Technical methods

A carbon-dioxide-in-air incubator

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A simple, inexpensive modification of a standard anhydric laboratory incubator is described which permits a steady inflow of 20 to 60 ml of CO_2 per minute and provides an atmosphere suitable both for the growth of CO_2 requiring bacterial cultures and of open-culture tissue cultures.

Method

A 12 mm hole was made from within the incubator[1] by drilling alongside the thermostat sensing bulb at the top, rear, inner corner which avoids the electrical wiring and another was made through the outer cabinet to coincide with it.

A flowmeter[2] was fixed byself-tapping screws in a convenient position on the outer cabinet (or an adjacent wall). This was connected by tubing[3] through to the interior of the incubator, protruding approximately 2 cm inside, and to the carbon dioxide cylinder[4] via a fine adjustment valve,[5] gas regulator, and cylinder adapter[6].

The rate of flow is governed by the fine adjustment valve and an initial period of four or five days is required for the flow to become stable. This precludes the use of an electric pump which would be subject to wear and tear and add considerably to the expense.

[1]Incubator LEEC 'Precision' Incubator P2, Laboratory & Electrical Engineering Co, Private Road No. 7, Colwick Estates, Nottingham. The model used was pre 1969 and had a mechanical catch. More recent models have a magnetic sealed door and may not give similar results.

[2]Flowmeter type 1100 S.S. 300. Calibrated to 150 ml per minute. Carbon dioxide. Scale on tube, 300 mm in length: Tube D3, Glass. Free float of stainless steel, inlet and outlet vertical nozzles. Rotameter Manufacturing Co. Ltd, 330, Purley Way, Croydon. Surrey CF9 4PG.

[3]Translucent vinyl tubing. 18H. shore 70. Portland Plastics Ltd, Hythe, Kent.

[4]Carbon dioxide 28 lb cylinder. The Distillers Company (Carbon Dioxide) Ltd.

[5]Fine adjustment single outlet control valve RH ref. 123882. British Oxygen Company.

[6]Gas regulator M.30.OG ref. 147328 Cylinder adaptor. ref. 147154, hose connexion nipple ⅜ in. ref. 032030, hose connexion nut ref. 032035, cyclinder stand 7 in. diameter ref. 32415. British Oxygen Company.

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To include an adjustable leakage aperture would also increase the cost of the modification which is essentially cheap and simple to carry out, and to 'swamp' the cabinet with a greater flow of gas would increase running costs and necessitate a more frequent change of the CO_2 cylinders with added inconvenience.

One 28 lb (12·7 kg) cylinder of carbon dioxide gas lasts approximately three months in continuous use. Cylinders are changed with as little variation of the fine adjustment valve as is needed to maintain the required flow.

For open-culture tissue cultures a moist atmosphere is maintained using metal boxes containing wet filter paper and with loosely fitting lids. (For example, microscope slide cultures can be laid on racks of glass tubing in Carbonet tulle gras dressing tins which are inspected daily and re-charged with distilled water as required).

This system allows tissue cultures to be maintained in the same incubator simultaneously with bacterial cultures and prevents corrosion of the incubator walls and fittings which would occur if the whole cabinet was humidified using a water bath on the bottom shelf.

CO_2 in Air Concentration

The incubator used was fitted with a glass inner door without a gasket and an outer door with a ribbed rubber seal mitred at the corners. This allowed leakage of the CO_2 air mixture out of the incubator and so prevented a build up of CO_2 to a high concentration.

Samples of gas were collected through one of the holes in the glass inner door of the incubator which are provided to hold a thermometer in place. A short piece of soft rubber tubing was pushed into the hole and a long, fine cannula passed through into the incubator cabinet; the position of the distal end of the cannula was adjusted as required. Gas sampling tubes with double oblique bore stopcocks (Gallenkamp GF-405) were filled by mercury displacement.

Estimations of the CO_2 concentration within the incubator were made by the method of Lloyd (1958) using the Lloyd gas analyser (Gallenkamp GC-400) involving absorbtion of CO_2 with half-saturated potassium hydroxide solution.

Twenty-four gas samples were taken at varying CO_2 flow rates (20 ml to 60 ml per minute) at varying

times after the incubator doors had been opened and closed (45 minutes to 45 hours), and in the centre of the highest and lowest shelf positions.

The CO_2 concentration varied between 3·1% and 7·2% and in practice a rate of between 20 and 60 ml per minute was found to support the growth of carbon dioxide requiring organisms such as newly isolated strains of *N. gonorrhoeae* and *Br. abortus* (including biotype 2).

The cost of the modification totalled £35 and the running costs are approximately 10p per week.

Reference

Lloyd, B. B. (1958). Development of Haldane's gas analysis apparatus. *J. Physiol. (Lond.)*, 143, 5-6.

An inexpensive and demountable walk-in incubator

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In many areas of scientific work there are advantages in the use of walk-in incubators or 'hot rooms'. Those models which are available commercially are, however, expensive and, in the event of the rearrangement of the laboratory in which they are housed are extremely cumbersome to dis-assemble. During the development of the virus laboratory in the Department of Microbiology at King's College Hospital Medical School considerations such as these led us to design an inexpensive and demountable hot room.

Structure of Hot Room

The room is 2·4 m × 1·75 m, with a sloping roof, the height of which ranges from 2·25 m to 2·6 m; its volume is 10·5 m^3. The roof slopes for ease of dusting and to avoid the collection of debris which often occurs on low-level flat roofs. The walls and roof consist of expanded polystyrene bonded to hardboard and are of about 5 cm thickness. They slot into and are supported by a framework of anodized extruded aluminium girders of a type specially prepared for and supplied by Murr Internal Constructions Ltd. These girders are H-shaped in cross section and the depth of the slot on each side is about 1·25 cm. The H shape is modified at corners and at roof-wall junctions.

A single standard-size door is fitted; this fits into an aluminium frame which has a 7·5 mm × 3 mm foam rubber sealing strip inserted, providing a tight fit at top and sides. At floor level we have found no need for a draught excluder (but one could easily be installed) and we consider that it would be dangerously easy to trip over a raised floor 'rebate'.

Heating Equipment

The source of heat is a 3 kw fan heater (type General Electrical Company H6448D) which is mounted in an upper corner of the room and set to direct the air flow downwards into the opposite lower corner. An Accuron unit controls the heat output of the fan *via* a thermistor probe which is mounted behind the

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