent) *vs* $1/W_T$ is also shown and was derived from the relation

$$100\ W_R/W_T = 15.75 - 0.1537\ (100\ W_L/W_T) \quad (3)$$

and equation (2), yielding:

$$W_R = 0.02363\ (W_T) + 0.01834 \quad (4)$$

and

$$W_R/W_T = 0.02363 + 0.01834\ (1/W_T) \quad (5)$$

For a given value of $1/W_T$, the vertical distance between the ordinate for W_L/W_T and that for W_R/W_T represents the concentration of water in the tissue.

It should be noted that presentation of the results as in Fig. 3 tends to emphasize that range of adipose tissue weights where the proportion of lipid varies most rapidly with W_T, *i.e.*, for $0.2 < W_T < 1.0$ or $1 < 1/W_T < 5$. On the other hand values of $1.0 < W_T < 5.0$, shown as $0.2\ 1/W_T < 1.0$, are in the range where W_L/W_T tends to be fairly constant over a relatively large range of W_T (see Fig. 2).

Discussion. The lipid content and fresh weight of epididymal fat are highly correlated above 0.5-1.0 g fresh weight and therefore may be used interchangeably above this

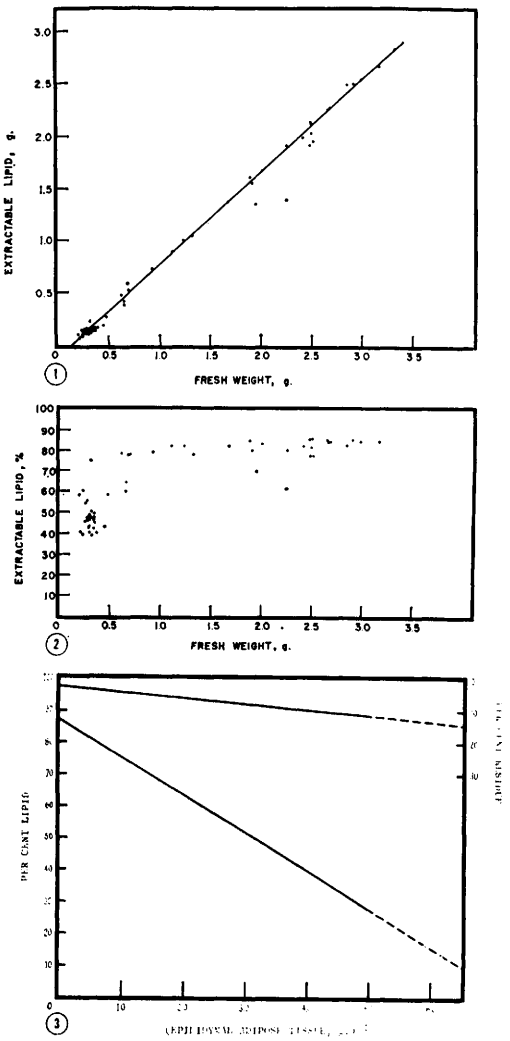

FIG. 1. Relationship between extractable lipid content and fresh wt of epididymal adipose tissue $W_L = -0.1193 + 0.871\ W_T$; $r = 0.9943$.

FIG. 2. Concentration of extractable lipid and fresh wt of epididymal adipose tissue.

FIG. 3. Concentrations of lipid, residue and water in rat epididymal adipose tissue.
$W_L/W_T = 0.871 - 0.1193\ (1/W_T)$
$W_R/W_T = 0.02363 + 0.01834\ (1/W_T)$

range. Lipid concentration, however, is nearly constant in tissue weighing more than 1.0 g. Rather large changes in W_T may occur with little effect on the value of W_L/W_T in the area of the plateau in Fig. 2. The opposite is true in tissue below 1.0 g, where differences of lesser magnitude in W_T reflect large differences in W_L/W_T.

If one were investigating nutritional, drug or hormone effects on epididymal adipose tissue, tissue weight or lipid content would reflect the treatment effect best above 1.0 g; below this value, percentage lipid would be a more sensitive measure.

Transformation of the data of this study to the form shown in Fig. 3 concisely illustrates the inter-relationships between lipid, water and residue concentration in the epididymal fat. However, it must be reemphasized that presentation in this form compresses the area of Fig. 2 where W_L/W_T is quite constant for a wide range of W_T values and tends to expand the changes in W_L/W_T for small differences in W_T values.

Residue and water appear to be present in epididymal fat tissue in constant amounts. Thus the concentrations of these constituents decrease with increasing adipose tissue fresh weight. Similar characteristics have been observed for perirenal adipose tissue of the rat (7).

Summary. A high positive correlation ($r = 0.9943$) was observed between fresh weight and lipid content of epididymal adipose tissue weighing more than about 0.5-1.0 g. Above this value lipid concentration in the tissue was 80-85% and was relatively constant over a wide range of weights. These relations suggest that for tissues weighing more than 0.5-1.0 g the weight or lipid content of the tissue is the most sensitive measure of treatment effects; lipid concentration is more sensitive below these values.

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1. Smith, T. C., Will, L., Oleson, J., Benitz, K.-F., Perrine, J., Ringler, I., *Am. J. Physiol.*, 1961, v200, 1277.

2. Tracht, M. E., Goldstein, M. S., Ramey, E. R., *ibid.*, 1956, v187, 2.

3. Levy, A., Ramey, E. R., PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 637.

4. ———, *Endocrinology*, 1959, v64, 586.

5. White, J. E., Engel, F. L., PROC. SOC. EXP. BIOL. AND MED., 1959, v102, 272.

6. Folch, J., Lees, M., Sloane-Stanley, G. H., *J. Biol. Chem.*, 1957, v226, 497.

7. Pandazi, A. A., Herrington, J. K., Schlueter, D. P., PROC. SOC. EXP. BIOL. AND MED., 1960, v103, 394.

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Effect of Exposure and Acclimatization to Cold on Susceptibility of Rats To Bacterial Endocarditis. (27610)

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The influence of exposure to cold on susceptibility to disease has been of concern for many years(1). Interest in this field has been renewed with the increasing use of hypothermia, particularly in surgery, and with increasing concern with the colder regions of the earth, outer space, and factors involved in acclimatization to cold. Previous studies have yielded conflicting results, which varied with the disease and the experimental conditions, and point to the need for further clarification.

Angrist, Oka, Nakao, and Marquiss(2) reported that rats given repeated 4-hour exposures to 4°C developed changes in the cardiac valves similar to those found in rats exposed repeatedly to simulated high altitude (3). Since we had found that bacterial endocarditis can be readily induced in rats exposed to high altitude(4,5), the present study was undertaken to determine if rats exposed