centages of the normal growth of the primary root in a water control. The phytotoxic activity of 1-alkylpyridinium bromides rises to a maximum around an alkyl chain length of 12 to 14. Lo Cicero *et al.* (*4*) found that the fungitoxicity of a related series of alkylpyridinium chlorides rose to a similar maximum at C_{14}. Examination of Table I indicates that branching of the alkyl chain does not have much effect on phytotoxicity. When the substituent on the pyridine nitrogen atom becomes an alkylbenzyl group, activity is still retained. For example, 1-(2,4-diisopropylbenzyl)-pyridinium chloride (C_{18}) has the same order of activity as 1-dodecylpyridinium bromide (C_{12}). Substituting alkyl groups for hydrogen around the heterocyclic ring does not seem to affect activity appreciably. Pyridine or alkylpyridines are not necessary for activity as shown by the phytotoxicity exhibited—e.g., by tri-(*n*-amyl)-3,5,5-trimethylhexylammonium bromide and similar quaternary salts.

The quaternary salts are nonselective in their phytotoxic action towards cucumber and wheat seeds, as evidenced by the data of Table I.

Table I (Continued)

	Percentage Root Growth			
	100 p.p.m.	10 p.p.m.	100 p.p.m.	10 p.p.m.
	Cucumber		Wheat	
Ammonium Bromide				
Phenyldimethyl-*p*-*sec*-amylbenzyl[c]	7	32	10	46
Tris-(β-hydroxyethyl)-*p*-*sec*-amylbenzyl[c]	27	78	41	77
Tris-(β-hydroxypropyl)-*p*-*sec*-amylbenzyl[c]	54	100	71	92
Dimethyl-β-hydroxyethyl-4,5,5-trimethylhexyl	21	79	12	79
Trimethyl-*p*-isopropylbenzyl-	50	84	32	58
Trimethyl-*p*-*sec*-amylbenzyl-	14	40	5	23
Others				
1-(3,5,5-Trimethylhexyl)-quinolinium bromide	7	48	4	51
2-(3,5,5-Trimethylhexyl)-isoquinolinium bromide	8	45	6	43
3-(3,5,5-Trimethylhexyl)-benzothiazolium bromide	58	86	47	102
1-(*p*-*sec*-Amylbenzyl)-quinolinium chloride	4	43	12	61

[a] *p*-Toluenesulfonate instead of bromide. [b] Iodide instead of bromide. [c] Chloride instead of bromide.

Experimental

Procedure for Herbicidal Assay. The method is substantially that described by Thompson *et al.* (*9*) with the modifications noted by Schlesinger and Mowry (*8*). Where compounds were tested with wheat, the procedure was the same except for substitution of wheat seeds for cucumber seeds in the Petri dishes. The data obtained are listed in Table I.

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MEASUREMENT OF PLANT PECTIC SUBSTANCES

Reaction of Hydroxylamine with Pectinic Acids. Chemical Studies and Histochemical Estimation of the Degree of Esterification of Pectic Substances in Fruit

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The reaction of the carbomethoxyls of pectinic acids with hydroxylamine in aqueous-alcoholic solutions was studied. The rate and extent of formation of pectin hydroxamic acid were followed by developing the colored spots of the ferric-pectin hydroxamic complex on filter paper strips and measuring the reflection densities. These series of reactions were applied to measure the degree of esterification of pectic substances in fruit tissue sections. This procedure introduces a new direct method for following the esterification of pectic substances in fruits at different stages of their development.

THE REACTION of the carbomethoxy groups of pectin with alkaline hydroxylamine produces pectin hydroxamic acids which react with ferric ion to form insoluble red complexes and has been used as the basis for a qualitiative histochemical test for pectic substances in tissue sections (*12*). When the reactions were conducted in an aqueous-alcoholic suspension they appeared to offer a quantitative means of determining directly the degree of esterification of pectic substances in tissue sections. The experiments reported here show some of the variables involved in the course of the reactions to produce pectin hydroxamic acids and the validity of reflection density measurements for the determination of the degree of esterification of pectic substances.

The degree of esterification is considered important in determining the solubility properties of pectins and, consequently, in understanding their probable role in fruit texture. Pectic substances extracted from fruits are known to have from 60 to 80% of their carboxyl groups esterified with methanol. The per cent esterification found in extracted pectins, however, depends

upon the condition and kind of fruit as well as on the procedures used for extraction and purification. Less than 50 to about 70% of the pectic substances can be extracted from most fruits (*8*, *10*). Conclusions regarding relationships between fruit texture and the solubility or chemical nature of the extracted pectins thus far have been based on only a fraction of the pectic substances present in a particular fruit.

Specific information is required on the locus and properties of the pectic substances as they exist in plant tissues. The degree of esterification of pectic substances in tissue sections of individual fruits of apples, peaches, and pears during growth and ripening was determined using this quantitative series of reactions.

Chemical Studies

Methods of Analyses. The titration and saponification of the citrus pectinic acids were conducted essentially as described (*14*). Free carboxyl groups were determined by titrating 50 ml. of a solution containing 0.200 gram of pectinic acid with 0.1*N* sodium hydroxide to a pH of 7.5. Esterified carboxyl groups were saponified in 30 minutes with 10 ml. of 0.5*N* sodium hydroxide. An equivalent of 10 ml. of 0.5*N* hydrochloric acid was added and final titration to pH 7.5 was made with 0.1*N* sodium hydroxide.

The total carboxyl content was the sum of those free plus those esterified. The value of $\frac{\text{esterified carboxyls}}{\text{total carboxyls}} \times 100 = \%$ esterification.

Pectinic Acids. Commercial citrus pectin (Pectinum NF 10 obtained from the Sunkist Growers, Ontario, Calif.), essentially free of acetyl groups, was the source material for preparation of samples of different per cent esterification.

Preparation of pectin of 25% esterification was made from a 1% solution of citrus pectin by treatment with sodium hydroxide at 15° C. at pH 11 for about 15 minutes (*11*). Then it was acidified with hydrochloric acid to a pH of 1 and precipitated in 70% ethyl alcohol. The wet, precipitated pectinic acid was washed and pressed several times with 60% aqueous ethyl alcohol until a negative test for chloride was obtained. The moist pectinic acid was washed once with 95% ethyl alcohol, dried in air, ground to pass 40 mesh, and analyzed.

Diazomethane esterified pectin was made as follows: A 1% solution of citrus pectin was acidified with hydrochloric acid to pH 1 and precipitated in 70% ethyl alcohol. The wet pectin was pressed and washed in 60% ethyl alcohol until the washings were free of chloride. Then it was washed with absolute ethyl alcohol and absolute methanol. The material was suspended in absolute methanol and cooled in a dry ice–ethyl alcohol bath to −20° C. and the suspension was treated with an excess of ethereal diazomethane (*16*). After 30 minutes the excess diazomethane and ether were allowed to evaporate. The product was washed once with methanol, pressed, and dried in air.

All the pectinic acid samples were ground to pass 40 mesh and were equilibrated to the same moisture content. They contained <0.1% ash.

Alkali-de-esterified, diazomethane-esterified, and the starting pectinic acids were 25, 94, and 71% esterified, respectively, as determined by titration.

Preparation of Pectin Hydroxamic Acid. A solution of 13 grams of potassium hydroxide pellets in 25 ml. of methanol was added to a solution of 12 grams of hydroxylamine hydrochloride dissolved in 60 ml. of methanol. The insoluble salts were removed by filtration and the filtrate added to a suspension of 8 grams of freshly prepared diazomethane-esterified pectinic acid in 500 ml. of methanol. The suspension was shaken continuously at 25° C. Hydroxylamine was added from time to time to ensure an excess of reagent. At time intervals of 7 minutes and 1, 6, 23, and 47 hours, samples were removed by filtration, and washed with acidified methanolic hydrochloric acid and with 60% aqueous methanol, until a negative chloride test with silver nitrate was obtained on the washings. The samples were given a final washing with absolute methanol, air dried, ground, and humidified to 10% moisture. The extent of conversion of the carbomethoxyls to hydroxamic acid was calculated from the decrease of the total free and esterified carboxyl groups. Pectin hydroxamic acids did not titrate at pH 7.5 and hence the titration method of analysis measured only free and esterified carboxyls. The degree of conversion as calculated by titration agreed with that from the increase in nitrogen. Hydroxamic acid nitrogen was determined by a Kjeldahl-iron digestion method (*1*).

Figure 1 shows the course and extent of reaction beyond 40 hours of reaction time under these conditions. The initial phase of the reaction is very rapid, but competing alkaline hydrolysis of the ester groups occurs and esters are not usually converted quantitatively to hydroxamic acids (*9*).

Reagents and Procedure

A. Fourteen grams of crystalline hydroxylamine hydrochloride were dissolved in 100 ml. of 60% aqueous ethyl alcohol.

B. Sodium hydroxide, 14 grams, was dissolved in 100 ml. of 60% aqueous ethyl alcohol.

C. One part of concentrated hydrochloric acid (specific gravity 1.188) was mixed with 2 parts by volume of 95% aqueous ethyl alcohol.

D. Two and one-half grams of ferric chloride lumps were dissolved in 100 ml. of 0.1*N* hydrochloric acid in 60% aqueous ethyl alcohol.

A sample of citrus pectin that had been converted to the hydroxamic acid to an extent of 34% was dissolved to make aqueous solutions of 0.25, 0.5. 1.0, 2.0, and 4.0% concentration. Ten-microliter spots were applied to Whatman No. 1 paper strips and then air dried. The color was developed by dipping the paper into the 2.5% solution of ferric chloride in 0.1*N* hydrochloric acid–60% ethyl alcohol. After 2 minutes the moist papers with the red spots of ferric hydroxamic acid complex were measured by determining the reflection density using a 515-mμ filter in the reflectance meter (Photovolt No. 501A with reflectance head No. 53). The reflection density values are the averages of four readings per spot on each of three replicate spots for each concentration. The mean reflection density values from 0.072 to 0.97, as absorbance readings, had an average standard deviation of 0.023. The relation between the reflection density reading (on the absorbance scale) and concentration of pectin hydroxamic acid is linear.

Per Cent Esterification from Reflection Density Readings. Solutions of pectinic acids of 25, 71, and 94% esterification were prepared to contain 1% of moisture-free anhydrouronic acid based upon titration of the free and esterified carboxyl groups. The total carboxyl content (free and esterified) per gram of commercial pectin was 5 meq. The carboxyl equivalent weight was 200 and the anhydrouronic content was therefore, $\frac{176 \times 100}{200}$, or 88%. The amount of material required to prepare 100 ml. of solution of 1% anhydrouronic acid was $\frac{100}{88}$, or 1.1137 grams.

A 2 × 7 cm. strip of Whatman No. 1 filter paper was passed rapidly through a 1% solution of the pectinic acid to be tested, blotted on a sheet of blotting paper, and then dehydrated by immersing it in 95% ethyl alcohol for 30 minutes. The strip was then placed in 10 ml. of the alkaline hydroxylamine reagent, prepared by mixing equal volumes of reagents A and B. After 7 minutes of reaction time the hydroxylamine reagent was acidified with 10 ml. of reagent C. The paper strip was then immersed in 10 ml. of the 2.5% ferric chloride reagent and after 2 minutes it was removed and mounted, in the moist condition, under a coverglass on a microslide. The average reflection density was obtained as described. The per cent esterification was calculated from the reflection density values as compared with the 94% diazomethane esterified standard by dividing the reading of the pectin sample by the reading for the 94% esterified sample and multiplying the quotient by

94. Values from the reflectance method on the two other pectinic acids of known constitution agreed with those determined by titration (Table I).

Discussion

The mechanism of the reaction of hydroxylamine with esters to produce hydroxamic acids is complex (*17*) and it may be further complicated in these tests because of the solubility properties of the polyester, pectinic acid.

The reactions of alkaline hydroxylamine with esters or lactones do not usually proceed to completion, but under selected conditions these compounds have commonly been determined quantitatively, by their reactions to form soluble, colored ferric hydroxamic acid complexes (*2, 3–5, 7, 9, 15*). Although less than 25% of the ester groups of the pectinic acids react with the alkaline hydroxylamine in 7 minutes under the reaction conditions used here, the series of reactions and measurements of maximum reflection densities are reproducible. Kaye and Kent (*7*) used the hydroxamic acid reaction, similarly, to estimate the total uronic acid content of some complex polysaccharides. These authors found the values, obtained from the hydroxamic acid reaction, in agreement with those obtained titrimetrically.

The competing reactions of the alkaline hydrolysis of the ester groups and the formation of hydroxamic acid with hydroxylamine explain the low degree of substitution in pectinic acids.

Although the extent of substitution is lower than desirable in the case of the pectic hydroxamic acids, the reactions are reproducible and proceed in proportion to the per cent esterification of the pectinic acid.

The pectin used here was free of acetyl groups, but some fruit pectins contain varying amounts, depending upon the source. Acetyl esters react with hydroxylamine to form soluble acetohydroxamic acid (*9*) which can be easily washed out of plant material. It has no tendency to adsorb on the insoluble cellular material (*12*). The test should be specific for the degree of esterification of pectin in plant material even in the presence of other ester groups.

Histochemical Estimation of Esterification of Pectic Substances in Fruits

Preparation of Sections. Rectangular blocks of tissue about 3/4 inch wide were cut from opposite sides of full-sized green and ripe fruit, proceeding from the skin to the center of the fruit at its greatest circumference. They were clamped in the specimen holder of a sliding microtome in a position to permit cutting of radial sections. Smaller and immature fruits were trimmed to fit similarly into the specimen holder and cheek sides of the fruit were used for peaches. Uniformly thick sections of

Table I. Per Cent Esterification of Pectinic Acids by Titration and Reflection

Pectinic Acid	Esterification % Titration	Esterification % Reflection
Commercial pectin (purified)	71	70
Alkali-de-esterified	25	27
Diazomethane-esterified	94	..

280 or 320 microns were cut from mature, ripe peaches by mechanically advancing the specimen holder after each section was cut. For immature peaches, sections of 200 microns in thickness were cut and, for very small fruit, sections of 120 microns in thickness were obtained.

Radial, rather than tangential sections were used, because in ripening peaches, the first tissue softening and ripening begins near the pits. Also, toward the pits of peaches and cores of apples and pears, the flesh parenchyma cells are elongated radially and present a structural condition different from that of the more spherical cells in the middle to the outer flesh regions. Test values representative of these different conditions can be obtained from a single radial section by taking five readings in each of the outer and inner flesh regions of the section.

The large intercellular spaces in apples required sections 400 to 500 microns thick. The desirable thickness of the section was based in part on the cell size in a given sample and on the convenience of manipulation in subsequent tests. Sometimes sections of a particular thickness could not be used because the ferric-hydroxamic acid color was too intense and gave reflection density measurements beyond the range of sensitivity desirable for these experiments.

After each section was cut, both the knife and the tissue block were flooded with water containing about 300 p.p.m. of sulfur dioxide. The cut section was removed with a brush and placed immediately in acetone. Sections from opposite and apparently greener and riper sides of individual fruits were dehydrated and stored separately in acetone at room temperature, which served to inactivate enzymes and extract most of the coloring matter. They were left so stored until ready for testing. All sections used for testing or further treatment were washed thoroughly three times with methanol to remove the acetone. Those receiving esterification treatments were removed and washed first. When these had been esterified and were ready for testing, replicate sections were removed, washed, and tagged by cutting tiny notches in the margins. Each series of tests for color development in a given fruit was then made simultaneously in the same container.

De-esterified Control Sections. Several acetone-free sections from each lot were soaked in 5 ml. of the 14% sodium hydroxide solution for 5 minutes, briefly rinsed in 60% ethyl alcohol, and then transferred with a section lifter to a Petri dish containing 10 ml. of the alkaline hydroxylamine reagent and treated as described for test sections.

Completely Esterified Sections. Ten or more acetone-free sections were stored overnight in 0.5*N* hydrochloric acid in dry methanol to fully esterify their pectic substances (*6*). After this treatment the sections were washed with methanol and tested for development of the ferric hydroxamic acid color.

Development and Measurement of Color in Test Sections. Five milliliters each of the hydroxylamine reagent and sodium hydroxide solution were mixed in a Petri dish and the acetone-free sections were placed in this mixture and gently stirred for 5 minutes along with an equal number of sections which had been fully esterified. Five milliliters of the 1 part of hydrochloric acid to 2 parts of 95% ethyl alcohol were then added and thoroughly mixed with the alkaline hydroxylamine. After the sections were gently stirred for 5 minutes in the acidified mixture, they were transferred with a section lifter to another Petri dish containing 15 ml. of 2.5% ferric chloride solution. Five to 10 minutes were allowed for the red color to develop. Then the sections were transferred singly to one end of a microslide and a cover glass was placed carefully to avoid entrapping air bubbles. Reflection density measurements were then made after properly placing the mounted section under the reflectance unit. When one section was so measured, the next was removed from the ferric chloride solution and measured.

The instrument was first set at zero absorbance, using a square of white paper on a microslide, under a cover glass. An average of 10 readings of the uncolored cell walls, in a control section, was determined. This average was then adjusted to zero as a basis for determining the reflection densities of color in the test sections. Ten readings each were made on five or more sections in each lot of esterified and non-esterified sections. Care was taken to avoid measurements of reflectance density in section areas containing vascular bundles which gave abnormally high values—i.e., only parenchyma tissue areas between vascular bundles were measured. Most of the readings were made in the range up to 0.7 on the absorbance scale of the photometer, depending on section thickness. From mean values of 0.23 to 0.77, as absorbance, the standard deviation was 0.032.

Microphotometric methods for measuring color intensities of stains and

chemical reactions in tissue sections have been applied directly at high microscopic magnifications of local intracellular areas (*13*). Such techniques might be useful in studies on the distribution of cell wall substances in the wall layers of different plant tissues and in thin sections. The large flesh parenchyma cells of fruits, however, require thick sections, and over all color reactions for esterification are best measured by the type of instrument chosen.

In this histochemical test procedure, a reaction time of the sections with alkaline hydroxlamine of five minutes is recommended. The reaction of only 25% of the ester groups with hydroxylamine occurred in 5 minutes, but when both the completely esterified and the test sections react simultaneously with exact timing in the same container, critical variations in the percentage of reacted ester groups are avoided. Replicate sections reacted for 1 hour gave essentially the same values for the per cent esterification as those obtained with sections carefully reacted for 5 minutes.

The rate of reaction to form pectin hydroxamic acid from citrus pectin was shown earlier in this paper. It appears that the reaction proceeds at a rapid rate for the first hour and then the rate of conversion of the ester groups to hydroxamic acid becomes slow. Competing reactions such as saponification of the ester groups and destruction of hydroxylamine in alkaline solutions occur and account for the incomplete substitution of ester carboxyls to hydroxamic acids. Sections, 2 × 4 cm., of Newtown Pippin apples 320 microns thick were dehydrated and stored overnight in acetone. After removal of acetone by repeated washing with methanol, the sections were reacted for 7 minutes and for 1, 6, and 24 hours. The alkaline hydroxylamine was replenished with freshly prepared reagent to ensure the presence of hydroxylamine. The sections were then removed and tested as described.

The course of the reaction of pectinic acid *in situ* up to 24 hours, under the conditions of this procedure, closely paralleled the results obtained with citrus pectin shown in Figure 1 of this paper.

Degree of Esterification. The quantitative test for the degree of esterification *in situ* depends upon the differences of the reflection density readings of the completely esterified sections, and of the test sections as compared with those of the de-esterified or control sections, respectively. Thus, the average reflection density of the test sections divided by the average obtained for the fully esterified sections times 100 is the per cent esterification. The assumption is made that free and esterified carboxyl groups present in the insoluble portion of the aqueous alcoholic medium of the reaction mixture are those associated with pectic substances.

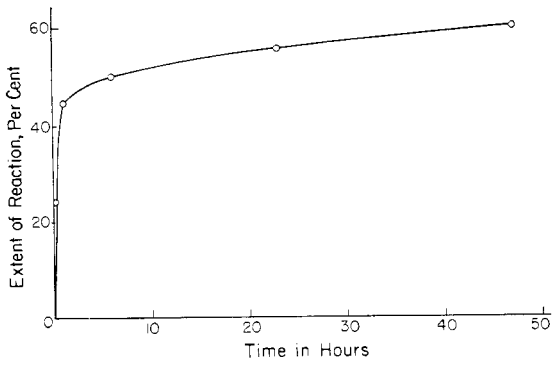

Figure 1. Rate of formation of hydroxamic acid from diazomethane-esterified citrus pectin

Table II. Degree of Esterification of Pectic Substances in Peaches

Variety	Esterification %: Green immature	Green mature	Ripening	Hard ripe	Firm ripe	Soft ripe
Freestone						
Early Elberta	81	86	96	97	85	...
Elberta	81	82	93	96	86	...
Nectar	81	85	98	96	84	58
Krummel	...	86	88	96	91	66[a]
Clingstone[b]						
Fortuna	...	85	91	99	88	...
Halford	80	86	95	102	90	...
Phillips	...	87	94	100	88	...

[a] Soft ripe fruits of the Krummel, a late variety, very mealy, 440-micron sections used.
[b] These varieties never reached the degree of softness exhibited by freestone varieties.

Results

Table II summarizes the results obtained (for 5 minutes of reaction time to form pectin hydroxamic acid) with several varieties of peaches during growth and ripening. The peaches were sampled from the same trees and the same locations on each tree at successive dates. Each value listed is the mean of 10 reflection density readings per section for five or more sections from each of two or three fruits. From mean absorbance readings of 0.23 to 0.77 the standard deviation is 0.032. The degree of esterification does not increase until the fruit is approaching full size. With the onset of ripening esterification increases to virtually 100%, and then decreases as the fruit softens. McCready and McComb (*10*) reported like results on extracted pectin of ripe Elberta peaches, but their value of 89% is lower than that obtained histochemically in the present study for hard, ripe fruit.

Results obtained on green and ripening apples and pears are listed in Table II. The degree of esterification as obtained histochemically increased to nearly 100% with the onset of ripening, but decreased markedly as ripening progressed and the fruit became softer. No pronounced differences were found in sections from opposite sides of the same fruit for either apples or peaches of superficially uneven ripeness.

Pectin and pectic acid in alcoholic suspension are difficult to esterify completely with dry hydrochloric acid in methanol (*6*). The pectic substances esterified *in situ* in chilled diazomethane and in dry methanolic hydrochloric acid appear to be completely esterified. They gave identical reflection density averages in all sections so prepared and tested. Replicate sections esterified for 20 minutes in hot methanolic hydrochloric acid gave the same test readings as those esterified at room temperature overnight. Likewise, replicate sections esterified for between 12 and 36 hours at room temperature in methanolic hydrochloric acid showed no increases in the intensity of the test color reaction.

Table III. Esterification of Pectic Substances in Apples and Pears

Fruit and Maturity	Esterification, %
Gravenstein apple	
Green, flesh hard, succulent	92
Coloring, flesh hard, succulent	96
Ripe, flesh firm, succulent	95
Ripe, flesh soft, slightly mealy	73
Golden Delicious apple	
Green, immature, 2-inch diameter	76
Green, immature, 2 3/4-inch diameter	81
Green, mature, flesh hard	84
Ripening, flesh hard, succulent	95
Ripe, flesh firm, succulent	93
Ripe, slightly mealy	85
Bartlett pears	
Green, immature, not juicy	85
Green, mature flesh hard, juicy	95
Ripe, firm to hard succulent	77
Ripe, soft and juicy	64

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FEED TECHNOLOGY

Handling Phosphoric Acid in Formula Feeds

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Phosphoric acid has proved acceptable as a nutrient in animal rations, but its handling in milling equipment has been questioned, because of its corrosive nature. A technique was developed for distributing acid uniformly throughout a commercial feed. Corrosion was observed as weight losses in mild steel strips secured to mixing, conveying, and pelleting equipment. Initially those areas in the mixer subjected most to abrasion were also affected most by corrosion from acid-containing feeds; however, areas of little friction eventually also showed considerable corrosion. With low-level (1.25%) acid addition, test strips showed weight changes normally suitable for materials used in feed milling equipment, except in the pellet mill conditioner chamber. When mixing high levels of acid (up to 35%) with limestone and soybean oil meal to form a dry premix, it was evident that the mixer should be made of resistant material. Once mixed, high-level acid carriers were used as an ingredient in feeds with no change in processing from the basal ration.

OF THE 37,000,000 TONS of formula feeds manufactured annually in this country, it is estimated (*1*) that about 1.8% are inorganic phosphorus additives. This is equivalent to 173,000,000 pounds of the element phosphorus. Defluorinated domestic rock phosphates, bone meal, colloidal phosphates, imported low-fluorine rock phosphates, and other phosphorus-containing materials are used to furnish the essential nutrient.

Phosphate supplements are commonly supplied as a dry material, in 50- or 100-pound bags with attendant problems of container disposal and material loss from breakage and incomplete emptying. Bulk handling is successfully used by some feed manufacturers and may be more generally accepted if attention is given to producing a granular product containing little dust. Liquid phosphorus supplements, as are used by the soft drink industry, have been proposed for formula feeds. The economic aspects both as to unit phosphate cost and handling cost have been covered by Maddy (*3*). The questions of nutritive value and availability have been answered for poultry by Titus (*4*) and for livestock by Menzies (*5*), and the effect on nutrients under adverse storage conditions by the Wisconsin Alumni Association, as reported by Mehring (*4*). The mechanics of handling liquid phosphoric acid have been studied in the pilot formula feed plant at the Kansas Agricultural Experiment Station.

Liquid additives supplementing dry feeds and even liquid feeds are not new to the industry (*2*). Cane molasses with a pH of 5.0 containing lactic acid, fish solubles with a pH of 4.5 containing sulfuric acid, and inedible animal and vegetable fats containing fatty acids, are in contact with a great deal of mixing and conveying equipment in certain feed mills. It has not been found necessary to replace equipment or specify stainless steel when using liquids containing weak organic acids or low concentrations of mineral acids buffered by proteins or their hydrolyzates.

Phosphoric acid is corrosive to mild steel, copper, brass, and bronze and is sufficiently hazardous to require precaution in handling. The common commercial products contain the equivalent of 75 to 85% orthophosphoric acid. Material used in this study contained 23.8% phosphorus and had a density of 13.3 pounds per gallon. Under the experimental conditions reported, about 1400 pounds of acid were added to feeds at an average level of 1.25%, with 90% of the feed being prepared in pelleted and crumbled form.

Equipment

Stainless steel holding and dispensing equipment was installed to supply acid to a batch horizontal mixer. Figure 1 shows a schematic drawing of the reservoir with connections to the mixer. Air pressure, superimposed on the liquid in the acid feed tank, was necessary to maintain a spraying action during the acid addition. An air bypass line, below the tank, permitted pressure to spray nozzles when liquid was not being dispensed. A header, to which were attached four spray nozzles, carried acid into the mixer either at position *A* or *B* as seen in Figure 2. Position *A* was above the agitators on the up side of the feed movement and in effect, feed was thrown against the header in this position. At *B*, directly across the mixer from *A*, the header was about 10 inches above the feed flow during mixing, because gravity and centrifugal movement of the spirals folded material into the mixer trough below this position.

Phosphoric acid was brought to the plant warehouse in lacquer-lined 55-gallon steel drums and transferred by air pressure and plastic tubing to 5-gallon carboys to be conveyed into the mill. Coveralls and goggles were used at all times. A shower stall was within 40 feet of the liquid acid for emergency use. The mixer was 15 cubic feet in capacity, 5 feet long × 24 inches wide with a double ribbon agitator, a 10-inch outlet, and operating at 69 r.p.m. with a 5-hp. motor. After discharge by the air