DETACHABLE TYPE MSM (NIIKHIMFOTO) EMULSION LAYERS FOR MICROAUTORADIOGRAPHY

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Detachable type MSM emulsion layers can be used for quantitative measurement of the content of labeled compounds in specimens. Details are given of the properties of MSM emulsion layers and the methods of work with them. For application to specimens the emulsion layer is cut into pieces and stripped from the backing with a scalpel. The detached layer is placed in distilled water with the emulsion side downward. The slide with the section is brought up to the layer from beneath. The specimen is dried at 20°C and exposed at 4°C. Development in amidol developer takes place at 15°C. All stages of the work are carried out in nonactinic yellow-green illumination.

Detachable emulsion layers were first used in autoradiography by Pelk in 1947 [2]. At the present time they are used in the West about equally with liquid emulsions. The AR-10 detachable layers manufactured by Kodak are the most widely used. Similar products are also manufactured by other Western firms.

The first attempts with the use of detachable emulsion layers in the USSR were made in 1957 [1]. However, the detachable NIKFI emulsions produced at that time had a number of considerable disadvantages: the large total thickness of the layer, fogging when the layer was removed from the backing, and so on. Recently type MSM detachagle layers, with properties very similar to those of the Western materials, have been developed by NIIKHIMFOTO in collaboration with the Institute of Oncology, Academy of Medical Sciences of the USSR. The main advantage of the detachable layer method over liquid micrograin emulsions is that the thickness of the layer is standardized, so that when they are applied to radioactive tissue sections it is possible to determine the content of labeled compounds in the biological objects quantitatively.

This paper describes the properties of MSM emulsion layers and the method of their application and photographic processing.

Characteristics of MSM (NIIKHIMFOTO) Detachable Layers. The structure of the detachable layers is shown schematically in Fig. 1. The detachable emulsion layer consists of a layer of gelatin 10 ± 1μ thick, poured on a glass backing measuring 9 × 12 cm. On top of the gelatin is poured a layer of photographic emulsion 5 ± 1μ thick. Between the carrier layer of gelatin and the glass backing a layer of hydrophobic material is introduced, to prevent the gelatin from sticking to the glass. The basic properties of the MSM detachable emulsion layers are given in Table 1.

Treatment of Tissue and Making Histological Preparations. The method of treatment and of making the histological sections for work with detachable emulsion layers is the same as during work with liquid emulsions [1].

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TABLE 1. Properties of MSM (NIIKHIMFOTO) Detachable Emulsion Layers

Index	Magnitude of index
Thickness of layer of photographic emulsion	$5 \pm 1\mu$
Thickness of gelatin carrier layer	$10 \pm 1\mu$
Sensitivity (number of grains of silver developed per 100 km trajectory of particle) of not less than relativistic energy	27 grams
Fogging in volume of 10^{-9} cm^3	2-3 grams
Concentration of silver halides	82 ± 0.5% (by weight)
Mean diameter of developed grain	$0.25 \pm 0.1\mu$
Decrease in sensitivity during 20 days	30%
Regression of latent image when kept for 45 days	40%

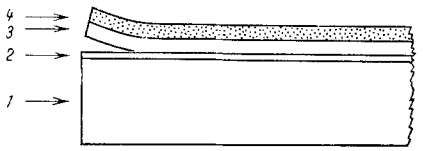

Fig. 1

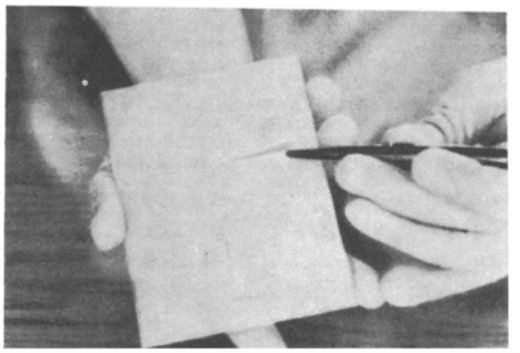

Fig. 2

Fig. 1. Detachable emulsion layer (scheme): 1) glass backing; 2) hydrophobic layer; 3) gelatin carrier layer; 4) emulsion layer.

Fig. 2. Separation of detachable emulsion layer from backing.

Pieces of tissue are fixed in Carnoy's fluid for 30 min to 2 h, depending on the size of the pieces, and embedded in paraffin wax. Other fixatives except mercuric chloride can be used for this purpose. Serial sections 5μ in thickness are mounted on slides.

Before the detachable emulsion layers are applied the specimens are dewaxed. Before application of the photosensitive layer the preparations are stained, usually with hematoxylin. Other stains not containing reducing agents may also be used [3]. In some cases the specimen can be stained through the emulsion layer after photographic processing. In such cases stains processing reducing properties can be used.

Application of Detachable Layers to the Specimens. During application of the detachable layers to the specimens the most convenient method is to cut the layer into pieces before removing it from the backing. An incision is made around the parameter of the plate with a razor or scalpel 1 cm away from the edge. One midline incision along the long dimension is then made within a circumscribed area, and two incisions are made along the small dimension so that the area of the glass is divided into six equal squares, each measuring approximately 3.5 × 3.5 cm. The subsequent operations with the detachable emulsion layers may be carried out in two different ways.

First Method. The plate on which the incisions have been made is passed through two portions of alcohol of increasing strength, for 5 min in 80° alcohol and 5 min in absolute alcohol. In absolute alcohol the layers are completely separated from the backing and transferred to distilled water.

Second Method. After the incisions are made the layer is separated from the backing at the edge of the incision and smoothly stripped off by gently pulling on one side (Fig. 2). The detached layer is placed with its emulsion side downward in distilled water at 20°C. After swelling in water for 20-30 sec, the slide with the section is placed underneath it and the layer is removed.

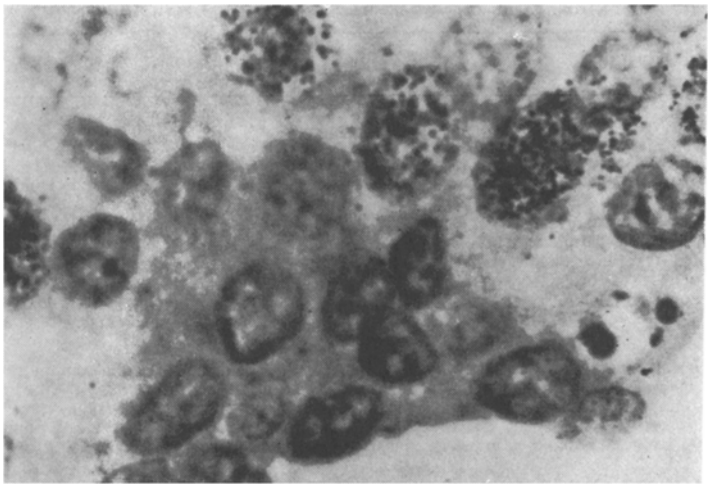

Fig. 3. Autograph of impression of tumor after incubation with thymidine-H^3. Hematoxylin-eosin, 700×.

If the emulsion does not swell sufficiently it forms folds on the slide and the layer does not lie in close enough contact with the specimen. Good contact between the layer and the slide, and firm fixation during subsequent processing take place only if the detached layer is slightly bent over the edge of the slide. Usually the swollen layer lies sufficiently evenly on the slide and requires no further manipulation. The excess of moisture is wiped off the slide with filter paper or the slides are allowed to stand for a short time in an inclined position.

The adherent layer is allowed to dry at 20°C. The specimens are exposed in a refrigerator at 4°C in lightproof boxes with a moisture absorber.

Removal of the detachable layer from the backing, its adhesion to the slide, and photographic processing are carried out in nonactinic yellow-green light (no. 117 or 118 filter).

Photographic Processing. The specimens are developed after exposure in a developer of the following composition: amidol 3 g, anhydrous sodium sulfite 120 g, distilled water to 1 liter. The developer is made up before use.

Stages of Processing of Detachable Layers in Amidol Developer

1. Cooled layers are soaked in distilled water at 18-20°C for 1 min.
2. Development in amidol developer at 20°C for 3 min.
3. Stop bath (1% aqueous acetic acid) at 5-7°C for 1 min.
4. Soaking in distilled water at 15°C for 1 min.
5. Fixation at 15°C in solution of the following composition: crystalline sodium thiosulfate 300 g, potassium pyrosulfate 1 g, distilled water to 1 liter. Duration of fixation 10-12 min.
6. Washing in tap water by decantation at 15°C for 10 min.
7. Drying at room temperature.

Chemically pure reagents must be used when making up all solutions. The solutions must be filtered through filter paper. Metol-hydroquinone developers are not recommended because they give a higher degree of fogging and they sometimes cause the emulsion to separate from the slides.

Conclusion and Analysis of Specimens. The developed specimens, after drying, are mounted in balsam. An example of an autoradiograph obtained by means of MSM (NIIKHIMFOTO) detachable emulsion layers is shown in Fig. 3. The quantity of radioactive isotopes in the tissue can be determined in relative units by counting the number of silver grains in the autoradiograph per unit area over the corresponding detail of the object (nucleus, cytoplasm, particular type of cells, etc.). If standard sources are used, the radioactivity can be estimated in curies.

LITERATURE CITED

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