Technical method

A simple method for incubating bacterial cultures in predetermined concentrations of carbon dioxide

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There is a surprising lack of data on the optimum carbon dioxide requirements of various CO_2-dependent organisms. Furthermore, there are no clear indications that the atmosphere of CO_2 obtained in a candle jar may not be suitable for culturing most CO_2-dependent organisms as only a few studies have been made on the subject. For *Brucella abortus* a concentration of 5 to 10% with a normal partial pressure of oxygen has been shown to be optimum (Wilson, 1931).

For *Neisseria gonorrhoeae* data are not unequivocal. Ferguson (1945) found no differences in growth in a wide range of CO_2 concentrations (2-22%); other data (James-Holmquest, Wende, Mudd, and Williams, 1973) indicate an optimum CO_2 concentration of about 3 to 4%, obtained by using a candle jar. Apart from these differences the use of candles in jars is somewhat untidy. Moreover, CO_2 concentrations in candle jars seem to be dependent on the size of the jar (Nye and Lamb, 1936; Ferguson, 1945).

The answer to these objections to candle jars may be found in the use of CO_2 incubators which are now commercially available. Most of these incubators fulfil the requirements in microbiological laboratories. Nevertheless, they have certain drawbacks of which the most important is the excessive use of CO_2 as the incubator is repeatedly opened during the day. In most diagnostic laboratories this is unavoidable. Consequently, cylinders of CO_2 must be frequently changed and full cylinders be immediately available. Another disadvantage may be the unequal distribution of the gas within the incubator. Finally, for some laboratories the high cost may be prohibitive.

The CO_2 dosage system described here lacks these drawbacks.

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Method

The principles of the system are: (*a*) the use of an expansion drum having the same volume as the container to be used for the incubation of the cultures; (*b*) leading CO_2 into this drum from a cylinder and obtaining an atmospheric pressure of the gas by means of a valve; (*c*) the exchange of a given volume of air in a sealed container for the same volume of CO_2 from the expansion drum.

For culturing CO_2-dependent organisms glass McIntosh jars are used (Baird & Tatlock (London) Ltd).

Binding clamps, metal covers with one outlet and valve, and the dosage apparatus were made in the

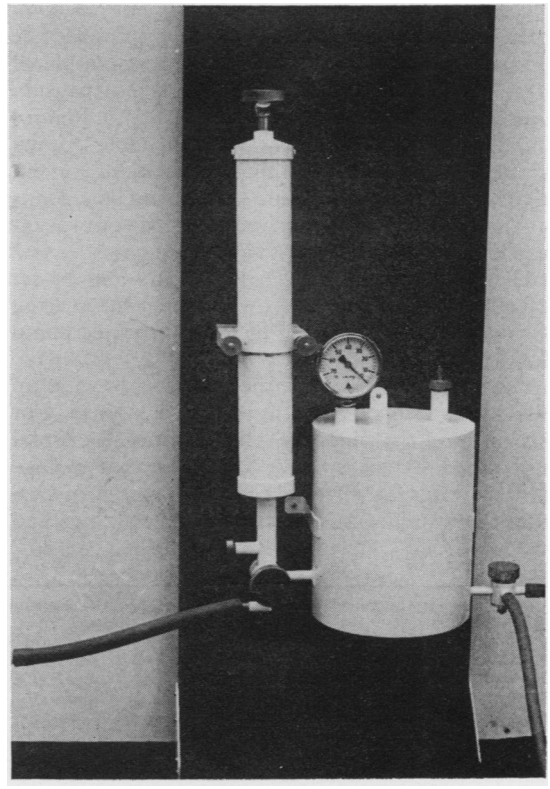

Fig 1 *Photograph of carbon dioxide dosage system.*

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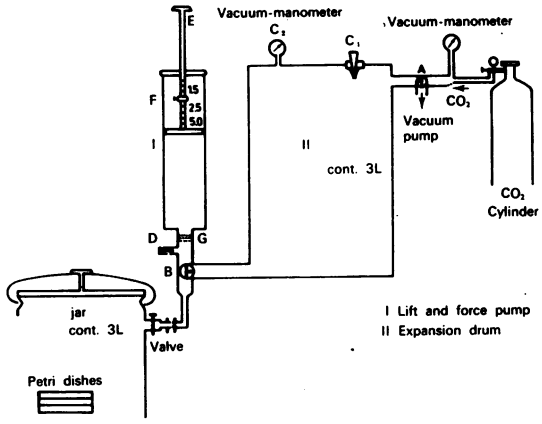

Fig 2 *Diagram of carbon dioxide system. A, B, and D, valves; C1, safety valve; E, plunger of lift and force pump; F, ring to adjust pump cylinder volume; G, screw-thread connexion of pump cylinder.*

Division of Mechanics of our department but commercial covers and clamps will serve as well. The apparatus itself was made of chromium-plated brass. The volumes of both jars and the expansion drum are approximately the same (3270 ml). Figures 1 and 2 illustrate the apparatus, and table I shows the steps required in obtaining the required content of CO_2. The whole procedure takes about 90 seconds of which about 60 seconds are required for evacuating the expansion drum with a vacuum pump.

The concentration of CO_2 in the jars may be set at will from $\pm$ 2 to 12% by changing the placing of ring F on the plunger of the hand-operated pump (fig 2).

The apparatus may also be used for drawing up CO_2 at atmospheric pressure with a syringe. For this purpose the pump-cylinder is screwed off and is replaced by an adaptor onto which a syringe is fitted. After drawing the required amount of CO_2 from the drum, a needle is fixed to the syringe and the gas injected into a culture bottle, which is sealed with a rubber cap. To prevent contamination of the medium in the culture bottle, a sterile paper filter is fitted into the adaptor. *Brucella abortus* has been isolated from Castañeda bottles to which CO_2 had been added in this way.

Concentrations of CO_2

Although for most bacteriological purposes it is not necessary to adjust the CO_2 content of culture jars precisely, it was interesting to determine the degree of accuracy attainable with the apparatus. Determinations of CO_2 were performed at several settings of the plunger in the pump-cylinder using the method of Haldane and the gas-analyzer described by Lloyd (1958) (Gallenkamp-Lloyd gas-analyzer GC-370, Gallenkamp London).

Concentrations of CO_2 in the jars were predicted by including the following data in the calculation: (1) expansion of the air and CO_2 respectively to subatmospheric pressure by lifting the plunger to its maximum at the set scale; (2) volume of the cylinder with lifted plunger at the set scale; (3) a 92% CO_2 content of the drum (obtained by introducing CO_2 after evacuating to $-$ 70 cm Hg).

Position of Ring F on Scale of Plunger	*Height of Gas Column in Pump Cylinder (cm)*[2]	*Volume of Pump Cylinder (ml)*	*CO_2 Content (%)*	
			Calculated[1]	*Observed*
4·2	23·9	400	10·25	10·25
				10·61
4·1	23·3	390	10·0	10·04
				10·09
2·1	12·0	201	5·38	5·9
				5·6
2·0	11·4	190	5·11	5·13
				5·1
1·9	10·8	181	4·84	4·81

Table II *Calculated and observed carbon dioxide contents of glass jars of 3270 ml volume*

[1]On the basis of 92% CO_2 concentration in expansion drum
[2]Inner diameter of cylinder 4·62 cm

1 Adjust pump-cylinder volume by fixing ring F at required scale number
2 Connect jar to apparatus with plunger E in down position
3 Check position of valves: valve A should connect expansion-drum with vacuum pump; valve B should connect pump-cylinder with jar and be closed to expansion-drum; close valve D; open valve of jar
4 Pump out air to $-$ 70 cm Hg as shown at C2
5 Connect expansion-drum with CO_2 cylinder by turning valve B
6 Let in CO_2 from CO_2 cylinder until excess pressure results in escape through safety valve C1, and close valve A to expansion-drum
7 Lift C1 for a second to establish atmospheric pressure in expansion-drum (II)
8 Lift plunger E as far as possible, close valve of jar
9 Open valve D and press down plunger E
10 Close valve D and turn valve B to connect pump-cylinder with expansion drum
11 Lift plunger E as far as possible
12 Turn valve B to connect pump-cylinder with jar only
13 Open valve of jar and press down plunger E
14 Close valve of jar and disconnect jar

Table I *Directions for use of CO_2-dosage apparatus*

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Table II lists the calculated and obtained concentrations of CO_2 in the jars. Most of the determinations show remarkable accuracy in the setting for the required CO_2 concentrations. The few exceptions were deviations of less than 10% of the required concentration. Calculating deviations in CO_2 content, caused by varying numbers of petri dishes in the jars, resulted in a maximum discrepancy of 7·3% from the set CO_2 concentration. If necessary, this may be corrected by adjusting the setting of the plunger. The use of somewhat smaller jars does not impair the performance of the apparatus. Calculation of the CO_2 content of a jar of 2500 ml, with a setting of the plunger adjusted to a 10% concentration for this size of jar, showed that with the expansion drum described, a concentration of 10·02% will be obtained.

These results indicate that the apparatus has a performance which is amply sufficient for the purpose intended. Handling the apparatus presents no difficulties for technicians.

I am indebted to Mrs J. A. van der Bult and W. A. van Beek of the Division of Mechanics, Department of Medicine, for their helpful advice and for the construction of the apparatus. I also wish to thank Drs R. J. M. de Leeuw and his technical staff for determining CO_2 concentrations by means of Lloyd's gas analyzer.

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Book reviews

Tumors of the Head and Neck: Clinical and Pathological Considerations By John Batsakis. (Pp. x + 388; illustrated. £9·25.) Baltimore: Williams and Wilkins Co. 1974.

This book in a relatively small compass has achieved the author's objective which was to produce a text for pathologist, otolaryngolist, and head and neck surgeon. In the preface he states . . . 'the pathologist must become an active and informed member of the diagnostic and therapeutic team and not retreat into his cloister of tissues and slides'. Amen, to that, and the surgeon likewise I would say.

No practical, problematic issue is glossed over in the traditional way ('regressing melanoma must be distinguished from inflamed naevus') and much of the writing bespeaks a forceful teacher. The bibliography is outstanding, the illustrations choice and in generous quantity. The only criticism the reviewer has is that apart from evidence of careless proof reading, the original manuscript shows no evidence of having been revised with a view to improving the crude syntax. Nevertheless, textual ambiguity is absent and the reader is assured of both a balanced account of tumour pathology and clinical behaviour as well as Batsakis's personal convictions, and they are worth having, since his experience is vast in a breadth and depth unobtainable anywhere in the British Isles.

If, after reading this account of common and rare tumours, the clinician does not grasp the import of a histological report, nothing will avail.

ARNOLD LEVENE

Manual of Histological Demonstration Techniques By H. C. Cook. (Pp. ix + 314; illustrated. £4·50.) London: The Butterworth Group. 1974.

Books on histopathological technique were few until comparatively recently, but now there are many that vary from good to excellent and this new manual enters a highly competitive field. The stated aim of the author is to provide a comprehensive coverage of methods for the demonstration of various tissues and cellular components. There are only four pages on commonly used haematoxylins after which the book is entirely devoted to what most people call 'special stains'. Chapters begin with brief notes on basic principles and there are useful comments on each method. These are carefully selected, well described and liberally sprinkled with tips and hints that come, one feels, from vast personal experience. The appendice include one on buffers, one on suggested control materials, and one on the history of the subject. There are 30 monochrome illustrations and a pretty comprehensive list of some 300 references is also supplied.

This work is clearly not intended to be a textbook on histopathological technology, but one misses, nevertheless, an introduction to the comparative merits of fixatives, processing techniques, and equipment. The plastic ring binding and soft covers are a weakness and are unlikely to stand up to bench top use for very long. The illustrations are poor and they might well have been omitted, making the book cheaper or the saving in cost used to provide a hard back cover.

None of these criticisms detract from the value of this book as an authoritative guide to special staining methods and it should prove useful to technicians and pathologists with some experience who wish to improve or perfect the standard of technical work in their laboratory.

P. P. ANTHONY

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