When benzaldoxime is chromatographed at either 200° or 250° over 2 feet of high vacuum-silicone grease on "Celite", the chromatogram obtained is similar to the chromatogram obtained over silicone rubber (Fig. 1), but the product eluted from the column is pure benzonitrile. Chromatography of salicylaldoxime at 200° over the same silicone grease column partially converts it to *o*-hydroxybenzonitrile and at 250°, the conversion is complete. At 200°, the chromatography of a mixture of (I) and (II) over the same silicone grease column gives a complicated nonreproducible chromatogram (Fig. 3). The infrared spectrum of the sum of the eluted compounds shows the presence of both unconverted oximes and the corresponding nitriles.

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A simple microtechnique for the preparation of biological samples for paper chromatographic analysis

In the course of our work on the biochemical analysis of insect tissues and body fluids, it was necessary to develop chromatographic microtechniques applicable to individual insects weighing as little as 2 mg and body fluid samples as small as 10 μl. Although the method described relates particularly to the analysis of amino acids in biological samples, the same method, with slight modifications, has been satisfactorily used in our laboratory for analyses of carbohydrates, organic acids, and nucleic acid components as well as to follow chromatographically such enzyme reactions as transamination.

The chemical procedures used for the extraction of amino acids are those of AWAPARA[1]. All steps in the extraction procedure are carried out inside a U-tube shown in Fig. 1(a). A small filter paper disc (0.5 cm diameter) is punched from Whatman No. 3 MM filter paper and is fitted snugly at the junction between the capillary and the wide tube inside arm A so that it covers the capillary and rests flatly on it. The mouth of arm B is fitted with a cork stopper which can be manipulated within limits without breaking the air seal. A known volume of any biological fluid to be analyzed is deposited on the paper disc by means of a micropipette. Eighty percent ethanol (0.3 to 0.5 ml) is then slowly added to the disc. The liquid is made to pass up and down through the disc a few times by manipulating the cork stopper

and then held there until extraction is complete (30 min–1 hour). The stopper is then released to let the alcohol extract descend into the capillary.

When whole insects are to be extracted, as was the case in our work on larval and adult mosquitoes, the specimens are lightly anesthetized with ether and a known number dropped on the filter paper disc. A small wad of glass wool is placed on the sample and it is macerated by means of a thin glass rod flattened at one end. The amino acids are then extracted with ethanol as described above. The precipitated proteins as well as the cell debris are left on top of the disc and when the stopper in arm B is released, a clear alcohol extract descends into the capillary part of the tube.

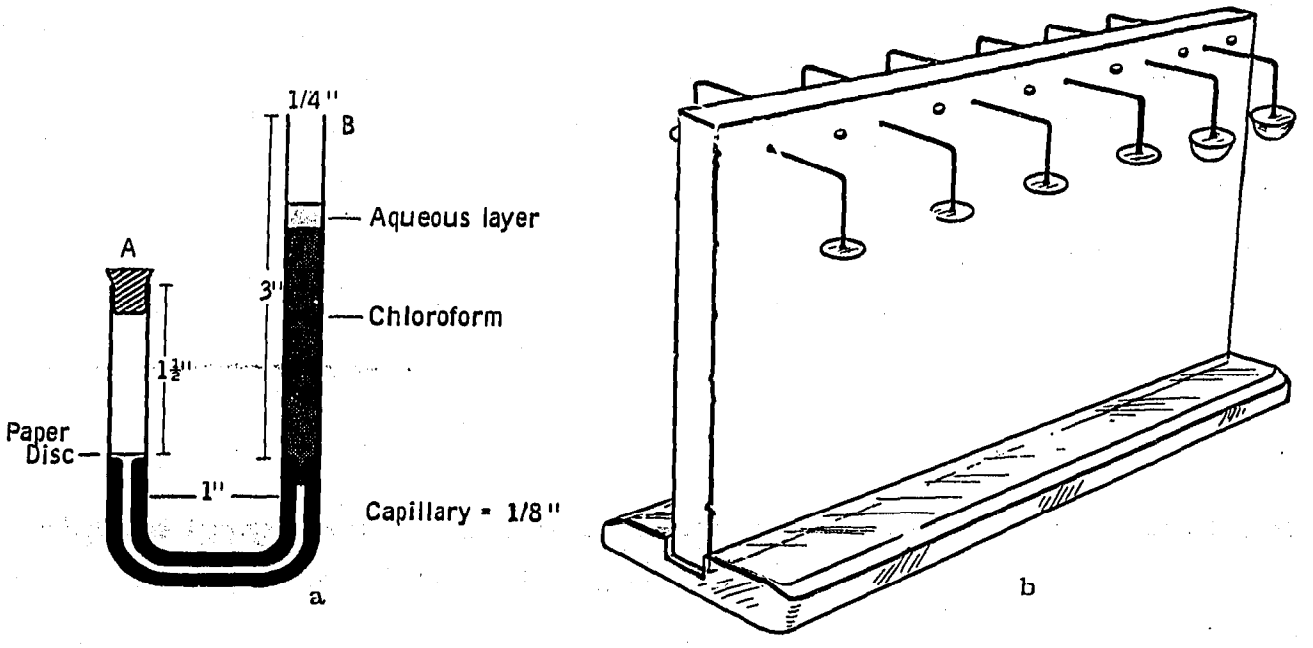

Fig. 1. (a) Apparatus for extraction of free amino acids from biological samples. (b) Preparation of filter paper discs for chromatography.

The stopper is now transferred to close the opening of arm A and it is pressed down so that most of the alcohol extract rises into the wide part of arm B. Three volumes of chloroform (up to 1.5 ml) are now added to the alcohol extract. Mixing and separating the layers is facilitated by manipulating the stopper. The clear aqueous layer which results can be drawn into a micropipette for spotting.

Fig. 1 (b) shows an arrangement used for depositing liquid samples on filter paper discs. Circular discs (0.5 cm in diameter) of Whatman No. 1 filter paper are punched out and suspended from the points of stainless steel pins attached to a cork board stand. This stand is placed inside a desiccator and the extract deposited on discs, a sufficient volume applied to each disc (50–70 μl) so as to hang down as a drop. The desiccator is then placed under vacuum. After the discs are dry, a second application of the extract is made, if necessary. The discs are then removed and attached to the filter paper by pushing them through two horizontal slits cut at the bottom of the paper.

Fig. 2 shows that the separation of amino acids by the U-tube filter disc tech-

nique (2 and 4) was as good or better than that obtained by the conventional procedure of extraction and direct spotting (1 and 3), and also seemed to result in a better quantitative yield, apparently due to the elimination of numerous manipulations.

Recovery was determined by applying aliquots of different dilutions of a test amino acid, alanine, to filter paper discs, and developing the chromatograms in a *n*-butanol–acetic acid–water solvent (4:1:5 v/v) on Whatman No. 3MM paper.

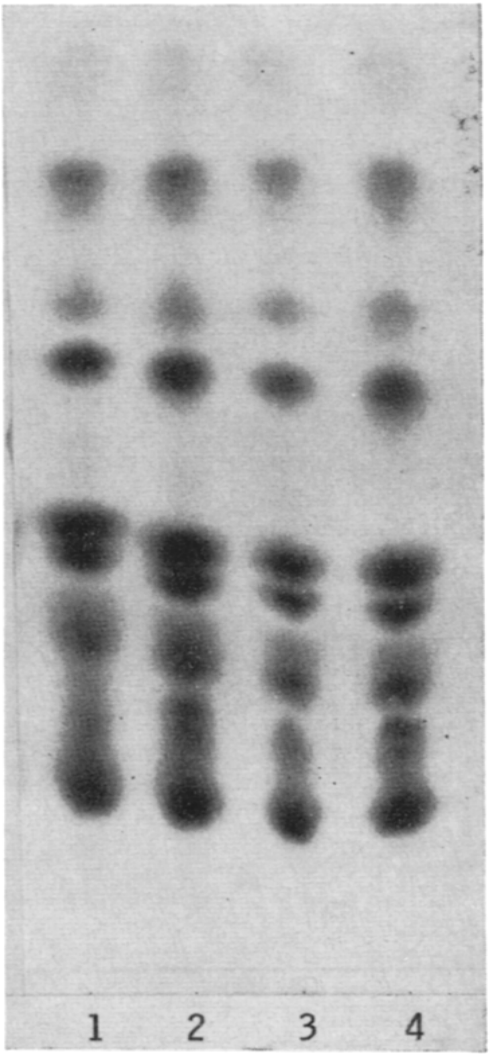

Fig. 2. Free amino acids of fourth stage *A. aegypti* larvae (UTMB strain). 1 and 3 = conventional extraction and direct spotting; 2 and 4 = extracted and spotted according to the technique described. Twice as much extract was spotted in 1 and 2 as in 3 and 4.

Recovery of alanine, as measured by the ninhydrin procedure, was highest (100 to 102 %) when the volume of the extract deposited was in the range 50 to 150 μl. Lower recoveries were obtained (91 to 97 %) when volumes less than 50 μl were employed. This is due to the fact that a small amount of the extract is also deposited on the part of the pin extending slightly below the disc and the resulting loss becomes significant when the extract is concentrated. This error can be greatly reduced by

taking care that the sharp end of the pin sticks into the paper disc just enough to hold it while spotting.

By using a series of filter paper discs, a large number of extracts can be spotted and dried simultaneously, resulting in a tremendous saving of time. In some of our experiments, as much as 0.5 ml of an 80 % alcohol extract was applied to each disc and as many as 100 samples of this size were prepared for analysis on chromatograms in a period of a few hours. In addition, all samples are thus confined to equal areas rendering corresponding amino acids on chromatograms more precisely comparable. Furthermore, while being spotted, the extracts are not subject to varying and high temperatures destructive to some of the components, as would be the case when the fluid is directly applied to the paper and is dried by a current of hot air. In the present technique, the temperature at which the spots are dried can be regulated, if necessary, by placing the evacuated desiccator in an incubator. Also, filter paper discs on which known quantities of amino acids, their mixtures, or hydrolysates of proteins have been deposited, have been stored in a desiccator in our laboratory without deterioration and have been employed satisfactorily as standard indicators in paper chromatography.

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The reaction of OsO_4 with iodide in acid solution
A chromatographic study

Paper electrophoresis and paper chromatography have lately contributed to the understanding of reactions of stable complexes of Rh, Ru and other elements (for a review see ref.[1]). A recent note by FENN *et al.*[2] describes the formation of several Os(IV) iodo- and chloro-complexes by the reaction of osmium tetroxide with potassium iodide in hydrochloric acid solution. Since the reaction as well as the processes involved in the isolation of the complexes appeared to be rather complicated we thought it would be of interest to study the reaction by chromatography.