The use of gas-liquid chromatography–mass spectrometry in biochemical pharmacology

H. R. HAZELTON, P. JOHNSON and B. C. WEATHERLEY, *Biophysics and Biochemistry Laboratory, Wellcome Research Laboratories, Beckenham, Kent*

Although the use of paper, thin-layer, ion exchange and conventional column adsorption chromatography has greatly facilitated the separation and identification of substances of biological interest, the accurate identification of closely related substances cannot be relied upon solely by R_F values even with the use of co-chromatography.

Mass spectrometry has been used for many years to identify compounds but requires isolation of individual substances. The advent of gas–liquid chromatography has allowed rapid separation of many closely related substances. The combined gas chromatograph–mass spectrometer therefore provides a sophisticated technique for identification of individual components in mixtures of biological substances.

We have coupled an F & M 5750 series gas chromatograph to an EAI Quad 300 mass spectrometer with fast ultraviolet recorder.

The fast scanning speed (total spectrum <1 s) of this particular mass spectrometer has been utilized to scan repetitively during the time taken for components to emerge from the gas chromatograph. The scanning is controlled by an optical switch on the GLC recorder which automatically activates the recorder of the mass spectrometer. This ensures an optimum mass spectral response for a given gas chromatographic peak and also facilitates resolution of inadequately resolved components from the chromatograph.

The combined instruments will be demonstrated in relation to the analysis of amino-acids and basic drugs.

Analysis of radioactively labelled compounds of pharmacological interest by gas-liquid radiochromatography

H. R. HAZELTON and P. JOHNSON, *Biophysics and Biochemistry Laboratory, Wellcome Research Laboratories, Beckenham, Kent*

A Perkin–Elmer/Berthold RGC 170 gas flow proportional counter has been modified to facilitate direct coupling to an F & M 5750 series gas chromatograph using the shortest possible heated transfer line. The effluent from the chromatographic column is passed through a two-way stream splitter, one part going to the flame ionization mass detector. The other part passes through the heated transfer line to the combustion furnace of the RGC 170 radioactivity detector. Here the sample may be converted either by oxidative combustion or hydrogenative cracking into a mixture of permanent gases for counting at room temperature in the proportional counter.

Such a continuous-flow system allows all emergent peaks containing enough radioactivity to be monitored whether or not the mass of the peak is sufficient to be detected by the flame ionization detector.

The ability to convert the chromatographic effluent to permanent gases either by hydrogenation or by oxidation means that ^{14}C compounds, for example, can be converted to $^{14}CO_2$ or $^{14}CH_4$ and ^{3}H-compounds to C^3H_4 and N^3H_3.

For ^{14}C compounds, oxidation is the normal method of choice, as our experiments with the analysis of ^{14}C-fatty acid methyl esters on diethylene glycol succinate columns, and of basic drugs on methyl phenyl silicone (OV17) columns, have shown that a slight loss of sensitivity occurs when hydrogenative cracking is used. This may be explained by the decreased residence time of the radioactive sample in the proportional counter due to an overall increase in gas flow rate which is necessary for the hydrogenative procedure.

The use of this equipment in the analysis of labelled fatty acid esters, drugs and other compounds of pharmacological interest will be demonstrated.

The study of antimalarial compounds *in vitro*

W. H. G. RICHARDS and S. G. WILLIAMS (introduced by P. J. GOODFORD), *Department of Protozoology, and Biophysics and Biochemistry Laboratory, Wellcome Research Laboratories, Beckenham, Kent*

Improvements in the culture medium (Cohen, Butcher & Crandell, 1969) have made it possible to culture the intra-erythrocytic forms of malarial parasites through a complete asexual reproductive cycle. The demonstration will show how the effect of drugs on this life cycle can be studied in such a defined medium *in vitro*.

Infected erythrocytes are incubated in the medium containing ^{3}H-labelled leucine with and without drug being present. At intervals small portions of the culture are removed and the radioactivity in the protein fraction which is insoluble in trichloracetic acid is determined by scintillation counting (Cohen *et al.*, 1969; Byfield & Scherbaum, 1966). In this manner the effect of the drug on the plasmodial protein metabolism may be quantitated. Antimitotic effects of the drugs are determined histologically from smears of the culture cells taken at intervals during the incubation.

To gauge the effect of the drug on the metabolism of the erythrocytes, which are host cells to the parasite, the ATP and K^+ content of these cells is determined. The latter assay is performed by atomic absorption spectroscopy and the former by the fire-fly luciferase method. Optimal use of the automatic scintillation counter for this assay is demonstrated (Stanley & Williams, 1969).

REFERENCES

BYFIELD, J. E. & SCHERBAUM, O. H. (1966). A rapid radioassay technique for cellular suspensions. *Analyt. Biochem.*, **17**, 434–443.

COHEN, S., BUTCHER, G. A. & CRANDELL, R. B. (1969). Action of malarial antibody *in vitro*. *Nature, Lond.*, **223**, 368–371.

STANLEY, P. E. & WILLIAMS, S. G. (1969). Use of the liquid scintillation spectrometer for determining adenosine triphosphate by luciferase enzyme. *Analyt. Biochem.*, **29**, 381–392.

Methods for the study of nucleotide, nucleic acid and protein metabolism

I. C. CALDWELL† (introduced by P. J. GOODFORD), *McEachern Laboratory, University of Alberta, Edmonton, Alberta, Canada*

Many drugs, particularly those acting on proliferative tissues, are known to have effects upon the formation and metabolism of nucleotides, nucleic acids and proteins. A number of newly developed methods applicable to studies of the effects of drugs upon these metabolic parameters will be demonstrated.