

Apparatus for the Maintenance of Bacterial Cultures in the Steady State

II. Improved turbidity control and culture cell

JOHN H. NORTHROP

ABSTRACT (1) A more convenient method for maintaining constant turbidity in the culture cell is described. (2) An improved culture cell and cell wipers which do not cause foaming are described.

The "steady state" apparatus as originally described (Northrop, 1954, 1958) has been found to have two disadvantages. The light system for maintaining constant turbidity is sometimes troublesome to adjust and this must be done whenever a light bulb is replaced. The cell wipers and the air stream may cause foaming and the organisms sometimes collect in the shoulder of the culture vessel.

The turbidity control system shown diagrammatically in Fig. 1 is much more easily adjusted and also more sensitive than the original method.

Turbidity Control—Fig. 1

The galvanometer is removed from the Klett photoelectric colorimeter. A 1 mm. hole is bored in the cover and a 3 mm. hole in the base so that a beam of light passing through the galvanometer from above is interrupted by the galvanometer needle. The galvanometer is securely mounted, as shown in the figure, between the photoexciter lamp and the photocell. The lamp housing and photocell housing should be easily removable and adjustable so that the lamp and photocell may be replaced and the housing returned to its original position. When the galvanometer needle is displaced so as to intercept the light beam, owing to a change in the density of the suspension in the cell, the photocell activates the electronic relay which, in turn, starts the time delay relay. The solenoid then opens the flow of the culture medium for the length of the timing interval of the time delay relay. This device obviates chattering of the relays and allows the determination of the growth rate from the kymograph record by inspection, since the time of flow of the culture medium is always in multiples of the time delay relay setting, usually 5 minutes.

From the Laboratory of The Rockefeller Institute, the Donner Laboratory of Biophysics and Medical Physics, and the Department of Bacteriology, University of California, Berkeley.

Received for publication, May 4, 1959.

Cell and Wipers—Fig. 2

The culture is saturated with air by the motion of the wipers, instead of by bubbling the air through it. This avoids foaming. A constant slow flow of disinfectant is maintained through the side arm into the shoulder and outflow of the cell. This prevents the accumulation of organisms in the outflow and also prevents contaminants from growing back into the culture.

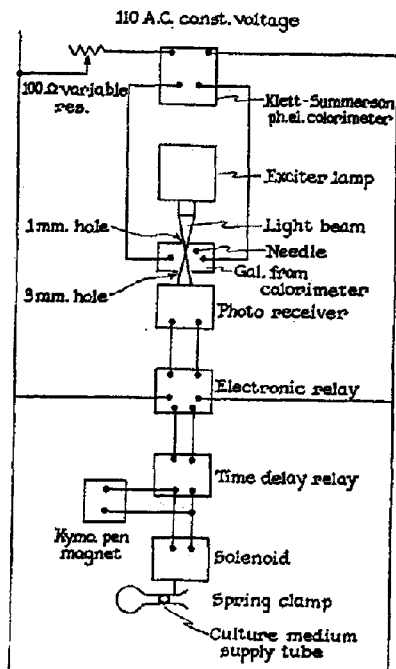


FIGURE 1. Turbidity control. Diagrammatic.

The wipers are cut from a 15×110 mm. "lusteroid" test tube. They are cemented to a No. 16 stainless steel wire by means of a solution of lusteroid in acetone. These wipers are operated by a motor at 300 to 500 strokes/minute with a 1 cm. stroke.

Assembly and Sterilization of the Apparatus

All glass to rubber connections are made by coating the glass with rubber cement, before connecting to the rubber tubes or stopper. After the apparatus is completely assembled, the cell is filled with formalin and allowed to stand 24 hours. The culture medium reservoir is connected to the culture medium intake tube and 1 to 2 liters of culture medium run through the cell to remove the formalin. The cell is inoculated by means of a fine hypodermic needle through the rubber sleeve of the wiper wire.

The galvanometer is adjusted to maintain the desired turbidity by means of the Klett colorimeter. The apparatus should maintain any desired turbidity within the range of the colorimeter indefinitely, except for replacement of the colorimeter and exciter lamp bulbs.

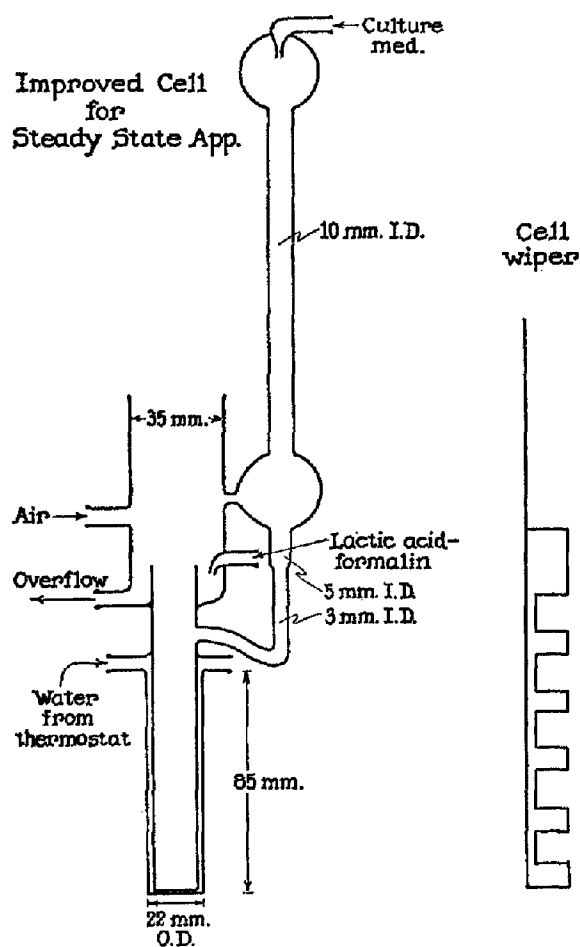


FIGURE 2. Cell and wipers.

Apparatus Needed

1. Photoelectric colorimeter, model 900-3, Klett-Summerson
2. Exciter lamp, model 33-3, Worner Electronic Devices, Rankin, Illinois
3. Photoreceiver, model 23, Worner Electronic Devices
4. Photoelectric relay, model 63, Worner Electronic Devices
5. Timoflex time delay relay, form 42, Eagle Signal Corporation, Moline, Illinois
6. Solenoid R9503-209C, General Electric Co., Schenectady, New York

7. Kymograph, Phipps and Bird, Richmond, Virginia, modified so that the pen drops 5 mm. every rotation of the drum
8. Motor, NS1-33R, 1/20 horse power, continuous duty, 5:1 reducing gear, 350 R.P.M., Bodine, Chicago, Illinois

BIBLIOGRAPHY

- NORTHROP, J. H., 1954, *J. Gen. Physiol.*, **38**, 105.
NORTHROP, J. H., 1958, Symposium on Continuous Cultivation of Microorganisms, Czechoslovak Academy of Sciences, Prague, 106.
Experimental results obtained by the use of this apparatus are reported in:
NORTHROP, J. H., and MURPHY, J. S., 1956, *J. Gen. Physiol.*, **39**, 607.
NORTHROP, J. H., 1957, *J. Gen. Physiol.*, **40**, 547.
NORTHROP, J. H., 1957, *J. Gen. Physiol.*, **41**, 131.
NORTHROP, J. H., 1958, *J. Gen. Physiol.*, **42**, 329.