A Simple Culture Vessel for Turbidimetric Studies of Anaerobic Growth of Microbes

Growth curves for microbial cultures in liquid media can be conveniently obtained by turbidimetric measurements, performed after various times of incubation. For studies of anaerobic growth, however, the culture vessel must offer the following possibilities: (1) to establish anaerobic conditions before the start of the experiment (i.e. before inoculation of the medium) and to maintain these conditions continuously until the end of the experiment; (2) to perform turbidimetric analysis at time intervals without interrupting the anaerobic state; (3) to release from the culture vessel excess gas, evolved in microbial metabolism during the experiment, without admitting air and (4) to agitate the liquid culture continuously during incubation.

The culture vessel to be described here fulfills these demands. It consists of a culture flask of any convenient type and size (e.g. a Florence flask of 100 ml), to the neck of which a side tube of uniform optical quality has been fused (Figure 1). The vessel is closed by a rubber stopper which has passages for entry and exit of gas, respectively. The culture vessel with appendages can be sterilized by wet heat in the state, indicated in Figure 1. After sterilization, the main compartment is filled aseptically with the sterile liquid culture medium and an inoculum of washed cells is brought into the side arm by means of a pipette with bent tip. The stopper is firmly attached to the flask by means of copper wire. Anaerobic conditions inside the culture vessel can now be secured by flushing with purified cylinder nitrogen from which traces of oxygen have been removed by leading the gas over a palladium-asbestos catalyst in a tube (not shown in Figures 1 and 2) as described by WIKÉN et al.[1]; the gas stream is sterilized by passage through a cotton-wool filter. This purified nitrogen gas first flushes the side arm and then passes through the flask, which is shaken during the flushing period. The gas stream can escape freely by way of a second filter of sterile cotton-wool. After a flushing period of 40 min, the exit tube is closed by means of a bicycle valve (Figure 2), permitting only the exit of gas at an overpressure of about 0.6 at., depending on the thickness and flexibility of the rubber tube closing the valve. Immediately afterwards the nitrogen supply is cut off by bending the entrance tube and fixing it firmly with a string; the entrance filter can then be removed. The appendages are connected to the vessel in order to prevent excessive motions during subsequent incubation on a shaking machine.

The growth experiment can now be started by washing the inoculum into the culture liquid, tilting the vessel several times. The turbidity of the culture can be read directly after mixing by inserting the side tube, containing part of the liquid culture, into a suitable colorimeter. In the same manner further turbidimetric readings can be made at time intervals during the period of incubation, which is performed on a shaking machine at constant temperature.

By means of flasks containing a decolorized alkaline solution of glucose and methylene blue[2] instead of a microbial culture, we could demonstrate that by the procedure described above anaerobic conditions can be maintained in the culture vessels for more than 4 days. In measuring anaerobic microbial growth by the present method we have obtained quite satisfactory results.

It may be remarked that this procedure allows for measurement of anaerobic growth in the presence of

[1] T. WIKÉN, W. A. SCHEFFERS and A. J. M. VERHAAR, Antonie van Leeuwenhoek *27*, 401 (1961).
[2] P. FILDES and J. MCINTOSH, Br. J. exp. Path. *2*, 153 (1921).

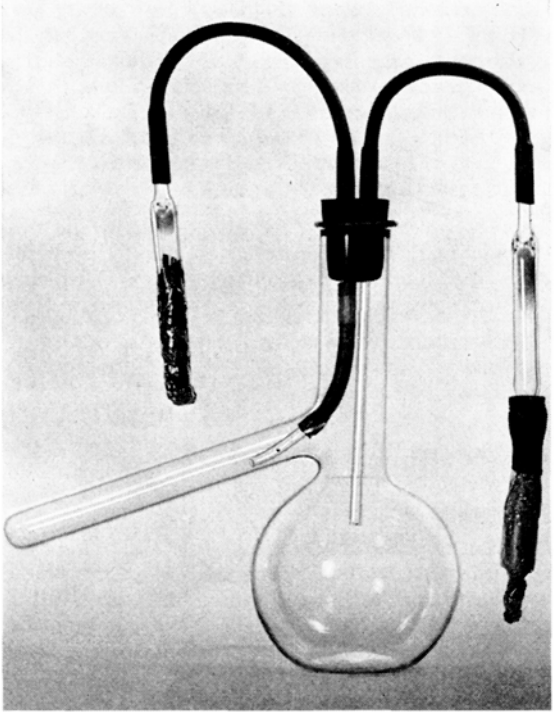

Fig. 1. Sterilized culture vessel, with entrance filter (left) and exit valve (right) wrapped in aluminium foil.

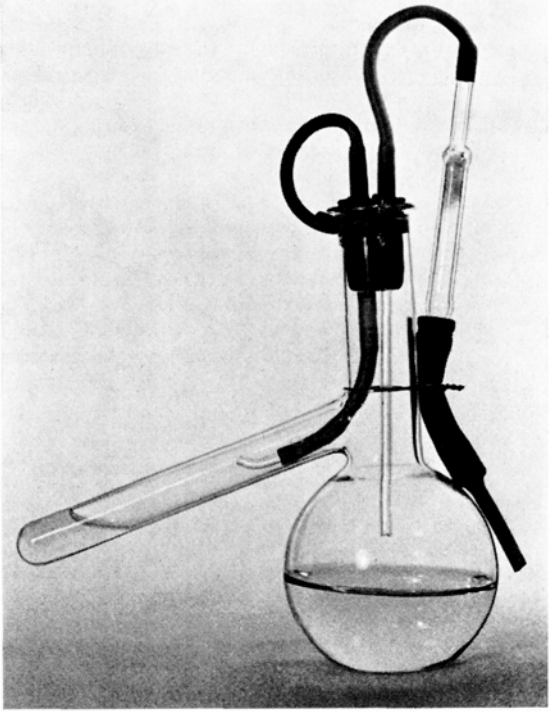

Fig. 2. Culture vessel containing liquid medium (main flask) and inoculum (side arm); entrance tube closed, entrance filter removed and exit valve closed by flexible tube after flushing with nitrogen gas.

carbon dioxide. This is of definite importance in those cases where CO_2 may influence growth, as for instance in lactic acid bacteria. If desired, effects of CO_2 on growth can be established by comparing parallel runs in culture vessels with and without an additional side bulb containing KOH, respectively.

Résumé. La fiole de culture décrite permet le mesurage par voie turbidimétrique de la croissance microbienne en culture anaérobie et agitée. Du gaz, produit par la culture, peut s'échapper par une valve. Par l'application additionnelle d'un bulbe latéral contenant de la potasse, la comparaison est possible entre des cultures parallèles, croissant soit en présence soit en absence de CO_2.

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Measurement of Blood Flow by Heated Thermocouple with Feedback Controlled Current

The principle of the heated thermocouple method[1] for regional blood flow measurement is that the degree of heat transmission from the hot junction varies with blood flow.

Slow response due to the heat capacity of blood and difficulty of calibration are disadvantages of this method. But this has been widely used for measurement of blood flow in various tissues, such as skin, muscle[2], liver[3], kidney[4] and bone marrow[5], partly because (1) its electrode is simple and flexible, (2) it can be buried in any tissue with little damage, and (3) it can afford continuous recording as well as separate recording from closely located spots.

However, as reported, the current required to raise the temperature of a thermocouple is kept constant irrespective of local tissue temperature so that the effect of local heat accumulation on vasomotor action[6] and nerve conduction velocity[7] cannot be neglected. Consequently, the error of measurement caused by such environmental changes cannot be avoided.

In the present study, a very low DC current is supplied to the heater in order to minimize such tissue reactions. Feedback control system is employed for automatic regulation of temperature difference between the hot and cold junctions to keep constant by adjusting heating current depending upon the change of flow rate. The characteristics of the constant current (CC) heating-method and the self-adjusting current (SC) heating-method have been compared with regard to their manner of response in application to animals and the linearity in response to the flow rate change in model experiment.

The structure of the electrode and the block diagram of the feedback control system are shown in Figure 1. The measuring element consists of a constantan wire (0.1 mm in diameter, 10 mm in length) to which 3 copper leads (0.1 mm in diameter) are connected at its middle (b in Figure 1) and both ends (a and c in Figure 1). The heater is a constantan wire (0.8 mm in length) coiled around the middle part of measuring element, and is insulated only electrically. Dimension of electrode should be appropriately chosen depending upon the flow rate. DC heating current is initially (during no flow) supplied to the heater so as to keep temperature difference of 0.35 °C. During the measurement, this temperature-difference is maintained constant by the feedback control system as mentioned above.

As the main purpose of this feedback control system is to measure the change of the flow rate, heating current is fed back proportionally to the change of a temperature-difference and an increase of this current is recorded. Loop gain of the system should be raised as much as possible within the stable range to reduce the temperature offset. The decrease of temperature-difference between the hot and cold junctions is also checked under the CC heating-method applied to this electrode.

Figure 2 shows a comparison of the CC and the SC heating-methods applied to the renal cortex of a dog. In the SC heating-method the rapid decrease of heating current occurs to compensate the total increase of temperature-difference caused by a temporary occlusion of renal artery. Prompter response and less influence of temperature-change to the tissue are observed in the SC heating-method than in the CC heating-method.

In the model system, the SC heating-method gives higher linearity and applicability over wider range of flow than the CC heating-method (Figure 3B). In the CC heating-method, as the flow rate increases, the decrement of temperature-difference decreases and gives a value nearly

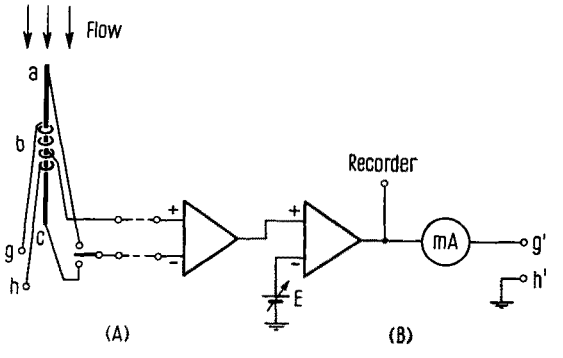

Fig. 1. (A) Structure of the electrode and (B) block diagram of the feedback control system. E, the reference voltage to set the temperature difference; a and c, both ends; b, central portion of constantan wire of measuring element; g and g', h and h' are connected respectively during the measurement.

[1] F. A. Gibbs, Proc. Soc. exp. Biol. Med. *31*, 141 (1933).
[2] H. Hensel, J. Ruef und K. Golenhofen, Z. Kreislaufforsch. *43*, 756 (1954).
[3] K. Graf, K. Golenhofen und H. Hensel, Pflügers Arch. ges. Physiol. *264*, 44 (1957).
[4] Y. Hagihara and I. Takahashi, J. Jap. Coll. Angiol. *8*, 125 (1967).
[5] K. Graf und E. Stein, Z. ges. exp. Med. *129*, 1 (1957).
[6] D. I. Abramson, L. S. W. Chu, S. W. Lee and S. Tuck jr., Vascul. Dis. *4*, 205 (1967).
[7] D. I. Abramson, L. S. W. Chu, S. Tuck jr., S. W. Lee, G. Richardson and M. Levin, J. Am. med. Ass. *198*, 1082 (1966).