

For  $^{14}\text{C}$  compounds, oxidation is the normal method of choice, as our experiments with the analysis of  $^{14}\text{C}$ -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***

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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  $^3\text{H}$ -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 trichloroacetic 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  $\text{K}^+$  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**

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

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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.

Nucleotides in neutralized acid extracts of 1 g or more of tissue are resolved and isolated by application of the extracts to long ( $1 \times 100$  cm) columns of DEAE-Sephadex A-25, acetate form, followed by elution of the nucleotides with consecutive concentration gradients of triethylammonium (TEA) acetate, pH 4.7, pooling of appropriate portions of the eluate and removal of the volatile TEA salt at reduced pressure. This procedure affords a high degree of resolution of tissue nucleotides in a single chromatographic run and provides salt-free samples suitable for further analysis (Caldwell, 1969).

When smaller amounts of tissue or tissues with relatively low nucleotide content (for example uterus) are analysed, chromatography on thin layers of polyethylenimine (PEI)-cellulose is utilized. The extracts are concentrated and partially purified on small ( $0.5 \times 2$  cm) columns of PEI-cellulose, the nucleotides being eluted with solutions of TEA bicarbonate, pH 8.5. These final eluates are dried at reduced pressure to remove the volatile eluant, the residues are dissolved in minimal volumes of water and applied to commercially available thin layers of PEI-cellulose. The chromatograms are then developed by the procedure of Randerath & Randerath (1964).

A procedure which permits the facile isolation of nucleotides, several species of nucleic acid and protein from single tissue samples by a phenol-detergent technique will also be demonstrated. Nucleotides and the bulk of the cellular RNA are selectively extracted at low ionic strength, followed by specific precipitation of the RNA with cetyltrimethylammonium bromide and resolution of the RNA into low- and high-molecular weight fractions at high salt concentrations. DNA (and closely associated RNA) are then extracted from the cellular residue at high ionic strength; alkaline hydrolysis of this extract followed by precipitation of the DNA yields the DNA-bound RNA as constituent nucleotides. The residual cellular material is then treated with organic solvents to remove lipid, leaving total cellular protein as the final residue.

A number of supplementary procedures which extend the usefulness of the above techniques will also be demonstrated.

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#### **An all-glass small animal metabolism unit with "fail safe" air supply and $^{14}\text{CO}_2$ collection device for long term continuous radiorespiration studies**

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The following criteria should apply to an effective small animal metabolism unit:

1. The physiological state of the animal should be as near normal as possible, with the provision of free access to food and water, minimal restriction on movement and the avoidance of abnormal environmental conditions.