[Contribution from the Department of Physiological Chemistry of The University of Chicago]

STUDIES IN PROTEINS. III. THE UNIFORM SOLUBILITY OF THE PROTEIN FRACTION OF ORANGE SEED MEAL IN SOLUTIONS OF VARIOUS SODIUM SALTS[1]

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Introduction

The solubility of certain proteins in neutral salt solutions was first observed by Denis[2] in 1859. The method received little attention until 1876 when Weyl[3] used it in extracting seeds from which he obtained a product which resembled the globulins found in animal tissues. Previously, Ritthausen[4] had obtained products similar to these plant globulins by extraction with dilute alkaline solutions, but it was maintained by Weyl that these products differed from globulins. Ritthausen, however, was able to show that the compounds which he obtained were similar whether extracted by weakly alkaline solutions or by neutral salt solutions. The value of his work was still questioned.

Because of the confusion that existed in reports on vegetable proteins, T. B. Osborne[2] undertook an extensive study of the subject and as a result has given us much information concerning the plant globulins. In practically all of the studies of Osborne and of the other workers in the field, sodium chloride was used in various concentrations as the extracting agent. In 1905 Osborne and Harris[5] determined the solubility of proteins in solutions of various salts. Edestin was used as a typical crystalline globulin. It was observed that the chlorides of univalent metals such as sodium and potassium had practically the same power to dissolve the edestin, while the chlorides of divalent metals extracted about twice as much as those of the univalent. A normal solution of sodium sulfate dissolved the globulin readily, while a molar solution dissolved very little. Sodium and potassium iodides were alike in having the greatest solvent effect on the protein. It was also observed that the basic salts in general had a greater solvent power than the acid salts.

Other workers than Osborne have investigated the solvent effect of various salts and have obtained varied results depending upon the salt

[1] From the thesis presented by Louise Knight Rotha in partial fulfilment of the requirements for the degree of Master of Science, 1931.

[2] P. S. Denis, *Mémoire sur le sang*, etc. (1858) as quoted in T. B. Osborne, "The Vegetable Proteins," Longmans, Green and Co., New York, **1919**, p. 21.

[3] T. Weyl, *Z. physiol. Chem.*, **1**, 72–100 (1877).

[4] H. Ritthausen, *Pfluger's Archiv.*, **15**, 269–288 (1877); **21**, 81–104 (1880).

[5] T. B. Osborne and I. F. Harris, *Am. J. Physiol.*, **14**, 151–171 (1905).

used, the concentration of the solution, the material being studied, and various other factors. Particularly outstanding in this connection are the studies of Gortner[6] and his co-workers. In an experiment on twelve kinds of wheat flour it was observed that the salt solutions of various concentrations showed a difference in their solvent or "peptizing" action toward the proteins. A definite lyotropic series was noted. From this they concluded that such effects were due to the properties of both of the cations and the anions of the salts, and that apparently none of the salts used was extracting a chemical entity that could be designated as a globulin.

In a more recent work, Gortner and Staker[7] have observed a lyotropic series in the case of many seeds. However, with sorghum, millet, corn and teosinte there was little evidence of a lyotropic series of anions, in fact sorghum showed none at all.

In 1929 Saunders[8] isolated from orange seed meal a crystalline globulin, pomelin. He obtained this globulin by extracting the meal with a warm solution of salt. Extractions of the meal were made with normal solutions of the chlorides, bromides and iodides of lithium, sodium and potassium with quite uniform results. The salt solutions were found to be extracting repeatedly an average of 73.4% of the total nitrogen of the meal. He concluded that there was no evidence for the existence of a lyotropic series in the extracting power of the different alkali halides on orange seed meal.

With such uniform results shown by the extraction of pomelin with normal solutions of alkali halides, a study of the extracting ability of other normal salt solutions was undertaken. The following pages give the results of these experiments.

Material.—Orange seed meal which contains the globulin, pomelin, was used. This material had been obtained by grinding the whole orange seeds[9] and removing the oil with benzene. The meal after being air-dried was sifted and the 20–40 mesh portion was used. Normal solutions of salts of high chemical purity were used for the extractions.

Method.—A 6-g. sample of the orange seed meal was mixed with 50 cc. of the salt solution being studied in a 250-cc. centrifuge bottle. This was shaken for twenty minutes in a mechanical shaker, centrifuged for twenty minutes until the material was well packed and then the supernatant liquid was filtered through cotton into a Kjeldahl flask. The solution was filtered rather than decanted in order to prevent any of the fine particles of meal, difficult at times to pack, from passing into the ex-

[6] R. A. Gortner, W. F. Hoffman and W. B. Sinclair, *Cereal Chem.*, **6**, 1 (1928); "Colloid Symposium Monograph," The Chemical Catalog Co., Inc., New York, **1928**, Vol. V, p. 179.

[7] E. V. Staker and R. A. Gortner, *J. Phys. Chem.*, **35**, 1565 (1931).

[8] Felix Saunders, THIS JOURNAL, **53**, 696 (1931).

[9] We are greatly indebted to the California Fruit Growers' Exchange for the seeds used in this study.

tract. Three extractions were carried out by the method described and the filtrates were combined. The Kjeldahl–Gunning method was used to determine the nitrogen in the combined filtrates. At least four determinations were run with each salt. Blanks were run on the salt solutions.

Experimental.—The experimental results are shown in Table I.

TABLE I

SOLUTION OF THE PROTEIN OF ORANGE SEEDS BY SODIUM SALTS

Salt	Mg. of N from 6 g. of meal	% of total N extracted
Sodium chloride	106	53.5
Sodium bromide	104	52.5
Sodium iodide	105	53.0
Sodium bicarbonate	104	52.5
Sodium acetate	102	51.5
Sodium benzoate	102	51.5
Sodium salicylate	101	51.0
Sodium carbonate	102	51.5
Sodium sulfate	106	53.5
Sodium borate	103	52.0
Sodium tartrate	103	52.0
Disodium phosphate	104	52.5
Trisodium phosphate	100	50.5
Sodium citrate	102	51.5
Sodium dihydrogen phosphate	81	40.9

The greatest discrepancy in this table is in the sodium dihydrogen phosphate extraction. The explanation probably lies in the fact that this was the only acid solution used and the presence of the acid might possibly have a denaturing effect on the protein. A compound such as a protean might be formed which would be less soluble in the salt solution than the original protein. In order to study further the effect of acid on pomelin, a mixture of equal parts of normal sodium chloride and normal hydrochloric acid was used as the extracting agent. As a result it was found that only about one-half as much nitrogen was obtained as would be expected. This gave more conclusive evidence that the presence of acid decreases the amount of nitrogen extracted. The slight variation observed for the other salts in the amount of nitrogen extracted might easily be considered within the range of experimental error.

Discussion

The experimental data show that not only are the alkali halides alike in their ability to extract this protein, but that this property is characteristic of many other salts as well. It is of particular interest to observe that the globulin is uniformly soluble in salts of a strong base and weak acids as well as in neutral salts. Previous definitions of globulins have stated only that they are soluble in salts of strong bases and strong acids. The fact that the salts are alkaline does not seem to decrease the amount of protein extracted as in the case of acid salts. The uniformity of the results obtained leads one to believe that a definite globulin is being extracted.

As stated before, this set of extractions shows no lyotropic series, indicating that the various anions are alike in their solvent effect upon the globulin. This evidence does not agree with that obtained by Gortner[6] in his studies on wheat and other seed proteins. However, in most cases he was probably working with a mixture of substances and might easily be extracting other substances with the globulin. This would account for the conflicting results. On the basis of these results he has raised an objection to the present definitions of globulins, which is justified by his experiments. Different salts may show a difference in their "peptizing" ability toward certain protein systems. In the case of orange seeds, however, uniformity seems to be the rule. Further studies along these lines are in progress.

Conclusions

1. Normal solutions of the salts used in this study extract the same amount of nitrogen from the orange seed meal and there is no evidence for the existence of a lyotropic series in this particular system.
2. Pomelin is soluble equally as well in normal solutions of salts of strong bases and weak acids as in solutions of neutral salts.
3. The presence of acid during the extraction apparently decreases the amount of nitrogen extracted.

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SOME REARRANGEMENT REACTIONS OF BENZYLMAGNESIUM CHLORIDE

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Introduction

Organomagnesium halides are very widely used in studies of the mechanisms of organic reactions and in the proof of structure of compounds. Such utility would be markedly circumscribed by uncertainties concerning rearrangements of the Grignard reagents. The present study is a continuation of earlier work[1] on the mechanism of such rearrangements and a classification of reactants which induce such transformations, primarily with a view to learning which simple compounds might be used with confidence in studies of Grignard reagents.

[1] (a) Gilman and Harris, THIS JOURNAL, **49**, 1825 (1927), and (b) Gilman and Kirby, *ibid.*, **51**, 3475 (1929).