Regulatory Toxicology and Pharmacology **31,** S15–S21 (2000)
doi:10.1006/rtph.2000.1381, available online at http://www.idealibrary.com on IDEAL®

Quantitative Whole-Body Autoradiography: Recommendations for the Standardization of the Method

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Received February 10, 2000

The objective of this article is to identify the best conditions for preparing, handling, and exposing radioactive sections by using the Fujix BAS 2000 system for quantitative radioluminography. Regarding the influence of thickness of sections, thicker sections may allow shorter exposure times due to the increased radioactivity, but on the other hand they take more time for the freeze-drying process, resolution will be reduced, and the recovery of radioactivity will be lower due to increased self-absorption particularly in organs like bones or teeth. The pretreatment of the sections should depend on the method of exposure. Powdering with talcum is the most recommendable method when vacuum packaging the imaging plates and sections. Spraying with Nobecutan is recommended when using the cartridge method. Particularly for low concentrations, the vacuum-contact method should be the method of choice. To avoid a flare effect, the geometrical arrangement of the sections on the imaging plate (IP) should always be vertical to the scanning direction of the laser. An exposure time longer than 10 days is not recommended and the time between the end of exposure and start of scanning should be as short as possible. In order to reduce the background signal, it is necessary to expose the IPs in a shielding box in a cold environment. No positive chemographic effects of sections were found. © 2000 Academic Press

INTRODUCTION

The "imaging plate" is a new film-like highly sensitive radiation image sensor composed of specifically designed phosphors that trap and store the radiation energy (Miyahara, 1989; Hamaoka, 1990). The stored energy is stable until scanned with a laser beam, which releases the energy as photostimulated luminescence (PSL). This new technology, launched in its first application to the medical X-ray diagnostic field, portends great promise in a wide range of newer scientific and technological applications.

One of these new applications is the whole-body autoradiography (WBA): This method, developed by Ullberg (1954), traditionally uses X-ray films for the detection of radioactive labeled compounds in the organism. Quantification of radioactivity has always been hampered by the small linear range of X-ray films and the laborious procedures involved in densitometry measurement of the exposed film materials. The newly introduced radioluminography (RLG) offers a more reliable and a very fast way of quantifying the radioactivity distribution in whole-body sections.

The objective of these studies was to identify the best conditions for preparing, handling and exposing radioactive sections by using the Fujix BAS 2000 system for quantitative radioluminography. The influence of thickness of sections, pretreatment, and the geometrical arrangement of the sections on the imaging plate (IP) was examined. Furthermore, the influence of duration and temperature of exposure and of the time between the end of exposure and beginning of scanning was examined. It was also studied whether the image information is influenced by repeated scanning of the IP and whether shielding of the IP by Pb distinctly reduces the background level. The studies are part of the cooperative European multicompany validation process of RLG.

MATERIALS AND METHODS

Whole-body tissue sections were prepared by the method of Ullberg (1954). ^{14}C- or ^{3}H-labeled compounds (sp radioact 0.1–4. 2 MBq/mg) were injected to male Wistar rats (body weight 165–200 g), which were sacrificed after suitable time periods and immediately deep frozen. The carcass was embedded in aqueous carboxymethyl cellulose gel, and sections of a given thickness (25–75 μm) were cut with cryomicrotomes (Poly-Macrocut, Leica, Germany; or PMV 450, PMV, Sweden) and freeze-dried.

Amersham microscales or blood matrix calibration samples radiolabeled with either carbon-14 or tritium were used as standards.

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TABLE 1
Influence of the Section Thickness on the PSL Values

Section thickness (μm):	25	37	50	62	75
Liver	946	1151	1759	2019	2274
Salivary gland	809	1144	1575	2054	2407
Myocardium	625	800	1161	1352	1661
Skeletal muscle	313	424	590	815	880
Blood (ventricle)	229	354	528	674	799
Bones	210	211	299	328	358
Testis	27.7	36.0	49.6	62.9	78.9
Spinal cord	14.4	19.0	29.4	40.6	45.5
Brain	11.4	14.2	22.5	31.1	39.0

Note. PSL, background/mm^2, $n = 5$.

TABLE 2
Influence of the Section Thickness on the Detected Concentrations (μg Equivalents/g, $n = 5$)

Section thickness (μm):	25	37	50	62	75
Liver	291	249	244	225	212
Salivary gland	249	248	219	229	225
Myocardium	192	173	161	151	155
Skeletal muscle	96.1	91.7	81.9	90.7	82.1
Blood (ventricle)	70.2	76.5	73.2	75.0	75.0
Bones	64.2	45.5	41.5	36.4	33.4
Testis	8.20	7.63	6.79	6.92	7.24
Brain	3.16	2.91	3.02	3.37	3.51

The evaluation of the sections was performed using the Fujix BAS 2000 system (Fuji Photo-Film, Tokyo, Japan) with software Fujix BAS 2000 or TINA (Raytest, Straubenhardt, Germany). The scanner conditions for all scans were warming up, more than 30 min; gradation, 1024; sensitivity, 10,000; resolution, 100; latitude, 4.

For the "vacuum-contact method," a vacuum packaging machine type GM 2/2002 (Boss) and black PA/PE vacuum bags were used.

Detailed information regarding the methods of the various studies is given below, together with the results.

RESULTS

The Influence of Section Thickness

A total of 1.5 MBq of a carbon-14-labeled drug was administered intravenously to one rat. The animal was killed 5 min after administration. Whole-body sections of different thicknesses (25, 37, 50, 62, and 75 μm) were prepared. After drying and pretreatment with talcum powder, the sections were exposed to IP for 5 days in a cartridge under lead protection. The recorded PSL was measured in different organs and tissues (Table 1 and Fig. 1).

One advantage of thicker sections is the increased radioactive amount per area and thus of PSL, too. Between 25 and 75 μm section thickness, the PSL signal increases roughly proportional for most of the organs and tissues investigated. The relative recoveries related to 25 μm (=100%) deviate between 80 and 100% for all section thicknesses and organs. However, as expected, with the exception of bones which clearly reveal a less than proportional increase of the PSL signal and a thickness-dependent reduction of recovery to 57% with 75 μm, relative to 25 μm. The reason is concluded to be the high self-absorption of bone mate-

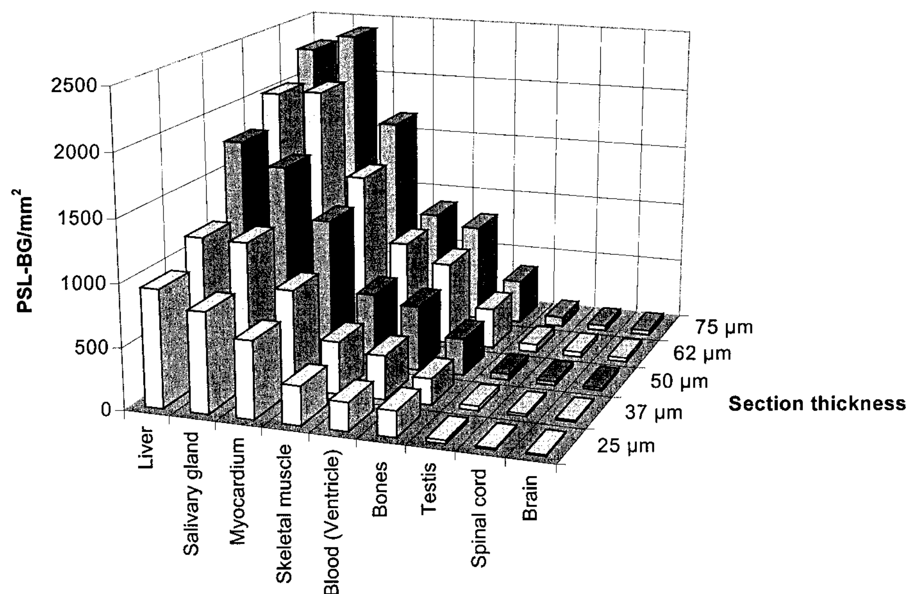


FIG. 1. Influence of the section thickness on the PSL values. Whole-body sections of different thicknesses (25, 37, 50, 62, and 75 μm) were prepared. After drying and pretreatment with talcum powder, the sections were exposed to IP for 5 days in a cartridge under lead protection. The recorded PSL was measured in different organs and tissues.

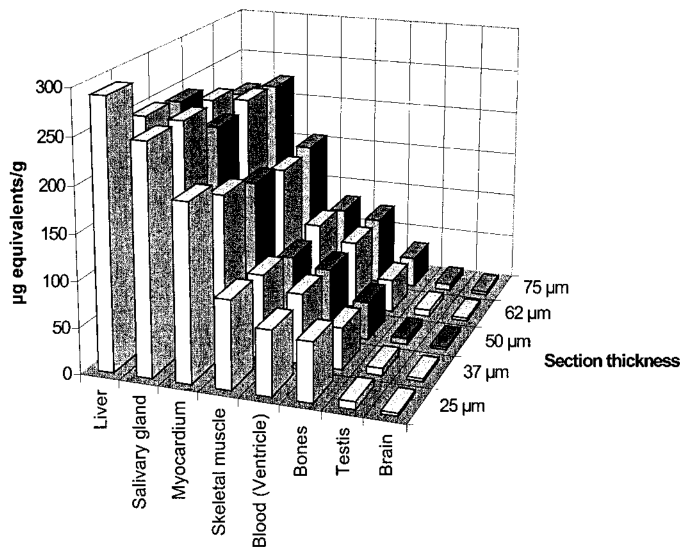


FIG. 2. Influence of the section thickness on the detected concentrations. Concentrations in the organs and tissues were calculated using calibration curves of internal blood standards.

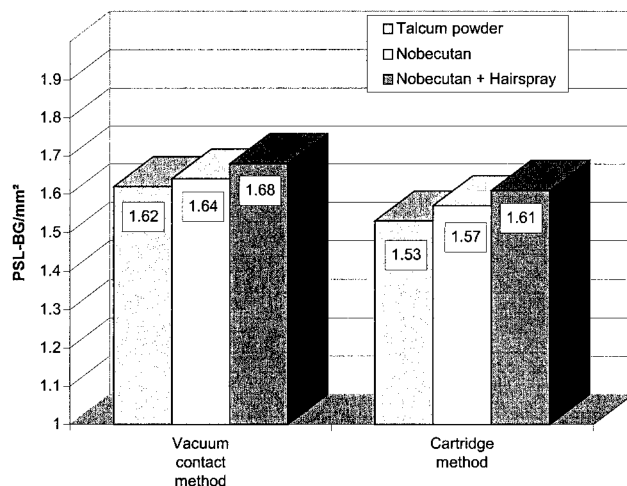


FIG. 3. Influence of pretreatment of the sections and method of exposure on the PSL values (consecutive sections). After freeze-drying of six consecutive sections, two of them were powdered with talcum, two were sprayed flimsily with Nobecutan spray, and the remaining two were sprayed with both Nobecutan and hair spray. All sections were exposed in a cartridge.

rial as described elsewhere (Klein *et al.,* 2000; Ahr *et al.,* 1994). In all organs and tissues tested including bones, the so-called "layer of saturation thickness" appears not to be reached up to 75 μm.

Concentrations in the organs and tissues were calculated using calibration curves of internal blood standards, i.e., radiolabeled blood calibration samples sectioned together with the animal body at the same thickness (Table 2 and Fig. 2).

As expected, the concentrations are roughly constant with a trend to higher values toward thinner sections. This observation was most pronounced with the concentrations in bones; the reason is the strong increasing self-absorption in this tissue. Small different self-absorption rates between blood (standards) and organs and tissues appear to be responsible for the observed small deviations. Consistent with that, the concentrations in ventricle blood calculated using blood standards keep constant for all section thicknesses.

Using thicker sections, the increased radioactivity may allow shorter exposure times, but thicker sections take more time for the freeze-drying process, resolution will be reduced, and the recovery of radioactivity will be lower due to increased self-absorption.

The Influence of Pretreatment of Sections

Six consecutive ^{14}C-labeled sections of 25 μm thickness were produced. After freeze-drying, two of them were powdered with talcum, two were sprayed flimsily with Nobecutan spray, a commercially available wound spray (Astra Chemicals, Wedel, Germany), and the remaining two were sprayed with both Nobecutan and hair spray. All sections were exposed in a cartridge and a second time by using the vacuum-contact method (Kloss *et al.,* 1973). The exposure time was 1 h under lead protection, in each case.

A tritium section of 25 μm thickness and a carbon-14 section of 50 μm thickness were firstly exposed by using the cartridge method and a second time by using the vacuum-contact method. Respective exposure conditions were 5 days (tritium) and 2 h ($_{14}$C) under lead protection.

Each section was evaluated by using standardized regions of interest (Fig. 3).

TABLE 3
Advantages and Disadvantages of Different Pretreatments of Sections

	Nobecutan	Talcum powder
Advantages	• Good fixation of the whole section, in particular git contents	• Very cheap
	• Reduction of freeze-drying artifacts	• Sections do not stick → No problems in reexposure
	• Good preservation of tissue colors	• Storage without problems
Disadvantages	• Sometimes sticky sections[a] → Reexposure with problems	• Change of section color → Evaluation more difficult
	• Problems in long-term storage	

[a] It was found that it is advantageous to use Nobecutan before freeze-drying of the sections. This reduces sticking of the sections to the IP.

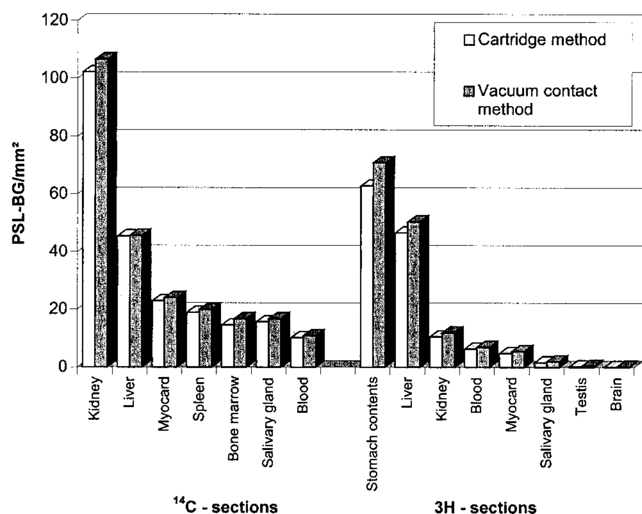


FIG. 4. Influence of the method of exposure on the PSL values (identical sections). Identical sections were exposed in a cartridge and a second time by using the vacuum-contact method.

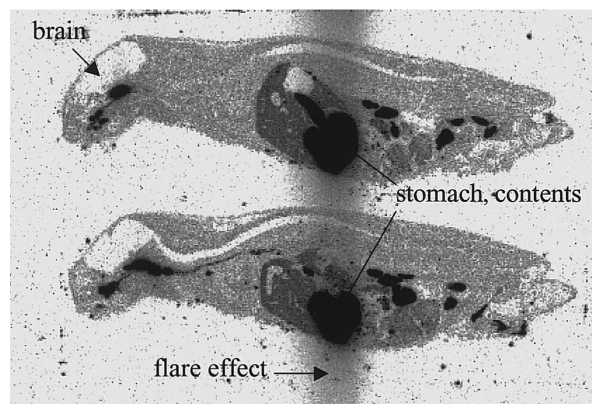


FIG. 5. Whole-body autoradiogram (radioluminogram) of a male mouse after oral administration of a ^{14}C-labeled compound. Note the high radioactivity concentration in the stomach contents which results in a strong flare effect in scan direction. In order to minimize the affected tissue areas, the geometrical arrangement of the sections on the imaging plate should always be vertical to the scanning direction of the laser, i.e., a horizontal arrangement on the imaging plate.

The pretreatment of the sections should depend on the method of exposure. Powdering with talcum is the most recommendable method when vacuum packaging the imaging plates and sections. Spraying with Nobecutan is recommended when using the cartridge method. Some advantages and disadvantages of the use of talcum and Nobecutan are given in Table 3. The pretreatment of the sections clearly affected the recorded information of the radioluminograms. Powdering with talcum powder seems to reduce the detected PSL values compared to the pretreatment with Nobecutan both in ^{14}C and in ^{3}H studies (Fig. 3). Therefore, an identical pretreatment of sections and standards (either talcum or Nobecutan) should be obligatory.

When using the vacuum-contact method, the recovery of radioactivity is increased compared with the cartridge method for both ^{14}C and ^{3}H imaging plates (Figs. 3 and 4).

Thus, for the measurement of low concentrations, the vacuum-contact method should be the method of choice; for higher concentrations, both methods can be used.

The Influence of the Geometrical Arrangement of the Sections

Parallel to the laser scanning direction, often a flare effect which is a scanning artifact is visible in the surroundings of high concentrations (e.g., stomach contents after oral administration, Fig. 5). Affected regions of low concentrations are impossible to validate and to quantify. Vertical to the scanning direction, this effect was barely observed. Based on these observations, the geometrical arrangement of the sections on the imaging plate should always be vertical to the scanning direction of the laser, i.e., a horizontal arrangement on the imaging plate.

The Influence of the Duration of Exposure

Amersham ^{14}C microscales were exposed to one imaging plate (cartridge method; lead protection) for different times from 1 to 21 days. The dependence of the PSL signal height on the exposure time was investigated (Fig. 6).

Although linearity with time is given throughout distinct ranges of exposure times, prolongation to 10 days and more leads to a less than proportional increase of PSL values. Approximately after 10 days, a plateau is reached and only a small further accumulation of PSL values is observed. Therefore, an exposure time longer than 10 days is not recommended.

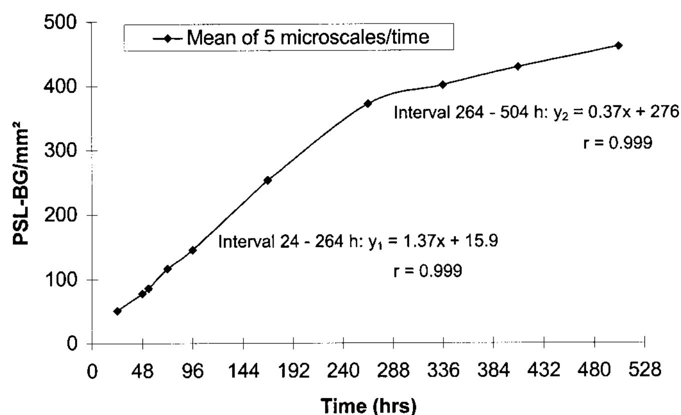
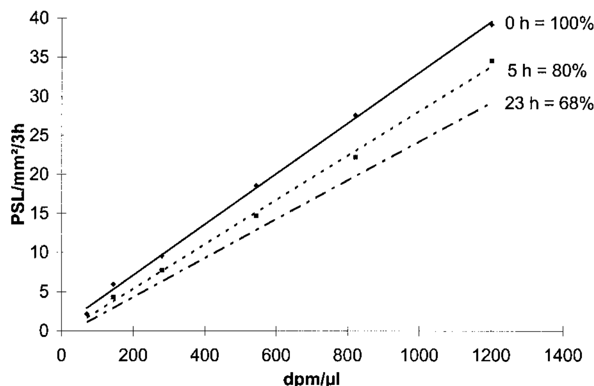


FIG. 6. Influence of exposure time on the PSL-BG/mm^2 values. After approximately 10 days, a plateau is reached and only a small further accumulation of PSL values is observed. Therefore, an exposure time longer than 10 days is not recommended.



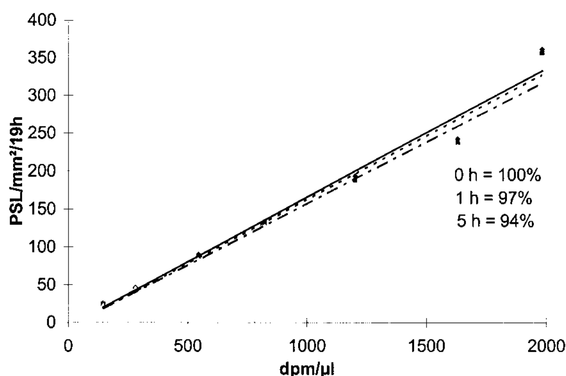


FIG. 7. Loss of information at room temperature after a 3- and after a 19-h exposure. The time between the end of exposure and start of scanning should be as short as possible; otherwise a loss of information is inevitable.

The Influence of the Time between the End of Exposure and Beginning of Scanning

^{14}C-labeled blood scales were exposed to imaging plates for 3 h at 4°C. The IPs were scanned immediately after exposure or 5 and 23 h after removing of the labeled blood scales. In a second study the blood scales were exposed for 19 h and scanned immediately, 1 h, or 5 h after removing of the labeled samples. After the sample exposure was stopped, the IPs were stored simulating usual exposure conditions at room temperature until the scan was started (Fig. 7).

Conclusively, the time between the end of exposure and start of scanning should be as short as possible; otherwise a loss of information is inevitable. After a 3-h exposure, 20% of the originally recorded signal was lost after 5 h, and more than 30% was lost after 23 h of storage, indicating a higher loss during the initial (shorter) storage period. Results obtained after the 19-h exposure are in good agreement with these observations. In this case, only 6% was lost after a 5-h

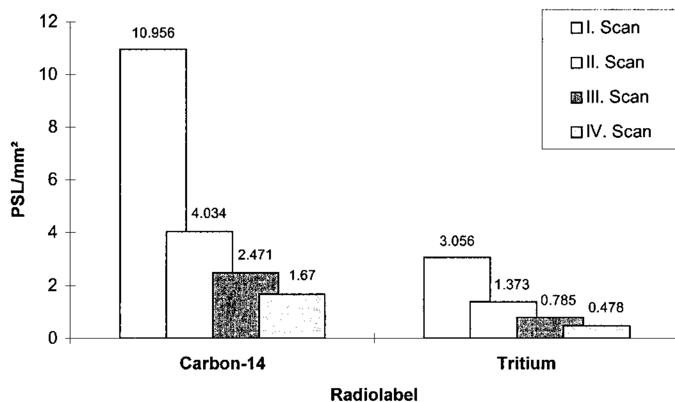


FIG. 8. Loss of information after repeated scanning of the same imaging plate. After repeated scanning of the same IP, the PSL signal height decreased with the number of scans.

storage. Obviously, this time-dependent fading of luminescence intensities occurs in nonlinear relation to time, but linear with respect to radioactivity concentrations. It is concluded that this fading effect also occurs during exposure.

The Influence of Repeated Scanning of the IP

Four sections of a rat treated with a carbon-14-labeled drug (thickness 25 and 50 μm) were exposed for 24 h on an IP, removed, and scanned four times. The same procedure was carried out with sections of a rat dosed with a tritium-labeled compound (Fig. 8).

After repeated scanning of the same IP, the PSL signal height decreased with the number of scans. A loss of around 60% is found after the first scanning procedure and up to 85% was lost from the first to the fourth scan for both ^{14}C and ^{3}H imaging plates. The reduction of the PSL signal height was independent of the radioactivity concentration.

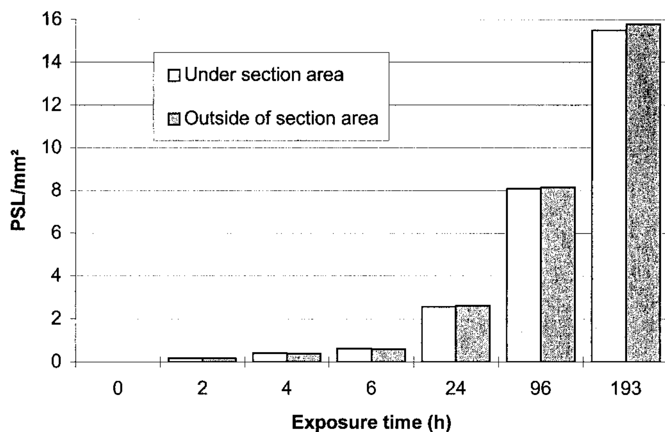


FIG. 9. Background under and outside of an unlabeled section (exposure temperature 4°C). The background was determined beside and under the sections to find out whether there is any recording of the IP due to chemographic effects of the tissue.

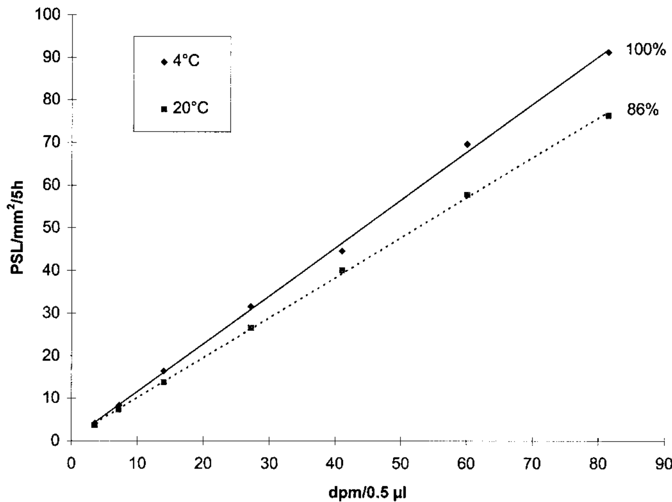


FIG. 10. Influence of temperature. Parallel to the increasing temperature, a loss of information was observed.

The Influence of Chemographic Effects of the Tissues

A nonlabeled section was exposed to the IP for different times up to 193 h. The background was determined beside and under the sections to find out whether there is any recording of the IP due to chemographic effects of the tissue (Fig. 9).

There was no difference in the background as determined either under or beside (outside) the section. No positive chemographic effects were found after exposure at room temperature and in a shielding box, either. Additionally, it can be concluded that the background signal which usually must be obtained outside the section areas of the IPs does not differ from the "real" background which is effective in the section area.

The Influence of Shielding and Temperature

Amersham microscales were exposed on a IP in a refrigerator (+4°C) or at room temperature for 5 h to assess the effect of temperature (Fig. 10). Background measurements were conducted after an exposure for 49 h inside and outside of the RAYTEST shielding box (+18°C) to visualize the effect of shielding (Fig. 11).

As a result of the increasing temperature, a loss of information was observed. On average the recovery was reduced to 86% (low-temperature exposure set to 100%); this occurred independently of the radioactivity concentration used. The reason is attributed to the fading effect already mentioned above which obviously is reduced at low temperatures.

In order to reduce the background signal, it is necessary to expose the IPs in a shielding box. Then the background was reduced by 70–80%. An increased sensitivity will result, if both methods, shielding and cold environment, are combined, e.g., exposure in a cooled shielding box. However, using low-temperature exposure may arise the problem of condensation of air humidity after exposure which affects the IP material, i.e., a certain temperature adaptation period of the IP must be included in the exposure process.

CONCLUSIONS

Based on the above described experiences it is necessary to thoroughly standardize the experimental procedures, particularly if quantitative evaluation will be done. Calibration samples should be as similar as possible to the organs and tissues with regard to self-absorption and preparation. Thus, blood samples are

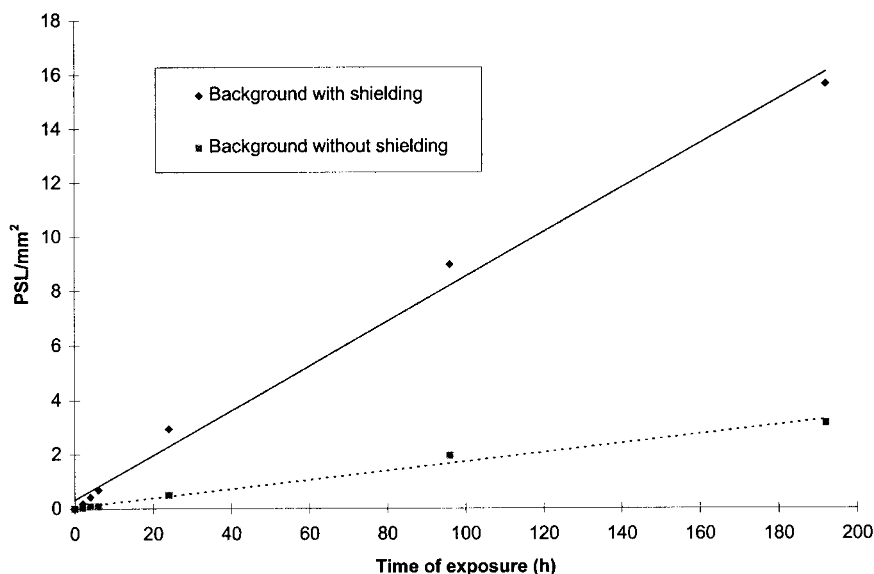


FIG. 11. Influence of shielding. In order to reduce the background signal, it is necessary to expose the IPs in a shielding box.

recommended for this purpose. The following further recommendations for the performance of whole-body autoradiography using imaging plates may be considered:

Section thickness: optimal 25–50 μm

Treatment of sections: using the cartridge method, Nobecutan or talcum powder; using the vacuum-contact method, talcum powder

Method of exposure: ^{14}C, cartridge method or vacuum-contact method; ^{3}H, vacuum-contact method

Geometrical arrangement of sections: always vertical to scanning direction

Duration of exposure: not shorter than 2 h, not longer than 10 days (^{14}C)

Temperature during exposure: keep exposure temperature constant (low if possible)

Time between exposure and scanning: as short as possible

Background reduction: use (cooled) shielding box

Calibration: use internal blood calibration standards.

ACKNOWLEDGMENTS

The authors thank Robert Braun, Dagmar Trummel, Heike Weigelt, Ingela Hillebrand, and Stephan Weber for their excellent technical assistance.

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