Predicting body cell mass with bioimpedance by using theoretical methods: a technological review

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De Lorenzo, A., A. Andreoli, J. Matthie, and P. Wathers. Predicting body cell mass with bioimpedance by using theoretical methods: a technological review. J. Appl. Physiol. 82(5): 1542–1558, 1997.—The body cell mass (BCM), defined as intracellular water (ICW), was estimated in 73 healthy men and women by total body potassium (TBK) and by bioimpedance spectroscopy (BIS). In 14 other subjects, extracellular water (ECW) and total body water (TBW) were measured by bromide dilution and deuterium oxide dilution, respectively. For all subjects, impedance spectral data were fit to the Cole model, and ECW and ICW volumes were predicted by using model electrical resistance terms R_e and R_i in an equation derived from Hanai mixture theory, respectively. The BIS ECW prediction bromide dilution was r = 0.91, standard error of the estimate (SEE) 0.90 liter. The BIS TBW prediction of deuterium space was r = 0.95, SEE 1.33 liters. The BIS ICW prediction of the dilution-determined ICW was r = 0.87, SEE 1.69 liters. The BIS ICW prediction of the TBK-determined ICW for the 73 subjects was r = 0.85, SEE = 2.22 liters. These results add further support to the validity of the Hanai theory, the equation used, and the conclusion that ECW and ICW volume can be predicted by an approach based solely on fundamental principles.

bioimpedance spectroscopy; extracellular water; total body water

THE FIRST SUCCESSFUL VALIDATION of extracellular water (ECW), TBW (total body water), and intracellular water (ICW) by using bioimpedance spectroscopy (BIS) methods was reported in 1992 (30). BIS, which implies fitting complex impedance (Z) data measured at multiple frequencies to a biophysical model, has been used extensively in biophysics and is the technique from which derive all the underlying theories for body impedance analysis (BIA) (48). Although a number of studies have been reported that used the methods employed in this study, the BIS principles and the rationale for the methods employed remain poorly understood. Partly because of the complex nature of BIS, there has been very little cross-transfer of BIS information to the field of human body composition; furthermore, these methods have not been fully reported by the investigators responsible for their development (30). As a result, these methods have been only partially reported with insufficient underlying discussion.

Several single-frequency Z studies have reported a prediction of body cell mass (BCM; Refs. 3, 42), but the scientific bases of the approaches used were not well supported, and the relationships reported may have simply been a result of high intercorrelation between variables (48). Results should be associated with ICW or total body potassium (TBK) rather than BCM, because BCM is a concept that can only be defined by ICW or TBK (33). Any relationship between Z and BCM can only emerge from the relationship between Z and cell volume. What becomes important then is the best approach of predicting cell volume with Z.

This study reports the relationship between a BIS-predicted ICW and a TBK-predicted ICW, and the relationship between a BIS-predicted volume of ECW, ICW, and TBW compared with dilution-determined volumes. This manuscript also provides a description of the principles of BIS and a discussion of the simplified single- and dual-frequency methods relative to well-known BIS principles.

Physical Principles

Variations in heterogeneous tissues cause interfaces, separating regions of different properties, to trap or release electrical charge as a stimulus potential is changed. The time lag between the stimulus potential and the change in charge in these interfaces creates a frequency (f)-dependent Z (i.e., dispersion). The dispersion found in the low-frequency (LF) radio range (1 kHz to 100 MHz), which is of interest for predicting ECW and ICW volume, is known as β-dispersion and is caused by cell membrane capacitance (C_m) (40) (Fig. 1).

With direct current (DC), there is no conduction through a capacitor. Thus, in the LF range of β-dispersion, there is minimal conduction through the cells because of the high Z of the C_m and conductivity is governed primarily by the properties of the ECW. As f increases into alternating current (AC), the Z of the C_m decreases, allowing more current to flow into the ICW compartment. Because of the change in polarity that occurs with AC current, the cell membrane charges and discharges the current at the rate of the f. The Z decreases with f, because the amount of conducting volume is increasing. At higher frequencies (HF), the rate of charge and discharge becomes such that the effect of the C_m diminishes to insignificant proportions, and the current flows through both the ECW and ICW compartments in proportions dependent on their relative conductivity and volumes (5) (Fig. 2). Thus, at both very low and very high frequencies, the overall Z is essentially independent of the C_m whereas at the mid- or characteristic frequency (f_c), the dependence of the value of the C_m is at a maximum.

If the complex Z [resistance (R) and reactance (X)] of skeletal muscle tissue is measured, and the f varies from low to high, a series of values is derived that can be represented by complex points. The curve formed by these points is called an impedance locus, and its shape is a result of the electrical and structural characteristics of the tissue (5). The mathematical model that is
used most often to describe both theoretical and experimental data on skeletal muscle tissue is known as the Cole model. It produces a semicircular relationship between $R$ and $X$, with a depressed center when plotted (5) (Fig. 3). Modeling is considered essential, because it is the only means of independently analyzing the individual components of a heterogeneous material system (5, 25). The Cole model can be viewed as the equivalent electrical circuit shown in Fig. 4.

To accurately predict volume, the mixture effects need to be accounted for, because the relationship between $R$ and body water volume is nonlinear (9, 17). These mixture effects are greater at LF because the conductor (i.e., ECW) represents only 25% of the total body volume compared with a concentration of nonconductor of 75%. At HF, the concentration of nonconductor is much less (e.g., 40%). Good examples of the mixture effects are the change in resistivity ($\rho$) that occurs with a change in hematocrit (14) and that plasma (i.e., ECW) is four- to sixfold more conductive than is skeletal muscle tissue measured at 1 kHz (14).

At 1 kHz, there is little conduction through the cells; thus the $\rho$ should be similar to that of plasma. It is dramatically increased because the cells are nonconductive at LF and restrict the flow of current. Hanai (17) developed a theoretical equation that describes the effect on the apparent conductivity of a conducting material having a restricting concentration of nonconductive material in suspension. Hanai postulated that the theory could be applied to tissues with nonconductive concentrations ranging from 10 to 90%. To employ his theory, we have constructed an equation that considers the ECW to be such a medium at LF, where the ECW is the conductive material, and all remaining items (including ICW because it is surrounded by cell membrane) in the body are the restricting nonconductive material. At HF, the combination of both ECW and ICW forms the conductive medium, and all remaining items in the body form the restrictive material. Previous results have supported that this theory can be used in vivo to predict ECW, TBW, and ICW volume (35, 47).

**SUBJECTS AND METHODS**

A group of 87 healthy Italian men ($n = 77$) and women ($n = 10$), ages 21–57 yr, volunteered to participate in this study. Written informed consent was obtained from all participants.
The study protocol was approved by the Medical Ethical Committee of the University of "Tor Vergata" in Rome. On arriving in the morning in an overnight-fasted state, subjects were weighed in swimming clothes, and body weight (Wt) was measured with a standard balance to the nearest 0.05 kg. Body height (Ht) was measured with a stadiometer to the nearest 1 mm. After the measurements of Wt and Ht were made, and still in the fasting state, all 87 subjects had their \( ^{40} \text{K} \) measured by a whole body counter, formed by a cell 2.5 m wide and 3 m high of 10-cm-thick lead bricks, the door to which was formed by a 22-cm-thick iron slab. The room was continuously ventilated. A single 20.3 \( \times \) 10.2 thallium-activated sodium iodine crystal was positioned above the subject, who was measured in a sitting position and dressed in only paper pajamas. TBK was calculated as \( ^{40} \text{K} = 8.474.6 \) (11). The correlation of variation (CV) for TBK was found to be between 2 and 3%. ICW was computed from TBK, assuming that potassium is only present in the intracellular fluid and assuming a potassium concentration in the intracellular fluid of 150 mmol/l (11).

For 14 of the men, out of the total sample of 87 men and women, TBW and ECW were measured by dilution methods. Deuterium oxide (D\(_2\)O) was used for the determination of TBW, and sodium bromide (NaBr) was used for the determination of ECW. While still in a fasted state and after the \( Z \) measurements were taken, the subjects drank 50 g of a solution containing 10 g of D\(_2\)O (99.8%; Carlo Erba) and 1.3 g of NaBr (Carlo Erba) in 38.7 g of tap water. After a 3-h equilibration time, urine was collected for the determination of NaBr (Carlo Erba) in 38.7 g of tap water. After a 3-h equilibration period and refrained from voiding. No correction was made for orthostatic fluid shifts. The measurements were taken on the left side of the body, with the use of disposable electrocardiogram electrodes (5 cm\(^2\); 3M, Minneapolis, MN) and in accordance with the standard wrist-to-ankle protocol (47). Data were transmitted directly from the analyzer into an ASCII format file via an RS232 interface to a personal computer and controlled by the software program supplied with the device.

The Z and \( \iota \) spectra data were fit to an enhanced Cole model (5) to account for any time delay (\( T_\text{d} \)) effects (see APPENDIX A), using the nonlinear curve-fitting software developed for the device. The ECW and ICW volumes were predicted by using modeled \( R_E \) and \( R_I \) values in equations formulated previously (47) from Hanai mixture theory (17) (APPENDIX B). The BIS TBW was calculated as ECW + ICW. The constants used for \( R_{ECW} \) (i.e., men = 0.306, women = 0.316) and \( k_\text{ICW} \) (men = 3.82, women = 3.40) had been scaled to D\(_2\)O and NaBr data collected in a previous study (47). Although only the constants for men were used in this study, the constants for women were included for discussion purposes because a previous study discovered a gender difference (47). When reference ECW and TBW data are available for only one gender, the software automatically and arbitrarily scales the other gender’s constant by the same percentage difference discovered previously. Further research needs to be done to determine whether there truly is a gender difference in these terms. Expressing the above \( k_{ECW} \) and \( k_\text{ICW} \) terms as apparent ECW and ICW resistivity (\( \rho_{ECW} \) and \( \rho_{ICW} \), respectively), they become 214 and 206, and 824 and 797 for \( \rho_{ECW} \) and \( \rho_{ICW} \) in men and women, respectively. These terms will be expressed as apparent \( \rho \) in the remainder of the document. The male constants from a previous study (47) listed above were used to predict the dilution ECW, TBW, and ICW of the 14 men. The TBK-determined ICW of these 14 men was also predicted by both BIS- and dilution-ICW volumes. Then, new constants for men were computed from the dilution volumes measured on the sample of 14 men, as described in APPENDIX B. The new \( \rho_{ICW} \) constant was then used to predict the BIS-ICW volume for the other 73 men and women and cross-validated against their TBK-determined ICW.

The Excel program was used for the statistical analysis. In addition to the descriptive statistics, the Pearson's product moment correlation (r) and standard error of estimate (SEE) statistics were computed. Bland-Altman plots were also constructed to display the individual subject differences between the BIS-predicted water volumes and those determined by TBK and dilution methods.

RESULTS

Table 1 displays the physical characteristics of the two subject groups. Table 2 provides the Cole modeling results for the two subject groups. Of the 87 subjects, according to the criteria rating the fit to the Cole model (APPENDIX A), 21 subjects were rated as 0, 64 were rated as 1, and the remaining 2 were rated as 2. The mean...
41.02 liters and a mean difference of 0.87, SEE remained identical for ECW (i.e., 0.90 liter) with no mean difference. For TBW, the constants for women became 167.8 and 1,139.34 for TBK, respectively. For discussion purposes, the computed dilution sample of 14 men were 174.32 and 1,177.94, respectively. For TBK, ICW is effectively a scalar, since changing it only slightly alters the prediction of ICW because the nonlinearity is slight. Figures 5-7 display the plotted differences between the BIS-predicted ECW, ICW, and TBW volumes and the dilution-predicted volumes. Figure 8 displays the plotted differences between the BIS and the TBK-predicted ICW. The ECW prediction was achieved by using the exponent 1.5 predicted by Hanai theory.

**DISCUSSION**

Effects of mixture, scaling, and reference methods. We constructed an equation from Hanai theory (17) because we wanted to account for as many error sources as possible, and we believed the relationship between R and volume should be explained scientifically rather than randomly through multiple-regression analysis. ρ is dependent on the concentration of nonconductor present in a mixture, giving rise to an empirical exponent ranging from 1.43 for very small spheres to 1.53 for packed cylinders (7, 17). Hanai theory predicts an exponent, 1.5. The exponent 1.5 was recently confirmed in vitro in human blood (9). A linear equation computed from multiple-regression analysis is not well

| Table 1. Descriptive characteristics of TBK and dilution study groups |
|---------------------------|---------------------------|---------------------------|---------------------------|
|                          | TBK                        |                           |                           |
|                          | Men                        | Women                     | Combined                  | Dilution, Men |
| n                         | 63                        | 10                        | 73                        | 14            |
| Age, yr                   | 37.92 ± 9.60              | 25.33 ± 3.29              | 36.20 ± 9.99              | 28.57 ± 6.64  |
| Height, cm                | 174.23 ± 7.43             | 164.55 ± 7.88             | 172.91 ± 8.20             | 175.25 ± 6.50 |
| Weight, kg                | 74.99 ± 8.71              | 56.40 ± 4.86              | 72.44 ± 10.47             | 74.80 ± 8.83  |
| TBK-ICW, liters           | 25.08 ± 3.19              | 17.54 ± 2.60              | 24.05 ± 4.06              | 28.35 ± 3.19  |
| Potassium, g              | 147.13 ± 18.72            | 102.90 ± 15.23            | 141.07 ± 23.78            | 166.30 ± 18.71|
| ECW, liters               | 18.34 ± 10.46             | 202.79 ± 1,020.42         | 1,504.73 ± 742.81         | 71.70 ± 10.46  |
| TBW, liters               | 45.48 ± 6.36              | 4.37 ± 0.39               | 81.33 ± 7.27              | 52.37 ± 6.27  |
| ICW, liters               | 27.13 ± 3.87              | 2.04 ± 0.03               | 9.55 ± 0.37               | 7.34 ± 0.03   |

Values are means ± SD. TBK, total body potassium; n, no. of subjects; ICW, intracellular water; ECW, extracellular water; TBW, total body water.

The correlation of fit to the Cole model, using scalar Z, was 0.998. With the use of the constants for men from a previous study (47), the dilution ECW was predicted as r = 0.91, SEE = 0.90 liters, with a mean of 21.03 liters and a mean difference of 2.69 liters. Dilution TBW was predicted as r = 0.95, SEE = 1.33 liters, with a mean of 41.02 liters and a mean difference of −4.46 liters. Dilution ICW (as TBW − ECW) was predicted as r = 0.87, SEE = 1.69 liters, with a mean of 19.99 liters and a mean difference of −7.14 liters (Table 3).

The new pECW and pICW constants computed from the dilution sample of 14 men were 174.32 and 1,177.94, respectively. For discussion purposes, the computed constants for women became 167.8 and 1,139.34 for pECW and pICW, respectively. Using the new pICW constant for men, the prediction of the TBK ICW for the 73 subjects with BIS ICW was r = 0.85, SEE = 2.22 liters, with virtually no mean difference (i.e., 0.08 liter). When the TBK ICW was predicted by gender (men = 63, women = 10) the correlations and SEE were similar to that of the total group, but there was a slight mean difference (i.e., 0.15 and −0.38). For the 14 men, the correlation and SEE values for the BIS- and dilution-predicted ICW and the TBK-predicted ICW were r = 0.56 and 0.57, and SEE = 2.68 and 2.32 liters, respectively.

To determine the effect that scaling of pECW and pICW had on the correlation and SEE, the new constants for men were used to repredict the dilution ECW, TBW, and ICW on the 14 male subjects. The correlation and SEE remained identical for ECW (i.e., r = 0.91, SEE = 0.90 liter) with no mean difference. For TBW, the correlation decreased slightly (i.e., r = 0.95–0.94) and the SEE increased slightly (i.e., 1.33–1.41 liters) with no mean difference. For ICW, the correlation decreased slightly (i.e., r = 0.87–0.80) and the SEE decreased very slightly (i.e., 0.01 liter) with no mean difference.

Thus, pECW is purely a scalar and has no affect on correlation or SEE for ECW. Similarly, pICW is effectively a scalar, since changing it only slightly alters the prediction of ICW because the nonlinearity is slight. The correlation and SEE values for the BIS-predicted ECW, ICW, and TBW volumes and the dilution-predicted volumes. Figure 8 displays the plotted differences between the BIS-predicted ECW, ICW, and TBW volumes and the dilution-predicted volumes. Figure 8 displays the plotted differences between the BIS and the TBK-predicted ICW. The ECW prediction was achieved by using the exponent 1.5 predicted by Hanai theory.

| Table 2. Cole modeling characteristics of TBK and dilution study groups |
|---------------------------|---------------------------|---------------------------|---------------------------|
|                          | TBK                        |                           |                           |
|                          | Men                        | Women                     | Combined                  | Dilution, Men |
| n                         | 63                        | 10                        | 73                        | 14            |
| r of fit                  | 0.9978 ± 0.001             | 0.9974 ± 0.001             | 0.9978 ± 0.001             | 0.9970 ± 0.001 |
| Ref, Ω                    | 582.09 ± 47.86             | 742.81 ± 44.56             | 604.13 ± 72.81             | 577.71 ± 44.87 |
| Ref, Ω                    | 1,109.04 ± 138.72          | 1,504.73 ± 209.42          | 1,163.20 ± 202.79          | 1,020.42 ± 81.33 |
| Cm, nF                    | 2.32 ± 0.43                | 1.27 ± 0.26                | 2.18 ± 0.55                | 2.24 ± 0.30    |
| α                         | 0.70 ± 0.02                | 0.68 ± 0.03                | 0.70 ± 0.03                | 0.68 ± 0.01    |
| f0, kHz                   | 57.02 ± 8.39               | 80.14 ± 17.22              | 60.19 ± 12.83              | 61.96 ± 5.95   |
| Ta, ns                    | 20.66 ± 8.46               | 32.40 ± 9.43               | 22.26 ± 9.49               | −3.27 ± 4.37   |

Values are mean ± 1 SD. n, No. of subjects; r, correlation of fit; Ref and Ref, Cole model electrical resistance terms used to predict volume of ECW and ICW, respectively; Cm, membrane capacitance; α, exponent; f0, characteristic frequency; Ta, time delay.
suited to nonlinear effects. Thus it did not seem prudent to solve for a five-dimensional nonlinear biophysical model (i.e., Cole) and then use an overly simplistic volume theory (i.e., \( \text{Ht}^2/\text{R} \)) that assumes only one material is being measured. Predicting volume with an equation formed by scientific principles would enhance its utility and address the error sources directly rather than accounting for them statistically, which offers no scientific explanation.

Although the successful prediction of ECW and ICW volume with the equation used in this study has been reported (35, 47), it had not been reported that when we regressed an exponent against NaBr space (47), the highest correlation was achieved by using the exponent 1.5 predicted by Hanai theory. This finding strongly suggests the presence of mixture effects, and the strong predictions by using the exponent 1.5 (10, 35, 47) support the validity of Hanai's theory. As a spherical theory developed in the emulsion sciences, the reasons why Hanai's theory should not work are many, but they do not explain the strength of prediction this theory provides or the emergence of the exact theoretical exponent. In the absence of a more applicable theory, we use the developed equation, because we believe the errors of not accounting for mixture effects are greater than the inadequacy of the theory.

Because the strength of the BIS prediction (r and SEE) is independent of scaling, and TBW is determined by ECW + ICW, a good prediction of a dilution TBW would suggest a strong ECW and ICW prediction. This would only not be the case if there were exactly offsetting errors in the ECW and ICW prediction. Thus it is probable that the BIS prediction of ICW was better than that of dilution because the r and SEE were better for the BIS prediction of TBW than that of ICW. The inaccuracies of determining ICW by dilution have not been adequately discussed, nor is it clear whether the errors are additive or propagated. For the 14 subjects, both the dilution and BIS-predicted ICW were poorly correlated to the TBK-ICW. That the TBK-ICW predic-

### Table 3. ECW, TBW, and ICW determined by \( \text{D}_2\text{O}, \text{NaBr}, \) and BIS

<table>
<thead>
<tr>
<th>Subject</th>
<th>Br</th>
<th>BIS-ECW</th>
<th>( \text{D}_2\text{O} )</th>
<th>BIS-TBW</th>
<th>( \text{D}_2\text{O}-\text{Br} )</th>
<th>BIS-ICW</th>
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<td>21.03 ± 2.02</td>
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<td>41.02 ± 3.84</td>
<td>27.13 ± 2.73</td>
<td>19.99 ± 2.34</td>
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BIS, bioimpedance spectroscopy.

Fig. 5. Residual extracellular water (ECW) values plotted against mean values for bioimpedance spectroscopy (BIS) prediction and reference method (sodium bromide). Heavy horizontal line, mean difference (bias); lighter lines, mean ± 2 times SD of differences.

Fig. 6. Residual total body water (TBW) values plotted against mean values for BIS predictions and reference method (deuterium oxide). Heavy horizontal line, mean difference (bias); lighter lines, mean ± 2 times SD of differences.
tion improved substantially for the larger sample of 73 subjects suggests that the poor correlations were caused by outlying data in a small sample. That both BIS and dilution ICW were highly correlated with each other, as well as both poorly correlated to the TBK ICW in the 14 subjects, suggests that the lower correlation between BISICW and TBKICW in the entire sample was due to the error in TBK rather than BIS. Nevertheless, the strength of the discovered relationships among the BIS ICW and dilution and TBK ICW suggests that BCM can be determined by BIS.

The only other equation derived from Hanai theory, or any mixture theory for that matter, was never validated, and its sensitivity to change was poor (9). We believe the results achieved in this and other studies can be attributed to viewing the body as having three compartments (i.e., ECW, ICW, and the remainder) rather than only the two used previously (i.e., ECW, ICW) (9). We also believe this is to be attributed to the fact that the Hanai equation describes the effect on conductivity of the material, not the overall conductance. Thus, its use is volume dependent. To apply mixture theory, total volume must be known and is provided by body Wt/body density (D0). D0 varies between individuals, but the range is generally within 1–1.07 kg/l (20). The effect on ρECW in this range is ±1%, because it is only dependent on the cube root of D0. We do not use body Wt as a fudge factor to improve the correlation (8) but rather as a theoretically required term to measure of total body volume. Like D0, body Wt is expressed in cube root form; thus its contribution to the prediction of body water is reduced by 2/3.

The fact that changing ρECW had no effect on correlation or SEE for ECW, and only slightly for ICW when ρICW was changed demonstrates the scaling nature of these constants and that they have no effect on the scientific relationship between the BIS and dilution volumes. Large offsets were observed when the constants derived from a previous study were used (47), but these constants, which were derived from D2O and NaBr (47), have been cross-validated (10, 35). As such, it is troubling that ρECW would decrease by 19% and ρICW increase by 30%. This difference could have been caused by error in the BIS method. However, the high correlations and low SEE values observed, and the fact that the sample studied was similar to those samples used to calibrate (47) and cross-validate (35) these terms, suggest otherwise. Because subjects were healthy, it is unlikely that there were any major differences in ion concentration. Even so, a 5 mmol change in ion has been found to affect ECW only 1–2% and ICW only 4–5% (38).

Different dilution methods produce differently sized ECW and TBW spaces, e.g., sulfate (35SO4) space being typically 20% smaller than NaBr space (11). Thus, a ρECW calibrated to NaBr would predict an ECW space scaled 20% larger than 35SO4 space. We have noted (28) that Van Marken Lichtenbelt et al. obtained slightly higher r2 and lower SEE values by using the methods described in this study, but D2O space was underpredicted by −6.3 liters, and the NaBr space overpredicted by 3.0 liters. The NaBr-to-D2O space ratio was in the expected range of 0.40 and 0.42 in this study (Table 1) and the study reported by Van Marken Lichtenbelt (48), respectively. In contrast, the BIS ECW-TBW ratio predicted in this study by the previous constants was 0.51 (Table 3). This supports that the ρECW constant computed previously (47) may be scaling ECW too large, but this would equally occur if ICW were underestimated. That ICW, and thus TBW, may have been scaled too low was supported by the finding that the percent TBW of body Wt was 61% by dilution and 55% by BIS (Tables 1 and 3). Further evidence that the new ρICW constant may have validity was that TBK-ICW, which was independent from dilution, was predicted with very little mean difference. It is of concern that the
previously determined constants are predicting ECW and TBW with little mean difference at some laboratories but not others. There is nothing apparent in the dilution methods used in this study, by Van Marken Lichtenbelt et al. (48) or Van Loan et al. (47). For D2O, each used accepted protocols (e.g., fasted state, dosage, and equilibration time). Both we and Van Loan et al. (47) analyzed D2O enrichment with an accepted infrared spectrophotometry method and Van Marken Lichtenbelt et al. (48) with an accepted isotope-ratio mass spectrometry approach. All three laboratories corrected for isotope fractionation. The only variable identified was that we did not make a baseline measure of D2O, which potentially could lead to an underprediction of TBW space. However, this is unlikely to explain such a large scaling difference. We also did not account for D2O lost in the urine, but the subjects were measured in a fasted state and refrained from drinking or eating; thus, this is unlikely to explain such an offset. Similarly, for NaBr, all three laboratories used accepted administration and analytical protocols, including 10% corrections for nonextracellular distribution and 5% Donnan equilibration. As did Van Marken Lichtenbelt et al. (48), we measured NaBr concentrations with the accepted anion-exchange chromatographic method (32, 50), and Van Loan et al. (47) used a fluorescent excitation technique. The only variables not accounted for were basal NaBr concentrations and NaBr lost in the urine during the equilibration. However, over such a short period of time and with the subjects being in a fasted state, the loss in NaBr in the urine would be small, as would be the error caused by not subtracting baseline NaBr from the plasma after administration (32). There are other variations (such as hydration status and metabolic rates) (11), but these also would not explain such large offsets. As such, there was little difference in the methods used. The offset could be attributed to the small sample size (n = 24) originally used to compute persistently and previously reported in biophysics (5, 14), respectively. These findings have been replicated in a pediatric sample where the computed persistently was within 5% (49) of the value discovered in healthy adults (47). It is uncertain how the mixture effects will be adequately accounted for with a segmental measurement.

Effects of geometry on ρ. A wrist-ankle measurement would be inappropriate for patients with ascites, but the vast majority of subjects do not have such conditions (29). The good predictions of body water reported by this and many other studies using a wrist-ankle measurement supports that body water is evenly distributed in healthy subjects. If not, good predictions of body water would not be possible. To evaluate the error caused by making a wrist-ankle measurement and determine the validity of ρECW and ρICW computed previously (47), we compared the values of these terms to results reported in biophysics for plasma and ICW. First, we used standard anthropometric values for the ratios of arm, leg, and trunk lengths and girths (45) in the equation listed in APPENDIX C to compute a geometry constant Kβ and remove the geometry effects on ρ. Albeit a rough approximation, because the arms, legs, and trunk are not perfect cylinders and the fraction of ECW and ICW is not constant between segments, an approximation should be possible. The value for Kβ was computed to be 4.3. The longitudinal ρ of human skeletal muscle tissue measured at 1 kHz has generally been reported to be 200–300 Ω·cm. (14). These reported measures for apparent ρ were obtained from direct measurements on skeletal muscle tissue (14) and thus were corrected for the mixture effects caused by the ICW contained in that muscle. Assuming the fluid distribution found in a previous study (47) to be representative of healthy adults (45% ECW in TBW and 73% TBW in FFM), the concentration of nonconductive material in the skeletal muscle tissue was estimated to be 67.2%. By using Eq. C4, this yielded a ρ for ECW of nominally 250·(1 − 0.672)1.5 or ρECW of 47 Ω·cm. Our results indicated that kecw was nominally 0.311; by using a nominal D of 1.05 kg/l, this relates to a ρECW of 41 Ω·cm, which is in reasonable agreement with both the ρ calculated from skeletal muscle tissue measurements and to the ρ for pure ECW reported (50–60 Ω·cm)(14). Thus the distribution of ECW throughout the body was very consistent, and there was no significant error caused by making a wrist-ankle measurement. Similarly, the values found for k (i.e., 3.6) and α (i.e., 0.7; Table 2) were in reasonable agreement with the values of 0.3 and 0.6 previously reported in biophysics (5, 14), respectively. These findings have been replicated in a pediatric sample where the computed pECW was within 5% (49) of the value discovered in healthy adults (47). It is uncertain how the mixture effects will be adequately accounted for with a segmental measurement.

Principles of fitting data to a biophysical model. Plots are used to construct an applicable physical or mathematical model. Once a model has been constructed, computing the components of the model becomes the focus (5, 25, 40). The Cole model can be computed graphically or mathematically by drawing or fitting the best fitting curve through the data and extrapolating each end of the curve to where it intercepts the resistance axis (known as R0 and R∞) (Figure 3). R is equal to R; thus, once R0 or R∞ are known, R can be determined by 1/R0 = 1/R∞ R fitting is generally performed mathematically because it is far more pre-
cise (25). Modeling can also be performed either manually or mathematically by simply fitting a circle through the measured R and X data (6, 43) but this approach does not include f and thus is two dimensional, using one-third less data to determine and cross-check the best fit. This method also provides no estimate of Cm but most importantly does not allow for the effects of Td to be removed. Network analysis is a well-known analytical technique, and the common method used for fitting data to a network model is to simultaneously fit f and weighted Z and θ data, using nonlinear least squares curve fitting (25). The important data are not at LF and HF but in the middle surrounding f, because these data have greater certainty. To accurately fit and extrapolate a curve requires adequate data on either side of f. Properly weighting the raw data is essential for obtaining the most accurate fit to the model (25) because measurements may have greater uncertainties at LF and HF. By weighting, the data with more certainty (i.e., middle) make a greater contribution to the overall fit to the model (25). Determining what weights to use is not easily decided (25), and simultaneously fitting a multidimensional nonlinear equation is an art, with the raw data having a very complicated relationship to the final fitted parameters. Evaluating the accuracy of fit should be determined by comparing the offset to fit to the expected measurement uncertainty at each f (25). Because of weighting and the Cole model being multidimensional, evaluating the fit with a two-dimensional statistical analysis (e.g., root mean square error (RMSE) of R and X) would be meaningless (8, 27, 43). Weighting can be determined by measuring the range of error at each f, according to the expected error in the measured quantities (e.g., accuracy specifications of the device), or as we use by weighting the error rather than the data by comparing the expected error with the actual error (25). Limitations must be enforced to prevent the software from forcing a fit. As many frequencies as possible should be used because solving for five unknowns requires at least five data, and all data are potentially contaminated with error (e.g., interference) and thus are uncertain (25). The ability to delete data that are significantly decreasing the overall accuracy of fit is a highly desirable capability (25). The accuracy of resolving a model is a function of the square root of the number of extra data pairs (i.e., Z and θ) over the number of variables in the model. A 16:1 increase in data provides a 4.1 improvement. However, processing time is effectively the square of the number of data pairs. Thus, a 16:1 increase in data takes 256 times longer to compute. We presently measure at 50 frequencies logarithmically spaced from 5 kHz to 1 MHz to balance between accuracy and processing time. It is important to space the frequencies logarithmically to ensure a proper density of data. We fit with both Z and θ (rather than Z alone, or R and X) to ensure the best possible accuracy of fit. If only Z is modeled (8), the overall accuracy is reduced (25). Using Z and θ provides twice the amount of data, and θ is an extremely important discriminating variable because it has a much broader range of sensitivity to change than Z. However, using θ requires that the time delay (Td) effects must be accounted for (37). We fit with Z and θ vs. R and X because the weighting introduced by X would enhance the importance of frequencies furthest away from f, and thus emphasize the opposite to what is needed. Furthermore, fitting against R and X is more complex and considerably slower because X is nonlinear.

Effects of f invariant Td. As published (30) and provided in Xitrion’s product literature since 1992, we recommend investigators extend the HF range of the measurement by removing the effects of Td by multiplying the Cole equation by the factor e\(^{-j\omega T_d}\), where e is natural number, j is \(-1\), and w is f in radians/s. Despite the confusion this parameter has caused (8, 43), all conductors exhibit a Td that causes a linear θ shift with f. Conductor length would be an obvious cause for Td (copper wire having a Td of ~1.2 ns/ft), whereby an 8-ft conductor length (e.g., wrist to ankle) would produce 10 ns of delay (37). However, a longer Td of 32.4 ns was observed in the female subjects (Table 2). This is because Td can also be caused by interaction between contact R, stray capacitance and transmission line effects, with the latter including conductor length (wrist to ankle) and the conductor (i.e., body) position relative to ground (floor, bed, table, and so on) (15). Only conductor length is a true Td effect, but in the 1 kHz-1 MHz f range the other effects also give rise to a linear θ shift with f and thus can be approximated as a Td. For simplicity, all effects will be labeled as Td throughout the discussion. The various causes of Td were simulated by using the widely available SPICE circuit-simulation software program (Intusoft, San Pedro, CA) and using the SPICE file shown in Table 4. Td were modeled by a high-Z transmission line with conductor length set by Td, and the body Z was modeled by a simple three-element model (no α) using an R_E = 680, R_i = 900, and C_m = 2.8 nF. Figure 9 shows a plot of θ vs. f in the 1 kHz-1 MHz f range for body Td of 0, 15, and 30 ns of Td, and a characteristic transmission line impedance (Zc) of 300 Ω. Because the body is usually suspended some distance from a ground plane, the body behaves like a transmission line (15). Figure 10 shows θ vs. f by using the same three-element values for R_E, R_i, and C_m, a fixed conductor length Td of 15 ns, and a Zc of 150, 300, 450, and 600 Ω, respectively.

For convention, θ in these plots is expressed as having positive polarity. As shown in Figs. 9 and 10, there is a significant linear θ shift with f caused by both conductor length (wrist to ankle) and conductor (body) relative to ground. Adjustment of the various parameters (e.g., distance from ground plane) in the simulation circuit changed the magnitude and frequency at which Td effects emerged. As shown in Figs. 11 and 12, there can be considerable variations in Td between subjects. Different devices, subjects, and environments will cause different responses to the variables causing Td (15). The interaction between variables resulted in an effect larger than their sum and begin to affect Z slightly over 1 MHz. As shown in Fig. 10, the effects caused by conductor relative to ground can cause a negative Td and opposite effect on θ, thus explaining the negative Td shown in Table 2. Although less accu-
rate than modeling, the $T_d$ effects can be removed manually by the methods described in Appendix D.

It is disappointing that several investigators would not use or even mention our suggested methods and information on $T_d$, then cause undue confusion by reporting an intermediate result and incorrectly attributing the deviation in the raw X data from the Cole model to measurement error (8, 43), particularly when these methods had been disclosed and used to successfully predict ECW and ICW volumes for the first time using Cole model terms $R_e$ and $C_m$ (30, 47). The effects of $T_d$ are linear on $\theta$ not on $X$, and in the frequency range of interest $T_d$ only significantly affects HF $\theta$ shifts not $Z$ (37). If $T_d$ were not a valid term, the correlation of fit of $Z$ (which is the $R^2 + X^2$) would also be seriously reduced, whereas it is not (i.e., 0.998; Table 2). Stroud et al. (43) should have questioned their conclusions, because if the measurement was poor, the data would not have corresponded so well to an electronic circuit. Similarly, Deurenberg et al. (8) should have questioned their conclusions when there was no deviation of $Z$ from the model at HF (8), and as stated, the final fit to the model included data up to 500 kHz. To review Appendix A, it can readily be seen that any $f$ significantly affecting the overall fit will be deleted.

The practical reasons for modeling for $T_d$ are simple. There are $T_d$ effects in all raw data to varying degrees (15); thus as much of the $T_d$ effects as possible should be removed from the analysis. Fortunately, in the frequency range of interest, all the effects of $T_d$ cause a linear $\theta$ shift with $f$. Because the Cole model (i.e., $C_m$) causes a nonlinear $\theta$ shift with $f$, the effects of $T_d$ can be effectively modeled and significantly removed. Our modeling program and information on $T_d$ were widely distributed in 1992. As such, it would not be difficult to adjust the various parameters to push out to higher frequencies where the effects of $T_d$ become dominant. However, as discovered by Stroud et al. (43), without removing the effects of $T_d$, only useful data up to 500–600 kHz will be obtained (43). Table 5 is an output file (.MDL file) generated from our fitting software on one of the subjects of this study. As shown, few data were deleted from the final fit, and
frequencies up to 1 MHz were included. Without removing the effects of $T_d$, inclusion of such HF data would not be possible. The problem with not modeling for $T_d$ is that there are variations in the environment in which measurements will be performed, and it is not uncommon, particularly in the clinical setting, to observe $f_c < 500$ kHz. With usable data only to 500–600 kHz, it would not be possible to accurately fit for $R_I$. Although the physics underlying $T_d$ is important, what is important to the prediction of ECW and ICW is to remove the effects of $T_d$ so the highest possible $f$ range can be included. Even with lower $f_c$, the closer the actual data are to $R_e$, the better the calculation of $R_I$ will become.

Dual- and single-frequency measurements. Biophysicists have been fitting $Z$ data to models since the early 1920s. However, before 1963 when a dual LF-HF $Z$ approach was first introduced as a measure of ECW and TBW (44), measuring $Z$ was much more difficult. Additionally, solving a five-dimensional nonlinear equation by hand without a microprocessor would have been extremely tedious. Although pragmatic at the time, it can readily be demonstrated (Figs. 1 and 13) that for some subjects where the current is fully conducting through the ICW does not occur until $>10$ MHz. A $f$ of 10 MHz is 100-fold away from 100 kHz. Thomasset (44) reported in 1963 that 100 kHz was too low a $f$. Even if higher frequencies could be measured, the effects of gamma dispersion must still be avoided (Fig. 1). Similarly, the effects of $\alpha$-dispersion become dominant near 1 kHz and must be avoided (Fig. 1). Most importantly, the proportion of current conducting through the cells at "any" single $f$ is not fixed but varies with $f_c$ and $f_c$ varies between individuals, as well as in the same individual when $R_e, R_i, C_m,$ or $\alpha$ is altered (5, 22, 26, 40; Figs. 1, 13, and 14). By fitting the data to the $R_e$ and $R_i$, the above error sources are removed. Jaffrin (19) reported that the overestimation of $R_I$ can be as high as 200% by not using $R_e$ (19).

Fig. 11. Plot of $\theta$ vs. $f$ of measured data (raw) and data fit to Cole model on 1 subject with calculated $T_d$ of ~1.3 ns.

Fig. 12. Plot of $\theta$ vs. $f$ of measured data (raw) and data fit to Cole model on subject with calculated $T_d$ of 36.1 ns.
As shown in Figs. 1 and 13, a 50-kHz measurement is neither a measure of ECW or TBW but rather some of both. No single HF measurement is a measure of TBW, including $R_e$ and $Z$ at $f_c$ as promoted by Cornish et al. (6, 27), but rather a measure of two significantly different fluids. The $\rho_{ECW}$ has been reported to be 50–60 $\Omega \cdot \text{cm}$ (14) and the $\rho_{ICW}$ to be 200–300 $\Omega \cdot \text{cm}$ (5). It has been previously assumed that the $\rho$ of TBW is constant. Obviously this assumption is invalid because a simple change in the ECW-ICW ratio would dramatically change it. This error can be reduced, as we have done, by using the measured $R_e$ and $R_i$ with the previously established constants $\rho_{ECW}$ and $\rho_{ICW}$ to determine the actual relative proportions of ECW and ICW. From this, one can establish the $\rho$ of TBW. The equation shown uses a linear mixture effect; however, in practice a

Table 5. Modeling software output file (MDL) displaying measured versus calculated data fit to the Cole model

<table>
<thead>
<tr>
<th>Deleted KHz</th>
<th>Fit r</th>
<th>Recf ohms</th>
<th>Rief ohms</th>
<th>R0 ohms</th>
<th>Rinf ohms</th>
<th>Cm nF</th>
<th>Td ns</th>
<th>alpha</th>
<th>Fc KHz</th>
</tr>
</thead>
<tbody>
<tr>
<td>none</td>
<td>0.9916</td>
<td>574.5</td>
<td>848.1</td>
<td>574.5</td>
<td>342.5</td>
<td>2.8</td>
<td>-1.3</td>
<td>0.7</td>
<td>55.7</td>
</tr>
<tr>
<td>1248</td>
<td>0.9940</td>
<td>573.0</td>
<td>878.2</td>
<td>573.0</td>
<td>346.8</td>
<td>2.8</td>
<td>0.1</td>
<td>0.7</td>
<td>53.8</td>
</tr>
<tr>
<td>74</td>
<td>0.9945</td>
<td>575.6</td>
<td>861.9</td>
<td>575.6</td>
<td>345.1</td>
<td>2.8</td>
<td>-0.3</td>
<td>0.7</td>
<td>53.4</td>
</tr>
<tr>
<td>1</td>
<td>0.9950</td>
<td>575.6</td>
<td>855.8</td>
<td>575.6</td>
<td>344.1</td>
<td>2.8</td>
<td>-0.6</td>
<td>0.7</td>
<td>54.4</td>
</tr>
</tbody>
</table>

Fig. 13. Resistance vs. frequency of 3 patients pre- and postdialysis. Data provided by and used with permission of Krassimir Katzarski, Dept. of Renal Medicine, Karolinska Institute, Stockholm, Sweden.
nonlinear ECW-ICW mixture effect was used. The difference is insignificant in healthy subjects or when small changes are not of concern.

The prediction of ECW is inherently better than ICW and TBW for both technical and theoretical reasons. The prediction of ECW is achieved directly from model term $R_e$, whereas ICW is predicted effectively by the difference between two large numbers ($R_e$ and $R_0$). Thus, a 0.1% error in $R_e$ is $<0.5\%$ error in the predicted ICW. Although there is a call for a return to a HF and LF approach because $R_e$ is more variable than a fixed HF (8), the above error sources can never be resolved with a fixed-f nonmodeling approach; thus, it is fraught with error. On the other hand, the repeatability and accuracy of solving for $R_e$ and thus ICW is technical rather than theoretical in nature. Improvements in the measurement should reduce the variability in predicting ICW.

Parallel reactance, phase angle, and cell membrane capacitance. Series reactance at 50 kHz ($X_s$) had been proposed as a measure of ECW (41) and as a measure of the extracellular mass/BCM ratio (42). A 50-kHz parallel X model ($X_p$) has now been proposed as a measure of BCM (3, 23). To support this proposal, Lukaski (23) performed a progressive potato study to demonstrate the $f$ dependence of biological tissue (23) and drew on the statement by Foster et al. (12) that $Z$ can be interpreted as either a parallel or series circuit and both resulting in two final elements (real and imaginary). The later is absolutely true for any single $f$ measurement, but biological tissue consists of more than two elements. $Z$ at any single $f$ can be interpreted as a parallel or series circuit, but the field is concerned with how to interpret the $Z$ of biological tissue. According to Fricke (13), Schwan (40), and Cole (5), single biological cells can be represented as a series-parallel network having three elements: $R_e$ in parallel with a series $C_m$ and $R_1$. Cole (5) added an exponent ($\alpha$) to the model to represent the distribution effects observed on biological cell suspensions and tissues. The Cole model is used most often to interpret $Z$ measured on biological tissue and consists of four elements (31). Based on the belief that how biophysicists interpret $Z$ measurements has merit, we use the Cole model. To do this, we fit all real and imaginary data (i.e., corresponding $Z$ and $\phi$) to the Cole model to discriminate the component parts of the tissue.

If previous work in biophysics does have validity, the use of $R$ and $X$ at any single $f$ to predict ECW or ICW would be an oversimplification and is dependent on the elements in the tissue having relative uniformity between individuals. Any relationship between BCM and $X$ is likely a function of the relationship between $X$ and $C_m$ because ICW is a resistive not capacitive medium. It has been suggested that as the cell swells, the membrane becomes thinner and $C_m$ increases, and the opposite occurs when the cell shrinks (16). However, $X$ at any single $f$ is not merely $C_m$, and $C_m$ can only be computed by modeling for all the elements in the Cole model (5, 25, 40). Which variable is affecting $X$ at any single $f$ cannot be determined. However, since $R_e$, $R_1$, and $\alpha$ tend to be tightly regulated and vary within narrow limits, $X$ would tenuously reflect $C_m$ and give rise to a correlation to cell volume.

To investigate the strength of the relationship between $C_m$ and the variables related to $C_m$ and BCM as defined by TBK, we investigated their correlation to TBK. As shown in Table 6, weight was strongly correlated to $C_m$, but variables other than BCM can cause a change in weight. $C_m$ alone was strongly correlated to TBK and improved when expressed as $Ht^2 \times C_m$. There was a poor direct relationship between $X_s$ and $X_p$ and TBK and $C_m$, respectively. $X_s$ and $X_p$ were moderately correlated to TBK when expressed as $Ht^2/X_s$ or $Ht^2/X_p$, respectively, but $Ht$ alone was highly correlated. $f_c$ was

<table>
<thead>
<tr>
<th>Variable</th>
<th>$Ht$</th>
<th>$Wt$</th>
<th>$C_m$</th>
<th>$f_c$</th>
<th>$Ht^2 \times C_m$</th>
<th>$Ht/\phi$</th>
<th>$Ht/X_s$</th>
<th>$Ht/X_p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBK</td>
<td>0.58</td>
<td>0.64</td>
<td>0.50</td>
<td>-0.39</td>
<td>0.73</td>
<td>0.62</td>
<td>0.43</td>
<td>0.69</td>
</tr>
<tr>
<td>$C_m$</td>
<td>0.33</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-0.81</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$Ht$, height; $Wt$, weight; $X_s$, series reactance; $X_p$, parallel reactance.
also correlated to TBK, but mathematically \( f_c \) is dominated by \( C_m \) and \( R_E \), and since this healthy population would have a narrow range in \( R_E \) in relation to the other model parameters, the relationship between \( f_c \) and TBK was most likely dominated by \( C_m \). As shown in Table 6, there was a strong relationship between \( f_c \) and \( C_m \). Use of \( f_c \) cannot be supported, because it is affected by all the variables in the model, but as expected, \( f_c \) predicted TBK better than \( X \) at 50 kHz. The mean \( f_c \) for this healthy sample was \( \approx 60 \) kHz (Table 2); thus, \( X \) would approximate \( C \). Out of the variables in the model, \( X \) is more closely related to \( C \) than \( X \) simply because \( f_c \) more closely reflects \( C_m \), whereas the strength of the relationship between \( X \) and \( C_m \) varied with \( f_c \), which ranged in this sample from 43 to 110 kHz. The strong relationship between \( C_m \) and TBK was expected because \( C_m \) is a function of cell surface area. The moderate relationship between \( C_m \) and weight reflects this dependence. However, \( C_m \) is also affected by the aspect ratio (length to cross-sectional area) of the body’s conductor. With an identical total cell volume, a greater conductor length would cause less \( C_m \), whereas a greater conductor cross-sectional area would cause higher \( C_m \). Although error caused by aspect ratio is removed by length\(^2 \times \) \( C_m \), this is only true for a uniform cylinder. Further refinements in \( C_m \) would need to be made by accounting for \( K_B \) (Appendix C). As discussed above, \( C_m \) is also affected by the thickness of the cell membrane. With the errors caused by aspect ratio and cell membrane thickness, it is unclear why a surface measurement (i.e., \( C_m \)) would be used to reflect what is inside the cells when it can be determined more directly by a resistive-predicted ICW (ICW\(_R \)).

A single 50-kHz-frequency measure of \( X \) and \( \theta \), and an R-X graph have been proposed as measures of fluid distribution and discriminating indexes of health and disease (2, 36). Recently, \( \theta \)-angle spectrum analysis (that is, \( \phi \) vs. \( f \)) has been proposed as descriptive of body water and body composition (4). \( \theta \) angle is a function of the ratio of \( R \) and \( X \); thus, both \( \theta \) and an R-X graph would be sensitive to the same errors and uncertainties as any single frequency of \( R \) and \( X \). The sensitivity of \( \theta \) is extremely dependent on \( X \), which in turn is extremely dependent on the relationship between the frequency of measurement and \( f_c \), and is symmetrical about \( f_c \). \( X \) and \( \theta \) at 50 kHz change dramatically when \( f_c \) changes, simply because 50 kHz is a fixed point on the changing curve (22, 26) (Fig. 14). \( X \) simply changes more than \( R \) because \( X \) is only 5% of the total Z; thus, a slight change in \( f_c \) causes a greater percentage change in \( X \). In 1974 (Fig. 14; see Ref. 34), it was observed that dialysis patients had a lower \( X \) and \( \theta \) measured at 50 kHz predialysis and that it returned to that observed in healthy subjects postdialysis. Lofgren (22) attributed the cause of this to the change in \( f_c \) 23 yr previously. The change in \( X \) and \( \theta \) with a change in fluid distribution has given the incorrect impression that \( X \) and \( \theta \) are somehow directly related to fluid distribution. A change in fluid distribution does change \( f_c \), which in turn changes \( X \) and \( \theta \), but this is principally caused by a change in ECW (and \( C_m \), as discussed above). Again, the problem with using \( X \), \( \theta \), or \( f_c \) to reflect any body composition parameter is that these variables are affected by all the elements in the tissue (Appendix A). One can question the utility or need for \( X \), \( \theta \), \( f_c \), or an R-X graph when which variable is causing their change cannot be determined and they have no theoretical basis. On the other hand, \( R_E \) and \( R_I \) at least relate in theory to a physical object (i.e., ECW and ICW).

On the same individual, \( C_m \) is determined by total cell volume and membrane thickness and porosity (5). Thus, any \( HT^2 \times C_m \) relationship would be a function of these three parameters. An ICW\(_R \) can be used to predict total cell volume, and then if removed from the \( K_B \)-corrected \( Ht^2 \times C_m \) relationship by using \( HT^2 \times C_m / ICW_R \), the remaining index would reflect cell membrane thickness and porosity. Such a measure might have several applications. Scheltinga et al. (39) observed that as the severity of sepsis increased, \( C_m \) decreased to the point where there was virtually no \( \beta \)-dispersion. This corresponds to what Lukaski (23) observed on a cooked potato. It is well known that with cell death or cell destruction, the cell membrane loses its high resistive properties. During dialysis, ECW changes are on the order of 20–30%, \( R \) varies little, but both \( F_c \) and \( C_m \) can change by as much as 2:1 (1, 19) (Fig. 14). As shown in Table 2, the mean \( C_m \) for the male subjects of this study was 2.32 nF. Bestoso et al. (1) discovered a mean increase of \( C_m \) in men from pre- to postdialysis of 64% (1.64–2.47 nF), with the postdialysis \( C_m \) being quite close to that measured in the men in this study. Thus, if Scharfetter’s (38) estimates are correct that a 5-mmol change in ion affects the ICW 4%, the error caused by ion on ICW\(_R \), as well as the error in predicting ICW, would be insignificant compared with the percentage change in \( C_m \). Use of \( C_m \) for studying cell membrane health is an exciting area of research that awaits further investigation.

In conclusion, there has been very poor cross-transfer of information from the fields of physics and engineering to the field of human body composition. It does not help that the principles of BIS and mixture theory are rather complicated for many investigators. However, Z is an engineering- and physics-based technique, and the principles and merits of modeling and mixture theory have been known for a very long time. Multiple-frequency devices safe for human studies and modeling programs have now been available for >4 yr; thus, the lack of appropriate equipment is no longer a valid reason for not using modeling. Due to the high intercorrelation (48) among ECW, ICW, and TBW, one can always correlate a limited single- or dual-frequency measurement to body water, but this leads to population-specific equations and a reduced sensitivity to change, which is why Z measurement has not yet reached its full potential. Until investigators begin using the proven and accepted fundamental techniques used in other fields of science (i.e., modeling) that use Z measurements (e.g., biophysics, advanced materials research, and chemical engineering), the use of Z in clinical medicine will remain what it is today—a tech-
APPENDIX A

Modeling

The Z and \( \theta \) spectra data were fit to the Cole-Cole model (5), Eq. A1, using iterative nonlinear curve-fitting software. The modeling program evaluated the weighted least square error of both Z and \( \theta \), where the weighting is established by the published accuracy specifications of the instrument, and removed any f that would significantly decrease the total weighted least square error. In addition to the correlation of fit using scalar Z, the program established the accuracy of fit to the model as follows:

1) Mean offset to fit \(<\frac{1}{2}\) the instrument measurement specifications.
2) Mean offset to fit \(<2\times\) the instrument measurement specifications.
3) Mean offset to fit \(<5\times\) the instrument measurement specifications.
4) Mean offset to fit \(<10\times\) the instrument measurement specifications.
5) Mean offset to fit \(>5\times\) the instrument measurement specifications.

To prevent the program from deleting frequencies solely to “force” a fit to the model, the following limitations were enforced in the software:

1) A maximum of 25% of the frequencies (f) may be deleted.
2) Within any 3:1 range of f, at least one f must remain.
3) Only f whose Z and \( \theta \) lay farther than the instrument specification from the curve may be deleted.
4) Only one f is deleted per iteration of fitting.
5) A f is only deleted if it results in the maximum improvement in resultant fit; this is not necessarily the f whose Z and \( \theta \) lay farther from the fit.

The Cole model was extended to allow for the f invariant time delay (\( T_d \)) caused by the speed at which electrical information is transferred through a conductor (15, 37). The error introduced by this fixed \( T_d \) was modeled as a \( \bar{\theta} \) error that increases linearly with f. This linear \( \bar{\theta} \) error was mathematically modeled by multiplying Eq. A1 by the factor \( e^{j\bar{\theta}f} \). Thus the overall modeled equation was

\[
Z_{\text{obs}} = \left( \frac{R_E}{R_E + R_i} \right) |R_i + \frac{R_E}{1 + j\omega C_m(R_E + R_i)j}| (e^{j\bar{\theta}f}) 
\]

(A1)

where \( Z_{\text{obs}} \) is the observed complex Z; \( R_E \), \( R_i \), and \( C_m \) are the component values of this circuit; \( \omega \) is in radians/s (\( = 2\pi \times f \)); and \( j \) is \(-1\).

\( f_c \) was computed after the model components (\( R_E \), \( R_i \), CM, \( T_d \), and \( \alpha \)) had been determined by solving the equation

\[
\frac{\partial X(f_c)}{\partial \omega} = 0 \quad \text{(A2)}
\]

where \( X(f_c) \) is the imaginary part of Eq. A1 at \( f_c \).

APPENDIX B

Theoretical Volume Equations

The ECW and ICW volumes were predicted from the modeled \( R_E \) and \( R_i \) by using equations formulated from Hanai’s theory, which describes the effect that a concentration of nonconductive material has on the apparent resistivity \( (p) \) of the surrounding conductive fluid, and is

\[
p = \frac{p_0}{(1 - C)^{\frac{3}{2}}} \quad \text{(B1)}
\]

where \( p \) is the apparent \( p \) of a conductive material; \( p_0 \) is the actual \( p \) of a conductive material; and \( C \) is volumetric concentration of the nonconductive material contained in the mixture.

From Eq. B1, with the following assumptions, we derived a set of equations as follows

\[
V_{ECW} = k_{ECW} \left( \frac{L^2 \sqrt{W}}{R_E} \right)^{2/3} \quad \text{(B2)}
\]

where \( V_{ECW} \) is the predicted total extracellular fluid volume (in liters)

\[
k_{ECW} = \frac{1}{1,000} \left( \frac{K_{BP}^2 \rho_{PECW}}{D_b} \right)^{1/3} \quad \text{(B3)}
\]

\( W_t \) is body weight (kg); \( L \) is height (cm); \( R_E \) is the value from the model fitting (\( \omega \)); \( K_{BP} \) is a factor correcting for a whole body measurement between wrist and ankle, relating the relative proportions of the leg, arm, trunk, and height (see APPENDIX C). \( P_{ECW} \) is the \( p \) of extracellular water (\( \Omega \cdot \text{cm} \)); \( D_b \) is body density (kg/l)

\[
\frac{(1 + \frac{V_{ICW}}{V_{ECW}})^{25/6}}{(1 + \frac{V_{ICW}}{V_{ECW}})^{25/6}} = \left( \frac{R_E + R_i}{R_i} \right) \left( 1 + k_{p_{ECW}} \right) \quad \text{(B4)}
\]

where

\[
k_{p_{ECW}} = \frac{p_{ICW}}{p_{PECW}} \quad \text{(B5)}
\]

\( R_i \) is the value from the model fitting (\( \omega \)).

The following assumptions were made:

1) The volumetric concentration of nonconductive elements in the body at low frequencies (LF) is given by

\[
1 - \frac{V_{ECW}}{V_{Tot}}
\]

where \( V_{Tot} \) is the total body volume.

2) The volumetric concentration of nonconductive elements in the body at high frequencies (HF) is given by

\[
1 - \frac{V_{ECW} + V_{ICW}}{V_{Tot}}
\]

3) \( V_{Tot} \) is body weight/D_b.

4) The total volume of a body fluid can be described by

\[
V_F = k_{WF} \left( \frac{L^2}{R} \right) \quad \text{(B6)}
\]

where \( V_F \) is the total volume of the fluid in the body; \( K_{WF} \) is a factor relating the relative proportions of the leg, arm, torso, and height; \( p_F \) is the \( p \) of the water; \( L \) is body height; and \( R \) is the measured resistance between wrist and ankle.

5) The factors \( D_b \), \( K_{WF} \), and \( p_F \) can be considered largely constant.

6) The Hanai equation is applicable at HF and LF to mixtures found in the human body.

By using Eqs. B2 and B4, predicted \( V_{ECW} \) and \( V_{ICW} \) were computed, from which predicted TBW and computed by using
the following equations

$$TBW = V_{ECW} + V_{ICW}$$  \hspace{1cm} (B7)$$

Computing the Constants

$k_{ECW}$ is established as the mean value of

$$V_{ECW} \left( \frac{L^2}{W \cdot T^2} \right)^{2/3}$$

using a standard spreadsheet program. The method used to derive $k_{ECW}$ is to repetitively predict $V_{ICW}/V_{ECW}$ and adjust $k_{ECW}$ until a minimum mean error between the predicted and measured ratio is obtained.

**APPENDIX C**

**Derivation of $K_B$**

It should be noted that the derivation for $K_B$ shown here is only an approximation for the purposes of confirming whether its use results in a $\rho_{ECW}$ value that is within the range measured by other investigators.

The resistance ($R$) of a cylinder, measured longitudinally, is given by

$$R = \frac{L}{\rho A}$$ \hspace{1cm} (C1)

where $\rho$ is the resistivity of the material; $L$ is the length of the cylinder; and $A$ is the cross-sectional area of the cylinder.

Restating Eq. C1 in terms of the cylinder length and circumference

$$R = \frac{4\pi L}{C^2}$$ \hspace{1cm} (C2)

where $C$ is the circumference of the cylinder. The volume of the cylinder is given by

$$V = \frac{LC^2}{4\pi}$$ \hspace{1cm} (C3)

If we consider the body to be formed by five cylinders (the legs, the arms, and the trunk), then the volume of the body is given by

$$V = 2 \left( \frac{L_a C_a^2}{4\pi} \right) + 2 \left( \frac{L_1 C_1^2}{4\pi} \right) + \left( \frac{L_2 C_2^2}{4\pi} \right)$$ \hspace{1cm} (C4)

where $L_a$ and $C_a$ are the length and circumference, respectively, of an arm; $L_1$ and $C_1$ are the length and circumference, respectively, of a leg; and $L_2$ and $C_2$ are the length and circumference, respectively, of the trunk.

When we measure the $Z$ between the wrist and the ankle, the measured value will be

$$R = \left( \frac{4\pi L_1}{C_1^2} \right) + \left( \frac{4\pi L_2}{C_2^2} \right) + \left( \frac{4\pi L_a}{C_a^2} \right)$$ \hspace{1cm} (C5)

But in Eq. B6 we assumed that $R$ is given by

$$V = K_B \frac{L^2}{R}$$ \hspace{1cm} (C6)

where $L$ is the height.

Combining Eqs. C4, C5, and C6 yields

$$K_B = \frac{1}{L^2} \left[ \frac{L_1}{C_1^2} + \frac{L_2}{C_2^2} + \frac{L_a}{C_a^2} \right] \left[ 2L_a C_a^2 + 2L_1 C_1^2 + L_2 C_2^2 \right]$$ \hspace{1cm} (C7)

If we relate $L_1$ and $C_1$ to height by factors $K_{il}$ and $K_{ic}$ respectively (i.e., $L_1 = K_{il}L_1$, Eq. C7 becomes

$$K_B = \left[ \frac{K_{il}}{K_{ic}^2} \right] + \frac{K_{il}^2}{K_{ic}^2} + \frac{K_{il}}{K_{ic}} \left( 2K_{il}^2 K_{ic} + 2K_{il} K_{ic}^2 + K_{il} K_{ic}^3 \right)$$ \hspace{1cm} (C8)

Thus $K_B$ can be set according to standard anthropometric ratios and is independent of any electrical parameters of the body.

**APPENDIX D**

**Removing the effects of $T_d$**

$T_d$ can be removed through modeling, but it is only applicable when the actual measured data are used as input to fitting a program against a model. In this case, an additional multiplicative term must be added to the model (Eq. A1), which yields no change in amplitude but instead yields a linear $\phi$ shift with increasing $f$. As shown, the user will have one more “constant” term ($K$ in this example) in the model

$$\text{Multiplicative term} = 1 + i(1 + Kf)/1 + Kf + 1$$

where $K =$ constant to be modeled; $f =$ frequency; and $i =$ complex unity.

The modeling approach to removing $T_d$ performs the best, allowing the user to extend the usable range up to ~1 MHz, thus allowing for higher accuracy modeling. However, this technique is the most complex and may take excessive computing knowledge or time. The user can also approximate the observed $\phi$ error manually and recalculate the set of measurement data including a correction for this effect. It should be noted that, without employing the full model as outlined above, it is not possible to separately model this effect alone because the biological effects will “skew” the results. The manual method does offer the advantage of being very fast to implement, and it may be performed either by computer or by hand. The technique does not offer the accuracy of this first method, however, because the correction is only optimized over relatively few data points. These data do, however, increase the useful range of the measured data up to several hundred kilohertz.

Because there is little biological $\phi$ shift caused by $C_m$ at HF, choose a HF and assume that all the observed $\phi$ shift is caused by $T_d$. If 1-MHz data (as an example) are selected, note the observed $\phi$ shift at 1 MHz as $\phi_{1 \text{MHz}}$, then correct the measured $\phi$ shifts by subtracting $\phi_{1 \text{MHz}} \times f/1,000$ from all measured $\phi$ shifts. The measured $Z$ data need no correction. The final, corrected, resistance ($R$) and reactance ($X$) data may be computed as follows

$$\text{resistance} = \text{impedance} \times \cos^{-1} \text{(corrected phase)}$$

$$\text{reactance} = \text{impedance} \times \sin^{-1} \text{(corrected phase)}$$

Because this manual method is only an approximation, and the resultant data is dependent on a single measured data point, the user may wish to try varying the $f$ of the measured data point (change the divisor in the $\phi$ correction for the $f$ of the point used) or may wish to try taking the average of several HF data points as shown in the following example of
averaging data collected at 700, 800, 900, and 1,000 kHz
phase correction = 0.25 \times \text{frequency} \times [ (\Phi_{700}/700) + (\Phi_{800}/800) + (\Phi_{900}/900) + (\Phi_{1000}/1000) ]

These manual techniques only offer advantages when the user is interested in fitting the measured data to a multi-element “circuit” model or in using the measured \( \theta \) or X data within a statistically produced model. If only the Z or R is of significant interest, then these corrections offer little because \( T_0 \) only affects \( \theta \) and has no effect on Z.

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Received 23 May 1996; accepted in final form 13 November 1996.

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