

Table III. Tritiated Water in the Heifer at Slaughter

tissue	dpm/g of wet tissue ^a			% ³ H ₂ O of total radioact
	combustion	³ H ₂ O	total ^b	
liver	5140	303	5443	5.6
kidney	601	329	930	35.4
muscle	288	303	591	51.3
fat	3158	4	3162	0.1
urine	—	353	4186	8.4
rumen	—	228	803	28.5

^a Average of three heifers; 6 h posttreatment.

^b Schoniger value (after drying and combustion) plus ³H₂O obtained by distillation of ³H₂O from frozen samples. Urine was counted directly in Ditol. Rumen fluid was digested by the perchloric acid technique.

SUMMARY AND CONCLUSION

Tissues from heifers fed nonradioactive MGA for several months (~0.5 mg head⁻¹ day⁻¹) followed by radioactive MGA and then slaughtered on-treatment contained varying amounts of radioactivity (MGA and metabolites). The highest level of radioactivity was found in liver. The levels in liver were based on three 21-dose tritium studies (12.1 ppb of MGA equiv) and on one 7-dose carbon-14 study (8.2 ppb of MGA equiv). The average MGA per se was 29% for the tritium study (3.5 ppb) and 37% for the carbon-14 study (3.0 ppb).

Peripheral fat contained an average of 7.7 and 3.6 ppb of MGA equiv of radioactivity for the tritium and carbon-14, respectively, of which nearly all was intact MGA. The average MGA per se level in fat was 86% (6.6 ppb) and 75% (3.0 ppb) for tritium and carbon studies, respectively. The higher level of MGA in fat relative to liver established fat as the "target tissue".

The non-MGA fraction in liver was separated on thin-layer plates of silica gel and found to be composed of three or more metabolites, none of which were greater than 1 ppb or 10% of the total radioactivity in the sample.

These data established MGA as the "marker compound" and demonstrated that fat, because of its high concentration of MGA, was the "target tissue". Currently, this method is applicable for the analysis of MGA in fat, liver, kidney, and muscle that is sensitive to 10 ppb which has AOAC final action status. Since there were no residues of metabolites greater than 1 ppb, no other residue methods were required.

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Preparation and Characterization of FeH₃(PO₄)₂•2CO(NH₂)₂

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TVA has been investigating the production of urea phosphate in a crystallization process. A contaminating precipitate that was detected in the product was identified as poorly characterized crystals of the compound FeH₃(PO₄)₂•2CO(NH₂)₂. Homogeneous crystals of the compound were prepared and its properties were characterized. These fine-grained crystals precipitate when the iron content of the recycle urea phosphate mother liquor increases to ~0.7% iron (1.00% Fe₂O₃) and are retained in the purified urea phosphate product, thus limiting the effectiveness of the process. Tests have shown that increasing the acidity of the urea phosphate process with either phosphoric or sulfuric acid will increase the solubility of the iron salt, decrease the solubility of urea phosphate, and extend the useful life of the urea phosphate recycle liquor. Studies on the spent liquor from urea phosphate purification have shown that ammoniation to pH 6 will allow recovery of a highly purified liquid fertilizer of 14-12-0 grade and a solid fraction containing the impurities, including uranium and heavy metals, at a concentration level suitable for separation and purification of the individual components.

Precipitated Solids in Recycled Mother Liquor from Urea Phosphate Process. A poorly characterized crystalline material, first detected as a solid component in the urea phosphate purification process, has been pre-

pared as a homogeneous product from simulated urea phosphate mother liquor (Tennessee Valley Authority, 1978) to characterize its precise chemical and physical properties.

The original solids as precipitated from recycled mother liquor from the urea phosphate process were filtered, washed with acetone, and air-dried at ambient temperatures. The chemical analyses of solids from three different mother liquors made from commercial acids are presented

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Table I. Solids from Urea Phosphate Mother Liquor

sample	composition, wt %											
	total							CaO	citrate-insoluble			composition of water-insoluble P ₂ O ₅ , % of total
	N	P ₂ O ₅	Fe ₂ O ₃	Al ₂ O ₃	MgO	F	SO ₄		P ₂ O ₅	NH ₃ -N	biuret	
A	11.5	29.1	10.3	6.8	3.8	6.9	6.3	0.13	nil	0.5	1.1	58.1
B	12.2	33.3	17.0	4.6	2.2	5.3		0.11	nil	0.6	1.6	66.2
C	14.4	37.0	19.1	2.3	1.0	1.6						

Table II. Composition of Recycled Mother Liquor, Weight Percent

Al ₂ O ₃	Fe ₂ O ₃	MgO	F	SO ₄	urea N	P ₂ O ₅
3.0	1.6	1.0	1.6	6.8	7.0	26.0

Table III. Precipitates Formed in 7-26-0 Simulated Urea-H₃PO₄ Mother Liquor

sample	initial concentration, wt %					precipitate ^a
	Al ₂ O ₃	Fe ₂ O ₃	MgO	F		
1	3.0	1.6	1.0	1.6		A and B
2	5.0	3.0	2.0	3.0		A
3	3.0	5.0	2.0	3.0		A
4	3.0	5.0	0.0	3.0		A
5	3.0	5.0	2.0	6.0		A and C
6	5.0	0.0	0.0	3.0		NP
7	0.0	5.0	0.0	3.0		A and B
8	5.0	3.0	0.0	3.0		A
9	3.0	7.0	0.0	3.0		A
10	8.0	0.0	0.0	0.0		B
11	0.0	8.0	0.0	0.0		A
12	0.0	12.0	0.0	0.0		A

^a A = unknown crystalline material; B = CO(NH₂)₂·H₃PO₄; C = MgF₂; NP = no precipitate.

in Table I. The composition of a typical mother liquor when using one of these commercial acids (Florida acid) is listed in Table II.

Petrographic examinations showed that samples A, B, and C were not homogeneous, but sample C did comprise a bulk phase of an unknown, relatively water insoluble crystalline material along with a very minor contaminating phase of Ca₄SO₄AlSiF₁₃·12H₂O (chukhrovite). X-ray and infrared analyses also confirmed the presence of the unknown crystalline material in all three recovered samples. Infrared analysis detected the presence of urea nitrogen, which was of considerable interest because of the relatively water-insoluble nature of the compound, suggesting its possible utilization as a slow-release nitrogen fertilizer. Chemical analysis (Table I) of the solids confirmed that the phosphate is a water-insoluble form, with the water-insoluble P₂O₅ being 58.1 and 66.2% for the first two samples. Likewise, the nitrogen was confirmed as being the urea form; for example, at 11.5 and 12.2% total nitrogen, the respective urea nitrogen values are 9.9 and 10.0. Also, the data showed that the unknown was an iron-containing compound with ~19% Fe₂O₃ (sample C).

Since none of the recovered samples of the unknown were homogeneous, their conditions of formation were studied in an attempt to prepare the compound in pure form to obtain precise characterization data regarding their composition, optical properties, X-ray diffraction pattern, and infrared spectrum. The chemical analysis of a typical mother liquor from the urea phosphate process (Table II) was used as a starting point for a factorial study of impurity concentrations necessary for precipitation of the unknown compound. The levels of concentrations used in the factorial study and the precipitating phases are listed in Table III. The simulated liquors were prepared by

Table IV. Solids from Simulated Urea Phosphate Mother Liquor

sample	composition, wt %					
	total N	total P ₂ O ₅	Fe ₂ O ₃	Al ₂ O ₃	MgO	F
3	15.0	37.2	21.2	0.15	0.12	0.30
9	15.3	36.8	19.7	0.58		0.20
12	14.9	36.2	19.5			
theor for FeH ₃ (PO ₄) ₂ ·2CO(NH ₂) ₂	15.18	38.48	21.68			

Table V. X-ray Powder Diffraction Data for FeH₃(PO₄)₂·2CO(NH₂)₂^a

d, Å	I/I ^o	d, Å	I/I ^o
7.52	100	2.093	3
4.882	42	2.012	2
4.727	23	1.965	8
4.295	26	1.954	1
3.929	12	1.930	8
3.805	44	1.904	6
3.770	33	1.874	14
3.658	9	1.755	3
3.143	4	1.699	8
3.329	10	1.674	3
3.058	38	1.665	7
2.962	28	1.638	5
2.901	92	1.633	3
2.819	4	1.618	3
2.604	8	1.599	3
2.415	14	1.573	3
2.261	2	1.540	2
2.239	4	1.483	3
2.214	8	1.453	4
2.128	10		

^a Sample side was loaded with silicon powder (NBS No. 640) as the internal standard. Data obtained with X-ray diffractometer with Cu K α radiation, $\gamma = 1.54178$ Å. Intensities were measured as peak heights above background and expressed as percent of strongest line.

using reagent-grade phosphoric acid, urea, ferric sulfate, aluminum hydroxide, hydrofluoric acid, and magnesium carbonate. It was apparent from samples 11 and 12 in this factorial design that impurities other than ferric iron were not essential constituents of the new compound. Likewise, sample 10 showed that the aluminum end member, if it existed, could not be formed at concentrations of Al₂O₃ up to 8%.

Characterization Data. The chemical compositions of typical, well-crystallized, homogeneous precipitates of the unknown crystalline material obtained from this factorial study are given in Table IV. These compositional data do not indicate significant substitution of Al³⁺ for Fe³⁺ (<5 mol % in product 9). Furthermore, no significant variations were observed in the optical, infrared, and X-ray diffraction data obtained on these three products. Therefore, the formula FeH₃(PO₄)₂·2CO(NH₂)₂ was assigned to this unknown compound. A further effort to prepare an aluminum phosphate-urea adduct at aluminum concentrations as high as 12% Al₂O₃ was not successful.

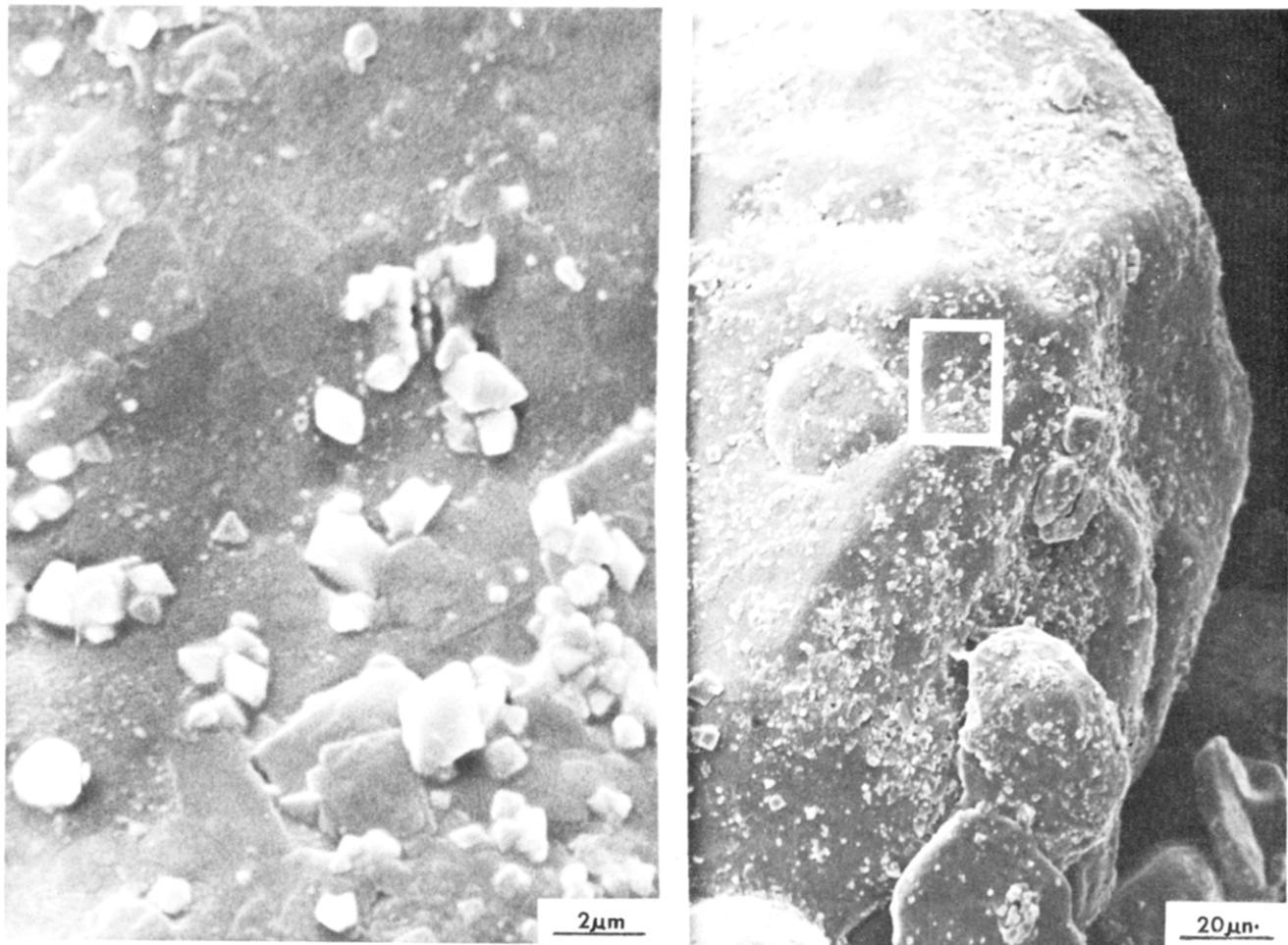


Figure 1. Fine-grained crystals of $\text{FeH}_3(\text{PO}_4)_2 \cdot 2\text{CO}(\text{NH}_2)_2$ on coarse $\text{CO}(\text{NH}_2)_2 \cdot \text{H}_3\text{PO}_4$ crystals.

Table VI. Infrared Absorption Spectrum of $\text{FeH}_3(\text{PO}_4)_2 \cdot 2\text{CO}(\text{NH}_2)_2$

cm^{-1}	I^a
3475	s
3420	s
3365	s
3325	s
3220	sh
1635	s
1588	sh
1475	w
1195	sh
1080	s
1010	s
920	m
758	w
585	w
525	s

^a s = strong; m = medium; w = weak; sh = shoulder.

The $\text{FeH}_3(\text{PO}_4)_2 \cdot 2\text{CO}(\text{NH}_2)_2$ crystallizes as small orthorhombic bipyramids exhibiting {111}. Optically, the crystals are biaxial (-), with $2V = 65^\circ$ (calcd) and refractive indexes $N_\alpha = 1.570$, $N_\beta = 1.625$, and $N_\gamma \sim 1.650$. The interfacial angles show that $a:b:c = 2.145:1:1.502$, where $a = Y$ and $b = X$. The density calculated by the Gladstone-Dale relationship is 2.10 g/cm^3 .

The X-ray powder diffraction data are shown in Table V, and the characteristic infrared absorption bands are listed in Table VI.

Significance in Urea Phosphate Process. The compound $\text{FeH}_3(\text{PO}_4)_2 \cdot 2\text{CO}(\text{NH}_2)_2$ has been detected not only in the recycled mother liquor from the urea phosphate

Table VII. Analysis of Liquid Phases in Equilibrium with $\text{FeH}_3(\text{PO}_4)_2 \cdot 2\text{CO}(\text{NH}_2)_2$

sample ^a	composition wt %, of Fe	pH
A	0.63	0.91
A + 4% H_3PO_4	0.70	0.75
A + 8% H_3PO_4	0.74	0.60
A + 12% H_3PO_4	0.73	0.50
A + 4% H_2SO_4	0.80	0.45
A + 8% H_2SO_4	0.92	0.19
A + 12% H_2SO_4	1.05	0.00
A + 4% $\text{CO}(\text{NH}_2)_2$	0.57	1.00
A + 8% $\text{CO}(\text{NH}_2)_2$	0.50	1.12
A + 12% $\text{CO}(\text{NH}_2)_2$	0.46	1.20

^a A = North Carolina byproduct mother liquor.

process but also as a significant contaminating phase in several pilot-plant urea phosphate products. The presence of this solid phase indicates that the centrifuging step to recover the coarse urea phosphate product leads to some "blinding" or partial retention of these fine-grained crystals. Figure 1 shows the fine-grained crystals of $\text{FeH}_3(\text{PO}_4)_2 \cdot 2\text{CO}(\text{NH}_2)_2$ on the coarser urea phosphate crystals.

In the factorial study to determine the parameters affecting the formation of $\text{FeH}_3(\text{PO}_4)_2 \cdot 2\text{CO}(\text{NH}_2)_2$ in simulated mother liquor of fixed P_2O_5 content, it was quickly apparent that an increase in iron increased the quantity of adduct that precipitated. However, it was observed that operation of the urea phosphate purification process at a higher P_2O_5 content in the recycled mother liquor would suppress the formation of $\text{FeH}_3(\text{PO}_4)_2 \cdot 2\text{CO}(\text{NH}_2)_2$. Thus, increasing the P_2O_5 concentration would extend the life

Table VIII. Byproduct Mother Liquor, Solids and Filtrate from Ammoniated Byproduct Mother Liquor, and Commercial Filter-Grade Acid

sample	composition, wt %											Mn	V	
	P ₂ O ₅	Al	Mg	Ca	Fe	Ni	Cr	U	Pb	Cd	Cu			Zn
byproduct mother liquor	22.2	0.45	1.09	0.04	1.40	0.0060	0.0420	0.0155				0.0886	0.0141	0.0079
solids from ammoniated	22.2	1.38	2.99	0.16	4.22	0.0171	0.1366	0.0454				0.2467	0.0399	0.0248
byproduct mother liquor	^a 39.7 ^b	7.3 ^a	9.3 ^a	1.2 ^a	8.4 ^a	9.5 ^a	10.4 ^a	9.1 ^a	^a		^a	9.5 ^a	8.5 ^a	8.9 ^a
filtrate from ammoniated	13.4	0.0008	0.026	0.001	0.004	0.00008	0.00026	94.8 ^b	^b		^b	86.0 ^b	97.8 ^b	98.9 ^b
byproduct mother liquor								0.0008				0.0124	0.00033	0.00009
filter-grade acid	26.76	0.19	0.32	0.13	0.50	0.0018	0.0131	0.0050	0.0			0.0259	0.0047	0.0028
byproduct mother liquor	22.96	1.19	0.54	0.022	1.61	0.0052	0.0165	0.0230	0.0006			0.0149	0.0779	0.0328
solids from ammoniated	23.43	4.54	1.99	0.040	6.23	0.0139	0.0677	0.0873				0.0597	0.3075	0.1333
byproduct mother liquor	^a 40.8 ^b	7.6 ^a	8.0 ^a	^a	8.5 ^a	9.3 ^a	15.7 ^a	6.6 ^a	^a		19 ^a	18.6 ^a	14.5 ^a	14.3 ^a
filtrate from ammoniated	13.59	0.006	0.048	0.001	0.013	0.0022	0.0004	80.9 ^b	^b		33.3 ^b	89.3 ^b	95.1 ^b	94.5 ^b
byproduct mother liquor								0.0044			0.0006	0.0016	0.0038	0.0018
filter-grade acid	28.46	0.60	0.25	0.48	0.73	0.0015	0.0043	0.0133	0.0002		<0.0001	0.0032	0.0212	0.0093
byproduct mother liquor	23.93	1.05	0.79	0.037	1.15	0.0228	0.0735	0.0187				0.1899	0.0146	0.1772
solids from ammoniated	26.72	3.30	2.12	0.10	3.28	0.0618	0.2362	0.0599	0.0023		0.0019	0.5367	0.0409	0.5145
byproduct mother liquor	^a 48.6 ^b	7.5 ^a	7.6 ^a	2.6 ^a	10.6 ^a	7.0 ^a	7.2 ^a	8.7 ^a	7.7 ^a		^a	7.4 ^a	5.9 ^a	8.1 ^a
filtrate from ammoniated	12.3	0.003	0.039	0.002	0.004	0.0008	0.0006	96.8 ^b	^b		95.8 ^b	91.1 ^b	98.6 ^b	99.1 ^b
byproduct mother liquor								0.0004	0.00009		0.00013	0.0169	0.0002	0.0016
filter-grade acid	28.6	0.44	0.28	0.038	0.31	0.0088	0.0327	0.0069	0.0003		0.0003	0.0721	0.0069	0.0635

^a Concentration effect of solid fraction compared to that of filter-grade acid. ^b Percent constituent (originally present in byproduct mother liquor) in solid fraction.

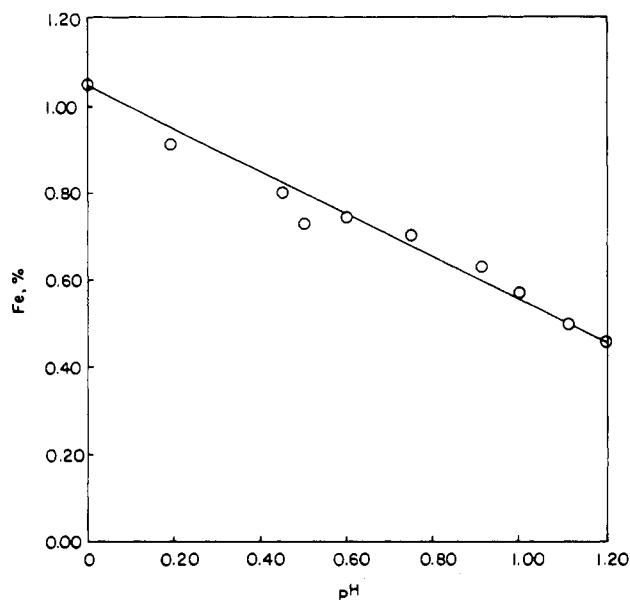


Figure 2. Relationship of percent Fe vs. pH in North Carolina byproduct mother liquor.

of the recycled mother liquor by allowing a buildup in iron content before reaching saturation and the onset of precipitation of $\text{FeH}_3(\text{PO}_4)_2 \cdot 2\text{CO}(\text{NH}_2)_2$ in the urea phosphate process.

In a further search of factors affecting the solubility of $\text{FeH}_3(\text{PO}_4)_2 \cdot 2\text{CO}(\text{NH}_2)_2$, a study was conducted using a byproduct mother liquor recovered from a batch crystallization of urea phosphate from urea and North Carolina green acid, which was saturated with respect to $\text{FeH}_3(\text{PO}_4)_2 \cdot 2\text{CO}(\text{NH}_2)_2$. Reagent-grade H_3PO_4 , $\text{CO}(\text{NH}_2)_2$, and H_2SO_4 were added at different concentration levels to aliquots of the byproduct mother liquor and then equilibrated at room temperature for 2 weeks. The compound $\text{FeH}_3(\text{PO}_4)_2 \cdot 2\text{CO}(\text{NH}_2)_2$ was maintained as the equilibrating solid phase during this time period. The filtrate was analyzed for Fe, and the results are shown in Table VII. It is apparent that H_2SO_4 has a greater effect on the solubility of $\text{FeH}_3(\text{PO}_4)_2 \cdot 2\text{CO}(\text{NH}_2)_2$ than does $\text{CO}(\text{NH}_2)_2$ or H_3PO_4 . A plot of the iron concentration vs. pH in the filtrate of the North Carolina byproduct mother liquor (Figure 2) shows a linear relationship, as represented by the equation

$$\text{wt \% Fe} = -0.456(\text{pH}) + 1.0167 \quad (1)$$

which has a correlation coefficient of 0.98. The corresponding equations for other byproduct mother liquors produced from different wet-process phosphoric acids should be similar to eq 1.

The increase in solubility of $\text{FeH}_3(\text{PO}_4)_2 \cdot 2\text{CO}(\text{NH}_2)_2$ is not the only advantage gained by adding H_2SO_4 to the urea phosphate process. Vidzhesingh et al. (1976) show that the solubility of urea phosphate in the system $\text{CO}(\text{NH}_2)_2\text{-H}_3\text{PO}_4\text{-H}_2\text{O}$ is also reduced significantly by adding H_2SO_4 . The region of $\text{CO}(\text{NH}_2)_2$ crystallization decreases, while the region of $\text{CO}(\text{NH}_2)_2 \cdot \text{H}_3\text{PO}_4$ crystallization increases. The influence of H_2SO_4 is strongest in the range of urea concentrations from 10 to 55%. For example, in an equilibrium-saturated solution at 20% $\text{CO}(\text{NH}_2)_2$, the H_3PO_4 content is 31% in the absence of sulfuric acid but decreases to 29.5% in the presence of 4% H_2SO_4 , and 27.5% in the presence of 5.5% H_2SO_4 . At 40% $\text{CO}(\text{NH}_2)_2$ the corresponding H_3PO_4 contents in the equilibrium solutions are 25.2, 23.5, and 21.5%, respectively.

A third advantage of adding H_2SO_4 to the urea phosphate process would be that the extra heat generated upon

ammoniation of the byproduct mother liquor would help in drying and granulation of the fertilizer product.

Analysis of Byproduct Mother Liquor from Urea Phosphate Process. Impurities that have not been reported in previous urea phosphate studies are the heavy metals and uranium. However, due to interest in the potential recovery of uranium and vanadium as byproducts in the wet-process phosphoric acid industry, a study was made of uranium and heavy metal concentrations in urea phosphate and urea phosphate byproduct mother liquors. Urea phosphate crystallized from a typical central Florida acid contained 40 ppm of U, whereas the byproduct mother liquor from this urea phosphate contained 200 ppm of U. For comparison, a 30% filter-grade acid from central Florida contains 115 ppm of U. Thus, extraction and recovery of the more highly concentrated uranium values in the byproduct liquor stream appear attractive.

Use of the byproduct mother liquor to produce a suspension or granular fertilizer may, however, raise environmental issues, particularly with western phosphoric acids where the Cd content is increased from 53 ppm in the filter-grade acid to 170 ppm in the byproduct mother liquor, the V content from 635 to 1772 ppm, the Cr content from 327 to 735 ppm, and the Ni content from 88 to 228 ppm. Urea phosphate crystallized from a typical western acid contains 31 ppm of Cd, 182 ppm of V, 94 ppm of Cr, and 23 ppm of Ni. From these analyses it is evident that the urea phosphate process is an excellent purification process for removing these potential environmental pollutants from the feed wet-process phosphoric acid in the production of urea phosphate. This could become a viable process for western producers, provided that the increased concentrations of heavy metals in the byproduct mother liquor were at acceptable levels in the final fertilizer products.

Previous Tennessee Valley Authority research by Potts et al. (1962) showed that by proper control of ammoniation of filter- or merchant-grade acid, a clear liquid fertilizer and a filter cake or slurry which contains the precipitated impurities could be produced without major filtration problems. Since Fe, Al, and Mg are essentially insoluble in ammoniated wet-process orthophosphoric acid (Akiyama and Ando, 1972), ammoniations of urea phosphate byproduct mother liquors were conducted to determine solubilities of uranium and heavy metals in the filtrate and filter cake. Table VIII lists the compositions of the original filter-grade acid, the byproduct mother liquor, the solids from ammoniated byproduct mother liquor, the filtrate from ammoniated byproduct mother liquor (pH 6.1), and the concentration effect of the solid fraction (compared to the original filter-grade acid) and the percent of each constituent from the byproduct mother liquor that is present in the solid fraction. These data show that the solid fraction contains 80–100% of the impurities, with a concentration effect of 7–10 times the impurity levels found in the original filter-grade acid. The liquid fraction produces a highly purified liquid fertilizer of approximately 14–12–0 grade, suitable as a starter fertilizer product or for mixing with UAN solutions. The solid fraction recovered by this purification step appears to provide an excellent source for recovery of metals such as Ni, Cr, Cd, Zn, Mn, V, and U, since they occur in a much more highly concentrated state than in the original wet-process phosphoric acid.

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Fatty Acids and Sterols Associated with Citrus Root Mycorrhizae

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The structures of four fatty acids present in citrus roots infected with VA-mycorrhizal fungus but not in noninfected roots were shown to be 11c-16:1, 6c,9c,12c-18:3, 8c,11c,14c-20:3, and 5c,8c,11c,14c-20:4. Compositional differences of these four acids between seedlings infected with three *Glomus* species were slight. These acids were present in the triglycerides and to a lesser extent in three phospholipids and one glycolipid of the fibrous roots. They were absent in the tap roots and leaves. Supplemental phosphorus failed to produce these acids in seedlings grown in noninoculated soil. Levels of these acids were lower in mature trees than in seedlings. Lipids from *Glomus mosseae* chlamydospores contained 11c-16:1 as well as a 20:5 fatty acid and almost exclusively a 24-methylcholesterol in its free sterol fraction.

Mycorrhizal fungi have a growth-stimulating effect on citrus seedlings (Marx et al., 1971; Kleinschmidt and Gerdemann, 1972; Nemeč, 1978) by enhancing nutrient uptake (Gerdemann, 1968). When these endomycorrhizal fungi are killed by heat treating or fumigating the soil in which the citrus seedlings are grown, stunting and chlorosis of the plants result. These effects are most pronounced in phosphorus-deficient soils. Lipids have been shown by microscopic examination (Cox and Sanders, 1974; Cox et al., 1975) and by solvent extraction (Cooper and Losel, 1978) to be more abundant in mycorrhizal-infected than in noninfected fibrous roots. When labeled lipid precursors were fed to onion seedlings, greater amounts of the label were observed in the infected roots (Losel and Cooper, 1979). Many hypotheses have been made as to the role lipids play in this growth stimulation (Cox et al., 1975; Harley, 1975). Citrus roots infected with *Glomus mosseae* were shown to contain three fatty acids that are unusual to higher plant material and which constituted over 40% of the total fatty acids (Nagy et al., 1980); a fourth unusual acid not reported with those three was present at less than 0.5%. These four acids, however, were not detected in control citrus roots. The acids were concentrated to the greatest extent (46-52%) in triglycerides but were also found in the glyco-polar lipids (7-20%). Also significant was the finding that campesterol/free demethyl sterol ratios were higher for the infected citrus roots than for the noninfected. For possible clarification of the role lipids, and specifically the role these unusual fatty acids play in this growth-stimulating effect of mycorrhizal fungi, a series of experiments were designed to answer the following

questions: (1) What are the structures of these unusual fatty acids? (2) Are these acids, as well as sterols, present in the fungus itself? (3) Are these acids found in roots infected with mycorrhizal fungi other than *G. mosseae*? (4) Are these acids found in roots of citrus seedlings grown in sterile soil supplemented with superphosphate to the extent that the growth of the seedlings is comparable to that of the infected seedlings? (5) Are these acids limited to the arbuscules, vesicles, and intercellular and external hyphae regions of the fibrous roots (Cox and Sanders, 1974; Cox et al., 1975) or may they be present in other tissues, such as the tap roots or leaves of the infected plant? (6) Are these acids preferentially found in certain phospholipids or glycolipids? (7) Are these acids found in roots of citrus plants in later stages of growth, e.g., 2 and 9 years of age?

EXPERIMENTAL SECTION

Preparation of Plant and Fungal Material. *Experiment 1.* Lipids from which concentrates of the four unusual fatty acids were prepared were from our first study (Nagy et al., 1980) and from tissues obtained from experiments 3-7 of this study.

Experiment 2. Individual chlamydospores of *G. mosseae* from the Sudan grass host (Nemeč, 1978) were removed under microscopic examination with tweezers and stored in a bottle of ethanol-benzene (1:4 v/v) under refrigeration.

Experiment 3. The three rootstocks used in this study were Rangpur lime (*Citrus reticulata* var. *austera* Swing.), sweet orange (*Citrus sinensis* L. Osb.), and sour orange (*Citrus aurantium* L.). Plants were germinated in a greenhouse from seeds planted in flats of steamed Astatula fine sand subsoil low in phosphorus (<20 ppm). Methods for fertilization, inoculation with *Glomus fasciculatus*, and transplantation were previously detailed (Nemeč, 1978). The seedlings were 186 days old when the fibrous roots were excised.

Experiment 4. Sour orange plants were grown as in experiment 3 but were inoculated with *Glomus etunicatus*. The noninoculated citrus plants were grown in soil en-

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