

Nucleotides in neutralized acid extracts of 1 g or more of tissue are resolved and isolated by application of the extracts to long (1×100 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 (0.5×2 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.

* Present address: Biophysics and Biochemistry Laboratory, Wellcome Research Laboratories, Beckenham, Kent.

REFERENCES

- CALDWELL, I. C. (1969). Ion-exchange chromatography of tissue nucleotides. *J. Chromat.*, **44**, 331-341.
- RANDERATH, E. & RANDERATH, K. (1964). Resolution of complex nucleotide mixtures by two-dimensional anion-exchange thin-layer chromatography. *J. Chromat.*, **16**, 126-129.

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

W. G. DUNCOMBE and P. JOHNSON, *Chemical Research Laboratory and Biophysics and Biochemistry Laboratory, Wellcome Research Laboratories, Beckenham, Kent*

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.

2. The separation and collection of urine and faeces should be efficient with minimal risk of cross-contamination.

3. Quantitative measurement of food and fluid intake should be possible, with minimal contamination of excreta by solid or liquid diet.

For radiorespiration work, the following additional requirements should be met:

4. The animal must be housed in a completely closed system.

5. Efficient and continuous trapping of $^{14}\text{CO}_2$ should be possible.

6. The air flow through the unit should be sufficient to prevent accumulation of expired CO_2 or water vapour.

7. An emergency air supply should be automatically switched to the cage in the event of failure of the normal supply.

8. It should be possible to wash residual urine from the cage into the collection flask without interruption of CO_2 collection.

9. Easy decontamination of all parts of the unit must be possible.

These criteria have been met in the unit which will be demonstrated.

An all-glass urinary/faecal separator (Draper & Robbins, 1956) and food and water reservoirs are connected by ground glass joints to a glass metabolism cage. Expired CO_2 is absorbed in a train of three scintillation vials containing organic base compatible with liquid scintillator for direct counting. The expired air may be switched at any time through a parallel series of vials or through larger absorbers for overnight collection.

A vacuum pump provides an air-flow of 500 ml/min. The incoming air passes through a CO_2 trap and drying agent before entering the metabolism cage. The vacuum manifold may be connected simultaneously to six parallel metabolism units, each one equipped with a flow controller. In the event of failure of vacuum or electrical supply, a supply of air from a cylinder is switched on automatically *via* solenoid valves.

REFERENCE

- DRAPER, H. H. & ROBBINS, A. F. (1956). A uricofecal separator for laboratory rat. *Proc. Soc. exp. Biol. Med.*, **91**, 174-175.

Drug resistance in *Babesia rodhaini*

ELIZABETH BEVERIDGE, *Department of Protozoology, Wellcome Laboratories of Tropical Medicine, Beckenham, Kent*

The rodent piroplasm *Babesia rodhaini* is normally maintained in rats (or in mice) by serial, twice weekly, intraperitoneal inoculations of parasitized blood. Three lines of this rat strain were made drug-fast to the babesicidal compounds Diminazene, Amicarbalide and Imidocarb by injecting subcutaneously at the time of inoculation of parasites a dose level of drug which would allow the development of a parasitaemia roughly equal to half that of the normal strain (ED50). As resistance to the drug developed, the dose levels were increased until the maximum tolerated level was reached.