Quantitative determination of tyrosinase activity has been made by chronometric (6), Warburg (1), and colorimetric (2, 8) techniques. Chronometric analysis has the disadvantage that it uses the measurement of a secondary reaction to determine indirectly the tyrosinase activity. The Warburg technique on the other hand is a direct measurement of tyrosinase activity using tyrosine substrate, but much equipment is needed to analyze several replications when many treatments are tested simultaneously. The colorimetric method of analyzing enzyme activity is also a direct method for determining the effect of tyrosine as a substrate, but when testing the effects of variations in permanent gas content on enzyme activity the equilibration of the liquid substrate to different gas contents during analysis is difficult, time consuming, and hard to maintain. Duplication of the exact conditions in subsequent trials is difficult at best. Also, in the liquid medium usually used for determination of tyrosinase activity only small quantities of tyrosine are soluble. Solubility could be increased by raising the pH or increasing the normality of NaOH, but enzyme activity would be simultaneously decreased.

It has been shown (4, 7) that substrates used by the polyphenol-oxidase enzymes could be detected on paper chromatograms by spraying them with crude tyrosinase extracts. Inhibitors of polyphenol oxidase have also been determined by similar paper chromatography techniques (5). This paper chromatography technique has not been made quantitative. The purpose of this study was to utilize this paper technique to develop a test for determining the activity of the tyrosinase enzyme that would be rapid, able to evaluate many treatments simultaneously, capable of being easily duplicated, able to detect minor changes in enzyme environment, and quantitatively accurate.

**Materials and Methods**

*Preparation of paper discs.* L-tyrosine (Eastman Organic Chemicals) was dissolved in 0.1N NaOH (0.1 ml = 500 μg). Using a 1 ml syringe with Chaney adapter aliquots of tyrosine were placed on Whatman #1 paper discs 1.3 cm in diameter set on the sharp ends of common pins held upright by being pushed through a thin piece of styrofoam. One-tenth ml of solution was sufficient to saturate the disc. Additional 0.1 ml aliquots could be added after drying in a stream of oxygen-free nitrogen to increase the tyrosine content of the discs. The dried paper discs were then placed in closed jars containing calcium chloride and held under
These tyrosine-treated discs did not turn dark if held under nitrogen. If oxygen was permitted to reach the discs they would darken and a lack of uniformity of color on the paper discs used as standards would not permit accurate quantitative evaluation.

**Apparatus and Analytical Procedures**

Holes 1.4 cm in diameter were drilled 0.2 cm apart in 0.2 x 3.0 x 42.0 cm plastic strips (Fig. 1A). A piece of clean scotch tape was placed across one side of the strip covering all of the holes. One of the strips with tyrosine impregnated discs was placed into a plastic developing cylinder 20.5 cm long and 3.2 cm in diameter (Fig. 1B). This cylinder had 0.4 cm injection holes (Fig. 1E) 1.3 cm apart. These holes were covered with scotch tape just before the test was begun. A rubber stopper containing a section of glass (Fig. 1C) was placed in each end of the tube. Water-saturated air at 20 ml/min (Fig. 1D) was delivered into one end of the cylinder and allowed to equilibrate for 15 min prior to applying the enzyme solution.

Crude enzyme extract was prepared by grinding 2 gr of fresh potato tuber tissue in cold 0.1 M phosphate buffer (pH 6.5) in a 5 ml Servall Omnimixer container held in an ice bath. The macerated extract was then filtered through a cold Buchner funnel, centrifuged in a cold room (5 C) for 5 min at 2000 g, made up to 10 ml with 0.1 M phosphate buffer, pH 6.5, and kept in an ice bath until used. Using a 100 μl syringe with Chaney adapter, 20 μl of enzyme solution were applied to the paper discs through the tape covered holes in the top of the developing cylinder. Twenty minutes after adding the enzyme solution the plastic strip containing the developed paper discs was evaluated with an Analytrol, model RB recording densitometer modified to take the 0.2 cm plastic strips. All treatments were run in triplicate. Boiled enzyme solution from the extract being evaluated was used as a control.

To determine the quantitative accuracy of the test a dilution series of 75, 50 and 25% of the enzyme solution was made and tested as described above.

**Results and Discussion**

Table 1 shows the effect of 500, 1000, and 1500 μg of tyrosine on tyrosinase activity. There was a slight decrease in activity at the 1500 μg concentration although very little difference could be observed between 500 and 1000 μg. It may be that in adding 0.3 ml of 0.1N NaOH to make paper discs with 1500 μg of tyrosine, the increased Na residue on the discs could have slightly increased the pH of the reaction solution and slightly reduced tyrosinase activity. This theory has not been tested because the decrease was slight and completely reproducible results were obtained using 500 μg of tyrosine. The discs treated with 500 μg of tyrosine gave a rate of change in enzyme activity of sufficient magnitude to accurately differentiate small variations in enzyme activity in different samples. The 500 μg of tyrosine was much in excess of that which could be dissolved in the buffer solutions used in standard colorimetric tests (2,8). Excess tyrosine reduces the possibility of a reaction being substrate limiting.