

TEXAS AGRICULTURAL EXPERIMENT STATIONS

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BULLETIN NO. 156

MARCH, 1913

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DIVISION OF CHEMISTRY

TECHNICAL BULLETIN

THE PHOSPHORUS COMPOUNDS OF COTTON SEED  
MEAL AND WHEAT BRAN

BY

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POSTOFFICE

COLLEGE STATION, BRAZOS COUNTY, TEXAS

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1913

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# PHOSPHORUS COMPOUNDS OF COTTON-SEED MEAL AND WHEAT BRAN.

BY

J. B. RATHER, ASSISTANT CHEMIST.\*

In a previous publication (Bulletin 146, Texas Experiment Station) we showed that the phosphorus compounds of cottonseed meal were nearly all organic in nature and not inorganic, as has been claimed, and especially that cottonseed meal does not contain meta- or pyrophosphoric acid. The work presented in this bulletin is a continuation of the above study of the forms of phosphorus compounds in cottonseed meal, together with an investigation of the acid-soluble phosphorus compounds of wheat bran.

## REVIEW OF LITERATURE.

Hardin states (Bulletin 8, new series, South Carolina Experiment Station) that cottonseed meal consists largely of meta- and pyrophosphoric acids. Crawford (Jour. Phar. & Expt. Therapeutics 1,151) claims that "the chief poisonous principle of cottonseed meal is a salt of pyrophosphoric acid." It has been shown by the writer (Bulletin 146) that the product which these writers thought was inorganic phosphoric acid was of organic nature and with very similar properties to pyrophosphoric acid.

In Technical Bulletin No. 25 of the New York (Geneva) Experiment Station, R. J. Anderson reports some work on meta- and pyrophosphoric acid in cottonseed meal and concludes that "The aqueous solution of the free acid gives all those reactions which have been attributed previously to the presence of meta- and pyro-phosphoric acid in cottonseed meal."

Bulletin 146, of the Texas Experiment Station, published ten months before the New York Bulletin, deals primarily with the question of the presence of the above named inorganic forms of phosphorus in cottonseed meal and the conclusions were as follows: "The principal compounds containing phosphorus were separated which gave the same reactions as those relied upon for proving the presence of meta- and pyrophosphoric acid. We have no evidence that the samples of cottonseed meal examined contain either pyrophosphoric acid or metaphosphoric acid."

The only difference between our work and Anderson's on the subject of meta- and pyrophosphoric acid was a difference in the method of preparation of the free acid tested and a much more complete duplication on our part of those tests on which were based the claims that cottonseed meal contains the above named inorganic acids.

Anderson further concludes that "cottonseed meal contains an organic phosphorus acid which is very similar to phytic acid. Whether the substance is identical with phytic acid could not be determined."

Posternak (Rev. Gen. Bot. 12, 5 and 65; Compt. Rend. 137, 3, 5, 8)

\*Under the general direction of G. S. Fraps, Chemist.

obtained an organic phosphorus acid from the seeds of peas, beans, pumpkins, lupine and other plants, which gave inosite as a cleavage product when heated in a closed tube with acid. He called this acid phytic acid, or anhydro-oxymethylene-diphosphoric acid, and proposed the formula  $C_2H_8P_2O_9$ .

Winterstein (Ber. Deut. Chem. Ges. 30, 2299) separated a phosphoryzed product from the seeds of a black mustard, which also gave inosite as a cleavage product.

Patten and Hart (New York [Geneva] Experiment Station, Bulletin 250) studied the acid-soluble phosphorus compounds of wheat bran, and state that the principal compound corresponds to the formula  $C_2H_8P_2O_9$ , and that it is probably identical with Posternak's "phytic acid."

Hart and Tottingham (Wisconsin Experiment Station Research Bulletin 9) claim that "phytin" is present in the seeds of maize, oats and barley.

Suzuki and Yoshimura (Bulletin College Agriculture [Tokyo] 7, 498) Neuberg (Biochem. Ztschr. 6, 405), and Forbes (Ohio Experiment Station Bulletin 215) regarded the above compound as phytin, but do not report complete analyses of the product.

Levine (Biochem. Ztscher. 16, 390) describes a semicrystalline barium salt of an organic phosphorus acid. Vorbrodtt (Anzeiger. Akad. Wiss. Krakau, 1910, Series A. 414) separated an organic phosphorus compound, to the barium salt of which he ascribed the formula  $C_{12}H_{26}P_{11}Ba_7O_{46}$ .

A number of constitutional formulas have been proposed by various investigators and several attempts have been made to synthesize "phytic acid" in the laboratory.

Rising (Svensk Kemisk Tidskrift 22, 143 [1910]) prepared a silver salt of "phytic acid" for which he proposes the formula  $C_6H_{14}P_5Ag_6O_{22}$ .

Anderson (New York [Geneva] Agricultural Experiment Station Technical Bulletins 19 and 21) studied the salts of "phytic acid" obtained from commercial phytin. Among others, he reports the analyses of two silver salts of the acid, one of which he termed octa-silver phytate and the other hexa-silver phytate. Carbon and hydrogen in the silver salts were not reported.

More recently, Anderson (New York [Geneva] Station Technical Bulletin 22) reported a study of the acid-soluble organic phosphorus acid of wheat bran, in which he states he prepared salts which corresponded to the formulas  $C_{25}H_{55}P_9Ba_5O_{54}$  and  $C_{20}H_{45}P_9Ba_5O_{49}$ . He claims that the latter compound is formed from the former by the splitting off of one pentose group. He concludes that wheat bran does not contain "phytin."

#### PRELIMINARY STUDIES.

The first step in the identification of an unknown compound is to establish its purity. Unless this is done the analysis is of doubtful value.

In the purification of the organic phosphorus acids from feeding materials, it is necessary to remove, besides other substances, the inorganic bases iron and aluminum, calcium, magnesium, potassium and sodium, and the inorganic phosphoric acid which is always present to a greater or less extent.

The method of purification used by Posternak (loc. cit.) and modified by Patten and Hart deserves especial attention, because of the fact that

upon analyses of products prepared by this method rests the theory that phytic acid has the formula  $C_2H_5P_2O_6$ , or a multiple thereof. The modification of Patten and Hart consists in the substitution of barium chloride for calcium chloride. They offer no evidence that inorganic bases have been removed, nor do they appear to have taken into consideration the possibility of the presence of inorganic phosphorus in the final product.

A product prepared from wheat bran by the method of Patten and Hart was dried at  $110^\circ$  and analyzed. The results were as follows:

Iron and aluminum.....	3.33 per cent
Calcium .....	0.30 per cent
Magnesium .....	0.18 per cent

The method was followed as carefully as possible.

In our previous work on this subject, we concluded from qualitative tests that lime and magnesium were practically eliminated by the method of Patten and Hart, but quantitative examinations proved that, although the amount of these bases was relatively small, the total basic impurities was sufficient to render any formula erroneous if it were based on the analysis of the product prepared in this way. The mixed iron and aluminum phosphates were assumed to be half and half; calculated to iron, it would be 3.13 per cent, and calculated to aluminum, 3.53 per cent. Inorganic phosphorus was determined according to the method of Forbes et al.; 0.32 per cent was found. This would correspond to 1.01 per cent ortho-phosphoric acid. The total impurities in this product are, therefore, about 4.7 per cent.\* The oxygen in the product would accordingly appear 4.7 per cent too high and the carbon too low; the apparent percentage of phosphorus would also be too high. It is obvious that a formula based on the analysis of such a product would be erroneous.

Another product prepared in a similar manner gave the following results:

Iron and aluminum.....	3.26 per cent
Calcium .....	0.38 per cent

Another product was prepared in a similar manner, but made alkaline with ammonia after the first hydrogen sulphide treatment to remove inorganic bases and the resulting precipitates filtered off, the excess of ammonia removed by evaporation, and the purification completed as usual. Found:

Iron and aluminum.....	1.85 per cent
Calcium .....	0.51 per cent

Another product was analyzed after a treatment similar to the above, involving the addition of ammonia in the proportion of about 10 to 1 and filtration from the resulting precipitate, the removal of ammonia and completion as usual. Found:

Iron and aluminum.....	1.93 per cent
Calcium .....	0.50 per cent

\*We have assumed that the iron and aluminum were present as phosphates in the ignited product. Should this not be the case, the total inorganic impurities would be about 10 per cent.

Still another product contained 1.75 per cent iron and aluminum and 0.30 per cent calcium.

It is evident, therefore, that the assumption that the method of Patten and Hart removes inorganic bases completely is not justified by the facts. The amount present is enough in most cases to seriously interfere with the accuracy of the work and any formula based on these analyses is undoubtedly wrong. The oxygen content would appear too high by several per cent and the carbon, hydrogen, and phosphorus contents too low. This effect on the phosphorus would be offset by the increase due to the presence of inorganic phosphorus. It is noteworthy that although the calculated percentage of carbon in the compound  $C_2H_8P_2O_9$ , or "phytic acid" of Posternak, Patten and Hart, Hart and Tottingham, and Anderson is 10.08, the American investigators *invariably* secured higher results on analysis, ranging from 10.22 to 10.76 per cent and averaging 10.50 per cent.

Patten and Hart state that their reason for using barium chloride instead of the calcium chloride used by Posternak was that the lime was much more difficult to remove. If this is correct, then the method of Posternak is of still less value than that of Patten and Hart, which we have already shown left in the product, in one case, 4.7 per cent inorganic impurities.

The Patten and Hart method has been used by Hart and Tottingham (*loc. cit.*) on corn and other grains. They make no mention of tests for the completeness of the removal of inorganic bases and their work is open to the same objections as that of Posternak, and Patten and Hart.

Anderson (*loc. cit.*), who studied a large number of the salts of phytic acid, used the method of Patten and Hart to prepare free phytic acid and based the calculated composition of these salts on the theory that phytic acid has the formula  $C_6H_{18}P_6O_{27}$ , a multiple of the  $C_2H_8P_2O_9$  of Posternak. In a large number of cases he made no carbon and hydrogen determinations in these salts, and as far as we have been able to find has made no tests to see if inorganic bases were absent. We will show that the acid does not correspond to the above formula, and it is obvious that all of the above work of Anderson is open to the same objections as that of Posternak, Patten and Hart, and Hart and Tottingham.

From a review of the literature it is evident that the theory that phytic acid has the formula  $C_2H_8P_2O_9$ , or a multiple thereof, rests entirely on the work of Posternak, Patten and Hart, Hart and Tottingham, and Anderson. The methods used were very much alike. Rising, Verbrodt, and the writer (this bulletin) used different methods and obtained different results.

As far as we have been able to find, few of the investigators of the so-called "phytin" have made any attempt whatever to prove that the products analyzed were homogeneous. This was apparently assumed after the completion of the various methods of purification used.

#### SEPARATION AND PURIFICATION OF THE PRINCIPAL ORGANIC PHOSPHORUS ACID OF COTTONSEED MEAL AND WHEAT BRAN.

In our previous work on cottonseed meal we found that more than twice as much phosphorus was soluble in water as in 0.2 per cent hydro-

chloric acid, and that after extraction with acid the remaining phosphorus was no longer soluble in water, but could be dissolved in 0.2 per cent ammonia.

The following method was designed to separate the water-soluble phosphorus in two fractions, the acid-soluble and the ammonia-soluble after acid extraction.

*Method.*—The method finally adopted for this work was a modification of the modified Patten and Hart method used by us in previous work on the phosphorus compounds on cottonseed meal.

The purpose of the precipitation with ammonia and with alcohol was to remove inorganic phosphorus and inorganic bases. It was found by analysis (see below) that this result was accomplished.

The method is as follows:

Two kilograms of cottonseed meal were digested with 8000 c.c. 0.2 per cent hydrochloric acid for three hours with frequent shaking. The extract was strained through cheesecloth and the residue washed well with water. The residue was digested with 8000 c.c. 0.2 per cent ammonia for three hours with frequent shaking and allowed to settle, and washed with water by decantation. Copper acetate was added to the acid extract of the cottonseed meal in sufficient quantity to precipitate most of the phosphorus compounds, the precipitate was washed well with water, decomposed with hydrogen sulphide, filtered, and evaporated to a sirupy consistency.

The product was dissolved in a small amount of water and about ten times the volume of ammonia added. The mixture was allowed to stand over night. The precipitate was filtered off. The filtrate from the precipitation with ammonia was evaporated on the water-bath to remove the ammonia and taken up with water. Barium chloride was added and the resulting precipitate was filtered and washed with water. The barium salts were decomposed with sulphuric acid and filtered. The filtrate was again made alkaline and precipitated with barium chloride. This process was repeated two or three times and the acid finally precipitated with copper acetate in acid solution, the copper salt decomposed with hydrogen sulphide, filtered and evaporated to a small volume.

A large volume of alcohol was then added and the resulting precipitate filtered off. The alcohol was evaporated from the filtrate and the product taken up in a small volume of water.

The ammonia extract of the cottonseed meal was made acid with hydrochloric acid, the precipitate allowed to settle and the liquid decanted through a filter. This extract was precipitated with copper acetate, ammonia, barium chloride and alcohol exactly as described for the acid extract.

In the examination of the wheat bran, the ammonia extraction of the feed was omitted.

Analysis of the wheat bran product, dried at 110° C, gave the following results:

0.53 gram gave .0011 gram combined iron, aluminum, lime  
and magnesium phosphates.

0.10 per cent inorganic phosphorus.

The above amount of phosphates would correspond to 0.08 per cent iron. It is evident that this amount of inorganic phosphorus, calcu-

lated as silver phosphate in the silver salt subsequently analyzed, would not change the composition of the organic silver salt beyond the limit of analytical error.

Analysis of the products from the extractions of cottonseed meal gave the following results:

- .6675 gram of the product from the acid extraction gave .0020 gram combined iron, aluminum, lime and magnesium phosphates.
- .3830 gram of the product from the ammonia extraction gave .0015 gram combined inorganic phosphates.

This would correspond to 0.11 per cent and 0.14 per cent iron, respectively. Inorganic phosphorus was found to be 0.12 and 0.10 per cent in the products.

The above percentages of inorganic phosphorus was calculated from analysis of silver salts hereinafter described. The method for the preparation of the acid product is therefore satisfactory, as far as the removal of inorganic impurities is concerned.

#### HOMOGENEITY OF THE PRODUCTS.

In order to see if the products were the same, the following experiments were made:

Portions of the acid products prepared as described above were made up to a volume of 200 c.c. with water, and aliquots titrated with standard potassium hydroxide and phenolphthalein. The rest of the solution was evaporated to about 50 c.c. and cooled to room temperature, an amount of standard ammonium hydroxide calculated to make a neutral ammonium salt was added to each. They were then diluted to make the concentration of the salts the same, and the theoretical amount of standard silver nitrate solution required to form a neutral salt was added. The resulting precipitates were filtered and washed with equivalent amounts of water. The washed precipitates were then dried in air, powdered and dried in a vacuum desiccator over sulphuric acid.

The filtrates from the silver precipitates were made slightly acid with nitric acid (two or three drops) and precipitated with copper acetate. The copper was removed from the washed precipitate with hydrogen sulphide and the resulting filtrate was evaporated to a small volume. The acids were then precipitated with silver nitrate under exactly the same conditions as to concentration, temperature, alkalinity, etc., as before. Unfortunately the amounts of the second fractions of the silver salts secured were too small to obtain good results with, and the analyses were not considered.

The silver was determined in 0.2000 grams of the dried first fractions described above, with the following results:

Cottonseed meal:	Grams silver.
Acid extraction .....	.1224
Ammonia extraction .....	.1237
Wheat bran:	
Acid extraction .....	.1206



The results agree closely and it appears probable that the products were the same, since the fact that the salts contain the same amount of silver when precipitated under the same conditions is evidence that they are identical.

One-half gram of each salt, dried in a vacuum over sulphuric acid, was digested at room temperature in 200 c.c. of water for five hours with frequent shaking. The solutions were then filtered and the residues washed with equal volumes of water. Silver was determined in the filtrates. The results are as follows:

Cottonseed meal:	Grams silver.
Acid extraction .....	.0178
Ammonia extraction .....	.0172
Wheat bran:	
Acid extraction .....	.0162

These results agree very closely, all of them being well within the limit of error. It is evident, therefore, that the compounds are the same.

The above is evidence that the compounds are pure, for it would be very improbable for them to be impure and each have the same amount of impurities and of the same solubility. Further evidence that the compounds are pure, will be mentioned later.

In our previous work on cottonseed meal (*loc. cit.*) we made a large number of tests on the products of the acid and ammonia extracts and in every case the reactions were the same. This is further proof that the cottonseed meal products are the same.

#### PREPARATION OF THE SILVER SALTS OF THE PRODUCTS.

Aqueous solutions of the acid products prepared as already described were made alkaline with ammonia and boiled until the excess of ammonia was expelled. Silver nitrate solution was added in slight excess and the resulting precipitates washed well with water. (Fractions 1.)

The filtrates from the above precipitation were made slightly acid with nitric acid (two or three drops) and precipitated with copper acetate. The precipitates were washed well with water, suspended in water, decomposed with hydrogen sulphide and the copper sulphide filtered off. The acid filtrates were then evaporated to remove excess of hydrogen sulphide and taken up with water. The products were then precipitated with silver nitrate in neutral solution exactly as described above. (Fractions 2.)

The silver salts were precipitated in warm solution and an excess of silver nitrate was generally used. The portion which went into solution was therefore more than in the homogeneity tests described before, and no difficulty was found in getting sufficient amounts of the second fraction to analyze.

The silver salts were dried in air, powdered, dried in a vacuum desiccator over sulphuric acid and analyzed.

Products were also prepared from the hydrochloric acid extract of cottonseed meal by a method similar to the above, but no ammonia was used to precipitate inorganic phosphates, etc. The crude free acid was precipitated with magnesia mixture as suggested by Forbes et al. (Ohio

Experiment Station Bulletin 215), and washed with 0.2 per cent nitric acid in alcohol. The magnesium was removed by precipitation of the acid with copper acetate in acid solution and the subsequent purification and the preparation and the fractionation of the silver salts was the same as described above. (Mg).

The silver salts prepared from the acid and ammonia extracts of cottonseed meal and from the acid extract of wheat bran were found on analysis to be free from nitrogen and pentosans. We have already shown that the amount of inorganic impurities is negligible.

The analyses of the silver salts and the formulas corresponding to them are shown in Table 1, together with those of the salts reported in our previous work on cottonseed meal.

TABLE 1.

## Percentage Composition of the Silver Salts of the Phosphorus Compounds.

		Carbon	Hydrogen	Silver	Phosphorus
Cotton Seed Meal Acid Extraction	Fraction 1, found.....	4.60	0.91	61.79	9.54
	$C_{12}H_{24}Ag_{17}P_9O_{42}$ , Calc.....	4.88	0.81	62.10	9.45
	Fraction 2, found.....	4.87	0.74	59.86	10.06
	$C_{12}H_{25}Ag_{16}P_9O_{42}$ , Calc.....	5.06	0.88	60.66	9.81
	Fraction 1, (Mg), found.....	5.46	0.94	56.15	10.73
	$C_{12}H_{25}Ag_{16}P_9O_{42}$ , Calc.....	5.70	1.11	55.57	11.04
	Fraction 2, (Mg), found.....	5.06	0.88	60.37	10.00
	$C_{12}H_{25}Ag_{16}P_9O_{42}$ , Calc.....	5.06	0.88	60.66	9.81
	Product B (Bull. 146), found.....	5.42	0.86	61.40	9.82
	$C_{12}H_{25}Ag_{16}P_9O_{42}$ , Calc.....	5.06	0.88	60.66	9.81
Cotton Seed Meal Ammonia Extraction	Fraction 1, found.....	4.78	0.78	62.68	9.40
	$C_{12}H_{24}Ag_{17}P_9O_{42}$ , Calc.....	4.88	0.81	62.10	9.45
	Fraction 2, found.....	4.97	1.08	57.15	10.20
	$C_{12}H_{27}Ag_{14}P_9O_{42}$ , Calc.....	5.47	1.03	57.41	10.59
	Product C (Bull. 146), found.....	5.09	1.14	60.03	10.15
$C_{12}H_{25}Ag_{16}P_9O_{42}$ , Calc.....	5.06	0.88	60.66	9.81	
Wheat Bran Acid Ex- traction	Fraction 1, found.....	5.21	0.82	61.86	9.67
	$C_{12}H_{21}Ag_{17}P_9O_{42}$ , Calc.....	4.88	0.81	62.10	9.45
	Fraction 2, found.....	4.75	0.97	59.27	10.12
	$C_{12}H_{26}Ag_{16}P_9O_{42}$ , Calc.....	5.25	0.95	59.10	10.18

The products correspond to the formulas given in the table. It is evident that the products from the acid and ammonia extracts of cottonseed meal and the acid extract of wheat bran are all salts of the same acid, but containing different quantities of silver. The latter is to be expected as no attempt was made to precipitate the salts under identical conditions, for it has already been shown that the salts have the same amount of silver when they are so precipitated. The free acid corresponds to the formula  $C_{12}H_{41}P_9O_{42}$ .

These results are not in accord with the conclusions of Patten and Hart, who claim that wheat bran contains an inosite-phosphoric acid corresponding to the formula  $C_2H_8P_2O_9$ , nor with those of Anderson, who claims that cottonseed meal contains an acid of similar composition, nor with those of Anderson, claiming that wheat bran contains an inosite-phosphoric acid of the formula  $C_{20}H_{55}P_9O_{45}$ .

In our previous work on cottonseed meal it was shown that more than twice as much phosphorus was soluble in water as in 0.2 per cent hydrochloric acid, and that after extraction with acid the remaining phos-

phorus was no longer soluble in water, but soluble in 0.2 per cent ammonia.

The principal acid-soluble and ammonia-soluble compound have been shown to be the same acid, an inosite-phosphoric acid corresponding to the formula  $C_{12}H_{41}P_9O_{42}$ . The amount of ammonia soluble inosite-phosphoric acid is then about twice as much as that soluble in 0.2 per cent acid. The difference in solubility of the salts as they occur in the meal is a point which is as yet unexplained, but on which we hope to work in the future.

It appears from the work on cottonseed meal that the amount of inosite-phosphoric acid present is not necessarily limited to that in the acid extract as has apparently been assumed. The behavior of cottonseed meal in regard to acid and water is similar to that of oats, malt sprouts, and wheat bran, for Patten and Hart found nearly twice as much water-soluble as acid-soluble phosphorus in oats and appreciably more in the case of the last named materials. It is probable, therefore, that phytin is the principal constituent not only of that part of the material soluble in 0.2 per cent acid, but is the principal phosphorus compound in the entire feeding material.

DECOMPOSITION OF THE PHOSPHORUS COMPOUNDS OF COTTONSEED MEAL INTO INOSITE AND PHOSPHORIC ACID.

Unweighed portions of the crude free acids extracted from cottonseed meal by the acid and ammonia treatment were heated in sealed tubes for three hours at 160-65° C. After cooling, the tubes were opened, the products removed, the sulphuric and phosphoric acids precipitated by barium hydroxide and carbon dioxide added to remove excess of barium hydroxide. The filtrates were evaporated nearly to dryness, taken up with hot water and the remaining barium carbonate filtered off. The filtrate was dissolved in purified alcohol, and ether was added until a cloudiness was produced. The liquid was then cooled in ice water and allowed to stand. A crystalline precipitate separated in both cases. The amount of the precipitates was small and they were not recrystallized.

Dried in a vacuum over sulphuric acid, the substances gave the following results on combustion:

	Carbon.	Hydrogen.
Product of acid extraction.....	38.96	6.86
Product of ammonia extraction.....	40.55	7.18
Calculated for inosite.....	40.00	6.66

The products gave the reaction of Sherer and Gallois. The product of the acid extraction melted at 220° C (uncorrected) and the product of the ammonia extraction melted at 225° C (uncorrected). Inosite melts at 218° C (uncorrected). Although the above products are impure they are undoubtedly inosite.

The product from what bran was not decomposed into inosite and phosphoric acid, this already having been done by Patten and Hart on a sample prepared in a similar manner to ours.

EXAMINATION OF THE AMMONIA AND ALCOHOL PRECIPITATES OBTAINED  
IN PURIFYING THE PRODUCTS.

The ammonia precipitates from the acid extract of cottonseed meal were powdered, dried in a vacuum desiccator over sulphuric acid, and analyzed. Found:

Carbon .....	8.24 per cent
Hydrogen .....	3.71 per cent

The substance was rich in phosphorus and inorganic bases. It is evidently composed of inorganic phosphates, salts of inosite-phosphoric acid and unknown substances. Its carbon and hydrogen content agrees roughly with that of a calcium-magnesium-potassium salt of inosite-phosphoric acid. The substance was not examined further.

The alcohol precipitate obtained in the purification of the product from wheat bran was powdered, dried as above and analyzed. Found:

Carbon .....	9.24 per cent
Hydrogen .....	3.42 per cent

This product contained phosphorus and inorganic bases and is apparently largely organic in nature. It comprised about one-fourth of the purified wheat bran product. It evidently consists of salts of inosite phosphoric acid and other substances. No further examination was made.

## IDENTITY OF THE COMPOUNDS.

To summarize the above work, we have the following evidence that the compounds are salts of the same acid: (1) the free acids yield inosite on decomposition with sulphuric acid; (2) the silver salts correspond to salts of the same acid; (3) silver salts having the same amount of silver are formed under identical conditions; (4) the silver salts, prepared under identical conditions, have the same solubility in water; (5) the qualitative reactions of the products from cottonseed meal are the same.

The facts that the various fractions of the silver salts correspond to salts of the same acid, and that the silver salts prepared under identical conditions have the same solubility, is evidence that the products are pure.

We are therefore justified in concluding that the inosite-phosphoric acids of wheat bran and cottonseed meal are the same, and that the free acid corresponds to the formula  $C_{12}H_{41}P_9O_{42}$ .

These results are not in accord with those of Patten and Hart, and of Anderson, whose work has already been discussed on preceding pages.

We have already shown that the method of Patten and Hart would give erroneous results, since we found as much as 4.7 per cent inorganic impurities present in a product prepared by their method.

Anderson's method for the preparation of the organic phosphorus acid from wheat bran, given in detail on a following page, consists in repeated precipitation of the crude salt with alcohol from acid solution and finally with barium hydroxide. It is a well known fact that calcium and magnesium phosphates are precipitated under the same conditions, and unless

the inorganic acid is present in a free state, which is not likely, this purification process would remove very little of the inorganic phosphoric acid or inorganic bases.

This method of Anderson is open to a still more grave objection, which will be discussed on a following page.

The method used by Anderson for the preparation of the inosite-phosphoric acid of cottonseed meal was similar to that used by him in his work on wheat bran, the only essential difference being the crystallization of a barium salt from neutral solution and from acid-alcohol. He obtained crystals of constant composition, but he apparently assumes that iron, alumina, lime and magnesium were absent, for he offers no analytical evidence concerning them. His product is therefore liable to contain these substances for the reasons we have already stated.

#### THE QUESTION OF THE PRESENCE OF PENTOSE IN THE INOSITE-PHOSPHORIC ACID OF WHEAT BRAN.

Anderson (*loc. cit.*) claims that wheat bran does not contain phytin, but that it does contain an acid, the barium salt of which corresponds to the formula  $C_{25}H_{55}O_{54}P_9Ba_5$ , and that this compound is easily transformed into a compound, the barium salt of which corresponds to the formula  $C_{20}H_{45}O_{49}P_9Ba_5$ , and that it loses one pentose group,  $C_5H_{10}O_5$ , in so doing.

Anderson digested the bran with 0.2 per cent hydrochloric acid, precipitated the strained extract with alcohol, and purified the resulting precipitate by reprecipitating six times with alcohol from 0.2 per cent hydrochloric acid; .3887 gram of the dried substance gave .0367 gram phlorglucid when distilled with 12 per cent hydrochloric acid.

Anderson dissolved a portion of the above substance in water and precipitated hot with barium hydroxide. The washed precipitate was dissolved in 0.5 per cent hydrochloric acid and reprecipitated with barium hydroxide, the resulting precipitate dissolved by addition of dilute hydrochloric acid, and then precipitated by the addition of a like volume of alcohol. The filtered substance, washed in dilute alcohol, was again dissolved in 0.5 per cent hydrochloric acid and reprecipitated in the same manner as before. These operations were repeated four times. It was then dissolved in the same strength hydrochloric acid, precipitated with alcohol, filtered, washed in dilute alcohol, alcohol, and ether, and dried in a vacuum over sulphuric acid; 1.2124 grams of this substance gave 0.0053 grams phlorglucid when distilled with 12 per cent hydrochloric acid. From analyses Anderson concludes that the formula is  $C_{25}H_{55}O_{54}P_9Ba_5$ .

The above salt was suspended in water, decomposed with dilute sulphuric acid, filtered from the barium sulphate and precipitated with copper acetate. The copper was removed by hydrogen sulphide and the filtered solution concentrated, dried and analyzed. The formula  $C_{20}H_{45}P_9O_{49}$  is proposed for this product.

The following is quoted from the summary, page 2:

"The purified barium salt of the compound corresponded to the following formulas:  $C_{25}H_{55}O_{54}P_9Ba_5$  and  $C_{20}H_{45}O_{49}P_9Ba_5$ . Attempts to isolate the free acid corresponding to the first salt did not succeed. From both salts the same acid, corresponding to that of the second salt,

$C_{20}H_{45}O_{40}P_9$  was obtained. This acid is apparently formed from the first by the splitting off of the elements of one pentose. . . . The conclusion seems justified that wheat bran does not contain phytin."

The following review of the evidence offered by Anderson will show that his conclusions are not justified by the facts:

According to theory, if the product to which he assigns the formula  $C_{25}H_{55}O_{54}P_9Ba_5$  contained one pentose group, this salt would contain 6.87 per cent pentose. But by his analysis 1.2124 grams yielded 0.0053 grams phlorglucid, which would correspond to 0.88 per cent pentose. This is 12.8 per cent of 6.87, or *only one-eighth as much as his theory requires.*

From the phosphorus-pentose ratio in the theoretical barium salt one can get an idea of what the lime-magnesium-potassium salt of the acid should contain. In the pure salts the ratio would be the same regardless of the amount of lime, magnesium, potassium or barium present. The theoretical percentage of pentose in the barium salt is 6.87 and the percentage of phosphorus 12.76. The amount of phosphorus found in the crude lime-magnesium-potassium salt was 14.42. Then if X represents the per cent of pentose theoretically present in this salt, 6.87:12.76::X:14.42, whence X equals 7.76. The amount of phosphorus in the crude salt, then, corresponds to 7.76 per cent pentose according to theory.

Anderson's analysis shows that 0.3887 grams of substance yielded 0.0367 grams phlorglucid, which would correspond to 10.94 per cent pentose, yet by theory 7.76 per cent is present in the salt, a difference of 3.18 per cent. *His crude salt, then, contains 41 per cent too much pentose and his barium salt 87 per cent too little.* It is evident from Anderson's results only one-eighth of the barium salt can be in the form claimed by him.

This matter was tested further in the following manner:

Twenty-five grams of wheat bran and of cottonseed meal were digested for three hours with 250 c.c. 0.2 per cent hydrochloric acid, filtered, and 200 c.c. precipitated with alcohol, just as described in the method used by Anderson. The precipitate was filtered and washed with alcohol, washed from the filter with hot water, boiled and some insoluble matter filtered off. (Anderson claims that the crude salt is readily soluble in water.) The filtrate was heated nearly to boiling and precipitated with barium hydroxide, filtered hot and washed with hot water. The phosphorus and pentosans were determined in the precipitate. The preparation of the barium salt was exactly the same as that used by Anderson except that we precipitated the product only one time with each precipitant.

The filtrate from the barium precipitate was freed from barium, concentrated and precipitated with alcohol. The precipitate was filtered on acid-digested asbestos and washed with alcohol. Phosphorus and pentosans were determined.

The results are shown in Table 2.

TABLE 2.

Products of Acid Extracts of Wheat Bran and Phosphorus and Pentoses in Cotton Seed Meal  
Percentage of Original Substance.

	Phos- phorus	Pentoses	Pentose Phosphorus
Barium Salt:			
Wheat Bran.....	0.314	0.144	0.459
Cottonseed Meal.....	0.085	0.111	1.307
Alcohol Precipitate from filtrate from Barium Salt:			
Wheat Bran.....	0.001	0.065	65.000
Cottonseed Meal.....	0.001	0.044	44.000
Theoretical: (According to Anderson's Proposed Formula).....			0.539
Sum of Pentoses and of Phosphorus:			
Wheat Bran.....	0.315	0.209	.....
Cottonseed Meal.....	0.086	0.155	.....
Percentage lost by treatment with Barium Hydroxide:			
Wheat Bran.....	3.	31.	.....
Cottonseed Meal.....	12.	28.	.....

From the table it is evident that wheat bran and cottonseed meal contain soluble pentoses that are associated with very little, if any, phosphorus, and that these pentoses are insoluble in alcohol. The evidence on this point is positive. The barium salt from the wheat bran contains not quite enough pentoses to satisfy the theory, and the cottonseed meal product contains more than twice as much as is required. The barium hydroxide treatment removed 31 per cent of the pentoses, from the crude salt from wheat bran and 28 per cent from that of cottonseed meal, while the amount of phosphorus removed in the same fractions was only 3 per cent and 12 per cent, respectively. Anderson's barium salt purified by repeated treatment contained only one-eighth as much pentoses as is required by his theory. So it is evident that pentoses are removed in each successive treatment with barium hydroxide. Anderson's crude salt contained 1.25 per cent nitrogen. He does not report the nitrogen in the barium salt, so we do not know what other non-phosphorised impurities it contained.

The above results, and those of Anderson show that *we have no evidence that wheat bran contains an inosite-phosphoric acid with pentose in the molecule.* There is also no evidence that cottonseed meal contains such an acid.

The method of Anderson described above for the preparation of the lime-magnesium-potassium salt of phytic acid was the same as that used by Patten and Hart. The latter workers analyzed a product prepared in this way and which they regarded as an impure lime-magnesium-potassium salt of phytic acid. This salt contained by analysis 17.30 per cent carbon; the free acid, according to their theory, contains 10.08 per cent carbon. The lime-magnesium-potassium salt, then, contained 70 per cent more carbon than the free acid, and at least twice as much as their theory would require for such a salt. This abnormal result can be explained by our work described above. They merely reprecipitated water soluble, alcohol insoluble pentosans, by their method, and obtained a product highly contaminated with those substances.

## COMPARISON OF FORMULAS PROPOSED FOR INOSITE-PHOSPHORIC ACID OF WHEAT BRAN AND COTTONSEED MEAL.

In Table 3 is shown the percentage composition of inosite-phosphoric acid of wheat bran and cottonseed meal, as calculated from the formulas proposed by different investigators.

TABLE 3.

Percentage Composition of Inosite-Phosphoric Acid of Cotton Seed Meal and Wheat Bran, Calculated from Formulas Proposed.

	Proposed Formula	Carbon	Hydrogen	Phosphorus
Patten and Hart.....	$C_2H_4P_2O_9$ .....	10.08	3.36	26.07
Anderson.....	$C_{20}H_{55}P_9O_{49}$ .....	17.67	4.05	20.54
The writer.....	$C_{12}H_{41}P_9O_{42}$ .....	12.68	3.61	24.56

We have already shown that the acid would apparently contain less carbon and more phosphorus than it really contained if prepared and analyzed by the method of Patten and Hart. The product of Anderson was obviously not pure, as it lost by simple separation as the free acid enough of some substance to change the calculated formula by  $C_5H_{10}O_5$ . It was not purified further and no homogeneity test was made. Since we have shown that our product was homogeneous and probably pure, we believe that the formula  $C_{12}H_{41}P_9O_{42}$  is more nearly the truth than the others proposed.

## THE COMPOSITION OF PHYTIC ACID.

The theory that the inosite-phosphoric acid, or so-called "phytic acid" of feeding materials has the composition represented by the formula  $C_2H_8P_2O_9$ , or a multiple thereof, rest upon the work of Posternak, Patten and Hart, Hart and Tottingham, and Anderson, all of whom used similar methods and analyzed, in most cases, the free acid. We have shown that grave errors are introduced by this method. Patten and Hart, Hart and Tottingham, Anderson, and as far as we have been able to find Posternak made no attempt to prove that their products were homogeneous and free from inorganic substances. The analysis of the free acid is attended with much risk, as gummy substances of this nature are liable to contain water which is imperfectly removed on drying.

The method used by Rising provides for the removal of inorganic bases but not for inorganic phosphorus. His method involves precipitation of bases with alcohol and ether, and from our results with alcohol it appears that it accomplished its purpose. Rising analyzed the silver salt of his product and his calculated formula agrees better with our formula than does that of any other investigator.

Verbrodt, with the details of whose work we are unacquainted, prepared and analyzed a barium salt of inosite-phosphoric acid, and assigned the formula  $C_{12}H_{40}P_{11}O_{46}$  to the free acid.

The percentage composition (calculated) of inosite-phosphoric acid, or so-called phytic acid, according to these investigators, is shown in Table 4.

Our formula is  $C_{12}H_{41}P_9O_{42}$ , Rising's multiplied by 2, would be  $C_{12}H_{40}P_{10}O_{44}$ , Verbrodt's is  $C_{12}H_{40}P_{11}O_{46}$ , while the formula proposed by Posternak, and agreed to by other investigators, multiplied by 6, would



be  $C_{12}H_{48}P_{12}O_{54}$ . Both the formula and percentage composition of our acid agree fairly well with that of Rising, and since he made no effort to remove inorganic phosphorus, the slightly lower content of carbon and higher content of phosphorus could be explained in this way. At any rate, the preponderance of evidence is in favor of a carbon content higher than 10.08 per cent, the results of Patten and Hart, Hart and Tottingham, and of Anderson, themselves being always higher than this figure.

TABLE 4.

Calculated Percentage Composition of Inosite-Phosphoric Acid According to Proposed Formulas.

	Proposed Formula	Carbon	Hydrogen	Phosphorus
Posternak, Patten and Hart; Hart and Tottingham; and Anderson.....	$C_2H_3P_3O_9$ .....	10.08	3.36	26.07
Verbrodt.....	$C_{12}H_{20}P_9O_{42}$ .....	11.42	3.17	27.04
Rising.....	$C_6H_{10}P_5O_{42}$ .....	12.02	3.34	25.88
Anderson (synthetic).....	$C_{12}H_{32}P_{10}O_{41}$ , calculated.....	12.60	2.80	27.14
	Found.....	12.62	3.24	26.51
The writer.....	$C_{12}H_{41}P_9O_{42}$ .....	12.68	3.61	24.56

We believe our formula,  $C_{12}H_{41}P_9O_{42}$ , more nearly represents the truth and propose it as the empirical formula of inosite phosphoric acid, or the so-called "phytic acid" of feeding materials.

It will be noticed that the calculated composition of our acid is similar to that of the di-inosite-tripolyphosphoric acid ester Anderson claims to have synthesized from inosite and pyrophosphoric acid, the phosphorus, however, being about 2 per cent higher in the product of Anderson. Anderson states that "the substance was isolated as the barium salt in exactly the same way . . . as described for the dipolyphosphoric ester." The preparation of this last named ester involved precipitation from phytic acid solution with alcohol. Since the phytic acid had not been shown to be free from inorganic bases, and since they have been found by us in considerable amounts in products prepared as Anderson prepared the phytic acid, and since these are precipitated by alcohol as salts of phytic acid, Anderson's products are necessarily impure.

#### SUMMARY AND CONCLUSIONS.

1. The method for the preparation of the so-called phytic acid, on which rests the theory that this acid corresponds to the formula  $C_2H_3P_3O_9$  or a multiple thereof, have been shown to produce an impure product containing notable amounts of iron and aluminum, calcium, magnesium and inorganic phosphorus. Formulas based on analyses of products prepared by these methods are therefore erroneous.

2. The principal organic phosphoric acids of cottonseed meal and wheat bran were separated and purified and the silver salts fractionated and analyzed.

3. Upon hydrolysis both the acid and the ammonia-soluble organic phosphorus compounds of cottonseed meal were found to yield inosite.

4. The inosite phosphoric acids of wheat bran and cottonseed meal were found to be identical and correspond to the formula  $C_{12}H_{41}P_9O_{42}$ .

5. Probably two-thirds of the inosite-phosphoric acid of cottonseed meal is not soluble in 0.2 per cent acid, but is soluble in water. It is not soluble in water after extracting the material with acid, but may be dissolved in 0.2 per cent ammonia. The inosite-phosphoric acid of feeding materials is therefore not necessarily confined to the acid extract, which has formerly been assumed.

6. We have no evidence that wheat bran contains an inosite-phosphoric acid with pentose in the molecule.

7. The formula  $C_{12}H_{41}P_9O_{42}$  is proposed for inosite-phosphoric acid, or the so-called "phytic acid" of feeding materials.