gains of three times that of the controls. The weight gain of the vitamin B_{12} group was almost eight times that of the negative control group. An analysis of the data showed that the increased weight gain of the B_{12} control over that of the negative control group was highly significant, but that the increased weight gains of the experimental groups over that of the negative control group could not be considered significant due to the wide variation in responses of the individual animals to their particular regimen. None of the *o*-phenylenediamines investigated here were found to possess vitamin B_{12} activity or inhibitor properties.

DEPARTMENT OF PHYSIOLOGY AND VITAL ECONOMICS
THE UNIVERSITY OF ROCHESTER
SCHOOL OF MEDICINE AND DENTISTRY
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The Deamination of Crystalline Egg Albumin. IV. Side Reactions

BY PAUL H. MAURER[1] AND MICHAEL HEIDELBERGER

It is well known that during the deamination of proteins by nitrous acid other reactions occur in addition to the removal of free amino groups. In the reports from this Laboratory on the deamination of crystalline egg albumin (Ea)[2-4] discussion of the side reactions other than oxidation of –SH to –S–S– was inadvertently omitted. While conditions may be chosen, as in this study,[2] so that oxidation and the introduction into the tyrosine and phenylalanine residues of chromophoric –NO and diazo groups are minimal[5-7] attempts were made to arrive at information on the extent of these reactions under the conditions chosen.

Results and Discussion

(1) **–SH and –S–S– Groups.**—Except in instances in which it was specifically desired to study the –S–S– form of the deaminated protein, all products were routinely reduced with thioglycolic acid.[2] The effects of thioglycolic acid on the viscosity and nitroprusside tests of the partially deaminated fraction were discussed in the previous papers.

(2) **Folin Colors.**—The Folin–Ciocalteau phenol reagent[8] was employed to detect changes in the reducing power of the deaminated Ea fractions. The introduction of –NO and diazo groups often alters the Folin color, but such effects as are noted are difficult to evaluate quantitatively.[6,7] In the case of the deaminated Ea fractions no detectable alteration in Folin color was observed (Fig. 1).

(3) **Color of Deaminated Preparations.**—Deaminated proteins in which substitution has occurred have colors ranging from light yellow to yellow-red depending upon the extent of the secondary reactions.[6,7,9,10] The deaminated Ea preparations described in the previous papers were straw yellow in color.

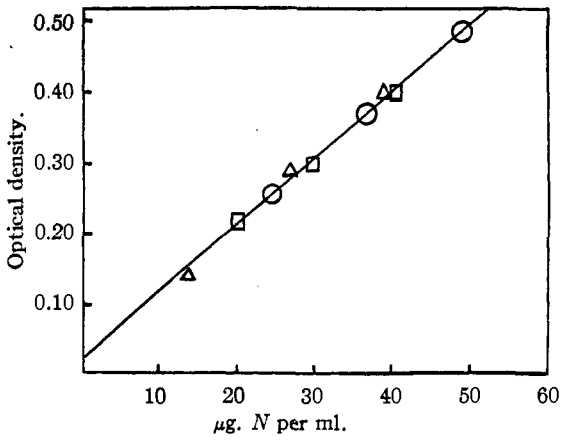

Fig. 1.—Folin colors: O, Ea; □, 3A; △, 3B.

For comparison tyrosine was deaminated as was crystalline Ea in acetate buffer at *p*H 4.0 with 1 *M* $NaNO_2$ at 0° for 8 hours.[2] The deaminated and partially substituted tyrosine, which was deep yellow in color, gave the absorption indicated in Fig. 2 when measured at 25° with a Beckman model DU spectrophotometer. Not only had the absorption peak shifted from 285 to 265 mμ, but there was a decrease in absorption at 285 mμ and appearance of a new absorption peak at 400 mμ.[6]

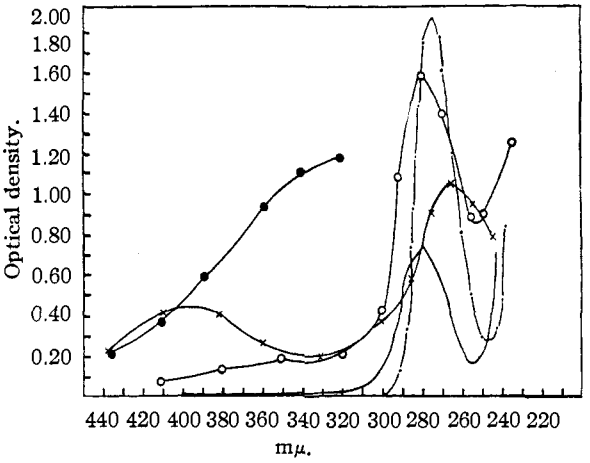

Fig. 2.—Absorption spectra: —·—·—· tyrosine (0.25 mg./ml.); —x—x—, deaminated tyrosine (0.05 mg./ml.); —o—o—, deaminated Ea 3B (0.39 mg. N/ml.); —●—●—, deaminated Ea 3B (1.86 mg. N/ml.); ———, Ea (0.15 mg. N/ml.).

Deaminated Ea 3B had its maximum absorption at 280 mμ as did Ea itself. However, from 300 to 440 mμ there was general absorption due to the introduction of chromophoric nitroso, azo and/or diazo groups into the aromatic acid residues.[5] The absorption at 411.5 mμ has been used as a measure of the extent of this reaction,[5,11,12] although the validity of any quantitative comparisons may be legitimately questioned. If one assumes 4% of tyrosine and 15.5% of nitrogen in Ea, it is evident that the deaminated Ea 3B exhibits the same color intensity as deaminated tyrosine at nine times its concentration. Therefore the second-

(1) University of Pittsburgh, School of Medicine, Pittsburgh, Penna.
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ary reactions which occur with tyrosine may have taken place, under the conditions chosen, to the extent of very roughly 10% with Ea during the deamination. The indications are, therefore, that in the deamination of Ea under controlled conditions the main reaction is a removal of a portion of the free $-NH_2$ groups and that the extent of the secondary reactions is minimized, though not eliminated.

(4) **Immunological Specificity.**—The classical studies of Obermayer and Pick[13] and of Landsteiner[14] demonstrate that the specificity of proteins is strongly modified by the introduction of substituents into the tyrosine residues, although exceptions are known.[15] The virtual identity of the specificity of fraction B with that of unmodified crystalline Ea may therefore, be interpreted as supplementing the other evidence that in this undenaturated fraction, at least, the effects of substitution are minimal. Whether or not substitution plays any part in the immunological differences observed between Fraction A, insoluble at its isoelectric point, and acid-denatured Ea is less certain.

Moreover acid denatured egg albumin (DnEa) which had been deaminated 58% by means of free nitrous acid was deep orange in color, yet its reactivity with anti-serum to DnEa was only slightly less than that of DnEa itself (Paper III, Fig. 3 ◀▶). Similarly the reactivities of the orange Ea FNA (Fig. 3 ◐) and of 8A FNA (◪) were only slightly less than that of the corresponding Fraction 6A (□). These data indicate that the specificity of DnEa is not greatly influenced either by the removal of roughly one-half of its free $-NH_2$ groups or by the substitution primarily of aromatic amino acid residues by nitrous acid.

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DEPARTMENT OF BIOCHEMISTRY AND MEDICINE
COLLEGE OF PHYSICIANS AND SURGEONS
COLUMBIA UNIVERSITY, NEW YORK

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The Self-Interaction of Mandelic Acid as Determined from Solubilities in Salt Solutions

BY W. F. MCDEVIT[1] AND F. A. LONG

One of the most common ways to determine the effects of salts on the activity coefficients of non-electrolytes is to compare solubilities in salt solutions with those in pure water. The data usually obey the Setschenow equation, $\log S_0/S = KC_s$, where S_0 and S are molar solubilities in pure water and salt solution, respectively, C_s is molar salt concentration and K is a constant, and it is common to identify $\log S_0/S$ with $\log f_i$ (where f_i is the molar activity coefficient of the non-electrolyte) and call K the salting out parameter. However, in general two types of interaction enter, ion–non-electrolyte interaction and self-interaction, *i.e.*, interaction of the non-electrolyte with itself. If one wishes to compare salt effects with theory or salt effects for two non-electrolytes of different solubility with each other, it is necessary to make a distinction between these two. In particular most theories of salt effect[2] deal only with the ion–non-electrolyte interaction. The contribution from the two types of interaction is shown by the following.

The activity coefficient of a species in solution, referred to the infinitely dilute solution, can usually be well represented by a power series in the concentrations[3] which for dilute solutions of a neutral molecule reduces to

$$\log f_i = k_s C_s + k_i C_i \qquad (1)$$

where the subscripts s and i refer to salt and non-electrolyte, respectively, and k_s and k_i are the parameters for salting out and for self-interaction. Combining this with the usual equation for equilibrium between saturated solutions

$$f_i S = f_i^0 S_0 \qquad (2)$$

and utilizing the fact that

$$\log f_i^0 = k_i S_0$$

one obtains

$$\log \frac{f_i}{f_i^0} = \log \frac{S_0}{S} = k_s C_s + k_i (S - S_0) \qquad (3)$$

When the solubility of the non-electrolyte is quite low, f_i^0 is unity and the term $k_i(S - S_0)$ is negligible. Here the salting out parameter k_s can indeed be obtained from a plot of $\log S_0/S$ against C_s. However, when the solubility is high the last term of Eq. (3) can be large and only with a knowledge of k_i can the value of k_s be determined.

A very interesting study by Ross, Morrison and Johnstone[4] on the influence of salts on the solubilities of racemic and optically active mandelic acids can be used to evaluate both k_s and k_i for this particular non-electrolyte and hence indicate the order of magnitude of the latter. The solubilities of these two forms of mandelic acid in water are high and quite different; at 25° the solubility of the racemic acid is 1.335 molal whereas that of the active form is only 0.738 molal. The striking features of the salt effects reported by the above authors for the two forms are: the observed salt effects are quite different for the active and racemic forms being considerably larger for the latter; the experimental values of the Setschenow parameters are very large when compared with similar molecules of low solubility (*e.g.*, benzoic and phthalic acids).

Table I gives for several salts the observed values of the Setschenow parameters, K^r and K^a for the racemic and active forms respectively. The other columns of the table give values of k_s and k_i calculated on the assumptions that the observed results are due to a combination of ion–non-electrolyte interaction and self-interaction and that the

(1) Chemical Department, Experimental Station, E. I. du Pont de Nemours and Company, Wilmington, Del.

(2) (a) P. P. Debye, *Z. physik. Chem.*, **130**, 56 (1927); (b) J. G. Kirkwood in Cohn and Edsall, "Proteins, Amino Acids and Peptides," Reinhold Publishing Corp., New York, N. Y., 1943, Chap. 12.

(3) Ref. 2b, Chap. 3.

(4) J. D. M. Ross, T. J. Morrison and C. Johnstone, *J. Chem. Soc.*, 264 (1938).