Technical Papers

Low Energy Counting with a New Liquid Scintillation Solute[1]

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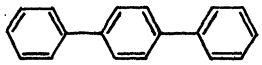
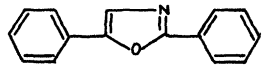
Scintillation studies involving low energy β-emitters such as C^{14} and H^3 require serious consideration of factors rarely encountered in high energy counting.

Conventional photomultipliers with low work function Cs-Sb cathodes (*1, 2*) give large counting rates of dark current in the low amplitude pulse region. Furthermore, Cs-Sb and Ag-Mg (*1*) dynodes are known to emit light on electron bombardment, some of which may not only produce a small amount of regeneration in the originating tube but may also pass over into a coincidence-arranged second tube. These practically coincident pulse phenomena can be referred to as "light dark current."

Our program on C^{14} and H^3 scintillation counting has made use of both coincidence circuitry and refrigeration to decrease dark current. Operation of the photomultipliers and scintillator at 0° C and below does not allow the use of the relatively insoluble *p*-terphenyl (*3*) (I), and therefore has forced us to investigate new and more soluble solutes.



I



II

2, 5-Diphenyloxazole (*4*) (II) has a solubility of 300 g/liter in toluene at room temperature, over forty times as great as *p*-terphenyl. As judged from its variation of RCA 5819 anode current production vs. concentration, 3 g/liter in toluene makes a suitable solution for counting.

This solution will absorb, per centimeter of path through it, more than 10% of light with wavelength less than 368 mμ. It gives a radium-excited scintillation spectrum ranging from 340 to 460 mμ, with maximum intensity at 380 mμ. Similar spectral values for 0.5% *p*-terphenyl in toluene are a range of 320–450 mμ and a maximum of 352 mμ.

The counting apparatus employs a supported removable Pyrex cell viewed by 2 RCA 5819 tubes selected for high signal-to-noise ratio and oriented at 90° to each other in a horizontal plane. This assembly, together with subminiature preamplifiers and 4 in. of iron shielding, was placed in a 6 cu ft refrigerator. The separate outputs were fed into wide band amplifiers and then into fast discriminators with delay line shaped outputs of 0.2 μsec duration. A separate output from one of the amplifiers was taken into a high level discriminator with a pulse width of 4 μsec. The three discriminator outputs were combined in a fast coincidence-anticoincidence circuit, giving passage of low level coincident pulses and rejection of pulses originating in the scintillator of amplitude high enough to be passed by the high level discriminator.

With 30 ml of 0.3% II in toluene and a total amplification of 2500, 35% of the disintegrations from dissolved C^{14}-benzoic acid are recorded as pulses of amplitude between 0.5 and 15 v. Counts/min of total noise include 1–2 of dark current, 20–25 of "light dark current," and 40–60 of radiation background. Use of the dioxane-water solvent and *p*-terphenyl described by Farmer and Berstein (*5*) gave only 7% efficiency with this instrument.

Comparison of compounds for their quenching action on the toluene solution of II gave the order: piperidine > phenol >> pyridine >> cyclohexanone > chlorobenzene >> acetic acid >> chlorocyclohexane = cyclohexanol > cyclohexane > toluene at 10 mole per cent.

A detailed single and mixed solvent study on this compound (II) together with similar studies on more than thirty new solutes will be published shortly.

[1] Work done under the auspices of the Atomic Energy Commission.

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Body Build and Body Composition

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Sheldon's system of somatotyping was originally proposed as a means for describing the over-all body "type," conceived as a fairly fixed, "constitutional" characteristic (*1*). However, it has been clearly shown (*2*) that the somatotype ratings are markedly affected by changes in nutritional status and, in fact, may be considered as partial measures of nutriture. Although this may have eliminated the supposed virtue of the system as providing a permanent (constitutional) index, its possibilities as a measure of nutriture deserve to be critically examined.

Stuart and Sobel (*3*) noted, in passing and without quantitative documentation, the positive relationship between endomorphy ratings and fatness. An individual predominantly endomorphic in his body type has a heavy *panniculus adiposus,* whereas an ecto-

TABLE 1

COEFFICIENTS OF CORRELATION (r) BETWEEN RATINGS OF SOMATOTYPE COMPONENTS AND SPECIFIC GRAVITY. $N = 62$ PAIRS OF VALUES FOR LASKER AND THE HARVARD GROUP

Somatotype component	Lasker	Harvard group
Endomorphy	−.716	−.637
Ectomorphy	+.582	+.601

morphic individual will tend to have a light or thin fold of subcutaneous tissues. Dupertuis *et al.* (*4*) obtained a high negative correlation between specific gravity and the endomorphy ratings in a group of 81 healthy male subjects, selected to include representatives of the extremes of body build. Round and soft, "fat" individuals had a low body density, whereas "lean" people tended to have higher body density. The following coefficients of product-moment correlation were obtained between specific gravity and the ratings of somatotype components: for endomorphy, −0.853; for mesomorphy, +0.167; and for ectomorphy, +0.369.

The present communication is concerned with the relationship between somatotype ratings and specific gravity in a group of individuals studied on two occasions. The subjects were 31 young men, examined under control conditions and after 24 weeks of semi-starvation associated with a loss of one quarter of body weight and a marked loss of body fat (*5*). The men were somatotyped, on the basis of photographs, by Gabriel W. Lasker, Department of Anatomy, Wayne University, and by a group directed by James M. Andrews, IV, at Harvard University (*2*).[1] The ratings were carried out independently and without knowledge of the subject's nutritional status. Both sets of ratings indicated a marked mean decrement in endomorphy, slight decrease in mesomorphy, and a marked increase in ectomorphy. The coefficients of correlation between the two sets of ratings of the three components of the somatotype (endomorphy, mesomorphy, and ectomorphy) were +0.856, +0.622, and +0.862, respectively.

[1] The authors alone are responsible for the utilization and interpretation of the data in this communication.

The coefficients of correlation between the somatotype ratings and specific gravity, with the volume of the body determined by underwater weighing, are presented in Table 1. The correlation is statistically highly significant for endomorphy (negative r) and ectomorphy (positive r). There are factors inherent in the sampling and in the method of measurement which tend to increase the correlation (a marked heterogeneity of the nutritional status), as well as to decrease it (inaccuracy of specific gravity determinations resulting from the use of average correction for residual air). Nevertheless the values are in essential agreement with Dupertuis' observations except for mesomorphy ratings, which yielded low negative values in our material and low positive r values in the sample of Navy men.

Table 2 contains predicted values of specific gravity (and estimated body fat [*6*]) corresponding to different ratings of endomorphy and ectomorphy. This is to indicate in a more concrete way the meaning of the correlation coefficients.

It is not our intention, however, to suggest using somatotype ratings for estimation of body fat. This appears to be a devious and inefficient route, except under special conditions in which direct measurements on the living man were not or could not be made. For these conditions a system of ratings and measurements (based on photographs) is needed that is more directly focused on the evaluation of individual differences in the basic anatomical components of the body than are Sheldon's "components" of the body type. It is the estimation of the absolute and relative amount of fat—which accounts for the largest part of the differences among adult individuals—of muscles, and of bones, which is the principal concern of nutritionally oriented anthropometry.

Elsewhere (*7*) equations were developed for predicting total fatness on the basis of measurements of skinfolds, varying in thickness as a result of different amounts of subcutaneous adipose tissues. This is a simple, objective, and rapid procedure. For younger men the coefficient of multiple correlation between

TABLE 2

PREDICTED VALUES OF SPECIFIC GRAVITY AND BODY FAT FOR YOUNG MEN (AV AGE, 26 YEARS) RATED WITH REFERENCE TO SOMATOTYPE COMPONENTS. THE PREDICTION EQUATION WAS BASED ON COMBINED LASKER AND HARVARD RATINGS ($N = 124$)

	Endomorphy ratings						
	1	2	3	4	5	6	7
Sp gr	1.0938	1.0857	1.0776	1.0695	1.0614	1.0533	1.0452
Fat (%)	2.8	6.6	10.5	14.4	18.3	22.3	26.4
	Ectomorphy ratings						
	1	2	3	4	5	6	7
Sp gr	1.0598	1.0659	1.0720	1.0780	1.0841	1.0902	1.0962
Fat (%)	19.1	16.0	13.1	10.3	7.4	4.7	1.9

specific gravity and skinfolds was 0.871, for older men 0.743, using skinfolds measured at 3 and 4 points of the body surface, respectively. The standard errors of estimate of the specific gravity are 0.0072 and 0.0086.

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Specific Volumes of Proteins and the Relationship to their Amino Acid Contents

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The specific volume of a protein is essential for calculating its molecular weight in solution and for relating the composition of a protein crystal to its density. Values for specific volumes are obtained experimentally from density measurements. Cohn and Edsall (*1*) have, however, described a method for calculating the specific volume of a protein from its amino acid composition, the volume of the protein molecule being considered to be the sum of the volumes of its component groups or atoms. At the time of publication of this method for calculating specific volumes of proteins from their amino acid compositions, the data on the amino acid composition of proteins were incomplete and unreliable. During the past ten years, new methods, such as the use of isotopes, bacteria, and chromatography, in the determination of amino acids have led to reliable and fairly complete amino acid analysis on a large number of proteins. It became of importance and interest, therefore, to test the method for calculating specific volumes of proteins using recent quantitative amino acid composition data. Values obtained for the specific volume of a number of proteins calculated from their amino acid composition are compared in Table 1 with the observed values obtained by density measurements. It may be noted that in most cases the values calculated from the amino acid composition are in excellent agreement with the observed values. The differences between the observed and calculated values for the last three proteins in the table are greater than might be expected in view of the other results and suggest that the amino acid composition and specific volume for these three proteins be redetermined.

The method for calculating a specific volume from the amino acid composition neglects electrostriction that is due to charged groups in the protein molecule; consequently, it might be expected that the calculated value for the specific volume would be higher than that observed. Cohn and Edsall (*1*) calculated that the value of the specific volume of egg albumin in solution would be reduced by 2.4% because of electrostriction. The value for electrostriction in other proteins would vary slightly owing to the number of charged groups in the molecule. Linderstrøm-Lang (*21*) observed that the initial enzymic hydrolysis of a protein involves a large change in volume per mole of peptide bond split (−50 cc). The preponderance of the peptide bonds in the protein, however, was found to give the normal contraction in volume when split (−20 cc); accordingly, the total effect of this volume factor on the specific volume of the protein would not be expected to be large. The excellent agreement between the calculated and observed values for the specific volumes of proteins may be due in part, therefore, to a compensation of variables.

The fact that the values for the volumes of proteins obtained by these two methods agree for such a wide variety of proteins is considered to be good evidence that the volume of a protein molecule in solution is essentially equal to the sum of the volumes of its component groups and that the method of Cohn and Edsall for calculating specific volumes is reliable.

[1] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

TABLE 1

SPECIFIC VOLUME OF PROTEINS

Protein	Sp vol observed* (cc/g)	Sp vol calcd from amino acid composition† (cc/g)
Silk fibroin (suspended in H_2O)	0.701 (*2*)	0.689 (*3*)
Ribonuclease	.709 (*4*)	.703 (*3*)
Wool (suspended in H_2O)	.716 (*5*)	.712 (*3*)
Lysozyme	.722 (*6*)	.717 (*7*)
Fibrinogen (human)	.725 (*8*)	.723 (*3*)
α-Casein	.728 (*9*)	.725 (*9*)
Chymotrypsinogen	.73 (*10*)	.734 (*3*)
Casein (unfractionated)	.731 (*9*)	.731 (*9*)
Serum albumin (bovine)	.734 (*11*)	.734 (*12*)
Insulin (Zn)	.735 (*13*)	.724 (*3*)‡
D-glyceraldehyde phosphate dehydrogenase	.737 (*11*)	.743 (*11*)
Aldolase	.740 (*11*)	.743 (*11*)
β-Casein	.741 (*9*)	.743 (*9*)
Ovalbumin	.745 (*14*)	.738 (*3*)
Hemoglobin (horse)	.749 (*15*)	.741 (*3*)§
β-Lactoglobulin	.751 (*16*)	.746 (*17*)
Botulinus toxin	.75 (*18*)	.736 (*18*)
Gelatin	.682 (*19*)	.707 (*3*)
Edestin	0.744 (*20*)	0.719 (*3*)

* These values were determined at 20° C, or close thereto.
† With the exception of references (*9*), (*11*), and (*18*), the specific volume values have been calculated from the amino acid compositions given in the cited reference. A value of 0.63 cc was used for the volume of the cystine residue instead of 0.61 cc, as given in Cohn and Edsall (*1*).
‡ The specific volume of zinc is not included.
§ The specific volume of hemin is not included.