mula is a quintupling, whereas the designated formula is a doubling, of the average of the compositions of many preparations, $Ca_{1.4}H_{1.2}P_2O_7.2H_2O$, but structural considerations indicate that a compound with Pahl's formula would have either an unusually large unit cell or an inordinately high density. Van Wazer (*13*) has suggested that the variations in the reported compositions of this compound indicate that the material is a solid solution, but results of x-ray examinations rule out this possibility.

Boullé and Dubost (*4*) describe as "calcium pyrophosphate-α" a preparation with the empirical formula $Ca_{2.74}H_{2.52}(P_2O_7)_2.2H_2O$ and with an x-ray pattern very similar to that of the compound designated $Ca_3H_2(P_2O_7)_2.4H_2O$. Boullé and Dubost concluded that their product was a double salt with the composition $2CaH_2P_2O_7.Ca_2P_2O_7.3H_2O$ or $Ca_4H_4(P_2O_7)_3.3H_2O$.

All the preparations listed in Table VII are essentially identical optically, morphologically, and in x-ray pattern. These preparations then must represent a single crystallographic species that is unaltered structurally by small changes in composition. The assigned formula is based on the supposition that a small deficiency in calcium can be compensated by substitution of hydrogen in a lattice in which the packing is dominated by phosphorus-oxygen tetrahedra.

Weissenberg single-crystal studies were made to establish the correct empirical formula. The study was complicated by the lamellar twinning that the crystals invariably displayed—the polysynthetic twinning occurs on the tabular (100) plane, which is also the composition plane, and the crystals frequently have such a hyperfine structure that the twinning is very difficult to detect optically. Because of the twinning, the x-ray results yielded two possible sets of cell constants. The volumes of the two mirror-image unit cells are identical, however, and each cell accommodates precisely one formula weight of the composition $Ca_3H_2(P_2O_7)_2.4H_2O$. The computed cell density is 2.46, which agrees reasonably well with the density, 2.41, calculated from the refractive indices and chemical composition. The tentative results of the Weissenberg study support the empirical formula $Ca_3H_2(P_2O_7)_2.4H_2O$.

In the region of composition in which $Ca_3H_2(P_2O_7)_2.4H_2O$ appears at room temperature, a lower hydrate with otherwise the same composition, $Ca_3H_2(P_2O_7)_2.H_2O$, appears at 50° to 75° C. The monohydrate has higher crystal symmetry than the tetrahydrate and crystallizes in well-formed, untwinned units. The mole ratio $CaO:P_2O_5$ in the monohydrate is consistently close to 1.50. The lower mole ratios $CaO:P_2O_5$ in the tetrahydrate probably result from lattice defects caused by hyperfine polysynthetic twinning that is shown by nearly all the preparations of the tetrahydrate.

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DEFOLIANT METABOLISM

The Fate of Cyanamide in Cotton

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CYANAMIDE is a colorless, crystalline solid which melts at 41° to 42° C. and is soluble in water, alcohol, and ether. It polymerizes at temperatures above its melting point and will react with weak acids to form salts and with strong acids to form urea.

Calcium cyanamide was patented in 1910 for use as a fertilizer (*9*) and was used in Germany before 1913 to kill mustard weeds in oats. Calcium cyanamide was first noted to induce cotton defoliation by Hall and Harrell in 1938 (*4*), and has since been used widely as a defoliant for cotton, soybeans, and for nursery stock (*1*).

Previous studies of the metabolism of cyanamide include the work of Hofmann *et al.* (*7*) who reported the enzymatic decomposition of cyanamide by an enzyme termed cyanamidase which was extracted from soybeans. He also noted that barley and corn plants cultured with cyanamide-C^{14} expired part of their carbon dioxide as $C^{14}O_2$.

Later, Hofmann *et al.* (*6*) stated that oats, barley, wheat, and rye converted cyanamide to dicyandiamide which could be detected after 2 to 3 days. Alanine, tryptophan, lysine, leucine, phenylalanine, and valine were present in higher concentrations in cyanamide-fed plants than in plants fed calcium nitrate as nitrogen source. Both alanine and aspartic acid had C^{14} activity when plants were fed barium cyanamide-C^{14}. Alanine displayed the greatest activity.

Latzko (*8*), in a review article, stated that Rathsack found dicyandiamide, guanidine, and guanyl compounds as intermediary decomposition products of cyanamide. In a previous report of limited distribution (*5*), the authors have summarized the results of studies conducted with mature, field-grown Deltapine 15 cotton. These plants were sprayed at rates equivalent to 2 and 4 gallons of 25% concentrate per acre of cyanamide-C^{14}. Carbon-14, which was originally contained in the cyanamide, was translocated from the sprayed leaves to the seed of developing bolls. Paper chromatographic procedures established that this seed did not contain cyanamide, but the character of the carbon-14 containing products was not established.

This study was initiated to discover

Studies were made of the fate of cyanamide applied in sublethal doses to cotton plants to determine if harmful residues might enter the seed. The cyanamide was rapidly complexed or destroyed in the leaves and in the injected bolls. The first product produced in the leaves was tentatively identified as urea. No cyanamide was found in any of the tissues which were extracted more than 8 hours after application. Evidence is presented which indicates that cyanamide was utilized as a substrate for growth and was not converted to dicyandiamide, azide, or cyanide.

the fate of cyanamide when applied to cotton plants. It concerns only the partial metabolism of cyanamide with special emphasis on probable toxic constituents in the seed.

Materials and Methods

Cotton plants were grown in potting soil in the greenhouse for use in these experiments. Plants were treated in one of two ways, with a water solution of cyanamide-C^{14} which was prepared by the action of carbon dioxide on barium cyanamide-C^{14} and which had a measured specific activity of 0.09 mc. per mg.

Boll Injection Experiment. A hypodermic syringe was used to inject selected bolls of two fruiting Deltapine 15 cotton plants with 0.05 ml. of aqueous cyanamide-C^{14} solution. Four bolls of these plants were injected with a total of 0.068 mg. of cyanamide which contained a total measured activity of 15.5×10^6 counts per minute. The bolls were 10, 20, 30, and 35 days old at the time of injection, and were left on the plants for 9 days before they were harvested. As the bolls were succulent and green when harvested, seed and lint were removed by cutting the carpels with a razor blade. Seed samples from treated bolls were frozen and stored overnight at −20° C., then dried in vacuo for 12 hours prior to the removal of the lint with tweezers.

The kernels were removed from the hulls with a scalpel and then ground slightly with a glass mortar and pestle before extraction.

Entire Plant Treatment. Eight greenhouse-grown cotton plants in the early fruiting stage were treated with 3.66 ml. of the cyanamide-C^{14} solution which contained 1.36 mg. of cyanamide per ml. of solution and had a measured specific of 0.121 millicurie per milliliter. The solution was applied as evenly as possible to the leaves of the plants with a small camel-hair brush. The young bolls on these plants were allowed to mature in the greenhouse before harvesting. Maturation required 47 days for completion. After harvest, lint was removed from the seed with tweezers, and the seed were excised with a scalpel to remove the kernels from the hulls. One-gram aliquots of

Table I. Comparison of Carbon-14 Distribution in Cotton Bolls from Plants Treated with Cyanamide-C^{14}

(Based on radioactivity measurements and expressed as p.p.m.)[a]

Distribution	Entire Plant Treatment, P.P.M.[b]	Boll Age at Injection, Days 10	20	30	35
Lint	6.6	..	1.7	1.7	1.1
Kernels	11.0	4.7	6.8	19.2	3.4
Burs	..	19.0	5.0	7.0	12.0
Kernel Fractions	**% Distribution of C^{14} in Kernels Extracted as Shown in Figure 1**				
Oils, fats, and pigments	3.0	3.0	3.4	0.2	0.7
Amino acid	28.0	16.0	10.0	11.0	13.0
Organic acid	1.0	1.0	0.5	0.4	0.7
Sugar	2.0	3.0	2.6	2.7	2.5
Protein	7.0	13.0	11.0	9.0	10.0
Cellulose, lignin, etc.	25.0	21.0	33.0	27.0	26.0

[a] Chemical structures of the products were not established; therefore, calculations are based on assumption that products have same molecular weight as cyanamide.

[b] $$\text{P.P.M.} = \frac{\text{total c.p.m. in sample} \times 1{,}000{,}000}{\text{wt. of sample in mg.} \times \text{specific activity of } H_2NC = N \text{ in c.p.m./mg.}}$$

$$\text{Total c.p.m.} = \frac{\text{observed c.p.m. (corrected for background)} \times \text{(self-absorption coefficient)} \times \text{(sample wt. in mg.)}}{\text{sample area in sq. cm.}}$$

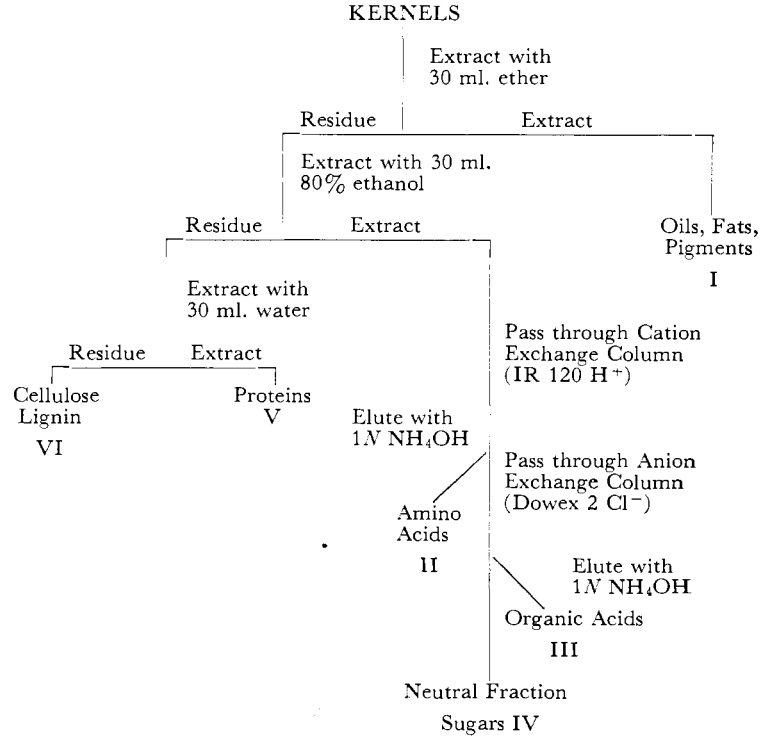

Figure 1. Fractionation scheme

each sample of lint, hulls, or kernels were pressed into a wafer and counted on both sides with a Nuclear-Chicago Model 183B Scaler and D-47 Geiger Tube to determine the amount of radioactivity in the samples (Table I). Samples were counted for a time sufficient to obtain statistical counting accuracy of ±5% on an average of four samples per treatment. A dilution series using carbon-14 labeling was conducted in ground cotton seed kernels. Infinitely thick samples, i.e., 1.5 grams with 6.6 cm. square area, were used for the determination of the self-absorption coefficient. Since the radioactivity was not absolute as measured by the National Bureau of Standards, a relative self-absorbent coefficient was obtained which was not significantly different from 0.29 (average for barium carbonate), therefore 0.29 was used for calculation purposes.

After counting, the wafers made from the ground kernels were extracted as shown in Figure 1.

Metabolite Sequence Study. Selected leaves of two cotton plants in the 10-leaf stage of growth were painted with 1 ml. of the original cyanamide-C^{14} solution. The cyanamide-C^{14} was applied early in the morning to ensure sufficient sunlight for photosynthesis during the early treatment period.

One leaf was harvested 1, 2, 4, and 8 hours and 1, 2, 4, and 8 days after treatment. Each leaf was quick-frozen and stored at −20° C. until all subsequent leaves were harvested. Leaves were extracted separately in a Waring Blendor with 30 to 35 ml. of water for a period of 3 to 5 minutes. Aliquots of the water extract from each leaf were chromatographed simultaneously in three different solvent systems (Table II).

The purpose of this study was not to establish the exact sequence of incorporation of the carbon-14 from cyanamide into each subsequent compound, but rather to establish if harmful products were produced.

Analytical Procedures

Extraction. All of the kernel samples were extracted as shown in Figure 1. After each extraction, the residues were pressed into the form of a wafer, weighed, and counted on both sides. This procedure effectively separated or fractionated the seed into six parts: oils, fats, and pigments; amino acids; organic acids; sugars; proteins; and cellulose, lignin, etc.

Extractions, volume reductions, and other operations of the experiments were conducted at temperatures below 40° C. to prevent dimerization of the cyanamide to dicyandiamide in vitro.

Chromatography. An aliquot of each extract that contained sufficient radioactivity was chromatographed on Whatman No. 1 filter paper. Reference spots of cyanamide consisted of comparable extracts from nontreated tissues which were overspotted rather than chromatographed alone to correct for any changes in R_f values that might have occurred due to the presence of salts or other interfering materials in the extract.

Each extract was chromatographed together with standards on the same paper in the following three solvent systems: *n*-butyl alcohol–acetic acid–water (100:22:50), 77% ethyl alcohol, and isopropyl alcohol–ammonia–water (80:5:15). Chromatograms were cut into strips and passed under a Geiger tube connected to a Nuclear-Chicago Model 1620A rate meter and recorder. The resultant radiochromatoscans were used as the basis for R_f calculations of all of the carbon-14-containing compounds since their amounts were below the limits of colorimetric determinations.

Dicyandiamide and sodium azide standards were detected on the chromatograms with an alkaline-nitroprusside spray reagent (*2*). The urea

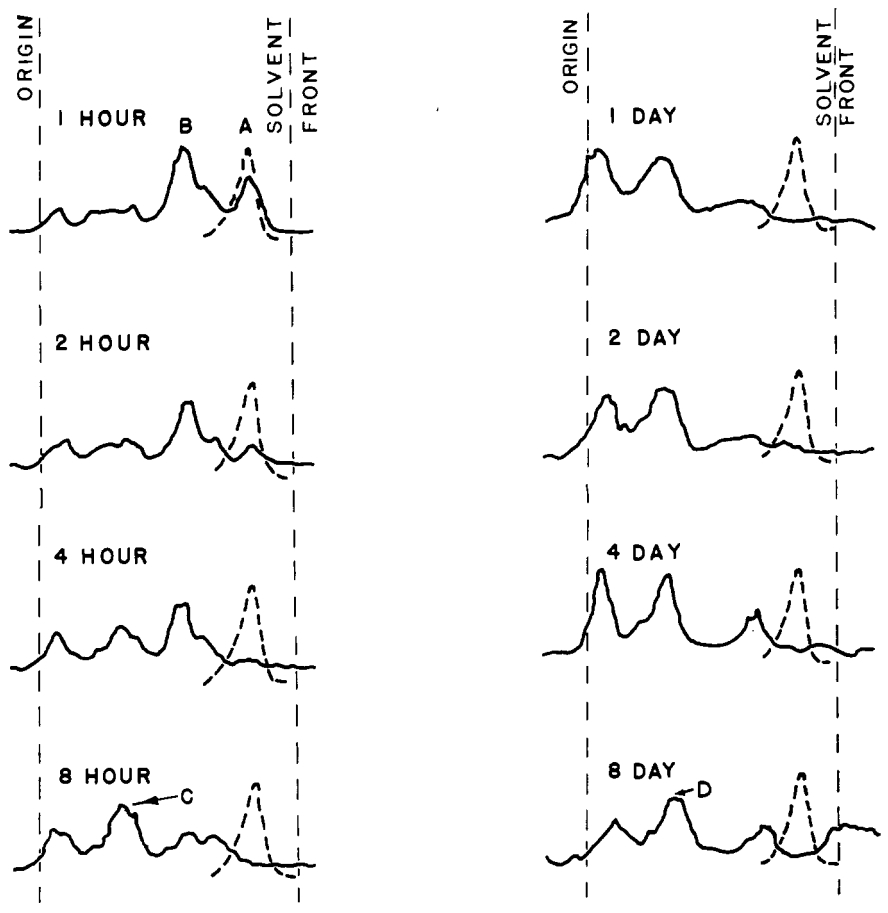

Figure 2. Radiochromatoscan of water extracts of cyanamide-C^{14}-treated cotton leaves

Chromatograms developed in 77% ethyl alcohol. Dashed line peaks represent reference cyanamide-C^{14}. Peak A: cyanamide-C^{14} extracted from cotton leaf. Peak B: chromatographically identified as urea. Peak C: mixture of two substances, one of which resembles alanine chromatographically. Peak D: major residual component after 8 days

Table II. R_f Values of Cyanamide-C^{14} Products

Products	Solvent Systems[a] I	II	III
Cyanamide-C^{14}	0.69	0.81	0.81
Dicyandiamide	0.56	0.61	0.61
Sodium azide	..	0.45	0.29
Urea	0.42	0.55	0.50
Potassium cyanide	0.31	0.18	0.10
Products extracted from leaves of Figure 2			
Peak B	0.46	0.57	0.48
Peak C	0.40	0.30	0.16
Peak D	0.33	0.34	0.20
Entire plant treatment kernel products			
Ether soluble	0.06	0.57	0.09
Alcohol soluble	0.38	0.32	0.23
Water soluble	0.05	0.24	0.07
Major products of kernels from injected bolls			
Alcohol soluble	0.25	0.36	0.18
Water soluble	0.36	0.28	0.18

[a] I. *n*-Butyl alcohol–acetic acid–water, 100:22:50. II. 77% Ethyl alcohol. III. Isopropyl alcohol–ammonia–water, 80:5:15.

Table III. R_f Values of Cationic Constituent of Peak C of Radiochromatoscans in Figure 2 and Standards

	Solvent Systems[a]			
	I	II	III	IV
Peak C cationic constituent	0.50	0.72	0.14	0.73
Alanine	0.52	0.74	0.16	0.64
Cyanamide	0.82	0.92	0.71	..
Urea	0.66	..	0.49	0.74
Potassium cyanide	0.35	0.46	0.33	0.55
Sodium azide	0.41	0.82	0.34	0.51
Dicyandiamide	0.70	0.86	0.55	0.67

[a] I. Pyridine–acetic acid–water, 50:35:15. II. Acetone–urea–water, 60:05:40. III. *n*-Butyl alcohol–acetic acid–water, 100:22:50. IV. 80% Aqueous phenol.

standard was detected with a phenol, chlorox reagent spray (*3*). When potassium and sodium cyanide were chromatographed separately in various solvent systems, spraying of the chromatograms with 10% bromocresol green indicator in absolute ethyl alcohol revealed spots believed to be cyanide. The alanine standard was detected with 0.25% ninhydrin in acetone.

Results

Boll Injection Experiment. Carbon-14 was found in each of the six-kernel fractions (Table I) as well as the lint, hulls, and burs of plants harvested 9 days after injection of the bolls. Radiochromatoscans revealed at least one compound was derived from the cyanamide in each of the extracts, but no free cyanamide was detected on the radiochromatoscans. None of the products derived from cyanamide and extracted from the lint, kernels, or burs compared chromatographically with dicyandiamide, urea, or azide. Different products in the kernels resulted after the injection of cyanamide-C^{14} directly into the boll from those found in the kernels when the leaves were treated (Table II).

Entire Plant Treatment. Cyanamide was not detected in any extracts from seeds which were allowed to mature on the treated plants. Carbon-14 was distributed throughout the six fractions of the kernels. Results of fractionation were very similar to those obtained in the boll injection experiment (Table I). The major differences were in the relative amounts of carbon-14 in the protein and amino acid fractions.

Chromatography of the ether, alcohol, and water extracts of the kernels revealed at least one derivative of cyanamide-C^{14} (Table II) in each extract. Based on their R_f values in three solvent systems, the derivatives could not have been dicyandiamide, urea, sodium azide, or sodium or potassium cyanide. These derivatives were different from those produced in 9 days in the injected bolls.

Metabolite Sequence Study. The sequence study indicated that free cyanamide-C^{14} was converted to other products in the cotton leaves within 8 hours after application (Figure 2). As the leaves were not washed prior to extraction, Figure 2 illustrates the disappearance of both internal and external free cyanamide. Cyanamide-C^{14} was converted to at least three major products in the leaves within 4 hours after treatment.

The first major product (Peak B of Figure 2) was tentatively identified chromatographically as urea since its R_f values compared favorably in the three solvent systems, shown in Table II, and in three other solvent systems: (the first R_f value quoted is for the metabolite, the second for urea); methyl alcohol–ammonium hydroxide–water, 80:5:15 (0.69 *vs.* 0.69); 70% isopropyl alcohol (0.47 *vs.* 0.49); and ethyl alcohol–*n*-butyl alcohol–water, 60:15:15 (0.54 *vs.* 0.52).

Two components were found in Peak C of Figure 2. One component adsorbed to IR 120 (H^+) cation exchange resin while the other did not. The cationic component was either alanine or a closely related product since its R_f compared favorably in three of the four solvent systems (Table III) and in 77% ethyl alcohol. Differences in R_f values in the phenol solvent are believed to be the result of interfering constituents from the leaf extract since the alanine R_f values were determined colorimetrically by chromatographing alanine alone.

Discussion

These studies indicate that cyanamide-C^{14} was converted to several products in the leaves of the cotton plant when applied in sublethal amounts. Many more carbon-14-containing compounds were found than are apparent in Figure 2 and Tables I and II. It is not certain which of the compounds were derived from the enzymatic incorporation of the carbon from cyanamide since heat-inactivated leaves were not included as a control. The occurrence of numerous products, however, suggests that an enzymatic incorporation may be involved and that the pathway of degradation was through urea to other products. The urea was believed to be utilized in the production of amino acids in a manner similar to that reported for beans (*10*). If phytotoxic constituents, such as dicyandiamide, azide, or cyanide, were involved in the sequence, it is doubtful that the conversion would have occurred to the extent observed.

The metabolism sequence study revealed only those products which remained in the treated leaves and did not account for products which may have been translocated to other parts of the plants. Therefore, the degradation of cyanamide-C^{14} within the injected bolls is believed to be significant since it indicates that any cyanamide which might be translocated from the leaves to these structures would be degraded or metabolized within the bolls and thus not be accumulated in the seed. This was, in fact, the result of the entire plant treatment. Degradation of cyanamide within the boll sap would also prevent accumulation of cyanamide which might enter through the carpels of the young bolls.

Thus, the cotton plant apparently contains a mechanism for the prevention of accumulation of cyanamide in the seed. Cyanamide applied to leaves is degraded; any that escapes degradation in the leaves would be destroyed after translocation to the bolls, and cyanamide entering through the carpels would also be converted to products.

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