rétention est de 173 sec pour O_2, 300 sec pour N_2, et 400 sec pour NO. L'analyse quantitative de NO est plus délicate car son élution n'est pas totale. Il reste environ 40 mm³ sur la colonne adsorbés de façon très durable (plus de deux heures dans nos conditions). Pour avoir des résultats quantitatifs très reproductibles, il faut fournir à la colonne une certaine quantité de NO pour atteindre un pseudo équilibre. On fait donc passer 200 mm³ de NO quatre fois avant d'utiliser la colonne pour l'analyse désirée. On peut alors opérer dans les deux heures qui suivent et obtenir une reproductibilité parfaite. Le pic est dissymétrique et il convient de mesurer sa surface. La méthode est d'autre part plus précise si on opère sur des quantités supérieures à 150 mm³. On a donc intérêt à ajouter aux échantillons à analyser de 100 à 200 mm³ de NO préalablement mesurés. La Fig. 2 montre d'ailleurs l'étalonnage donnant la surface des pics en fonction du volume en mm³ introduit sur la colonne et mesuré préalablement à la jauge de Mac Leod sous pression réduite.

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Reçu le 5 novembre 1958

Homogenization and extraction of biological material for chromatographic purposes

The preparation of biological samples for chromatographic analysis usually involves homogenization and extraction. These procedures are very laborious and they expose the investigated samples to loss and enzymic changes. Moreover they require at least decigram amounts of the preparation, whereas for the paper chromatographic analysis a hundredth of this amount suffices.

A simple method was elaborated in our laboratory for the homogenization and extraction of small amounts of biological material directly onto the chromatographic paper.

Homogenization

The new homogenization procedure is based on the new method of botanical investigation devised by MEDVEDEV[1,2] and the method for studying simple compounds in plants described by GREENSHIELDS[3], as well as on the method of MORGAN AND WICKSTROM[4]. The chromatographic paper is prepared as for one-dimensional, two-dimensional or circular paper chromatography. The preparation to be investigated (hyphae, a small piece of leaf, corola, thick section of fruit, stems, etc.) is placed on the starting point; plastic foils (6 × 6 cm) are placed under the chromatographic paper

and over the preparation and then the whole is placed between two polished steel plates (Fig. 1) and submitted to transient high pressure by means of a blow with a hammer or a suitable spring apparatus. The transient high pressure causes the plant cells to burst so that their contents flow onto the paper where they are instantly absorbed. The spot is then dried with a hair dryer to interrupt the enzymic processes in the juice. To prevent loss of juice by absorption on the upper plastic foil the sample can be placed between two chromatographic papers. After pressing, the two spots

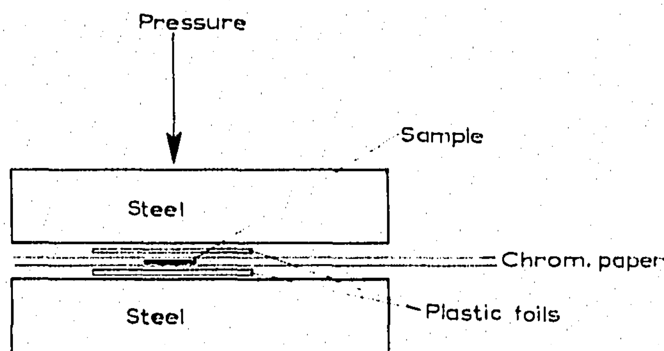

Fig. 1

obtained are transferred onto one chromatogram by some method of rechromatography[5,6].

By the isotopic method using ^{32}P it was confirmed that less than 0.1% of phosphorus contained in plant juice is absorbed on the plastic foil, if the sample is placed between two papers.

In the investigation of virus necrosis on leaves, infiltrated spaces of leaf etc., small pieces of leaf can be cut out with a cork borer or other perforator (1–4 mm in diameter).

It is also possible to apply this method to the investigation of zoological preparations, such as whole small insects, parasites or separate organs (glands, nerves etc.), small sections of muscle, skin, etc.

Extraction

If the total juice pressed out of the preparation cannot be analysed because of contaminating or inhibiting substances, the analysis can be accomplished after extraction of the desired ingredients by a suitable solvent. The extraction can also be carried out on the chromatographic paper.

For this purpose the biological sample is homogenized on the chromatographic paper as described above. When the sample has been subjected to pressure at the starting point, the ingredient of the sample can be extracted by allowing a suitable solvent, instead of the developer, to flow along the chromatogram.

The extracted substances can be collected in a tube according to the method of DENT[7] or on a small tongue of chromatographic paper according to the method of

OERTEL[8]. The small tongue of paper with the collected substances can be used as the spot of origin for the new chromatogram[5], or the substances can be extracted with a suitable microextractor for further analysis[9].

If the substance to be investigated is present in dilute solution, it can be concentrated by the method devised by KRZECZKOWSKA[10]. This method can be combined with that of GREGORY[6] for transferring a spot from one chromatogram to the origin of another. The combination of these two methods is illustrated in Fig. 2.

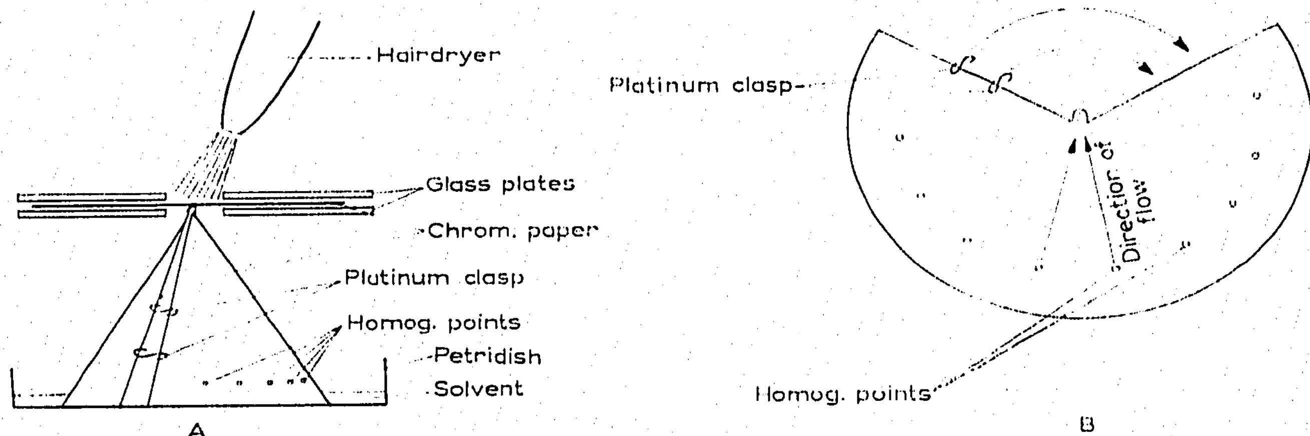

Fig. 2

The same preparation can be extracted several times by using different specific solvents for the various compounds[11].

The method described is especially convenient for radiochromatographic analysis of biological material, because it offers to the possibility of analysing small amounts of the sample and because of the simplicity of the technique.

Acknowledgements

The author wishes to express his thanks to Professor Dr. W. MOYCHO for his helpful criticism of this work and to Mrs. E. ANTOSZEWSKA for her kindness in supplying the necessary equipment.

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Received October 29th, 1958

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