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Quantification of Tissue Self-Absorption of Weak β-Radiation in Lyophilized Whole-Body Sections of Rats

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Whole-body autoradiography has been widely used in the investigation of the distribution of radiolabeled compounds in animals. The newly introduced radioluminography offers a reliable way of quantifying the radioactivity distribution within whole-body sections. Since the radioactivity is distributed over the entire depth of the section, self-absorption of β-radiation in tissues is supposed to relevantly affect the detection of radioactivity at the section surface. The self-absorption of radiation energy (^{14}C) was investigated in 28 organs/tissues of routinely produced lyophilized rat sections. Nonradioactive whole-body sections with different thickness between 20 and 120 μm were placed between a homogeneous ^{14}C source and the imaging plates to detect the transmitted radioactivity. The self-absorption was expressed in terms of percentage of transmission of the radioactivity through the sections. Transmission decreased with increasing section thickness, e.g., from 44% (20 μm) to 28% (120 μm) for blood. Comparison of three complete sets of data disclosed intertissue variations of up to about 30% (i.e., ±15%) disregarding bone. A defined bandwidth of ±15% around the blood transmission would cover the transmission of almost all tissues. Thus, for most organs radioactivity can be quantified by direct comparison with radioactive blood calibration samples.

INTRODUCTION

Since its introduction by Ullberg (1954) whole-body autoradiography has been widely used to investigate the distribution of radiolabeled compounds in animals. However, quantification of the radioactivity has always been hampered by the small linear range of the X-ray films and the tedious procedures involved in densitometric measurement of the exposed film materials. The new imaging plate technique offer a more reliable way of quantifying the radioactivity distribution in whole-body sections. A comprehensive validation of the radioluminography is presented in several articles of this issue. Validation was conducted as a joint multicompany effort. This article presents the specific aspect of the self-absorption of the weak beta emitter ^{14}C in routinely produced lyophilized rat sections of different thickness.

MATERIALS AND METHODS

Whole-body autoradiography was carried out according to Curtis *et al.* (1981). The sections were attached to adhesive tape, lyophilized for at least 24 h, and placed between a homogeneous ^{14}C source (Amersham, UK) and the imaging plate (Fuji Photo Film Ltd., Tokyo, Japan). The source was a [^{14}C]polymethyl methacrylate sheet, 250 × 200 × 1 mm; its total radioactivity was 329 MBq (8.9 mCi) with a specific radioactivity of 5.37 MBq/g (145 μCi/g). This arrangement was exposed under lead shielding at ambient temperature for 10 min. After exposure the imaging plates were scanned using the Fuji BAS 2000 image analyzer (Hamaoka, 1990; Miyahara, 1989). Radioactivity was quantified in 28 organs and tissues using the Fuji software package Image Analyse in the following manner. In each organ or substructure 10 definite regions were set, whenever possible, and integrated. The resulting "photostimulated luminescence" per area (PSL/S) is proportional to the amount of radioactivity which had transmitted the particular tissue. In order to check the homogeneity of the source the same procedure was applied to the bare source. Since all rat sections adhered to tape, the transmission of this tape was also measured. Transmission data obtained for the organs were expressed as percentage of transmission in relation to the adhesive tape; i.e., the transmission of the tape was set to 100%. The whole experiment was conducted in triplicate to estimate the variability. Additionally, the correlation between section thickness and the relative response (PSL/area for section thickness of 30 μm was set to 1) was investigated for various tissues by direct measurements in radiolabeled sections from ^{14}C-dosed rats, i.e., without use of an external radiation source.

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TABLE 1

Transmission of Radioactivity of a ^{14}C Source through Nonradioactive Whole-Body Sections of Male Rats. Influence of Section Thickness and Tissue Type

Section thickness (μm):	20	40	50	60	80	100	120
^{14}C source:	728	665	683	681	687	701	704
Adhesive:	100	100	100	100	100	100	100
Lungs	41.5	37.1	39.1	35.6	36.7	35.2	38.1
Renal medulla	49.7	38.0	38.8	34.6	33.0	29.6	29.3
Pineal body	nd	41.7	37.4	34.7	37.5	31.0	28.3
Renal cortex	48.7	37.0	36.6	33.2	30.5	27.7	26.9
Small intestine	46.8	36.1	35.7	32.3	28.7	27.3	26.3
Intestinal mucosa	49.0	36.8	35.7	31.6	30.1	28.0	26.3
Spleen	49.8	36.1	35.6	32.2	29.9	27.2	25.4
Skeletal muscle	47.9	35.7	35.5	32.3	29.4	27.8	25.6
Testes	43.4	37.4	35.1	35.7	35.4	33.3	32.1
Pancreas	nd	34.7	34.1	30.2	28.1	26.0	24.0
Hypophysis	nd	34.9	33.9	32.2	30.6	24.2	25.9
Brain	40.5	34.3	32.8	31.6	28.1	24.5	24.7
Thymus	42.5	33.8	32.7	31.6	28.0	26.5	25.1
Blood	44.3	34.0	32.5	32.6	29.1	26.6	27.5
Liver	46.1	33.4	32.3	31.1	27.9	26.1	24.7
Heart	41.9	35.6	31.6	31.8	28.2	25.8	24.7
Colon contents	41.5	34.0	31.6	30.5	29.8	26.3	24.7
Thyroid	nd	34.3	31.3	30.4	23.1	24.1	23.8
Skin	36.9	32.9	30.4	26.1	24.5	22.5	20.5
Spinal cord	41.3	32.2	29.9	28.0	25.1	22.4	22.5
Adrenal gland	49.4	31.2	29.7	29.2	26.1	24.0	23.5
Bone marrow	44.4	32.1	29.1	27.9	25.0	21.9	22.0
Renal adipose tissue	42.2	28.5	27.5	24.8	24.1	21.1	20.5
Eye lens	45.0	32.4	27.0	22.9	22.1	21.1	21.4
Cartilage	34.7	30.5	26.7	26.3	22.5	20.9	27.7
Brown adipose tissue	36.6	30.9	24.0	22.5	19.8	21.0	19.1
Skull	34.0	25.2	21.9	22.5	20.8	19.7	17.3
Bone (femur)	31.8	16.4	17.8	15.4	15.8	15.3	15.9

Note. Transmission through the adhesive tape was set to 100% (means of $N = 3$). nd, not determined.

RESULTS AND DISCUSSION

Since the radioactivity is distributed over the entire thickness of the section, self-absorption of the β-radiation is expected to occur during its movement through the section and may significantly hamper the detection of radioactivity at the surface of the section. The degree of self-absorption or transmission is dependent on the thickness and on the specific absorption coefficient of the various tissues and organs. Therefore, the self-absorption was investigated in sections of different thickness (20 to 120 μm). The data are expressed as degree of transmission, i.e., radiation energy which had passed through the entire section thickness. On the other hand, the difference to 100% is equivalent to the degree of radiation energy which was absorbed from the tissue. The results are given in Table 1.

A typical transmission image of 50-μm sections which were exposed to the ^{14}C source is shown in Fig. 1. The transmission has also been plotted as a function of (fresh) section thickness (Figs. 2 to 6). As expected, the transmission decreased with increasing section thickness, e.g., from 44% (20 μm) to 28% (120 μm) for blood. A sharp drop was observed from 20- to 40-μm sections in almost all tissues investigated. The further decline varied among the organs. The lowest decline was found for lung tissue owing to its relatively low density. In the bone for example, the relative transmission had reached a value of below 20% at 40 μm and remained almost constant between 40 and 120 μm (Fig. 3). This can be explained by its relatively high density owing to its mineral consistency, which transforms the β-radiation to X-ray at higher thicknesses. An example for the opposite extreme is skin (Fig. 4), where the so-called layer of saturation thickness was still not reached at 120 μm. In most organs, however, saturation thickness was apparently not reached up to 100 μm of section thickness.

With sections obtained from ^{14}C-dosed rats a linear relationship between thickness and the amount of radioactivity measured was observed in the range of 10 to 60 μm (Fig. 7) for blood, liver, lungs, skeletal muscle, kidneys, and testes. In contrast to that, in adipose tissue no further increase of radioactivity was visible in a section thickness higher than 40 μm.

Intratissue variation of transmission was shown to

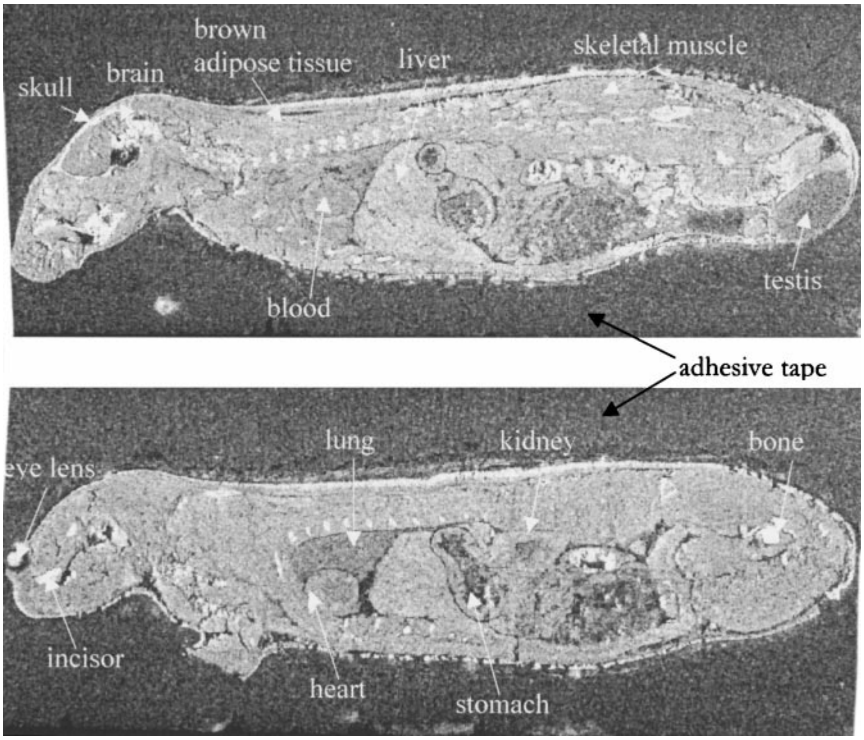

FIG. 1. Typical image showing absorption of radiation energy in organs and tissues of nonradioactive whole-body sections (50 μm) of a male rat after exposure to an homogeneously ^{14}C-labeled polymethyl methacrylate sheet as radiation source. Transmitted radiation was measured by imaging plates. The darker the blackening the higher the transmitted radioactivity.

be less than 10% independent of the section thickness. This was not true for bone and bone marrow, which are known to be inhomogeneous in structure, and small organs like thyroid, pineal body, and adrenals where only one or two regions of integration could be placed.

The homogeneity of the radiation source varied by 3.4%. This variation was not diminished by the adhesive tape covering the rat section.

With the exception of bone, the maximum interorgan differences in transmission or self-absorption were in the

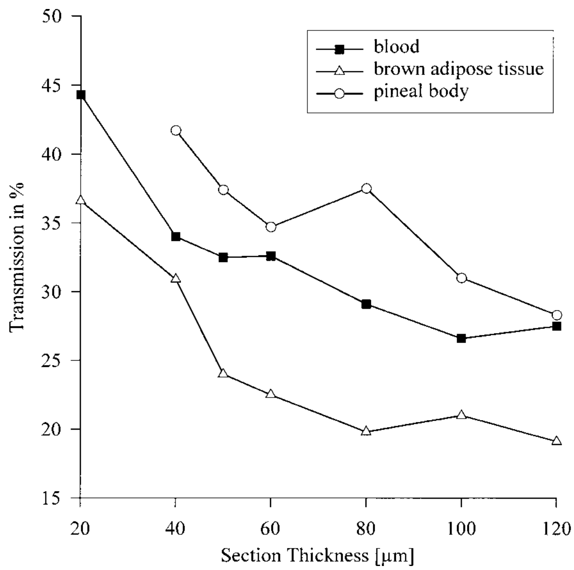

FIG. 2. Transmission of ^{14}C radiation energy across nonlabeled whole-body sections of male rats in dependence on section thickness and tissue types. Means of $N = 3$.

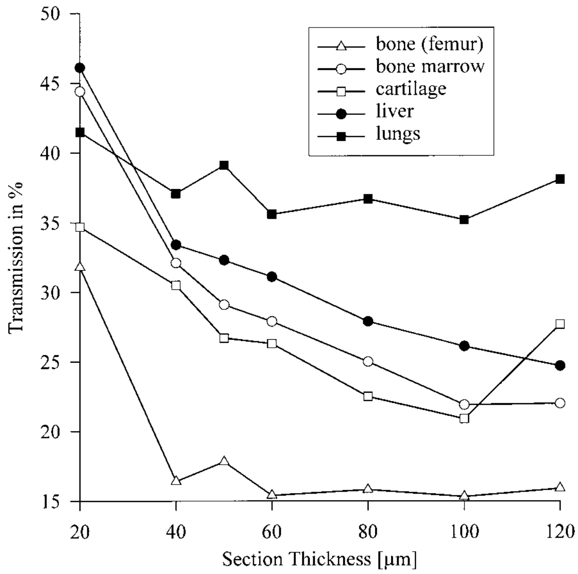

FIG. 3. Transmission of ^{14}C radiation energy across nonlabeled whole-body sections of male rats in dependence on section thickness and tissue types. Means of $N = 3$.

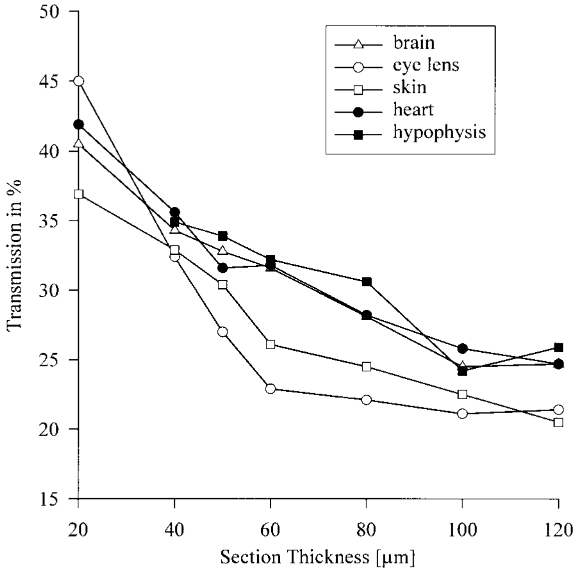

FIG. 4. Transmission of ^{14}C radiation energy across nonlabeled whole-body sections of male rats in dependence on section thickness and tissue types. Means of $N = 3$.

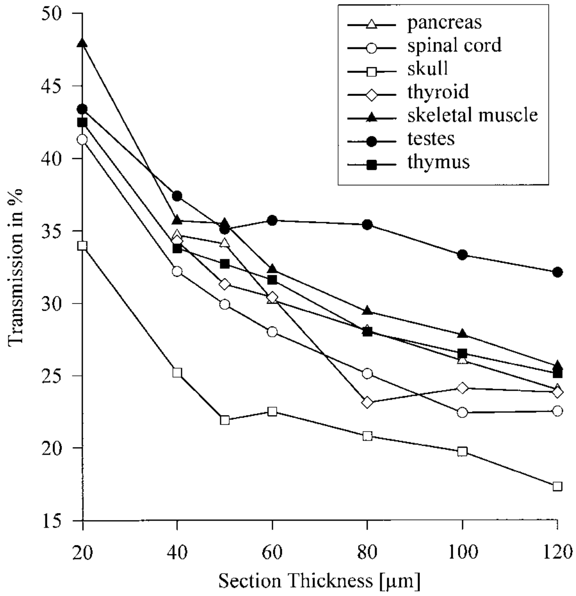

FIG. 6. Transmission of ^{14}C radiation energy across nonlabeled whole-body sections of male rats in dependence on section thickness and tissue types. Means of $N = 3$.

range of 13 to 19% (Table 1). A comparison of three complete sets of data disclosed intertissue variations of up to about 30% (±15%), disregarding bone. For the majority of tissues and organs, however, the variation was less than 10%. Even for the unobstructed radiation source differences up to 6% were observed. Inhomogeneities of the imaging plates have to be considered as well. Blood showed a medium transmission which appeared to be most representative for most organs and tissues. Using blood as a basis for calibration and an "equivalence" range of ±15% of the blood transmission, only a few tissues were not covered. Lungs had a higher transmission compared to blood while adipose tissue, skull, and bone exhibited distinctly lower values (for details see Table 2).

CONCLUSIONS

The self-absorption or transmission of β-radiation in blood corresponds well with most other organs and tissues. Based on these finding, it is suggested to use blood as representative tissue for calibration. The ra-

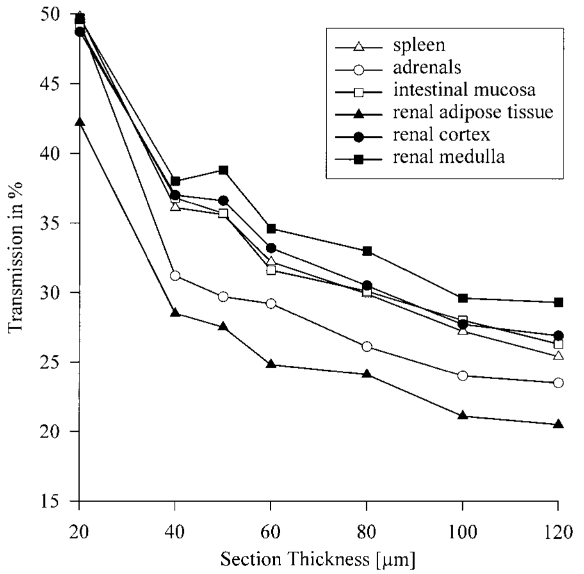

FIG. 5. Transmission of ^{14}C radiation energy across nonlabeled whole-body sections of male rats in dependence on section thickness and tissue types. Means of $N = 3$.

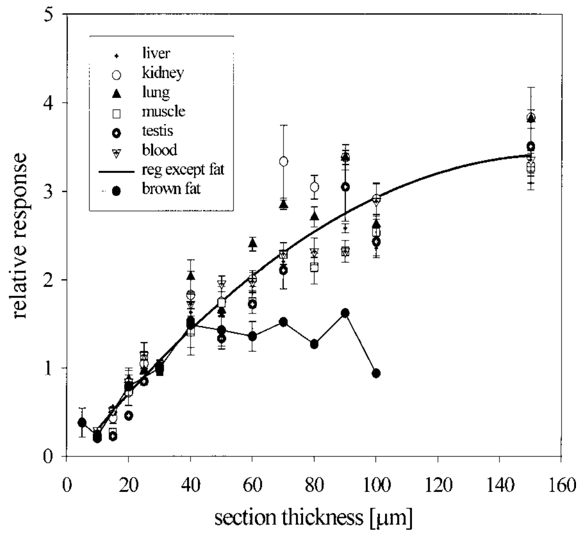

FIG. 7. Effect of section thickness on the relative response (PSL/area for section thickness of 30 μm was set to 1) measured in whole-body sections from ^{14}C-dosed rats, without external radiation source.

TABLE 2
Organs and Tissues Which Revealed a Transmission Deviating More Than ±15% from Blood Transmission (as Derived from Table 1)

Section thickness (μm):	40	50	60	80	100	120
Lungs		−20		−26	−32	−39
Renal adipose tissue	16		24	17	21	26
Brown adipose tissue		26	31	32	21	31
Skull	26	33	31	29	26	37
Bone (femur)	52	45	53	46	43	42

Note. Negative values indicate a higher transmission and positive values indicate a lower transmission than in blood.

dioactivity concentration in all tissues, except lungs, adipose tissues, skull, and bone, can be quantified by direct comparison with the blood standards. For the quantitative calibration in lungs, adipose tissues, skull, and bone individual correction factors for self-absorption may be appropriate.

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