A METHOD OF DOUBLE AUTORADIOGRAPHY

V. E. Zaichik and A. V. Tkachev

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By means of the method of double autoradiography described, combined autographs can be obtained of two different isotopes in the same specimen.

In the course of cytological and histochemical investigations it is often necessary to obtain two superposed autographs from the same specimen containing two different radioactive isotopes. Sometimes it would also be an advantage to obtain a single autograph without any manipulation of the specimen, yet retaining access to it.

The known method of double autoradiography is as follows. A photosensitive layer is applied to the specimen and after appropriate exposure and processing, the first autograph is obtained. This specimen and autograph are then isolated by a layer of celloidin, the surface of which is again covered with photosensitive emulsion, and a second autograph is obtained [4]. This method is not without important disadvantages: 1) The second layer of emulsion is separated from the specimen by a distance equal to the thickness of the first layer of emulsion and the protective layer, so that resolution of the second autograph is considerably impaired [3]; 2) observations cannot be made separately on the specimen and second autograph, because the first autograph lies between them; 3) while the first autograph is being obtained the specimen is in contact with photographic processing solutions; because of the possible extraction of water-soluble compounds, the scope of use of the method is limited; 4) it is impossible to stain the specimen after both autographs have been obtained.

The writers set out to develop a method of double autoradiography in order to obtain superposed autographs of two different isotopes contained in the same specimen. The suggested method allows observations to be made separately on both autographs, excludes contact between the specimen and processing solutions before the final information is obtained, and enables unfixed specimens to be studied and then stained after both autographs have been obtained.

This principle is put into practice by using both sides of the specimen, which is isolated during photochemical processing of the first autograph. The four stages of the method of double autoradiography are illustrated in Fig. 1.

The first stage (I) is that of preparation of the transparent water-impermeable film which serves simultaneously as the backing and protective layer for the specimen. A solution of nitrocellulose in amyl acetate is poured onto a carefully cleaned glass slide laid on a horizontal platform and it is allowed to dry in a dust-free container. After the solvent has evaporated, a nitrocellulose film is formed on the slide. By varying the concentration of nitrocellulose in the solution and the volume of solution poured onto the slide, the thickness of the films can be regulated. A thickness of 5μ was chosen so as to obtain adequate resolution of the autograph and satisfactory mechanical properties of the film itself. The slide with the film is then heated for several hours at 80° or kept in a vacuum for several days. In this way all traces of

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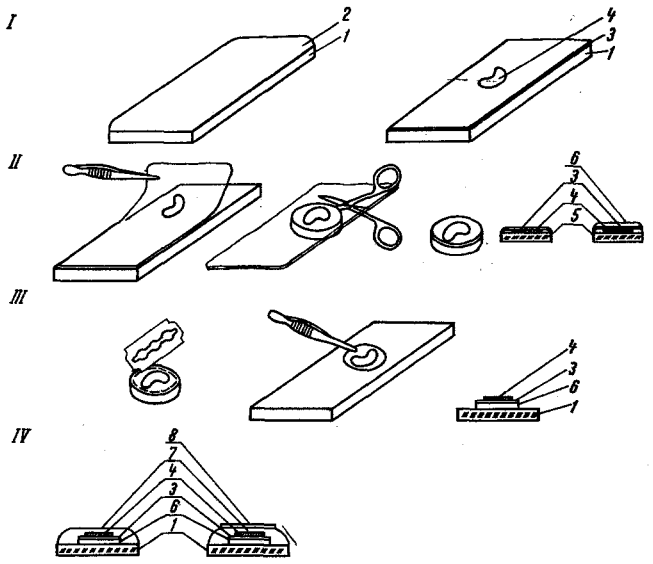

Fig. 1. Order of operations in preparation of a double autograph: 1) glass slide; 2) nitrocellulose solution in amyl acetate; 3) nitrocellulose film; 4) section of frozen tissue; 5) transparent plastic backing; 6) removable emulsion; 7) layer of liquid emulsion; 8) cover slip.

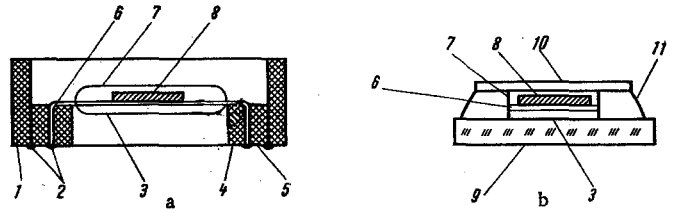

Fig. 2. Scheme of preparing (a) and mounting (b) double autograph by the second method: 1) outer ring; 2) place of gluing; 3) first layer of photographic emulsion; 4) inner ring; 5) middle ring; 6) Lavsan film; 7) second layer of photographic emulsion; 8) tissue section; 9) slide; 10) cover slip; 11) Canada balsam.

solvent are removed from the film and the possibility of development of artefacts during subsequent contact with emulsion is ruled out. A section of freshly frozen tissue obtained on a cryostat is mounted directly on the film surface.

The method can be used with sections obtained from material embedded in paraffin wax or other substances.

The second stage (II) is that of preparation of the first autograph. The film is undercut with a razor blade at the edge of the slide and is then easily removed together with the section by means of forceps. The area of the film backing is greater than the area of the section. A little nitrocellulose solution in amyl acetate is applied around the edges of a prepared firm celluloid or transparent plastic backing with a smooth surface. The detached film is mounted with the tissue section facing the backing. Care must be taken that the solution causing the film to adhere to the backing does not spread as far as the section. After the film has been glued to the backing, the excess film is cut off. It was verified experimentally that if the temperature was kept constant during mounting and subsequent photochemical processing, the necessary isolation of the section is maintained. Removable or liquid emulsion is applied on top of the nitrocellulose film. After suitable exposure, photochemical processing is carried out in the usual way. After drying, a circular incision is made in the film so as to remove the specimen.

In the third stage (III), freed from the firm backing, the histoautograph is transferred by the emulsion to a glass slide which is first smeared with fresh egg albumin to obtain better fixation of the autograph.

The fourth stage (IV) is that of preparation of the second autograph. After decay of the isotope producing the first autograph, the layer of photographic emulsion is applied directly to the specimen. In this way the second autograph of the isotope with a longer half-life period is obtained. After appropriate exposure and photochemical processing the specimen is stained through the top layer of photographic emulsion, taken to xylol, and then mounted in Canada balsam and a cover slip placed over it.

A parallel study was made of the microdistribution of iodine-131 and native iodine-127, introduced into a section of unfixed thyroid gland tissue [2, 5]. To obtain the first histoautograph, the section was irradiated in a reactor with epicadmium neutrons, as a result of which native iodine-127 was converted into its radioactive isotope iodine-128 ($T_{1/2}$ =25 min). The activation and exposure conditions for preparing the first autograph and the quantity of iodine-131 introduced were chosen so that the contribution of β radiation of iodine-131 in the first autograph was negligible. After complete fission of the iodine-128, the second autoradiograph was prepared from iodine-131, which has a longer half-life ($T_{1/2}$ =8.14 days).

In this experiment a restriction was imposed by the size of the backing (not more than 15 mm in diameter) on which the specimen was irradiated with epicadmium neutrons in the reactor channel. When the size of the backing was not so rigidly limited, another procedure was used, avoiding the use of an intermediate backing. This prevents possible deformation of the backing film together with the specimen at the time of mounting. This alternative ensures controllable isolation of the specimen during photochemical processing of the first autograph. In addition, in this alternative, Lavsan film of standard thickness is used as backing film, an important feature for the production of serial autographs. It is also important to note that Lavsan films, even when very thin, possess the necessary mechanical qualities.

The double autograph (Fig. 2) is obtained by means of a cassette, consisting of three concentric transparent plastic rings fitting snugly together. The internal diameter of the smallest ring is determined by the size of the specimen. The height of the walls of the two inner rings is the same, while the outer ring is slightly taller. The thickness of the rings is determined by their strength. The Lavsan film (in this case 4μ thick), using as isolating backing film, is placed on the inner ring and pressed tightly from above by the middle ring. The projecting edges of the film are stretched and cut off, and the rings glued together at their free end with dichloroethane. The section of unfixed tissue from a cryostat (the use of fixed specimens is also possible with this method) is then mounted on the smooth surface of the film. The outer ring of the cassette is then placed over, and this is glued to the two inner rings.

The first autograph is obtained on the side of the Lavsan film, and reagents for photochemical processing are poured directly into the "vessel" formed by the film and edges of the cassette. The specimen is then completely isolated. The second autograph is obtained directly on the side of the specimen, and the necessary procedures are carried out in such a way that the corresponding reagents are poured directly into the cassette from the opposite side.

With this method, histochemical reactions can be carried out on the tissue section using the first three stages. This modification has certain advantages over known methods [1]. The method of double autoradiography envisages the use of the most suitable modern materials for histoautoradiography: sections of unfixed tissue can be used without contact with water, good resolution is possible (because of the excellent mechanical properties of Lavsan film, its thickness can be reduced considerably), and insulation of the specimen from the processing solutions is guaranteed. In addition, the method is much simpler, more reliable, and less laborious.

The suggested method can be used not only for recording and cytological localization of an introduced radioactive isotope and its stable analogue after neutron activation of the latter, but it can also yield superposed autographs of isotopes with a short and long half-life, and also of a single radioactive isotope in an organic and inorganic form, because after the first autograph has been obtained, the inorganic form can be eluted. By using emulsions with different thresholds of sensitivity, and also by varying the thickness of the isolating backing film, a double autograph of radioactive sources with different linear energy losses can be obtained.

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