[CONTRIBUTION FROM THE DEPARTMENT OF MEDICINE, COLLEGE OF PHYSICIANS AND SURGEONS, COLUMBIA UNIVERSITY, AND THE PRESBYTERIAN HOSPITAL]

The Viscosity–Fluidity Relations of Proteins[1]

BY HENRY P. TREFFERS

In the course of an investigation on the properties of antibody solutions, comparison has been made with the viscosities of related constituents in normal animal sera. Numerous empirical equations have been proposed[2] to account for the variation of the viscosity with protein concentration but singularly enough, in spite of its wide application elsewhere,[3] little or no serious use of the fluidity function has been made in protein chemistry. As this relation, in addition to its advantages of simplicity and ease of application, fits the available data quite well, it would seem to deserve further mention.

If the relative viscosity of a solution be expressed by η/η_0, where η is the viscosity of the solution and η_0 the viscosity of the solvent, the relative fluidity $\varphi_{rel.}$ is defined by the reciprocal

$$\varphi_{rel.} = \eta_0/\eta \qquad (1)$$

The fluidity is thus obtained directly from the experimental measurements with no more calculation than is required for the relative viscosity.

In Fig. 1 data on two proteins of rather widely different viscosity–concentration relations, egg albumin,[4] and a horse serum globulin, PII,[2c] have been plotted according to three representative equations. Curves 1 and 2 represent the fluidities, as in equation (1), curves 3 and 6 the logarithms of η/η_0 (Arrhenius relation[2a]), as plotted against the protein concentration. Curves 4 and 5 are a modification of the Einstein equation in which $\eta/\eta_0 - 1$ has been plotted against the quantity KVC, in which K is a constant, V the partial volume, and C the concentration.[2c,5] The abscissas have all been set equal, which necessitates, however, that curve 5 be shorter than curve 4.[6]

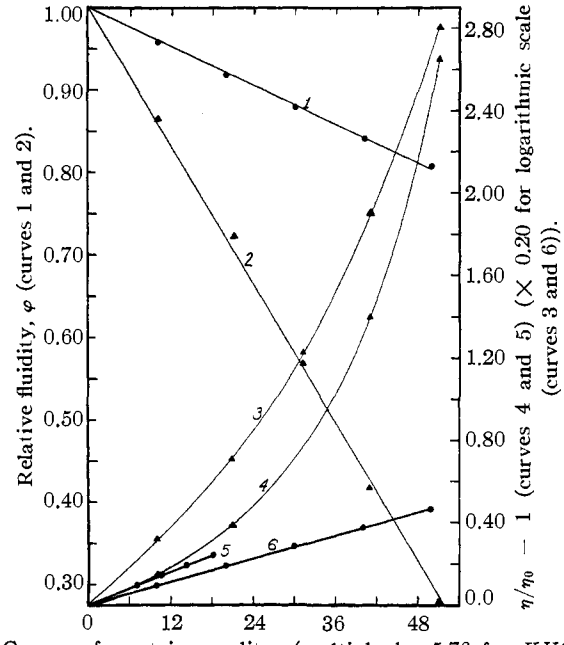

Fig. 1.—Viscosity and fluidity of egg albumin and horse serum globulin using three different equations: Curve (1) fluidity of egg albumin; (2) fluidity of serum globulin; (3) log η/η_0, globulin; (4) $\eta/\eta_0 - 1$, globulin; (5) $\eta/\eta_0 - 1$, egg albumin; (6) log η/η_0, egg albumin.

The utility of the fluidity function is seen from the fact that practically all protein solutions for which data are available in the literature yield a satisfactory linear plot for the relative fluidity φ against the protein concentration expressed in grams per liter of solution. The examples presented in Fig. 2 were selected for their representative slopes rather than for their agreement with the relation presented; nevertheless, the average deviation of all points from their respective "least square" lines is only 0.54%. In this calculation a deviation of the experimental fluidity 0.805 from the calculated 0.809 is taken as 0.5%.

Unfortunately only a few measurements are available at relatively high protein concentrations. Several of these, over their linear range, are given in Fig. 3. At sufficiently high concentrations of some proteins the measured fluidities lie above the line drawn through the lower points, but a comparison of the figures shows that the linearity

(1) The work reported in this communication was presented before the Division of Biological Chemistry at the Boston meeting, September, 1939, and was carried out under the Harkness Research Fund of the Presbyterian Hospital.

(2) Literature reviewed in (a) Cohn, *Physiol. Rev.*, **5**, 423 (1925); (b) Bredée and de Booys, *Kolloid-Z.*, **79**, 31 (1937); (c) Fahey and Green, THIS JOURNAL, **60**, 3039 (1938); (d) Greenberg, in "Chemistry of the Amino Acids and Proteins," ed. Schmidt, C. C. Thomas, Springfield, Ill., 1938, p. 452.

(3) Bingham, "Fluidity and Plasticity," McGraw-Hill Book Co., Inc., New York, N. Y., 1923.

(4) Polson, *Kolloid-Z.*, **88**, 51 (1939).

(5) Daniel and Cohn, THIS JOURNAL, **58**, 415 (1936).

(6) (a) Cohn and Prentiss, *J. Gen. Physiol.*, **8**, 622 (1925–1928); (b) Kunitz, Anson and Northrop, *ibid.*, **17**, 365 (1934); (c) Achard, Boutaric and Thevenet, *Compt. rend.*, **204**, 928 (1937).

covers a useful working range which compares favorably with that provided by other relations. Deviations at very high concentrations may represent a failure of the relation, or perhaps a departure of the behavior of the solution itself from that of true viscous flow. For some reason which is not clear, the data on casein solutions do not pass through the origin. Tobacco mosaic virus protein solutions give a linear relation for six out of the seven points given by Lauffer,[7] *i. e.*, up to 1.0 mg. of protein per cc.[8]

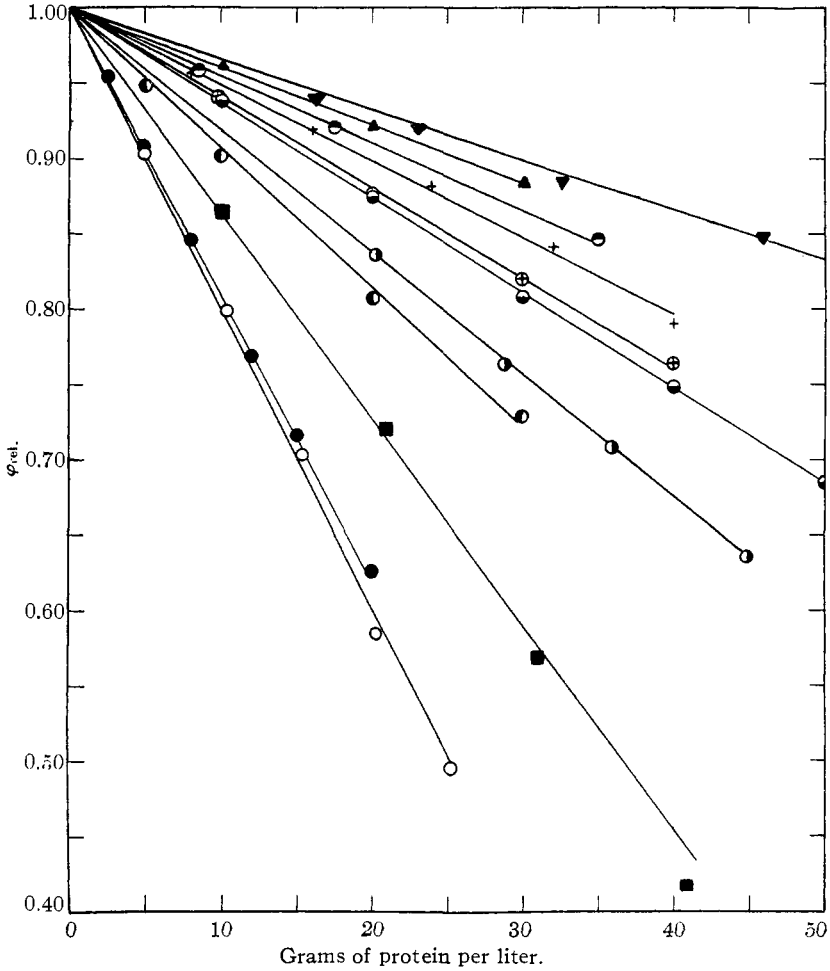

Fig. 2.—Fluidities of representative protein solutions. ▼, oxy-hemoglobin[6a]; ▲, CO-hemoglobin[4]; ◓, homarus hemocyanin[4]; +, trypsin[6b]; ⊕, octopus hemocyanin[4]; ◒, thyroglobulin[4]; ◑, horse pseudoglobulin, ■, PII, ○, PIII (horse globulin fractions)[2c]; ◐, gliadin[4]; ●, beef globulin.[6c]

The concentration of protein has been expressed in all cases in grams per liter rather than as the volume fraction, since the necessary density data were not always available. The volume fraction is probably the more general function on both theoretical and experimental grounds,[8] but since the concentration in grams per liter is proportional to it, it may be used in practice, provided that the partial specific volume of the protein remains constant. This constancy may be verified by plotting the specific volume against the weight fraction, which should yield a linear plot.[9]

Since the origin is always unity, each line may be determined in principle by a single accurate measurement, permitting the fluidity or, if desired, its reciprocal the viscosity, to be determined easily for any other concentration. However, two or more additional points are desirable to ensure greater accuracy and to establish the validity of the linear relation over the range in question.

That the linear relation holds for denatured as well as for native proteins, in spite of the change in absolute values, is seen when the viscosity data of Neurath and Saum[10] are converted to the corresponding fluidities. The resulting family of lines represents the behavior of horse serum albumin dissolved in urea solutions, a denaturing solvent (Fig. 4).

The relation holds as well for a protein which has been modified by the introduction of new chemical groupings, in this case a phosphorylated egg albumin[11] (Fig. 5).

In addition to providing a convenient interpolation formula, the fluidity method appears to offer a convenient tool in studying protein mixtures.

Experimental

Globulin prepared by half-saturation of diluted normal rabbit serum with ammonium sulfate was dialyzed for several days against frequent changes of distilled water.

(7) Lauffer, *Science*, **87**, 469 (1938).
(8) Loeb, *J. Gen. Physiol.*, **4**, 73 (1921–1922).
(9) Lewis and Randall, "Thermodynamics," McGraw-Hill Book Co., Inc., New York, N. Y., 1923, pp. 38–40.
(10) Neurath and Saum, *J. Biol. Chem.*, **128**, 347 (1939).
(11) Heidelberger, Davis and Treffers, in press.

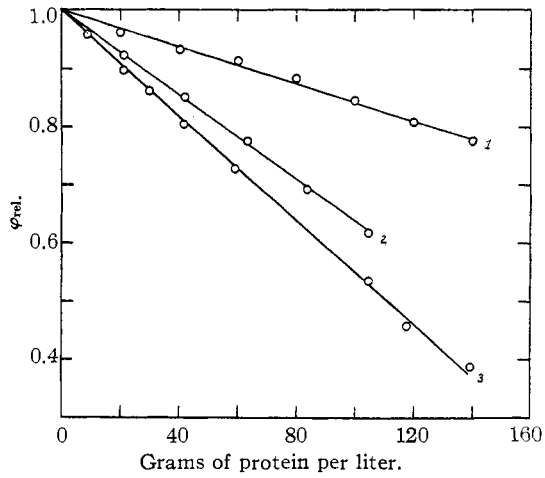

Fig. 3.—Fluidities at higher protein concentrations: Curve 1, egg albumin[8]; curve 2, CO-hemoglobin[6b]; curve 3, serum albumin.[20]

The portion which precipitated is designated euglobulin, the soluble material as pseudoglobulin. In order to avoid effects due to variation in the *p*H and salt concentration, both fractions were arbitrarily made up in solutions at *p*H 5.70 and 0.15 *M* potassium chloride. Several mixtures of the two solutions were made and the observed fluidities, compared with the calculated values from the fluidities of the separate components, are given in Table I.

As an albumin-globulin system was desired, a rather weak immune horse serum was centrifuged clear of lipid at 0° and an equal volume of half-saturated ammonium

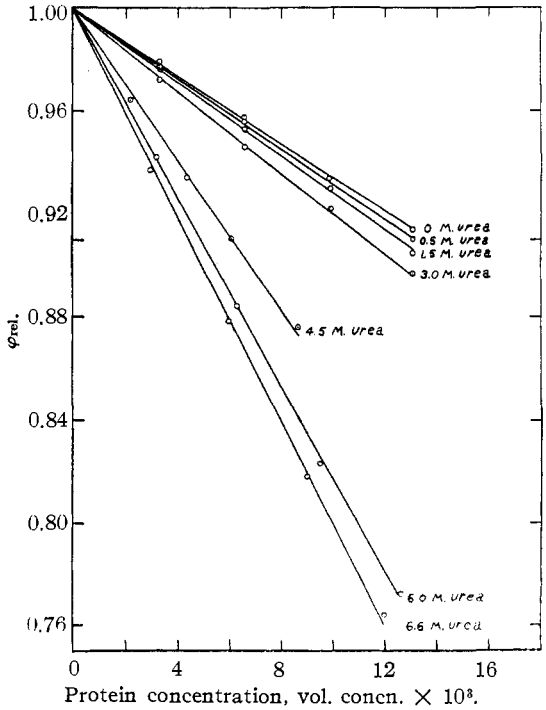

Fig. 4.—Fluidity of horse serum albumin in various urea solutions (Neurath and Saum[10]).

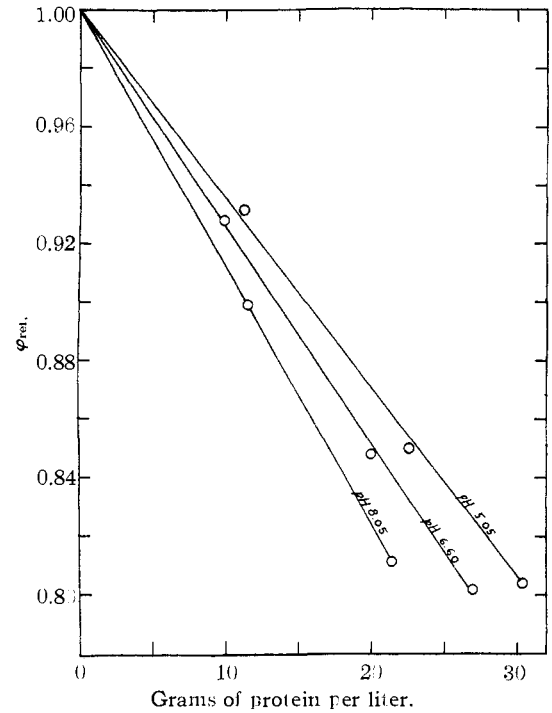

Fig. 5.—Fluidity of phosphorylated egg albumin at various hydrogen-ion concentrations.[11]

TABLE I

FLUIDITIES OF RABBIT PSEUDO- AND EUGLOBULIN MIXTURES AT 25.0°

Pseudoglobulin stock solution, 2.89 mg. of N/ml., rel. density 1.004. Euglobulin stock solution, 3.07 mg. of N/ml., rel. density 1.004.

Ratio pseudo/euglobulin solutions	Fluidity found	Fluidity calcd. eq. (4)
100 : 0	0.883	...
80 : 20	.862	0.867
64 : 36	.858	.855
53.3 : 46.7	.848	.847
43 : 57	.842	.838
0 : 100	.807	...

sulfate solution added. The supernatant albumin solution and the redissolved total globulin precipitate were dialyzed separately against 0.15 *M* potassium chloride until free of sulfate, and then adjusted to *p*H 5.70. Two series of mixtures were made, one from a concentrated albumin solution A and the globulin solution C, and the other from a more dilute albumin solution B with the same globulin solution C (Fig. 6). As Bingham has pointed out,[3] the fluidities of an ideal mixture do not in general lie exactly along a line joining the fluidities of the two separate components because of the difference between weight and volume concentrations. When the densities of the two component solutions are very close, however, the correction may be quite negligible, as is the case here.

Discussion

From the linear relation presented, the fluidity

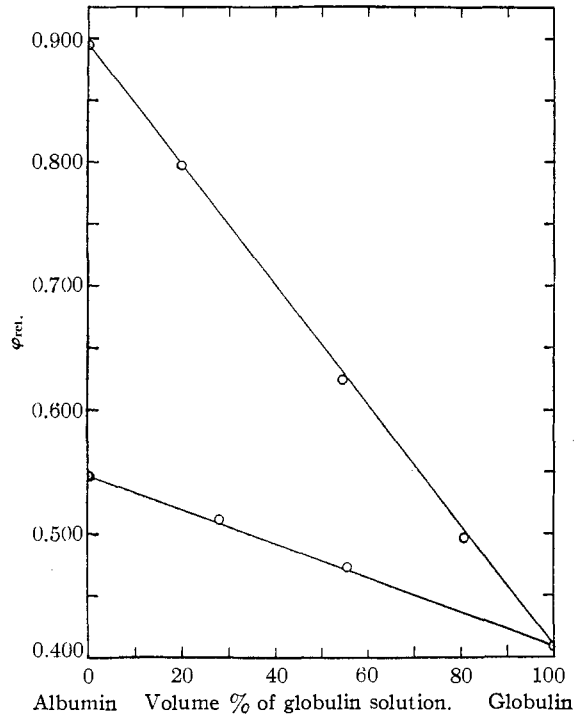

Fig. 6.—Fluidities of mixtures of a horse globulin solution with a horse albumin solution of two different concentrations.

of solutions of the water soluble globulin of concentration C_1 may be expressed as

$$\varphi_1 = 1 - k_1C_1 \qquad (2)$$

and that of the water insoluble euglobulin as

$$\varphi_2 = 1 - k_2C_2 \qquad (3)$$

where the k's are the respective slopes.

In the absence of any specific interaction between the two proteins the fluidity of a mixture of the two is therefore given by

$$\varphi_{mix.} = 1 - k_1C_1 - k_2C_2 \qquad (4)$$

Alternatively, for mixtures of protein solutions which do not differ greatly in density, the fluidities may be represented directly by a graphical method, as in Fig. 6.

Nugent and Towle[12] have outlined a method for the determination of albumin/globulin ratios—a quantity of some clinical importance—by the comparison of the viscosity at known total protein concentrations with that of standard curves for known mixtures. The modification employing fluidities, as shown here, should be somewhat more direct in application since an implicit value for the ratio is given by solving eq. (4) for the ratio C_1/C_2, which gives

$$\frac{C_1}{C_2} = r = \frac{A}{1 - A} \qquad (5)$$

where

$$A = \frac{\frac{1 - \varphi_{mix.}}{C_{total}} - k_2}{k_1 - k_2}$$

The ratio is thus given directly from the fluidity of the mixture, and the total protein concentration when the slopes of the fluidity lines of the separate components are known. For simplicity only two components have been assumed but there is nothing in the treatment to limit extension to systems of three or even more components.

Although no completely satisfactory theoretical justification is available at present for the use of the fluidity rather than the viscosity, comparison of the figures indicates the marked regularities which obtain with the fluidity. The graphical expression of experimental data is thus much easier, especially if it is desired to calculate a least square fit.[13]

It is not without interest that this single, simple relation should hold for such a diversity of protein solutions, and up to concentrations as high as 14% in some cases. The examples presented in Figs. 2 and 3 cover an important range of molecular weights, from that of gliadin 27,400, to that of octopus hemocyanin 2,785,000. The molecular shape factor, as given by the axial ratio, likewise varies from a value of 3.84 for egg albumin to 10.64 for gliadin.[4] Some of the solvents represented are half-saturated magnesium sulfate (trypsin), 0.85 M sodium chloride (beef globulin), and 61 vol. % alcohol (gliadin). Other examples, not given here, show that the same protein solution may give linear fluidity curves at several temperatures and, as indicated in Fig. 5, at various hydrogen-ion concentrations.

The ultimate dependence of the viscosity on the volume occupied by the protein molecules has been pointed out by numerous authors.[3,4] Furthermore, Kuhn, Polson and others have demonstrated that there is a close connection between the axial ratio and the viscosity.[14] It might be expected therefore that if a compound were to be

(12) Nugent and Towle, *Proc. Soc. Expll. Biol. Med.*, **33**, 374 (1935).

(13) For this special case, in which the line passes through the origin, the least square slope is given by the expression

$$\text{slope} = [\Sigma(C\varphi) - \Sigma(C)]/\Sigma(C^2)$$

in which each term denotes the summation of the corresponding column of data.

(14) A preliminary study indicates that, like the Polson relation for a viscosity function, the relation between the *slope* of the fluidity line and the square of the axial ratio may be a linear one for most proteins.

formed between two proteins, or a change in the state of aggregation were to occur, the volume fraction, or more especially the effective shape of the protein components, would be changed with a consequent deviation of the fluidity from that predicted on an additive basis (*cf.* Chap. V, ref. 3). The data in Table I and in Fig. 6 do not indicate such deviation, within the experimental error (*cf.* also ref. 2a). Nor do density determinations on beef albumin–globulin mixtures show any variation from strict additivity.[15] Even such a complicated mixture as serum yields a constant average partial specific volume, over the entire range, if one plots the density data of Chick and Lubrzynska.[16] A fair linear fluidity curve up to and including physiological concentrations of protein may also be obtained for this system.

Further discussion of this question, especially as to its bearing on the interesting effects observed in the ultracentrifuge by McFarlane[17] and by Pedersen[18] must await more accurate fluidity data, particularly on protein containing systems in which known compound formation and dissociation occur.

The author wishes to express his appreciation to Dr. Michael Heidelberger for his aid in the preparation of this paper.

(15) Nugent and Towle, *J. Biol. Chem.*, **104**, 395 (1934).
(16) Chick and Lubrzynska, *Biochem. J.*, **8**, 59 (1914).
(17) McFarlane, *ibid.*, **29**, 407 (1935).
(18) Pedersen, *Compt. rend. trav. lab. Carlsberg*, **22**, 427 (1937).

Summary

1. It has been found that the fluidity, or reciprocal of the relative viscosity, is linear with the protein concentration over a wide range of proteins and concentrations.

2. The fluidity provides a convenient interpolation formula for converting the fluidity or viscosity obtained at one concentration to that at any other.

3. It is shown that the fluidity is additive for mixtures of rabbit eu- and pseudoglobulin, and for horse albumin and globulin fractions.

4. The relation of additive fluidities to the presence of compounds in mixtures of proteins is discussed.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, COLUMBIA UNIVERSITY]

Ascorbic Acid Oxidase from Summer Crook-neck Squash (*C. pepo condensa*)

BY P. L. LOVETT-JANISON AND J. M. NELSON

In 1928 Szent Györgyi[1] observed that certain plant tissues catalyzed the aerobic oxidation of ascorbic acid, and in 1930[2] he postulated the existence of a specific enzyme in cabbage leaves. Since then, other investigators[3,4,5,6,7] have reached the same conclusion working with different plant sources, such as apples, Hubbard squash, Drumstick, cauliflower, bananas, cabbage, carrots, cucumbers, potatoes and string beans.

Barron, DeMeio and Klemperer[8] were among the first to show that the aerobic oxidation of ascorbic acid was catalyzed by ionic copper, and in a later paper[9] they questioned the enzymic nature of ascorbic acid oxidase. Stotz, Harrer and King,[10] working upon this problem, were inclined to attribute the catalytic activity of squash juice to copper in combination with protein material, rather than to a specific oxidase. On the other hand, Spruyt and Vogelsang[11] working with fruit juices concluded that, "ascorbic acid oxidation and copper do not go hand in hand and that this oxidation is certainly not exclusively to be ascribed to the presence of copper."

From the above, it is obvious therefore that some confusion arises. It is the object of this paper to show that ascorbic acid oxidase is indeed a true enzyme, consisting of a copper-protein, similar in type to tyrosinase (polyphenol oxidase), which has been recognized as a true enzyme.

Purification of Enzyme from Summer Squash.—After experimenting with cucumber, cauliflower, summer squash, apples, parsnips, carrots, spinach, string beans, cabbage,

(1) A. Szent Györgyi, *Biochem. J.*, **22**, 1387 (1928).
(2) A. Szent Györgyi, *Science*, **72**, 125 (1930).
(3) S. S. Zilva, *Biochem. J.*, **28**, 663 (1934).
(4) H. Tauber, I. S. Kleiner and D. Mishkind, *J. Biol. Chem.*, **110**, 211 (1935).
(5) M. Srinivasan, *Biochem. J.*, **30**, 2077 (1936).
(6) F. G. Hopkins and E. J. Morgan, *ibid.*, **30**, 1446 (1936).
(7) W. Stone, *ibid.*, **31**, 508 (1937).
(8) E. S. G. Barron, R. H. DeMeio and F. Klemperer, *J. Biol. Chem.*, **112**, 625 (1935–1936).
(9) E. S. G. Barron, A. G. Barron and F. Klemperer, *ibid.*, **116**, 563 (1936).
(10) E. Stotz, C. J. Harrer and C. G. King, *ibid.*, **119**, 511 (1937).
(11) J. P. Spruyt and G. M. D. Vogelsang, *Arch. Nêrland Physiol.*, **23**, 423 (1938).