A simple control system for CO₂ incubators

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Incubators in which a concentration of 3-4% CO₂ in the atmosphere is maintained are commonly used in virological work, but no entirely satisfactory system seems to have been developed for keeping the concentration within the required limits for long periods. The obvious solution, a device which measures the concentration and feeds in CO₂ as required, is relatively complicated and expensive and requires a certain amount of skilled maintenance which is often not available. Another solution which is commonly employed is to supply a flow of CO₂/air mixture of the required concentration to flush out the incubator. This has the disadvantage that such mixtures are relatively expensive to prepare, and if the CO₂ concentration in the incubator is to be brought rapidly to the required level every time it has fallen as a result of the door being opened, a large volume of mixture is required.

An operational analysis of the behaviour and use of such an incubator showed that in practice only a very simple system should be necessary.

To maintain the desired CO₂ concentration when the incubator is closed required only a slow steady flow of CO₂ sufficient to make up losses due to leakage, which, in a well-sealed incubator should be so small as to require a flow of only a few millilitres of CO₂ per minute.

More important, this flow should be quite constant, because the leakage from the incubator should not change appreciably from day to day; if it does, it is better

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Liquid nitrogen storage of haemoglobin variants—cont.

vital staining. These inclusion bodies which arise from the denaturation of haemoglobin H, however, developed after two hours' incubation with cresyl blue as would normally occur with fresh cells. Red cells from a carrier of the sickle-cell trait retained the sickling phenomenon after freezing in liquid nitrogen and subsequent thawing. The stored solutions of haemoglobins A+G, A+D, and A+G respectively had identical electrophoretic properties to fresh solutions. Electrophoresis was also performed on haemoglobin solutions prepared from normal red cells which had been stored at -196°C. for two years. The results were indistinguishable from those obtained with fresh solutions, and in particular the small A₂ component was as clearly visible as with freshly prepared haemolysates. This technique appears of value for the storage of human haemoglobins for reference purposes.

REFERENCE

- Huntsman, R. G., Hurn, B. A. L., Ikin, E. W., Lehmann, H., and Liddell, J. (1962). *Brit. med. J.*, 2, 1508.

to improve the sealing rather than to try to follow the changes by adjusting the CO₂ feed. Since the leakage of CO₂ takes place by diffusion it is proportional to the concentration which will not therefore build up excessively if the CO₂ feed rate is greater than required. In other words, it is an inherently stable system for which continuous automatic control is unnecessary.

Similarly, replacement of the loss of CO₂ resulting from opening the incubator does not require CO₂ concentration to be measured, because in practice it is found that the CO₂ concentration falls almost to zero every time the door is opened. Restoration of the CO₂ concentration is therefore simply a matter of putting in the correct volume of CO₂, calculated from the volume of the incubator and the concentration required.

As a result of this operational analysis a system of CO₂ control has been developed over the past two years which is simple to make and use, and yet gives quite satisfactory control of the CO₂ concentration at all times.

The principle of the system is as follows:

A slow steady flow of CO₂ into the incubator is maintained at a rate which is found by experiment to keep the concentration within the required limits, so long as the incubator is closed. To replace the CO₂ lost when the door has been opened a flow of 200 ml./minute is maintained for a variable period, controlled by a time valve, the period required being calculated from the volume of the incubator and the CO₂ concentration desired. The time valve is operated manually by the user each time the incubator opens and shuts the incubator.

The control system consists of the following components, which may be arranged in relation to the incubator, as shown in the Figure.

THE CONTROL SYSTEM

STAND FOR THE GAS CYLINDER AND OTHER COMPONENTS The stand is important because CO₂ cylinders are otherwise liable to be laid down in such a position that the liquefied gas enters the reducing valve and damages it. Neglect of this point is one of the commonest reasons for failure to maintain a steady flow of gas. The base of the stand serves as a convenient attachment for the time valve, so that it can be operated with one hand.

CO₂ CYLINDER FITTED WITH A PRESSURE-REDUCING VALVE, PRESSURE GAUGE, AND NEEDLE VALVE A cylinder of 7 lb. (3.19 kg.) capacity is usually a convenient size and lasts about three weeks. The pressure in the cylinder is fairly constant at about 60 atmospheres, so a cheap and simple single-stage valve will reduce the pressure with sufficient precision. The pressure gauge is of value, in spite of the fact that the cylinder pressure is nearly constant, because it gives warning when the liquid is exhausted. With a 7 lb. cylinder the remaining gas will usually last for 24 hours with normal use, and longer at weekends when the incubator is not being opened. The needle valve, which controls the rapid flow rate, is fitted with a shrouded screw head so that its adjustment cannot be altered by casual visitors.

TIME-CONTROLLED GAS VALVE WITH FINE ADJUSTMENT NEEDLE VALVE The time-controlled valve is made from

FIG. 1. Diagram of the CO₂ incubator control system:
 1 Kitagawa CO₂ tubes
 2 Time valve
 3 Securing screw for timer (when this is undone the timer can be swung round to give access to the fine adjustment needle valve at the back).
 4 Pressure gauge
 5 Pressure reducing valve
 6 Coarse adjustment needle valve
 7 Inlet tube to incubator
 8 Sampling tube
 9 Cutter for CO₂ indicator tubes
 10 Sampling pump

The valve assembly on the cylinder and the time valve may be turned round so that the cylinder can be placed either to the right or the left of the incubator. The only important requirement is that the time valve shall be readily accessible near the incubator door.

a kitchen timer to which an on/off gas valve is fitted, operated by the alarm. With it is incorporated a bypass needle valve similar to that on the cylinder, but smaller and with a finer taper, for controlling the slow flow rate. Provided that an efficient filter, *e.g.*, of packed cotton-wool, is fitted, the needle valve will maintain a constant flow of a few millilitres indefinitely so long as the pressure remains constant.

WASH BOTTLE WITH A FLOW-INDICATING DEVICE It is desirable to humidify the CO₂ because most CO₂ incubators also require an atmosphere saturated with water vapour, but the main purpose of the wash bottle is to act as a flow indicator. A standard screw-capped bottle of convenient size is used. The inlet pipe, which is of perspex, dips into the water and carries a marking ring a few centimetres below the surface. A hole about 0.5 mm. in diameter below the surface allows the gas to bubble up intermittently when the flow is only a few millilitres per minute. The frequency of bubbling can be used as a measure of flow rate. When the high flow rate is turned on, gas bubbles continuously from the hole, and the

water in the pipe is depressed to the marking ring, indicating that the correct flow is being delivered. The calibration of this device is not affected by the water level so long as the hole is submerged.

CO₂ SAMPLER This uses a standard Kitagawa¹ type A CO₂ indicator tube. The tube is intended for the range 0 to 2.5%, so a special sampling pump is provided of only 50 ml. capacity instead of the standard 100 ml. pump. The indicator tubes are relatively expensive, but once steady conditions are established sampling should only be required about once a month, and the method has the advantage of being very simple, reliable, and sufficiently accurate for the purpose.

PROCEDURE

The apparatus is connected to the incubator by means of a length of butyl rubber tubing passing in through the thermometer inlet. If no such inlet is provided it may be necessary to make one, which must be wide enough to accommodate a sampling tube as well.

The time required to reach the desired concentration is calculated, taking into account the volume of the incubator, with a flow rate of 200 ml./minute. The timer is set to this period and the CO₂ run in. After allowing five minutes for equilibration a sample is taken to check the calculations. The slow flow is set to bubble about six times a minute, and the incubator is left closed for several hours, preferably overnight, before sampling again. The slow flow rate is adjusted accordingly, and the procedure repeated daily until the desired concentration level is maintained. Provided the incubator is efficiently sealed, the system should then run indefinitely without further adjustment.

SAMPLING

Samples should be taken from a sampling tube inserted with the feed tube into the incubator, but separate from it. The tube should be sucked through to flush it out before attaching it to the indicator tube. Detailed instructions for the use of the sampling pump and tubes are supplied with the tubes.

This system has been in use on two incubators in this Institute for about two years and has been found to be quite satisfactory.

Constructional details have not been given but are available, with working drawings, if required.

SUMMARY

A slow steady flow of CO₂ into the incubator is maintained at a rate which is found by experiment to keep the concentration within the required limits, so long as the incubator is closed.

To replace the CO₂ lost when the door has been opened a flow of 200 ml./minute is maintained for a variable period, controlled by a time valve, the period required being calculated from the volume of the incubator and the CO₂ concentration desired. The time valve is operated manually by the user each time he opens and shuts the incubator.

Continued on page 102.

¹Obtainable from Minerva Detector Company Ltd., Lower Mortlake Road, Richmond, Surrey.

Book Reviews

THE PATHOLOGY OF THE TUMOURS OF CHILDREN. By Rupert A. Willis. (Pp. ix+200; 79 figures. 42s.) Edinburgh and London: Oliver & Boyd. 1962.

Professor R. A. Willis on tumours requires no introduction to readers of this journal. The clarity of his writing makes any book written by him a delight to read. The present book is relatively small, just under 200 pages, and is one of a series of monographs on pathology appearing under the editorship of Sir Roy Cameron and Dr. G. Payling Wright.

For this book, Willis has had the great advantage of being able to use the material that became available through the Manchester Children's Tumour Registry, from which Dr. J. K. Stewart has made what is probably the best comprehensive study of malignancies in children. Thus, the amazing collection of rare and problematic material that Willis referred to him is balanced by this recent comprehensive survey.

Realizing, perhaps, the limitations of the possible, the author has not attempted in his illustrations to give a series of photographs of sections from the most difficult tumours in children to diagnose but illustrates tumours that the general pathologist will see rarely and the paediatric pathologist occasionally. It is regrettable that the publishers have not permitted him photomicrographs of the standard set by the American Armed Forces Institute of Pathology.

A limitation of this book is in the field of the very common small 'lumps and bumps' that the surgeon removes from children, almost none of them malignant tumours, to which Willis's other book 'The Borderland' is an excellent introduction.

This book should be used as a supplement to the well-known 'Pathology of Tumours' and 'Borderland', and the volume's chief value, in your reviewer's opinion, is in the extensive list of references to articles on tumours that it contains with Willis's usual cryptic comments. It can thus be recommended as a useful addition to any pathologist's library shelf.

JOHN L. EMERY

Ciba Foundation Study Group No. 14. **INTESTINAL BIOPSY.** Edited by G. E. W. Wolstenholme and Margaret P. Cameron. (Pp. 120; 53 Illustrations. 15s.)

A simple control system for CO₂ incubators—continued

The concentration is checked from time to time by means of indicator tubes.

I am much indebted to Dr. D. M. Chapronniere for her assistance in developing and testing this system, and to Mrs. E. Malik for the illustration.

ADDENDUM

Since the above was written five more sets of control systems have been made and installed in a number of laboratories, and are giving satisfactory service.

J. & A. Churchill Ltd., 104 Gloucester Place, London, W.1. 1962.

This little book contains the papers presented at a Ciba Foundation study group under the Chairmanship of Professor A. C. Frazer. It provides an up-to-date account of developments in the field of intestinal biopsy, particularly in relation to the different types of malabsorption syndrome. Accounts are given of the morphology of normal and abnormal small intestinal mucosa, studied by the dissecting microscope, the light microscope, the electron microscope, and by histochemical methods.

For many readers the main attraction must be the informal discussions which follow each paper and add considerably to the ground covered so that the whole field of intestinal biopsy is surveyed, including even the vexed question of terminology. Where further study is necessary this is indicated.

This slender stiff-backed volume slips easily into a pocket. There are 53 illustrations, most of which are excellent, but one or two are, perhaps, unworthy of the production as a whole. An admirable book at a modest price.

G. STIRLING

BILIARY CIRRHOSIS. Pathological Monographs. Number One. By Sir Roy Cameron and P. C. Hou. (Pp. 216; 80 figures. 63s.) Edinburgh and London: Oliver and Boyd. 1962.

If succeeding monographs in this series maintain the high standards of the first, both as to content and production, a corpus of pathological literature of great value will be provided. To recommend this outstanding monograph to pathologists and to those interested in liver disease would do scant justice. For this is academic pathology at its finest. The authors have blended their ripe scholarship, great knowledge and experience, and their wisdom with an alert receptivity to all the information that can be derived from a wide range of modern experimental methods. This, while instructing us in what is known and paying due attention to the often overlooked observations of earlier workers, is focused especially on problems the solution of which still eludes us. Constantly attention is drawn to the lacunae in our knowledge and the authors are fertile in their suggestions for future research. Seeing things with a wide gaze from opposite sides of the earth, the world's literature is amply covered and the scope and extent of the references are noteworthy in all sections.

The first five chapters deal with the structure and function of the biliary apparatus and these are explored in detail. The remaining eight chapters deal with all types of biliary cirrhosis. Each reader will pick out the plums that appeal to him from the harvest provided. To one the section on primary biliary cirrhosis without obstructive lesions, and the relationship of this to that vexed entity, Hanot's hypertrophic cirrhosis, was the most interesting, as was the discussion of intrahepatic bile duct atresia. The