ENDERS, R. K. 1952. Reproduction in the mink (*Mustela vison*). Proc. Amer. Philos. Soc., 96: 691–755.

FRILEY, C. E., JR. 1949. Age determination, by use of the baculum, in the river otter, *Lutra c. canadensis* Schreber. J. Mamm., 30: 102–110.

HAMILTON, W. J., JR. AND A. H. COOK. 1955. The biology and management of the fisher in New York. N. Y. Fish and Game J., 2: 13–35.

HAMLETT, G. W. D. 1935. Delayed implantation and discontinuous development in mammals. Quart. Rev. Biol., 10: 432–447.

HOOPER, E. T. AND B. T. OSTENSON. 1949. Age groups in Michigan otter. Occ. Papers Mus. Zool. Univ. Michigan, No. 518: 1–22.

LIERS, E. E. 1951. Notes on the river otter (*Lutra canadensis*). J. Mamm., 32: 1–9.

———. 1958. Early breeding in the river otter. J. Mamm., 39: 438–439.

STEPHENS, M. N. 1957. The natural history of the otter. Univ. Federation for Animal Welfare, London, England. 88 pp.

WILSON, K. A. 1959. The otter in North Carolina. Proc. 13th Ann. Conf. S. E. Assoc. Game and Fish Comm., 267–277 (mimeo).

WRIGHT, P. L. 1942. Delayed implantation in the long-tailed weasel (*Mustela frenata*), the short-tailed weasel (*Mustela cicognani*), and the marten (*Martes americana*). Anat. Rec., 83: 341–353.

——— AND R. RAUSCH. 1955. Reproduction in the wolverine, *Gulo gulo*. J. Mamm., 36: 346–355.

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DENSITY STUDIES OF MULE DEER BODY FAT

BY FLOYD W. WHICKER

ABSTRACT: This paper reports on density measurements at temperatures ranging from 10–35°C of internal, subcutaneous and bone-marrow fat sampled from north-central Colorado mule deer. Density measurements were made on the ether extracts using the method of pycnometry. Regression equations for fat density as a function of temperature were estimated as $1/D = (1.4813 \times 10^{-3})T + 1.0385$ for internal fat, $1/D = (1.4075 \times 10^{-3})T + 1.0461$ for subcutaneous fat and $1/D = (1.4878 \times 10^{-3})T + 1.0371$ for bone-marrow fat. Application of the density measurements to the quantitative estimation of body fat for mule deer is discussed.

Numerous investigators have employed the technique of measuring the specific gravity of the whole animal body to estimate total body fat. Rathbun and Pace (1945) presented equations to relate the specific gravity of the whole body to the percentage of body fat for humans and guinea pigs. Morales *et al.* (1945) have shown that

$$\% \text{ Fat} = 100\left[\frac{D_f}{D_{ff} - D_f}\right]\left[\frac{D_{ff}}{\text{sp gr}} - 1\right], \qquad (1)$$

where D_f and D_{ff} are the densities of the body fat and the fat-free body, respectively, and sp gr is the experimentally determined specific gravity of the

whole body. The specific gravity of the whole body is usually estimated by weighing the subject while completely immersed in water. From this weight and the ordinary weight as measured in air, the specific gravity is calculated by use of the Archimedes' principle. Values for the density of body fat may be obtained by physical measurements on ether extracts. Estimates of the density of the fat-free body are more difficult to obtain, especially on large animals, but values for several species including man, guinea pig and cattle have been reported in the literature (Behnke, 1961: 129).

Attempts are being made by the Colorado Department of Game, Fish, and Parks to use per cent body fat as a criterion for physiological condition in mule deer (*Odocoileus hemionus*). Application of the specific gravity technique for estimation of body fat required an investigation of fat density since, as far as the author is aware, data on body fat density are unavailable for the mule deer.

The purpose of this paper is to report density measurements at temperatures ranging from 10–35°C on internal, subcutaneous and bone-marrow fat sampled from north-central Colorado mule deer.

MATERIALS AND METHODS

The procedures used for the determination of fat density were modified from those described by Fidanza *et al.* (1953). Samples were obtained from animals which had been sacrificed the same day. Internal fat consisted primarily of that which was incorporated with the mesentery and did not include renal or cardiac fat. Subcutaneous fat samples were prepared by pooling material from the rump area, along the back and along the upper and lower areas of the rib cage. Bone marrow was obtained from the entire length of the large metacarpal.

Approximately 10 g of sample were finely diced under cold acetone and dried for 2 hours under vacuum at 60°C. The samples were cooled to room temperature and transferred to paper extraction thimbles, then extracted continuously on a Soxhlet apparatus for about 8 hours, using anhydrous ethyl ether. Extraction was carried out rather rapidly with the chamber siphoning over about every 10 min. Most of the ether was driven off the extract using the heating element of the Soxhlet apparatus. After drying for 30 min in a 50°C vacuum oven, the extract was suitable for density measurements.

A pycnometer fitted with a thermometer graduated in 0.2°C and having a volume capacity of about 5 ml was used. The pycnometer was standardized with kerosene over the temperature range 10–35°C. The relative density of the kerosene was determined by comparing its weight to that of distilled water in a 50-ml flask at given temperatures. Relative density values for water were taken from Lange's *Handbook of Chemistry* (1956: 1188). For each determination, the tared, calibrated pycnometer was filled ¾ full with melted fat (deer fat

melts at about 45–50°C), allowed to cool to room temperature and weighed. The pycnometer was then filled nearly to the mark with kerosene using a hypodermic syringe fitted with a thin, flexible piece of polyethylene tubing. The tubing was inserted through the neck of the pycnometer which was held at such an angle that air bubbles did not remain in the vessel. The pycnometer was then immersed in a constant-temperature water bath which had been previously adjusted to a desired temperature. It was assumed that thermal equilibrium had been reached when the thermometers of the pycnometer and water bath had been reading the same (± 0.2°C) for 5 min. The pycnometer was brought to the volumetric mark with kerosene of the same temperature, removed from the water bath, dried, allowed to achieve room temperature and reweighed. Fat density was calculated from the weight of the fat, the weight of the added kerosene, the weight of kerosene required to fill the pycnometer at the experimental temperature and the density of the kerosene at that temperature.

The density of each sample was determined at approximately five different temperatures over the range of 10–35°C. Because the fat was slightly soluble in kerosene, the pycnometer was adjusted and weighed at the higher temperatures first to allow addition rather than removal of kerosene upon cooling.

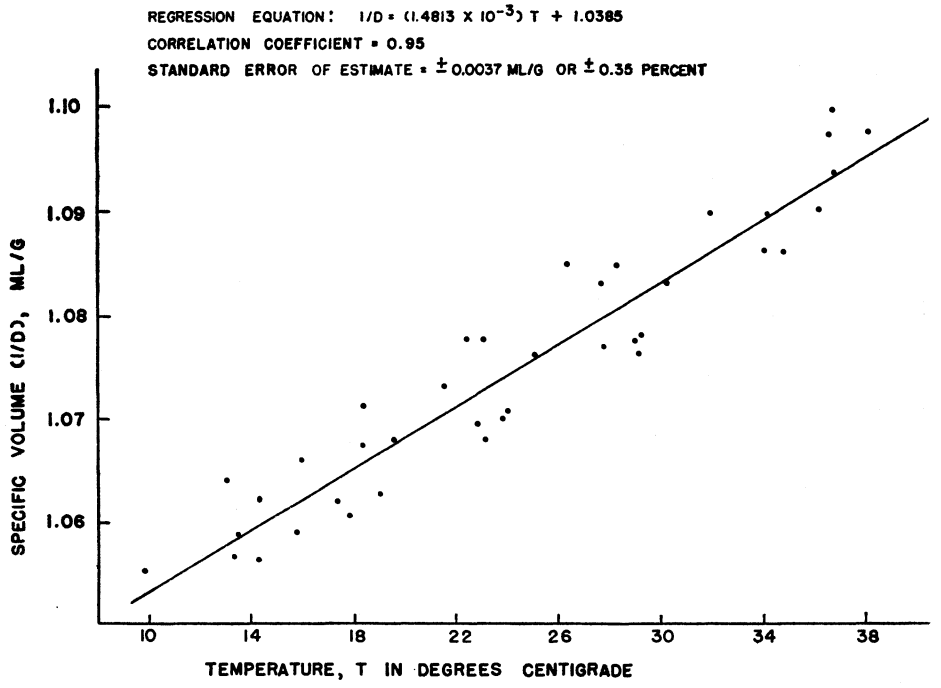

FIG. 1.—Internal fat, specific volume as a function of temperature.

RESULTS

The data obtained from the density measurements and supplementary information are summarized in Table 1.

Internal fat.—The results of density measurements on six individual samples (40 observations) at various temperatures are plotted in Fig. 1. A regression line fitted by the least squares method is drawn through the data. The regression equation is $1/D = (1.4813 \times 10^{-3})T + 1.0385$, where $1/D$ is the reciprocal of the density and T is the temperature measured in C. The correlation coefficient is 0.95 and the standard error of estimate is ± 0.0037 ml/g or ± 0.35% over the temperature range indicated.

Subcutaneous fat.—Density measurements on six individual samples (29 observations) are plotted as a function of temperature in Fig. 2. The line of least squares has the regression equation $1/D = (1.4075 \times 10^{-3})T + 1.0461$. The correlation coefficient was calculated as 0.89 and the standard error of estimate as ± 0.0043 ml/g or ± 0.40% over the indicated range of temperatures.

Bone-marrow fat.—Density measurements on two individual samples (9 observations) are plotted in Fig. 3. The line of least squares has the regression equation $1/D = (1.4878 \times 10^{-3})T + 1.0371$. The standard error of estimate was calculated as ± 0.0016 or ± 0.15% and the correlation coefficient as 0.99 over the indicated temperature range.

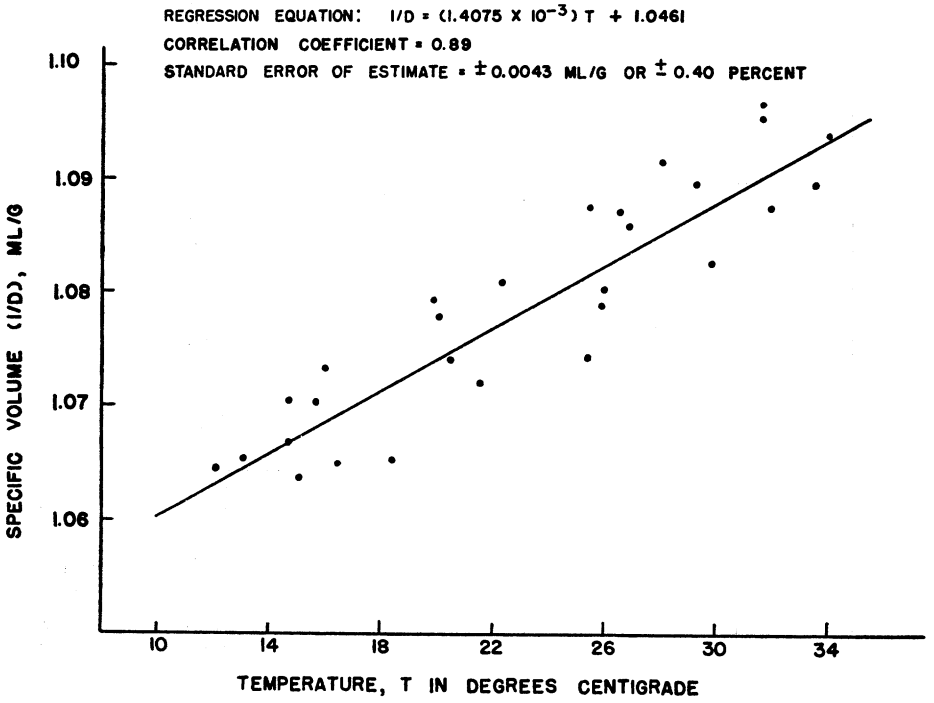

FIG. 2.—Subcutaneous fat, specific volume as a function of temperature.

Note that sampling of internal and subcutaneous fats was done at various seasons from both sexes and various age-classes. It is perhaps possible that slight variations in fat density may be caused by differences in season, condition, age or other variables. No relationships such as these are apparent from the limited data in this paper, however. In the case of the bone-marrow fat, the two samples were collected just a week apart and both animals were females of a younger age-class. The standard error of estimate for the bone-marrow fat determination is considerably lower than for internal and subcutaneous fats. Again, however, no conclusions of cause and effect can be inferred because of the small sample size.

TABLE 1.—*Density data on mule deer body fat and supplementary information*

Type fat	Deer no.	Sex	Est. age, months	Date of collection	Temperature, C	Density, g/ml
Internal	16	M	50	2 Aug. 1961	38.1	0.9119
					34.2	0.9179
					30.2	0.9230
					25.0	0.9295
					19.5	0.9365
					14.2	0.9414
	23	F	4	5 Oct. 1961	36.7	0.9095
					27.7	0.9235
					21.5	0.9320
					18.3	0.9369
					13.4	0.9446
	25	M	16	18 Oct. 1961	36.6	0.9117
					28.2	0.9220
					23.0	0.9281
					18.3	0.9334
					12.9	0.9397
	30	F	114	20 Nov. 1961	36.2	0.9180
					29.2	0.9284
					23.8	0.9348
					15.7	0.9447
					12.4	0.9504
	34	F	114	23 Jan. 1962	34.0	0.9213
					27.8	0.9292
					22.8	0.9354
					17.3	0.9419
					13.2	0.9466
	47	M	34	26 Apr. 1962	34.8	0.9214
					29.0	0.9284
					24.0	0.9344
					17.7	0.9426

TABLE 1.—*Continued*

Type fat	Deer no.	Sex	Est. age, months	Date of collection	Temperature, C	Density, g/ml
Sub-cutaneous	16	M	50	2 Aug. 1961	31.6	0.9117
					25.4	0.9194
					15.9	0.9316
					12.0	0.9394
	21	F	27	12 Sept. 1961	31.6	0.9125
					26.5	0.9199
					20.0	0.9279
					13.0	0.9387
	25	M	16	18 Oct. 1961	31.9	0.9195
					25.9	0.9259
					20.4	0.9310
					14.7	0.9374
	41	F	57	20 Mar. 1962	36.0	0.9015
					28.0	0.9162
					19.8	0.9264
					14.6	0.9341
	42	F	33	22 Mar. 1962	36.8	0.9122
					33.5	0.9178
					25.8	0.9270
					21.4	0.9329
					16.4	0.9393
	47	F	34	26 Aug. 1962	37.0	0.9147
					29.8	0.9238
					25.3	0.9312
					18.3	0.9390
Bone marrow	35	F	31	30 Jan. 1962	34.2	0.9185
					28.3	0.9273
					22.3	0.9352
					18.0	0.9403
					13.6	0.9456
	36	F	19	6 Feb. 1962	30.0	0.9247
					25.0	0.9305
					20.0	0.9362
					16.0	0.9418

DISCUSSION

In applying the above data to the densitometric estimation of total body fat for mule deer, it is necessary to know the approximate proportions of the various types of fatty material present so that a representative value for the density of fat can be assigned. If, however, the body density is determined on the skinned, evicerated carcass with all the internal or body cavity fat removed, only the values for bone-marrow and subcutaneous fat need be considered. In fact, it should be sufficient to use only the value for subcutaneous

fat since it seems to be present in considerably greater proportion than bone-marrow fat. In any case, an error of not more than about 0.7% would be introduced in the value of the representative density of the body fat by using only those values determined for subcutaneous fat.

Numerous investigators, including Rathbun and Pace (1945), Keys and Brozek (1953) and Kraybill *et al.* (1952) have given values for the density of the fat-free body in several animals, including man, guinea pig and cattle. The values presented are all very near 1.1 g/ml. This apparent uniformity seems remarkable, considering the difficulties involved in measuring the density of the fat-free body. Behnke (1961: 120) suggests that the value 1.1 may be a "biological constant" which varies within narrow limits. Using this figure and the density of subcutaneous fat (as determined at 20°C for example) in equation (1), the following working equation was obtained:

$$\% \text{ Fat} = 100\left[\frac{6.0598}{\text{sp gr}} - 5.5089\right]. \qquad (2)$$

Assuming values in the range of 1.03 to 1.08 for the estimated specific gravity of the whole body, an error of 0.7% in the value of the fat density would not

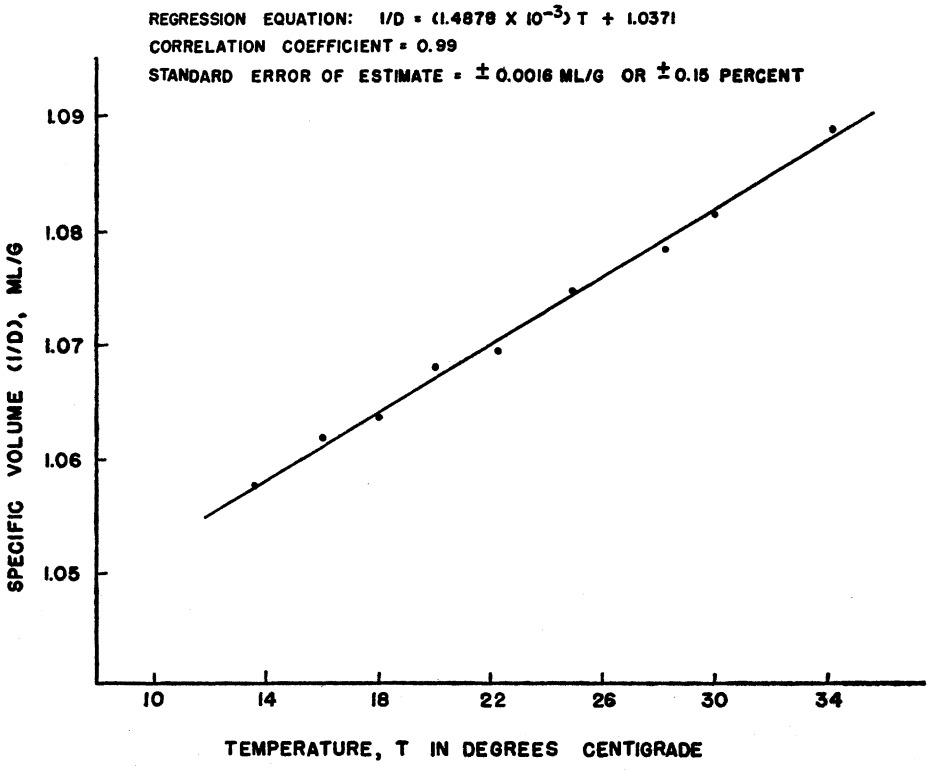

FIG. 3.—Bone-marrow fat, specific volume as a function of temperature.

ordinarily make a difference of more than about 2% in the calculated percentage of body fat.

The importance of having the entire deer carcass at thermal equilibrium at the time of the densitometric measurement may be assessed from the fact that a difference of 5°C in temperature could cause an error of about 3% in the estimation of total body fat.

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LITERATURE CITED

Behnke, A. R. 1961. Comment on the determination of whole body density and a resume of body composition data. *In* Techniques for measuring body composition. Proceedings of a Conference—Quartermaster Research and Engineering Center, Natick, Mass., 22–23 Jan. 1959. National Academy of Sciences–National Research Council, Washington, D. C., pp. 118–133.

Fidanza, F., A. Keys and J. T. Anderson. 1953. Density of body fat in man and other mammals. J. Appl. Physiol., 6: 252–256.

Keys, A. and J. Brozek. 1953. Body fat in adult man. Physiol. Rev., 33: 245–325.

Kraybill, H. F., H. L. Bitter and O. G. Hankins. 1952. Body composition of cattle, II. Determination of fat and water content from measurement of body specific gravity. J. Appl. Physiol., 4: 575–583.

Lange, N. A. 1956. Handbook of chemistry. Ninth ed. Handbook Publishers, Inc., Sandusky, Ohio. 1969 pp.

Morales, M. F., E. N. Rathbun, R. E. Smith and N. Pace. 1945. Studies on body composition. J. Biol. Chem., 158: 677–684.

Rathbun, E. N. and N. Pace. 1945. Studies on body composition. J. Biol. Chem., 158: 667–676.

College of Veterinary Medicine, Colorado State University, Fort Collins, Colorado. *Received 13 March 1963.*