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IMPROVED RESOLUTION IN AUTORADIOGRAPHY WITH RADIOIODINE USING THE EXTRANUCLEAR ELECTRON RADIATION FROM ^{125}I

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SUMMARY

In autoradiographic experiments with labelled micro test-charts, stair-cases, and histological sections, ^{125}I has proved to offer significantly better resolution than ^{131}I. Autoradiographic techniques favouring a registration of the low energetic internal conversion- and Auger-electrons emitted from ^{125}I are discussed.

INTRODUCTION

The longer half-life of ^{125}I, 60 days, compared to the 8-days half-life of ^{131}I, permits time-consuming synthesis procedures as well as storage of radioiodine-labelled compounds. This and other physical properties of ^{125}I have increased the interest for the applicability of this isotope in biological research.

^{125}I disintegrates by emission of electromagnetic radiation and extranuclear electrons[1-3]. The autoradiographic use of extranuclear electrons—internal conversion electrons and Auger electrons—theoretically offers possibilities of obtaining high resolution autoradiograms. This is of interest not only in autoradiography with ^{125}I but also in work with a great number of other nuclides emitting this type of radiation. During the last few years we have made some more or less preliminary autoradiographic studies[4-8] using some of these nuclides (^{55}Fe, ^{57}Co, ^{83m}Br, ^{85}Sr, ^{109}Cd, ^{125}I).

The emission of extranuclear electrons is always accompanied by X-radiation of various energies. The soft X-rays can be assumed to decrease the autoradiographic resolution to some extent. The resolution therefore is likely to vary considerably with changing experimental conditions, mainly thickness of sections and emulsions as well as the quality of the emulsions.

In order to try to elucidate the properties of ^{125}I in autoradiography we have made experiments with histological sections, labelled micro test-charts and ^{125}I stair-cases. The resolution in experiments with ^{125}I has been compared with that of ^{131}I.

METHODS

Micro test-charts

Micro test-charts were made on Kodak maximum resolution plates according to a technique previously described by STEVENS[9]. The type of the initial test-chart was

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Wild Heerbrugg. The external measurements of one total micro test-chart was 1 × 1 mm and the shortest distance between two separable lines was about 2 μ. Figures for the approximate distances between lines of different width are listed in Fig. 1. The micro test-charts were then labelled by immersion in an ^{125}I labelled iodide solution in a similar way as described by STEVENS[9].

Autoradiographic exposure of the labelled micro test-charts was made by apposition against various films listed in order of decreasing emulsion thickness: Agfa Printon, Ilford G5 plates, Kodak AR. 10 (stripping film), Kodak SWR (Schumann-type emulsion).

^{125}I stair-cases

^{125}I stair-cases were prepared using the gelatin layer of photographic films as an absorbent of the ^{125}I. Films with the emulsion thickness 20 μ and photographic plates with emulsion thickness 5 μ were used. The silver halides were removed by photographic fixation. Strips of the silver bromide-free films were immersed in water solution of [^{125}I]iodide in the concentrations 1:2:4:8:16. Carrier (KI) and wetting agent (Johnson 326) were added to the solutions in order to improve the evenness of the ^{125}I distribution and the [^{125}I]iodide was consequently taken up by the film.

Autoradiograms were made by apposition of the stair-cases against Structurix X-ray film (about 20 μ thick) or Ilford G5 emulsions (about 5 μ thick). As the emission of extranuclear electrons is always to some extent accompanied by electromagnetic radiation, it was of interest to investigate the relative contribution of these two types of radiation in producing the autoradiographic image. Mylar sheets, 9 mg/cm^2, which absorbed the emitted extranuclear electrons of ^{125}I but not the electromagnetic radiation, were put as intervening layers in a longitudinal direction leaving one half of each stair-case free. The combined thickness of the ^{125}I stair-case plus autoradiographic emulsion was either 5 + 5, 20 + 5, 5 + 20, or 20 + 20 μ.

Histological sections

Three pregnant mice weighing about 40 g each were injected in a tail vein with 375 μC carrier-free ^{125}I per animal. The animals were sacrificed after 30 min, 1 h and 24 h by immersion in a mixture of solid carbon dioxide and aceton (−70°). Sagittal sections through the entire frozen animals were taken from each mouse at −10° at various levels. The sections were 40, 20, 10 and 5 μ thick. The sections were dehydrated at −10° and pressed against the emulsions for 3–30 days of exposure. The following autoradiographic emulsions were used: Gevaert Structurix X-ray film, Kodak SWR, Ilford G5, and Ilford Q3. After exposure the sections and emulsions were separated and the emulsions were developed and fixed. The autoradiographic technique has been described in detail previously[10].

RESULTS AND DISCUSSION

Autoradiograms from micro test-charts

The most pronounced differences in the resolution between the autoradiograms from ^{125}I- and ^{131}I-labelled micro test-charts were observed in the experiments using Kodak SWR emulsion. Results from such an experiment are illustrated in Fig. 1. The probable explanation to these observations is that in the SWR and in other

Schumann-type emulsions the silver bromide grains are exclusively confined to the surface of the gelatin.

Autoradiograms from ^{125}I stair-cases

In the experiments with Mylar sheets as intervening layers it was observed that the relative contribution of the electromagnetic radiation was smallest when using

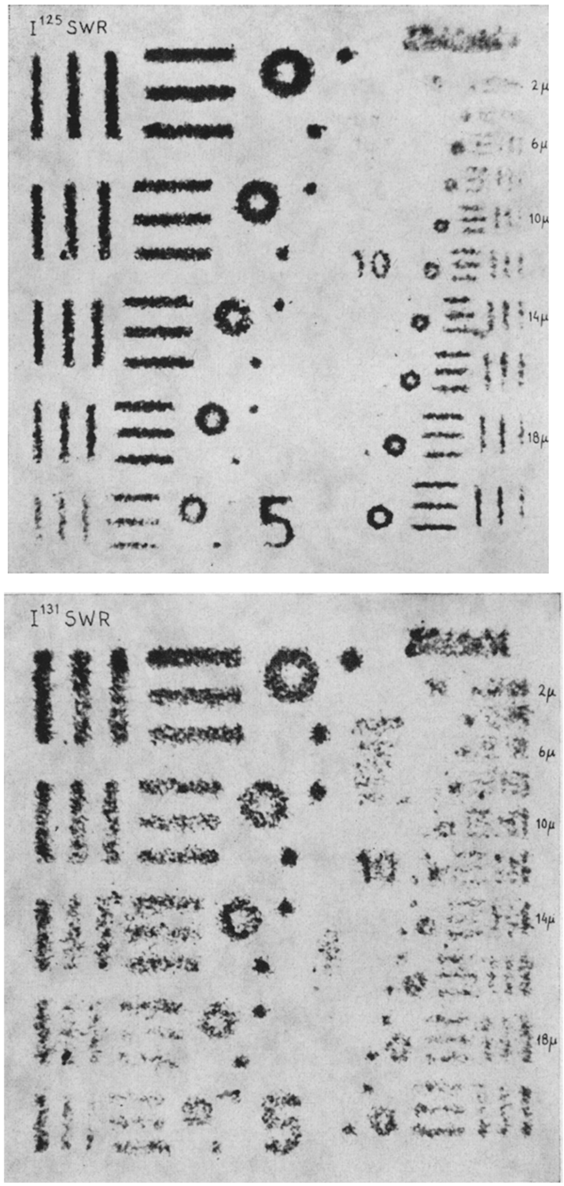

Fig. 1. Autoradiograms of micro test-charts on Kokak SWR film showing the different resolution with ^{125}I and ^{131}I.

thin emulsions. When using the 20 μ thick stair-case and 20 μ thick photographic emulsion the intervening Mylar sheet decreased the blackening response to a relatively small extent. In those experiments only when the total thickness of stair-case and emulsion was 10 μ, the electron radiation gave a stronger blackening response than the electromagnetic radiation. Typical examples of autoradiograms obtained are shown in Fig. 2.

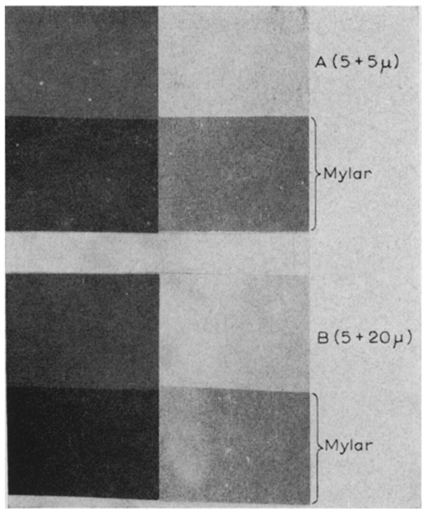

Fig. 2. Autoradiograms from an ^{125}I stair-case (2 steps) made by use of a 5 μ thick film and exposed on a 5 μ thick emulsion (A) and on a 20 μ thick emulsion (B). The lower parts of the emulsions have been covered with a Mylar sheet.

Autoradiograms from histological sections

In all the autoradiograms of histological sections of the mice injected with ^{125}I obtained in the various emulsions the resolution became significantly better than in the autoradiograms produced by ^{131}I under identical experimental conditions. Relatively, the resolution gains more by a decreasing thickness of the sections when using ^{125}I than using ^{131}I. Fig. 3, showing an abdominal detail of an autoradiogram from a pregnant mouse sacrificed 1 h after injection of the ^{125}I solution, illustrates the results obtained with a 5 μ thick section on Ilford Q3 emulsion after 7 days of exposure.

Detailed data concerning the radiation properties of ^{125}I and ^{131}I are to be found in nuclear data compilations[1,2] and in other papers[3,4]. The energies of the internal conversion- and Auger-electrons of ^{125}I are approximately 3, 25, 30, and 34 keV. The range in water is for 25 keV about 8 μ, for 30 keV about 15 μ, and for 35 keV about 20 μ. ^{131}I emits four negatrons and eight γ-rays. The dominating β-radiation has a maximum energy of 608 keV and an average energy of 188 keV. The range in water for the 608 keV β-rays is about 2100 μ and for the 188 keV β-rays about 380 μ. There are consequently great differences in the penetration abilities of the extranuclear electron radiation from ^{125}I and the β-rays from ^{131}I. Further discussions about the physical properties of ^{125}I and ^{131}I have previously been presented[3]. The internal conversion electrons with the energies of 30 keV and the Auger electrons with the

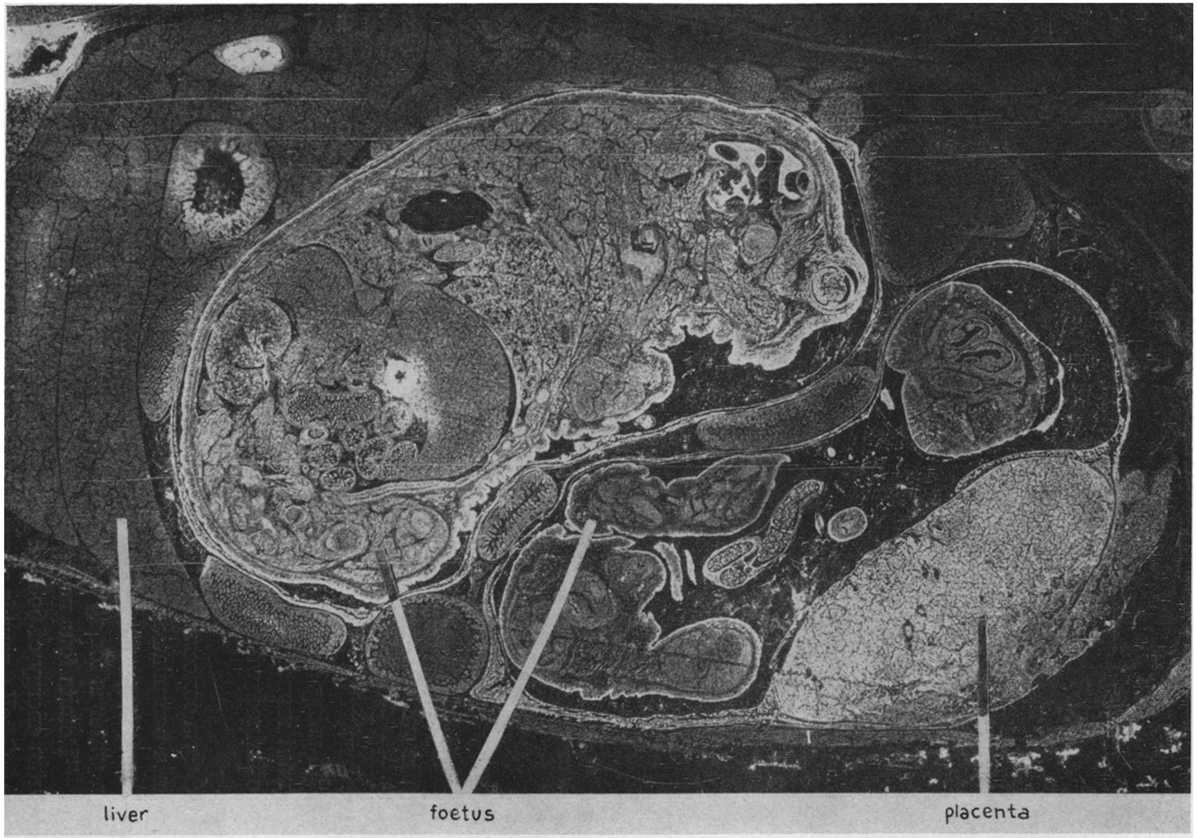

Fig. 3. Autoradiogram showing the distribution of ^{125}I in the abdomen of a pregnant mouse, 60 min after an intravenous injection of the ^{125}I solution. A 5 μ thick section was in contact for [illegible] days of exposure on Ilford Q3 emulsion.

energies between 22 and 32 keV are mainly responsible for the blackening in the autoradiographic emulsions. The relative contribution from the electrons with energies of 3–4 keV is probably significant only in experiments with ultrathin sections and very thin silver bromide layers (combined autoradiography and electron microscopy[11]). The blackening response due to the 35 keV γ-rays and the 27–32 keV X-radiation is probably very small. However, the X-rays with energies lower than 4.9 keV seemed to contribute to the blackening in the various emulsions used in the present experiments. It will also be noted that the autoradiograms obtained only with these soft X-rays of ^{125}I, where the electrons consequently were stopped, showed a very good resolution. This has also been found in our experiments with ^{55}Fe, ^{85}Sr, and other nuclides[6]. Recently FITCH *et al.*[12] also showed some autoradiograms obtained by means of ^{125}I. These autoradiograms also showed a high resolution. It can be assumed that the electromagnetic radiation of ^{125}I will cause relatively little blurring in the autoradiograms, especially when the sections and autoradiographic emulsions are thin.

In this connection it will be noted that development of production methods using thermal neutrons in bombardment of ^{124}Xe (see ref. 13) has permitted large scale production of highly purified ^{125}I at a moderate price[3].

Some other nuclides emitting internal conversion- and Auger-electrons of high intensities are under study for their properties for autoradiographic use. The results of these experiments will be published elsewhere[8].

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