PRO LABORATORIO

Commercially available equipment suitable for post-implantation embryo culture

P.K. Priscott

Clinical Research Centre, Watford Road, Harrow HA1 3UJ (England), 22 March 1979

Summary. There is a need for commercially manufactured equipment for use in post-implantation embryo culture techniques. A simple, inexpensive modification to an existing device is described that enables such an apparatus to be used for the roller-tube technique.

The several methods available for the cultivation of post-implantation rodent embryos have recently been reviewed[2]. These include the watch-glass[3], circulator[4-7], roller tube[8] and rotator[9,10] techniques. For in vitro teratological studies, where it is usually sufficient to observe treatment groups at the end of the culture period, the roller-tube technique has been found to be convenient[11-13]. Apparatus for this technique is not currently available commercially and therefore has to be specially constructed. This communication describes a simple, inexpensive modification to commercially manufactured equipment that enables its use in the roller-tube technique.

The apparatus and its modification. A 'Spiramix' haematological roller (Denley Instruments Ltd., Bolney, Sussex, England) was used. Spirally-wound wire around the rotating rollers had been incorporated into the design of this machine to facilitate thorough mixing of blood with anticoagulant. After cutting away the plastic protective sleeve, these spirals could be easily removed to leave a bare-metal roller and the whole apparatus was then placed inside an anhydric bench incubator (Laboratory & Electrical Engineering Company, Nottingham, England). The volume of the incubator was such that, even when left permanently switched on, heat generated by the roller motor was dissipated.

Culture system. For culture vessels, 30-ml wide-mouth all-glass reagent bottles were used. These were filled with up to 5 ml of medium and rotated at 36 rpm. Larger bottles could be used; 60-ml reagent bottles rotated at 30 rpm. Figure 1 shows the culture set-up.

Rat embryos were explanted by the method of New[14]. In this laboratory, most work has been done with 9- or 10-gestational-day embryos. Medium was prepared as described by New et al.[15] and was not replaced during the 2- or 3-day culture period. Figure 2 shows representative embryos before and after culture with yolk sacs intact. In this experiment embryos were explanted on the morning of the 10th day of gestation and cultured 42 h, regassing once at 24 h. Results compared favourably to those described with use of individually made, purpose-built apparatus (table). In the series reported here all embryos had vigorous heart beats and circulations, had turned to the ventrally-concave position, and were morphologically normal. Embryonic measurements given in the table were derived from control groups, cultured during a period of several months, without ensuring that all groups were of a similar developmental stage at the start of culture. Thus, the apparently large SE about the means were thought to reflect variation in embryos before culture from week-to-week (this was supported by the small SE calculated for each group). The lower values of crown-rump length and embryonic protein compared with those given by Cockcroft[16] probably result from differences in the gassing regimens which have been shown to be important in obtaining maximal growth[15,17].

A rotator has recently been described[10] that allows for the continuous gassing of cultures. It was shown that pO_2, pCO_2 and pH in the cultures all changed to a lesser extent than they did in the roller-tube cultures; whereas osmolarity was less affected in the latter[10]. There is currently no

Comparison of expected results after 2-day culture of 10.5-day gestation rat embryos

	No. of embryos	No. of somites	Crown-rump length (mm)	Embryonic protein (μg)*
'Spiramix'	18	34± 1.5	3.7±0.4	397± 62
New et al.[8]	15	28± 0.7	not given	333± 18
Cockroft[16]	not given	33–37	4.7–5.4	450–700

* Determined, after removal of extra-embryonic membranes, by method of Lowry[18].

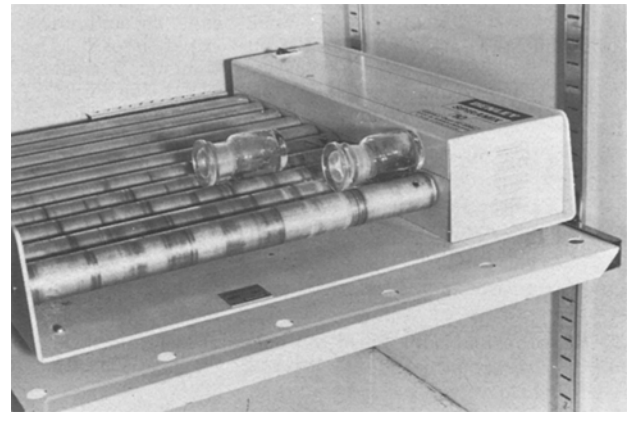

Fig. 1. Culture apparatus in situ with 2 30-ml culture bottles mounted.

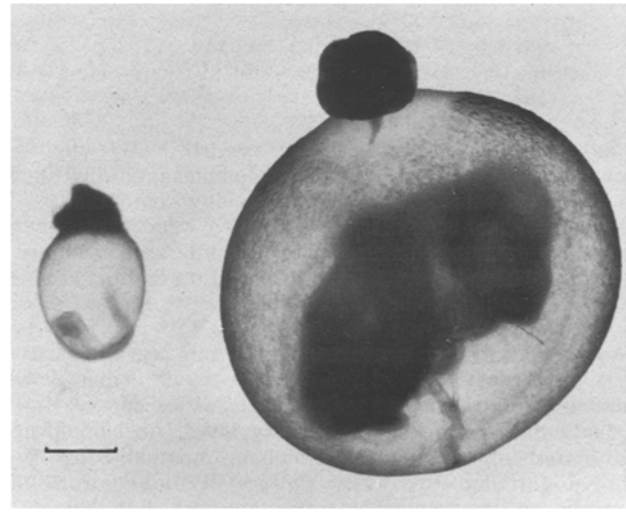

Fig. 2. 10-day rat embryos at explantation (left) and after 42 h roller-tube culture (after fixation in formal saline). Bar = 1 mm.

evidence to suggest that stabilising these factors significantly enhances embryonic growth and differentiation, although in certain experiments it would be advantageous to define them.

1 Acknowledgments. I would like to thank Drs R.D. Barnes and F.E. Hytten for much encouragement and provision of facilities. Also, the Obstetric Research Fund, University College Hospital Medical School for financial support.
2 D.A.T. New, Biol. Rev. *53*, 81 (1978).
3 D.A.T. New and K.F. Stein, J. Embryol. exp. Morph. *12*, 101 (1964).
4 J.S. Nicholas, Anat. Rec., *70*, 199 (1938).
5 D.A.T. New, J. Embryol. exp. Morph. *17*, 513 (1967).
6 A. Tamarin and K.W. Jones, Acta embryol. morph. exp. *10*, 288 (1968).
7 M.A. Robkin, T.H. Shepard and T. Tanimura, Teratology *5*, 367 (1972).
8 D.A.T. New, P.T. Coppola and S. Terry, J. Reprod. Fert. *35*, 135 (1973).
9 D.M. Kochar, Teratology *11*, 274 (1975).
10 D.A.T. New and D.L. Cockroft, Experientia *35*, 138 (1978).
11 D.A.T. New and R.L. Brent, J. Embryol. exp. Morph. *27*, 543 (1972).
12 D.L. Cockroft and D.A.T. New, Nature, Lond. *258*, 604 (1975).
13 D.L. Cockroft and P.T. Coppola, Teratology *16*, 141 (1977).
14 D.A.T. New, in: Methods in Mammalian Embryology. Ed. J.C. Daniel. W.H. Freeman & Co., London 1971.
15 D.A.T. New, P.T. Coppola and D.L. Cockroft, J. Reprod. Fert. *48*, 219 (1976).
16 D.L. Cockroft, in: Methods in Prenatal Toxicology. Ed. D. Neubert, H.-J. Merker and T.E. Kwasigroch. George Thieme Pub., Stuttgart 1977.
17 D.A.T. New and P.T. Coppola, J. Reprod. Fert. *21*, 109 (1970).
18 O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, J. biol. Chem. *193*, 265 (1951).

PRO EXPERIMENTIS

A simple procedure for the photometric evaluation of incidental findings on the recording strips of the Burkard pollen-and-spore trap[1]

G. Boehm* and Ruth M. Leuschner**[2]

** *Departement Forschung, Laborat. Dermatol./Allergol., Kantonsspital, Hebelstr. 20, CH-4031 Basel (Switzerland) and* * *Herrengrabenweg 51, CH-4054 Basel (Switzerland), 26 June 1979*

Summary. Reference is made to a procedure for evaluating certain incidental findings presented by particles of soot or dust on the adhesive strips of the Burkard pollen-and-spore trap or the slides of the similar Hirst apparatus. For this an evaluating device is recommended that is generally used for pherograms in large hospitals today. The often very noticeable streaks of soot or dust particles on the adhesive layer can be assessed with this method. Furthermore, it is possible to determine the time of the occurrence of the particles and possibly to relate them to the velocity and direction of the wind, and also eventually to detect the source of the polluting material.

The soot and dust content of the air can be monitored over an extended period, or occasionally, with apparatus of greatly differing construction. These include devices with continuous strips smeared with an adhesive medium on which the particles in question are caught. After exposure, these strips can, for instance, be evaluated densitometrically. (Apparatus of this kind are described by Leithe[3].)

In very many places, namely in Sweden, Finland, England, Denmark, Holland, Belgium, Austria, the USA, and Canada, and likewise in the Federal Republic of Germany and Switzerland, Burkard pollen-and-spore traps or older Hirst apparatus are in continuous operation. These devices work volumetrically, that is, they collect the floating living particles in a certain volume of air in the course of a day on an adhesive surface. Depending on the location of the apparatus and the prevailing direction of the winds, distinct streaks of soot and dust of very different origin occur as *incidental findings.* (The particles of these streaks can easily be identified under the microscope.)

In the Burkard pollen-and-spore trap (Leuschner[4]), a strip of Melinex® of 1.9 cm width, coated with vaseline as an adhesive medium, is rotated 2 mm each h past a suction slit of 14 mm width and 1.7 mm height. A volume of 10 l air is sucked in each min (Figure 1). (The Hirst apparatus works on a similar principle. Here a slide covered with an adhesive layer is drawn past a corresponding suction slit.) With the naked eye one can, for instance, estimate the thickness of the soot or the layer of dust on the strips or slides and determine the time of settling of these non-living particles polluting the air.

The evaluation of these specimens enclosed in Gelvatol (a polyvinyl alcohol) can today be simply and easily performed with the collaboration of a laboratory in a large hospital. This will be shown below.

Modern apparatus for the evaluation of ordinary discontinuous electrophoresis diagrams (= pherograms) of blood

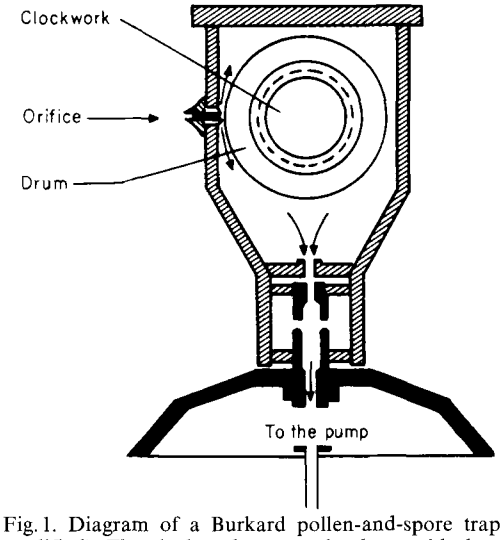

Fig. 1. Diagram of a Burkard pollen-and-spore trap (after Stix[6], modified). The clockwork rotates the drum with the adhesive tape 2 mm/h. ▨ Moving part; ■ fixed part.