Table I. Incorporation of ^{14}C from Different Substrates into Patulin

labeled compound[a]	patulin yield (mg/100 mL)	sp act. dpm/mg	sp act. μCi/mmol	% label conversion
sodium [1-^{14}C]acetate	455	0.16	0.011	0.338
	708	0.19	0.014	0.622
sodium [2-^{14}C]acetate	739	2.72	0.189	9.059
	872	2.39	0.166	9.387
[U-^{14}C]glucose	897	1.90	0.132	7.676
	691	1.93	0.134	6.023

[a] Obtained from New England Nuclear, Boston, MA.

Table II. Antibiotic Activity of [^{14}C]Patulin as Determined by Disc Assay[a]

patulin sample obtained from:	zones of inhibition, mm
standard	12
sodium [1-^{14}C]acetate	11
	11
sodium [2-^{14}C]acetate	9
	12
[U-^{14}C]glucose	11
	11

[a] Method of Stott and Bullerman (1975) using *E. coli*.

R_f comparable to known patulin standards. Recycle chromatography failed to show any evidence of an impurity.

The results shown in Table I indicate that sodium [2-^{14}C]acetate was the preferred labeling source with approximately 9% of the radioactivity incorporated into patulin. The preference of sodium [2-^{14}C]acetate over sodium [1-^{14}C]acetate was 15 to 1 and may be due to the conversion of the C-1 carbon to CO_2 by way of the Krebs tricarboxylic acid cycle. The preference of sodium [2-^{14}C]acetate over [U-^{14}C]glucose may exist for the same reason, or may be the result of specific utilization of glucose for growth rather than for toxin production.

The antibiotic activity of [^{14}C]patulin compared to a standard patulin sample is shown in Table II. All samples exhibited antibiotic activity comparable to the standard, suggesting that there was very little degradation of the biological activity of [^{14}C]patulin by the alumina column clean-up and TLC purification.

Based upon the results obtained from this study, the use of sodium [2-^{14}C]acetate incorporates a much greater efficiency of label into [^{14}C]patulin than does the use of other precursors (Bassett and Tannenbaum, 1960; Tannenbaum and Bassett, 1959; Nip and Chu, 1977). It is therefore concluded that large quantities of [^{14}C]patulin can be successfully biosynthesized with *Penicillium urticae* and sodium [2-^{14}C]acetate in a potato dextrose medium.

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Inhibition of [^{14}C]Glucose Uptake into Rat Liver Glycogen by Dietary Cyclotetraphosphate

A dietary intake of 0.1% P as cyclotetraphosphate (sodium tetrametaphosphate) for 2 weeks was shown to significantly decrease the uptake of [^{14}C]glucose into liver glycogen of fasted rats. Total liver glycogen was 39% less in these animals when compared to that from animals fed a comparable level of orthophosphate.

The cyclic phosphates, cyclotriphosphate and cyclotetraphosphate, comprise approximately 7–9% of the phosphorus of hexametaphosphate, one of the food additive condensed phosphates currently used (Martens and

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Rieman, 1961; Kalliney, 1972). In addition, cyclic phosphates are formed during the in vitro hydrolysis of hexametaphosphate (Van Wazer, 1958; McCullough et al., 1956). Enzymatic hydrolysis of cyclotetraphosphate has been observed in vitro in rat and pig intestines (Ivey and Shaver, 1977). However, a greater uptake of ^{32}P from an oral dose of [^{32}P]cyclotetraphosphate than from an oral dose of [^{32}P]orthophosphate has been observed in rat brain and ovaries. At the same time, cyclotetraphosphate appeared in the urine of these animals, indicating that some fraction of this phosphate might be absorbed intact (Allen and Smith, 1976). Rats fed cyclotetraphosphate also incorporated a greater percent of a pulse dose of ^{32}P from [^{32}P]cyclotetraphosphate into selected tissues than did rats which were fed orthophosphate, indicating a possible induction or activation by dietary cyclotetraphosphate of a mechanism which facilitates its uptake and utilization. In addition, this increased uptake was greatest for rat brain which had a higher percent incorporation of ^{32}P dose from [^{32}P]cyclotetraphosphate than from [^{32}P]orthophosphate (Allen and Smith, 1976, 1978). The importance of protein phosphorylation to metabolic regulation in rat brain (Roberts and Ashley, 1978) and other tissues (Greengard, 1978) has been suggested. In light of these findings and the previous data, this study was undertaken to investigate a possible effect of cyclotetraphosphate on the phosphorylation of protein by comparison of the uptake of [^{14}C]glucose into liver glycogen from fasted rats which had been fed cyclotetraphosphate with that from fasted rats which had been fed orthophosphate. If cyclotetraphosphate were involved in the activation of a protein kinase, the uptake of [^{14}C]glucose into the livers from rats which had been fed cyclotetraphosphate would be less than from rats which were fed orthophosphate.

PROCEDURE

Two groups of Sprague Dawley-Wistar-Long Evans adult male rats housed in galvanized cages were fed standard diets modified from Caputto et al. (1958) for 14 days. One group was supplemented with Na_2HPO_4 and the other group with $Na_4P_4O_{12}\cdot 4H_2O$ (sodium cyclotetraphosphate) at a 0.1% level of phosphorus. Sodium cyclotetraphosphate was prepared by hydrolysis of phosphorus pentoxide with ice and sodium bicarbonate according to the method of Bell et al. (1952) and purified by fractionation with methanol. The purity of the product was checked by paper chromatography suggested by Reiman and Barkenkamp (1961). The preparation used contained 81.0% cyclotetraphosphate, 9.5% pyrophosphate, 4.75% orthophosphate, and 4.75% tripolyphosphate. The diets and distilled water were provided ad libitum. At the end of the dietary period, the animals were fasted 24 h. Each rat then received 1.5 mL of [^{14}C]glucose solution containing 375 mg of glucose (sp act., 1.24×10^4 cpm/mg of glucose) by stomach tube. After 2 h, the animals were sacrificed by decapitation and livers removed to hot 30% KOH for isolation of glycogen according to the method described by Lifson et al. (1948). Glucose content of the glycogen preparation was determined using anthrone reagent (Carroll et al., 1956).

RESULTS AND DISCUSSION

Uptake of [^{14}C]glucose expressed as total counts per minute was decreased approximately 44% in the livers of rats fed cyclotetraphosphate compared to those fed orthophosphate (Table I). When expressed in terms of body weight, a comparable decrease in [^{14}C]glucose uptake in the livers of these rats was still evident. In addition, rats fed cyclotetraphosphate had 39% less total liver glycogen in milligrams of glucose than rats which were fed orthophosphate (Table II). The magnitude of decrease in total liver glycogen in milligrams of glucose was also approximately 39% when expressed relative to animal body weight. The decrease in total counts per minute and milligram of glucose were significant at $p < 0.01$. The level of significance is $p < 0.02$ when these parameters are expressed per 100 g body weight.

Table I. [^{14}C]Glucose Recovered in Rat Liver Glycogen

	dietary phosphate			
	orthophosphate		cyclotetraphosphate	
rat no.	total cpm × 10^{-5}	cpm × 10^{-4} / 100 g body weight	total cpm × 10^{-5}	cpm × 10^{-4} / 100 g body weight
1	2.89	7.12	1.71	4.61
2	3.96	10.53	1.77	4.02
3	2.41	5.86	1.63	3.99
4	2.88	6.48	1.68	4.11
av ± SE	3.03 ± 0.33[a]	7.50 ± 1.04[b]	1.70 ± 0.03[a]	4.18 ± 0.14[b]

[a] Means are significantly different ($P < 0.01$) by paired comparisons. [b] Means are significantly different ($P < 0.02$) by paired comparisons.

Table II. Total Liver Glycogen Recovered

	dietary phosphate			
	orthophosphate		cyclotetraphosphate	
rat no.	mg of glucose	mg of glucose / 100 g body weight	mg of glucose	mg of glucose / 100 g body weight
1	113.9	28.0	76.3	20.6
2	141.7	37.7	59.7	13.6
3	91.1	22.2	77.9	19.1
4	125.0	28.1	73.7	18.1
av ± SE	117.9 ± 10.6[a]	29.0 ± 3.2[b]	71.9 ± 4.6[a]	17.8 ± 1.5[b]

[a] Means are significantly different ($P < 0.01$) by paired comparisons. [b] Means are significantly different ($P < 0.02$) by paired comparisons.

It appears from these data that dietary cyclotetraphosphate may have an inhibitory effect on the uptake of glucose into liver glycogen. Though the [^{32}P]cyclotetraphosphate sample administered in this study contained other condensed phosphates as well as orthophosphate as impurities, neither [^{32}P]tripolyphosphate nor [^{32}P]-orthophosphate have been incorporated into rat brain and lipid to the extent of [^{32}P]cyclotetraphosphate when given orally to animals fed the respective dietary phosphate (Allen and Smith, 1977). Therefore, this effect may be attributed to the difference in dietary phosphate, lending support to previous results which indicated that some fraction of the cyclotetraphosphate might be absorbed unhydrolyzed (Allen and Smith, 1976). Since the synthesis of glycogen is inhibited by cyclic AMP mediated phosphorylation of glycogen synthetase, then inhibition of glycogen synthesis under conditions at which cyclic AMP levels would be minimal suggests that cyclotetraphosphate may be affecting synthesis of glycogen through interference with the mechanism of cyclic AMP mediation. Tetrapolyphosphate has been shown to inhibit phosphodiesterase in yeast (Speziali and Van Wijk, 1971). Cyclotetraphosphate may be affecting the synthesis of glycogen through an interference with the regulation of cyclic AMP levels.

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Dicamba in Lysimeter Runoff and Percolation Water

The postemergence herbicide dicamba was applied at 5.6 kg/ha on May 1, 1974, to Lysimeter Y101C and to an adjacent soil plot (4.1 m^2) at the North Appalachian Experimental Watershed at Coshocton, Ohio. An August storm produced runoff that contained 0.23 ppb of dicamba. We detected 1.0 ppb of dicamba in the percolate water at the 2.4-m depth 11 months after application. The data are compared with earlier measurements of 2,4,5-trichlorophenoxyacetic acid and picloram found in runoff and percolation water from this lysimeter. The results indicate that the loss of dicamba in runoff and percolate water will not be a source of groundwater pollution.

Dicamba (2-methoxy-3,6-dichlorobenzoic acid) is a postemergence herbicide used in the selective control of broadleaf and grassy weeds in cereal crops, pasture, and turf area. Studies on the movement of dicamba in runoff water (Trichell et al., 1968) and in leachate from soil columns (Harris, 1964) showed that the herbicide moves laterally, upwardly and downwardly in the soil. Dicamba degradation studies showed that the substance undergoes microbial breakdown in the soil with over 50% loss in 4 weeks (Smith, 1974). In the present work, dicamba residues were monitored for 1 year in runoff and percolate water from a field lysimeter to determine if the use of this herbicide would cause groundwater pollution.

EXPERIMENTAL SECTION

Lysimeter and Treatment. The soil on Lysimeter Y101C (4.26 × 1.89 × 2.44 m) at the North Appalachian Experimental Watershed at Coshocton, Ohio, is Muskingum silt loam with 1.72% organic matter and pH of 5.0. The predominant vegetation growing over the lysimeter was bluegrass and broadleaf weeds. On May 1, 1974, we applied the dicamba formulation Banvel D at a rate of 5.6 kg/ha of the active ingredient to the lysimeter and a plot of soil (4.1 m^2) adjacent to the lysimeter that was used for removing surface soil samples for residue analysis. The 5.6 kg/ha rate is three–ten times the commonly used rate for weed control. Monthly soil samples consisting of 20 cores (2.5 cm deep × 29 mm diameter) were collected, placed in polyethelene bags, and then frozen.

Runoff and Percolation Water. Samples from storm runoff were collected and stored automatically in an underground storage chamber. Percolation water samples were collected manually 2.4 m below the lysimeter surface. All chemical analyses were conducted at Beltsville, MD.

Dicamba Analyses. After acidification with 10 mL of 1 N H_2SO_4, dicamba was extracted from 250-mL aliquots of runoff and percolation water samples with 100 mL of ethyl ether. The ether extract was dried over anhydrous sodium sulfate and concentrated to a final volume of 2 mL. The methyl ester of dicamba was prepared and quantitated on a gas chromatograph equipped with an electron-capture detector as described by Woolson and Harris (1967). Protective gloves and a face shield are recommended for the handling of diazomethane and ethyl ether because of the highly explosive nature of these chemicals.

Soil samples from the plot adjacent to the lysimeter were first thawed, air-dried, and put through a 2-mm seive. Subsamples were removed for moisture determinations. To a 20-g soil sample we added 100 mL of methanol and 25 mL of 1 N H_2SO_4. The suspension was shaken for 1 h and centrifuged. The liquid phase was poured into 200 mL of distilled water. Dicamba was extracted from the aqueous methanol solution with ethyl ether and analyzed using the same procedure as described above for the water samples. Recoveries of dicamba from soils fortified at 2 ppm were between 85 and 90%. The minimum level of