

[CONTRIBUTION FROM THE DIVISION OF AGRICULTURAL BIOCHEMISTRY, MINNESOTA AGRICULTURAL EXPERIMENT STATION.]

## THE NITROGEN DISTRIBUTION IN PROTALBINIC AND LYSALBINIC ACIDS.<sup>1</sup>

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### Introduction.

In 1902 Paal<sup>2</sup> prepared two substances by the action of alkali upon egg albumen which he named "protalbinic" and "lysalbinic" acids. These preparations have become important in that they exert a powerful protective or stabilizing action when added to suspenoid sols.<sup>3,4</sup> This action is so pronounced that sols to which these "acids" have been added may be evaporated to complete dryness and even after long standing they again form colloidal solutions when water containing a little alkali is added to the dried residue.

These products are classified as semi-colloids<sup>5</sup> in that the properties of their solutions appear to lie in the border ground between the crystalloids and the true emulsoids. Thus, for example, a solution of lysalbinic acid diffuses slowly through parchment paper and shows a measurable depression of the freezing point indicating a molecular weight of 700-800.

While the colloidal properties of the substances have been studied rather extensively, we know of no data bearing upon their composition other than the figures given by Paal for their ultimate analyses. We have therefore prepared these substances and determined their nitrogen distribution by the method of Van Slyke.<sup>6</sup>

### Experimental.

**The Preparation of the Materials.**—In preparing the lysalbinic and protalbinic acids, we were careful to follow the directions exactly as outlined by Paal.

Fifteen grams of sodium hydroxide were dissolved in 500 cc. of water and 100 g. of powdered egg albumen added. The flask containing the alkali was strongly shaken during the addition of the protein so as to avoid the formation of lumps. The mixture was then heated on an actively boiling water bath for approximately one hour, or until all but a few

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<sup>2</sup> Paal, C. "Über die Einwirkung, ätzender Alkalien auf Eialbumen," *Ber.*, **35**, 2195-2206 (1902).

<sup>3</sup> Taylor, W. W. "The Chemistry of Colloids." Longmans, Green & Co., N. Y., 1915, pp. 130-131, 188-189, 263-264, etc.

<sup>4</sup> Freundlich, H. "Kapillarchemie." Leipzig. 1909, pp. 451-453, etc.

<sup>5</sup> Freundlich, *loc. cit.*, p. 436.

<sup>6</sup> Van Slyke, D. D. The analysis of proteins by determination of the chemical groups characteristic of the different amino acids. *J. Biol. Chem.*, **10**, 15-55 (1911).

flecks had dissolved. During this heating large amounts of ammonia were evolved. The solution was filtered and acetic acid added as long as a precipitate formed. Hydrogen sulfide was given off at this stage. After allowing the precipitate to stand 12 hours it was filtered off, washed with a little water, ground to a paste with additional water and dialyzed against distilled water until the solution in the dialyzer was no longer acid. The precipitate in the dialyzer was then filtered off, dried in a vacuum oven and powdered.

The protalbinic acid so prepared was an almost white powder, yield 35 g. from 100 g. of egg albumen.

The filtrate from the protalbinic acid contains the lysalbinic acid. However, when the filtrate is to be worked up for lysalbinic acid, Paal recommends that the acetic acid precipitation of the protalbinic acid be replaced by sulfuric acid. This was accordingly done. The filtrate from the protalbinic acid was neutralized with sodium hydroxide and dialyzed until practically free from sodium sulfate. This left a solution of "lysalbinic sulfate" in the dialyzer. Barium hydrate solution was added to this "lysalbinic sulfate" in sufficient quantity to exactly precipitate all of the sulfuric acid and the barium sulfate filtered off. The filtrate containing the free "lysalbinic acid" was concentrated to a thin syrup and poured into 4 or 5 volumes of alcohol. The lysalbinic acid separated as a white cheese-like precipitate which was filtered off, dried in a vacuum oven and powdered. Yield 20% of the original egg albumen.

**Method of Analysis.**—Van Slyke's<sup>1</sup> method of analysis is too well known to need description here. Duplicate portions of 3 g. each of the original egg albumen, the protalbinic acid and the lysalbinic acid were hydrolyzed for 48 hours with 1.115 sp. gr. HCl and the nitrogen distribution determined. The averages of these analyses, calculated in per cents. of the total nitrogen, are shown in Table I.

TABLE I.—SHOWING THE AVERAGE NITROGEN DISTRIBUTION IN PER CENTS. OF THE TOTAL NITROGEN OF EGG ALBUMEN, PROTALBINIC ACID AND LYSALBINIC ACID.

	Egg albumin.	Protalbinic acid.	Lysalbinic acid.
Ammonia N.....	9.08	5.08	8.21
Humin N.....	4.71	4.00	4.49
Cystine N.....	0.72	0.11	0.51
Arginine N.....	6.05	6.32	6.23
Histidine N.....	6.48	7.96	6.42
Lysine N.....	10.09	13.73	12.50
Amino N in filt. from bases.....	61.26	58.17	58.37
Non-Amino N in filt. from bases.	4.58	5.22	2.70
Total.....	102.97	100.59	99.43

It will be seen from the table that there are but slight differences be-

<sup>1</sup> *Loc. cit.*

tween the analysis of the original egg albumen and those of the protalbinic and lysalbinic acids. Indeed, the differences are so small as to be in most of the fractions well within the experimental error of the analysis. Protalbinic acid is significantly lower in ammonia N than is either the original egg albumen or the lysalbinic acid and both of the derived "acids" are higher in lysine content. It must be pointed out, however, that in all probability the figures in a Van Slyke analysis of materials prepared in this manner are misleading for if the guanidine group of arginine in the original egg albumen is attacked by the alkali, ornithine would result and this would be calculated as lysine in Van Slyke's method. To our mind the apparent increase in lysine in the protalbinic and lysalbinic acids is due to the presence of ornithine. This hypothesis, however, has not as yet been tested experimentally.

#### Summary.

Lysalbinic and protalbinic acids have been prepared from egg albumen by Paal's method and the nitrogen distribution of these preparations has been determined by Van Slyke's method and compared with a similar analysis of the original egg albumen. This comparison leads to the following conclusions:

(1) The nitrogen distribution in protalbinic and lysalbinic acids is not markedly different from that of the original egg albumen.

(2) Both protalbinic and lysalbinic acids show a somewhat greater apparent lysine content than does the original egg albumen.

(3) We believe that this apparently greater lysine content is due to the presence of ornithine, derived from arginine by the action of the alkali, inasmuch as ornithine if present would appear in the lysine fraction in Van Slyke's method.

(4) The analyses here recorded furnish no evidence as to whether or not the protalbinic and lysalbinic acids are true chemical compounds or as to whether or not their chemical structure is more simple than is that of egg albumen. It is extremely improbable, however, that either preparation has as low a molecular weight as 800.

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### THE EFFECT OF PROLONGED ACID HYDROLYSIS UPON THE NITROGEN DISTRIBUTION OF FIBRIN WITH ESPECIAL REFERENCE TO THE AMMONIA FRACTION.<sup>1</sup>

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#### Introduction.

In an article devoted to the study of "Different Forms of Nitrogen in

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