aspect of the problem which has been studied in proteins such as Ea, β-lactoglobulin and horse serum pseudoglobulin.[27] Only 70% of the amino groups of Ea are affected by PhNCO[28] and HCHO, whereas acetic anhydride blocks 85% of the amino groups.[13] Brand, *et al.*, noted a difference in the rates of deamination of proteins by HNO_2.[29] Differences in reactivity of the $-NH_2$ groups toward formalin have been observed with diphtheria toxin[30] and tobacco mosaic virus.[31]

The third factor influencing the removal of amino groups may be the *p*H. Kornblum and Iffland have shown that aliphatic primary $-NH_2$ groups[32] do not react below *p*H 3.0, indicating a narrow *p*H zone in which deamination is achieved. This probably explains the smaller extent of deamination of acid DnEa at *p*H 3.0 than at *p*H 3.5 (Table I).

Since more groupings are detectable in the denatured than in the native state,[33] it was hoped to obtain a highly deaminated protein by reacting free HNO_2 with acid DnEa at *p*H 3.0–3.5. However, deamination merely equalled that of Ea under the same conditions, but was greater than the deamination of Ea in the milder buffer mixture. Even 8A which had been deaminated 44% by the buffer mixture lost only an additional 23% of its $-NH_2$ groups after exposure to free HNO_2.

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[CONTRIBUTION FROM THE DEPARTMENTS OF BIOCHEMISTRY AND MEDICINE, AND THE ELECTROPHORESIS LABORATORY, COLLEGE OF PHYSICIANS AND SURGEONS, COLUMBIA UNIVERSITY]

The Deamination of Crystalline Egg Albumin.[1,2] II. Physical and Chemical Properties of the Soluble and Denatured Derivatives

BY PAUL H. MAURER, MICHAEL HEIDELBERGER AND DAN H. MOORE

The partially deaminated (27 to 36%) and soluble derivative of egg albumin, fraction B, has physical and chemical properties like those of native Ea and may therefore be termed an undenatured deaminated Ea. The insoluble derivative, fraction A, formed during deamination (33–56%) of Ea has many properties characteristic of the denatured state, but its configuration appears less extended than that of acid denatured Ea (DnEa) as indicated by nitroprusside tests, sedimentation and diffusion constants, and the reduced tendency to aggregate in salt solutions. This less completely unfolded protein is relatively stable to further treatment with acid at *p*H 1.5 whereas the B fraction is readily transformed into an acid DnEa derivative different from the one obtained by deaminating acid DnEa.

In the preceding paper[3] the preparation of various deaminated and denatured derivaties of crystalline egg albumin (Ea) was described. Since the solubility,[3] optical activity, viscosity, diffusion constant, sedimentation constant, electrophoretic mobility, ion binding and sensitivity to salt are all useful for the characterization of the protein molecule and detection of changes, these properties were studied.

1. **Optical Activity.**—The specific rotation of the protein solution was determined at room temperature, 22–25°, at about 1% concentration, after dialysis against phosphate buffer at *p*H 7.5, 0.05 ionic strength. The factor 6.45[4] was used to convert mg. N to mg. protein. An increase in negative rotation of Ea upon denaturation (Table I) is in accord with previous reports on this[5,6,6a] and other proteins[7], and has been ascribed to changes in the asymmetry of the "backbone" of the peptide chain[6,8] but may also be due to an unfolding of the molecule, as appears to be the cause of the parallel increase in ultraviolet absorption[9] and in the number of detectable -SH groups.[10]

The $[\alpha]_D$ of the soluble, or B, fractions resembled that of native Ea. After acid treatment of B at *p*H 1.5 $[\alpha]_D$ was like that of acid denatured Ea (DnEa) and of the A, or insoluble, deaminated fractions. No detectable change was observed after further acid treatment of A (Table I) nor was there significant change in the $[\alpha]_D$ of DnEa upon deamination (DnEa Deam -S-S-, for the oxidized form; -SH, for the reduced form (Table I)).

2. **Viscosity.**—Eight to ten ml. of a 1% protein solution were dialyzed at 0 to 5° for 4 to 5 days in the presence of toluene against two changes daily of 0.02 *M* phosphate buffer at *p*H 7.5, ionic strength 0.05, a concentration sufficient to suppress the electroviscous effect.[11] Viscosity determinations were made with 5 ml. of dialyzed solution in an Ostwald–Fenske type viscometer at 25 ± 0.5°.

(1) Submitted by Paul H. Maurer in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Faculty of Pure Science, Columbia University.
(2) Presented before the 40th Annual Meeting of the American Society of Biological Chemists, Detroit, Michigan, April 18–22, 1949, and before the 41st Annual Meeting of the American Society of Biological Chemists, Atlantic City, N. J., April 17–21, 1950.
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(8) W. Pauli and R. Weiss, *Biochem. Z.*, **233**, 381 (1931).
(9) J. L. Crammer and A. Neuberger, *Biochem. J.*, **37**, 302 (1942).
(10) M. L. Anson, *Adv. in Protein Chem.*, **2**, 361 (1946).
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TABLE I

PROPERTIES OF Ea, DEAMINATED Ea AND DENATURED Ea

Preparation	$[\alpha]_D$ pH 7.5	D_{20} × 10^7	S_{20}	f/f_0	Particle weight	$\eta_{rel.}$ at 2 mg. N/ml.
Ea	−28[a]	7.8[b]	3.55[b]	1.1	45,000	1.044
3A	−51	3.0	4.2	1.9	135,000[h]	1.136
3B	−40	8.0	3.1	1.1	38,000	1.056
3B(A)	−53					1.134
3B(B)	−37					1.051
4A	−51	3.9	3.0	1.8	75,000	1.136
4B	−36	8.8[c]	3.0	1.0	33,000	1.053
5A	−51	3.4	4.3	1.7	125,000[h]	1.181
5B	−36	7.8	3.3	1.1	41,000	1.058
6A	−52	3.2	4.4	1.8	135,000[h]	1.134
6B	−36	7.5	3.0	1.1	39,000	1.064
8A	−52	4.0	3.1	1.7	76,000	1.134[d]
8B	−37	7.3	3.2	1.1	43,000	1.047[d]
10A	−54					1.224
5A Dn	−57	2.7	3.9	2.1	140,000	1.186
6A Dn	−57	2.5	4.0	2.2	175,000	1.143
8A Dn	−55	3.5	6.2	1.5	175,000[h]	1.153
6B Dn	−63	2.6	7.8	1.7	290,000[h]	1.149
8B Dn	−62	3.1	8.9	1.4	280,000	1.149
DnEa 105	−53	1.5	9.2	2.3	600,000[h]	1.227[e]
DnEa 105 Deam (SH)	−60					1.315
DnEa 105 Deam (SS)	−50					1.428
DnEa 106	−55	2.0	10	1.8	490,000[h]	1.198[e]
DnEa 106 Deam (-SH)	−59	2.0	13	1.7	630,000[h]	1.398[f]
DnEa 106 Deam (SS)	−59	1.5	13	2.0	850,000[h]	1.575
8A FNA	−50	2.2	6.3	2.0	280,000[h]	1.145[d]
Ea FNA	−55	2.4	5.0	2.1	205,000[h]	1.160[d]
Heat DnEa 27[g]	−65					1.550

[a] Other values of $[\alpha]_D$ reported are: −37° (refs. 6, 8); −27° to −32° (ref. 6a); −31° (ref. 12). [b] From Svedberg and Pedersen, ref. 27. [c] The value closest to that of Ea is given. All other S_{20} values are averages of two or three calculations. [d] Same value for the -SH and -S-S- forms. [e] Value after three months of ageing. [f] Twice treated with thioglycolate. [g] Prepared by Dr. C. F. C. MacPherson, ref. 14. [h] Value given to nearest 5,000 particle weight.

Two concentrations of protein were used, the weaker being obtained by twofold dilution with buffer. The N content of one solution was determined by micro-Kjeldahl analysis.[12] The time of outflow for buffer alone was about 260 sec. No corrections were made for small differences in density between the protein solutions and the buffer as the over-all accuracy was limited to 1 to 2% by the method of analysis. Concentration in mg. N per ml. was plotted against relative fluidity. This yields a linear relation up to at least 2 mg. N/ml.[13,14]

The viscosity of a protein solution is believed to reflect the particle shape and extent of hydration of the protein.[15] A marked increase in viscosity, *i.e.*, a decrease in relative fluidity, would be indicative of the unfolding of the protein into a more asymmetric form characteristic of the denatured protein.[16,17] A decrease in viscosity,

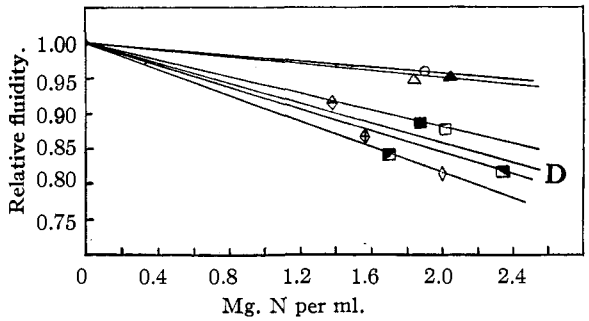

Fig. 1.—Comparison of fluidities of Ea, DnEa and deaminated fractions—key to chart: △ 3B, ▲ 4B, □ 3A, ■ 4A, ◹ 5A, ◩ 10A, ○ Ea, ⟐ DnEa 101, ⟐ DnEa 102, ◇ DnEa 105; line D represents average curve for DnEa 101 and 102.

such as that shown by acid DnEa and other DnEa preparations on "ageing,"[14] may be taken to indicate disaggregation. Although the A fractions resembled DnEa in many respects, their relative fluidity had not changed after a year. The relative fluidities of the B fractions resembled those of native Ea (Fig. 1), whereas those of the ADn preparations differed little from the fluidity of the original A fractions. On the other hand, when Fraction B was treated with acid its viscosity increased to about that of A and DnEa, indicating the denaturation confirmed by other tests. As for the deaminated denatured Ea, DnEa Deam, -S-S- form, its viscosity was greater than that of the -SH form, which, in turn, was greater than that of the DnEa used. The viscosity of DnEa Deam, -SH form was not reduced appreciably by a second treatment with thioglycolic acid. The difference in viscosity between the -S-S- and -SH forms of DnEa Deam is not exhibited by the -S-S- and -SH forms of the A and B fractions, perhaps because of differences in the nature of the -S-S- linkages. As will be shown in connection with the nitroprusside tests, the A fractions are not "unfolded" to the same extent as is acid DnEa. Therefore, fewer -SH groupings are available for intermolecular -S-S- formation. However, upon oxidation, the less exposed -SH groups in Ea and fraction A could undergo intramolecular -S-S- bond formation, and this would not change the viscosity.[18,19]

3. Electrophoresis.—Recrystallized Ea is electrophoretically homogeneous except between *p*H 5.0 and 10.0[20] where two as yet inseparable components appear. The ratio of these components varies with age, preparation, *p*H and medium.[21] The isoelectric point of Ea is between 4.6 and 4.8.[22] The electrophoretic properties of DnEa have also been investigated.[14,22]

(12) E. A. Kabat and M. M. Mayer, "Experimental Immunochemistry," C. C. Thomas, Springfield, Ill., 1948.

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(15) Reviewed by M. A. Lauffer, *Chem. Rev.*, **31**, 561 (1942).

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(17) A. E. Mirsky and L. Pauling, *Proc. Natl. Acad. Sci.*, **22**, 439 (1936); W. T. Astbury, S. Dickinson and K. Bailey, *Biochem. J.*, **29**, 235 (1935); H. B. Bull, *Cold Spring Harbor Symp. Quant. Biol.*, **6**, 140 (1938).

(18) M. L. Anson, *J. Gen. Physiol.*, **25**, 355 (1941).

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(22) H. A. Abramson, L. S. Moyer and M. H. Gurin, "Electrophoresis of Proteins," Reinhold Publ. Co., New York, N. Y., 1942.

TABLE II

MOBILITIES OF DEAMINATED Ea FRACTIONS AND Ea

Preparation	% deamination	Buffer	pH	$\mu \times 10^5$
		At 0.05 ionic strength		
4A	42	Citrate–HCl	2.9	+6.71
4A	42	Phosphate	6.2	−7.77
4A	42	Phosphate	7.5	−9.81
4B	31	Citrate–HCl	2.9	+6.28
4B	31	Phosphate	6.2	−7.38
4B	31	Phosphate	7.5	−9.49
		At 0.10 ionic strength[a]		
Ea		Glycine	3.05	+6.25
		Acetate–NaCl	3.62	+3.89
		Acetate	4.64	−0.20
		Acetate	5.65	−3.53
		Phosphate	6.12	−4.46
		Phosphate	6.80	−5.92
		Phosphate	7.10	−6.70

[a] Data taken from L. G. Longsworth, *Ann. N. Y. Acad. Sci.*, 41, 267 (1941).

Mobilities were determined in a modified compact Tiselius electrophoresis apparatus of 2 ml. capacity.[23] The buffers used were of 0.05 ionic strength (Table II), the low concentration being used for comparison with the salt sensitive acid DnEa investigated under the same conditions.[14] The protein concentration was approximately 0.5%.

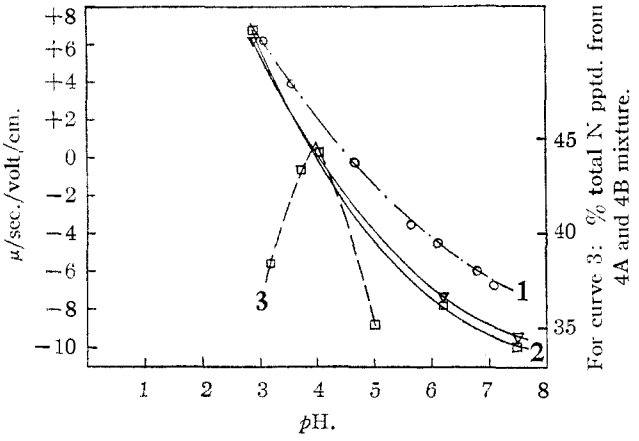

Fig. 2.—Mobilities of Ea (Curve 1) and fractions 4A and 4B (curves 2), and precipitation of A and B mixture (curve 3)—key to chart: O Ea, □ 4A, ▽ 4B.

Conductivities of protein solution and buffer were determined after 4 days' dialysis. At pH 2.9 where Ea is homogeneous both A and B were also homogeneous, and were much less heterogeneous at the other two pH values than is crystalline Ea. Two definite peaks were never obtained. No determinations were made between pH 3.5 and 5.5 as fraction A is insoluble in this range. The isoelectric points of both the A and B fractions had shifted to about pH 4 in agreement with the data on their separation by the method of minimum solubility (Table II and Fig. 2). A similar shift in isoelectric point upon deamination has also been observed with gelatin.[24]

(23) D. H. Moore and J. U. White, *Rev. Sci. Instr.*, **19**, 700 (1948).

(24) D. I. Hitchcock, *J. Gen. Physiol.*, **5**, 383 (1923); E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids and Peptides," Reinhold Publ. Co., New York, N. Y., 1945.

4. Diffusion.—One per cent. protein solutions were dialyzed as for viscosity. Measurements of diffusion were carried out with the usual modifications in the Tiselius apparatus at 1 ± 0.05°. The scanning method of Longsworth[25] was used. The diffusion constants tabulated are averages from the two arms of the U-tube and were calculated by the inflection points-maximum ordinate[26] method. The patterns obtained indicated a fair degree of homogeneity. However, diffusion constants are insensitive as an indicator of polydispersity, which is more readily detected by measurements of sedimentation properties.[27]

From Table I it will be seen that the D_{20} of Ea and the B fractions are about the same, *i.e.*, 7.8 × 10^{-7}. The A fractions, which are denatured by definition (see Paper I) and by other criteria, have diffusion constants of about 3.2. The ADn samples show a slight decrease in D_{20} which approaches that of acid DnEa. However, B, on conversion to DBn exhibits greatly altered D_{20} values in the range of those of DnEa and ADn. A decrease in diffusion constant is ordinarily ascribed to an increase in the asymmetry of the molecule and/or to association[14,26] rather than to hydration.[28]

5. Sedimentation.—Sedimentation runs were made in an air-driven ultracentrifuge[29] operating at 48,000 r.p.m. All runs were made in 0.02 M phosphate buffer at pH 7.5 with a 1% protein solution. Sedimentation patterns of the proteins were recorded by the Longsworth scanning method[25] as adapted to the ultracentrifuge.[30] Sedimentation constants were calculated from the equation of Svedberg and Pedersen.[27] It is evident from Table I that the soluble deaminated B fractions have sedimentation constants like that of native Ea, but slightly lower, possibly owing to the low salt concentration used.[27] The patterns (Fig. 3) show a sharp sedimenting boundary indicative of a monodisperse system.[27] The insoluble deaminated A fractions which show other physical properties similar to those of DnEa exhibit differences from DnEa in sedimentation. The S_{20} was not 9 to 12[14] as with the least aggregated acid DnEa preparations, but instead was 3 to 4, like that of native Ea. There was also less spreading of the sedimenting boundary than with DnEa (Fig. 3).

6. Particle Weights and Shapes.—The particle weights of the preparations were calculated from the sedimentation and diffusion constants at 20° according to the equation[27]

$$M = RTS_{20}/D_{20}(1 - V\rho)$$

From S_{20} and D_{20} it is possible to calculate the frictional ratio f/f_0 by the formula

$$f/f_0 = 10^{-8}\left(\frac{1 - V\rho}{D_{20}^2 S_{20} V}\right)^{1/3}$$

(25) L. G. Longsworth, THIS JOURNAL, **61**, 529 (1939).

(26) O. Lamm and A. Polson, *Biochem. J.*, **30**, 528 (1936).

(27) T. Svedberg and K. O. Pedersen, "The Ultracentrifuge," Clarendon Press, Oxford, 1940.

(28) H. Neurath and A. M. Saum, *J. Biol. Chem.*, **98**, 353 (1932).

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(30) D. H. Moore, *Rev. Sci. Instr.*, **14**, 293 (1943).

If this f/f_0 ratio is 1.0, the protein is a compact, spherical molecule and not appreciably hydrated. If f/f_0 is >1 the molecule is either hydrated or non-spherical or both.[27,31]

The molecular weights and f/f_0 values of the B fractions agreed with those of Ea within experimental error (Table I). The particle weights of the A fractions were about 2 to 3 times that of native Ea. Previously prepared DnEa preparations[14] were roughly 5 to 20 times the weight of native Ea owing to aggregation. If it be assumed that denaturation involves no change in molecular weight as distinguished from particle weight,[32,33] the A fractions represent polymers of 2 to 3 molecules, whereas acid DnEa preparations are polymers of more than 5. The f/f_0 values of both the A and acid DnEa preparations were 1.7–1.9, indicating an extensive unfolding of the molecule, $f/f_0 = 2.2$ being given[33] for a completely denatured, unfolded Ea molecule. Apparently both A and acid DnEa have unfolded into aggregates of roughly similar shape but different size. Exposure of A to *p*H 1.5 for 72 hours increased the particle weight about 20,000, whereas after a 144-hour treatment, it had increased 100,000 (Table I). This was also reflected in the greater salt sensitivity of the product ADn (see below).

Deamination of acid DnEa led to an increase in particle weight from about 490,000 to 850,000. This was reduced to 630,000 after thioglycolic acid treatment. These results supply additional evidence that HNO_2 oxidizes -SH groups.

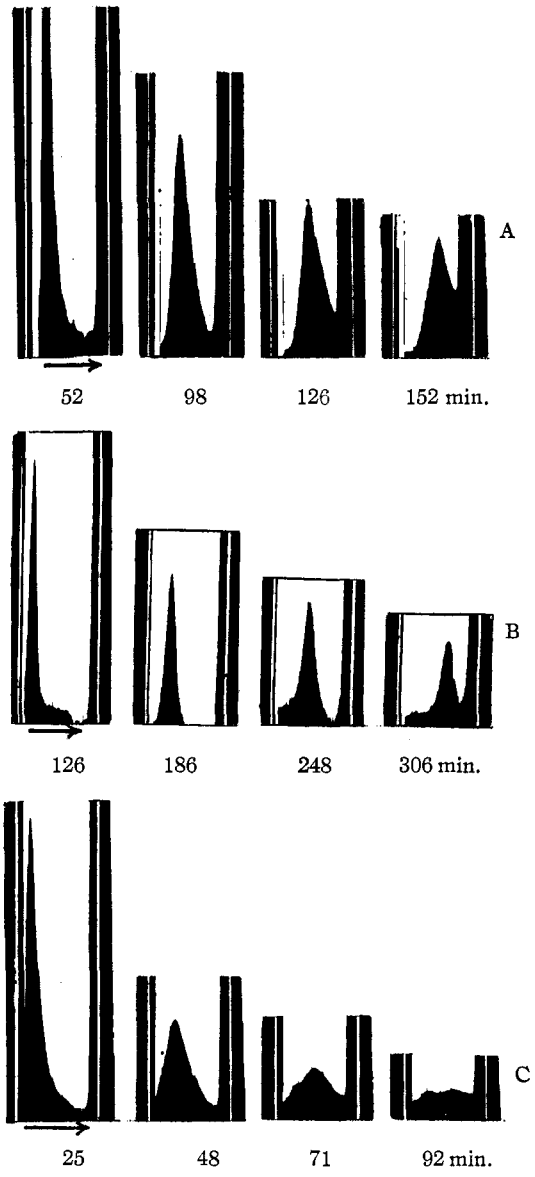
7. Nitroprusside Tests.—Qualitative nitroprusside tests were carried out on 1% protein solutions by recorded methods and are listed in Table III. All colors were compared with the color of maximum intensity produced by Ea denatured in the presence of guanidine. The liberation of -SH groups detectable by nitroprusside parallels the degree of unfolding of the Ea molecule.[34] However, different color intensities are obtained with different denaturing agents and in the presence or absence of the denaturing agent.[34,35] Definite differences between the various preparations are evident. In the absence of guanidine, acid DnEa gives a stronger test for -SH groups than does the reduced (-SH) form of 8A, even after the addition of 2 *N* NaCN. However, in the presence of guanidine both produce colors of like intensity. This would seem to indicate that the -SH groups of the A fraction are not exposed to the same degree as those in acid DnEa. A difference in the degree or mode of unfolding of the native Ea could account for this. The -S-S- forms of the A, B and DnEa Deam fractions give positive nitroprusside tests only in the presence of cyanide, which cleaves -S-S- linkages.

TABLE III

COMPARISON OF QUALITATIVE NITROPRUSSIDE TESTS

Preparation	Without guanidine	With guanidine	With 2 *N* NaCN	With 2 *N* NaCN and guanidine
Ea	−	++++	−	++++
Acid DnEa 105	++	++++	++	++++
8A (S–S–)	−	−	+	+++
8A (–SH–)	−	+++	−	++++
8B (S–S–)	−	−	−	++++
8B (–SH)	−	+++	−	++++
DnEa 105 Deam –S–S	−	−	+	+++
DnEa 105 Deam –SH	+	++	++	+++
Ea FNA (–S–S)	−	−	+	+++
Ea FNA (SH)	−	+++	−	++++

(31) J. L. Oncley, *Ann. N. Y. Acad. Sci.*, **41**, 121 (1941).
(32) N. F. Burk and D. M. Greenberg, *J. Biol. Chem.*, **87**, 197 (1930).
(33) A. Rothen, *Ann. N. Y. Acad. Sci.*, **43**, 229 (1942).
(34) A. E. Mirsky, *J. Gen. Physiol.*, **24**, 399 (1940).
(35) A. E. Mirsky, *ibid.*, **24**, 709 (1940); **25**, 355 (1941).



Figs. 3A, B, C.—Sedimentation patterns—Key to chart: A, prepn. 6A; B, prepn. 4B; C, DnEa 105.

8. Tests of Salt-sensitivity.—Since denatured Ea is readily rendered insoluble by exposure to salts, comparative information on this property was obtained with the various products which had been studied by other tests. One ml. of solutions containing about 1 mg. N/ml. in *p*H 7.5, 0.005 *M* phosphate buffer were treated at 37° with 1.0 ml. of NaCl solutions of varying molarities for 1 hr. After centrifugation the precipitates were analyzed for N. The results are summarized

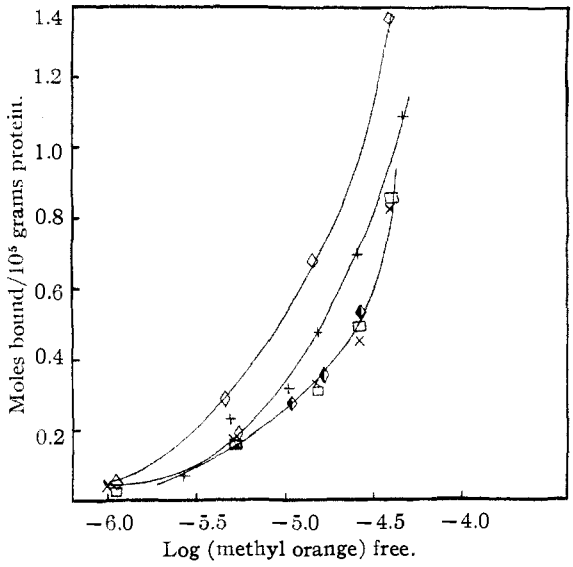


Fig. 4.—Methyl orange bound by DnEa and deaminated Ea fractions—key to chart: ◇ DnEa 105, ◐ DnEa 105 Deam-SH, □ 5A, × 5A Dn, + 8B Dn.

in Table IV. Here, too, a definite difference is shown between acid DnEa and heat DnEa on the one hand, and an A fraction on the other. In 1.5 *N* NaCl solution the A fraction remains soluble whereas the DnEa preparations precipitate to the extent of 60%. This would indicate that the A fraction does not exist in a form as fully extended or as subject to aggregation as the other DnEa preparations, in accord with the nitroprusside tests and the particle weights. In its salt sensitivity, also, fraction 5ADn appeared to be more unfolded or aggregated than 5A. Whereas 5A was opalescent in 2.0 *M* NaCl, 5ADn precipitated to the extent of 24%. Acid denatured 8B showed less tendency to aggregate than DnEa, in agreement with the viscosity data.

TABLE IV

EFFECT OF SODIUM CHLORIDE SOLUTIONS ON VARIOUS DENATURED PREPARATIONS. PER CENT. PROTEIN PRECIPITATED, 1 HR., 37°

	0.25 *M*	0.5 *M*	1.0 *M*	1.5 *M*	2.0 *M*	2.5 *M*
5A	*a*	*a*	*a*	*a*	*b*	63
5A Dn	*a*	*a*	*a*	*b*	24[c]	76
Acid DnEa 105	*a*	*b*	*b*	57	72	99
8B Dn	*a*	*a*	*b*	31[c]	69	88
Heat Dn 27	*b*	*b*	63	88	94	98

[a] Clear solution. [b] Opalescent solution. [c] Supernatant opalescent after centrifugation.

9. Binding of Methyl Orange.—The extent of binding of the anion of methyl orange by approximately 0.2% protein solutions was measured at *p*H 6.8 by differential dialysis.[36] The samples were dialyzed against dye solutions ranging in concentration from 0.165×10^{-5} to 8.40×10^{-5} *M*. The results are summarized in Fig. 4. Neither Ea nor 8B bound dye, but DnEa did, in agreement with results and statements of others.[37]

When acid DnEa is deaminated, it has a reduced binding ability similar to that of the A and ADn preparations. 8BDn, which can be considered in a sense "isomeric" with deaminated DnEa, binds less than DnEa and more than its deaminated form. It is difficult to decide what part in these relationships is played by the decrease in cationic nitrogen and what part is due to differences in molecular configuration.

(36) I. M. Klotz, F. M. Walker and R. B. Piven, THIS JOURNAL, **68**, 1486 (1946).

(37) I. M. Klotz and J. M. Urquhart, *ibid.*, **71**, 1597 (1949).

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The Deamination of Crystalline Egg Albumin.[1,2] III. Quantitative Immunochemical Studies on Crystalline Egg Albumin and its Denatured and Deaminated Derivatives

BY PAUL H. MAURER AND MICHAEL HEIDELBERGER

The serological specificity of Ea is not influenced by the removal of about one-third of the free $-NH_2$ groups when the protein remains undenatured. In the denatured form, which shows a specificity different from that of Ea, removal of up to *ca.* 60% of $-NH_2$ from DnEa or A resulted in a scarcely significant decrease in the nitrogen precipitated from their homologous antisera. Changes in immunolgical specificity of Ea, DnEa, and fractions A and B due to physical aggregation or change of -S-S- to -SH linkages are relatively minor. The quantitative immunochemical data supplement information gained by parallel chemical and physical studies of Ea and its deaminated and denatured derivatives and lead to the conclusion that the products described have definite form and structure.

In previous papers[3,4] it was shown that the fraction B of partially deaminated egg albumin (Ea) soluble at the isoelectric point (i.e.p.), had many properties resembling those of native Ea. On the other hand, Fraction A, which was rendered insoluble at the i.e.p. during deamination and acquired many of the properties of denatured Ea (DnEa), nevertheless showed definite differences from Ea denatured, for example, by acid.

These resemblances and differences are further illustrated in the accompanying quantitative immunochemical studies. These show both the wide applicability and the limitations of this technique.

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(2) Presented before the 34th Annual Meeting of the American Association of Immunologists, Atlantic City, N. J., April 17–21, 1950.

(3) P. H. Maurer and M. Heidelberger, THIS JOURNAL, **73**, 2070 (1951).

(4) P. H. Maurer and M. Heidelberger, *ibid.* **73**, 2072 (1951).