Efficiency of Grain Production and Latent Image Fading in F-18 Micro-autoradiography

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Yamada, S., Kubota, R., Kubota, K., Ishiwata, K. and Ido, T. Efficiency of Grain Production and Latent Image Fading in F-18 Micro-autoradiography. Tohoku J. Exp. Med., 1992, 167 (3), 181-184 —— In F-18 micro-autoradiography using a frozen section method, the grain production was measured relative to the exposure time and radioactivity. The grain production increased until 6-hr exposure, but 7-hr or longer exposure induced a characteristic of latent image fading. However, the ratios of the efficiency of grain production between two different F-radioactivities (11.9 and 2.3 mBq/100 μm²) were constant over experimental exposure times, thus indicating that quantitative analysis by grain counting is preserved. ——— micro-autoradiography; F radiopharmaceutical; efficiency of grain production; latent image fading

F Radiopharmaceuticals have been utilized as tracers for positron emission tomography to visualize the metabolic and functional activities. To obtain a further information of tracer distribution at cellular level, we developed an F micro-autoradiography (micro-ARG) technique in which a frozen section was directly mounted on a glass slide covered with stripping film (Yamada et al. 1990). In a frozen-section method, however, a latent image fading which reduces the developed grains is unavoidable (Boren et al. 1975; Rogers and Watanabe 1988). In the present study, we measured the efficiency of grain production relative to the exposure time and radioactivity, and assessed the effect of latent image fading on quantitative analysis by grain counting in F micro-ARG.

MATERIALS AND METHODS

Micro-ARG process

Two normal male C57BL/6 mice were injected intravenously with 277.5 and 62.9 MBq of 2-deoxy-2-[F]fluoro-D-glucose, respectively, and were killed 5 min later. As homogeneous in vivo samples, the livers were quickly removed, and frozen on a flat dry-ice. Under a safety light, the frozen liver blocks were sectioned in a cryostat (−20°C). Each 5 μm-thick section was directly mounted on a cooled glass slide coated with AR-10 stripping

Received June 19, 1992; revision accepted for publication July 9, 1992.

181
film (Kodak, London, U.K.) and exposed at $-70^\circ$C in an exposure box from 1 to 10 hr with 1 hr interval and for 30 hr. Other 5 $\mu$m-thick sections of the same samples were attached to thin polyethylene films and punched for radioactivity measurement. After exposure, the autoradiograms were developed in Konidol-X (Konica, Tokyo), fixed in Fuji general purpose fixer (Fuji, Tokyo).

Grain number and radioactivity measurement

Grain number was counted under a light microscope ($\times 1,000$) using a micrometer. Background was subtracted from each data. $^{18}$F radioactivity in the punched out section was measured with a gamma-counter. Cross calibration between the gamma-counter and a well-type dose meter was performed. The radioactivity per 100 $\mu$m$^2$ of the section was calculated and corrected for decay.

Efficiency of grain production

Efficiency of grain production can be defined by the grain number produced for every one disintegration in the specimen.

In $^{18}$F micro-ARG, the cumulative number of disintegrations during the exposure time $t$, $D(t)$, is written as

$$D(t) = \int_0^t A(0) \cdot \exp(-0.693t/T_{1/2})dt$$

where, $A(0) = ^{18}$F radioactivity at the starting time of exposure, $T_{1/2} =$ half life of 109.8 min.

Therefore, the grain production during the exposure time $t$, $G(t)$, is given by

$$G(t) = K \cdot D(t)$$

($K$ : constant).

The value of $K$ indicates an efficiency of grain production for exposure time $t$. The efficiency was calculated by solving the above equation using experimental data of grain numbers.

RESULTS

Mean $^{18}$F radioactivities of the punched sections of the mice injected with 277.5 and 62.9 MBq of $^{18}$F-FDG were 11.9 $\pm$ 1.2 and 2.3 $\pm$ 0.2 mBq/100 $\mu$m$^2$ at the starting time of exposure, respectively.

The grain number, the efficiency of grain production and the ratios between two radioactivities are given in Table 1. The ratios of the grain numbers were almost constant (mean $\pm$ s.d. = 5.14 $\pm$ 0.16), which were close to the ratio of the radioactivities (5.17). The efficiencies of grain production were constant during the first 6 hr (high-radioactivity : 0.32 $\pm$ 0.02, low-radioactivity : 0.33 $\pm$ 0.02), but they decreased with longer exposures. The ratios of the efficiency were constant (0.96 $\pm$ 0.02).

DISCUSSION

The latent image fading is due to the interference between the developed grains and the chemical reactive groups in the specimen, and is favored by the presence of moisture (Boren et al. 1975). If there is no latent image fading, the grain number increases with the extension of exposure time, and the efficiency of grain production is independent of the radioactivity and does not change with time. In this experiment, it is uncertain whether the latent image fading occur-
red or not during the first 6 hr, however, the apparent latent image fading occurred at 7 to 30 hr exposure. The moisture in the specimen might cause the latent image fading.

In AR-10 stripping film, the efficiency of grain production in $^{32}$P (maximum energy of beta particle: $E_{\text{max}} = 1.7$ MeV), $^{131}$I ($E_{\text{max}} = 0.82$ MeV), $^{59}$Fe ($E_{\text{max}} = 0.46$ MeV), $^{14}$C ($E_{\text{max}} = 0.14$ MeV) and $^3$H ($E_{\text{max}} = 0.018$ MeV) at room temperature are 0.39, 0.9, 0.8, 0.9, and 0.43 grains/disintegration, respectively (Rogers. 1979), however, the efficiency in $^{18}$F ($E_{\text{max}} = 0.625$ MeV) is not known. In this experiment, the average efficiency in $^{18}$F during the first 6 hr was $0.32 \pm 0.02$ grains/disintegration. It was lower than those in $^{131}$I and $^{59}$Fe whose maximum energies are close to that of $^{18}$F. Low temperature exposure might reduce the efficiency of grain production.

In conclusion, these results showed that the apparent latent image fading occurred with longer exposure than 6 hr, but the ratios of the efficiency of grain production were constant over experimental exposure times. Whether the latent image fading occurs or not, the quantitative analysis by grain counting is preserved when the series of experiment are performed with the same exposure time.

**References**


2) Rogers, A.W. (1979) *Technique of Autoradiography*, Elsevier/North Holland,