species (I) this volume was calculated on the basis of Chalkley's (*1*) measurements which give an average value of 0.15×10^{-3} µl per individual. For species (II) the volume was calculated from reduced weight measurements, asuming the relation 1 µg r. w. = 50 µl volume [Zeuthen (*7*)]. The volume of species (III) was calculated from direct measurements of dimensions.

As the Table shows, in organisms (I) and (II) the magnitudes measured correspond as closely as can reasonably be expected for intimately related species. As a matter of fact, the differences fall within the range of individual variations which can be found in one species. Organism (III), on the other hand, is decidedly different from the two others, at least with regard to the content of the two enzymes. This is most clearly seen by a comparison of the ratios given in the lower half of the table.

It might be thought that the remarkably low enzyme content of species (III) is not real, but rather due to inactivation during the homogenization prior to enzyme determination. However, this would not impair the validity of our comparison, since the homogenization and enzyme determinations were carried out exactly alike in all cases. Our figures thus indicate either a difference in enzyme content or in enzyme stability. Both properties ought to be equally suitable for the purpose of species comparison.

The result of our study is thus quite unambiguous: if biochemical features are considered as valid as morphological ones, organisms (I) and (II) ought to be placed in the same genus, while organism (III) seems rather different from the two others. Since nobody questions the validity of the name *Pelomyxa palustris* for species (III), we suppose that our results would support either the generic name *Amoeba* or *Chaos* for the other two. Not being taxonomists, we venture no suggestion as to which of these it should be.

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Separation of Chloride Group Anions by Partition Chromatography on Paper

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The separation of cations by partition chromatography was described in two previous papers (*3, 4*). These separations were only possible when hydrochloric acid was added to the solvent. This prevented both hydrolysis and the existence of ions in complex and simple form at the same time. The tailing or "comet" effect of Lugg and Overell (*5*) was thus avoided.

Westall (*7*) first measured the R_f value of the Cl^- ion in several solvents and observed that the addition of NH_3 to phenol inhibited the "comet" effect and gave Cl^- ions a constant R_f value, irrespective of the accompanying cation.

Partridge (*6*),, in a correction to his paper, observed that both Br^- and I^- travel at different speeds and interfered with the technique he used for the separation of sugars; but he did not give their R_f values or describe the shape of the spots obtained.

In this paper a new micromethod for the separation of Cl^-, Br^-, I^-, and CNS^- will be described. The solvent employed in the chromatograms is butanol saturated with I · 5N NH_4OH. This keeps the halides completely in the ionic form and appears to prevent any adsorption on the filter paper.

Separation is a simple matter in this method when compared with the technique required to show up the spots. After a trial of several techniques the simplest was found to be a method by which the paper was sprayed successively with two reagents and then washed and exposed to H_2S vapors.

The first method to be tried was an adaptation of that of Feigl (*2*). The silver chromate paper described there was prepared and the dried paper chromatogram was laid on top of it and sprayed with water. The halides were washed out and produced yellow spots on the red paper. This method was abandoned because the spots frequently moved on spraying with water, and also because the adsorption of halide ions on the paper might lower the sensitivity of the test.

The next method tried was spraying the chromatograms with silver nitrate solution, and then exposing to sunlight. This was found highly unsatisfactory, since a dark background formed which made the spots indistinct.

The most satisfactory method is as follows:

The paper is first sprayed with a mixture of ferric nitrate solution and hydrogen peroxide. The spraying is started from the liquid front and carried to within 4 cm of the starting point. This produces a red spot for CNS^-, R_f value = 0.42–0.48; and a blue spot for I^-, the R_f value being 0.29–0.32.

The whole of the paper is then sprayed with 0.1 N $AgNO_3$ solution, which discolors the spots originally formed and precipitates all four ions as insoluble silver salts inside the filter paper. This paper is now washed with very dilute HNO_3, so as to remove the excess of Ag^+ ions, two washes being sufficient. The paper is then held over H_2S and black AgS is formed wherever silver halides were precipitated. Chloride thus produces a brown spot (R_f = 0.1–0.11), and bromide a brown spot (R_f = 0.15–0.18).

In this paper the complete technique used will be described in detail, since a number of simplifications have been made to the method of Williams and Kirby (*8*), on which it is fundamentally based.

The aqueous phase is placed on the bottom of a 5-gal crock, where there is also a large Petri dish containing butanol. In the Petri dish is placed a measuring cylinder, fitted with a cork carrying a T-piece.

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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