
Inhomogeneous Deposition of Radiopharmaceuticals at the Cellular Level: Experimental Evidence and Dosimetric Implications

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We have undertaken an experimental examination of the conventional internal dosimetry assumptions of homogeneity of radionuclide deposition in tissues. The distribution of radiolabeled Microlite has been quantitated in mouse liver at the millimeter (multicellular) and the micrometer (cellular) levels. Measurements of radioactivity in 1-mm³ tissue samples indicate homogeneous radionuclide distribution; those derived from autoradiographs of 0.5- μ m tissue sections show that, relative to other cells, the colloid was concentrated 200- to 1000-fold in liver macrophages. The dosimetric implications of such inhomogeneous radionuclide distribution in human liver, where similar radionuclide distribution is expected, are discussed on the basis of a recently developed model for calculating the dose at the cellular level, and the estimates are compared to conventional internal dosimetry predictions. It is demonstrated that during routine diagnostic examinations with ^{99m}Tc-Microlite, conventional dosimetry underestimates the dose to labeled human liver cells by factors of 8–30.

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In estimating the hazards associated with the use of radiopharmaceuticals in nuclear medicine, conventional internal dosimetry (1,2) provides methods to calculate the average dose to individual human organs. An alternative parameter to the integrated organ dose, which may also be of importance, is the radiation dose to individual radiosensitive cells (3) or even to intracellular sites within such cells (4–7). The conventional methods for calculating the radiation dose delivered to human organs by radionuclides implicitly assume that

the dose to all cells of an organ is the same as the integrated organ dose (8). A basic requirement for the validity of this assumption is the fairly homogeneous distribution of the radionuclide and its emitted energy throughout the organ of interest. In some situations, this requirement is met, especially when the radionuclide is distributed throughout the extracellular space, e.g., sodium ortho[¹³¹I]iodohippurate used for dynamic renal-excretion-function imaging (9). In other instances, the radiolabeled compound may concentrate intracellularly, e.g., technetium-99m- (^{99m}Tc) labeled hexakis(carbomethoxyisopropylisonitrile) technetium(I) (10), hexakis(t-butylisonitrile) technetium(I) (11) and hexakis(isopropylisonitrile) technetium(I) (11) used for myocardial imaging, and N-isopropyl-p-[¹²³I]-iodoamphetamine (12) for brain imaging. Under such circumstances, the dosimetric consequences of assuming a homogeneous radionuclide distribution depend on the range of action of the emitted radiations. If penetrating gamma rays or energetic electrons with ranges far greater than the cell diameter are emitted, intra- or extracellular radionuclide concentration will not be a relevant factor since in either case the bulk of the individual cellular dose emanates from radioactive decays outside the cell whose exact origin is unimportant (8). However, for radionuclides whose decay results in the emission of low-energy electrons with cellular or subcellular ranges, situations may exist in which the homogeneity assumption of conventional dosimetry may be quite inadequate to describe the dose to individual cells (4–7).

To demonstrate the relevance of such considerations to current nuclear medicine practice, the distribution of radiolabeled albumin colloid (Microlite, Du Pont) in mouse liver was determined quantitatively at both the millimeter (multicellular) and the micrometer (cellular) levels. This radiopharmaceutical, when labeled with technetium-99m and used for hepatic scintigraphy (9),

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is rapidly cleared from circulation following intravenous (i.v.) injection and ~80% of the injected activity is taken up by the liver (13). Kupffer cells, which line hepatic sinusoids, fix and ingest circulating foreign material (14,15) and are thought to be the main site of ^{99m}Tc uptake (16). The decay of this radionuclide produces both penetrating gamma rays (140 keV) and very low-energy electrons with cellular and subcellular ranges (8), making it a suitable radionuclide with which to test the validity of the dosimetric assumptions of conventional dosimetry.

The results obtained at the multicellular and cellular levels were radically different: a nearly homogeneous radionuclide distribution was obtained in the former case while an extremely inhomogeneous distribution was seen in the latter case. The consequences of these findings for the calculation of the radiation dose to human liver Kupffer cells labeled with ^{99m}Tc -Microlite were evaluated on the basis of a recently developed model for dose calculation at the cellular level (17). This model describes adequately the cellular geometry of human tissues and accounts for the effects of changing the intracellular-to-extracellular radionuclide concentration, the labeled cell density, and the cell size factors that conventional dosimetry ignores. A comparison of the radiation dose estimates for ^{99m}Tc -Microlite made with both the cellular dosimetry model and the conventional dosimetry model reveals large differences. The implications of these results for radiation protection are discussed.

MATERIALS AND METHODS

Preparation of Tritiated Microlite

Commercially available Microlite (human serum albumin colloid), obtained from Du Pont (North Billerica, MA) was radiolabeled with tritium (^3H). Tritiation was preferred to ^{99m}Tc labeling because of the inconveniently short half-life of the latter radionuclide for adequate autoradiographic exposure. The Microlite was suspended in phosphate-buffered saline (PBS), pH 7.2, centrifuged at $100 \times g$, and the colloidal pellet washed once with PBS to remove additives. The purified Microlite pellet (M) was resuspended in 2 ml of PBS. A tritiated solution (185 MBq) of N-succinimidyl-propionate (NSP) (Du Pont) was placed in a 5-ml Reacti-vial and the solvent evaporated under nitrogen. To the solid residue of NSP, 0.3 mg of purified M in 0.6 ml of PBS was added, and the mixture allowed to react for 2 hr with occasional shaking. The reaction mixture was centrifuged at $1000 \times g$ for 20 min and the ^3H -M pellet washed twice with PBS.

Biodistribution Studies

One-month-old female mice (weighing ~20 g) were anesthetized with phenobarbital administered intraperitoneally and injected through the tail vein first with ~6.66 MBq of ^3H -M in PBS and 20 min later with 10 ml of formalin-glutaraldehyde-picric acid (FGP). The in situ fixed liver was excised, sliced, fixed for an additional 24 hr first in FGP and then in

osmium tetroxide. The liver slices were sectioned at either the millimeter (multicellular) level or the micrometer (cellular) level.

Liver Sectioning

Multicellular Level. Sectioning into 1-mm³ pieces was achieved using a specially designed cutter containing parallel blades at a distance of 1 mm from each other. Each sample was then digested for two days at 50°C in 1 ml of NCS tissue solubilizer (Amersham, Arlington Heights, IL), decolorized by adding 1 ml benzoyl peroxide in toluene, and counted in 10 ml of scintillation fluid (Econofluor, Du Pont).

Cellular Level. Sectioning for autoradiography was performed by embedding the liver samples in epon-araldite (plastic) and cutting them into 0.5- μm thick sections. The specimens were coated with Ilford L4 emulsion (Polyscience, Worthington, PA). Exposure varied from one day up to eight weeks. Autoradiographs were developed for 2-3 min in Kodak D19 (Kodak, Rochester, NY), stained with 1% toluidine blue, pH 7.2, dried, mounted in immersion oil, and examined by light microscopy.

Autoradiographic Quantitation

The intracellular-to-extracellular radioactivity concentration ratio was derived from the autoradiographs using a quantitative video imaging system (DMD2, Imaging Technology, Inc., Woburn, MA). This system digitizes the microscopic images obtained from a Zeiss Universal microscope fitted with Planapo objectives (6.3x-100x, oil immersion). Autoradiographic slides were placed on an illumination apparatus consisting of a light box and a mounted camera. The video signals were digitized in real time (0.033 sec/frame) into 640×480 (=307,200 pixels) images that were corrected for uneven illumination and variable video target response by a decalibration process in which each pixel was individually corrected using stored bright- and darkfield reference images. Multiple regions of interest could be selected either automatically or manually, and the associated number of blackened grains in selected intracellular or extracellular regions was stored for subsequent analysis on an IBM-AT microcomputer system (7 Mbytes memory, 6-image frames, a flash A/D digitizer and Dage MTI Model 61 and 81 vidicon target cameras, running under the Xenix operating system). Using the DMD2 video imaging system, the density of the blackened emulsion grains inside and outside the Kupffer cells was quantitated. For this purpose, slides exposed for two days were used. Slides exposed for longer periods of time were found to be unsuitable for quantitation studies, because the number of emulsion grains that are available for blackening around the radioactive decay sites is limited (saturation effect). An estimate of the background in any particular slide was made by scanning within the same slide portions of emulsion containing no tissue sample. After background subtraction, the intracellular-to-extracellular blackened grain density ratio was derived. This value is assumed to be an approximate measure of the intracellular-to-extracellular radioactivity concentration ratio (k).

To determine the fraction of mouse liver volume occupied by the radiolabeled Kupffer cells, photographs were taken of randomly selected areas of the autoradiographic slides, and the ratio (R_s) of the blackened area to the total photograph area was measured for each sample. The ratio of the corresponding volumes (f) was then approximated as: $f = R_s^{1.5}$.

RESULTS

Multicellular Level Distribution

The distribution of Microlite at the millimeter level is shown in Figure 1. A typical 1-mm thick liver section is assumed to be placed on the XY plane at $Z = 0$ and is subdivided into 1-mm² areas. The radioactivity associated with each of the corresponding 1-mm³ pieces is displayed on the vertical Z-axis. The relative standard deviation of the mean radioactivity associated with these liver pieces is $\pm 20\%$. Considering the experimentally introduced uncertainties (due to uneven liver sectioning, scintillation counting statistics, etc.), it can be concluded that the true variation of the radioactivity associated with these liver pieces is $< 20\%$. This variation is attributed to certain circumstances; for example, some of the tissue pieces measured will contain blood vessels with little or no radioactivity present.

Cellular Level Distribution

A typical autoradiograph of tritiated Microlite uptake and distribution at the cellular level is shown in Figure 2. The autoradiographic reaction is present as numerous small silver grains that appear black. High concentrations of radioactivity are indicated by the intense reactivity over the Kupffer cells which line most of the sinusoidal lining. These phagocytic cells, the expected sites of uptake (14,15), were recently shown to be the cells in rat liver that take up ^{99m}Tc-colloid (13). The rest of the liver seems to be depleted of radioactivity. The intracellular-to-extracellular radioactivity concentration ratio (k) for the Kupffer cells was found to be

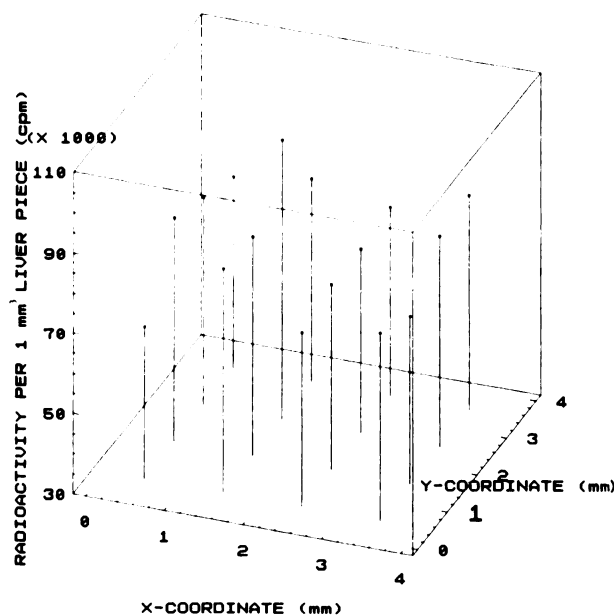


FIGURE 1
Measured distribution of radiolabeled Microlite at millimeter (multicellular) level: Typical 1-mm thick mouse liver section is assumed to be placed on XY plane at $Z = 0$ and is subdivided into 1-mm² areas. Radioactivity associated with each of corresponding 1-mm³ liver pieces is displayed on vertical (Z) axis.

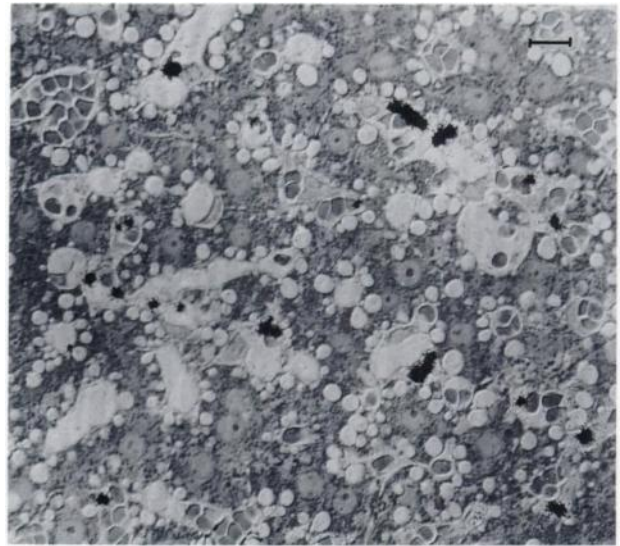


FIGURE 2

Ratio of radiation dose estimate to individual ^{99m}Tc-labeled cells (8- μ m diameter) in human liver derived with cellular dosimetry model to that derived by conventional (MIRD) dosimetry, as function of fraction of human liver volume occupied by labeled cells (f) and intracellular-to-extracellular radionuclide concentration (k). Curve 1: $k = 1$; curve 2: $k = 100$; curve 3: $k = 1000$. For technetium-99m-labeled albumin colloid (Microlite), corresponds to $f = 0.001-0.01$ and $k = 200-1000$. Thus conventional dose estimate for labeled cells is 8-30 times lower than that predicted by present model.

in the range of 200-1000, depending on the particular sample examined and on the background radioactivity. The fraction of the liver volume occupied by the radiolabeled Kupffer cells (f) was measured and found to be between 0.001 and 0.01, corresponding to average distances between labeled Kupffer cells of 25 to 50 μ m. Indeed, the fraction of liver volume occupied by the phagocytic Kupffer cells is typically around 0.01 to 0.02. Those Kupffer cells closer to the main liver vessels are more likely to be labeled than others and, therefore, only a fraction of these phagocytic cells would be expected to be labeled. Hence, the f value of 0.001 to 0.01 derived from the autoradiographs is as expected.

DISCUSSION

Radiation dose estimates for individual radiolabeled cells in human liver can be obtained only from autoradiographic studies. The distribution of radiolabeled Microlite in mouse liver measured at the millimeter (multicellular) and the micrometer (cellular) levels is strikingly different. In the former case, an approximately homogeneous radioactivity pattern is obtained (Fig. 1) and thus the assumptions of conventional dosimetry are correct at this level. In the latter case, an extremely nonhomogeneous radionuclide deposition is revealed and nearly all of the radioactivity is contained in a small fraction of the liver cells.

The experimental data obtained in the mouse liver for tritiated Microlite should apply to ^{99m}Tc -labeled Microlite, as it is not anticipated that the choice of the label (i.e., ^3H or ^{99m}Tc) will influence the ability of Kupffer cells to engulf such large particles. Furthermore, the intracellular-to-extracellular radioactivity concentration ratio (k) is not expected to depend on the actual values of the administered activity or on the liver size (e.g., mouse liver versus human liver). Although the actual total circulating radioactivity available for phagocytosis is different in the two cases, it is anticipated that the ratio of intracellular-to-extracellular radioactivity concentration (k) will remain the same; this is a reasonable assumption as long as mouse Kupffer cells have a phagocytic capacity similar to that of human Kupffer cells. Similarly, the fraction of the organ volume occupied by the labeled Kupffer cells (f) is assumed to be the same in mice and humans. Hence the experimental results of the mouse liver study should also apply to humans.

For such an inhomogeneous distribution of radioactivity at the cellular level in human liver, it is conceivable that the conventional dosimetry assumption of homogeneity may introduce high uncertainties in the derived dosimetric estimates for patients undergoing imaging with ^{99m}Tc -labeled Microlite. To verify and quantitate this, a recently developed model for dosimetry at the cellular level was employed (17). A computer program that models the liver as a multicellular cluster of cells (represented by spheres) arranged in a hexagonal geometry was used. Indeed, histologic studies indicate that liver lobules are structured in a hexagonal geometry thereby maximizing cell contact (15). This program assumes a given labeled cell cluster volume fraction (f) to describe the concentration of the radiolabeled compound over a small fraction of cells in the cluster (i.e., a small labeled cell density). When the labeled cell cluster volume fraction is small ($f \ll 1$), the labeled cells appear to be in a random arrangement similar to that revealed by autoradiography in Figure 2. The program also accounts for changes in the intracellular-to-extracellular radionuclide concentration (k) and the cell size (the average diameter of Kupffer cells in human liver (15) is $\sim 8 \mu\text{m}$).

The total radiation dose to an individual sphere (i.e., cell) from ^{99m}Tc is due to photons and electrons produced from radioactive decays in the extracellular medium, in other cells, and within the radiolabeled cell itself. Specification of the exact origin of radiation is unnecessary for the emitted photons (140 keV), whose range of action is much greater than the cell diameter (i.e., homogeneity is valid here). Thus, for the photon dose, the conventional dosimetry estimate for human liver was adopted (18,19). However, calculation of the dose due to the emitted electrons was made as a function of the parameters f and k , and the total radiation

dose to individual cells (labeled or nonlabeled) per unit administered activity in the cellular cluster was derived. The results were then compared to the predictions of conventional dosimetry (1), which assumes a homogeneous distribution of the radionuclide in the multicellular cluster (human liver), with photons contributing about 52% of the total radiation dose to all liver cells (18,19).

Figure 3 presents the cellular-to-conventional dosimetry ratio for ^{99m}Tc in a multicellular cluster with a volume equal to that of human liver as a function of the labeled cell cluster volume fraction (f) and for various intracellular-to-extracellular radionuclide concentrations (k). In the case examined here, f is in the region 0.001 to 0.01 and k is in the region 200 to 1000. The results indicate that conventional dosimetry calculations for ^{99m}Tc -Microlite underestimate the dose delivered to the labeled Kupffer cells of human liver 8- to 30-fold. The conventional dose estimate for the dose to the liver by i.v. administration of 8 mCi of ^{99m}Tc -labeled Microlite is 2.7 cGy (9); hence, according to the present study, the radiation dose to the labeled Kupffer cells per examination varies from 20 cGy to 90 cGy.

The nonlabeled cells of the liver (hepatocytes) concomitantly receive a lower radiation dose, since most of the electrons emitted by ^{99m}Tc decays in Kupffer cells have cellular or subcellular ranges (8). The total dose

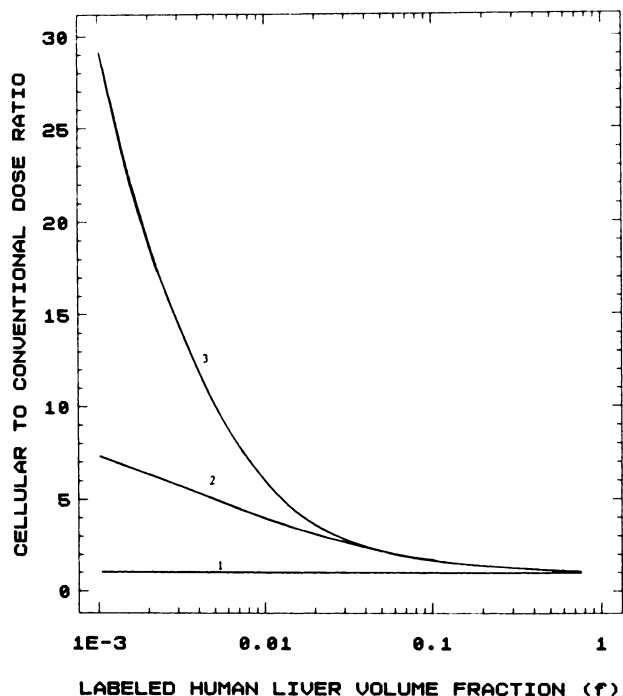


FIGURE 3
Distribution of radiolabeled Microlite at micrometer (cellular) level: Typical autoradiograph of 0.5- μm thick mouse liver section is shown. Radiolabeled Microlite (blackened grains) is highly concentrated in specific cells (Kupffer cells). Bar = 20 μm .

to these cells is estimated as 52% of the conventional dosimetry estimate depending on the distance of the nonlabeled cells from the Kupffer cells. Hence, for the hepatocytes of the liver, conventional dosimetry overestimates the delivered dose.

The results of the present investigation may generate a certain degree of concern regarding the possible radiologic consequences of elevated radiation doses to liver macrophages during diagnostic examinations. Fortunately, human Kupffer cells are not very sensitive to radiation: recent epidemiologic studies (20) have shown that the highly elevated doses delivered by alpha particles to human Kupffer cells during diagnostic examinations with Thorotrast (a ^{232}Th -containing agent used in the past for angiography) did not produce any observable Kupffer cell malignancies. In most instances, the malignancies produced were of endothelial cell origin. Thus, for diagnostic examinations with $^{99\text{m}}\text{Tc}$ -labeled Microlite, the dosimetric findings of the present paper have a rather unclear radiologic significance. However, the experimental model and the calculations demonstrate the presence of instances where the conventional dosimetry assumption of homogeneity of radionuclide distribution may lead to gross miscalculation of the dose delivered to individual cells. Such dosimetric inaccuracies occur only when the radionuclide (a) is taken up by a small percentage of cells in the organ of interest ($f < 0.2$), (b) concentrates intracellularly ($k \gg 1$), and (c) emits short-range radiations. Nevertheless, these situations may not be uncommon in current nuclear medicine practice. In addition to the liver macrophages that have been examined in this paper, the macrophages of spleen, lung, and bone marrow are also expected to receive a much higher dose from $^{99\text{m}}\text{Tc}$ -labeled albumin colloids than that assumed by conventional dosimetry. In fact, the dose underestimation by the MIRD approach in these cases is expected to be even higher than that for liver Kupffer cells, as macrophages in lung, spleen and bone marrow occupy even smaller organ volume fractions (f) than in liver.

Further cases exist where inhomogeneity of radiopharmaceutical deposition at the cellular level is evident:

1. Technetium-99m-labeled macroaggregated albumin used for pulmonary microcirculation imaging is known to become trapped in the capillaries and precapillary arterioles. It has been estimated that only one in a million capillaries traps an aggregated particle (9). Therefore, the radionuclide distribution and the resulting dose pattern to individual lung cells are expected to be very inhomogeneous.
2. The labeling of blood elements with ^{111}In or $^{99\text{m}}\text{Tc}$ is commonly used for diagnostic procedures. A large fraction of these labeled blood elements is

known to concentrate in the liver and spleen a few hours after injection. In this case, the conventional dosimetry assumption that the radionuclide concentrates homogeneously over the organ of interest (21,22) is also inaccurate, as only a small fraction of the organ volume is labeled.

3. Recently published radiation protection studies on the dosimetry of protein-bound tritium have shown that the intranuclear localization of this radionuclide results in the underestimation of the dose by a factor of 3 to 5 in comparison with the case of an assumed homogeneous distribution to the whole cell (23).
4. An inhomogeneous and selective radionuclide distribution is expected to occur in the diagnostic use of antibodies labeled with ^{123}I , ^{111}In , or $^{99\text{m}}\text{Tc}$ (24-28).

Inhomogeneity of antigenic expression or poor regional blood supply in tumor cells can cause a very irregular and heterogeneous distribution of the radiolabeled antibody at the cellular level. It can be shown that even if the radionuclide emits energetic electrons (^{131}I or ^{90}Y), situations may exist where the radiation dose to individual cells is very inhomogeneous (29), and this has a particular significance for prospective therapeutic antibody uses. Furthermore, internalization of the antibody by tumor and/or normal cells may produce high intracellular-to-extracellular radionuclide concentrations. Application of the conventional dosimetry approach to radiolabeled antibodies is thus bound to introduce high uncertainties in the dosimetry estimates to individual cells.

The possible biologic consequences resulting from such dosimetric underestimations depend on the radiosensitivity of the labeled cells. As the question of the health effects of low radiation levels in the general population remains unanswered for most labeled cells (30), it is still unknown what mutagenic or transformational changes, if any, may be produced by radiation doses elevated by factors of 8 to 30. A further issue of radiobiologic significance for very low-energy electron emitters relates to the intracellular distribution of radiopharmaceuticals (4-7). In fact, when the decay of an Auger electron emitter occurs in the vicinity of DNA, the dose to microscopic volumes within the DNA may prove to be even more meaningful than the dose to the whole cell (4,7). Elucidation of such considerations becomes increasingly important, especially with the generation of new radiolabeled diagnostic agents.

In conclusion, although it is recognized that the conventional dosimetry approach is still valid in the majority of nuclear medicine applications, it is important to understand that certain limitations are present in specific situations. Dosimetric treatments that encompass such situations are needed which in combi-

nation with biologic data could lead to more realistic dose estimates.

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