A SIMPLE AND CHEAP METHOD FOR TISSUE CULTURES

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The deteriorated laboratory glassware are broken into small pieces and the fragments are well washed. After rinsing them with distilled water and drying them, the glass pieces are introduced into Erlenmeyer flasks to form an inclined layer of 0.5 cms in height on one side and 3 cms on the opposite one.

The liquid culture medium is added so that half of the broken glass layer will be under half above its surface.

The callus develops at the zone of contact between the liquid medium and broken glass surfaces.

The organ-, tissue- and cell-culture, a relatively new technique of the modern sphere of genetic engineering [13] sets up fascinating theoretical and practical questions.

Being an unconventional artificial modality (of apomictic type) of the plant reproduction, the organ-, tissue- an cell-culture „in vitro" has philosophical, general biological and productive implications. Only one individual can be multiplied in millions „alter-ego"-s. At the same time, „in vitro", the callus can be maintained in an undifferentiated state and with a remarkable potency of division for an unlimited time. Does this mean that death was or will be vanquished ?

Another question raised by this culture technique is that of the integrality of the individual level system. No doubt there is a difference between the integrality „in vivo" and „in vitro". In the latter case, the interactions and the cell interdeterminations have a different intensity. Then, can we speak about a new state of existence of the species, not yet met with in nature ? Can we elucidate by means of cell-culture techniques, aspects of the process of cell differentiation and specialization not known yet ? What are the causes and the mechanisms of cell differentiation and redifferentiation ? Will the interspecific barriers be broken, by means of somatic hybridization between protoplasts [4, 7, 8, 10, 11, 12], and will the discontinuous character of the species disappear ? Will it be possible to produce „in vitro", on a large scale and with economic efficiency, biomass, proteins, enzymes, other known active principles or some other new ones, giving up the classical methods of culture of the plants in the fields ?

These are some of the questions set up by this new field of science, that rouses more and more interest, all over the world, in the world of both specialists and non specialists. Consequently, numerous laboratories have taken over, developed and diversified this new technique [1, 2, 9, 14].

Briefly, the organ- and vegetable cell cultures imply the existence of a complex nutritive medium, aseptic conditions and adjustible conditions of medium [3, 6].

The culture medium is divided, except for the criterion of the composition in nutritive elements (this composition is different according to various authors, both qualitatively and quantitatively), into two large categories liquid and solid (with agar). The culture media are inconvenient, for the moment, from the point of view of the price (of the cost). For the solid medium, the acquisition and price of agar set up special problems. For the liquid medium, similar problems, perhaps even more acute, are set up the obtaining of the shake and their maintenance in function, that presupposes a consumption of energy, too. On the other hand, the liquid medium is more efficiently utilized by the callus.

In the general effort of the falling down of the research price and of the raising of the economic efficiency of the culture technique „in vitro", we experimented a series of ingredients that were added to the culture medium in order that agar may be substituted. We have obtained the best results with broken glass, a material that is available everywhere and which is extremely cheap.

The glass preparation

Deteriorated laboratory dishes are broken until fragments up to 0.5 cm^2 are obtained. After breaking, the resulted fragments are washed well, if needed with detergent. Then they are rinsed with distilled water and are dried, dust protected, in free air or in a drying closet.

The glass so prepared is introduced into Erlenmeyer flasks, forming a 0.5—3 cm thick layer. It is advisable that the layer of brocken glass should not be horizontal but inclined so that its surface with that of the liquid medium should form an angle of 145°—160° (Fig. 1). It is enough if on the surface of the glass layer there is a swelling above the surface of the liquid medium.

Sterilization is assured normally in autoclave for any culture medium.

Callus starting is obtained by introducing steam-, root- and leaf-explants or stamens or any other organ in the dish with medium at the interface liquid-glass (broken).

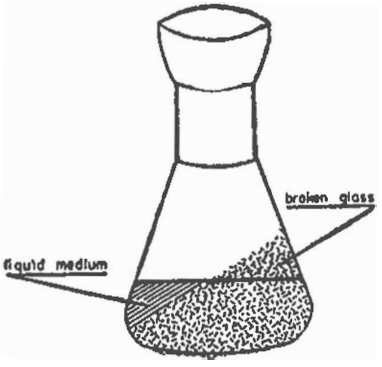

Fig. 1 — The Erlenmeyer flask with liquid medium and broken glass

A part of the explant will be introduced in liquid and the rest remains on the glass layer. The rough glass layer prevents the tissue from sliding down the surface of the medium. The callus appears on the surface of the liquid medium and right under it. For instance, in the case of *Matricaria chamomilla* L. species the callus appeared faster and more abundant. on Gamborg and co. (1968) medium with broken glass than on the same medium with agar (Tab. 1).

TABLE

The nutritive medium	The average weight of plantless at the transfer on medium	The days needed for callus starting	The average weight of callus after 20 days (g)
Gamborg and co. (1968)			
with agar	0.0123	20	0.2877
with broken glass	0.0161	17	0.2989

The advantages of our method are the low price and its easy use. Unlike the agar medium only one sterilization is needed here — the medium and the broken glass at the same time.

The callus can be easily and quickly separated and transfered. The weight of the callus formed in a certain period of time does not contain agar residues. Finally, the entire liquid can be analysed to detect all substances produced by the callus.

The method combines the advantages of solid and liquid medium in the sense that it assures a better diffusion of the secretion products from the callus into the medium. Each time the broken glass can be used again.

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