TECHNIQUES FOR DETERMINING SPECIFIC GRAVITY OF LIVE ANIMALS

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The use of specific gravity in determining body composition is based on the promise that the major body components - namely lean, bone, water and fat - differ in density. Vierordt (1906) reported the panniculus adiposus has a specific gravity of 0.971 compared to 1.0414 (range 1.0382 to 1.0555) for striated muscles and to 1.717 for the bony cranium. Various investigators (Ashworth and Cowgill, 1938; Pace and Rathbun, 1945; McNally, 1955) have shown body composition to be relatively constant when reduced to the fat-free basis for rats, guinea pigs and chickens. Pace and Rathbun (1945), working with guinea pigs, reported water and nitrogen contents were relatively constant fraction of live weight when expressed on the fat-free basis with average values of 72.4 and 3.52 per cent, respectively. From such evidence the so-called "lean body mass" concept was developed, which in essence states that the gross chemical composition of the body is relatively constant with fat acting as a diluent agent.

There are some indications (Page and Babineau, 1953; Light et al, 1934; Annegers, 1954) that abnormal conditions such as withholding water or subjecting animals to cold will upset the normal balance of body water and fat.

Water Displacement Method

Early work on specific gravity dates back to Robertson in 1757 reviewed by Boyd (1933). Attempts were made to determine specific gravity of human subjects by submerging the body in water. This method is based on the Archimedean principle that the body immersed in water loses weight by an amount equal to the water displaced by it. The early attempts of Robertson were unsuccessful due to failure to correct the weight of the submerged body for air in the lungs and air passages. Vierordt (1906) quoting the work of Krause for values obtained with a so-called "normal adult man" reported a specific gravity of 1.0551 after a moderate expiration, but when corrections were made for air in the lungs and for gas in the intestines, the value rose to 1.1291.

Successful application of the water displacement method of determining specific gravity as a measure of fatness for human subjects is credited to Behnke (1941-42). He estimated the specific gravity of the human body, including fluids and soft tissues, bone and essential lipids, but excluding "excess fat" at 1.099. The addition of excess adipose tissue in the amounts of 10, 20 or 33.3 per cent of the total body weight was calculated to give specific gravity values of 1.080, 1.062, and 1.036 respectively.

Behnke et al. (1942) described the method they used in determining the specific gravity of human subjects by underwater weighing. Weights were

taken with a spring balance scale to the nearest ounce by means of a line from the subject to the balance. A weighted lead belt was used to maintain negative buoyancy for all types of persons. In order to make necessary calculations and corrections for air in the lungs and respiratory passages, it was necessary to weigh once at the end of maximum inspiration and again at the end of maximum expiration. The difference in the two weights records hydrostatic displacement, which serves as a measure of vital capacity. By correction for the effect of mean hydrostatic pressure on the thoracic volume, values comparable to those obtained by standard spirometry methods are possible. The following problem taken from their data will tend to illustrate the principle:

Wt. in air	183 lbs.
Wt. in H_2O - full inspiration	14.2 lbs.
Wt. in H_2O - full expiration	23.2 lbs.
Vital capacity - computed from volume H_2O displaced	4090 cc.
Volume residual air - computed	1200 cc.
Wt. of belt	13.75 lbs.

<u>Corrections applied</u>

Gross wt. in H_2O	23.2 lbs.
Wt. of belt (in H_2O)	13.75 lbs.
Gross Wt. minus wt. of belt	9.45 lbs.
Corrected for residual air (1200/453)	2.65 lbs.
Corrected Wt. in H_2O	12.10

Wt. in Air - Wt. in H_2O = Body Volume

$$\text{Specific Gravity} = \frac{\text{Wt. in Air}}{\text{Volume}} = \frac{183}{170.9} = 1.071$$

Behnke et al. (1942) and Welham and Behnke (1942) presented data showing the relationship between certain physical measurements and specific gravity for the human as measured by water displacement. Rathbun and Pace (1945) have evidence using eviscerated guinea pigs that the water displacement method is valid. DaCosta and Clayton (1950) using albino rats on different nutritional levels have verified the relationship between specific gravity and fat content. Correlations calculated from DaCosta and Clayton's data by Brozek and Keys (1950-51) and reported in their excellent review are $r = -0.73$ for specific gravity and fat content of the carcass, and $r = -0.87$ for specific gravity and water content of the carcass.

Unfortunately, the water displacement method has not been readily adaptable to animals other than man. Not only is the method cumbersome and

slow, but breathing cannot be readily controlled in laboratory or farm animals. However, the principles involved are sound.

Helium Dilution Method

This method makes use of the dilution technique, in which a measured volume of helium is injected into a chamber of known volume containing the experimental animal. After allowing time for the gases in the chamber to reach equilibrium, a sample is removed and analyzed for helium by a Cambridge Analyser, which utilizes differences in thermal conductivity of helium and dry air to give the amount of helium. This method has been described by Walser and Stein (1953) and they have reported good agreement between this method and specific gravity as determined by water displacement of the eviscerated carcass.

Siri (1953) simultaneously has reported successful results using the helium dilution technique. The exact details of this method have not been published.

This method would appear to be most promising for studying body composition of the intact animal. Not only is the method adapted to humans, but in principle could be applied to either laboratory or farm animals.

Air Displacement Method

Wedgewood and Newman (1953) have proposed an air displacement method for determining the specific gravity of the intact animal. This method is based on the measurement of the volume of gas displaced by the animal body. The method reported by Wedgewood and Newman (1953) has not been described in detail, but as shown in a picture by courtesy of the authors it apparently makes use of bellows.

At the Michigan Station, working in cooperation with Dr. E. P. Reineke of our Physiology Department, we have been working independently on a similar principle. Our device consists of two chambers of known volume connected together by means of rubber tubing with a vacuum pump and menometer linked into the system. The animal is introduced into one chamber and a vacuum is drawn on the other chamber, which is closed off by means of a two-way stop-cock. The amount of the vacuum is recorded and the two chambers are connected. Then by measuring the change in pressure, it is possible to calculate the volume occupied by the animal. We believe this method is sound in principle, but the effect of a number of variables, such as temperature of the chamber, vapor pressure and changes in the gas content of the animal itself must be established before it can be applied with precision.

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MR. AUNAN: Thank you, Dr. Pearson, for that fine presentation on technics for determining specific gravity of live animals.

At this time we will hear from Professor C. P. Wilder on "Probing as a Means of Determining Fatness."

MR. WILDER: Some time ago when I was assigned this topic I figured it would be a cinch just to get up here and review some of the probing work. Then I was told that it included the lean-meter which I had seen demonstrated but had never used. So I think that I have learned a lot in the last week, since I have been preparing this talk both from the standpoint of review of the literature and from learning to use the lean-meter.

I tried the lean-meter on my fingers this morning and it didn't register. Right away I figured that I was dead. But Ed Kline came over and assured me I was alive. So I guess everything is all right.