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EGG ALBUMIN.

BY THOMAS B. OSBORNE.

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HOPKINS has recently shown[1] that the crystallization of egg albumin is greatly facilitated by the addition of acetic acid to the half saturated ammonium sulphate solution. I have found that crystallization is thus promoted, because the crystallized egg albumin is a compound of the protein substance with acid.

When egg white is first mixed with half saturated ammonium sulphate solution an alkaline reaction towards litmus can be detected and a decided odor of free ammonia develops. After this solution has stood for some hours, all evidence of free ammonia disappears and the solution is then perfectly neutral to litmus and continues neutral during the gradual separation of the albumin. The deposited substance, whether in the form of spheroids or of crystals, when filtered out and dissolved in water, reacts distinctly acid with litmus, as well as with phenolphthalein.

In order to obtain the albumin in crystals, it has heretofore been necessary to precipitate it several times, evidently because, during the earlier evaporations, an insufficient amount of acid

[1] *Jour. Physiology*, **23**, 131.

is formed to produce the crystalline compound. It is also for this reason that, if acetic acid be added as Hopkins directs, the albumin is obtained completely crystallized by a single precipitation, and that too without any concentration by evaporation.

I have found that if, instead of acetic acid, a molecularly equivalent quantity of hydrochloric acid be added, the separation takes place even more quickly and, so far as my experience has as yet gone, within a short time is more complete than with acetic acid during the same time. Thus, I prepared from two portions of 1500 cc. each of perfectly fresh egg white a quantity of crystallized egg albumin by aid of each of these acids, with the following results:

After adding to one-half of the egg white, acetic acid in the proportion and manner directed by Hopkins, and a molecularly equivalent quantity of hydrochloric acid mixed with 300 cc. of half-saturated ammonium sulphate solution to the other half, the two solutions were set aside to deposit albumin. After three hours a very large crystalline precipitate had separated in the portion with hydrochloric acid. This precipitate was then filtered out, but the portion with acetic acid was allowed to stand for twenty-four hours, because the separation appeared to be much less than that in the hydrochloric solution.

These two precipitates were each twice recrystallized, freed as completely as possible from mother-liquor, by pressing out with filter-paper, dissolved in water, and the solutions dialyzed for ten days, until wholly freed from sulphate, when they were filtered clear and evaporated at about 50°. The residue left by the acetic acid solution, A.1, weighed twenty-nine grams; that from the hydrochloric acid, H.1, fifty-nine grams.

The filtrates from the several crystallizations of these two preparations yielded a second crop of completely crystallized albumin; that from the acetic acid solution, A.2, weighing forty-three grams; that from the hydrochloric acid solution, H.2, seven and nine-tenths grams. Similarly, from the mother-liquors from these preparations, two other entirely crystalline products were obtained, weighing respectively, A.3, eight grams and H.3, four and nine-tenths grams. From the finally remaining acetic acid solutions another preparation separated, consisting wholly of spheroids, A.4, which weighed nine and one-tenth grams.

There were thus secured from 1500 cc. of egg white, by adding acetic acid, 80 grams of wholly crystallized albumin, and from 1500 cc., with hydrochloric acid, 73.2 grams, or 5.30 and 4.90 grams respectively per cubic centimeter of egg white.

The crystallized albumin, like all the other protein preparations which I have as yet examined, is a compound of a protein substance with an acid. In order to neutralize to litmus and to phenolphthalein the solutions of one gram of each of these preparations of albumin, it was necessary to add the following quantities of decinormal potassium hydroxide solution:

	A.1.	A.2.	A.3.	A.4.	H.1.	H.2.	H.3.
To phenolphthalein.....	2.05	2.30	2.30	2.35	2.05	2.25	2.20
To litmus	1.30	1.60	1.65	1.55	1.30	1.60	1.50
Difference.....	0.75	0.70	0.65	0.80	0.75	0.65	0.70

If, as pointed out in another paper, the molecular weight of the protein substance is about 15,000,[1] one gram would react with 0.67 cc. of a decinormal solution, a quantity nearly equal to the difference in acidity shown by these two indicators. Three molecules of acid reacting with one of albumin would be equal to two cc. of decinormal solution per gram of albumin, a quantity in very close agreement with that found for the two fractions constituting the greater part of all the albumin, A.1, and H.1., and which also differs but little from that required to neutralize one gram of all the other fractions.

When the albumin, dissolved in water, was neutralized with decinormal potassium hydroxide, the solution evaporated to dryness and the proteid matter burned off, an ash was left containing potassium carbonate almost molecularly equivalent to the acid of the albumin originally neutralized. From this it appears that the acid is mostly, if not wholly, organic.

It has been, thus far, impossible to discover what acid or acids were united to the albumin. Neutralization of the albumin suspended in fifty per cent. alcohol resulted in the formation of a gummy mass difficult to filter and wash, and from which none of the products of neutralization could be separated. Neutralization of a solution of ten grams of the albumin and dialysis in distilled water, failed to give enough salts in the diffusate to shed light on the nature of the acid. Neutralization with baryta of a

[1] Sabanejeff: *Chem. Centrbl.* (*1891*), 10, found the molecular weight of purified egg albumin by determining the lowering of the freezing-point to be 15,000.

solution of two grams of the albumin gave a very slight precipitate, which after standing some days, was filtered out, washed and ignited, but only four milligrams of mineral matter were obtained.

The preparations showed no excess of sulphur over that usually found in coagulated and thoroughly washed albumin prepared without the use of sulphuric acid or sulphates. Determination of total phosphorus showed A.1 and H.1 to contain 0.38 and 0.40 per cent. phosphorus pentoxide respectively. These preparations contained 0.87 and 0.69 per cent. of ash which was almost wholly insoluble in water and appeared to consist chiefly of calcium phosphate. The total phosphorus in these preparations was equal to 0.59 and 0.64 per cent. of tricalcium phosphate respectively.

Towards lacmoid these preparations reacted alkaline, about one cc. of decinormal acid being required to neutralize the solution of one gram, and three cc. to give an acid reaction. When one gram was treated with decinormal hydrochloric acid, no evidence of free acid was shown with tropæolin, until eight or nine cc. were added.

When pure water solutions containing two and five-tenths per cent. of my albumin preparations were heated they all became turbid at 58°–59° and separated a minute quantity of flocks at 59°–60°. On gradually raising the temperature the coagulum slowly increased until at 70° much of the dissolved albumin had coagulated. The solutions heated for some time at 74° and filtered still contained a little proteid which even on heating at 99° did not separate until some salt was added. No break in this gradual coagulation of the albumin was detected, the solutions when filtered after partial coagulation yielding a coagulum on again heating up to the temperature to which they had been developed at previously heated.

When solutions of pure ten per cent. sodium chloride brine containing two and one-half per cent. of each of these preparations except A.3, H.3 and A.4 were slowly heated, turbidity developed at 56°–59° and flocks at 56°–60°.

Only a trace of coagulum was obtained, however, below 64°, and the solutions filtered from this remained perfectly clear until heated to nearly or quite 70°, when the albumin began to coagulate. It was, however, found necessary to heat the solution to nearly 84° before most of it was separated.

The three preparations A.3, H.3, and A.4, behaved as just described, except that below 64° each yielded a relatively considerable coagulum. These preparations, it is to be noted, are final fractions obtained in small quantity and it seems probable that this coagulum obtained at 60°–64° is due to the presence of a different substance from that constituting the chief part of the other fractions. This is the more probable as A.3 and A.4 also showed a difference in specific rotation, as well as in composition.

The degree of acidity was found to have much influence on the coagulation of the albumin. Exact neutralization to phenolphthalein, as might be expected, entirely prevented coagulation, even on boiling. When the acid of the albumin was neutralized so that the acidity was equal to one and two-tenths cc. of decinormal solution per gram of albumin, a solution containing two and five-tenths per cent. of the proteid became slightly opalescent on heating to 72° and remained otherwise unchanged, even after heating for a long time in a boiling water-bath. If, however, the acidity was but one-tenth cc. greater, that is, equal to one and three-tenths cc. per gram of albumin, the solution became turbid at 70°, and very opaque after heating in the water-bath at 99°. The difference between the two solutions was marked and it is evident that the additional one-tenth cc. had caused a change in the condition of the albumin. An acidity of 1.33 cc. per gram is almost exactly equal to two molecules of acid per molecule of albumin, assuming the latter to have a molecular weight of 15,000. From this it would seem to be necessary to add three molecules of acid to one of albumin, in order to form the coagulable substance.

The specific rotation of these preparations was approximately determined by means of a Schmidt and Haensch polarimeter using a 200 mm. tube. The readings on the sugar scale were converted into degrees of circular polarization by multiplying by 0.346. The formula used in calculating the results was

$$(a)_D = \frac{a \times 100}{p \times d \times l} \text{ where}$$

a = observed rotation,
p = per cent. of albumin in the solution,
d = density of the solution,
l = length of tube in decimeters.

The results obtained were as follows:

Preparation.	Per cent. of dissolved albumin.	Solvent.	Rotation.	Average.
A.1	5.861	Water	−29° 48′	−29° 17′
	6.670	"	−28° 46′	
A.2	3.422	"		−29° 23′
A.3	3.273	"		−33° 3′
A.4	3.404	"		−41° 45′
H.1	3.425	10 per cent. NaCl	−29° 0′	−28° 35
	3.237	Water	−28° 33′	
	6.478	"	−28° 1′	
H.2	1.699	"		−28° 14′
H.3	3.205	"		−39° 31

As the results obtained on A.1, A.2, H.1, and H.2, agree closely and as these preparations represent very different proportions of the total albumin of the egg white, it seems probable that we have in these fractions but one substance.

Bondzynski and Zoja, working with solutions containing ammonium sulphate, obtained similar but somewhat lower figures for the specific rotation of their least soluble fractions; namely, 25° 8′ and 26° 2′, duplicate determinations on the same fraction. Two other fractions gave them 34° 18′ and 42° 54′, figures agreeing fairly with those obtained by me for my more soluble fractions. They determined the albumin in the polarized solution by coagulation, a process which does not admit of so exact a determination of the dissolved albumin as that employed by me, which consisted simply in evaporating the pure water solution to dryness, drying to constant weight at 110° and deducting ash. A slight error in determining the dissolved albumin causes a considerable error in the specific rotation.

The effect of acid and alkali on the rotation of the albumin solutions is shown by the following results, obtained by dissolving one gram of A.2, in twenty-five cc. of water and treating with the given quantities of acid or of alkali:

1 gram A.2	+ nothing		−29° 17′
	+ 0.8 cc.	N/10 HCl	−29° 5′
	+ 8.0 cc.	"	−33° 46′
	+ 1.4 cc.	N/10 KOH	−28° 45′
	+ 2.7 cc.	"	−30° 20′
	+ 4.2 cc.	"	−32° 30′

It is to be noted that by eight cc. of the acid and by four and two-tenths cc. of the alkali a rotation was produced about ten

per cent. higher than with the smaller quantities of acid or alkali. This increase may well be due to a local overreaction taking place on mixing the acid and alkali with the proteid solution, it having been demonstrated that large proportions of acids and alkalies yield products of high specific rotation.

Panormoff[1] has studied the specific rotation of fractionally precipitated crystallized egg albumin and concludes that there are two albumins present in egg white, one with a specific rotation of —23.6° and the other —46.2°. The albumin with the lower rotations he obtains from the so-called egg globulin precipitated by adding an equal volume of saturated ammonium sulphate solution to the egg white. This he succeeded in crystallizing and, so prepared, finds it to have the properties and composition of albumin. He considers, therefore, the egg globulin to be a compound of egg albumin with some unknown substance.

As the egg white is alkaline to litmus and ammonia is set free on adding to it a saturated solution of ammonium sulphate, it is not surprising that a product should be produced of different solubility from that of the albumins which we have been considering.

Panormoff converted his crystallized albumin into a chloride by dialysis against two-tenths per cent. hydrochloric acid. He analyzed the product obtained and it is interesting to note that, if calculated free from hydrochloric acid, the figures for the albumin are in exceedingly close agreement with the average of the best analyses of albumin. Furthermore, the proportion of hydrochloric acid in the compound is exactly the same as that which I found with tropæolin to be fixed by the albumin; that is, I found that one gram of albumin united with eight cc. of decinormal acid or 0.0292 hydrochloric acid to form a compound showing no free acid with tropæolin, while Panormoff's chloride contained 2.92 per cent. or exactly the same quantity.

In regard to the composition of egg albumin, confusion has recently been caused by Hofmeister, who states[2] that he has found in repeatedly crystallized egg albumin 1.01 and 1.18 per cent. of sulphur and that Dr. F. N. Schulz, in his laboratory, has obtained 1.24 and 1.27 per cent. He consequently calls in ques-

[1] Ref. in *Chem. Centrbl.* (*1898*), II, 358 and 487.

[2] *Ztschr. physiol. Chem.*, 24, 166.

tion the purity of the samples of crystallized albumin, analyzed with great care by Bondzynski and Zoja. As Hofmeister's figures for carbon are higher and for nitrogen lower than those of Bondzynski and Zoja, as well as of other investigators who have analyzed *amorphous* egg albumin, the whole question of the composition of this substance is again thrown into confusion.

Having at hand a sample of egg albumin, which had been three times recrystallized in the manner described by Hofmeister as necessary for its purification, and obtained in the same proportion from the egg white as stated by him to be the usual yield after thorough purification, and which had been coagulated with alcohol and thoroughly washed until all the ammonium sulphate had been removed, I analyzed it, dried at 110°, with the result given under No. 1.

Analyses of the seven fractionally crystallized preparations A.1–4, and H.1–3, were made after drying them to constant weight at 110°.

	No. 1.	H.1.	H.2.	H.3.
Carbon	52.18	52.85	52.33	51.72
Hydrogen	6.91	6.92	6.90	6.90
Nitrogen	15.67	15.66	15.77	15.26
Sulphur	1.70	1.572	1.644	1.958
Oxygen	23.54	22.998	23.356	24.162
	100.00	100.00	100.00	100.000
Ash	0.56	0.69	0.67	0.59
Total phosphorus pentoxide	....	0.40	0.21	trace

	A.1.	A.2.	A.3.	A.4.
Carbon	52.60	52.61	52.33	51.44
Hydrogen	7.02	6.94	6.93	6.88
Nitrogen	15.54	15.76	15.40	15.20
Sulphur	1.610	1.612	1.778	1.912
Oxygen	23.230	23.078	23.562	24.568
	100.000	100.000	100.000	100.000
Ash	0.87	0.65	0.67	0.40
Total phosphorus pentoxide	0.37	0.28	trace	trace

There can no longer be question about the amount of sulphur in albumin being greater than that stated by Hofmeister. My sulphur determinations were made with extreme care, fusing more than a gram of the substance over an alcohol lamp with

pure sodium hydrate and peroxide in a nickel crucible,[1] dissolving the fusion in an excess of hydrochloric acid, neutralizing most of the excess of acid and precipitating with barium chloride from a boiling solution of at least 800 cc. volume. Blank determinations showed no trace of sulphur in the reagents and also that none was absorbed during the fusion over the alcohol lamp. These results agree with those obtained by Bondzynski and Zoja, though the difference in composition between their extreme fractions was not quite so great as found for my preparations.

The composition, rotation, heat-coagulation points and reactions of the crystallized egg albumin obtained by aid of hydrochloric or acetic acids show this to be the same substance as that which has in the past been regarded as egg albumin.

My results, those of Bondzynski and Zoja, and of Panormoff, make it plain that there are two protein substances in the egg white, which are commonly obtained admixed when preparing egg albumin by the usual processes. Whether the extremes of my fractional precipitations of these two albumins consist wholly or even largely of each one of these bodies requires further investigation of large quantities of egg white. This work we now have well under way.

Moerner[2] has described ovomucoid as identical with Neumeister's pseudopeptone,[3] and states that it constitutes about one-eighth of the organic substance of the egg white. As this substance is described as largely, though not wholly, precipitated by two-thirds saturation of its solution with ammonium sulphate, it ought, if present as such in the egg white, to be found among the more soluble fractions thrown down by successive additions of ammonium sulphate. It is intended to direct especial attention to the isolation of this substance and to determine if possible in how far it may be admixed with the albumins.

LABORATORY OF THE CONNECTICUT AGRICULTURAL EXPERIMENT STATION, NEW HAVEN, CONN.

[1] I have found many times when using a nickel crucible that, on dissolving the fusion, there was a black substance present (this looks and behaves like nickel sulphide, but it seems hardly possible that nickel sulphide could escape oxidation), which, when filtered out and oxidized, was found to contain sulphur. This black substance should be dissolved by the chlorine liberated from the hydrochloric acid by the peroxide of hydrogen, otherwise too low results will be obtained.

[2] *Ztschr. physiol. Chem.*, 18, 525.

[3] *Ztschr. Biologie*, 9, 369.