The paper is formed into a cylinder around the measuring cylinder, and supported at the top by perforating it with the arms of the T-piece. Narrow pieces of paper can be folded longitudinally and likewise supported. A lid is then placed on the crock to maintain a saturated atmosphere.

The development is an ascending one and the speed of the liquid front seems to decrease the farther it goes. The container was not altogether airtight, and to keep the solvent from varying in composition, it was renewed every day. In this apparatus the liquid front travels about 25 cm in 24 hr.

To prepare the solvent, 10 ml of 15 N NH_4OH was mixed with 90 ml of distilled water; this was shaken in a separating funnel with 100 ml of butanol.

The mixture separates after a very short time; the lower layer is run into the bottom of the crock and the upper layer into the Petri dish in the center of the crock.

The mixture of ammonium and potassium chlorides, bromides, iodides, and thiocyanates is concentrated or diluted so as to obtain the approximate concentration of 0.1 N. A drop of this solution is placed on the paper by dipping a thin stirring rod (2 mm diam) into the solution and then touching the paper with it about 2.5 cm away from the lower end. The adhering drop is absorbed by the paper and its position marked with pencil. The paper is then placed in the developing crock for 24 hr. Immediately on removing the chromatogram from the container, the liquid front is marked in pencil. The developed paper is dried by hanging for about 10 min in the air. This removes the ammonia and most of the butanol.

An AGLA atomizer was used for spraying reagents on the chromatograms. It produces a reasonably fine spray, but is a little too small for satisfactory use.

I^- and CNS^- travel faster than Cl^- and Br^- and can be revealed by spraying a mixture of 3% H_2O_2 and 0.1 N $Fe(NO_3)_3$ solution on the upper portion of the chromatogram. The lower 4 cm must not be sprayed with this reagent since a distortion of the Cl^- and Br^- spots may occur. The red spot of $Fe(CNS)_3$ is reasonably stable, but the blue spot of I_2 may fade in a few minutes.

The whole of the paper is then sprayed with 0.1 N $AgNo_3$ and washed twice in 0.1 N HNO_3, and the surface moisture is removed by pressing between filter papers. It is then exposed to H_2S, producing a brown spot for CNS^- and I^-, and an oblong spot of adjacent Cl^- and Br^- when both these ions are present. If only one is present a round spot is formed, which cannot be mistaken because it has different R_f measurements.

The R_f values are in reference to the liquid front, so that $R_f = \frac{\text{distance traveled by ion}}{\text{distance traveled by liquid front}}$ (1).

In the separation of cations two liquid fronts were noticed. This is not the case here and after drying there is little indication of the liquid front.

This new chromatographic separation of the halide groups is, in the author's opinion, highly suitable for ultimate organic analyses, since only 0.1 mg of the halide ion is sufficient to be identified.

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2,4-Dichlorophenoxyacetic Acid Inhibition of Castor Bean Lipase[1]

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The discovery of selective herbicides has aroused considerable interest in the mechanism of hormone activity. Since the growth and metabolism in the growing plant is disrupted, one might expect the enzyme systems to be affected.

Smith (*4*) showed appreciable effect on oxygen uptake of tissue slices from plants treated with 2,4-dichlorophenoxyacetic acid. One may presume that the sections of the plant functioned as entireties, and the action of the herbicide on any of the enzyme systems involved in growth and respiration would affect the observed oxygen consumption. On the other hand, we have observed no inhibition on polyphenol oxidase, α-hydroxy acid oxidase, or catalase in the presence of 2,4-D, using the tissue homogenate technique.

Preliminary studies have indicated that certain hydrolytic enzymes are affected by 2,4-D. The activity of castor bean (*Ricinus communis*) lipase was depressed by the sodium salt of 2,4-dichlorophenoxyacetic acid. The procedure used was essentially that of Longenecker and Haley (*3*). Two grams of olive oil substrate and 0.05 g of solvent-extracted castor bean meal, as the enzyme source, were weighed into 50-ml Erlenmeyer flasks. One ml of water was added to the control flasks, and 1 ml of recrystallized 2,4-D monohydrate sodium salt of the desired concentration was added to the inhibition flasks. In all cases the enzyme was activated by the addition of 0.6 ml of 0.1 N acetic acid. The flasks were incubated in a 37° C water bath. After desired reaction time, 50 ml of 95% ethyl alcohol was added to stop reaction. The degree of hydrolysis was determined by potentiometric titration of the liberated acids with NaOH to an indicated pH of 8.5. Under these conditions, slightly over half of the control substrate was utilized in a 6-hr period.

The effectiveness of the 2,4-D inhibition is of the order of 10% by 3.03×10^{-4} M. Slightly less than 70% inhibition was noted in the presence of 0.03×10^{-3} M. Harris *et al.* (*1*) have shown that sodium dichlorophenoxy acetate

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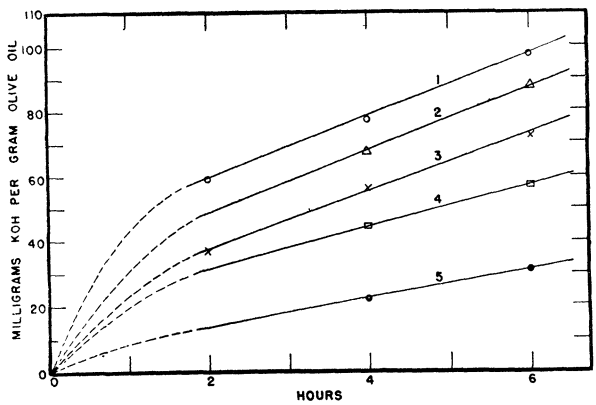

FIG. 1. 2,4-Dichlorophenoxy sodium acetate inhibition of castor bean lipase on the hydrolysis of olive oil. The curves represent varying concentration of the 2,4-D inhibitor. (1) Control, H_2O; (2) 3.03×10^{-4} M; (3) 6.05×10^{-4} M; (4) 1.52×10^{-3} M; (5) 3.03×10^{-3} M.

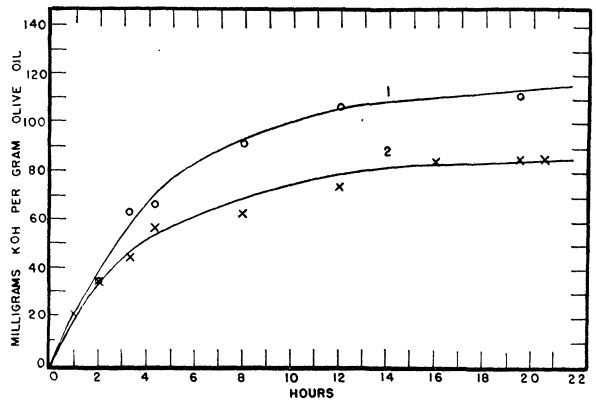

FIG. 2. 2,4-D butyl ester hydrolysis by castor bean lipase to the active 2,4-D-free acid in the presence of olive oil. Sodium oleate emulsifier added to both series of flasks. (1) Control, H_2O; (2) 0.051 M 2,4-D butyl ester.

acts as a protein precipitant at relatively low concentration. The ether-extracted castor meal contains most of the protein originally present in the beans. Since the 2,4-D would be expected to combine with other proteins as well as the lipase, the effective concentration necessary for inactivation is probably considerably lower.

In a kinetic study of this system, conditions were followed which allowed graphical determinations as developed by Lineweaver and Burk (*2*). By varying the substrate concentration, and keeping other conditions constant, an apparent increased activation occurred upon dilution of the substrate.

2,4-D is available commercially in four chemical forms: free acid, sodium or ammonium salts, amine salts, and esters. The sodium, ammonium, and amine salts, in all probability, could be converted to the 2,4-D free acid at a rapid rate by simple dissociation, *in vivo* and *in vitro*. However, the ester conversion would be more difficult to explain by this mechanism.

The possibility of castor bean lipase acting as a typical esterase on 2,4-D esters was selected for study in an attempt to explain such a conversion mechanism. The procedure used was essentially the same as described above. The reaction flask contained 8.5 ml of solution; 0.063 M with respect to 2,4-D butyl ester, 5.7×10^{-4} M sodium oleate as an emulsifier, 5.3×10^{-2} M acetic acid as activator, and castor meal as the enzyme source. The reaction was arrested and titrated as described here.

That castor bean esterase does hydrolyze the butyl ester is indicated by the titration of liberated acid. The calculated effective concentration of 2,4-D acid product of hydrolysis, showing complete inactivation of castor bean lipase, is approximately 4.00×10^{-3} M. Thus, the 2,4-D acid approaches the same concentration, by the end of 21 hr, as shown necessary for inactivation of the lipase by the sodium salt of 2,4-D in Fig. 1.

The reaction was followed in the presence of a natural glyceride substrate. To all flasks 0.50 g of castor meal, 2.0 g olive oil, and 4.5 ml of 0.1 N acetic acid was added. The control and inhibitor series were brought to an effective concentration of 4.6×10^{-4} M sodium oleate. Butyl ester of 2,4-D was added to the inhibitor series to a concentration of 0.051 M. Total volume for all flasks was 10.5 ml. The reaction was arrested and titrated as previously described.

From Fig. 2, it becomes apparent that even in the presence of a competing substrate, the butyl ester is not effective as an inhibitor until the hydrolysis to the 2,4-D acid occurs. The time for complete inactivation of the enzyme appears to follow closely the time of inactivation shown in Table 1.

This investigation has shown that 2,4-D effectively inhibits the activity of castor bean lipase at very low concentrations. Also, through a study of the kinetics, it appears activation occurs by dilution of the substrate concentration. The butyl ester of 2,4-D has been shown to be inactive as an inhibitor for castor bean lipase, and must first be hydrolyzed to the 2,4-D acid for activity.

TABLE 1

2,4-D BUTYL ESTER HYDROLYSIS BY CASTOR BEAN LIPASE, AND ULTIMATE INHIBITION OF LIPASE BY THE HYDROLYTIC PRODUCT, 2,4-D FREE ACID

Grams of castor meal	% of butyl ester converted to free acid	
	21 hr	46 hr
0.05	6.32	6.13
	5.86	....
0.10	11.15	11.43
	9.76	11.43
0.30	29.74	31.97
	32.81	31.88
0.50	48.19	52.05
	48.61	....

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