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AUTORADIOGRAPHY OF LIVE AND DEAD MAMMALIAN CELLS WITH ^{99m}Tc-TETRACYCLINE

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Considering the diagnostic significance of a few ^{99m}Tc-chelates in the diagnosis of myocardial infarct, we have recently developed a necrosis model in tissue culture obtained from mammalian cells, where the dead cells showed enhanced binding of ^{99m}Tc-tetracycline like the necrotic cells of infarcted myocardium. Technical details of the principles of labeling, fixing, washing and drying, dipping in emulsion, exposure, development and staining of mammalian cells have been described. Considering the short half-life of ^{99m}Tc-radioisotope, the recently available techniques have been modified to make autoradiography possible in a reasonably short period of time. Technetium-99m in different physical and chemical states is playing a very important role in a variety of diagnostic procedures. The autoradiography of ^{99m}Tc-labeled cells and tissue will shed further light on the mechanism of cellular uptake and subcellular distribution in normal and pathologic states. Presently no information is available regarding autoradiography with ^{99m}Tc-chelates of mammalian cells obtained from tissue culture.

In spite of its short half-life, ^{99m}Tc is an ideal radioisotope for autoradiography. The 2.2-keV conversion electron obtained from the decay of 142.7–140.5-keV level has short range in emulsion to delineate the subcellular localization. Because of the increasing role of ^{99m}Tc-chelates in a variety of diagnostic procedures, the understanding of the mechanism of cellular uptake and subcellular distribution in normal as well as pathologic states will be useful. Autoradiography of the tissues of the brain and bone with $^{99m}TcO_4^-$ ion and ^{99m}Tc-polyphosphate was reported by Baum (*1*) and Tilden, et al (*2*). The myocardial uptake of ^{99m}Tc-tetracycline and its analogs has been presented (*3–8*). We have recently developed a necrosis model in tissue culture obtained from mammalian cells (*9–11*) and used the model successfully for screening a variety of radiopharmaceuticals in terms of their affinity for necrotic tissue. Details of this autoradiographic technique have been described (*12,13*). In this investigation, a faster procedure for autoradiography of necrotic cells with ^{99m}Tc-tetracycline will be described.

MATERIALS AND METHODS

Fixation of cells. Glass slides with frosted ends were washed with distilled water and ethanol and dried with a hair dryer. The live and dead cells were labeled according to the method reported previously (*4,5*). Technetium-99m-tetracycline-labeled cells were smeared with a paint brush in thin layers. The slides were air-dried for 15–20 min (slow-blown with a hair dryer). The cells in slides were then fixed in Bouin's fixative containing a mixture of 5 ml of glacial acetic acid, 75 ml of saturated picric acid solution, and 25 ml of 36.6% formalin, rinsed in three changes of distilled water and three changes of 95% ethanol, and dried with a hair dryer for 40–45 min in a slide box. In order to insure proper staining of the cells, the removal of picric acid by washing is essential.

Dip-coating of slides. In the darkroom, NTB-2 emulsion (Eastman Kodak Co.) was melted in a glass beaker in a constant temperature water bath of 40–45°C for approximately 40–45 min and diluted with equal volume of distilled water. The slides, mounted on a slide holder, were dipped vertically for 10 sec in molten emulsion in the beaker and allowed to stand vertically for 45 min for drying. The slides were placed in a Bakelite slide box con-

Received Aug. 29, 1974; revision accepted Nov. 18, 1974.

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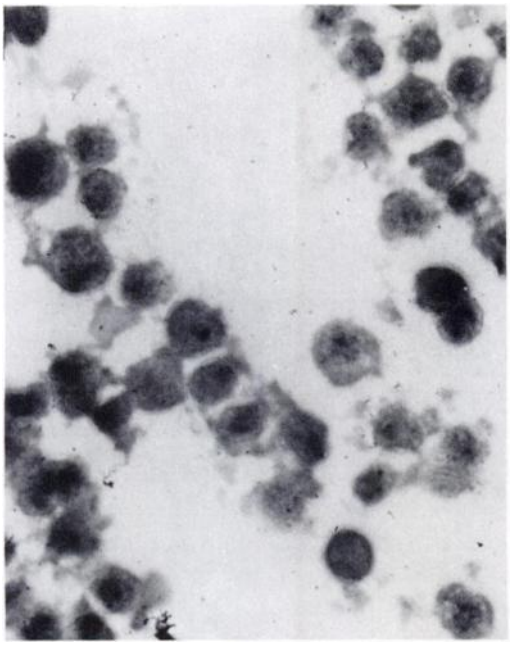

FIG. 1. Autoradiography of live mammalian cells (LICH) with ^{99m}Tc-tetracycline (×3200). Few silver grains are associated with live cells.

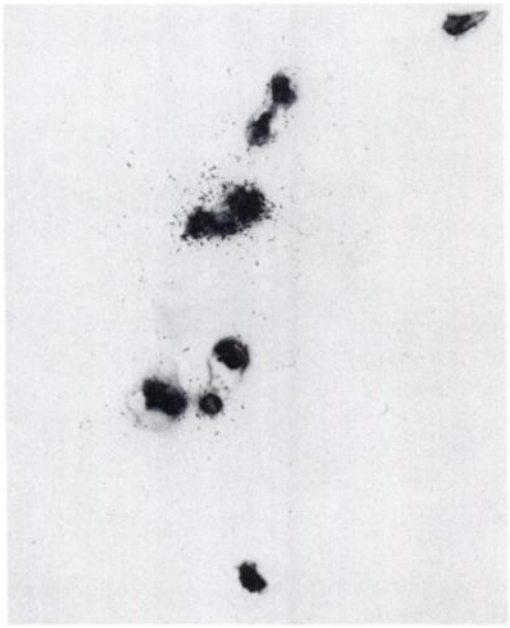

FIG. 2. Autoradiography of necrotic cells with ^{99m}Tc-tetracycline (×3200). In contrast to live cells, dead cells are loaded with silver grains. Nuclei of dead cells are pyknotic.

taining a small package of Drierite in gauze. The box was taped (two to three layers) with Scotch brand pressure-sensitive tape (No. 672 black tape from 3M Co.). Sparking should be avoided during taping. The Bakelite boxes were put inside used x-ray film envelopes and stored in the refrigerator for 48–72 hr. A long exposure will increase the contribution of grain counts from the high-energy beta ray (E_{max} = 298 keV) from the decay of ground-state technetium isotope.

Developing and fixing. The procedures were carried out at 18°C. All reagents, D-19 developer (30 gm/190 ml), Kodak fixing bath (30 gm/150 ml) and distilled water were cooled to 18°C. Filtered developer solution was diluted (1:2) with distilled water. The slides were developed for 3 min with occasional agitation, rinsed briefly in water for 30 sec, and fixed for 5 min in dilute fixer with occasional agitation. The slides were rinsed in running tap water for 1 hr and allowed to dry by standing 40–65 min on absorbent paper. Excess of emulsion from the back of the slide was removed with a razor blade.

Staining. After photographic processing, the slides were placed in removable trays of staining dishes and stained with Harris-Lillie (*14*) nuclear stain (Fisher Scientific Co.), for 5 min and rinsed in water. The slides were placed for 1 min in the 50, 70, 95, and 100% ethanol, xylene-ethanol mixture (1:1) and then in the xylene (100%) for drying. The coverglass was mounted on a coated slide with Permount (Fisher). The slides were allowed to dry for 24–48 hr. The autoradiographs were then ready for study under the microscope. Photomicrographs of live and dead cells were taken and are shown in Figs. 1 and 2, respectively.

RESULTS AND DISCUSSION

The live cells did not retain much radioactivity as was observed by determining gamma-ray activity and the grain counting in the photomicrograph (Fig. 1). Less than 15% of activity was observed in the washing and fixing baths. The grains in the outer edge of the cells in Fig. 2 are probably partly due to the high-energy beta rays. The nuclei of the dead cells are pyknotic. The subcellular localization is in agreement with our finding of biochemical localization study with modified Schmidt-Thannhauser procedure (*15*). These simplified techniques could be applied for autoradiography of short-lived radioisotopes. This evidence of enhanced retention of ^{99m}Tc-tetracycline in the dead cells as well as the present information in our laboratory confirming the poor affinity of the same agent for normal and ischemic tissue does indicate that ^{99m}Tc-tetracycline will be a valuable agent for myocardial infarct scanning.

ACKNOWLEDGMENTS

The author deeply appreciates the suggestions of S. J. Adelstein and the cooperation of E. Prince-Bradley and Paul C. Kahn in this investigation. Thanks are due to Marcia Barron for excellent typing. This work was partly supported by USPHS Grants 1-S01-RR05598-09 and GM18674.

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