cell (1 cm. light path, 1.75-ml. capacity) contained 50 μmoles of potassium phosphate buffer, *p*H 7, 60 γ of xanthine oxidase, guanine, adenine and water in a total volume of 1.525 ml. The optical density at 290 mμ was measured and found to be stable. Purified adenase (0.005 ml., 60 γ protein) was then added. This resulted in a linear increase in density which came to a halt in 10–20 minutes. Finally, 0.005 ml. of guanase (0.25 mg. protein) was added and the increase in optical density at 290 mμ was again measured. The results of one such experiment are plotted in Fig. 2. From the density change (corrected for enzyme blank) the concentration of base was calculated according to Kalckar.[19] Recovery figures are given in Table III. It was Kalckar who introduced this general technique for spectrophotometry of purines. However, at that time he was not able to apply the method for the determination of adenine and mixtures of adenine and guanine, because a specific adenase was not available.

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TABLE III

THE ENZYMATIC DETERMINATION OF ADENINE AND GUANINE IN SOLUTIONS OF THE MIXED BASES[a]

Sample	ΔE_{290} after adenase	Adenine found, μmoles/ml.	Adenine added, μmoles/ml.	ΔE_{290} after guanase	Guanine found, μmoles/ml.	Guanine added, μmoles/ml.
1	0.726	0.059	0.059	0.097	0.0155	0.016
2	.715	.058	.059	.180	.030	.032
3	.378	.031	.030	.362	.060	.064

[a] The experimental procedure is described under "Coupling to Xanthine Oxidase." ΔE_{290} is the net change in optical density at 290 mμ after subtraction of an enzyme blank.

BETHESDA, MARYLAND

[CONTRIBUTION FROM THE NORTHERN UTILIZATION RESEARCH BRANCH[1]]

Phytin Elimination in Soybean Protein Isolation

BY ALLAN K. SMITH AND JOSEPH J. RACKIS

RECEIVED JULY 5, 1956

Phytin accounts for about 70% of the phosphorus in soybean meal. In extracting proteins from the meal, the phytin reacts with the proteins to form complex products of varying composition. In the water extract of the meal, which has *p*H of about 6.6, the reaction is limited but increases as the *p*H is lowered for precipitation of the protein. It has been demonstrated that phytin can be eliminated from water extract of soybean meal by a combination of dialysis and treatment with the anionic-exchange resin Dowex-1-X10. Electrophoretic studies have shown that one minor component of the acid-precipitated protein is a protein–phytin reaction product, and two other minor components are affected by the presence of phytin. Removing the phytin raises the isoelectric point of the acid-precipitated protein by 0.8 unit, and increases the *p*H range of complete dispersibility on the acid side of its isoelectric point.

It has been reported by many investigators[2,3] that the principal form for storage of phosphorus in seeds is phytin, the calcium–magnesium–potassium salt of phytic acid. Other phosphorus compounds known to be present are phospholipids, inorganic phosphorus and nucleic acids.

Earle and Milner[4] studied the distribution of phosphorus in a single variety of soybeans, Dunfield. They accounted for 92% of the total phosphorus and found that about 11% was phosphatide phosphorus, 4.5% inorganic phosphorus and 71% phytin phosphorus, with 5.5% of unknown composition remaining in the residue. According to Earle and Milner, the petroleum ether extracted approximately 0.5% of the total phosphorus, showing that hexane-defatted meal contains more than 99% of the original phosphorus of the beans. Recently DiCarlo, Schultz and Kent[5] found that defatted and dehulled soy flour contains 1.3% ribonucleic acid. Their results indicate that one or more of the proteins of the soybean may belong to the class of nucleoproteins.

The presence of various phosphorus compounds in the soybean meal introduces a series of complicating factors to the problem of isolation and purification of homogeneous proteins from the soybean. For example, it has been demonstrated by Fontaine, *et al.*,[6] that the solubility of the phosphorus compounds in the water extract of soybean meal varies with the *p*H of the solution, in a manner similar to the solubility of the major protein components. Smiley and Smith[7] have shown that the acid-precipitated protein of the soybean is low in nitrogen as compared to proteins extracted with salt solutions. McKinney, Sollars and Setzkorn[8] showed that phosphorus compounds in the acid-precipitated protein are largely responsible for the low nitrogen values.

Bourdillon[9] obtained a crystalline protein–phytic acid complex from *Phaseolus vulgaris*, whereas Barré, *et al.*,[10–12] have reported a reaction between phytin or phytic acid and the basic groups of proteins, the extent of the reaction being controlled by the *p*H of the system. Smith, Schubert and

(1) One of the Branches of the Agricultural Research Service, U. S. Department of Agriculture, Peoria, Illinois. Presented at the ACS meeting, Atlantic City, September 16–21, 1956.

(2) E. B. Earley and E. E. DeTurk, *J. Am. Soc. Agron.*, **36**, 803 (1944).

(3) J. E. Webster, *J. Agr. Res.*, **37**, 123 (1928).

(4) F. R. Earle and R. T. Milner, *Oil and Soap*, **15**, 41 (1938).

(5) F. J. DiCarlo, A. S. Schultz and A. M. Kent, *Arch. Biochem. Biophys.*, **55**, 253 (1955).

(6) T. D. Fontaine, W. A. Pons, Jr., and G. W. Irving, Jr., *J. Biol. Chem.*, **164**, 487 (1946).

(7) W. G. Smiley and A. K. Smith, *Cereal Chem.*, **23**, 288 (1946).

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(9) J. J. Bourdillon, *ibid.*, **189**, 65 (1951).

(10) R. Barré, J. E. Courtois, P. Delrieu and R. Perles, *Ann. biol. clin. (Paris)*, **12**, 501 (1954).

(11) R. Barré, J. E. Courtois, P. Delrieu and R. Perles, *Ann. pharm. franc.*, **12**, 601 (1954).

(12) R. Barré, J. E. Courtois and G. Wormser, *Ann. Acad. Sci. Fennicae*, [A II] **60**, 104 (1953).

Belter,[13] in studies on soybean protein isolation and fractionation, concluded that elimination of the phytin from soybean meal extracts is an essential preliminary step to the study of the individual proteins of the soybean.

Preparation of the Meal and its Water Extract.—Adams soybeans, 1954 crop, were used throughout this study. The soybean meal was prepared by cracking the beans between corrugated rolls into 6 to 8 parts, and removing the hulls by aspiration and screening. The grits were tempered to a moisture level of about 15% and converted to flakes by means of smooth rolls. The excess moisture was removed by a stream of air and the oil extracted with hexane.

The initial protein extracts were prepared by adding 10 parts of water to 1 part of the defatted flakes, stirring for one hour at room temperature and removing the undissolved material in a centrifuge. The residue was reëxtracted with another 5 parts of water and the two extracts were combined. These extracts had a final *p*H of about 6.6. This solution contains about 90% of the meal protein as well as a major part of all the other water-soluble components. About 88% of the nitrogen in the solution is precipitated as protein by adjusting the *p*H of the above extract to 4.5; this fraction is known as acid-precipitated protein and the remaining solution as soybean whey solution. The whey contains about 12% of the extracted nitrogen, including the non-protein nitrogen. The acid-precipitated protein contains 0.5 to 0.8% phosphorus or about 90% of the phosphorus extracted from the meal.

Preliminary Experiments

The whey solution was clear when first recovered, but on standing a precipitate slowly formed. We found that the precipitates which formed in 24 and 48 hours had the same analysis of 0.5% phosphorus and 14.25% nitrogen. All analytical values were calculated on a moisture-free basis. The formation of the precipitate continued after 48 hours, but its composition was different, containing 0.072% phosphorus and 14.7% nitrogen. However, if the whey solution is immediately recovered from the acid-precipitated protein and is adjusted to *p*H 8.0 with NaOH, a precipitate is formed containing 13.8% phosphorus and 1.7% nitrogen. Analysis indicated this precipitate to be mostly a calcium–magnesium phytate. If the whey is made alkaline with $Ca(OH)_2$, analysis of this precipitate is 7–10% phosphorus and 3.96% nitrogen. When the precipitate which forms at *p*H 8.0 is removed and the solution is readjusted to *p*H 4.5, no further precipitation occurs.

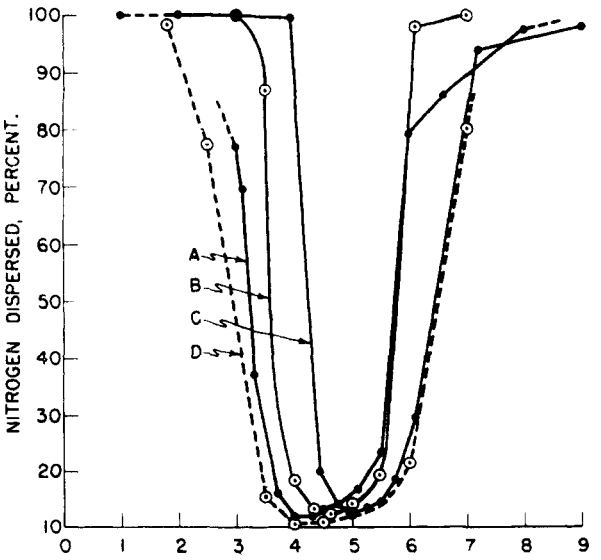

Fig. 1.—The effect of various treatments of the water extract of the soybean meal on the dispersibility of the protein at various *p*H values. A is dispersibility of protein in a water extract without any special treatment; B, the water extract was dialyzed for 24 hours; C, the water extract was dialyzed 24 hours and treated with Dowex-1-X10; and D, was the same as C, except that sodium phytate was added to treated extract.

(13) A. K. Smith, E. N. Schubert and P. A. Belter, *J. Am. Oil Chemists' Soc.*, **32**, 274 (1955).

Another method of removing a substantial part of the phosphorus from the water extract of the meal or from the whey is to adjust these solutions to *p*H 2.2. The precipitates which are formed in the two systems contain, respectively, 2.5 and 1.6% phosphorus, and 14.01 and 13.0% nitrogen. These precipitates are formed by a reaction between phytin and protein, as indicated by the isolation of crystalline sodium phytate from each of them.

The nature of the reaction that occurs between phytin and protein has not been studied. The original water extract appears to be quite stable since no insoluble products are formed. As the *p*H is lowered, the amount of interaction would appear to depend upon the number of positively charged groups in the proteins that are free to react with the ionized phosphate groups of phytin. This ionic interaction is very rapid and is followed by what appears to be a non-ionic irreversible reaction. A small portion of the phytate–protein complex remains insoluble when brought back to the *p*H of the initial water extract. By repeating this process, increased amounts of the product are insoluble even at higher *p*H values.

These preliminary experiments show a reaction between phytin and protein in the water extracts of soybean meal to form products of varying composition, and the need to eliminate phytin from the extract before proceeding with a study of the protein. The present investigation has identified one of the five electrophoretic components described by Smith, *et al.*,[13] in the acid-precipitated protein as a protein phytin complex and has found two additional electrophoretic components, one of which also appears to be a reaction product of protein with phytin. The effect of removing phytin on the dispersion characteristics of the proteins in the water extract of the meal has been determined.

Removal of Phytin and Other Compounds.—The removal of phytic acid and inorganic phosphorus from the water extract of the meal was accomplished (1) by dialysis, (2) by use of an anion-exchange resin, and (3) by a combination of these two procedures. The dialysis method was investigated at several *p*H values and in the presence of added salt. The ion-exchange studies were made at several different *p*H values by a batch method using the acetate form of Dowex-1-X10 resin, with granulation of 50–100 mesh. The extract from 25 g. of meal was treated with 35 g. of resin. A *p*H of 7.0 was found to be the most effective for removing phosphorus with a minimum removal of nitrogen from the solution. The resin treatment removed approximately 2.5% more nitrogen than dialysis. The results of these experiments are shown in Table I. From these data, it was concluded that the most satisfactory way of removing phytate and inorganic ions was by using a combination of dialysis followed by treatment with an anion-exchange resin with

TABLE I

REMOVAL OF PHOSPHORUS AND NITROGEN COMPOUNDS FROM WATER EXTRACT OF THE MEAL BY DIALYSIS AND ANION EXCHANGE

	Phosphorus removed, %	Nitrogen removed, %
Dialysis 24 hr., *p*H 7.2	40.5	7.5
Dialysis 48 hr., *p*H 6.5	72.0	7.0
Dialysis 48 hr., *p*H 8.7	52.0	28.0[a]
Dialysis 48 hr., *p*H 8.7 in *N* NaCl	37.0	..
Anion exchange,[b] *p*H 7.5	56.5	4.0
Anion exchange, *p*H 7.0	65.0	7.5
Anion exchange, *p*H 6.5	82.3	24.0
Dialysis + anion exchange,[c] *p*H 7.0	78.2	10.1
Dialysis + anion exchange,[c] *p*H 7.0	78.5	9.2

[a] Precipitate formed in dialysis bag. [b] Dowex-1-X10. [c] Dialysis at *p*H 7.2 (24 hours) followed by Dowex-1-X10 at *p*H 7.0.

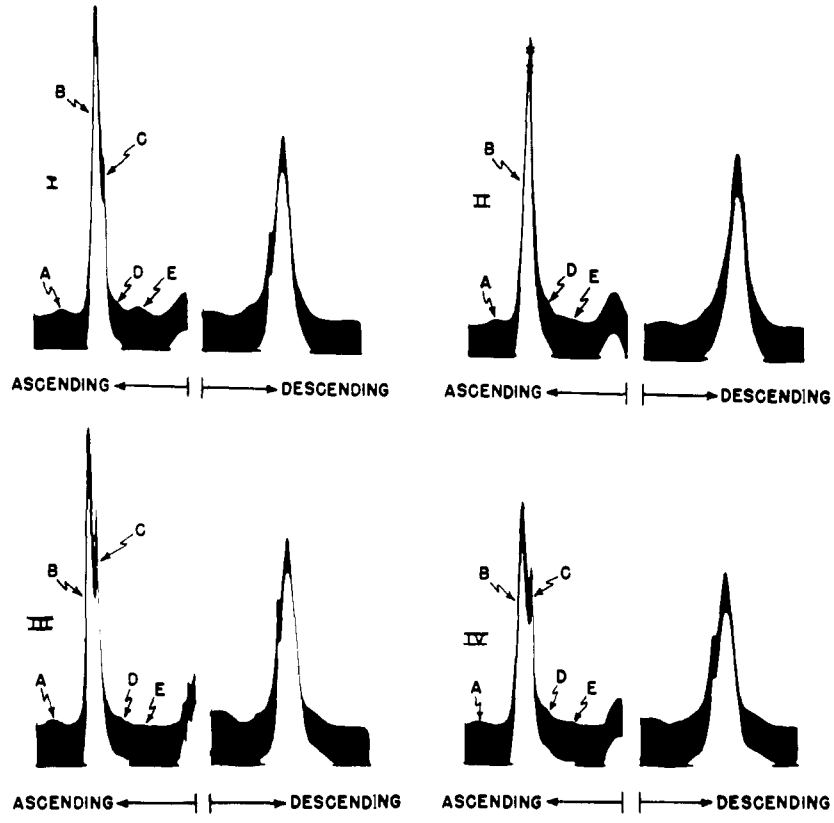

Fig. 2.—Electrophoretic patterns showing the effect of various treatments of water extract of soybean meal on presence or absence of component C in the acid-precipitated protein. Conditions, 1.2% protein concentration in *p*H 7.6 phosphate buffer, 0.1 μ, field strength 6.2 volts per cm., and 120 minutes. Pattern I, of protein precipitated at *p*H 4.4 from the water extract of the meal, II the water extract was dialyzed and treated with Dowex-1-X10 before precipitation of protein at *p*H 5.1, III same as II except that sodium phytate was added after resin treatment, IV protein was prepared as in II, then dissolved at *p*H 7.6, the sodium phytate added, and reprecipitated at *p*H 4.4.

both steps at *p*H 7.0. Dialysis at higher *p*H values appeared to favor the formation of calcium–magnesium salts of phytic acid and retard the rate of removal of phytin. Added salts also decreased the rate of removal of phosphorus. Seventy-five to 78% of the phosphorus, which has been shown by earlier workers to be mostly phytin phosphorus, was removed by the combined dialysis and resin treatments. The nature of the remaining phosphorus will be treated in another publication and shown to occur as nucleic acid phosphorus combined probably as nucleoprotein.

Effect of Phytin Removal on Protein Dispersibility.—The reaction of proteins with negative ions is known to modify their dispersion characteristics. Therefore, the effect of dialysis and of anion exchange treatments on the *p*H–dispersion characteristics of the acid-precipitable protein in the water extract was determined. These experiments were carried out by adjusting the original extract and the dialyzed or resin-treated water extract to various *p*H values and determining the amount of nitrogen which was left in solution. After *p*H adjustment, the solutions were shaken for 30 minutes, the precipitated protein removed in a centrifuge and a sample of the supernatant taken for Kjeldahl nitrogen. Solubility of the nitrogenous compounds is expressed as the per cent. of nitrogen remaining in solution after *p*H adjustment and 30 minutes shaking. The solubility results for (1) untreated extract, (2) dialyzed extract and (3) combined dialyzed and resin-treated extract are shown in Fig. 1. Curve A is for the untreated water extract of the meal, curve B is for a water extract which was dialyzed for 24 hours, and curve C is for an extract which was dialyzed and resin treated. The dialysis and resin treatments made two notable changes in the dispersion characteristics of the protein, (1) they increased the dispersibility of the protein in the acid range, where complete dispersibility was attained at *p*H values of 3 and 4, respectively, whereas untreated protein is only 80% dispersed at *p*H 3.0, and (2) they raised the *p*H of minimum dispersibility by 0.4 and 0.8 unit, respectively. These or similar effects would be anticipated by removal of any large anion such as phytate from the system.

Effect of Added Phytic Acid.—It is apparent that the water extract of soybean meal contains many negative ions beside the phytate, each of which will have some effect on the properties of the protein. In order to determine whether the phytate ion could be a major contributor to the change in properties of the protein, it was decided to add calculated quantities of sodium phytate to a resin-treated extract and measure the effect on dispersion and electrophoretic properties of the proteins.

Preparation of Sodium Phytate.—For the proposed experiments, crystalline sodium phytate was prepared from commercial calcium phytate. The commercial product was washed with hot water, recovered and dried in a vacuum oven at 50°. By a batch method, 20 g. of the washed phytate was treated with 200 g. of Dowex-50(H) in 300 ml. of water and the phytic acid solution recovered by filtering. The solution was adjusted to *p*H 11.0 with sodium hydroxide, heated to 60° and methanol added to 50% concentration by volume. A brown, gummy precipitate which formed was removed by filtration and discarded to give a clear solution. The solution was reheated to 60°, methanol added to the cloud point, and then warm water added to clear the solution. After standing several days at room temperature, another gummy precipitate formed which was converted to crystalline sodium phytate by dissolving in water and adding methanol to the cloud point. While maintaining a cloudy solution, water and methanol were alternately added until a condition was attained which gave prompt crystallization. The sodium phytate in the mother liquor was also crystallized by warming, adding methanol and storing in the refrigerator for several days. Plate-like, weakly birefringent sodium phytate crystals were obtained. By another crystallizing procedure with more dilute solutions, weakly birefrin-

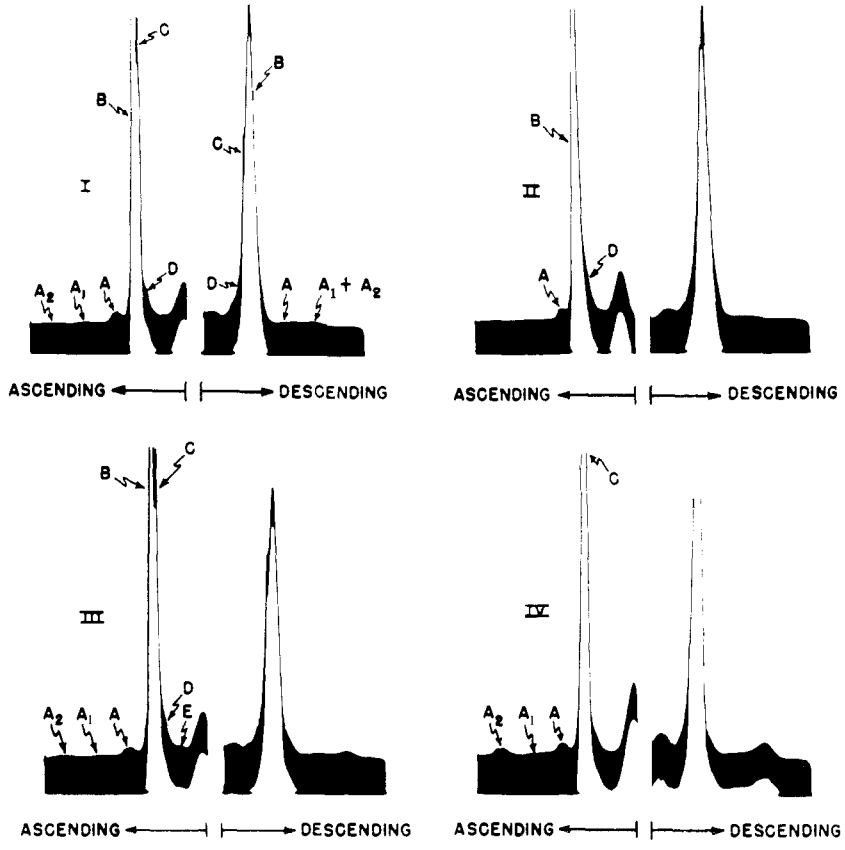

Fig. 3.—Electrophoretic patterns showing the effect of various treatments of water extract of soybean meal on presence or absence of components A_1 and A_2. Conditions: 1.2% protein concentration, *p*H 7.6 phosphate buffer, 0.1 μ, field strength 6.2 volts per cm., and 60 minutes. Pattern I, of protein precipitated at *p*H 4.5 from water extract of the meal, II water extract was dialyzed and treated with Dowex-1-X10 before precipitation at *p*H 5.1, III same as II except that sodium phytate was added after the resin treatment, and IV protein was prepared as in II, then dissolved in *p*H 7.6 buffer, sodium phytate added, and protein reprecipitated at *p*H 4.5.

gent, lath-shaped crystals were formed, but the first method gives the higher yield.

On a moisture-free basis, the analysis of phosphorus for two different samples was 20.60 and 20.22; these analyses agree most closely with the formulas $C_6H_7O_{24}P_6Na_{11}$ and $C_6H_6O_{24}P_6Na_{12}$, respectively.

Effect on Dispersion Properties of the Protein.—Sodium phytate was added to a dialyzed and resin-treated water extract of the meal in an amount equivalent to the phytate removed as calculated on a phosphorus equivalent basis. The results on dispersion characteristics of the protein are shown in Fig. 1, curve D. The dispersibility of the protein now approximates that in the original untreated extract shown in Curve A.

Effect of Phytin on Electrophoretic Patterns.—The preceding experiments have demonstrated that a protein–phytic acid complex of varying composition occurs in the water extract of soybean meal, the next step was to investigate the reaction product by boundary electrophoresis. The work of Smith, Schubert and Belter[13] had demonstrated the presence of five electrophoretic components in the acid-precipitated fraction of the soybean protein. We anticipated that one or more of these components would be a reaction product of protein with phytin.

In searching for the protein–phytic acid components by the boundary electrophoresis method, the acid-precipitated proteins were prepared by four different methods. Protein no. I was precipitated from the water extract of the meal at *p*H 4.4 and washed with water. Protein no. II was prepared by the same procedure as for no. I except that the water extract of the meal was dialyzed against distilled water and treated with Dowex-1-X10 to remove phytin and other negative ions before precipitation of the protein at *p*H 5.1. Protein no. III was prepared the same as no. II except that sodium phytate was added to the resin-treated solution at the rate of 200 mg. of sodium phytate per 100 ml. of solution and the protein precipitated at *p*H 4.4. This amount of sodium phytate is slightly more than that removed by the resin treatment when calculated on a phosphorus equivalent basis. For no. IV the protein was prepared the same as in no. II, then it was dissolved in *p*H 7.6 phosphate buffer and sodium phytate added at a level equivalent to twice the phosphorus content in solution no. I and then reprecipitated at *p*H 4.4. Protein no. I contained 15.0% nitrogen and 1.04% phosphorus, whereas Protein No. II contained 15.84% nitrogen and 0.33% phosphorus. No analysis was made on proteins no. III and IV. The Tiselius patterns for all four proteins were made by dissolving in *p*H 7.6 phosphate buffer of 0.1 ionic strength; the solutions were dialyzed against the same buffer and diluted to 1.2% protein concentration using a nitrogen to protein conversion factor of 6.0. Solutions I, III and IV were clarified in a Servall type SSIA centrifuge at 7600 r.p.m. (about 8600 × *g*) for 2 minutes; this removes a small but undetermined amount of protein–phytate complex.

Tiselius patterns for the four proteins after 120 minutes of electrophoresis are shown in Fig. 2. Patterns I, III and IV show the five electrophoretic components numbered A, B, C, D and E which were previously described by Smith, *et al.*[13]; however, in pattern no. II, from which the phytin was removed by the resin and not re-added, the C-component is absent. Thus this group of Tiselius patterns, which has been duplicated several times, support the conclusion that the C-component represents a protein–phytic acid reaction product.

An attempt was made to increase the proportion of the C-component by adding increasing amounts of sodium phytate to protein solution no. IV described above. Component C

was increased only a limited extent because the increased phytic acid precipitated part of the protein and reduced the concentration of the total protein below the desired level of 1.2%. The precipitation of protein by phytin was confirmed by adding sodium phytate to a protein solution at a ratio of 1:3 on a dry weight basis and adjusting the system to the *p*H range of 2.0–3.0. About 96% of the protein was precipitated having a composition of nitrogen 14.52% and phosphorus 3.06%.

Two New Components.—Examination of the electrophoretic pattern of solution I of the above experiment taken after 60 minutes showed two fast-moving minor components which had not been identified in previous work. These two components are not in the photographs taken at 120 minutes, as they have moved outside the field of the camera. Also, comparison of the 60-minute patterns of solutions I and II, in this experiment indicated that another protein–phytin reaction was occurring. To examine this reaction, the experiment described in Fig. 2 was repeated, except that the four photographs were taken after 60 minutes of migration. The 60-minute photographs are in Fig. 3 where pattern no. I shows the two new fast-moving components A_1 and A_2, whereas in pattern no. II of dialyzed and resin-treated protein the A_2 component is not apparent. Pattern no. III, made from a dialyzed and resin-treated extract followed by the addition of sodium phytate to a level of about 50% above the original level of phytin as determined by phosphorus analysis, shows component A_2 is as prominent at least as in the original solution. Pattern no. IV is protein prepared as described for no. IV of Fig. 2, except that a slightly higher level of sodium phytate was used. This pattern also shows component A_2 in a much higher ratio than for sample no. I.

This series of patterns seems to indicate that component A_2, as it occurs in the acid-precipitated protein from the original untreated protein solution, may be a reaction product of protein and phytin, and that dialysis and resin treatment of the original water extract removes the phytin before a detectable reaction has occurred. There is some indication that component A_1 also reacts with phytate, but the differences between the patterns are not large enough for a satisfactory conclusion.

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PEORIA, ILLINOIS

[CONTRIBUTION FROM THE FULMER LABORATORY, DEPARTMENT OF CHEMISTRY, STATE COLLEGE OF WASHINGTON]

The Oxidative Cleavage of Phenylhydrazide Groups from Carboallyloxy-α-amino Acid Phenylhydrazides and Carboallyloxydipeptide Phenylhydrazides[1,2]

BY H. BAYARD MILNE, JOHN E. HALVER, DON SO HO AND MICHAEL S. MASON

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A method is described for the quantitative estimation of phenylhydrazide groups in carboallyloxy-α-amino acid phenylhydrazides. A number of carboallylloxyamino acid phenylhydrazides have been oxidized with ferric chloride to yield the corresponding carboallyloxyamino acids. The enzymatic synthesis of L-leucyl-L-leucine is reported.

One of the limitations of the enzymatic synthesis of dipeptides by means of the Bergmann reaction is the difficulty of removing an amide or anilide group without splitting the peptide bond.[3] However, Waldschmidt-Leitz and Kuhn[4] prepared glycylglycine by the following reactions. They incubated carbobenzoxyglycine with glycine phenylhydrazide in the presence of papain and obtained carbobenzoxyglycylglycine phenylhydrazide, which reacted with hydriodic acid in the presence of acetic acid to give glycyclglycine phenylhydrazide. The phenylhydrazide was refluxed with copper acetate to yield glycylglycine.

It was of interest to see if the phenylhydrazide oxidation could be used with carboallyloxy-α-amino acid phenylhydrazides, which may be prepared by an enzymatic synthesis,[5] without oxidizing the carboallyloxy groups.

It was decided to try several oxidizing solutions to determine the best procedure for the oxidation of carboallyloxyamino acid phenylhydrazides. As the earlier work indicated that liberated nitrogen could be used as a measure of the extent of the reaction, a modified Van Slyke–Koch[6] micro-nitrogen apparatus was built.

In preliminary experiments using this apparatus, the nitrogen liberated from the oxidation of hippuric acid phenylhydrazide was measured. The hippuric acid phenylhydrazide was dissolved in a number of solvents and the solutions mixed with several different oxidizing agents. The highest yield of nitrogen was obtained with potassium permanganate using methyl Cellosolve as a solvent for the phenylhydrazide.

In the cases where the yield of nitrogen was low, an amorphous white solid precipitated from the reaction mixtures when they were diluted with water. This material would not react further with the oxidizing agent even upon refluxing for several hours. This product was not studied further, but is thought to be a diphenylhydrazide as reported by Tafel.[7]

As the potassium permanganate would probably react with the allyl group in the carboallyloxyamino acids, an attempt was made to improve the yields of nitrogen when copper acetate and ferric chloride were used as oxidizing agents. It was found that increasing the temperature of the reaction mixture greatly increases the yield of liberated nitrogen. When a representative group of carboallyloxyamino acid phenylhydrazides was oxidized (Table I) with copper acetate or ferric chloride at 96 to 97°, the

(1) This investigation was supported in part by a Grant-in-aid from The State College of Washington Research Fund.

(2) Presented in part at the St. Louis Meeting of the American Chemical Society, September 8, 1948.

(3) J. S. Fruton, "Advances in Protein Chemistry," Vol. V, Eds. M. L. Anson, J. T. Edsall and K. Bailey, Academic Press, Inc., New York, N. Y., 1949, p. 1.

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