relative to DNA. If this structural proposal is the correct one, it may be possible for ribonucleic acid, also, to assume a double-stranded configuration in solution through pairing of only the adenine nucleotides.[6]

(6) We are very much indebted to Mrs. Elizabeth Klemperer who was reponsible for many of the measurements in this investigation.

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INCORPORATION OF THE CARBON CHAIN OF METHIONINE INTO SPERMIDINE[1,2]

Sir:

The cumulative work of several laboratories has indicated the metabolic importance of the polyamines putrescine, spermidine and spermine in diverse biological systems. Tabor, *et al.*,[3] have shown that putrescine is the source of the four-carbon chain of spermidine and spermine. Recent results obtained by the author show that the side chain of methionine is an efficient precursor of the three carbon chain of spermidine in *Neurospora crassa*.

Neurospora crassa strains 74A-3b (wild type) and 38706 (blocked in the conversion of homocysteine to methionine) were grown at room temperature on Fries minimal medium supplemented with 50 μg./ml. of DL-methionine-2C^{14}. The spermidine was isolated by grinding the mold with sand and 5% TCA[4] and chromatography of the extract on 0.6 × 20 cm. columns of Dowex 50-X2 using an HCl gradient. The spermidine containing fractions were combined and dried; spermidine trihydrochloride was crystallized from methanolic hydrogen chloride and ethyl acetate. The specific activities of the added methionine and the isolated spermidine are compared in Table I. Amine concentrations were determined as described by Rosenthal and Tabor.[5] Radiopurity of the isolated spermidines was checked by paper ionophoresis in *p*H 8.2 borate buffer and by descending paper chromatography (3 parts 1-propanol:1 part 0.2*M* sodium acetate buffer). In all cases the radioactivity moved in one spot and was in the same position as added authentic spermidine trihydrochloride.

TABLE I

Strain	Specific Activities: Methionine (initial) c.p.m./μmole	Specific Activities: Spermidine (isolated) c.p.m./μmole	Ratio Spermidine/ Methionine
74A-3b	6.36×10^4	3.38×10^4	0.53
38706	11.4×10^4	6.34×10^4	0.56

Spermidine was degraded by oxidation with alkaline permanganate. The small yield of succinate, from the four carbon chain, was isolated by chromatography on Dowex 1[6] and on paper using aqueous phenol.[7] The succinate isolated from the oxidation product of synthetic spermidine,[8] labeled in the four carbon chain, contained about 33% of the radioactivity put on the Dowex 1 column, while less than 0.5% of the radioactivity was found in the succinate from the oxidation products of isolated spermidine and synthetic spermidine[8] labeled in the three carbon chain. The low level of activity in the succinate shows that the isolated spermidine is not labeled in the four carbon chain and is consistent with labeling in the three carbon chain.

TABLE II

*p*H	Total radioactivity in spermidine fractions, c.p.m. + ATP	− ATP
7.2	2.1×10^4	520
8.2	2.5×10^3	795

Incubation, four hours at 37°; incubation mixture, 100 μg. 2C^{14}-DL-methionine (3.8×10^5 c.p.m.) per vessel, 0.01 *M* putrescine, 0.008 *M* glutathione, 0.003 *M* $MgCl_2$, 0.00001 *M* pyridoxal phosphate, 0.1 *M* buffer, 2.5 ml. 1:1 extract of 74A-3b, 0.02 *M* K_4ATP as indicated, total volume 5 ml.; buffers are *p*H 7.2, potassium phosphate + Tris hydrochloride (1:1), *p*H 8.2, Tris hydrochloride.

A possible mechanism for this reaction is the transfer of the methionine side chain from S-adenosyl methionine to putrescine in a manner similar to the transfer of the methyl group of this compound first observed by Cantoni.[9] The almost absolute ATP requirement for the incorporation of C^{14} methionine into spermidine by a cell free extract of 74A-3b as shown in Table II is consistent with this hypothesis. Further evidence for the presence of radioactivity in the spermidine from the *p*H 7.2 incubation mixture was obtained by paper ionophoresis in *p*H 8.2 borate buffer. Tabor, *et al.*,[10] have extended these studies in extracts of *E. coli* and have found further evidence that S-adenosylmethionine is an intermediate.

(1) A preliminary report of this work was presented before the American Society of Biological Chemists; *Federation Proc.*, **16**, 189 (1957).

(2) This work was supported in part by Research Grant C-3436(A) from the National Cancer Institute, National Institutes of Health, Bethesda 14, Md.

(3) H. Tabor, S. M. Rosenthal and C. W. Tabor, *Federation Proc.*, **15**, 367 (1956).

(4) Abbreviations used are: TCA, trichloroacetic acid; ATP, adenosine triphosphate; Tris, tris-hydroxymethylaminomethane.

(5) S. M. Rosenthal and C. W. Tabor, *J. Pharmacol. and Exptl. Ther.*, **116**, 131 (1956).

(6) J. K. Palmer, Bull. 589 Conn. Agric. Expt. Station (1955).

(7) H. K. Berry, H. E. Sutton, L. Cain and J. S. Berry, Biochemical Institute Studies IV, No. 5109, Univ. of Texas, Austin, Texas, 1951, p. 22.

(8) Kindly supplied by Dr. E. Jackson of the National Institute of Arthritis and Metabolic Diseases.

(9) G. L. Cantoni, "Phosphorus Metabolism," Vol. II, Johns Hopkins Press, Baltimore, Md., 1952, p. 129.

(10) H. Tabor, S. M. Rosenthal and C. W. Tabor, personal communication.

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THE HETEROGENEITY OF POLYSACCHARIDES AS REVEALED BY ELECTROPHORESIS ON GLASS-FIBER PAPER

Sir:

Glass-fiber paper[1] has been used in conjunction with a borate buffer *p*H 9–10 for the electrophoretic separation[2] of a wide variety of organic substances

(1) M. J. O'Leary, R. B. Hobbs, J. K. Missimer and J. J. Erving, *Tappi*, **37**, 446 (1954).

(2) D. R. Briggs, E. F. Garner, R. Montgomery and F. Smith, *Anal. Chem.*, **28**, 1333 (1956).

including polysaccharides[3] and it has been reported that the method can be used for differentiating certain specimens of glycogen from various sources[4]; dextrans and other bacterial polyglucosans also may be distinguished.[4] The advantage of using the glass-fiber paper rather than cellulose paper is that complexes between the polysaccharide and the fiber are avoided[5] and the location of polysaccharides of all types is no longer a problem.[3,4] While it is possible to separate certain polysaccharides such as fructans, glucans and pentosans which have entirely different structures, using a borate buffer,[6] the inhomogeneity of a single polysaccharide cannot be ascertained in the same way, probably because of molecular association. We have found, however, that by replacing the borate buffer with $2N$ sodium hydroxide considerable information can be obtained. By this technique it has been shown that many polysaccharides hitherto assumed to be essentially homogeneous can be separated into two or more components.

All glycogen specimens from a wide variety of sources[7] thus far encountered have been found to be composed of two fractions, the major one moving at about ten times the rate of the other. In all cases the slow-moving component moved at the same rate but some of the glycogen specimens can be differentiated by the rate of movement of the faster moving component. Thus a specimen of liver glycogen obtained from a pregnant rabbit[8] can be distinguished by the fact that the faster moving component moves more slowly than the corresponding component of normal rabbit liver glycogen.

Amylopectins from a wide variety of starches have not only been found to be heterogeneous but also to differ from one another; three components have been detected in corn amylopectin, one of which corresponds to one of the components of corn amylose which is likewise heterogeneous. Repeated precipitation fails to separate all the amylose component(s) from amylopectin and both corn and potato amylose display heterogeneity even after repeated crystallization. The reason for this is not yet clear, but it is of interest to note that amylitol, obtained from corn amylose by sodium borohydride reduction,[9] moves on the glass paper largely as a single component and shows a mobility quite distinct from that of amylose.

Gums from *Acacia arabicum*, *A. pychnantha*, *A. senegal*, *A. seyal*, *Anogeissus latifolia* (gum ghatti) and gum tragacanth (*Astragalus sp.*) were all found to be heterogeneous whereas Black Wattle gum from *A. decurrens* and mesquite gum from *Prosopis juliflora* appeared to be homogeneous. Immunological examination has recently indicated the heterogeneity of gum arabic.[10]

The gums from the seeds of guar (*Cyamopsis tetragonaloba*), locust bean (*Ceratonia siliqua L.*), Kentucky coffee bean (*Gymnocladus dioica*), ivory nut (*Phytelephas macrocarpa*), tara (*Caesalpinia spinosa*), and those from flat (*Linum usitatissimum*) and *Plantago lanceolata*, all proved to be heterogeneous, two components being detected in each case.

The hemicelluloses separated from corn hulls, wheat straw, flax straw and ramie grass, and the so-called hemicelluloses from aspen wood, slash pine, loblolly pine and Western Hemlock all proved to be heterogeneous in nature. On the other hand the crystalline xylans[11] prepared from birch wood and barley straw by partial hydrolysis and also a specimen of the galactoaraban of sugar beet pulp proved to be homogeneous. These two crystalline xylans had almost identical mobilities on glass-fiber paper while the galactoaraban moved most rapidly, probably because of the more readily ionizable character of the primary alcoholic groups. Potassium hydroxide has been found to be a better electrolyte than sodium hydroxide for the separation of certain hemicelluloses; this observation may be related to the long-established fact[12] that it is better to use potassium hydroxide than sodium hydroxide for methylating hemicelluloses by the methyl sulfate method.

The fructans from rye flour (graminin), dahlia and perennial rye grass which have relatively low molecular weights were found to be homogeneous while the fructans from the ti (*Cordyline terminalis*) root, and from cocksfoot (*Dactylis glomerata*) contained small amounts of a second slow moving component.

Carraggenin already recognized as being heterogeneous[13] was found to consist of several components; one of the components corresponded to the K-fraction precipitated with potassium chloride, which was found to be homogeneous. Similarly agar has been found to be a mixture, a result in agreement with the recent report[14] that it consists of a neutral (agarose) and an acidic (agaropectin) component. Floridean starch also exhibits heterogeneity as also does laminarin from both *Laminaria digitata* and *L. cloustoni*. The reduced ($NaBH_4$) laminarin from *L. cloustoni* displays homogeneity.

Glass-fiber paper electrophoresis has also shown that yeast mannan and the glucomannans from Konjak mannan and Iles mannan are heterogeneous while the three mucopolysaccharides, heparin, hyaluronic acid, and chondroitin sulfate which individually behave as homogeneous polymers can be separated from each other with relative ease.

The movement of carbohydrate polymers on the glass paper appears to depend on the ionization of the alcoholic groups under the influence of the alkaline electrolyte. This is supported by the

(3) D. R. Briggs, E. F. Garner and F. Smith, *Nature*, **178**, 154 (1956).

(4) J. A. Cifonelli, B. A. Lewis, R. Montgomery and F. Smith, p. 30, of Abstracts 129th A.C.S. meeting, Dallas, Texas, 1956.

(5) *Cf.* E. J. Bourne, A. B. Foster and P. M. Grant, *J. Chem. Soc.*, 4311 (1956).

(6) *Cf.* K. W. Fuller and D. H. Northcote, *Biochem. J.*, **64**, 657 (1956).

(7) M. Abdel-Akher and F. Smith, THIS JOURNAL, **73**, 994 (1951).

(8) S. Peat, P. J. P. Roberts and W. J. Whelan, *Biochem. J.*, **51**, XVII (1952).

(9) M. Abdel-Akher, J. K. Hamilton and F. Smith, THIS JOURNAL, **73**, 4691 (1951).

(10) *Cf.* M. Heidelberger, J. Adams and Z. Dische, *ibid.*, **78**, 2853 (1956).

(11) A. P. Yundt, *ibid.*, **71**, 757 (1949).

(12) H. A. Hampton, W. N. Haworth and E. L. Hirst, *J. Chem. Soc.*, 1739 (1929).

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(14) C. Araki and K. Arai, *Bull., Chem. Soc. Japan*, **29**, 543 (1956).

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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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 *p*H 4. The reaction is slow enough to permit spectroscopic and titrimetric observation of both the native and the altered form near *p*H 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 *p*H 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

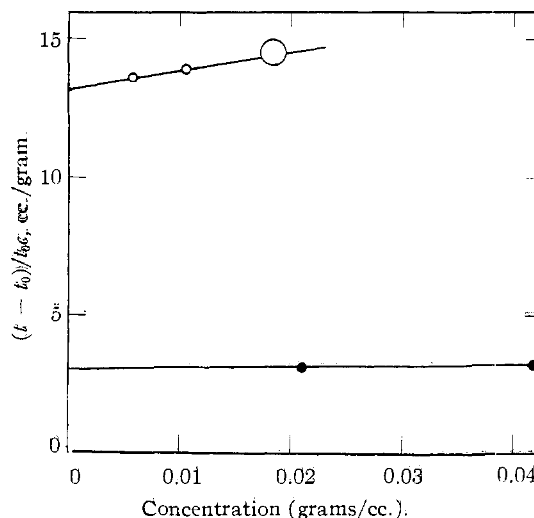

Fig. 1.—Viscosity data for native ferrihemoglobin (●) and for the same protein exposed to *p*H 3.5 for about 10 to 15 minutes (○). t represents flow time for the solution of concentration c, and t_0 flow time for solvent. All measurements were performed at ionic strength 0.04 at 25°.

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 *p*H 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 *p*H 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.

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).

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(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).