fact that the non-reducing neutral oligosaccharides, sucrose, raffinose, stachyose, and the α- and β-Schardinger dextrins display considerable mobility, and like the free sugars, glucose and maltose, show no evidence of heterogeneity.

Electrophoretic separations were carried out on strips of glass-fiber paper (7 × 40 cm.) at 120 volts and 110 milliamperes for 10 hours:[15] The mobilities of the polysaccharide components are expressed conveniently as the ratio of the distance of migration of the component from the origin to the distance moved by the faster and major component of calf liver glycogen whose mobility is taken as 100. In a typical experiment the two components of glycogen moved 4.0 and 0.4 cm., thus having mobilities of 100 and 10, respectively. Likewise the three components of the seaweed polysaccharide from *Laminaria cloustoni* had mobility values of 10, 105 and 130 whereas the apparently homogeneous polysaccharides, birch wood xylan, sugar beet galactoaraban, Dahlia inulin and levosine (from rye flour) have mobility values of 110, 160, 130 and 140, respectively.

The components of a number of the above polysaccharides are now being separated on a larger scale with the object of ascertaining the structural significance of the phenomenon of heterogeneity as revealed by glass paper electrophoresis. It seems evident even at this early stage of the work that heterogeneity in polysaccharides especially those of fairly high molecular weight may be the rule rather than the exception. It is also conceivable that this phenomenon is analogous to the heterogeneity already recognized in proteins[16] and it may have some bearing on the specificity of the chemical components of the various species of plants and animals.

(15) The glass fiber sheets were obtained through the courtesy of Dr. R. B. Hobbs of the Bureau of Standards, Washington, D. C., to whom the authors express their thanks.

(16) J. R. Colvin, D. B. Smith and W. H. Cook, *Chem. Rev.*, **54**, 687 (1954).

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RECEIVED FEBRUARY 7, 1957

THE ACID DENATURATION OF FERRIHEMOGLOBIN[1]

Sir:

A series of important studies by Steinhardt and Zaiser[2] has demonstrated that hemoglobin undergoes a drastic configurational change below pH 4. The reaction is slow enough to permit spectroscopic and titrimetric observation of both the native and the altered form near pH 3.5. It is characterized by a change in absorption spectrum, and by the binding of about 36 protons per molecule (mol. wt., 67,000); *i.e.*, near pH 3.5 the "denatured" form contains about 36 more bound protons than the native form.

This communication reports the viscosity change accompanying the reaction. Figure 1 shows the data obtained from flow times in an Ubbelohde viscometer. To obtain intrinsic viscosities ($[\eta]$) from these data one must add 0.5 to the limiting value of the ordinate at zero concentration, so as to correct for the density difference between solution and solvent[3] and for the kinetic energy of the effluent solution. One obtains $[\eta]$ = 3.5 cc./gram for the native protein in neutral solution, in good agreement with a determination by Cohn and Prentiss.[4] At pH 3.5, after the configurational change has occurred, $[\eta]$ = 13.5 cc./gram. The configurational change thus clearly involves a marked expansion of the protein molecule, much like that observed for serum albumin.[5] Hemoglobin dissociates into half-molecules, at a pH much higher than that at which the configurational change occurs.[6] If this dissociation is assumed to occur without appreciable change in $[\eta]$, then, by Einstein's equation, the radius of an equivalent sphere (for half-molecules) is 26.5 Å. in the native state and 41.5 Å. in the expanded state.

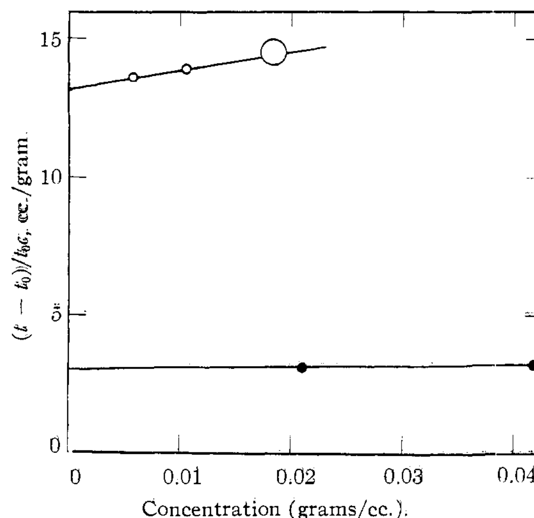

Fig. 1.—Viscosity data for native ferrihemoglobin (●) and for the same protein exposed to pH 3.5 for about 10 to 15 minutes (O). t represents flow time for the solution of concentration c, and t_0 flow time for solvent. All measurements were performed at ionic strength 0.04 at 25°.

The finding here reported is of special interest because it suggests a simple explanation for the uptake of protons observed by Steinhardt and Zaiser.[2] For expansion implies penetration of solvent into the molecular domain, which profoundly affects the titration curve by reducing all electrostatic interactions.[7] Specifically, the factor w, which occurs in the usual equation for the titration curve,[5,7] becomes much smaller and the titration curve itself becomes steeper. Equations derived earlier[7] enable this effect to be calculated if a spherical model is assumed. For spherical half-molecules with the radii given above we get, at 25°

(1) This work was supported by research grant RG-2350 from the National Institutes of Health, Public Health Service.

(2) Summarized by J. Steinhardt and E. M. Zaiser, *Advances in Protein Chem.*, **10**, 186 *et seq.* (1955).

(3) C. Tanford, *J. Phys. Chem.*, **59**, 798 (1955).

(4) E. J. Cohn and A. M. Prentiss, *J. Gen. Physiol.*, **8**, 619 (1927).

(5) J. T. Yang and J. F. Foster, THIS JOURNAL, **76**, 1588 (1954); C. Tanford, J. G. Buzzell, D. G. Rands and S. A. Swanson, *ibid.*, **77**, 6421 (1955).

(6) E. O. Field and J. R. P. O'Brien, *Biochem. J.*, **60**, 656 (1955).

(7) C. Tanford, *J. Phys. Chem.*, **59**, 788 (1955).

and ionic strength 0.02, $w = 0.065$ in the native state and $w = 0.023$ in the expanded state. These values of w require that the configurational change, at this temperature and ionic strength, at pH 3.5, should be accompanied by the binding of 29 protons per 67,000 g. If allowance is made for the fact that the radius of the expanded form is probably larger at ionic strength 0.02 than at ionic strength 0.04 (*cf.* serum albumin[5]), then the calculated proton uptake is increased to about 34. It thus appears that most, and perhaps all of the observed uptake of 36 protons can be accounted for.

Ferrihemoglobin used in this study was prepared from crystalline horse oxyhemoglobin (kindly furnished by Dr. J. H. Wang) by the method of Steinhardt and Zaiser.[8] The author is indebted to Dr. E. P. Geiduschek and Mr. G. Saliba for invaluable assistance in performing these measurements.

(8) J. Steinhardt and E. M. Zaiser, THIS JOURNAL, **75**, 1599 (1953).

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RECEIVED JUNE 3, 1957

S-(DICHLOROVINYL)-L-CYSTEINE: AN AGENT CAUSING FATAL APLASTIC ANEMIA IN CALVES[1]

Sir:

We are reporting the synthesis of the compound S-(dichlorovinyl)-L-cysteine which upon oral administration produces a fatal aplastic anemia in young calves similar to that which we have observed with toxic trichloroethylene-extracted soybean oil meal (TESOM).

S-(Dichlorovinyl)-L-cysteine was synthesized by treating equimolar quantities of trichloroethylene (TCE) with the disodium salt of L-cysteine in liquid ammonia by a modification of the method of du Vigneaud.[2] The product precipitated on adjusting the pH to 5.0 with acetic acid and was crystallized from water–ethanol, 60–70% yield; m.p. 155–156° (decomp.). *Anal.* Calcd. for $C_5H_7O_2NSCl_2$: C, 27.29; H, 3.27; N, 6.48; S, 14.84; Cl, 32.82. Found: C, 27.83; H, 3.29; N, 6.47; S, 14.9; Cl, 32.8. *Ultraviolet absorption* in water: λ max. 210 mμ, $\epsilon = 8600$; λ max. 258 mμ, $\epsilon = 3200$. *Titration.* Neut. equiv., calcd. 216; found, 216; pI', 5.4. *Paper chromatography.* 70:30 1-propanol–H_2O on Whatman No. 1. R_f 0.72; positive ninhydrin, 4-(p-nitrobenzyl)-pyridine (4-NBP),[3] ultraviolet and iodoplatinate tests.

Preliminary data indicate that the compound is S-(1,2-*trans*-dichlorovinyl)-L-cysteine.

The biological response of young calves to oral administration of S-(dichlorovinyl)-L-cysteine was studied by the methods established for the toxicity assay of TESOM.[4] The assay results are shown in Table I. The 500 and 200 mg./100 lb./day dosage levels were not tolerated by the assay calves and had to be discontinued after 5 and 7 days, respectively; however, all dosage levels tested produced the complete clinical, hematologic and postmortem picture of severe aplastic anemia of the bovine.[4]

TABLE I

BIOLOGICAL RESPONSE OF CALVES TO ORAL ADMINISTRATION OF S-(DICHLOROVINYL)-L-CYSTEINE

Dosage mg./100 lb./day	Days to develop						
	Thrombocytopenia[a]	Leucopenia[b]	Lymphocytosis[c]	Elev. temp.	Fecal blood	Visible hemor.	Death[d]
500	10	9	13	13	13	11	14
200	12	10	10	11	11	13	14
50	14	19	21	21	17	19	23
20	18	22	22	25	25	22	27
10	20	27	31	51	27	26	60

[a] Platelet count below 200,000. [b] Leucocytes below 5000. [c] Over 85% lymphocytes. [d] Necropsied when moribund. Typical severe lesions of aplastic anemia.

We recently reported[5] on fractionation studies of toxic TESOM which indicated that the aplastic anemia causing entity is associated with the protein component of the meal. Analyses indicated that toxic TESOM contained 0.5 mole less sulfhydryl groups per 10^6 g. and about 25 p.p.m. more chlorine than did hexane-extracted meal from the same beans. In addition evidence that TCE would react with cysteine was obtained from sealed tube experiments. Of fourteen amino acids tested, only cysteine gave more than one ninhydrin spot by paper chromatography and one of these absorbed ultraviolet light and gave a positive 4-NBP test. Likewise reduced glutathione liberated chloride equivalent to its sulfhydryl content in sealed tube reactions and gave strong spots of R_f 0.50 and 0.70 which absorbed ultraviolet light and were 4-NBP positive. These facts and the knowledge that heat is required for the apparent interaction of TCE with soybean flakes to form toxic TESOM suggested the attempt to interact TCE with cysteine on a larger scale suitable for characterization and assay of the product.

Oral administration of S-(dichlorovinyl)-L-cysteine at the 20 and 10 mg./100 lb./day levels produces the typical syndrome of aplastic anemia in calves consuming levels of toxic TESOM'S ranging from $^3/_4$ to $^1/_5$ lb./100 lb./day and to samples of our isolated toxic protein at levels ranging from $^3/_8$ to $^1/_{10}$ lb./100 lb./day.

Paper chromatograms of fractions from a proved toxic pancreatic digest of TESOM's protein show ultraviolet absorbing 4-NBP and S positive spots with an R_f 0.45 and 0.70 with 70–30 1-propanol–H_2O on Whatman no. 1 paper. Similarities of the chemical and physical characteristics of these spots with those of S-(dichlorovinyl)-L-cysteine and its derivatives (TCE + glutathione) plus the observation that S-(dichlorovinyl)-L-cysteine pro-

(1) A report of work done, in part, under contract with the U. S. Department of Agriculture and authorized by the Research and Marketing Act. Contract supervised by Northern Utilization Research and Development Division, Agricultural Research Service.

(2) V. du Vigneaud and W. F. Patterson, *J. Biol. Chem.*, **114**, 533 (1936).

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