[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF CHICAGO]

Studies in Proteins. VI. The Solubility of the Nitrogenous Constituents of Certain Seeds in Sodium Chloride Solutions

BY L. P. O'HARA AND FELIX SAUNDERS

Introduction

These studies were undertaken principally in the hope that a more precise and explicit definition of globulins might be proposed. At present no two writers of textbooks on biochemistry define globulins in the same way. For example: Globulins are "soluble in dilute solutions of neutral salts, not soluble, however, in concentrated solutions; precipitated by half-saturated ammonium sulfate" (Williams)[1]; "simple proteins insoluble in pure water but soluble in neutral solutions of salts of strong bases with strong acids" (Bodansky)[2]; "insoluble in water, but soluble in neutral dilute solutions of salts of strong bases and acids, such as sodium chloride" (Mathews).[3] According to the "Joint Recommendation of the Physiological and Biological Committees on Protein Nomenclature"[4] globulins should be defined as "simple proteins insoluble in pure water but soluble in neutral solutions of salts of strong bases with strong acids." Gortner[5] has questioned the validity of these definitions. He says, "a definition which is based upon solubility, *i.e.*, peptization in a dilute salt solution, is so ambiguous as to be absolutely meaningless."

In the preceding papers of this series the following facts have been brought out. (1) As would be expected from the usual definitions of globulins, normal alkali halide salts extract from orange-seed meal the same amount of protein (pomelin) and nitrogenous material.[6] (2) Normal solutions of various sodium salts, some neutral and some alkaline, extract the same amount of nitrogenous material, presumably protein, from orange-seed meal.[7] The present work was undertaken in order to determine how much nitrogenous material is extracted by different concentrations of sodium chloride from various seed meals so that we may test our definition of globulins on another point, that of solubility in concentrated salt solutions.

Experimental

The seeds were ground, defatted and sifted in the manner already described.[7] The orange-seed meal was 60–80 mesh, the peanut meal and the flaxseed meal were 80–100 mesh. Total nitrogen was determined for the air-dried seed meal. A weighed amount[8] of the seed meal was placed in a 250-cc. centrifuge bottle and 50 cc. of solution added. The bottles were shaken for thirty minutes in a mechanical shaker, centrifuged for twenty minutes and the supernatant liquid decanted directly into a Kjeldahl flask. The extraction was repeated two more times and the total nitrogen determined in the combined solutions by the Kjeldahl macro method.

As long as the scope of the investigation had to be limited it seemed best to select seeds representing as many different types as possible. Consequently, the following seeds were chosen: orange and flax, which yield crystalline globulins; peanut, which gives a well-defined but non-crystalline globulin; and rye, which probably does not contain any globulin. Sodium chloride was chosen because it is the salt most commonly used in protein-extraction studies.

In order to study the effect of time a series of extractions was run on flaxseed meal with 0.37 N sodium chloride. The time intervals for extraction were one-half, two, and four times the usual interval. The results as shown in Table I indicate that time is a negligible factor within the limits chosen for this experiment.

TABLE I

THE EFFECT OF TIME UPON THE AMOUNT OF NITROGENOUS MATERIAL EXTRACTED FROM FLAXSEED MEAL BY 0.37 N SODIUM CHLORIDE

Time (x = usual time interval)	Total N extracted, %
$0.5x$	60.77
$1.0x$	60.46
$2.0x$	59.82
$4.0x$	60.68

Several times, especially during the summer months, discordant results were obtained. It was finally concluded that the deviations might be due to temperature changes which are often very marked and sudden in Chicago. In order to check this hypothesis, several extractions of seed meal with 4 N sodium chloride were run at various temperatures. The results (Table II) clearly show a temperature coefficient. Temperature changes are probably negligible during the winter when buildings are steam heated, but during the summer temperature must be con-

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(1) Williams, "Introduction to Biochemistry," D. Van Nostrand Company, Inc., New York, 1931.

(2) Bodansky, "Introduction to Physiological Chemistry," 3rd ed., John Wiley and Sons, Inc., New York, 1934.

(3) Mathews, "Principles of Biochemistry," William Wood and Co., Baltimore, 1936.

(4) *J. Biol. Chem.*, **4**, xlviii (1908).

(5) "Outlines of Biochemistry," John Wiley and Sons, Inc., New York, 1929.

(6) Saunders, THIS JOURNAL, **53**, 696 (1931).

(7) Rotha and Saunders, *ibid.*, **54**, 342 (1932).

(8) The weight of a sample was always the same for any given meal (Table III).

trolled. After the importance of temperature variations was demonstrated, extractions were run at constant temperature.

TABLE II

EFFECT OF TEMPERATURE UPON THE AMOUNT OF NITROGENOUS SUBSTANCE EXTRACTED FROM ORANGE-SEED MEAL BY 4.0 *N* SODIUM CHLORIDE

Temperature, °C.	Total N extracted, %
39.7	48.65
26.0	41.43
−2.5	35.92

The two findings reported above were only incidental to the main problem, namely, to determine how the amount of nitrogen extracted from seed meals would vary with changes in the concentration of the extracting sodium chloride solution which ranged from 0 to saturation. The data (Table III) demonstrate clearly that (1) the most efficient extraction of nitrogenous material occurs when the concentration of the sodium chloride is between 0.25 and 3.0 *N* and (2) even a saturated sodium chloride solution will extract almost as much as a normal solution. Because of this fact, extraction with saturated sodium chloride has been made our standard procedure for the isolation of crystalline globulins, since the extracted material is less likely to be contaminated with gummy substances which interfere with further purification.[9]

TABLE III

EFFECT OF CONCENTRATION UPON THE AMOUNT OF NITROGENOUS SUBSTANCE EXTRACTED FROM VARIOUS SEED MEALS BY SODIUM CHLORIDE

	—Total N extracted from seed meals, %—			
Normality of NaCl soln.	Flax 3-g. sample	Orange 3-g. sample	Peanut 1.5-g. sample	Rye 5-g. sample
Distilled water	24.27	32.24	52.86	14.81
0.125	41.73	35.59	71.15	20.32
.25	58.63	51.10	91.76	32.45
.375	64.59	58.12	94.85	34.75
.50	64.50	65.54	94.55	34.13
.75	64.48	70.22	93.88	26.69
1.0	64.59	73.34	94.42	25.00
2.0	65.19	72.38	95.37	17.03
3.0	63.75	71.59	94.14	18.57
4.0	61.71	67.62	95.89	14.49
5.0	56.29	67.82	92.26	11.23
6.0	50.12	65.84	87.68	7.65
Saturated	45.53	64.92	81.23	7.65

Discussion

If we make the assumption that most of the nitrogen extracted represents protein, then the data of Table III show that in solutions of sodium chloride greater than 0.5 *N* the concentration of the sodium chloride has little effect upon the solubility of the protein. This assumption is probably justified (except in the case of rye flour) since it can be shown that as much as 90% of the total nitrogen extracted by *N* sodium chloride can be recovered as crystalline globulin (unpublished results) and, as mentioned above, crystalline globulin can be extracted from some seed meals by saturated sodium chloride solutions. The crystalline globulins obtained by extraction with either *N* or saturated sodium chloride solutions have the same crystalline form and give the same analytical figures for total sulfur, total nitrogen and nitrogen distribution.

It has also been shown[7] that *N* solutions of strong bases and weak acids such as sodium benzoate and sodium acetate will extract just as much nitrogen from orange-seed meal as will solutions of salts of strong bases and strong acids.

The data in Table III show that the proteins of rye flour, in which gliadin predominates, show a type of solubility curve entirely different from those of the other three seeds in which globulin predominates. In the case of the rye flour, which contains little or no globulin, the curve rises to a definite peak and then falls off rapidly. In the case of the globulin-containing seeds the curve rises steeply and then remains almost horizontal.

The findings reported above show that the ordinary definitions of globulins are false and misleading, at least by implication. Certainly some globulins are soluble in saturated salt solutions and they are also soluble in solutions of salts of strong bases and weak acids. A satisfactory definition of globulins must take these facts into consideration explicitly.

Several workers have questioned the advisability of basing definitions and classification of proteins on solubility. Staker and Gortner,[10] referring to an earlier paper, state, "Gortner, Hoffman and Sinclair[11] were the first to object to a system of protein classification based upon 'solubility'." These authors[11] also state, "It may be that only 'crystalline' globulins are chemical entities and that the amorphous globulin preparations recorded in the literature are non-homogeneous fractions whose chemical and physical properties depend upon the technic employed in their isolation."

It is interesting to note that McCalla and Rose,[12] working with the gliadin–glutenin system of wheat flour, have also come to the conclusion

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[9] *Cf.* Weyl, *Z. physiol. Chem.*, **1**, 72 (1877); Osborne, *Am. Chem. J.*, **14**, 629 (1892); THIS JOURNAL, **19**, 487 (1897).

[10] Staker and Gortner, *J. Phys. Chem.*, **35**, 1565 (1931).

[11] "Colloid Symposium Monograph," Vol. V, The Chemical Catalog Co., Inc., New York, 1928, p. 179.

[12] McCalla and Rose, *Can. J. Research*, **12**, 346 (1935).

that, "definitions of flour proteins based on solubility in a solution such as 70% alcohol are purely arbitrary." Their work has no bearing on the specific problem of the definition of globulins but is of importance in the general problem of protein classification.

Undoubtedly the solubilities of different proteins are a reflection of the kind and arrangement of their constituent amino acids. Any definition must necessarily be inadequate and empirical until more is known about the composition of proteins.

Conclusions

1. The amount of nitrogenous material extracted from certain seed meals by saturated sodium chloride solutions is almost as large as that extracted by N solutions. In those seed meals where this conclusion is true, the material which is extracted appears to consist almost wholly of a crystalline or hemi-crystalline protein having the characteristics of a globulin.
2. When a solution of given concentration is used for the extraction of nitrogenous material, a marked temperature coefficient is found.
3. The ordinary textbook definitions of globulins do not adequately consider the solubility properties of globulins.

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[A CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY OF RUTGERS UNIVERSITY]

The Mechanism of Coprecipitation of Anions by Barium Sulfate[1]

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Five theories[3] have been advanced to account for the contamination of precipitates, *viz.*, formation of mixed crystals, occlusion, surface adsorption after the formation of the precipitate, compound formation and postprecipitation. If coprecipitation is defined as the retention of soluble substances by a precipitate, compound formation and postprecipitation cannot be regarded as true coprecipitation. Surface adsorption is important only in the cases of amorphous and flocculent precipitates. Therefore, coprecipitation by barium sulfate is due either to the formation of mixed crystals or to occlusion. By occlusion is meant the adsorption of foreign ions during the growth of the crystals of the precipitate and subsequent entrapping of these foreign ions by the growing crystals.[3]

All conditions of precipitation such as temperature, concentration, time of addition of reagents, time of digestion, etc., being the same, the amount of coprecipitated ion in a precipitate should depend upon some specific property of this ion. As stated by Paneth[4] and Fajans,[5] the occlusion theory maintains that this determining factor is the solubility of the compound formed by the coprecipitated ion and the ion of opposite charge of the precipitate. On the other hand, the mixed-crystal theory of Grimm[6] and coworkers holds that this property is the similarity in crystal structure of the precipitated and coprecipitated substances. Otto Hahn[7] was among the first to recognize that both explanations might be correct. He also stated that mixed crystals may be formed even when Grimm's[8] conditions are not fulfilled. His rules, however, are not very clear and are apparently contradictory in certain respects.

A study of the coprecipitation of various anions by barium sulfate throws considerable light upon this subject. Weiser and Sherrick[9] determined the quantity of coprecipitated anions under identical conditions of precipitation. They did not use the Paneth–Fajans rule to interpret their results and hence were somewhat at a loss to explain them. Sofin[10] repeated their work under more carefully controlled conditions. Using iodide, chloride, bromide, nitrite, nitrate and chlorate, he found that, in accord with the Paneth–Fajans rule, the lower the solubility of the barium

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[1] Presented before the Division of Physical and Inorganic Chemistry at the 92d Meeting of the American Chemical Society, Pittsburgh, September 7-11, 1936.

[2] Abstracted from a thesis by Frank Schneider, presented to the Graduate Faculty of Rutgers University in partial fulfilment of the requirements for the Ph.D. degree.

[3] For a discussion of these theories see Kolthoff, *J. Phys. Chem.*, **36**, 860 (1932).

[4] Paneth, *Physik. Z.*, **15**, 924 (1914).

[5] Fajans and Beer, *Ber.*, **46**, 3486 (1913).

[6] Grimm and Wagner, *Z. physik. Chem.*, **132**, 131 (1928).

[7] O. Hahn, Erzbacher and Feichtinger, *Ber.*, **59**, 2014 (1926).

[8] Grimm, *Z. Elektrochem.*, **30**, 467 (1924).

[9] Weiser and Sherrick, *J. Phys. Chem.*, **23**, 205 (1919).

[10] Sofin, Master's Thesis, Rutgers University, 1934.