

stopper, was set in the ultrasonic cleaner for 1 min. Uniform size of the sonicated mycelial fragments was achieved by filtration through sintered glass (40–90  $\mu\text{m}$ ) under reduced pressure (Figure 1). The density of the resulting suspension was checked by spectrophotometer and, if necessary, diluted with sterile distilled water, in order to obtain a standard mycelial suspension. Finally the tube containing the standard mycelial suspension was sealed with a special plug (Figure 2) consisting of a cotton filter and silicone septum (standard type used in gas-liquid chromatography) which allows sterile withdrawal by a syringe. All procedures were carried out under aseptic conditions.

The viability of dermatophytes in sterile distilled water has been evaluated for one year<sup>7</sup>, thus we expect the septum assembly can be used for long term stocking of standard mycelial suspensions.

**Résumé.** Une méthode simple de préparation des suspensions mycéliales standardisées de dermatophytes a été établie. Le mycélium fut dispersé à l'aide d'ultrason et les particules mycéliales homogénéisées par filtration sous pression réduite; la suspension mycéliale fut standardisée par spectrophotométrie. Les suspensions uniformisées ont pu être conservées dans des récipients munis de garde à coton.

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<sup>7</sup> A. CASTELLANI, *J. trop. Med. Hyg.* 64, 60 (1961).

## A New Culture Flask for Plant Tissue Suspension Cultures

The growth of plant tissue in vitro is generally measured by parameters, such as dry weight, fresh weight, or total nitrogen of the tissue<sup>1</sup>. In all such measurements the material, or a portion thereof, must be sacrificed.

A culture vessel especially designed for the measurement of growth rates of suspension cultures of plant tissue is described. The use of this flask allows the preservation of the tissue and is based on the relationship found between settling volume and dry weight of tissue. It is basically a modified 250 ml Nephelo flask with a side arm that is covered with a Morton Closure and is sufficiently high to allow shaking of the cultures (Figure 1). Because the extension tubes of these flasks are of uniform diameter (gauge material), determinations of volume may be made directly on changes in height of the settled tissue. Height determinations are made with a millimeter rule. It is feasible to add graduations to the extension tube but this would add 25% to the cost of the flask.

Flasks, containing 100 ml of modified White's 10  $\times$  salts liquid medium<sup>2</sup> with 2% sucrose and pH adjusted to 6.3 with NaOH prior to autoclaving, were inoculated with 10 ml of a 45-day-old suspension culture of *Vinca rosea* L. VA6 crown gall tissue. Cultures were incubated at 27 °C on a gyrotary shaker at 150 rpm. Periodically, 5 randomly selected flasks were inverted for 1 h and the settling

heights determined to the nearest millimeter. The tissue had settled after 20 min at which time measurements could have been made. Tissues from the individual flasks were washed on filter paper with five 100 ml portions of distilled water. Dry weights were determined after drying at 80 °C for 48 h.

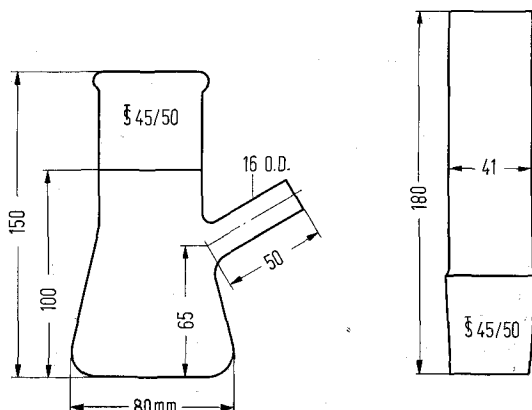


Fig. 1a. Culture flask components.

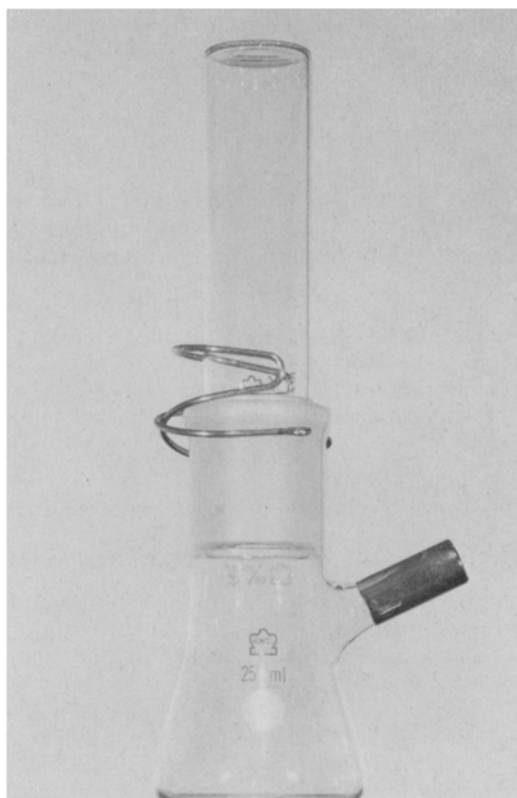


Fig. 1b. Assembled culture flask.

<sup>1</sup> M. C. LANCE, *Rev. gen. Bot.* 64, 123 (1957).

<sup>2</sup> J. LIPETZ, *Am. J. Bot.* 49, 460 (1962).

On a dry weight basis plant tissue in suspension culture exhibits a lag period during onset of growth, which is followed by an exponential phase (Figure 2). A similar pattern is evident when settling volumes are measured.

Particularly during the logarithmic phase the relationship between volume and dry weight is linear, with dry weight (mg) =  $12.3 \times$  height (mm) for VA6 tissue. During the exponential phase of growth, regression of the means is significant at the 10% level. The overall regression of the data is significant at the 0.5% level with a correlation coefficient of 0.965. Linearity has similarly been observed in *V. rosea* VB6 tissue<sup>3</sup> although the ratio of weight to settling height is appreciably lower. During the lag and stationary phases of growth this value is lower for both

tissues than during exponential growth, e.g. dry weight =  $8.4 \times$  height for VA6 tissue.

The use of this flask permits the entire growth cycle of cell populations to be monitored within individual flasks without sacrificing tissue. When changes in height are determined throughout the cycle on the same flasks rather than different ones estimations of growth rate should be even more accurate, since the same cell populations will be followed continuously. It is possible to differentiate the individual portions of the growth cycle by measuring heights of settled tissue. One can, therefore, accurately determine growth rates of suspension cultures during the exponential phase by computing the slope of the linear portion of the curve.

This culture flask simplifies the procedures for the determination of growth rates of various plant tissues in suspension culture and allows the estimation of the effect of different media and metabolic inhibitors on the growth of these tissues<sup>4,5</sup>.

**Résumé.** Description d'un flacon pour mesurer la croissance des cultures de tissus végétaux, sans les sacrifier.

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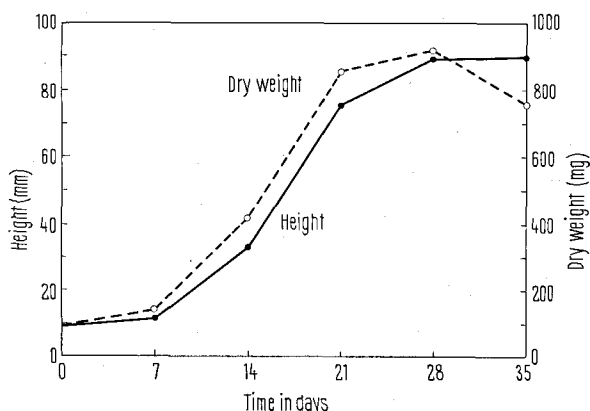


Fig. 2. Growth cycle of *V. rosea* VA6 tissue as measured by dry weight and settling volume.

<sup>3</sup> R. J. MANASSE and J. LIPETZ, Can. J. Bot. 49, 1255 (1971).

<sup>4</sup> This work was supported in part by grant No. E-570 from the American Cancer Society.

<sup>5</sup> The author wishes to thank Dr. D. D. C. McCUNE for the statistical analyses and Miss MAUREEN CUSACK for excellent technical assistance.

## CONGRESSUS

### USA

#### 3rd Congress of the International Society on Thrombosis and Haemostasis, in conjunction with the Council on Thrombosis, American Heart Association

in Washington, D.C., 22–26 August 1972.

The Congress will be held at the Mayflower Hotel in Washington. The topics for the plenary sessions include the following: Control mechanisms in hemostasis. Cell membranes: structure and function; platelets. Molecular

biology and pathophysiology of fibrinogen. Vessel wall and thrombogenesis.

Further information by Dr. Harold R. Roberts, Chairman of the Organizing Committee, Box 630, Chapel Hill, N.C. 27514, USA.

## ACTUALITAS

### International Cell Research Organization (ICRO)

1. *Training Courses.* One of the main activities of ICRO is the organization of training courses on topics of high novelty and on modern techniques in cellular and molecular biology: Principles and techniques of tissue and organ culture; Genetics and Physiology of Bacterial viruses; Energy transducing systems on the sub-cellular level; Methods in mammalian cytogenetics; Membrane Biophysics; DNA-RNA Hybridization; Biogenesis of Mitochondria; Embryology and Epigenetics; Interaction between Animal Viruses and host cells, application of computers to experimental work in biology and chemistry; Methods in molecular biology, etc. The courses generally last 3–5 weeks, and include 16–20 young participants (sometimes more). The ICRO courses are fully inter-

national, both the teaching staff and the participants coming from the largest possible number of countries.

2. *The Problem of Developing Countries.* Most of the past ICRO courses have been organizing in European countries – east and west – but the demand from developing countries is increasing steadily. ICRO activities in developing countries may tend to give preference to topics of potential economic usefulness, such as applied microbiology, microbial protein production, fermentation industries, soil microbiology, plant genetics, etc.

Inquiries for more information should be addressed to: Dr. Adam Kepes, International Cell Research Organization, c/o Unesco – AVS, Place de Fontenoy, 75 Paris 7e, France.