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.

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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.

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A Note on the Phosphorescence of Proteins

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As the literature on the fluorescence and phosphorescence of proteins is scanty, and since it is of interest to know more about this subject and its relation to protein denaturation, an investigation of the visible light emitted by proteins under ultraviolet excitation has been carried out.

Wels (*1*) and Vlés (*2*) reported that a blue fluorescence was observed when proteins were irradiated with ultraviolet light at room temperature. The intensity of the fluorescence (which is not strong) depends on the pH and the oxygen content of the solution and on the irradiation time. It can be excited by many different wavelengths of the ultraviolet region.

With compact animal materials such as nails, tendons, and cartilage, a distinct blue phosphorescence which lasts about 0.2 sec at room temperature has been reported (*3, 4*). The globular proteins and noncompact body materials such as muscle did not exhibit this phosphorescence.

We have found that many proteins emit a brilliant blue phosphorescence at low temperatures. However, no fluorescence in the visible range has been observed in any of our experiments.

The proteins used in this study were bovine serum albumin, egg albumin, gelatin, human γ_2-globulin, zein, human fibrinogen, silk fibroin, and keratin (human nail). Material containing protein such as bacteria (*Escherichia coli*), commercial yeast, "Witte" peptone, agar, and dehydrated beef muscle show the same phosphorescent properties as the individual proteins. The emission was observed with solid protein, with suspensions, and with solutions.

In order to find out which groups in the proteins are active, 18 amino acids were investigated. Of these, only the 3 common aromatic amino acids (tyrosine, tryptophane, and phenylalanine) gave indications of characteristic emissions. However, the remaining 15, including histidine, showed weak blue emissions which had the characteristics of those from tyrosine and tryptophane. Since it was found that as little as 10^{-9} g of tyrosine gives a discernible blue phosphorescence, it is our opinion that the blue emissions of these 15 are caused by trace amounts of the aromatic amino acids. Indeed, it seems that phosphorescence is a sensitive detector of certain impurities.

These experiments, unless stated otherwise, were carried out at the temperature of liquid nitrogen (77° K). A General Electric AH-6 mercury-vapor arc was used as the source of ultraviolet light. For the kinetic studies, an RCA 5819 multiplier phototube and either an oscillograph or a galvanometer have been employed, depending on the rate of decay. The spectra were determined with a Hilger constant deviation spectrograph and Eastman Kodak spectrographic plates.

At any particular pH, there are at least two exponential decay emissions from the majority of the proteins. Results with the oscillograph, although complicated, indicate that the lifetimes[3] are about 3 sec. Some experiments at the temperature of dry ice (193° K) were less complicated, and it was found possible to prove the monomolecular nature of the decay, the semilog plots being consistent and the decay constants being reproducible at that temperature.

The amino acid tryptophane has a bluish-white phosphorescence with a lifetime of about 3 sec at all pH values. The phosphorescence of tyrosine is brilliant and deep blue; it has a lifetime of about 3 sec in neutral and acid solutions, whereas the lifetime in alkaline media is 0.9 sec. The emission of phenylalanine also seems to be bluish-white, but its lifetime is much shorter, probably being less than 0.1 sec.

The visible spectrum of the protein phosphorescence is dependent on the pH, which fact may be attributed to the association of protons to the aromatic amino acids (*5*). In Table 1, some of the features of protein phosphorescence in alkaline media are pre-

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[3] The mean lifetime of an exponential decay is that amount of time necessary for the phosphorescence to fall to $1/e$ of its initial intensity.