AN INCUBATOR FOR HISTOCHEMICAL REACTIONS WITH SMALL VOLUMES OF MEDIA

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In connection with the development of new histochemical methods and, in particular, with the detection of the respiratory enzymes (DPN- and TPN-transaminases, dehydrogenases, oxidases), economy in the use of reagents which are in short supply is important. The synthesis and production of reagents such as the tetrazolium compounds and some substrates of the oxidative enzymes (di- and triphosphopyridine nucleotides, especially their reduced forms) involves considerable difficulty. Histochemists therefore work with minimal quantities of reagents, making do with a few drops of solution covering the section, impression, film, slide with tissue culture, and so on.

For a normal enzymic reaction to take place, and also for a correct histochemical technique of detection of enzymes, it is necessary to incubate biological materials at 37°. For short periods of incubation (less than 10 minutes) it is quite possible to use the ordinary dry incubator at 37°. For longer periods of incubation (1 hour or longer), such as may be required for the detection of enzymes of low activity, the substrate solution evaporates and its concentration is altered. Either the solution must be added periodically, or the slides with the objects must be placed in humid chambers (for example, in Petri dishes with moist filter paper) and then incubated.

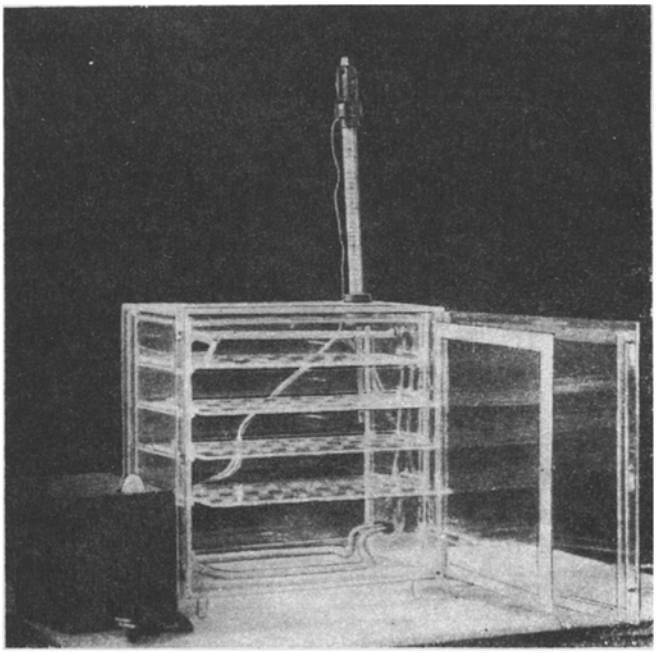

Fig. 1. General view of the incubator. On the left – relay with signal lamp.

We have constructed an incubator suitable for the prolonged incubation of biological objects at 37° in 1 or 2 drops of substrate without evaporation of the solution. This is attained by saturating the atmosphere inside the incubator with water vapor. The incubator also has several other features which make the operation of detection of enzymes much easier.

The incubator is made of organic glass and has double walls on all sides and double doors. Because of the transparency of the walls, the course of the reaction can easily be watched by the intensity of staining of the sections. The air space between the walls of the incubator keeps the interior at a given temperature, which is regulated by a contact thermometer and relay. Heat is produced by two electric nichrome coils inside the chamber at the top and bottom. The lower coil is enclosed in a glass tube filled with vaseline oil to prevent overheating. This tube is placed in water covering the base of the chamber. The second coil, mounted on a glass rod, is below the ceiling of the chamber, and under it is placed a vessel with water. The two coils are connected in parallel. They must not be heated above 45-50°, at which level the temperature of the incubator is raised to 37° for $1\frac{1}{2}$-2 hours. So that this temperature may be reached more quickly, the reservoir should be filled with water heated to 60-65°. If the coils are overheated excess of water vapor is formed and condensation is observed. At the base of the chamber, in one corner, a tube is present, closed with a cork, for removal of the water. The inner chamber must be airtight and must have a tightly fitting door to prevent water vapor from penetrating into the space between the incubator walls.

Slides with biological objects, for example, with tissue sections prepared in a cryostat, are placed on parallel laths of the movable shelves. These shelves have holes to permit the circulation of water vapor in the chamber. After the temperature inside the incubator has reached 37°, the biological specimens are covered with 1 or 2 drops of the substrate solution and the shelves with the slides are placed in the incubator. If the heating arrangements are properly made, ensuring the necessary saturation of the chamber with water vapor, not a single drop of substrate solution will evaporate and its concentration will remain unchanged during several hours of incubation at 37°.

The external appearance and scheme of the incubator are shown in Figs. 1 and 2. The dimensions of the incubator may be varied. In our model the dimensions of the inner chamber were 30 × 30 × 15 cm, the distance between the movable shelves was 5 cm, and the distance between the walls of the outer and inner chambers 1 cm.

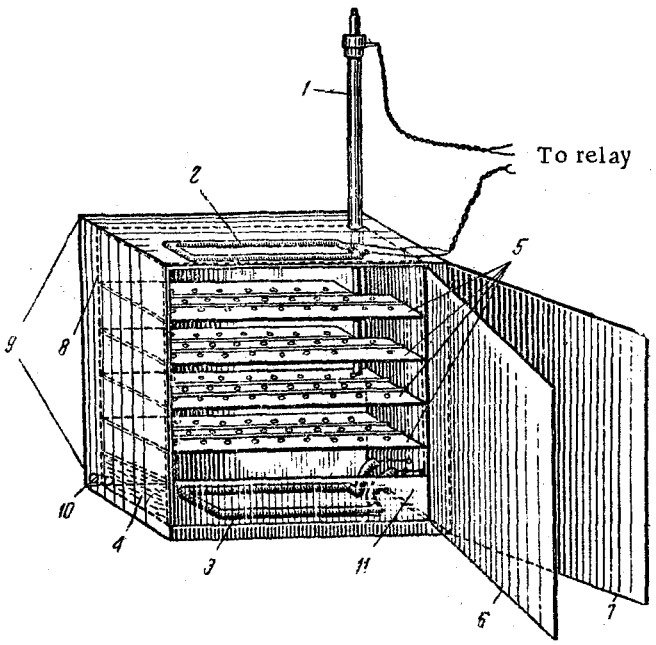

Fig. 2. Scheme of the incubator. 1) Contact thermometer; 2) upper coil; 3) lower coil; 4) water reservoir; 5) movable shelves; 6) inner door; 7) outer door; 8) body of the inner chamber; 9) body of the outer chamber; 10) opening of water drainage tube; 11) front wall of water reservoir.

In conclusion we must point out that the incubator we have described can be made easily in any laboratory. This particular apparatus was made to our design in the workshops of the Institute of Experimental and Clinical Oncology of the Academy of Medical Sciences of the USSR. It was tested by us for one year during work on the histochemical detection of respiratory enzymes (transaminases, dehydrogenases, oxidases) in normal and tumor tissues, and also in tissue cultures. This work proved the value of the apparatus, easing the work of the histochemist and enabling economy to be practised with expensive reagents. The incubator speeds the rate of work and enables tens of histochemical preparations to be produced in a day.

This incubator may be used in any research necessitating the incubation of biological objects in solutions containing substances in constant concentration.