Acta Medica Okayama

Volume 46, *Issue* 2 1992 *Article* 3

APRIL 1992

Rapid fixation of whole organ specimens and attendant problems.

Behice Durgun-Yucel[*] Fahri Dere[†]

Ahmet H. Yucel[‡] Ozkan Oguz[**]

[*]Cukurova University,

[†]Cukurova University,

[‡]Cukurova University,

[**]Cukurova University,

Rapid fixation of whole organ specimens and attendant problems.*

Behice Durgun-Yucel, Fahri Dere, Ahmet H. Yucel, and Ozkan Oguz

Abstract

Gross specimens are valuable sources in morphology education. In this study, we investigated how the fixation of gross specimens may be accelerated. For this purpose, whole organ specimens from freshly killed rabbits: extremities, kidney, heart, liver, stomach and uterus were fixed in a mercaptoethanol-formaldehyde mixture for 3-3.5h under the following conditions: 1, at room temperature; 2, at gradually increasing temperatures up to 45 degrees C; and 3, at a gradually increasing vacuum ranging from 20 kPa to 40 kPa. The results were compared with those of formaldehyde-fixed controls, and the mercaptoethanol-formaldehyde mixture was found to be useful in shortening the fixation time and providing good fixation. Both heat and vacuum enhanced these phenomena.

KEYWORDS: rapid fixation, mercaptoethanol-formaldehyde mixture, aldehyde, whole organ speciments

---

*PMID: 1575061 [PubMed - indexed for MEDLINE]

Acta Med Okayama 46 (2) 75-81 (1992)

Rapid Fixation of Whole Organ Specimens and Attendant Problems

Behice Durgun-Yücel*, Fahri Dere, Ahmet H Yücel and Ozkan Oguz

Department of Anatomy, Faculty of Medicine, Cukurova University, Adana, Turkey

Gross specimens are valuable sources in morphology education. In this study, we investigated how the fixation of gross specimens may be accelerated. For this purpose, whole organ specimens from freshly killed rabbits: extremities, kidney, heart, liver, stomach and uterus were fixed in a mercaptoethanol-formaldehyde mixture for 3-3.5h under the following conditions: 1, at room temperature; 2, at gradually increasing temperatures up to 45°C; and 3, at a gradually increasing vacuum ranging from 20 kPa to 40kPa. The results were compared with those of formaldehyde-fixed controls, and the mercaptoethanol-formaldehyde mixture was found to be useful in shortening the fixation time and providing good fixation. Both heat and vacuum enhanced these phenomena.

Key words : **rapid fixation, mercaptoethanol-formaldehyde mixture, aldehyde, whole organ specimens**

The process of fixation forms the foundation for subsequent stages in prepartion of tissue sections. Tissues and organs, which have been fixed, should be as close to their living state as possible.

Current methods of fixation rely on chemical agents. The most frequently used fixatives are aldehydes: such as formaldehyde and glutaraldehyde (1,12,14,19). Various conclusions have been drawn about which of these fixatives is most appropriate for a particular study (1,4,6,8,11, 15).

Although the details of the chemical bases for fixation are not yet known, the general principles behind them are expressly understood. The most important reaction is the stabilization of proteins which is associated with disulphide bond formation and a decrease in the solubility of proteins. As soluble proteins become fixed to structural proteins, whole tissue hardens, facilitating subsequent procedures (*e. g.*, dehydrating, embedding, sectioning, and staining) (7,9,10,14). In biochemical studies, mercaptoethanol (HS, CH_2 CH_2OH) is used to stabilize disulphide bonds of proteins (23). Disulphide group serves as a covalent cross-link between two polypeptide chains or between two points in a single chain. Disulphide cross-links can be cleaved by the action of mercaptoethanol (a reducing agent). Thus intrapeptid and interpeptid disulphides within proteins are reduced rendering them more stable (23).

Fixation and fixatives have become more interesting parallel to the demands of cell biology (1,2,5,6,14,15,20 - 22). Recently, microwave irradiation was used for the rapid fixation of surgical and autopsy specimens (3,13,16-18).

* To whom correspondence should be addressed.

However, studies regarding rapid fixation of whole organ specimens are very limited.

Rapid fixation of gross specimens is valuable in histopathologic and morphologic studies. Long fixation time in formaldehyde may even be harmful. The purpose of this investigation was to shorten the fixation time of the whole organ specimens. Whole organ specimens from freshly killed rabbits were fixed in a mercaptoethanol-formaldehyde mixture and compared with formaldehyde-fixed specimens.

Materials and Methods

Organs. Animal organs were obtained from freshly killed rabbits at the Animal Unit, School of Medicine, Cukurova University. Rabbit samples included the uterus extremities, kidneys, hearts, and stomachs. The volumes of these samples are given in Table 1.

Fixative solutions. Thirty-seven to forty percent formaldehyde was obtained from Pur Kimya and mercaptoethanol was obtained from Merck. Thirty-seven to forty percent formaldehyde was diluted 1:10 vv with distilled water to give 10 percent formalin.

Mercaptoethanol-formaldehyde mixture was prepared as follows: 0.025 ml of mercaptoethanol was added to 1 liter of 10 % formalin.

Experiments. The rabbits, weighing 2.4–3.2 kg were killed by decapitation under anesthesia. General anesthesia was performed by intraperitoneal injections of sodium pentobarbitone of 40 mg/kg body weight. Then they were fastened on a wooden operation table. The abdomen was opened by a midline incision, which continued beneath the left costal arch and the anterior thoracic wall was cut away. The organs were immediately excised and rinsed with the fixativc solution in which they were to be placed. The organs were then subjected to the following procedures:

1. As controls, 3.5 h of immersion in 10 % formalin at room temperature.

2. Immersion in the mercaptoethanol-formaldehyde mixture for 3.5 h at room temperture.

3. Three hour fixation in 10 % formalin and mercaptoethanol-formaldehyde mixture at gradually increasing temperatures up to 45°C. A heat cabinet (Electro-mag) was used with an output of 220 V at a frequency of 50 Hz.

4. Fixation in the same solutions for 3.5 h at a gradually increasing vacuum ranging from 20 kPa to 40 kPa. The vacuum was decreased gradually. Vacuum fixation continued at 20 kPa for 2 h, at 40 kpa for 0.5 h and at 20 kPa for lh. The vacuum was checked at 0.5 h intervals and adjusted if necessary. A vacuum cabinet (Forma Scientific) and vacuum pump (Pfeiffer 220/380 V, 50/60 Hz) were used for vacuum fixation. The vacuum cabinet operated at 50 Hz and had an output of 220 V. It also had a heating system to 100°C.

In all cases, the volume of fixative solution was approximately 10–20 times larger than that of the organ. After fixation, the tissue slices (approximately 0.8–1.0 cm^3) were cut with a very sharp knife or razor. All tissue slices that were obtained from the deep zone of these specimens were transferred to an autoprocessor (Fisher, Model 166 MP) and processed through an overnight cycle in the normal manner. The tissues embedded in paraffin and sectioned, were then stained with haematoxylin eosin. Light microscopic observations were made at a x40 magnification with Olympus BH-2 microscope.

Table 1 The volume of the organs from rabbits used in experiments

The volume of organs (mm^2)					
Extremities	Kidney	Heart	Liver	Stomach	Uterus
60	10	20	70	40	6

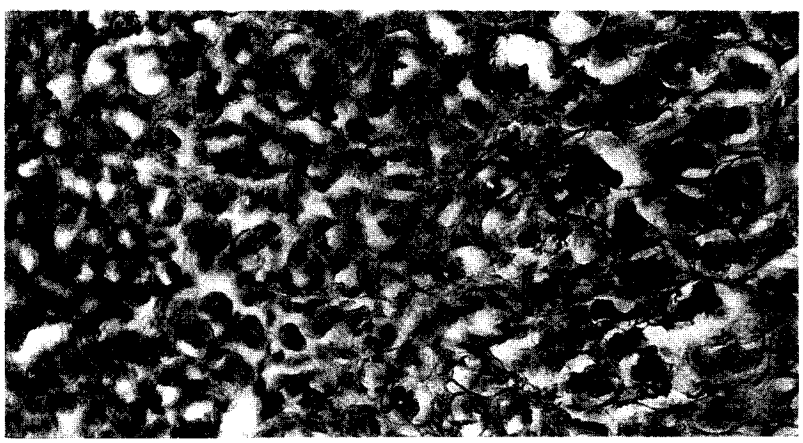
The volume was measured by buoyancy principle of Archimedes.

Results

Gross appearance of the specimens and light microscopic examination were used as criteria to determine if specimens were well fixed.

Control specimens immersed in 10 % formalin at room temperature for 3.5 h were found to be soft. Peripheral zones of these specimens were fixed, but deep zones were not. Light microscopic observations also confirmed that morphologic preservation was poor and unsatisfactory (Figs. 1, 2). Similar results were obtained from the gross specimens fixed in the same solution at gradually increasing temperatures.

Immersion in the mercaptoethanol-



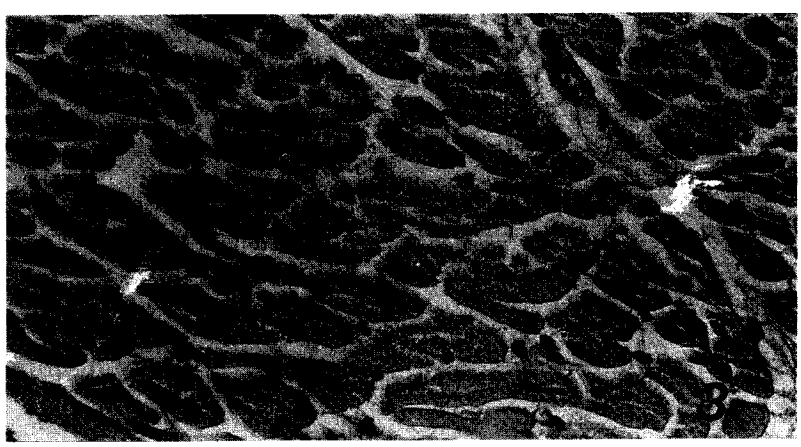


Fig. 1 Rabbit heart immersed in 10 % formalin for 3.5 h. Haematoxylin and eosin. × 40.

Fig. 2 Rabbit stomach immersed in 10 % formalin for 3.5 h. Haematoxylin and eosin. × 40.

Fig. 3 Rabbit heart immersed in the mercaptoethanol-formaldehyde mixture for 3.5 h. There is good morphological preservation compared with that immersed in 10 percent formalin in Fig. 1. Haematoxylin and eosin. × 40.

formaldehyde mixture for 3.5 h at room temperature produced good morphological preservation. The specimens were adequately hardened and fixed by this solution.

In the stripped muscle, striated appearance and ovoid or elongated nuclei at the periphery of the fibers were clearly evident.

In the kidney, the preservation of glomerular morphology was good. The proximal and distal convulated tubules and endothelial cells were distinct. Fibrous tissue was seen in some regions because the rabbit kidney used in this experiment was pathologic.

In the heart, there was good morphologic preservation compared with that immersed in 10 % formalin (Fig. 3).

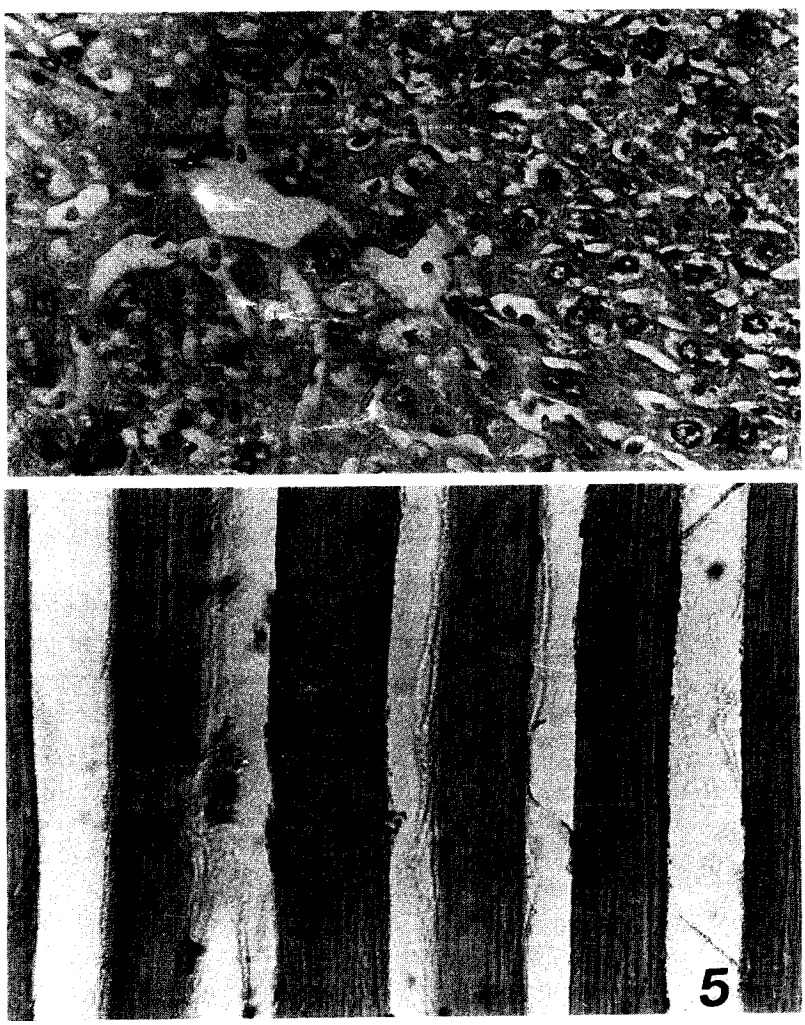


Fig. 4 Rabbit liver immersed in the mercaptoethanol-formaldehyde mixture for 3.5 h. Paraffin section stained with Haematoxylin and eosin. × 40.

Fig. 5 Rabbit stripped muscle fixed in the mercaptoethanol-formaldehyde mixture at increasing temperature up to 45°C for 3 h gave better results than that of fixed in 10 % formalin. Haematoxylin and eosin. × 40.

In the liver, hepatic lobuli, hepatic cells, branches of portal vein, hepatic artery and bile ducts were seen very clearly (Fig. 4)

In the stomach, epithelial cells in the mucosa were apparent, but the parietal cells were swollen.

In the uterus, smooth muscles, nuclei and arteries at the myometrium were easily distinguishable. In the endometrium, columnar epithelial cells, tubular uterine glands, arteries and lamina propria were maintained very well.

The rabbit's extremity and kidney, which were subjected to 3h of fixation in the

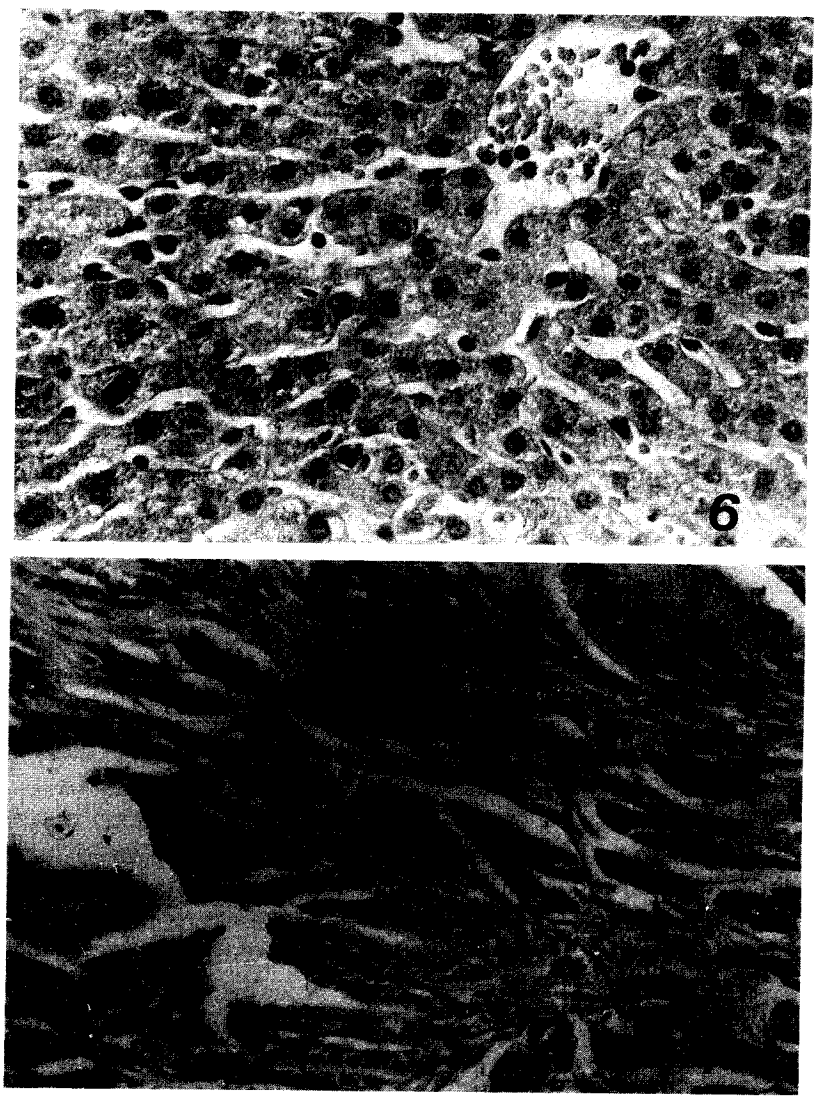


Fig. 6 Rabbit liver fixed in 10% formalin by gradually increasing vacuum for 3.5h Haematoxylin and eosin. × 40.

Fig. 7 Rabbit stomach fixed for 3.5h in the mercaptoethanol-formaldehyde mixture at gradually increasing vacuum. Haematoxylin and eosin. × 40.

mercaptoethanol-formaldehyde mixture at gradually increasing temperatures, also revealed good preservation of cell morphology (Fig. 5).

Vacuum fixation in 10 percent formalin gave the best results when compared with control specimens, but cytological details were poor, when compared with tissues which were immersed in the mercaptoethanol-formaldehyde mixure at room temperature for 3.5 h (Fig. 6). Formalin pigment was observed in the uterus.

Vacuum fixation in the mercaptoethanol-formaldehyde mixture for 3.5 h, produced sections which were almost identical in quality to those subjected to 3.5 h immersion in the mercaptoethanol-formaldehyde mixture. In fact, vacuum fixation in the mercaptoethanol-formaldehyde mixture produced better results in some tissues such as stomach, liver and stripped muscle (Fig. 7).

Discussion

Conventional formalin fixation of whole organ specimens which is routinely used in histopathology laboratories takes a day or more. Fixation of specimens as large as the human liver requires 2–3 weeks.

Heat is sometimes used to accelerate the fixation of biopsy specimens, though this increases tissue distortion. Perrachia and Mittler (22) produced evidence that fixation of tissue with glutaraldehyde, at increasing temperature up to 45 °C, satisfactorily fine structural preservation. The use of even higher, but controlled, temperatures for speeding fixation with a mixture of ethanol, formaldehyde and acetic acid at 80 °C, has been described by Ni *et al.* (21) for the preparation of rapid paraffin sections for histopathology. However, Dutt (5) reported that liver subjected to prolonged (12 h) fixation, in concentrated formaldehyde at increasing temperatures, revealed a progressive reduction in nuclear staining. Hopwood (14) found that mammalian tissues were severely damaged above 60 °C, even in the presence of formaldehyde. In our laboratory, we investigated effects of increasing temperatures up to 45 °C in 10 % formalin and mercaptoethanol-formaldehyde mixture, because protein denaturation may occur at temperatures above 60 °C. We found that whole organ specimens fixed in 10 % formalin gave worse results than those fixed in the mercatoethanol-formaldehyde mixture. Gross specimens were fixed well in the mercaptoethanol-formaldehyde mixture within 3 h. These results agree well with Perrachia and Mittler's (22) findings.

Microwave irradiation, as a method of rapid fixation, was introduced by Mayers (18). Long *et al.* (16) found that after heating tissues submerged in normal saline to 58 °C, very good cellular and nuclear morphology were maintained. Tissue blocks of the size up to 2 cm × 2 cm × 2 cm may be fixed uniformly in 2 min by microwave irradiation (13,14,16). Although microwave fixation is a more rapid method, various researchers reported that microwave irradiation tends to produce various artifacts (3,13,14,16). In this respect, chemical fixation appears to be an efficient alternative to physical methods.

In our laboratory, we found that 0.025 ml mercaptoethanol in 1 liter of 10 % formalin produced rapid fixation. It was also found that morphological preservation of all gross specimens fixed in this solution for 3–3.5 h was good. Vacuum and heat reinforced these phenomena.

It has been previously demonstraed that formaldehyde penetrates rapidly, but appears to fix slowly. Our experiments, which produced poor fixation in 10 % formalin confirmed the findings of Ericsson and Biberfeld (6), Dawson (4), Hopwood (12), and Larrson (15).

It has been reported previously, that materials were lost from tissue during formaldehyde fixation (1). Baker and McCrae (2) noted that fixation in formaldehyde for long periods of time decreased the staining ability of tissues. It is also stated that prolongd fixation in formaldehyde caused shrinkage and hardening of tissues (12). Our observations confirmed that rapid fixation

may eliminate these harmful effects of prolonged formaldehyde fixation.

Vacuum fixation proved to be successful, not only in the mercatoethanol-formaldehyde mixture but also in 10% formalin. Vacuum enhances penetration by forming negative pressure reducing fixation time.

Mercaptoethanol appears to be a relatively useful fixative for the preservation of gross morphology for observation by light microscope. Unfortunately, however, it has a distinctly unpleasant odor. The effects of mercaptoethanol on protein retention wers outside the scope of this study and require further investigation.

References

1. Artvinli S: Biochemical aspects of aldehyde fixation and a new formaldehyde fixative. Histochem J (1975) **7**, 435-450.
2. Baker JR and McCrae JM: The fine structure resulting from fixation by formaldehyde: The effects of concentration, duration and temperature. JR Microsc Soc (1966) **85**. 391-394.
3. Bernard GR: Microwave irradiation as a generator of heat for histological fixation. Stain Technol (1974) **49**. 215-224.
4. Dawson IM: Fixation: What should the pathologist do? Histochem J (1972) **4**, 381-385.
5. Dutt MK: Fixation of mammalian liver in concentrated neutral formaldehyde at different temperatures. Acta Histochem (1974) **51**, 140-142.
6. Ericsson JLE and Biberfeld P: Studies on aldehyde fixation: Fixation rates and their relation to fine structure and some histochemical reactions in liver. Lab Invest (1967) **17**, 281-298.
7. Hardy PM, Hughes GJ and Rydon HN: Formation of quaternary pyridinium compounds by the action of qlutaraldehyde on proteins. J Chem Soc Chem Commn (1976) 157-160.
8. Hommes OR, Warshawsky H and Leblond CP: Fixation of brain and incisor teeth for light mircoscopy: Gluratalde-hyde perfussion followed by immersion in Bouin's fluid. Stain Technol (1966) **41**, 261-265.
9. Hopwood D: Fixatives and fixation: A review. Histochem J (1968) **1**, 323-360.
10. Hopwood D, Allen CR and McCabe M: The reactions between glutaraldehyde and various proteins. An investigation of their kinetics. Histochem J (1970) **2**, 137-150.
11. Hopwood D: Theoretical and practical aspects of glutaraldehyde fixation. Histochem J (1972) **4**, 267-303.
12. Hopwood D: Fixation and fixatives; in Theory and Practice of Histological Techniques, Bancroft and Stevens eds, Churchill-Livingstone, Edinburgh (1977) pp16-28.
13. Hopwood D, Coghill G, Ramsay J. Milne G and Kerr M: Microwave fixation: Its potential for routine techniques, histochemistry, immunocytochemistry and electron microscopy. Histochem J (1984) **16**, 1171-1191.
14. Hopwood D: Cell and tissue fixation, 1972-1982: A review. Histochem J (1985) **17**, 389-442.
15. Larsson L: Effects of different fixatives on the ultratructure of developing proximal tubule in the rat kidney. J Ultrastruct Res (1975) **51**, 140-151.
16. Leong ASY, Daymon ME, Millios J: Microwave irradiation as a form of fixation for light and electron microscopy J Pathol (1985) **146**, 313-321.
17. Login GR: Microwave fixation versus formalin fixation of surgical and autopsy tissue. Am J Med Technol (1978) **44**, 435-437.
18. Mayers CP: Histological fixation by microwave heating, J Clin Pathol (1970) **23**, 273-275.
19. McManus JFA and Mowry RW: Staining Methods, Histologic and Histochemical A Hoeber International Reprint, Japan (1964) pp8-36.
20. Moller JC, Skriver E, Olsen S and Maunsbach AB: Perfusion fixation of human kidneys for ultrastructural analysis. Ultrastruct Pathol (1982) **3**, 375-385.
21. Ni C, Chang TC, Searl SS, Coughlin-Wilkinson E and Albert DM: Rapid paraffin fixation for use in histologic examination. Ophtalmology (Rochester) (1981) **88**, 1372-1376.
22. Perrachia C, Mittler BS: New glutaraldehyde fixation procedures. J Ultrastruct Res (1972) **39**, 57-64.
23. Walker JM: Methods in molecular biology; in Protein, Vol 1. Humana Press-Clifton, New Jersey (1984).

Received February 25, 1991; accepted April 17, 1991.