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**The Forms of Phosphorus in Cotton  
Seed Meal**

BY

J. B. RATHER, Assistant Chemist.



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# THE FORMS OF PHOSPHORUS IN COTTON SEED MEAL

BY J. B. RATHER, ASSISTANT CHEMIST.\*

Cottonseed meal is used extensively as a feed and as a fertilizer. It is rich in phosphorus and that element is indispensable to animal and plant life.

Phosphorus exists in animal and plant tissues in both organic and inorganic forms. According to Forbes<sup>1</sup> "The principal need for phosphorus in the body is for inorganic phosphorus," but "organic phosphorus can supply all the bodily needs for phosphorus, both organic and inorganic," and "inorganic phosphorus can supply the needs of the body for inorganic phosphorus only." If the phosphorus in cottonseed meal is largely inorganic, as is claimed, then it is possible that it is deficient in organic phosphorus, from the standpoint of the student of animal nutrition.

Nearly all the phosphorus of cottonseed meal is found to be "available" when tested by the Official Method<sup>2</sup> for available phosphoric acid in fertilizers. This method does not distinguish between soluble organic and inorganic phosphorus, and the value of the former could quite possibly be less to plants than the latter.

Again, if the phosphorus were largely inorganic, the well known toxic effects of certain inorganic salts of phosphoric acid would render a knowledge of these constituents of the meal of much value.

*Review of the Literature.* The amount of work done on the nature of the phosphorus compounds in cottonseed meal is not extensive.

Gebeck<sup>2</sup> found .058 per cent phosphorus in the ether extract of cottonseed meal.

Stelwaag<sup>4</sup> found that 4.35 per cent lecithin was in the ether extract of cottonseed meal.

In 1892 Hardin<sup>5</sup> published the results of a study of cottonseed meal. He concluded that "all the reactions which have been described seem to show, beyond any reasonable doubt, the presence of both meta and pyrophosphoric acid in the aqueous solutions of the meals examined."

The more important criteria on which Hardin based his conclusions are as follows:

The phosphorus passed through a semipermeable membrane.

"In all the cases of direct precipitation (of the water extracts), if the filtrates were allowed to stand at 60° C., additional precipitation occurred, the quantity increasing with the time of standing."

A white precipitate soluble in nitric acid formed when the aqueous solutions of cottonseed meal were treated with silver nitrate solution.

The aqueous extracts coagulated albumen when acidified with acetic acid.

Magnesia mixture gave a precipitate largely soluble in an excess of ammonium chloride and wholly soluble in an excess of magnesium chloride.

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

A sample, prepared by diffusion, precipitation with lead acetate and decomposition of the lead salt with hydrogen sulphide, was strongly acid, coagulated albumen, and gave white precipitate with silver nitrate soluble in an excess of the acid. After nearly neutralizing with sodium hydroxide, silver nitrate gave a nearly pure white precipitate, leuto-cobaltic chloride a pale reddish yellow precipitate soluble in an excess of the solution. Magnesium sulphate produced no precipitate. Calcium salts produced a precipitate soluble in an excess of the solution. Ammonium molybdate gave a yellow precipitate only after standing for some hours at 60° C.

The product obtained by precipitation by boiling with magnesia mixture and an excess of magnesium sulphate and ammonium chloride, gave a white precipitate with silver nitrate, did not coagulate albumen, and did not precipitate with ammonium molybdate until after heating and standing.

“The facts that the original solution of the acids of the meal coagulated albumen, and that phosphoric acid in considerable quantity remained in the solution after the removal of the ortho and pyrophosphoric acid, and that this phosphoric acid was not readily precipitated by molybdate solution, show that the aqueous solution of cottonseed meal contains also metaphosphoric acid.”

The possibility that the cottonseed meal contained organic acids with phosphorus in the molecule does not seem to have been considered by Hardin.

More recently Crawford<sup>6</sup> reported a study of cottonseed meal from the standpoint of its toxicity and concluded that “the chief poisonous principle of cottonseed meal is a salt of pyrophosphoric acid.” The principal chemical experiments on which this conclusion was based were as follows:

He treated the digestion products of cottonseed meal with lead acetate and decomposed the lead precipitate with hydrogen sulphide. The filtrate from the lead sulphide was markedly acid, soluble in alcohol, but could be precipitated from alcohol solution by ether. On the removal of the alcohol a colored gum was obtained. This gum was treated with absolute alcohol to remove inorganic salts, the filtrate evaporated and taken up with water. Lead acetate was added and the lead precipitate decomposed with hydrogen sulphide. The filtrate from the lead sulphide was colorless and very acid in reaction. It gave no protein reaction and did not reduce Fehling's solution.

After neutralization the solution gave a white precipitate with silver nitrate, which was insoluble in an excess of sodium pyrophosphate but soluble in the free ammonia and nitric acid. Barium chloride gave a precipitate, ammonium molybdate gave a precipitate on heating, and magnesia mixture gave at once a white precipitate. The acetates of lead, mercury, iron and cadmium gave precipitates.

Copper sulphate gave a bluish white precipitate in the neutral solution of the acid, soluble in an excess of the mother fluid. Zinc sulphate gave white precipitate, likewise soluble in an excess of the solution. Egg albumen was precipitated by the acid solution, but after precipitating with magnesia mixture and dissolving the precipitate in acetic acid egg albumen gave only a cloud.



Copper acetate gave a bluish white precipitate, ferric chloride a white precipitate and uranyl acetate a yellow-white precipitate.

Suzuki and others claim that the principal acid-soluble phosphorus compound in rice bran,<sup>7</sup> wheat bran,<sup>8</sup> corn, oats and barley,<sup>9</sup> is a salt of an acid of the formula  $C_2H_5O_6$ , which was first described by Posernak and named phytic acid by him.

Quantitative studies of the inorganic phosphorus present in conc. feeding stuffs have been made in this country by Hart and Andrews<sup>11</sup> and by Forbes and associates<sup>12</sup>. So far as we have been able to find, no determinations of the inorganic phosphorus in cottonseed meal have been published except one reported by Forbes and associates<sup>12</sup>. They got .044 per cent phosphorus by the Hart and Andrews method and .118 per cent by one of their own which they subsequently declared unreliable.

### PRELIMINARY STUDIES.

*Effect of Washing With Ether Before Extraction.* In order to test if the fat in the meal would interfere with the extraction of the phosphorus the following experiment was made:

Three grams of meal were extracted six times with ether on a hardened filter, transferred to a 300 cc. flask and digested with 300 cc. 0.2 per cent hydrochloric acid for three hours with frequent shaking. The extract was decanted through a filter, 100 cc. pipetted off, evaporated, and ignited with magnesium nitrate solution and the phosphorus determined by the official volumetric method (A). The phosphorus was determined in three grams of meal as above except the ether washing was omitted (B).

TABLE 1—EFFECT OF WASHING WITH ETHER ON PHOSPHORUS EXTRACTED FROM COTTON SEED MEAL WITH 0.2 PER CENT HYDROCHLORIC ACID.

Laboratory Number.	Per Cent Phosphorus Soluble in 0.2 Per Cent Hydrochloric Acid.	
	(A)	(B)
	Washed Six Times With Ether.	Without Washing.
9196.....	.234	.258
9251.....	.166	.205
9298.....	.218	.227
9430.....	.179	.218
9468.....	.262	.288
9523.....	.221	.218
Average.....	.213	.235

The average amount of phosphorus extracted when the meal was washed with ether (A) was .213 per cent; when no ether was used (B) it was .235 per cent.

The results are slightly lower with the ether washing than without. The removal of the fat does not increase the amount dissolved under the conditions of the experiment. The fat did not appreciably interfere with the extraction of the phosphorus. The lower results with the ether washed meal are possibly due to the lecithin removed by the treatment.

*Solubility in water, acids, and ammonia.* The amount of phosphorus soluble in 0.2 per cent hydrochloric acid is much lower than published results for water soluble phosphorus in cottonseed meal. The following experiment was made to ascertain if the phosphorus not extracted by acid was soluble in water or ammonia:

Three grams of sample No. 9523 were extracted with 300 cc. of 0.2 per cent hydrochloric acid as already described. The residue from the extraction was washed with 0.2 per cent hydrochloric acid and then digested for three hours with 300 cc. of water. One hundred cubic centimeters of the filtered extract were evaporated, ignited with magnesium nitrate and the phosphorus precipitated as in the official volumetric method. Only a trace of the ammonium phosphomolybdate appeared and the determination was not completed. The phosphorus remaining from the extraction with acid was not soluble in water.

The residue from the extraction with water was extracted in the same manner with 0.2 per cent ammonia and the phosphorus determined in 100 cc. of the filtrate as described above. The amount of phosphorus found was .638 per cent. The amount remaining from the acid extraction was calculated to be .716 per cent. Two-tenths per cent ammonia extracted nearly all of the phosphorus remaining in the residue from the acid extraction.

A qualitative test on a portion of the filtered ammonia extract (above) showed that no precipitate formed when the solution was acidified with varying amounts of hydrochloric acid. The phosphorus not removed from the meal by the acid extraction was not precipitated from the ammonia extract on acidifying with hydrochloric acid.

*Precipitation of Meta and Pyro-Phosphoric Acids.* In order to determine how meta and pyro-phosphoric acids would act under the conditions of the determination of inorganic phosphorus by the method of Forbes and associates the following experiment was made: About 0.5 gram of meta-phosphoric acid and 1 gram of (Merck's Reagent) sodium pyrophosphate were dissolved in 200 cc. water in a graduated flask. Ten cubic centimeters of each solution were placed in beakers with 150 cc. water and precipitated with 20 cc. ammonia and 15 cc. magnesia mixture, adding the magnesia mixture drop by drop and allowing to stand over night. The resulting precipitates, which were scarcely visible in all cases, were washed on a filter with 2.5 per cent ammonia, and with alcohol, the filter and precipitate digested with 100 cc. of 95 per cent alcohol containing 0.2 per cent nitric acid and filtered. Seventy-five cubic centimeters of the filtrate were evaporated nearly to dryness, taken up with water and the phosphorus determined by the official volumetric method. Aliquots of the original solutions were digested with hot dilute nitric acid for about 16 hours and the phosphorus determined by the official volumetric method. The results are shown below:

	Milligrams Phosphorus		Percentage Phosphorus Recovered.	
	Added	Recovered.		
		1	2	
Meta-Phosphoric Acid	8.25	0.40	0.36	4.6
Pyro-Phosphoric Acid	7.10	0.03	0.00	0.2

The amount of phosphorus in solution was about the same as would be in 150 cc. of the 0.2 per cent hydrochloric acid extract of cottonseed meal. Four and six-tenths per cent of the meta-phosphoric acid was recovered and 0.2 of the pyro-phosphoric acid. These results may be due to ortho-phosphoric acid present in the salts as an impurity.

The amount of phosphorus recovered shows that the method of Forbes and associates will not include any appreciable amount of meta and pyro-phosphoric acid in the determination of inorganic phosphorus, if these acids are present in the plant tissue examined.

#### QUANTITATIVE STUDY OF THE PHOSPHORUS SOLUBLE IN WATER.

Six samples of cottonseed meal, average quality, probably of upland origin, and collected from different parts of the State were used in this investigation. For the determination of inorganic phosphorus in feeds, the method of Forbes and associates<sup>12</sup> was used. However, there are some objections to this method. It depends upon the assumption that the organic phosphorus precipitated by magnesia mixture is insoluble in 95 per cent alcohol containing 0.2 per cent nitric acid. Although it was found that magnesium phytate was insoluble under the above conditions, it has not yet been shown that phytin is the only acid soluble phosphorus compound in plants. Other organic phosphorus compounds may be present which are precipitated by magnesia mixture but behave differently towards the acid alcohol. Meta and pyro-phosphoric acid, if present, would be estimated as organic phosphorus, by this method. This should be borne in mind in considering the analyses by the Forbes method.

The amount and nature of the water soluble phosphorus were determined in the following manner:

*Determination of Total Phosphorus.* (A) One gram of cottonseed meal was ignited with 5 cc. magnesium nitrate solution, and the phosphorus determined by the official volumetric method.

*Phosphorus Soluble in Water.* (B) Three grams of cottonseed meal were digested in 300 cc. acid for three hours with frequent shaking, the extract decanted through a filter and the phosphorus determined in 100 cc. by evaporating, igniting with magnesium nitrate and completing as in the official volumetric method.

*Inorganic Phosphorus Soluble in Water.* (C) The solution was prepared as described above. One hundred and fifty cubic centimeters were precipitated in a beaker with 20 cc. strong ammonia and 15 cc. magnesia mixture. The precipitate was allowed to stand over night, filtered and washed several times with 25 per cent ammonia and with 95 per cent alcohol until free from ammonia.

After allowing to dry the precipitate and filter was transferred to an erlenmeyer flask containing 100 cc. of 95% alcohol containing 0.2 per cent nitric acid. The flask was closed with a rubber stopper, shaken vigorously and allowed to stand over night. The mixture was then filtered, 75 cc. of the filtrate evaporated almost to dryness, dissolved in dilute nitric acid and the phosphorus determined by the official volumetric method. The results are shown in Table 2.

TABLE 2—TOTAL, WATER-SOLUBLE, AND INORGANIC PHOSPHORUS SOLUBLE IN WATER.

Laboratory Number.	(A)	(B)	(C)
	Total Phosphorus.	Phosphorus Soluble in Water. (a)	Inorganic Phosphorus Soluble in Water.
9196.....	.886	.698	.042
9251.....	.786	.458	.023
9298.....	.947	.567	.039
9430.....	.978	.589	.035
9468.....	1.070	.698	.035
9523.....	.934	.568	.042
Average.....	.933	.563	.039
Average of 30 Texas Meals (b).....	1.148		

a. Under conditions of experiment.

b. Bull. 112 Texas Station.

The total phosphorus (A) varied from .786 per cent to 1.070 per cent and averaged .933 per cent. The average of 30 analyses of Texas meals made at this Experiment Station is 1.148. These samples are therefore low in phosphorus.

The phosphorus soluble in water (B) varied from .458 per cent to .698 per cent and averages about 60 per cent of the total phosphorus. Hardin<sup>5</sup> has shown that the amount of phosphorus removed by water depends on the conditions of the experiment and that by repeated washing with water most of the phosphorus goes into solution. The inorganic phosphorus (C) varies from .023 per cent to .042 per cent and averages .039 per cent. This does not include metaphosphoric acid and pyro-phosphoric acid if they are present. Hereafter when the word inorganic phosphorus is used the above limitations are implied. These analyses show that there is very little ortho-phosphoric acid in the water extracts of the meals examined.

#### PHOSPHORUS SOLUBLE IN ACID AND AMMONIA.

Based on the preliminary work described on a preceding page the following method of work was used:

*Acid Soluble Phosphorus.* Three grams of cottonseed meal were digested with 0.2 per cent hydrochloric acid as described in the study of the water extract. The total (A) and inorganic (ortho) phosphorus (B) were determined as described for the water extract. The acid-alcohol insoluble matter obtained in the determination of the inorganic phosphorus was washed with acid alcohol, ignited with magnesium nitrate, taken up with 10 cc. of nitric acid and 2 cc. of hydrochloric acid and the phosphorus determined by the official volumetric method (C).

*Ammonia Soluble Phosphorus.* The cottonseed meal residue from the extraction with 0.2 per cent hydrochloric acid was washed with water, put in a flask and digested for three hours with 0.2 per cent ammonia with frequent shaking. The extract was filtered and the total phosphorus determined as already described for the water and acid extracts (D). One hundred and fifty cubic centimeters of the extract were treated with 20 cc. strong ammonia and 15 cc. magnesia mixture. The precipitate was allowed to settle over night, filtered and washed with 4 per cent ammonia. The filter and precipitate were



ignited with magnesium nitrate and the determination completed as already described for total phosphorus (E).

The results of the work are shown in Table 3.

TABLE 3—PHOSPHORUS EXTRACTED FROM COTTON SEED MEAL BY ACID AND BY AMMONIA.

Laboratory Number.	Total Phosphorus.	1. Acid-Soluble Phosphorus.			2. Phosphorus Insoluble in Acid (by dif.).	2. Ammonia Soluble Phosphorus.	
		(A) Total.	(B) Inorganic (a).	(C) Organic Magnesia Ppt.		(D) Total.	(E) Magnesia Precipitate.
9196.....	.886	.258	.039	.144	.628	.668	-----
9251.....	.786	.205	.025	.144	.581	.600	-----
9298.....	.947	.227	.043	.144	.720	.729	-----
9430.....	.978	.218	.039	.144	.760	.740	.437
9468.....	1.070	.288	.056	.205	.782	.860	.725
9623.....	.934	.218	.051	.131	.714	.653	.603
<b>Average..</b>	<b>.934</b>	<b>.234</b>	<b>.042</b>	<b>.152</b>	<b>.698</b>	<b>.708</b>	-----

(a) According to method of Forbes et al.

The phosphorus soluble in 0.2 per cent hydrochloric acid (A) varied from .205 per cent to .288 per cent, and averaged about 25 per cent of the total phosphorus.

The total water soluble phosphorus (Table 2) is more than twice as great as the total acid soluble phosphorus, averaging .563 per cent and .235 per cent respectively.

*The Inorganic Phosphorus.* The inorganic phosphorus soluble in 0.2 per cent hydrochloric acid (B) varied from .025 per cent to .056 per cent and averaged about 4 per cent of the total phosphorus. Since meta-phosphoric acid and pyro-phosphoric acid are not precipitated by magnesia mixture under the conditions of the experiment they would not be included in these figures and the results would be too low if they were present. We will show on another page that we have no proof of their presence in cottonseed meal. The sum of the inorganic (B) and the organic phosphorous precipitated by magnesia mixture (C) subtracted from the total acid soluble (A) phosphorus represents nucleins, meta-phosphoric acid and pyro-phosphoric acid (if present), and unknown compounds. This averages .040 per cent. Assuming that this consisted entirely of meta and pyro-phosphoric acid the total inorganic phosphorus would then be .082 per cent, less than ten per cent of the total phosphorus. These results are not in accord with the theory that the phosphorous of cottonseed meal is principally inorganic.

A comparison of Table 3 and Table 2 brings out the fact that there is as much inorganic phosphorus soluble in water as there is in 0.2 per cent hydrochloric acid. The use of hydrochloric acid in the determination of inorganic phosphorus in cottonseed meal is therefore unnecessary.

*Magnesia Precipitate Insoluble in Acid Alcohol.* The phosphorus in the magnesia precipitate (C) insoluble in acid-alcohol varies from .131 per cent to .205 per cent and comprises the larger portion of the acid soluble phosphorus.



*Ammonia Soluble Phosphorus.* The phosphorus removed by the ammonia averaged .708 per cent while the phosphorus remaining from the hydrochloric acid extraction was .698 per cent (calculated by difference). Ammonia extracted all of the phosphorus remaining after the acid extraction. The differences in the table are probably analytical errors. The precipitates of ammonia-soluble phosphorus by magnesia mixture (E) were very difficult to filter. They ran through the filters repeatedly and finally clogged up the filters so filtration was impossible. With sample 9523 a clear filtrate was obtained. The other filtrates were opalescent and apparently were not free from the magnesia precipitate. With the exception of sample 9523 the determinations are therefore probably too low. Nevertheless, they indicate that most of the ammonia soluble phosphorus is precipitated by magnesia mixture. This precipitate is probably all organic.

#### PRECIPITATION BY COPPER AND LEAD.

The amount of phosphorus in cottonseed meal precipitated by lead subacetate and by copper acetate from water solution was ascertained in the following manner: Three grams of meal were digested for three hours with 300 cc. of water and 200 cc. of the extract precipitated with lead subacetate solution 100 cc. of the filtrates from the precipitations were freed from lead, evaporated, ignited with magnesium nitrate solution and the phosphorus determined by the official volumetric method.

The phosphorus not precipitated by lead averaged .028 per cent (average of 6 analyses).

Sample 9523 was treated as above, but copper acetate was used instead of lead subacetate.

The amount of phosphorus remaining in solution was .022 per cent. These reagents precipitated almost all of the phosphorus from water solution.

#### SEPARATION AND PURIFICATION OF PHOSPHORUS COMPOUNDS.

The meal was extracted in a manner similar to that described in the analytical work. The method of purification of the acids was similar to that used by Hart and Tottingham<sup>9</sup>, except that we used alcohol at one stage of the work to precipitate an alcohol insoluble compound. The method was as follows:

Two kilograms of cottonseed meal were digested with 8000 cc. of 0.2 per cent hydrochloric acid for 3 hours with frequent shaking. The extract was strained through cheese cloth and the residue washed well with water. The residue was digested with 8000 cc. 0.2 per cent ammonia for 3 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 syrupy consistency. The brownish syrup was dissolved in a small amount of water and filtered from an insoluble

brownish residue. Ninety-five per cent alcohol was then added to the water solution in sufficient amount to make the solution about 80 per cent alcohol. A white flocculent precipitate formed and quickly settled. It was filtered and washed with 95 per cent alcohol, dissolved in weak, hot ammonia, the ammonia removed by boiling and alcohol again added. The precipitate was again washed, dissolved in weak ammonia, made neutral with acetic acid and precipitated with copper acetate. The copper acetate precipitate was washed well with water, decomposed with hydrogen sulphide and filtered. The filtrate was concentrated and made alkaline with sodium hydroxide and barium chloride added in excess. The barium precipitate was washed free from alkali with water, suspended in water and the barium precipitated with sulphuric acid. The filtrate from the barium sulphate was precipitated with copper acetate, washed and decomposed with hydrogen sulphide. The filtrate from the hydrogen sulphide precipitate was evaporated to a syrupy consistency. (Product A.)

The filtrate from the first precipitation with alcohol in the preparation of Product A was evaporated on a water-bath to remove the alcohol, taken up with water and made alkaline with sodium hydroxide. Barium chloride was added and the resulting precipitate was 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 syrupy consistency. (Product B.)

The ammonia extract 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, alcohol and barium chloride exactly as described for the acid extract. (Product C.)

#### PROPERTIES OF THE PRODUCTS.

Product A (alcohol insoluble) was a colorless gummy substance with a strong acid reaction. It darkened on heating at 100° C.

It was precipitated by copper acetate, lead subacetate, barium chloride and silver nitrate. The silver salt had a pinkish tinge. The acid was precipitated from water solution by alcohol and the resulting precipitate was difficultly soluble in water and weak hydrochloric acid, and soluble in hot dilute ammonia and sodium hydroxide.

The product was separated from a sample which had been purified by precipitation with barium chloride and copper acetate. Since this bulletin went to press, further work has made it appear probable that Product A is the lime or magnesia salt of Product B.

An alcohol insoluble substance similar to Product A was found in the ammonia extract, but smaller in amount than that found in the acid extract.

*Properties of Products B and C.* Product B (acid soluble) and product C (ammonia soluble) had similar properties and will be described together. They were thick, almost colorless, gummy sub-

stances, which darkened in the air and on heating at 100° C. They were strongly acid. Million and Biuret tests gave no reaction for protein. Product B fused with sodium gave no reaction for nitrogen. Upon precipitating Product B with magnesia mixture and dissolving the precipitate in acetic acid the solution did not coagulate albumen until allowed to stand over night and then only slightly.

Portions of the acid and ammonia soluble products were neutralized with sodium hydroxide and placed in diffusion shells. After twenty-four hours the water on the outside of the shells gave yellow precipitates with ammonium molybdate when heated above 65° C.

Portions of the diffused substance gave white precipitates with silver nitrate and coagulated albumen.

In Table 4 are collected for comparison the above described reactions together with additional ones, and those of the products obtained by Crawford and by Hardin.

Silver Nitrate.	White precipitate soluble in nitric acid, in ammonia in excess of the phosphorus acid.	White precipitate soluble in nitric acid, in ammonia and in excess of the phosphorus acid.	White precipitate soluble in nitric acid and in excess of the phosphorus acid.
Magnesia Mixture.	Heavy white precipitate soluble in acetic acid.	Heavy white precipitate, soluble in acetic acid.	
Ammonium Molybdate.	No precipitate in cold, yellow precipitate when heated above 65°C, or allowed to stand at 65°C, for 2 or 3 hours, or on heating above 65°C.	No precipitate in cold but yellow precipitate on heating.	Yellow precipitate after stand some hours at 60°C.
Fehling's Solution.	No copper reduced.	No copper reduced.	
Barium Chloride.	White precipitate.	White precipitate.	
Lead, Mercury, Iron and Cadmium Acetates.	Precipitate.	Precipitate.	Lead acetate gave precipitate.
Cobaltamine.	Precipitate.	Precipitate.	Precipitate.
Copper Sulphate.	Heavy bluish white precipitate, soluble in excess of the phosphorus acid.	Heavy bluish white precipitate soluble in excess of the phosphorus acid.	
Albumen.	Coagulated at once.	Coagulated at once.	Coagulated.
Ether.	Precipitate (from alcohol solution).	Precipitate.	
Zinc Sulphate.	White precipitate soluble in excess of phosphorus acid.	White precipitate.	
Copper Acetate.	Heavy bluish white precipitate soluble in ammonia and hydrochloric acid.	Heavy bluish white precipitate soluble in ammonia and hydrochloric acid.	
Ferric Chloride.	White precipitate (soluble in excess of the phosphorus acid).	White precipitate soluble in excess of the phosphorus acid.	
Uranyl Acetate.	Nearly white precipitate soluble in an excess of the phosphorus acid.	Nearly white precipitate soluble in excess of the phosphorus acid.	
Precipitated with Magnesia Mixture, precipitate dissolved in acetic acid: (a) Albumen added. (b) Ammonium Molybdate.	Coagulated slightly after standing over night. Yellow precipitate when heated above 65°C. and allowed to stand.	Coagulated slightly.	Did not coagulate. Yellow precipitate after heating and standing.
Sodium salts of the acids diffused through semi-permeable membrane and tested: (a) Ammonium Molybdate. (b) Silver Nitrate. (c) Albumen.	Yellow precipitate above 65°C. White precipitate. Coagulated.		Yellow precipitate on heating and standing. White precipitate. Coagulated.



A possible explanation of the fact that the magnesia precipitated did not coagulate albumen when acidified with acetic acid is that acetic acid may not be strong enough acid to liberate the other acid from its salt.

The cobaltamine used in the test given in the table was prepared by us in the following way: A few grams of cobalt chloride were dissolved in about 500 cc. water, a little hydrochloric acid added; the solution made alkaline with ammonia and allowed to stand in a shallow dish for several days, ammonia being added occasionally. The precipitate which formed was washed with weak ammonia before using.

In the test with ammonia molybdate the precipitate began to form in about fifteen minutes and continued to precipitate for about three hours. At the end of that time the precipitation was not complete, as shown by heating the filtrate to a higher temperature (85-90°C) when an additional yellow precipitate formed.

Since Products B and C are both precipitated by magnesia mixture, and since most of the phosphorus in the acid and ammonia extracts was precipitated by magnesia mixture, it appears probable that these products are the principal phosphorus compounds in cottonseed meal.

#### ANALYSIS OF THE SILVER SALTS OF THE PRODUCTS.

The acids were diluted with water and neutralized with ammonia, boiled until free from ammonia and precipitated with silver nitrate solution. The silver salts were washed with water, dried in the air, powdered and dried for several days in a vacuum desiccator over sulphuric acid.

Product A was pinkish white, and Products B and C were white. Analysis of these salts gave the following results:

	Carbon.	Hydrogen.	Silver.	Phosphorus.
Product A (alcohol insoluble)	4.80	1.23	57.98	10.16
Product B (acid extraction)	5.42	0.86	61.40	9.82
Product C (ammonia extraction) .....	5.09	1.14	60.03	10.15

The empirical formulae corresponding to the above analyses are Product A:  $C_7H_{12}Ag_5P_3O_{15}$ ; Product B:  $C_6H_{10}Ag_7P_4O_{17}$ ; and Product C:  $C_4H_{10}Ag_5P_3O_{13}$ .

It appears that, since the compounds described on the preceding pages have properties very similar to meta and pyro-phosphoric acids, conclusions that the latter are present in cottonseed meal have no value when based on these reactions. Therefore we have no proof that cottonseed meal contains either pyro or meta phosphoric acid.

These results are not in accord with the conclusions of other investigators (5 and 6), based on qualitative reactions which are described in detail on preceding pages, that cottonseed meal contains pyro and meta-phosphoric acids in considerable amounts. However valuable the qualitative tests for phosphoric acids may be in a mixture of inorganic compounds, conclusions based on them in an unknown organic mixture should be drawn with caution.



From the work described above it appears that the acid and ammonia soluble products have very similar properties differing only in their solubility in hydrochloric acid and the percentage of silver in the silver salts.

We are not prepared to state at this time that the above products are pure chemical compounds, but it seems certain that they are free from all but traces of inorganic phosphates since the copper salts of these acids are soluble in water to some extent, in hydrochloric and sulphuric acids and the preparation of the products includes precipitation in dilute solutions of both of these acids. The small amount present would probably be removed by the treatment given, since over nine-tenths of the preparations were lost in purification. They also appear to be free from lime and magnesia since a test of the product obtained from the first precipitation with copper, after washing, decomposition with hydrogen sulphide and concentration of the filtrate from the copper sulphide, showed only traces of these metals and they would no doubt be removed by the second acid precipitation.

The study of the Products A, B, and C, is being continued.

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#### SUMMARY AND CONCLUSIONS.

1. The fat in cottonseed meal did not interfere with the extraction of the phosphorus with 0.2 per cent hydrochloric acid.
2. The phosphorus soluble in water was more than twice as much as that soluble in 0.2 per cent hydrochloric acid.
3. The same amounts of inorganic phosphorus (Forbes Method) were found in the acid and in the aqueous extracts.
4. The phosphorus soluble in 0.2 per cent hydrochloric acid was about 25 per cent of the total phosphorus.

5. The inorganic phosphorus (Forbes' Method), in the samples of cottonseed meal examined was less than 5 per cent of the total phosphorus.

6. The phosphorus in the hydrochloric acid extract of cottonseed meal capable of being precipitated by magnesia mixture and not soluble in acid-alcohol was about 16 per cent of the total phosphorus.

7. After extraction with 0.2 per cent hydrochloric acid the phosphorus remaining was *insoluble* in water but nearly completely soluble in 0.2 per cent ammonia.

8. Magnesia mixture precipitated most of the phosphorus extracted by ammonia.

9. Practically all of the phosphorus was precipitated from the aqueous extract of cottonseed meal by lead subacetate and by copper acetate.

10. The principal compounds containing phosphorus were separated, which give the same reactions as those relied upon for proving the presence of meta and pyro-phosphoric acid.

11. We have no evidence that the samples of cottonseed meal examined contain either pyro-phosphoric acid or meta-phosphoric acid.