Technical Note

Preparation of Solidified Biological Specimen by γ -Ray-induced Polymerization[†]

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Solidification of soft organisms at room temperature was achieved by γ -ray-induced polymerization of a hydrophilic liquid monomer exchanged with the inner water. Typical recipes are given for an egg of 2.5 cm in diameter and for a bulb of a plant. This technique would be applicable to preparation of the large permanent sections of biological specimens.

Key Words: solidification, gamma-ray-induced polymerization, biological specimen

1. Introduction

The technique of solidification applicable to soft organisms has long been desired in biological studies. Deep-freezing of a whole body for cryo-microtome can achieve tentative solidification, but obviously the sliced specimens can not be kept long. In microscopy the permanent solidification of a biological specimen is usually practised by embedding it in paraffin or epoxy resin after dehydration, but these methods are applicable only to a small cut of a whole body¹⁾. Flowers could be embedded in unsaturated polyester resin, but only in dry state²⁾.

In general the solidification of a soft organism like an egg which has neither inner cellular structure nor rigid skeleton is recognized to be difficult to perform, but it will help the studies as embryology profoundly if it becomes

possible.

We succeeded in complete and permanent solidification of whole bodies of soft biological organisms without causing appreciable damage even to the outer cell-membrane. This report describes the application of γ -ray-induced polymerization to the solidification of soft organisms in appreciably large size. The technique will be effectively usable to prepare large sections for inspection by optical or by other means.

2. Experimental

2.1 Materials

Eggs of quail (*Coturnix coturnix*) and hen (*Gallus gallus*) and bulbs of white amaryllis (*Zephyranthes candida*) were used for solidification. Formaline (37%) and ethanol (99.5%) were used for fixation and preliminary dehydration. As monomers, 2-hydroxyethyl methacrylate (HEMA) (b.p. 67 °C/460 Pa) and N-vinyl-2-pyrrolidone (VPY) (b.p. 94-96 °C/1 700-1 900 Pa) were used without further purification.

[†] γ 線重合による固化生物標本の作成。早川 淳, 平光厲司*: 工業技術院名古屋工業技術試験所, 462 名古屋市北区平手町1, *埼玉医科大学, 350-04 埼玉県入間郡毛呂山町毛呂本郷38。

2.2 Fixation and exchange of water with monomers

A typical procedure of fixation and exchange of water with monomer for eggs and bulbs was as follows.

A fresh egg of quail was dipped in 40 ml formaline for 7 days and then in fresh formaline for another 1 day. Then the egg shell was removed by hand. Alternatively, the shell could be removed by dipping the egg into 2 N acetic acid for 2 hours. The naked egg was successively immersed at room temperature in the solutions (40 ml), compositions of which are shown below in volume percentage:

- (1) 25 % Ethanol+75 % water for 2 days,
- (2) 50 % Ethanol+50 % water for 2 days,
- (3) 75 % Ethanol+25 % water for 2 days,
- (4) 90 % Ethanol+10 % water for 2 days,
- (5) 100 % Ethanol for 2 days,
- (6) 75 % Ethanol+25 % HEMA for 2 days,
- (7) 50 % Ethanol+50 % HEMA for 2 days,
- (8) 25 % Ethanol+75 % HEMA for 2 days,
- (9) 100 % HEMA for 7 days,
- (10) Fresh 100 % HEMA for 7 days.

From flickering by refraction in solution, the exchange of outer liquid for inner liquid through the outer membrane was considered to proceed rapidly and 1 day would be enough for completion, but for confirming the complete exchange, 2 days or more were adopted for each procedure.

The direct exchange of water with HEMA was also possible, when the following recipes were used (respectively 20 ml solutions):

- (1) 25 % HEMA+75 % water for 1 day,
- (2) Fresh 25 % HEMA+75 % water for 1 day,
- (3) 50 % HEMA+50 % water for 1 day,
- (4) Fresh 50% HEMA+50% water for 1 day,
- (5) 75 % HEMA+25 % water for 1 day,
- (6) Fresh 75 % HEMA+25 % water for 1 day,
- (7) 90 % HEMA+10 % water for 1 day,
- (8) Fresh 90 % HEMA+10 % water for 1 day,
- (9) 100 % HEMA for 2 days,
- (10) Fresh 100 % HEMA for 2 days.

Such repeated renewals of a smaller quantity of solutions within shorter periods are recom-

mended for economy of solutions.

Entirely the same procedures using VPY instead of HEMA were applicable to eggs and bulbs. Use of methanol instead of ethanol caused no problems on the result of exchange.

2.3 γ -Ray-induced polymerization of impregnated specimens

The egg or bulb impregnated completely with liquid monomers was placed in a 50 ml glass vessel with a lid, and fresh monomer was poured on the specimen so as to immerse it thoroughly. Irradiation was carried out with γ -rays from ^{60}Co at a dose-rate of 500 Gy/h for 20 hours at room temperature. The polymerization of monomer was perfect except for the surface layer in contact with air; sometimes the vessel was broken by the inner pressure increase during polymerization. Sliced specimens were prepared by cutting the whole polymer block without removing the surrounding matrix polymer.

When VPY was used instead of HEMA, homogeneity of polymerization and compatibility of the polymer with organisms looked to be better than when HEMA was used and particularly the yolk was more homogeneously hardened; this result would be due to the lower shrinkage of VPY during polymerization. The plastic matrix, however, seemed to be softer than when HEMA was used.

3. Results and Discussion

The reason for the use of γ -ray-induced polymerization for the preparation of solidified organisms is as follows.

(1) For biological and similar purposes like the observation of generation processes, the damage suffered by the process of solidification should be as little as possible. Therefore, heating, freezing, rapid dehydration, or treatment with hazardous chemicals which would cause the destruction or shrinkage of original organisms should be avoided if possible.

(2) For the perfect exchange of the water in a cell through a cell-membrane with an organic liquid material which can be hardened afterwards, the organic material should be of a low molecular weight.

Photograph 1 Sectional view of an egg of quail (ca. 2.5 cm) solidified with HEMA and stained lightly with methylene blue.

Photograph 2 Sectional view of an egg of quail (ca. 2.5 cm) solidified with HEMA and stained lightly with methylene blue (another section of the same sample with that of Photograph 1).

(3) The solidification should be homogeneous through a whole body, and the solidified specimens should be hard enough to allow slicing or grinding of the surface.

The γ -ray-induced polymerization of a hydrophilic liquid monomer was considered to satisfy these requirements. The penetrability of γ -rays especially fitted for inducing polymerization of the monomer present in an organism at room temperature. HEMA and VPY were selected as liquid monomers because of their intimacy with organisms. Besides these, some of methacrylic esters, vinyl esters, or vinylpyridines may be successfully used for the same purpose.

Photograph 3 Sectional view of a bulb of white amaryllis (ca. 1.5 cm) solidified with VPY.

Photograph 4 Sectional view of a bulb of white amaryllis (ca. 1.5 cm) solidified with HEMA.

In this experiment the hen eggs as large as 6 cm in diameter were solidified. The recipes described above are typical examples, and the procedures could be altered depending on the specimens used. The concentration of formaline could also be changed. Better results may be expected if the glass vessel containing the impregnated specimen is dipped into a thermostated bath during irradiation to avoid the possible temperature rise by heat of polymerization.

Supplied monomers usually contain a small

quantity of stabilizers, but in the present experiment the monomers were used without further purification because the spontaneous polymerization of impregnated monomers during a long period of the exchange process would be avoided and practically no trouble occurred in the polymerization process.

Photographs 1, 2, 3, and 4 show the sections of a solidified egg of quail (lightly stained with

methylene blue) and a solidified bulb of white amaryllis.

References

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