Transferrin-Mediated Uptake of Radionuclides by the Testis

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In an attempt to explain the deleterious effects of gonadal radionuclide localization, we examined the role of transferrin in testicular radionuclide uptake. **Methods:** In vivo testicular uptake and retention of the transferrin binding radionuclides ^{114m}In-citrate and ^{59}Fe-citrate were compared with that of the nontransferrin binding isotopes ^{137}Cs-citrate and Na^{125}I for 63 days postinjection. Isotope uptake mechanisms were investigated in vitro using isolated seminiferous tubules and Sertoli cell monolayers grown in bicameral culture chambers. **Results:** Indium-114m, ^{59}Fe and ^{137}Cs were localized in the testis by 24 hr postinjection, but accumulation of ^{125}I was minimal. Although testicular ^{114m}In remained constant, ^{59}Fe declined slowly over the following 63 days and ^{137}Cs fell very rapidly. When ^{114m}In- or ^{59}Fe-loaded testes were fractionated, and markedly more ^{114m}In was associated with the seminiferous tubules than ^{59}Fe, suggesting that ^{114m}In may be retained. In vitro uptake of ^{59}Fe, ^{67}Ga and ^{114m}In by isolated seminiferous tubules was inhibited by transferrin, but uptake of ^{137}Cs and ^{125}I was unaffected. Iron-59, ^{67}Ga and ^{114m}In were retained by isolated tubules in contrast to ^{137}Cs and ^{125}I. Whereas ^{137}Cs, ^{59}Fe and ^{114m}In all crossed Sertoli cell monolayers, the rate of transcellular transport of ^{137}Cs was faster than that of ^{59}Fe or ^{114m}In, suggesting differences in the intracellular processing of transferrin binding and nontransferrin binding radionuclides. **Conclusion:** These data suggest that some radionuclides may access the seminiferous epithelium through receptor-mediated endocytosis of transferrin. Such radionuclide localization could lead to continuous irradiation of the testes, resulting in mutagenic damage to spermatogenic cells.

Key Words: testis; transferrin; indium-114m; cesium-137-citrate; iodine-125; iron-59

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Localization of radionuclides in the testis offers a route for the impairment of spermatogenesis and the transmission of genetic damage by irradiated sperm (*1–8*). The available data on testicular uptake and retention of radionuclides are limited. Of 113 radiopharmaceuticals for which biokinetic models have been tabulated in ICRP Report 53 (*9*), data regarding testicular uptake are only included for 3. It is important to understand the localization, distribution and retention of radionuclides within the testes in order to make better assessments of the hazards presented by such radiation exposures.

Most toxicants are excluded from germ cells within the seminiferous tubule adluminal compartment by the blood-testis barrier formed by tight junctions between adjacent Sertoli cells (*10*). Sertoli cells also maintain the microenvironment within the seminiferous tubule by the synthesis of a number of proteins, including the iron-transport protein transferrin (*11*). Like all eukaryotic cells, germ cells have an obligate requirement for iron as a constituent of biological redox systems. As iron is constantly lost from the testicular iron pool by the passage of sperm to the reproductive tract, there must be continuous transfer of iron from the general circulation to germ cells within the tubule adluminal compartment. This is achieved by a two-stage process in which serum iron-transferrin binds to transferrin receptors at the basal pole of the Sertoli cell and is internalized (*12*). The iron then dissociates from the transferrin and is bound by immunologically distinct Sertoli cell transferrin for delivery to adluminal germ cells. The serum apotransferrin is recirculated back to the basal surface and released to the circulation (*12*).

Transferrin binding radionuclides are common in both medical and environmental situations. Indium and gallium isotopes (*13,14*) are widely used in diagnostic nuclear medicine whereas transferrin-binding actinides such as plutonium (*15*) and curium (*16*) are released from nuclear plants. The purpose of this study was to study the role of transferrin in testicular radionuclide uptake.

MATERIALS AND METHODS

Radionuclides and Dosimetry

Iron-59-chloride (110–740 MBq/mg), ^{125}I (3.7 GBq/ml) as sodium iodide, ^{137}Cs-chloride (37–370 MBq/mg) and ^{67}Ga-chloride (370 MBq/μg) were obtained commercially (Amersham International, Aylesbury, U.K.). Indium-114m-chloride (3.7 GBq/mg) was prepared as described previously (*3*).

The absorbed radiation dose to each testis was calculated from the average radionuclide content and listed radiation decay spectra (*17*) using standard MIRD formulations (*18*). The absorbed fractions of the emitted radiations were calculated to be less than 0.01 for x and gamma rays and unity for beta particles, Auger and Coster-Kronig electrons. All fluctuations in testicular weight were taken into consideration.

Organ Distribution Studies

Male Sprague-Dawley rats aged 8–9 wk and weighing approximately 200 g were used. Experimental procedures were performed in accordance with the Animals (Scientific Procedures) Act, U.K. Parliament, 1986. Animals were housed under normal laboratory conditions with free access to tap water and standard commercial diet. For the biodistribution studies, 14.8 MBq/kg ^{59}Fe, ^{114m}In, ^{125}I or ^{137}Cs diluted in rat plasma were injected intraperitoneally. At 24 hr, 7, 28 or 63 days postinjection, groups of five to six animals were killed by terminal anesthesia and exsanguination. Testes, epididymides, liver, spleen and a piece of gastrocnemius muscle were resected. Blood was collected by cardiac puncture. Tissues were weighed wet then assayed for radioactivity using an Autogamma counter; for those organs not dissected, whole tabulated normal weights were used (*19,20*). All samples were counted under the same geometry and activity measurements were corrected for background and physical decay.

Testicular Distribution Studies

Adult rats were injected intraperitoneally with 14.8 MBq/kg ^{114m}In or ^{59}Fe diluted in saline. The animals were killed after 7

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TABLE 1
Biodistribution of Radionuclides 24 Hours Postinjection

Radionuclide	Testis	Epididymis	Liver	Spleen	Muscle	Blood
^{59}Fe	0.1 ± 0.01*†	0.1 ± 0.02	10.5 ± 2.0	1.6 ± 0.2	3.1 ± 1.4	3.8 ± 1.2
^{114m}In	0.2 ± 0.03	0.1 ± 0.02	8.2 ± 0.7	0.6 ± 0.2	8.1 ± 1.9	4.7 ± 0.7
^{137}Cs	0.7 ± 0.1	0.6 ± 0.1	12.2 ± 1.4	0.6 ± 0.1	54.1 ± 6.8	2.0 ± 0.3
^{125}I	0.03 ± 0.01	0.01 ± 0.002	0.7 ± 0.02	0.01 ± 0.004	0.3 ± 0.04	1.1 ± 0.1

*Values show the percentage of injected activity per organ as the mean ± s.e.m. for six animals per group.
†Values were corrected for physical decay.

days. Testes were removed, weighed and gamma counted prior to removal of the tunica. The seminiferous tubule and interstitial compartments of the testis were separated by incubation in Eagles Minimal Essential Medium (MEM) containing collagenase (0.5 mg/ml) and DNase (20 μg/ml) for 20 min at 34°C in a shaking waterbath. The seminiferous tubules were obtained by coarse filtration and the pooled filtrate and washings represented the testicular interstitium.

Isolated Tubule Experiments

Seminiferous tubules were isolated as described previously (*21*). Isolated tubules were incubated with 1 × 10^5 cpm ^{59}Fe, ^{67}Ga, ^{114m}In, ^{125}I or ^{137}Cs at 34°C in MEM supplemented with L-glutamine (2 m*M*), sodium bicarbonate (0.85 g/liter), nonessential amino acids and vitamins with 5% fetal calf serum, buffered to pH7.4 with 15 m*M* 4-(2-hydroxyethyl)-1-piperazine-ethane sulfonic acid. This methodology resulted in intact tubules with a plug of spermatozoa at each end which were trypan blue impermeable for up to 6 hr.

To determine the effect of transferrin on radionuclide uptake, tubules were incubated with the radionuclides for 2 hr in the presence of 200 μg/ml diferric transferrin (*22*). At the end of the experiment, the tubules were washed with ice-cold Hanks Balanced Salts Solution (HBSS) and treated with 0.5 mg/ml proteinase K for 30 min on ice to remove any surface-bound transferrin. Tubules were then washed with HBSS and lysed with 1 N NaOH prior to gamma counting and protein determination.

Radionuclide retention was monitored by incubating the tubules with the radionuclides for 2 hr then washing with HBSS. The radionuclide-loaded tubules were then re-incubated in fresh (radionuclide-free) medium and aliquots of tubules were removed at regular intervals and treated as previously described.

Culture of Sertoli Cells in Bicameral Culture Chambers

Transcellular transport of the radionuclides was studied in primary cultures of immature rat Sertoli cells grown in bicameral (dual-compartment) culture chambers on filters coated with reconstituted extracellular matrix (*23,24*). Sertoli cells grown under these conditions display the morphological and functional characteristics of Sertoli cells in vivo, including tight junction formation (*25*) and polarized secretion of transferrin and other proteins (*25,26*).

Small aggregates of Sertoli cells (10–15 cells per aggregate) were isolated from 18-day-old Sprague-Dawley rats by sequential enzymatic digestions (*25,27*). The Sertoli cell aggregates were plated on 0.45-μm filters coated with reconstituted basement membrane in the apical chamber of HA bicameral culture chambers at a density of 1.8 × 10^6 cells/0.64 cm^2 well. The apical and basal chambers contained a total of 400 and 600 μl of serum-free defined medium (SFDM) (*23*), respectively. Culture medium was replaced after 24 hr, then every 2 days thereafter.

After 5 days, the permeability of the cell monolayers was determined by monitoring the free diffusion of [^{3}H]inulin from the basal to apical chambers (*25*). The passage of [^{3}H]inulin in control chambers containing coated filters but no cells was 4.9%/hr; when a confluent cell monolayer was present, the passage of [^{3}H]inulin was approximately 1.9%/hr. Cultures in which [^{3}H]inulin passage exceeded 2.5%/hr were excluded from further use.

To study transport of the radionuclides across the Sertoli cell monolayer, 1 × 10^5 cpm of ^{59}Fe, ^{114m}In or ^{137}Cs was added to the basal compartment of each bicameral chamber. Radionuclide passage into the apical chamber was monitored after 2, 4 and 6 hr at 34°C. At the end of the experiment, cells were removed from the filters by incubation with dispase and cell number and radioactivity were counted.

RESULTS

Organ Distribution Studies

The distribution of the radionuclides in the resected tissues at 24 hr postinjection is shown in Table 1. At this time, a substantial proportion of each radionuclide, with the exception of ^{125}I, was associated with the resected tissues. The highest specific activity of ^{59}Fe, ^{114m}In and ^{137}Cs was found in the liver and spleen, but a large portion of the ^{137}Cs was associated with muscle. The majority of the ^{125}I was localized in the thyroid (not shown).

At 24 hr, the specific activity of the testis ranged from 14.5 kBq/g for ^{137}Cs to 0.9 kBq/g for ^{125}I. Figure 1 shows the retention of the radionuclides by the testis for up to 60 days thereafter. Although testicular ^{114m}In stayed virtually constant

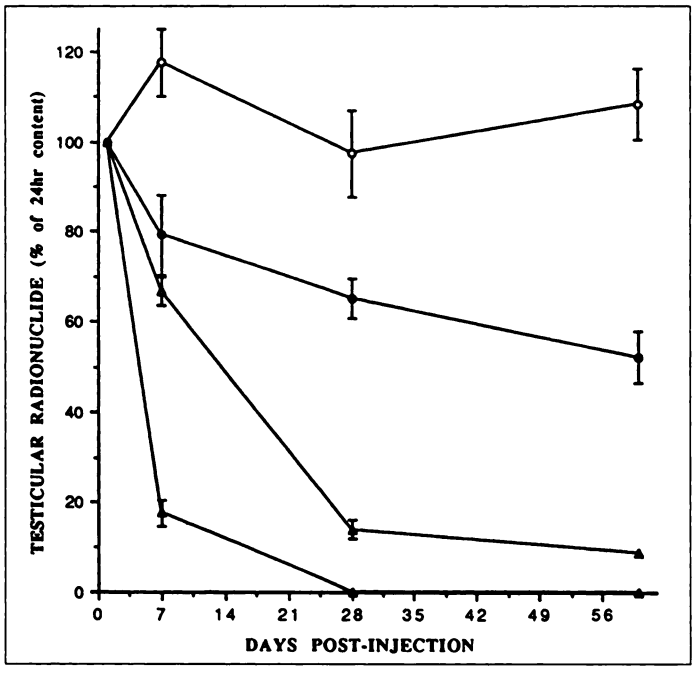

FIGURE 1. Testicular retention of ^{114m}In (○), ^{59}Fe (●), ^{137}Cs (△) and ^{125}I (▲) in adult rats. Data are expressed as a percentage of the 24-hr content and each point represents the mean ± s.e.m. for six animals.

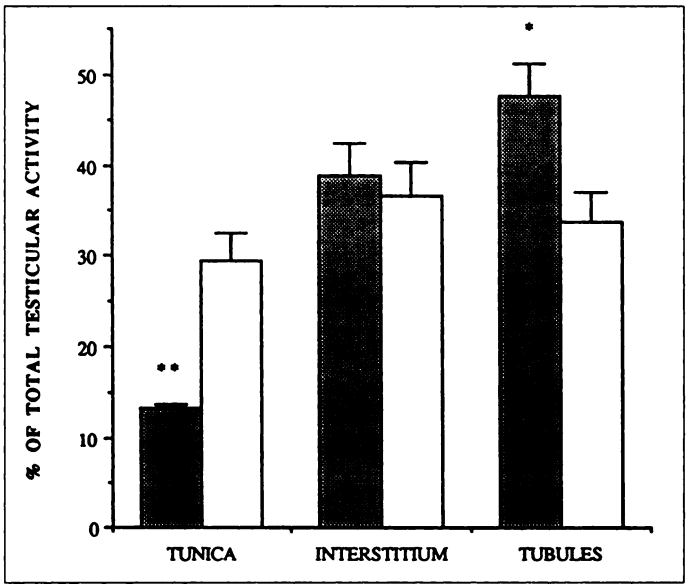

FIGURE 2. Intratesticular distribution of ^{114m}In (shaded bars) and ^{59}Fe (open bars). Data represent mean ± s.e.m. for six animals. *$p < 0.05$, **$p < 0.01$ as compared to ^{59}Fe content.

over the experimental period, the activity of the other transferrin binding radionuclide ^{59}Fe steadily declined so that approximately 60% of the 24-hr activity was present at 60 days. Of the nontransferrin binding isotopes, ^{137}Cs activity decreased rapidly so that by 28 days less than 20% of the 24 hr activity remained, while no testicular ^{125}I was detectable by 28 days postinjection.

The average absorbed radiation dose to the testis over the experimental period (63 days) was calculated to be 3.99 Gy for ^{114m}In, 1.24 Gy for ^{59}Fe, 0.82 Gy for ^{137}Cs and 0.0012 Gy for ^{125}I.

Testicular Distribution Studies

A possible explanation for the observation that ^{114m}In is retained by the testis to a greater degree than ^{59}Fe (Fig. 1) is that these isotopes are distributed differently in the testis. To investigate this theory, the testicular distribution of these two radionuclides was studied at 7 days postinjection. As shown in Figure 2, significantly more ^{59}Fe than ^{114m}In was localized in the tunica ($p < 0.001$), while the proportion of intratesticular

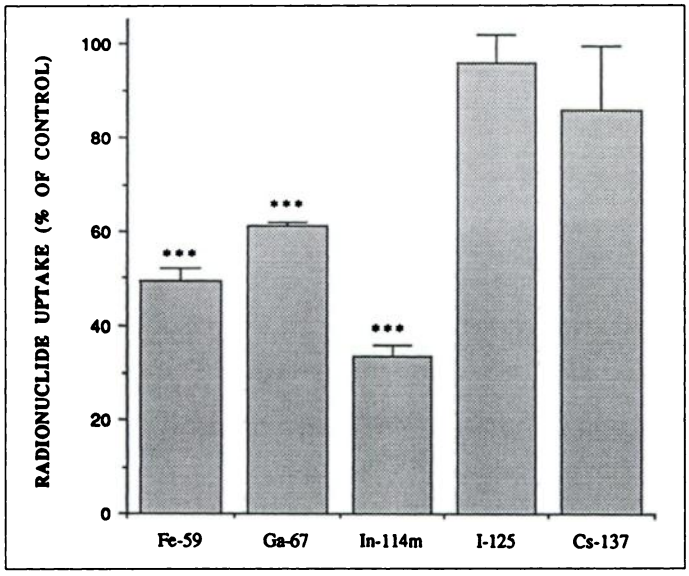

FIGURE 3. Effect of 200 μg/ml diferric transferrin on uptake of radionuclides by isolated seminiferous tubules. Data represent mean ± s.e.m. for quadriplicate samples. ***$p < 0.001$ as compared to control uptake.

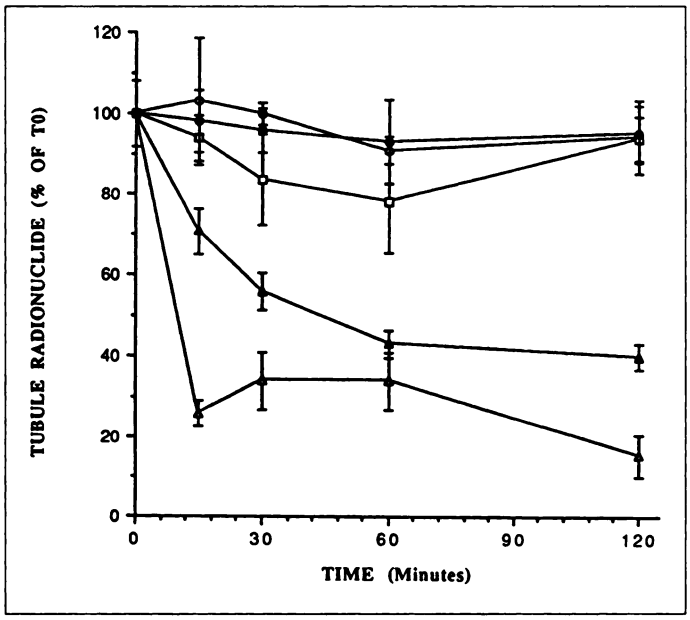

FIGURE 4. Retention of ^{114m}In (○), ^{59}Fe (●), ^{67}Ga (□), ^{137}Cs (△) and ^{125}I (▲) by isolated seminiferous tubules. Data are expressed as a percentage of tubule radionuclide content at T0, taken as the commencement of the reincubation period. Each point represents the mean ± s.e.m. for quadriplicate samples.

radionuclide localized in the interstitium was similar for both isotopes. By contrast, significantly more ^{114m}In was present in the seminiferous tubules than ^{59}Fe ($p < 0.05$).

Radionuclide Uptake by Isolated Seminiferous Tubules

All the radionuclides were accumulated by the isolated tubules at 34°C. When 200 μg/ml iron-saturated human transferrin was added to the incubation medium, tubular uptake of ^{59}Fe, ^{67}Ga and ^{114m}In was markedly decreased from the control values of 10,229 cpm/mg protein, 3917 cpm/mg protein and 4288 cpm/mg protein respectively ($p < 0.001$). By contrast, tubular uptake of ^{125}I and ^{137}Cs did not differ significantly from the control uptakes of 3050 cpm/mg protein and 2161 cpm/mg protein, respectively (Fig. 3).

Retention of the radionuclides by the tubules was studied by re-incubating radionuclide-loaded tubules in fresh radionuclide-free medium for another 2 hr. By this time, more than 50% of the ^{125}I and ^{137}Cs associated with the tubules at the start of the incubation had been released into the medium. By contrast more than 75% of the ^{59}Fe, ^{114m}In and ^{67}Ga remained tubule-associated (Fig. 4).

Transport of Radionuclides Across Sertoli Cell Monolayers

Transcellular transport of ^{137}Cs across Sertoli cell monolayers was significantly faster ($p < 0.001$) than that of ^{59}Fe or ^{114m}In (Fig. 5, Table 2). Furthermore, ^{59}Fe was transported across the monolayer significantly more rapidly than ^{114m}In ($p < 0.001$). These differences in transport rate were reflected by the ratio of radioactivity in the apical-to-basal chamber (A/B ratio) for each radionuclide at the end of the experiment.

Generally, only a small portion of the radionuclide was retained within the cells. After a 6-hr incubation, however, significantly more ^{114m}In was associated with the Sertoli cells than ^{59}Fe or ^{137}Cs ($p < 0.01$), and more ^{59}Fe was cell-associated than ^{137}Cs ($p < 0.01$) (Table 2).

DISCUSSION

Under normal physiological conditions, approximately one-third of plasma transferrin is iron-saturated, leaving the remainder free to bind other metals including transition elements such

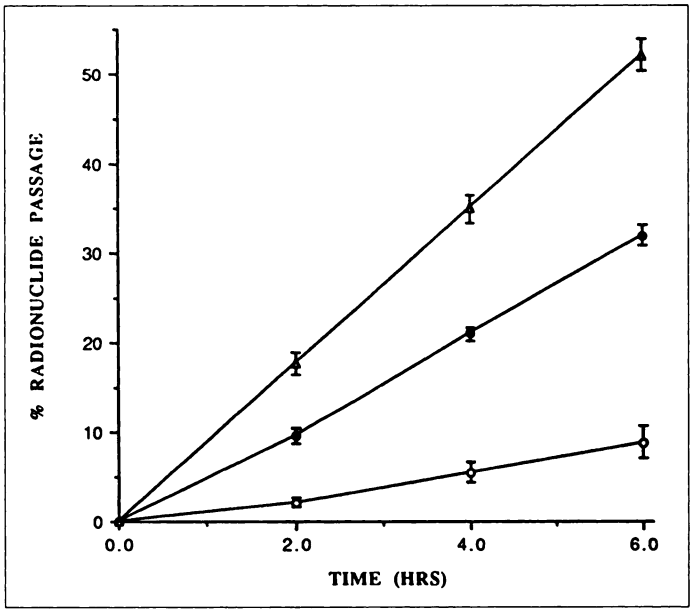

FIGURE 5. Passage of ^{137}Cs (△), ^{59}Fe (○) and ^{114m}In (●) across Sertoli cell monolayers grown in bicameral culture chambers. Each point represents mean ± s.e.m. for eight samples.

as indium, gallium and manganese (*13,14*) and actinides such as plutonium and curium (*15,16*). This ability of transferrin to bind metals other than iron will influence the in vivo distribution and retention of some radionuclides.

At 24 hr postinjection, the organ retention of ^{114m}In and ^{59}Fe were similar and contrasted to that of the nontransferrin binding isotopes ^{137}Cs and ^{125}I. The relative constancy of the testicular levels of ^{114m}In and ^{59}Fe, over the experimental period could be due to a continual supply of labeled transferrin from other body compartments such as the liver. As testicular ^{59}Fe declined more than ^{114m}In, it is probable that ^{114m}In is retained within one of the testicular compartments.

Although the organ distribution of ^{114m}In and ^{59}Fe was similar, the testicular distribution of these radionuclides was quite different, because more ^{59}Fe than ^{114m}In was localized in the tunica. Since the tunica is well vascularized, this may reflect circulating ^{59}Fe-labeled erythrocytes as well as ^{59}Fe-transferrin complexes. Because ^{114m}In is not incorporated into hemoglobin (*28*), circulating ^{114m}In is less abundant (*29*).

That more ^{114m}In than ^{59}Fe was associated with the seminiferous tubules supports the hypothesis that ^{114m}In is retained by the tubules. Although indium is similar to iron in that it binds to transferrin and enters cells through the transferrin-mediated endocytotic pathway (*14*), it is unlikely that indium could replace iron in intracellular processes (*28*). Consequently ^{114m}In may not be utilized by developing germ cells in the same way as ^{59}Fe, but may be contained in structures such as residual bodies (*30,31*) within the tubule boundaries. In contrast, ^{59}Fe would be utilized by developing germ cells in the normal way and subsequently lost to the testis when the resultant sperm passed out of the testis into the epididymis. It is probable that a small portion of testicular ^{114m}In is also lost to the epididymis through transferrin in the luminal fluid (*32,33*) and by ^{114m}In-transferrin bound to surface transferrin receptors on released sperm. Indeed, our previous autoradiographic observations have shown radiolabeled epididymal sperm after systemic ^{114m}In administration (*1*). This depletion of testicular ^{114m}In may be compensated for by a continual supply of circulating ^{114m}In transferrin, thus resulting in a net equilibrium of testicular ^{114m}In. Further experimentation should resolve this interesting issue.

TABLE 2
Distribution of Radionuclides across Sertoli Cell Monolayers

Radionuclide	% Passage hr^{-1} 10^6 $cells^{-1}$	A/B ratio†	% Cell-associated activity (10^6 $cells^{-1}$)
^{59}Fe	2.95 ± 0.10*	0.47 ± 0.02	0.32 ± 0.04
^{114m}In	0.81 ± 0.16	0.11 ± 0.02	0.59 ± 0.06
^{137}Cs	4.82 ± 0.16	1.11 ± 0.07	0.24 ± 0.05

*Data show the mean ± s.e.m. for eight samples at each point.
†Values show the ratio of radioactivity in the apical to basal chambers.

Indium-114m or ^{59}Fe localized in the interstitial fraction would most likely be bound to transferrin in the lymphatic and vascular space. As the interstitium comprises only 18% of the total testicular volume compared with 82% for the tubules (*34*), it can be calculated that the percentage of testicular activity per unit volume was 2.03 and 0.41, respectively, for interstitium and tubules for ^{59}Fe and 2.15 and 0.58, respectively, for ^{114m}In. Thus, the interstitial radionuclide is more concentrated than the tubular radionuclide, thereby illustrating the importance of the blood-testis barrier in protecting against nonspecific localization of protein-bound contaminants in proximity to adluminal germ cells.

The data from the isolated tubule studies highlight the importance of transferrin in testicular radionuclide delivery. The inhibition of ^{59}Fe, ^{114m}In and ^{67}Ga uptake by transferrin suggests competition for tubular transferrin receptors between iron-transferrin and radionuclide-labeled transferrin.

The finding that only the transferrin binding radionuclides were retained within the isolated tubules is consistent with our in vivo observations. These data concur with previous observations (*12*) that transferrin-mediated iron uptake across the blood-testis barrier is unidirectional. Moreover, our previous in vivo data show that ^{114m}In is not retained in the testes when the blood-testis barrier is absent as occurs in neonatal rats (*3*).

Many of the radionuclides used in diagnostic nuclear medicine (e.g., ^{111}In, ^{67}Ga, ^{201}Tl) decay by electron capture and internal conversion, which is characterized by the emission of numerous low-energy electrons with subcellular ranges. Such radionuclides will only present a mutagenic hazard to germ cells if the radionuclide is in close proximity to the target cell. In the testis, iron bound to testicular transferrin is secreted from the apico-lateral domain of Sertoli cells to developing germ cells and the tubule lumen (*12,35*). Our results using the bicameral chamber system show that ^{114m}In is also secreted from the apical pole of Sertoli cells, albeit at a slower rate than ^{59}Fe. The differing kinetics of ^{59}Fe and ^{114m}In passage may be attributable to differences in intracellular radionuclide processing by the Sertoli cell. In particular, these data suggest that ^{114m}In may be retained by the cell in contrast to ^{59}Fe, which complements in vivo observations suggesting tubular retention of ^{114m}In.

The fastest rate of transport across the Sertoli cell monolayer was observed with the nontransferrin binding isotope ^{137}Cs, a potassium analog which utilizes membrane Na^+/K^+ pumps to pass across cell membranes (*36*). Although ^{137}Cs is not commonly used in diagnostic nuclear medicine, large quantities of Auger-emitting cesium radionuclides are present in the environment from nuclear plant emissions. Furthermore, the frequent use of the Auger-emitting radionuclide ^{201}Tl as a myocardial imaging agent is also dependent upon its ability to act as a potassium analog (*37*). It is possible that transcellular transport of Auger-emitting radionuclides through the Sertoli cell into the tubule lumen could present a significant genotoxic hazard. Such transport mechanisms provide a likely explanation

for the enhanced testicular toxicity of some Auger-emitting radionuclides (*2,4–6*).

Previous studies with ^{114m}In have suggested that a minimum threshold dose of 2 Gy is necessary for spermatogonial cell killing in adult rats (*2*). In the current study, only ^{114m}In delivered a dose in excess of this threshold, and this was the only radionuclide that caused significant testicular cytotoxicity. By contrast, the induction of mutations in developing germ cells may be dependent upon dose-rate rather than absolute dose (*2*). Thus, it is possible that the localization of any single radionuclide in the testis may lead to mutagenic damage to germ cells. The radiotoxicity of Auger emitters is dependent upon their subcellular as well as their cellular distribution (*4–6,38*), but testicular retention of other radionuclides with longer range emissions such as alpha- and beta-emitting isotopes may pose a more substantial mutagenic hazard (*7,8*).

CONCLUSION

Our data support the hypothesis that some medically and environmentally relevant radionuclides may utilize the physiological serum and testicular transferrin pathway to cross the blood-testis barrier and so gain access to mutagenically sensitive germ cells. Since the transferrin pathway is ubiquitous among mammalian species (*39*), it is probable that transferrin-binding radionuclides also transcend the blood-testis barrier in the human. Because the human testes contain many more transferrin synthesizing Sertoli cells than the rat testis and have an increased ratio of parenchymal-to-spermatogenic tissue (*40*), it is possible that radionuclide uptake values from the rat underestimate human testicular uptake. Nevertheless these data point towards the possibility that other radionuclides may be deposited in the testes by similar mechanisms, offering a route for the impairment of spermatogenesis and transmission of genetic damage by radiation-induced mutations in emergent sperm.

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