[CONTRIBUTION FROM THE LABORATORY OF PHYSICAL CHEMISTRY, UNIVERSITY OF WISCONSIN]

The Physical Chemical Investigation of Certain Nucleoproteins. I. Preparation and General Properties

BY R. OWEN CARTER[1,2] AND JAMES L. HALL[1,3]

Nucleoproteins are present in all cells, occurring in highest concentration in the nucleoplasm. Since no systematic investigation of the physicochemical properties of this class of proteins has been made, it has been selected by us as the first class of tissue proteins to be studied. This report describes the preparation and some properties of two members of this group, the nucleohistone of the calf thymus, and the nucleoprotein of the hog thyroid. For the first of these are given the phosphorus and nitrogen contents, the apparent specific volume, and the refractive index increment, along with viscosity studies of its solutions and a titration curve.

Calf Thymus Nucleohistone

Preparation.—The calf thymus nucleohistone was prepared by a modification of the method of Huiskamp.[4] The glands, obtained directly from the animal carcasses, were reduced to a pulp, suspended in two to three times their volume of pure water, and extracted at 5° for twenty-four to thirty-six hours. The extract was filtered and clarified and the nucleohistone precipitated as the corresponding salt by addition of an equal volume of either 2% sodium chloride solution, or 0.2% calcium chloride solution. All work was done near the freezing temperature.

The sodium salt is a dense gel-like material which dissolves either in pure water or 5% sodium chloride solution. The calcium salt is insoluble in pure water but dissolves easily in solutions of 2% calcium chloride or 5% sodium chloride, though in the last case it is converted to the sodium salt.

Phosphorus Content of Sodium Nucleohistone.—Sodium nucleohistone which had been dialyzed against distilled water until the dialyzate gave no chloride test was frozen and dried to constant weight in a vacuum over phosphorus pentoxide. Samples of this dried protein were completely ashed with alcoholic magnesium nitrate and phosphate determinations were made colorimetrically with a small Duboscq calorimeter following the method of Fiske and Subbarow.[5] These colored solutions do not absorb light as required by Beer's law. The corrections for this deviation given by Bodansky[6] were found to apply and were used in the calculations.

TABLE I

PHOSPHORUS CONTENT OF CALF THYMUS NUCLEOHISTONE

Sample, g.	Color. reading,[a] mm.	Vol. of solution, cc.	Wt. P, g.	Per cent. P
0.0309	15.3	250	0.00137	4.43
.0510	9.3	250	.00238	4.67
.0241	18.6	250	.00109	4.52
.0174	10.8	100	.00081	4.66

Average 4.6 ± 0.1

[a] The standard containing 0.02 mg. P in 5 cc. was set at 20 mm.

Nitrogen Content of Sodium Nucleohistone.—This value was determined by the micro-Kjeldahl method of Pregl. Because of the deliquescence of the dried protein, these determinations were performed on aliquots of solutions.

TABLE II

NITROGEN CONTENT OF CALF THYMUS NUCLEOHISTONE

Mg. N/g. soln.		Mg. protein/g. soln.		N, %
3.105, 3.040, 3.063	3.069	18.39, 18.37	18.38	16.70
2.984, 2.984	2.984	17.81, 17.84	17.83	16.74
1.363, 1.368	1.366	8.15		16.76

Average 16.73 ± 0.02

Apparent Specific Volume of Sodium Nucleohistone.—For this determination a double bulb pycnometer of the Ostwald type was used. All measurements were made at 25 ± 0.005°. The protein solution was sterilized with merthiolate and completely dialyzed against distilled water. In two determinations made at a protein concentration of 1.7805%, the result was $V = 0.6575 \pm 0.003$. This is in contrast to the usual protein value of 0.73 to 0.75. Such a low value is reasonable, however, since nucleic acid, which makes up a large part of the nucleohistone molecule, has a density of about 2.15 g. per cc.[7] as compared to the usual protein density of 1.33 g. per cc.

Refractive Index Increment.—The refractive index of solutions of nucleohistone is a linear function of the protein concentration, permitting the use of refractive index methods for following concentration. As determined by use of a Bausch and Lomb immersion refractometer at 25.0°, the difference between the refractive indices of the protein solution and solvent, divided by the concentration of the protein, was found to be $(1.76 \pm 0.02) \times 10^{-3}$ per g. per 100 cc.

Viscosity of Sodium Nucleohistone Solutions.—Three series of viscosity measurements were made with the sodium nucleohistone solutions, (a) in dilute buffer,

(1) This work is taken in part from portions of the theses submitted in June, 1939, to the Faculty of the University of Wisconsin by R. Owen Carter and James L. Hall in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

(2) Present address: Research Laboratory, Procter and Gamble Co., Cincinnati, Ohio.

(3) Present address: Department of Biochemistry, Cornell University Medical College, New York, N. Y.

(4) W. Huiskamp, *Z. physiol. Chem.*, **32**, 145 (1901).

(5) C. H. Fiske and Y. Subbarow, *J. Biol. Chem.*, **66**, 375 (1925).

(6) M. Bodansky, *ibid.*, **99**, 197 (1932).

(7) G. Schmidt, E. G. Pickels and P. A. Levine, *ibid.*, **127**, 257 (1939).

0.005 *M* in KH_2PO_4 and 0.005 *M* Na_2HPO_4, at 25°; (b) in the same buffer at 0.7°; and (c) in the same buffer plus 5% sodium chloride at 25°. A Washburn–Williams viscometer was used. The densities were determined pycnometrically. The data of Table III show that the presence of 5% sodium chloride causes a great increase in the viscosities of these solutions. The concentration of protein, *c*, is given in g. per 100 cc. of solution; time of outflow, *t*, is in seconds; η/η_{H_2O} is the viscosity relative to water; and η/η_0 is the viscosity relative to solvent.

TABLE III

VISCOSITIES OF SODIUM NUCLEOHISTONE SOLUTIONS

Concn. of protein, %	Density	Time, sec.	η/η_{H_2O}	η/η_0
(a) In dilute phosphate buffer at 25°				
Water		168.0		
Solvent	0.9986	168.2	1.0027	
0.815	1.0013	247.8	1.4812	1.4772
.611	1.0006	220.2	1.3153	1.3118
.407	0.9999	204.1	1.2183	1.2150
.196	.9992	185.6	1.1071	1.1041
.098	.9988	177.6	1.0590	1.0561
(b) In dilute phosphate buffer at 0.7°				
Water		324.0		
Solvent	1.0007	327.4	1.0113	
0.815	1.0037	495.6	1.5354	1.5182
.611	1.0030	436.0	1.3498	1.3347
.407	1.0022	400.2	1.2380	1.2242
.196	1.0014	363.0	1.1220	1.1095
.098	1.0011	345.6	1.0679	1.0560
(c) In phosphate buffer containing 5% of NaCl at 25°				
Solvent	1.0326	176.3	1.087	
0.846	1.0355	937.5	5.796	5.332
.315	1.0338	387.5	2.392	2.201
.158	1.0333	288.1	1.777	1.635
.079	1.0329	220.9	1.362	1.253
.039	1.0327	197.5	1.220	1.122

Titration Curve for Sodium Nucleohistone.—A titration curve was obtained by using nucleohistone which had been twice precipitated with sodium chloride and then dissolved in and dialyzed against distilled water until the dialyzate was chloride free. The initial *p*H of this dialyzed solution was about 5.4. Either 0.1 *N* sodium hydroxide or hydrochloric acid was continuously titrated into a 4% protein solution. The *p*H was measured at intervals by means of a glass electrode. The results, calculated on the basis of moles of acid or base bound per gram of protein are shown in Fig. 1.

The curve is completely reproducible although the characteristics of the glass electrode make the *p*H values beyond *p*H 9.5 very doubtful. The apparent break in the curve just below *p*H 2 is possibly genuine, since the nucleohistone hydrolyzes in acid solution and releases the comparatively strong nucleic acid. The entire acid combining capacity may be accounted for on the basis of the phosphorus present in the protein molecule. Because of the insolubility of the protein over part of the range, and because it was not possible to determine an exact isoelectric point, no attempt is made to estimate individual amino acids from the curve.

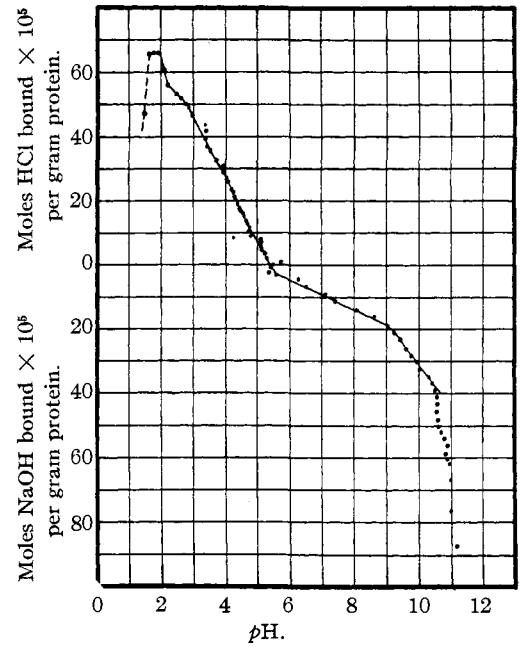

Fig. 1.—Titration curve of thymus nucleohistone: acid and base combining capacity.

Nucleoprotein from the Hog Thyroid

The nucleoprotein of the hog thyroid gland was prepared after the method of Heidelberger and Palmer.[8] It was found quite impossible to separate the thyroglobulin from the nucleoprotein by salting out at various concentrations of ammonium or sodium sulfates, thus completely confirming the work of Heidelberger and Palmer in this respect. Accordingly the nucleoprotein was precipitated and purified by precipitation at *p*H 4.9 with either 0.1 *N* hydrochloric or acetic acid. In the purification of the nucleoprotein a negative iodine test was used as a criterion of the absence of thyroglobulin.

Free diffusion and sedimentation velocity experiments indicate that the protein so prepared is more or less polydisperse, but electrophoretic studies indicate that the amount of nucleic acid present is independent of the preparational method. When the diffusion curves are analyzed by a statistical method, an average diffusion constant can be calculated. This value depends upon the previous treatment of the protein. Material prepared with acetic acid gives a much lower diffusion constant than that prepared with hydrochloric acid. Precipitation of purified nucleoprotein with ammonium sulfate also gives a material having a low diffusion constant.

The sedimentation, diffusion, and electrophoretic studies of these substances will be reported in later papers of this series.

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(8) M. Heidelberger and W. W. Palmer, *J. Biol. Chem.*, **101**, 433 (1933).

ration of the nucleoprotein of the hog thyroid. One of us (R. O. C.) wishes to acknowledge financial assistance received from the Wisconsin Alumni Research Foundation.

Summary

1. Certain striking physical and chemical properties of the calf thymus nucleohistone protein have been presented. The phosphorus and nitrogen contents were accurately determined. The specific volume, refractive index increment, and viscosity of this protein in solution, as well as a titration curve are given.

2. The effect of certain preparational methods on the centrifugal and diffusion properties of the hog thyroid nucleoprotein has been briefly discussed.

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The Calculation of Weight Average Molecular Weights during the Course of Decomposition of Homogeneous Linear Polymers

BY WALTER H. DURFEE[1] AND Z. I. KERTESZ[2]

For materials that occur naturally and that are composed of large molecules (in whatever sense the word molecule may be understood), some degree of heterogeneity as regards size of the individual molecules must be assumed. This natural heterogeneity is further increased upon progressing degradation until the lower polymers become dominant and, finally, by complete depolymerization, homogeneity is attained.

Such heterogeneity is obvious in any pectin isolated from plant material.[3] Its heterogeneity is increased by the chemical manipulations necessary for its isolation. On the other hand, there is ample evidence to show[4] that by extracting the crude pectic materials with 50% ethanol the lower polymers originally present (or formed during preparation) are removed, together with some other materials, usually accompanying pectins in nature. It is obvious, however, that even material prepared by this latter method contains a certain range of the various polymers and is not entirely homogeneous.

For several years one of us (Z. I. K.) has been engaged in research on the chemical and physical structure of the pectin molecule. One method of approach to this problem is through a study of the changes which take place in the chemical and physical structure of pectin upon enzymic hydrolysis. It is hoped that by such a study information can be gained concerning the naturally occurring pectic materials.

It has been reported recently[5] that when the decomposition of pectin is followed by various methods, the conclusions drawn concerning the average degree of polymerization are not in agreement. This led to an investigation of the number and weight averages[6] (M_n, M_w) for the "molecular weight" during the course of decomposition. During this work a formula was developed for the calculation of the M_w for any stage of progressive decomposition of a homogeneous linear polymer which, the authors feel, may also be useful to other workers dealing with such materials.

Several articles dealing with similar calculations already have appeared in the literature. W. Kuhn[7] made a study of the kinetics of the decomposition of chain-like polymers and calculated the proportions of various fission products occurring during the course of the hydrolysis. Dostal and Mark[8] investigated the heterogeneity of materials composed of macromolecules and proposed a method for the determination of the distribution of the proportion of fractions having various molecular weights in the mixture. More recently Lassatre[9] considered the degree of polymerization of cellulose during hydrolysis. All these authors approached the problem from the kinetical point of view and we feel that there is need to consider the basic relation between

(1) Department of Mathematics, Hobart College.

(2) Department of Chemistry, New York State Agricultural Experiment Station. Approved by the Director for publication as Journal Paper No. 369, January 30, 1940.

(3) Pectin is considered as a polymer of galacturonic anhydride residues in which the carboxyl groups are mostly changed to the methyl ester.

(4) Schneider, *et al.*, *Ber.*, **69**, 309, 2530, also 2537 (1936); **70**, 1611, also 1617 (1937); **71**, 1553 (1938), etc.

(5) Kertesz, THIS JOURNAL, **61**, 2544 (1939).

(6) Kraemer and Lansing, *J. Phys. Chem.*, **39**, 153 (1935).

(7) W. Kuhn, *Ber.*, **63**, 1503 (1930).

(8) Dostal and Mark, *Trans. Faraday Soc.*, **33**, 350 (1937).

(9) Lassatre, not yet published.