Body water compartments with human aging using fat-free mass as the reference standard

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LESSER, GERSON T., AND JULES MARKOFSKY. *Body water compartments with human aging using fat-free mass as the reference standard.* Am. J. Physiol. 236(3): R215–R220, 1979 or Am. J. Physiol.: Regulatory Integrative Comp. Physiol. 5(2): R215–R220, 1979.—Forty-five healthy men and women aged 16–39 and 59–89 yr were studied for total body water (TBW) and extracellular water (ECW); intracellular water (ICW) was calculated as the difference (ICW = TBW − ECW). An independent measurement of total body fat by inert gas uptake provided a value (±2%) for fat-free mass (FFB is wt minus body fat). Results agreed with observations by others that TBW and ICW are lower in the aged and lower in women, whether expressed as absolute volumes or per unit of weight, surface area, or height. However, with FFB as the reference standard, very different aging trends appeared. TBW/FFB remained constant to our oldest measured subjects (704 ± 7 ml/kg). ICW/FFB was slightly lower at advanced age, but the 4–5% decrease for each sex was within statistical variability. With age ECW increased slightly and its proportion within the fat-free body (ECW/FFB) was significantly higher. Based upon FFB, the distribution, proportions, and aging trends of body water compartments were similar for men, women, and male rats. Although its potential limitations must be appreciated, the FFB appears widely useful as a reference standard. The stability of ICW volume and of fat-free mass in aging man does not support the hypothesis that cellular mass is lost by healthy mammals with age.

total body water; extracellular water; intracellular water; aging in mammals; body composition; standard of reference

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REPORTS OF BODY WATER VOLUMES in man have almost universally indicated that total body water (TBW) and intracellular water (ICW) are lower in women than in men and lower with age in both sexes, whether expressed as absolute volume (liters) or in terms of body weight (TBW/wt or ICW/wt, 1/kg) (2, 11, 16, 30, 34, 38). However, problems inherent in cross-sectional study, particularly those related to subject comparability and selective longevity, raise questions as to the interpretation of these data. It is not known if the older study populations had, in their youth, been similar to the younger populations, or if the older subjects were representative random survivors of their contemporaries. Age-related differences of height and of fat content have proved particularly perturbing when comparing young and old populations. Almost without exception, average heights of older human subjects have been considerably less than those of the younger subjects, so that observed differences may reflect those of height rather than of age. Furthermore, the usual addition of storage lipid to the body with age, associated with little increment of water, serves to dilute nonlipid body components and thus to lower the ratio TBW/wt (or ICW/wt). In the available studies of human water volumes, the mass of body fat was either unknown or was measured by methods that have not proved reliable.

Absolute values for water volumes (or for any other body constituent) therefore require improved standardization to validate young-old comparisons. The need for such "normalization" to interpret data on body content of several elements was recently reemphasized by Ellis and associates (12). Surface area, body weight, or body height have traditionally been used as reference standards. Surface area has no rational basis for physiological or anatomic use, and weight and height have the serious limitations noted. The concept of interindividual comparability of the fat-free body (FFB) was suggested over 50 yr ago (31), and the FFB has been proposed as a reference standard (12, 17, 27, 37). In limited earlier reports, O_2 consumption and cardiac output correlated more closely with FFB than with body weight or surface area (25). However, the advantage of FFB as a reference could not be statistically documented, perhaps due to imprecision of the methods available for its measurement.

In the present study, the use of inert gas uptake to measure body fat provided a value for FFB mass (±2%) for each of the subjects, without the need for major assumptions as to body composition (18, 20, 23). The results were consistent with earlier findings in that TBW and ICW appeared lower in women than in men and lower at advanced age, both as to absolute volumes or when calculated per unit of weight, surface area, or height. However, the same water volumes, when calculated per unit fat-free body, showed no significant differences with age or between the sexes. In addition, and again in contrast to earlier interpretations, both men and women showed significant age-related increases of extracellular water (ECW) content if related to the fat-free body (ECW/FFB).

The age course of body water and its compartments of a colony of male rats was also followed by similar methods. The more detailed animal investigations, including histochemical analyses of carcass and individual tissues

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(19),[1] and concurrent longitudinal observations (21), not only confirmed the present human results but helped to clarify and explain the findings. Studies of these two mammalian species also permitted comparative evaluations of the standards of reference commonly used in mammalian biology, and reexamination of the hypothesis that decrease of fat-free mass is associated with the aging process.

METHODS

Subjects. Forty-five men and women were studied at ages 16–39 and 59–89 for each sex. Apart from three older subjects with mild, uncomplicated, untreated hypertension, all were free of apparent disease on examination, evidenced no fluid retention, and each had normal blood count, blood sugar, urea nitrogen, and urinalysis. None was on regular medication. Grossly obese subjects were excluded but no other selection was made for body habitus. All were active, working, or recently retired; none was institutionalized. All lived in New York City or nearby suburbs.

Basic body composition data on these subjects were presented in an earlier publication (20); those measurements essential to the present study are noted in Tables 1–4. Each individual was studied for body fat, total body water, and for extracellular water volumes on one or more occasions. Subjects were weighed before breakfast, postmicturition, and without clothing to the nearest 0.1 kg. Height was measured without shoes to the nearest centimeter. All procedures were initiated between 0700 and 0900 h.

Total body fat. Total body fat was measured by the prolonged uptake of cyclopropane and/or ^{85}Kr from a closed breathing circuit. Small, known volumes of one or both of these highly fat-soluble, inert gases were introduced at zero time and the rate of gas uptake by the subject monitored over the next 6.5–9 h. Details of the procedure, apparatus, analytic methods, solubility coefficients, calculations, and error analyses have been previously described (18, 20, 23). The mass of lipid measured by inert gas uptake agrees closely with the mass of all ether-extractable material in the body (20). Fat-free body was calculated as total body weight minus total body fat. It may be noted that FFB so defined differs from "lean body mass," which has been variously assumed to include differing small amounts of "essential" lipids, and also from "adipose free body," as the latter excludes total adipose tissue, which contains lean components as well as its lipids.

Total body water. Total body water was measured as the volume of dilution of tritiated water (^3H_2O). After a plasma sample was taken for background correction, 200–400 μCi of tritium, as ^3H_2O, were administered intravenously in 35–50 ml of 0.9% saline from a calibrated burette. Blood was sampled after 5–8 h and the plasma analyzed for tritium content. The procedure was as previously described (24) except that, for all but the first 15 subjects, samples were counted with a different liquid

TABLE 1. *Body composition of men 17–39 yr old*

Subj, Age	Ht, cm	Wt, kg	FFB, kg	TBW, liters	ECW_S, liters	TBW/FFB, ml/kg	ECW_S/FFB, ml/kg	ICW_S/FFB, ml/kg
MS, 17	176	59.1	48.1	34.8	10.5	723	218	505
EF, 22	177	70.0	56.5	34.2	7.0	605	124	481
CW, 23	177	77.2	65.3	47.0	13.1	720	201	519
SR, 25	189	85.9	71.2	45.9	12.5	645	176	469
JR, 27	182	78.8	68.2	45.5	12.2	667	179	488
FK, 29	170	73.3	57.4	41.2	10.7	718	186	532
SD, 32	172	75.2	58.7	40.2	9.0	685	153	532
GL, 33	189	75.4	68.1	50.1	13.1	736	193	543
GJ, 35	163	52.0	46.6	33.1	8.9	710	191	519
EB, 36	183	76.0	57.1	40.2	11.4	704	200	504
PD, 37	177	85.3	62.0	40.0	9.9	645	160	485
TJ, 39	168	73.2	58.5	41.3	12.5	706	214	492
Mean 29.6	176.9	73.45	59.81	41.13	10.90	688.7	182.9	505.8
±SE ±2.0	±2.3	±2.79	±2.21	±1.54	±0.56	±11.9	±7.8	±7.2

FFB, fat-free body is body weight minus body fat from inert gas absorption; TBW, total body water, measured as described in METHODS; ECW_S, extracellular water volume measured as volume of dilution of $Na_2{}^{35}SO_4$; ICW_S, TBW − ECW_S.

TABLE 2. *Body composition of men 59–89 yr old*

Subj, Age	Ht, cm	Wt, kg	FFB, kg	TBW, liters	ECW_S, liters	TBW/FFB, ml/kg	ECW_S/FFB, ml/kg	ICW_S/FFB, ml/kg
HB, 59	161	65.6	48.5	31.5	11.4	649	235	414
AB, 59	179	96.4	73.1	52.6	16.1	720	220	500
DS, 64	179	57.3	45.4	35.2	11.5	775	253	522
JB, 66	174	78.9	57.5	42.6	11.7	741	203	537
JA, 70	162	78.1	54.0	39.4	11.5	730	213	517
PL, 71	173	86.5	63.7	42.3	11.7	664	184	480
IR, 74	177	56.9	45.0	33.0	11.1	733	247	487
JG, 74	176	77.5	55.1	38.6	12.3	701	223	478
IS, 75	159	67.3	49.1	35.1	10.4	715	212	503
DM, 89	165	73.0	50.8	31.0	9.9	610	195	415
Mean 70.1	170.5	73.75	54.22	38.13	11.76	703.8	218.5	485.3
±SE ±2.8	±2.5	±3.94	±2.77	±2.08	±0.53	±15.5	±7.2	±13.0

For abbreviations see Table 1.

TABLE 3. *Body composition of women 16–38 yr old*

Subj, Age	Ht, cm	Wt, kg	FFB, kg	TBW, liters	ECW_S, liters	TBW/FFB, ml/kg	ECW_S/FFB, ml/kg	ICW_S/FFB, ml/kg
SZ, 16	151	51.3	35.3	25.4	6.0	720	170	550
KL, 17	173	66.0	47.9	34.7	11.6	724	242	482
VL, 19	161	52.4	38.5	28.7	8.0	745	207	538
JP, 22	170	60.4	43.7	32.7	8.6	748	197	551
SG, 24	165	54.3	45.9	32.3	11.3	704	246	458
CL, 24	178	63.4	50.8	35.1	9.7	691	191	500
FP, 25	168	56.3	43.6	30.6	7.5	702	172	530
JW, 25	172	53.7	42.1	32.2	7.8	765	185	580
RS, 27	171	79.1	59.5	37.1	10.4	624	175	449
GK, 38	156	53.3	42.6	29.9	8.3	702	195	507
Mean 23.7	166.5	59.02	44.99	31.87	8.92	712.5	198.0	514.5
±SE ±2.0	±2.6	±2.72	±2.13	±1.08	±0.57	±12.2	±8.8	±13.9

For abbreviations see Table 1.

[1] Cross-sectional and longitudinal observations were also carried out on a colony of male rats followed through life. Members were studied at regular intervals for body fat and ECW by methods similar to those described in this paper, and also for details of body composition by histochemical techniques. Certain aspects of these studies have been published (19, 21). Our findings for body water compartments, and those for whole-body and individual tissue composition, are included in a manuscript recently submitted for publication. Inasmuch as these studies in the rat often parallel those of the present paper for man, the results are cited frequently here to compare, clarify, and extend the human findings.

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TABLE 4. *Body composition of women 62–77 yr old*

Subj, Age	Ht, cm	Wt, kg	FFB, kg	TBW, liters	ECW_S, liters	TBW/FFB, ml/kg	ECW_S/FFB, ml/kg	ICW_S/FFB, ml/kg
KG, 62	154	74.2	50.4	33.1	10.6	657	211	446
SL, 62	149	58.9	39.2	26.6	6.9	679	176	503
MW, 65	158	69.0	47.9	27.8	9.6	580	200	380
AV, 66	158	62.8	34.4	25.5	6.9	741	201	540
AL, 67	160	62.2	46.5	30.0	8.2	645	176	469
LO, 68	162	84.1	50.5	35.1	11.9	695	236	459
HS, 69	166	56.7	37.5	26.1	9.0	696	240	456
BGO, 70	155	64.1	40.1	29.1	9.3	726	232	494
BGA, 70	146	54.6	34.5	25.0	7.3	725	212	513
DF, 71	163	56.4	39.2	30.0	10.9	765	278	487
FR, 73	156	75.5	51.0	36.7	12.2	720	239	481
LK, 75	162	58.5	35.2	29.1	9.0	827	256	571
AS, 77	159	74.8	36.5	29.2	9.4	800	258	542
Mean 68.8	157.5	65.52	41.76	29.48	9.32	712.0	224.2	487.8
±SE ±1.3	±1.6	±2.55	±1.81	±1.00	±0.48	±18.4	±8.9	±14.1

For abbreviations see Table 1.

scintillation counter (Nuclear-Chicago model 6725).

The volume of dilution of antipyrine had been developed and employed in this laboratory for the measurement of total body water (38) and for six of our early subjects, body water was measured with antipyrine (*subj FK, GL, EB,* and *PD* of Table 1, *subj VL* and *GK* of Table 3). It has generally been thought that isotope-labeled water overmeasures TBW by 2–3%, probably due to some exchange of the labeled material into nonaqueous compounds (16, 35). It is likely that the volume of dilution of antipyrine slightly undermeasures TBW because of the binding of a portion of antipyrine to plasma proteins. Comparisons of the two methods in different laboratories have given variable results, perhaps due to interspecies differences as well as to methodological differences. In man, the relative volumes of deuterated water:antipyrine were 1.09 (26) and 1.06 and 1.08 in two separate studies of Freeman et al. (15) and the volumes of ^3H_2O:*N*-acetyl-antipyrine were 1.05 (16). Culebras and Moore (8) recently calculated that theoretically the maximum possible nonaqueous exchange of hydrogen should be about 5%, but felt that actual exchange should be much less during the limited period of a dilution study. For 10 normal adults we found the volume of dilution of antipyrine averaged 4% less than that of ^3H_2O, a value quite compatible with the calculations of Culebras and Moore and with the results noted for man by Freeman et al. (15) and by Fryer (16). For the present study, we corrected all tritium volumes by the factor 0.98 and antipyrine volumes by the factor 1.02, on the assumptions that "true" body water volume is overmeasured 2% by volume of dilution of ^3H_2O and undermeasured 2% by volume of dilution of antipyrine.

Extracellular water. Extracellular water was measured as the volume of dilution of $^{35}SO_4$. Procedure was as previously described (22), with several modifications. After a blank plasma sample was taken for background correction, 17–25 ml of 0.9% saline containing 20–35 μCi of carrier-free ^{35}S as $Na_2{}^{35}SO_4$ was administered intravenously from a calibrated burette over a 1- to 2-min period. Four blood samples were taken at approximately 25, 32, 44, and 56 min from the midpoint of infusion (t_0) and the plasma analyzed for ^{35}S content by liquid scintillation counting (Nuclear-Chicago model 6725). In 20 human subjects we noted the time course of plasma decay of $^{35}SO_4$, observed in detail from 4 to 60 min, to rarely resemble a single exponential function before 20–24 min. The ECW was therefore calculated from the radioactivity in four samples of plasma between 25 and 60 min, plotted on semilog paper and extrapolated to t_0. Extended observations on four of the subjects with serial blood samples and urinary excretion up to 14 h indicated that plasma decay of ^{35}S follows another, slower rate during the period 60–400 min, consistent with prior results and suggesting several rates affecting plasma decay of sulfate due to exchange into body compartments other than rapidly equilibrating ECW.

The advantages and limitations of using the volume of distribution of radiosulfate (ECW_S) as a measure of extracellular fluid volume are considered below in DISCUSSION.

Intracellular water volume. Intracellular water volume (ICW_S) was calculated as the difference between the measured volumes of TBW and of ECW_S.

Statistical analysis. For all values, means and standard errors of the means were calculated. The Student *t* test was used to test for differences between population means.

From the observed data the variables sex, age, height, weight, body fat, TBW, and ECW_S were examined pairwise for correlations. Zero order correlations were computed for each pair of these variables as well as for the derived values for ICW and FFB. In addition, partial correlation coefficients were computed for each pair of the observed variables, holding the other five variables constant. To identify sources of variation among the variables, analysis of variance was performed in the form of stepwise multiple regression analysis.

RESULTS

Total body water. Average volume of TBW (in liters) was smaller for women at all ages and 7–8% smaller at advanced age for both sexes (Tables 1–4). As in most human studies, mean body height was appreciably less in the older subjects of both sexes, so it is not reasonable to compare the water volumes in absolute terms (liters) without some adjustment for size. Several techniques to improve young-old height comparability were examined: *a*) for the six young-old pairs of men and five young-old pairs of women who could be matched for comparable height (±2 cm), age-related differences of body water volumes were negligible (mean Δ for men, +1.3 liters; for women, −0.4 liters); *b*) no significant variability of TBW with age was noted by partial correlation analysis, controlling for height, weight, total fat, and extracellular volume.

With total body weight as the reference standard, water volumes (TBW/wt) of the older populations were 7% lower for men and 17% lower for women. These differences apparently reflected the higher fat contents of older subjects; when FFB was used as reference standard, no significant differences of FFB hydration were observed either with age or with sex (Tables 1–4). Mean water content of each of the four age-sex groups was not significantly different from any other, nor could signifi-

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cant differences be shown between all young vs. all old people, nor for all men vs. all women. We could therefore document no change of FFB hydration for men or for women from early adult life through the 8th decade. Water content of the individual fat-free body (TBW/FFB) ranged from 580 to 827 ml/kg; the mean value for all 45 subjects was 704.2 ± 7 (SE). Inasmuch as the present populations included only one subject beyond 80 yr, we cannot ignore the possibility that human FFB hydration, as that of the rat,[1] may change at very advanced ages.

Extracellular water volume. ECW_S was smaller in women than in men at all ages. Its absolute volume was 4–8% higher with age in both sexes (Tables 1–4). When the data were compared for subjects of similar height, the age differences became greater. For the six young-old male pairs (±2 cm ht), the older subjects' average ECW_S volume was 23% higher, for the five older paired women, 13% higher. For only 1 of the 11 pairs was the value lower for the older subject of comparable height. The partial correlation of age with ECW—holding height, weight, body fat, and TBW constant—was marginally significant for men ($P = 0.05$) and for women ($P = 0.04$), and highly significant if the sexes were combined to include all 45 subjects ($P < 0.003$). When referred to the FFB of each individual, the aging increment of ECW_S/FFB was 13% for women ($P < 0.05$) and 19% for men ($P < 0.001$); the young-old difference for both sexes combined was also highly significant ($P < 0.001$). It may be concluded, therefore, that the extracellular water volume (by radiosulfate) of healthy men and women at 70 yr is greater than at age 20–30 yr, and that the young-old difference becomes more obvious when adjusted for noncomparable heights or when related to the fat-free mass of the older individuals. In the absence of human data for the 5th and 6th decades, the age at which this change is first noted in man cannot be more closely defined.

Intracellular water volume. The absolute volume of ICW_S (TBW − ECW_S) was smaller in women at all ages and 12–13% smaller for older subjects of both sexes (Tables 1–4). When the data were inspected for subjects of similar height, the apparent differences with age were appreciably less. For the six young-old male pairs (±2 cm ht) the older subjects' ICW_S was 4.5% lower; for the five older paired females, 7% lower. For 6 of these 11 pairs, ICW_S was lower with age and for 5 pairs higher, suggesting a lack of directional change. When referred to the FFB of each individual, the male-female differences disappeared, and the aging decrement of ICW_S/FFB was 5% for women and 4% for men, both values without statistical significance. These results were verified by the lack of any significant differences for paired group comparisons, including testing for all possible groupings for sex and age. Furthermore, the tests showed no statistically significant differences of the variances between any pairs of groupings. We have, therefore, not been able to document a significant age-related change of intracellular water volume for men or for women.

DISCUSSION

As methods became available for in vivo measurements of body water volumes (4, 11, 29, 35, 38, 40, 41), it was noted that in older people, TBW/wt was lower whereas ECW/wt remained approximately stable (2, 9, 16, 30, 32, 34, 39). The apparent decrease of ICW volume, often coupled with supplementary information suggesting lower body K content, has been interpreted to indicate decreasing mass of the cellular compartment with aging (5, 13, 14, 22, 33, 36).

The present results agreed with these earlier observations of lower TBW/wt and lower ICW/wt in older people (Tables 1–4). However, when FFB was employed as the standard of reference, rather than the more commonly used weight or height or surface area, the data indicated very different aging trends. Body water content (TBW/FFB) remained constant to the oldest measured human subjects (8th decade). Extracellular water (ECW/FFB), rather than appearing stable through adult life, was significantly higher in older men and women. Intracellular water content (ICW/FFB) was slightly lower in the older groups, but the 4–5% fall for each sex could not be demonstrated to be of statistical significance. In a parallel study, the life course of body water and its compartments in the male Sprague-Dawley rat,[1] again based upon a fat-free body standard, closely resembled that observed for man at "comparable" stages of adult life (1).

These important differences in interpretation of observations resulted primarily from use of a different standard of reference. Body weight has obvious and serious drawbacks as a reference standard. The addition of body weight due to accumulation of body fat (storage lipid) associated with little or no increment of water, serves to dilute the stable mass of TBW, and thus to lower the ratio TBW/wt (or ICW/wt). Body height (length) has been assumed to be a more adequate standard. However, from height-weight tables for large populations it can be calculated that, at any given age, a height difference of 20% is associated with an average weight difference of about 34% (6). Thus the use of height as a reference standard can effect a spurious increment (or decrement) of any FFB constituent due solely to height differences among normal subjects. These standards of reference, commonly employed for young-old comparisons, tend to be quite misleading for older human populations, usually of lesser height and greater fatness. Recently, Ellis and associates (12) utilized a group of parameters—age, sex, height, and total body K—to "normalize" measurements of body Ca, P, Na, and Cl in men and women at different ages. The parameters were applied to equations with empirically derived constants (some of the "constants" are varied with sex and age) and the entire procedure appeared to strive for predictability rather than for the asserted "normalization."

There is probably no fully acceptable standard of reference for all purposes. All suffer, to a greater or lesser extent, from an implied but untenable assumption that each unit element is identical to every other element and is representative of the total. Because by definition the FFB contains no fat, it is not seriously perturbed by a wide range of change in nutrition. Within the present study population, there was less variability within each age-sex group when TBW, ECW, or ICW were referred to FFB rather than to weight or height. Furthermore, the

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male-female differences of body water and its compartments disappeared when the volumes were referred to FFB (Tables 1-4). Use of the FFB reference standard also permitted interspecies comparisons, inasmuch as the proportions of body water compartments were almost the same for similarly studied rats.[1]

Nonetheless, the FFB must be specifically evaluated as an appropriate standard for each particular measurement under study. Water contents of the various lean tissues fall within the relatively narrow range of 550–800 g/kg, the only important exceptions being blood lymph and bone. In addition, the composition of individual tissues was noted to be similar throughout the entire range of FFB size and to vary only slightly at all adult ages (19).[1] Thus, only an unusual change in circulating volume or a major alteration of the relative mass or the water content of a large tissue would change overall TBW/FFB sufficiently to be appreciated when measured. In serial observations, the only tissue alteration of consequence in the aging rat was a fall of some 10% of bone mass; this can be calculated to have a negligible effect on FFB water content. Male and female hydration should be similar despite some differences in relative proportions of body tissues. As water content of muscle is close to that of the overall FFB, a higher proportion of muscle tissue in the male can be shown to affect TBW/FFB minimally. Also, the differences in proportions of the various tissues of the rat as compared to man—larger viscera, more skin, slightly less bone—are of small relative magnitude and in varying direction (as to effect on hydration) so that TBW/FFB was quite similar for these two mammalian species (19). In light of these considerations and of the additional information derived from the tissue studies, it seems likely that apparent differences of body water in earlier human reports were not directly the result of age or of sex difference, but more probably were attributable to use of inadequate reference standards or to unsuspected disease processes in members of the older populations.

The proportions of intracellular and extracellular water—ECW_S/FFB and ICW_S/FFB—and their changes with age, were also strikingly similar in rat and man.[1] This result, however, might be less readily anticipated since the various tissues differ appreciably as to cellular and extracellular water contents. The interspecies similarity is in part fortuitous and, in part, a function of similar proportions of the large skeletal muscle mass. Fat-free muscle is 46–47% of the rat FFB and is probably in this range for man (28). In view of the rather wide variations of ECW and ICW concentrations within the individual tissues, it would be best to use caution in employing the FFB uncritically as a reference for these particular measurements in all species or under all circumstances of health, age, and sex.

The more frequent observations of rats in the parallel study helped to define that part of the life span in which the change of ECW content took place. The significant increment of ECW_S/FFB was observed between 362 and 579 days, indicating this change to be a phenomenon of rat "middle life" rather than of senescence. Furthermore, the increase could largely be accounted for by concurrent middle life changes of size and/or ECW content of a few tissues.[1] In the absence of data for human subjects in the 5th and 6th decades, we cannot similarly define the age for the increasing ECW in man. A suggestion that ECW increases in human middle life may be drawn from the data of Norris et al. (32): values for ECW/TBW (which should be proportional to ECW/FFB) increased by some 6% between the 4th and 6th decades in men, and then were approximately stable to the 8th decade, roughly parallel to our serial observations in the male rat.

The difficulties of defining an "extracellular water volume" and the theoretical and technical problems associated with each of the current methodologies have been well reviewed by several investigators (3, 7, 10, 42). The several tissues for which the distribution of sulfate unquestionably fails to define a reasonable extracellular volume are sufficiently small that calculation of body ECW_S is minimally affected; and we have observed these aberrant intratissue volumes to change little with age.[1] Plasma decay rates of radiosulfate were also similar for young and old rats and for young and old humans, suggesting stability of its handling and distribution through adult life and senescence. We have also noted *a*) that the volume of distribution of chloride (ECW_{Cl}) maintains a constant relationship to that of sulfate in young and old rats (ECW_S:ECW_{Cl} = 0.66 − 0.69) and *b*) that the magnitude of the aging increment of ECW_S/FFB was almost identical with that of ECW_{Cl}/FFB. Thus $^{35}SO_4$ appears to measure a physiologically useful volume that is likely to vary proportionally with non-cell water, which is highly reproducible, and which should not be spuriously altered by age-related metabolic changes. With these properties, ECW_S appears to have sufficient "empirical utility" (42) to be a reasonable method for observing changes of extracellular volume with age. The appropriateness, the validity, and the limitations of utilizing the distribution of radiosulfate and of the particular procedure used as a measure of extracellular fluid volume are considered in further detail in another study.[1]

The present body water data, based upon FFB as a standard, lend added support to an hypothesis of stability of FFB with age (21). Total water content was unchanged into the 8th decade. When related to the fat-free body, ICW_S/FFB was only 4% lower for older (vs. younger) men and 5% lower for older women; in both instances the difference with age was not significant (Tables 1–4). These findings in humans were again reflected by those of the male Sprague-Dawley rat suggesting that cell water, some 75% of total cell mass, had remained relatively stable. Therefore, we have not been able to document a loss of total "cellular" water, long a cornerstone of the cell loss hypothesis, either in healthy man into the 8th decade, or in the male rat to 888 days.

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