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Bromine-80m-labeled Estrogens: Auger-electron emitting, Estrogen Receptor-directed Ligands with Potential for Therapy of Estrogen Receptor Positive Cancers[1]

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Footnotes

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4. Deceased, to whose memory this manuscript is dedicated.

5. The abbreviations used are: Br-BHPE, 2-bromo-1,1,bis(p-hydroxyphenyl)-2-phenyl ethylene; Br-VE_2, 17α-bromovinylestradiol; 16α-BrE_2, 16α-bromoestradiol-17β; 16α-$BrME_2$, 16α-bromo-11β-methoxyestradiol-17β; 17α-$BrVME_2$, 17α-bromovinyl-11β-methoxyestradiol-17β; DES, diethylstilbesterol; E_2, 17β-estradiol; ER, estrogen receptor; I.P., intraperitoneal; sub Q, subcutaneous; I.V., intravenous; IUdR, 5'-iodo-2'-deoxyuridine; BUdR, 5'-bromo-2'-deoxyuridine; $TK_{10}E$, 10 mM Tris, 10 mM KCl, 1 mM EDTA, pH 7.4 buffer; DCC, dextran-coated charcoal.

6. O.T. DeJesus, R.C. Mease, P.V. Harper, G. Powell, E.R. DeSombre, A. Hughes, J. Schwartz, J. Rotmensch, S.J. Gatley and R.W. Atcher. In preparation.

ABSTRACT

A triphenylbromoethylene, 1,1 bis (p-hydroxyphenyl) 2-bromo-2-phenylethylene, Br-BHPE, and a bromosteroidal estrogen, 17α-bromovinylestradiol, $BrVE_2$, were labeled with the Auger electron emitting nuclide bromine-80m, prepared by the [p,n] reaction with ^{80}Se. To assess their potential as estrogen receptor (ER) directed therapeutic substrates the bromine-80m labeled estrogens were injected into immature female rats and the tissue distribution studied at 0.5 and 2 hours. Both radiobromoestrogens showed substantial diethylstilbesterol (DES)-inhibitable localization in the ER rich tissues, uterus, pituitary, ovary and vagina at both time points. While the percent dose per gram tissue was higher for the Br-BHPE, the $BrVE_2$ showed higher tissue to blood ratios, especially at 2 hr, reflecting the lower blood concentrations of radiobromine following administration of the steroidal bromoestrogen. Comparing intraperitoneal, intravenous and subcutaneous routes of administration for the radiobromine labeled Br-BHPE, the intraperitoneal route was particularly advantageous to provide maximum, DES-inhibitable concentrations in the peritoneal, ER-rich target organs, the uterus, ovary and vagina. While uterine concentrations after BrBHPE were from 10-48% dose/g and after $BrVE_2$ were 15-25% dose/g, similar treatment with ^{80m}Br as sodium bromide showed uniform low concentrations in all tissues at about the levels seen in blood. The effective specific activity of [^{80m}Br]BrBHPE, assayed by specific binding to ER in rat uterine cytosol, was 8700 Ci/mmole. This triphenylbromoethylene estrogen was shown to bind to the estrogen receptor by the interaction of the complex with a monoclonal antibody to the receptor. The substantial specific uptake of these [^{80m}Br]-labeled estrogens by ER-rich tissues, combined with previously reported strong evidence for the effective

radiotoxicity of Auger electron emitting isotopes in cell nuclei indicate significant potential for such ligands as therapy for ER rich cancers.

INTRODUCTION

Endocrine-based treatment for breast and endometrial cancers has been an important therapeutic approach for some time (1,2). With the recognition that the presence of estrogen receptors (ER)[s] in breast cancers is an important discriminant to differentiate patients who were good candidates for endocrine therapy from those who were unlikely to respond to such treatments (3,4), it became possible to direct such therapy to individuals most likely to benefit (5). However, reports from many institutions have clearly shown that while few breast cancer patients lacking ER respond to such therapy, only about half of those with ER+ lesions receive objective benefit (6). Likewise, while only about a quarter of endometrial cancers respond to endocrine therapy (7), a significantly greater proportion are found to contain ER. In the case of ovarian carcinomas, where few patients have been reported to obtain remission to endocrine therapy (7), up to half of the primary lesions are found to have ER (7,8).

In the case of breast cancers (9) and endometrial carcinoma (10) it has been found that ER+ cancers lacking progestin receptor, an estrogen-inducible protein, less frequently respond to endocrine therapy. While there have been some suggestions that the ER in such non-responsive cancers may be defective, there is no good evidence suggesting any consistent difference between the localization or affinity of ER in responding and non-responding cancers (6). Hence while assay for the progestin receptor content of breast and endometrial cancers may increase the accuracy of predicting which cancers will respond to endocrine therapies it cannot increase the proportion of cancers which respond to current modes of endocrine therapy. What would be useful, therefore, would

be a therapy based only on the presence of ER in the cancer, and not related to whether the cancer demonstrates growth dependence on estrogenic hormones.

We have suggested such an approach by incorporating the Auger-electron emitting nuclide bromine-80m into a ligand which retains high affinity for the estrogen receptor (11). This metastable bromine isotope has a half life of about 4.42 hours and emits, on average, 7-10 low energy Auger and Coster-Kronig electrons[6]. We have recently reported that the bromine containing triphenylethylene, 1,1-bis(p-hydroxyphenyl)-2-bromo-2-phenyl ethylene, (Br-BHPE), is estrogenic and competes effectively with estradiol for binding to the estrogen receptor (12,13). This report describes our initial findings on the in vivo distribution of bromine-80m labeled Br-BHPE and, for comparison, similar studies with the steroidal bromine containing estrogen, 17α-[^{80m}Br]bromovinyl estradiol, in the immature female rat.

MATERIALS AND METHODS

Production of ^{80m}Br.

Bromine-80m was prepared by irradiating a 0.15 mm layer of >99% enriched ^{80}Se on a cooled aluminum target at a 10° grazing angle with a 20 μA collimated 15 MeV proton beam using a CS-15 cyclotron at the University of Chicago with an end of bombardment yield of 1-2 mCi per μAmp-hour. After dissolving the Se target in 15 ml phosphoric acid and 55% peroxide the radiobromine was removed by steam distillation. The distillate was then made basic, pH > 8, evaporated to dryness and reacted directly with the precursor. Further details on the production will be reported elsewhere.

Synthesis of [^{80m}Br]Br-BHPE.

The no carrier added $^{80m}Br^-$ was evaporated to dryness in a nitrogen stream with heating in a 0.5 dram Kimble screw cap vial. After dissolving the bromide in 25 µl water, 100 µl of a 4.8 mM ethanolic solution of 1,1 bis(p-hydroxyphenyl)-2-phenyl-2-tri-n-butylstannylethylene (11) and 100 µl of a 2:1 mixture of 30% hydrogen peroxide:glacial acetic acid were added. The vial was sealed, shaken and allowed to stand at room temperature for 10 min. The reaction mixture was then injected onto a 0.46 x 25 cm C_{18} reverse phase HPLC column eluting with 40% acetonitrile/water. The [^{80m}Br]-Br-BHPE eluted at 33 min as 60% of the eluted radioactivity, and was concentrated by rotary centrifugation under vacuum.

Synthesis of [^{80m}Br]BrVE$_2$.

The no carrier added $^{80m}Br^-$ solution was placed into a 0.5 dram Kimble screw cap vial and concentrated to dryness using heat and a stream of nitrogen. Into this vial was then placed 30 µl water, 100 µl of a 3.0 x 10^{-2}M ethanolic solution of 17α-E-tri-n-butyl stannylvinylestradiol and 100 µl of a 2/1 v/v 30% hydrogen peroxide-glacial acetic acid solution. The vial was sealed, shaken and allowed to stand 10 min at room temperature. The entire contents of the vial were injected onto the radio-HPLC (0.46 x 25 cm, 5µ, C_{18} reverse phase column, mobile phase 60% water, 40% acetonitrile, flow rate 1.1 ml/min) and 1.1 ml fractions were collected. The 17α E-bromovinylestradiol eluted at 12.5 min. The product fraction contained 53% of the activity eluted from the column. The product fraction was concentrated at 40-45°C under a stream of nitrogen and reconstituted in 1/1 ethanol/water.

Sedimentation analysis of [^{80m}Br]Br-BHPE. For sedimentation analysis 75 µl (60 µCi) of [^{80m}Br]Br-BHPE was added to 75 µl of 10 mM Tris, 10 mM KCl, 1 mM EDTA, pH 7.4 buffer ($TK_{10}E$), to which was added 600 µl of rat uterine cytosol prepared by a 30 min 208,000 x g, 2°C centrifugation of a 1+4 homogenate of immature rat uterus in $TK_{10}E$. In parallel, 25 µl (20 µCi) of [^{80m}Br]Br-BHPE was added to 25 µl of 10 µM diethylstilbesterol in $TK_{10}E$ buffer, to which was added 200 µl of the same rat uterine cytosol. After mixing and incubation for 1 hr in shaved ice these mixtures were added to charcoal pellets from an equivalent volume of 1% Norite, 0.5% dextran T40 in $TK_{10}E$ buffer (DCC), the charcoal pellets resuspended on a vortex mixer, incubated 10 min in ice and centrifuged at low speed in the cold. The supernatant solutions were removed and stored in ice. Two hundred µl portions of the DCC supernatant incubated in the absence of DES were added to 50 µl (2.5 µg) of the monoclonal antiestrogen receptor antibody H222 or 50 µl of $TK_{10}E$, and the tubes incubated for another hour in the cold prior to sedimentation analysis on high salt, 10-30% sucrose gradients in 10 mM Tris, 400 mM KCl, 1 mM EDTA pH 7.4 buffer. Low salt gradients, i.e. 10-30% sucrose gradients in $TK_{10}E$ buffer, were used to analyze the amount of specific binding of the [^{80m}Br]Br-BHPE by layering 200 µl portions of the charcoal treated supernatants incubated with the radiolabeled Br-BHPE in the presence or absence of DES, described above, centrifugation for 14 hrs at 208,000 x g, 2°C, fractionation of the gradients and assay of the fractions for radioactivity. For determination of the effective specific activity of the [^{80m}Br]Br-BHPE the results of these low salt gradient assays were compared with a similar saturation assay for the estrogen receptor of the same rat uterine cytosol with ^{3}H estradiol. For that purpose 25 µl of 100 nM 6,7 ^{3}H-estradiol (Specific activity - 41 Ci/mmole, Amersham) was added to

either 25 µl of $TK_{10}E$ buffer or 25 µl 10 µM DES and 200 µl of the rat uterine cytosol extract added to each and mixed. After incubation for 1 hr in ice these mixtures were each added to the pellets of equal volumes of DCC, as above, mixed, incubated in ice for 10 min, centrifuged and decanted. Two hundred µl of these DCC-treated extracts were analyzed at the same time on similar low salt gradients. Following this analysis and the determination of the specific activity of the [^{80m}Br]Br-BHPE, we calculated that the actual concentration used for the [^{80m}Br]Br-BHPE incubations above was 9.1 nM. Considering that the affinity of Br-BHPE for the receptor is about 1/2 of that of estradiol (13), it is likely that saturating conditions were employed for these assays and therefore the specific activity calculated should be correct.

^{80m}Br-labeled estrogens in vivo. The ^{80m}Br labeled Br-BHPE and Br-VE_2 were diluted in isotonic saline containing 10% ethanol and by assay of the delivered injectants found to contain 12.4 µCi/200 µl, Br-BHPE alone; 14.3 µCi/200 µl, for Br-BHPE with 1 µg DES; 17.7 µCi/200 µl for $BrVE_2$ alone and 19.8 µCi/200 µl for $BrVE_2$ with 1 µg DES. It appeared that the presence of DES reduced the losses of radioactive estrogen on expulsion of the injectants from the syringes since both injectants for each estrogen were made by similar dilutions of a stock solution. For this first set of experiments we were unable to determine the specific activity of the [^{80m}Br]bromo-estrogens. Estimates from the UV tracing of the HPLC elution of the purified estrogens indicated approximate specific activities in the range of 4000 Ci/mmole. Inorganic ^{80m}Br, as sodium bromide in saline, was assayed at 111 µCi/200 µl. The injectants were administered I.P. to 22 day old female Sprague-Dawley rats and at 1/2 and 2 hours, 3 animals for each group were sacrificed by decapitation, the various tissues were removed, dissected free of extraneous tissue, gently blotted on

filter paper and rapidly weighed. For the studies comparing the route of administration, the [^{80m}Br]Br-BHPE for subcutaneous and I.V. injections were made up the same way, in isotonic saline, 10% ethanol, and 200 µl were administered to each animal. For I.P. injections two concentrations were prepared, both for administration in 1.0 ml of isotonic saline, 2% ethanol. In each case the injectants prepared to include DES contained 1.0 µg of DES in the volume to be injected. The assays for the injectants, taken from actual volumes delivered from the syringes were: high I.P. dose [^{80m}Br]Br-BHPE alone (1.0 ml) 61 µCi, with DES 69 µCi; low I.P. dose [^{80m}Br]Br-BHPE alone (1.0 ml) 9.2 µCi, with DES 16.1 µCi; subcutaneous and I.V. doses (0.2 ml): [^{80m}Br]Br-BHPE alone (0.2 ml) 7.3 µCi, with DES 14.5 µCi. For all experiments the tissue samples and 200 µl portions of blood were counted in a γ-counter set to record the high energy emission (i.e. 200-1000 keV) of the daughter ^{80}Br isotope ($t_{1/2}$=17 minutes), at equilibrium with the ^{80m}Br parent by the time of counting. Quantifying on this higher energy emission substantially reduced tissue quenching which can be a problem with the relatively low energy γ-emission of the ^{80m}Br. DPM were obtained by comparison with the counting efficiency for the 600 KeV peak of a sample of ^{80}Br related to a Cesium 137 standard, and because of the short half-life of ^{80m}Br, all tissue radioactivity and injectant DPM were decay corrected to the time of injection (time zero). The results were calculated as means and standard deviations for the percent of the dose present in each tissue divided by the tissue wet weight in grams, or as tissue to blood ratios (DPM per mg wet weight in the tissue divided by the DPM/µl of blood of the same animal), thereby allowing direct comparisons of the somewhat different amounts of radioactivity injected for the various injectants, as well as individual animal differences.

Because of the size of the counting chamber of the γ-counter, portions (generally 100-200 mg) of the larger tissues were assayed (liver, intestine, brain (cerebrum), muscle (femur)). The entire uterus, vagina, pair of ovaries, pair of adrenals, and hemi diaphragm were taken. Fat was taken from inguinal mammary fat pads using care to avoid the mammary epithelium. Stalk blood (200 µl) was taken directly for assay. Because of the small size of the pituitary and the rapid dehydration on excision, the entire tissue was placed directly into the assay tube without attempting to obtain a wet weight. The calculations all assumed a one mg wet weight for this tissue. In our experience under the experimental conditions used the errors in weighing the individual pituitaries are greater than the differences in weight among pituitaries of immature rats of the same age and size.

RESULTS

The specific incorporation of [^{80m}Br]Br-BHPE by various tissues of the immature rat, expressed as the % dose per gram wet weight of tissue, is shown in figure 1. It can be seen that there is a significant, DES-inhibitable uptake of [^{80m}Br]Br-BHPE in the usual estrogen target tissues, the uterus, pituitary, vagina, and ovary. No such DES-inhibitable uptake is seen in the other tissues assayed, i.e. fat, liver, adrenal, intestine, leg muscle, diaphragm, or brain. This pattern of uptake is the same at both 0.5 hour and 2 hours, with the amounts generally lower by 2 hours. The very substantial, specific uptake by the uterus at both times is evident in figure 1. Although the intestinal concentration is more than 10% dose/g at 0.5 hour there is no significant difference whether the radiolabeled estrogen was administered alone or along with DES. The same results, expressed as the tissue to blood

radioactivity ratio, are shown in figure 2. This manner of presentation minimizes animal variations in metabolic clearance or any differences in the amount of labeled estrogen each animal actually received, but, as can be seen, shows essentially the same pattern of specific uptake by estrogen target tissues. The only significant difference in the data evaluated in this way is that the relatively small difference in specific uptake of the vagina at the 0.5 hr time point is eliminated. However specific uptake of [^{80m}Br]Br-BHPE is seen in the vagina at the 2 hour time point. It is important to appreciate that substantial tissue to blood ratios are seen for the target tissues, as high as 20 for the uterus.

Similar studies with [^{80m}Br]Br-VE$_2$ are shown in figures 3 and 4. With this radiobromine-labeled estrogen we also found significant, DES-inhibitable uptake by the classical estrogen target tissues, uterus, pituitary, vagina and ovary. Although a relatively high 0.5 hr uptake, with a higher percent dose/g in the absence than in the presence of DES is seen for the liver (figure 3), it is clear from the very large standard deviations that this difference in the presence and absence of DES is not statistically significant. Nor is such a difference in liver uptake seen at 2 hours. When expressed as tissue to blood ratios, fig. 4, it is clear that there is no DES-inhibitable difference in ratio with liver at either time point.

Although the % dose/g of the bromotriphenylethylene localized in the uterus at 0.5 hr (fig. 1) is nearly double that seen with the steroidal estrogen (fig. 3), the localization expressed as tissue/blood ratios are very similar (fig. 2,4). Part of this difference may be due to the fact that reanalysis of the injected bromoestrogens showed that the [^{80m}Br]Br-BHPE, but

not the Br-VE_2, contained some free bromide, apparently formed during the evaporation of the elution solvent of the HPLC purification. Although there are only low tissue to blood ratios in target or nontarget tissues after administration of [^{80m}Br] sodium bromide, figure 5, the ratios do not change a great deal over the first 2 hours.

Thus, the patterns, showing specific uptake by estrogen target tissues for both the non-steroidal and steroidal bromine-containing estrogens, are significantly different than the pattern seen when inorganic radiobromide is injected, fig. 5. This clearly indicates that the radiobromine is not rapidly eliminated from the estrogens before the estrogens can be specifically taken up by the target tissues.

We intentionally explored I.P. administration of the radiobromine labeled estrogens as a first approach because of our interest in the applicability of the therapy to the intraperitoneal metastases often accompanying the spread of ovarian cancer. We were struck, however, by the disproportionate specific localization of Br-BHPE in the uterus and especially ovary, as compared with the pituitary. It seemed likely that this could be due to direct access of the radiolabeled estrogen to these tissues while the pituitary would not have access to such a high initial concentration of compound. Therefore we compared the distribution of [^{80m}Br]Br-BHPE administered I.V. (tail vein), subcutaneously (back) and I.P. at the same 2 time points. With improved production of ^{80m}Br for these studies, providing more significant amounts of ^{80m}Br to work with, we were able to directly assay the specific activity of the labeled estrogen by sedimentation analysis (fig. 6). A comparison of the DES-inhibitable binding of ^{3}H estradiol for a rat uterine "cytosolic" ER with

that for [^{80m}Br]Br-BHPE indicated that the [^{80m}Br]Br-BHPE had a specific activity of about 8700 Ci/mmole, about 200 times the specific activity of the ^{3}H E_2. We were also able to confirm that the [^{80m}Br]Br-BHPE actually bound to the estrogen receptor, seen by the downfield shift of the salt dissociated, 4S, form of the receptor complex incubated with monoclonal antibody to ER (fig. 7).

When the uterine uptake of this ^{80m}Br-BHPE was compared following the 3 routes of administration it was evident that there was a substantially higher uptake by the uterus following I.P. administration than by the other 2 routes at both 0.5 and 2 hours (fig. 8). In each case, nonetheless, most of the uptake was DES-inhibitable. Both the low and the high dose of [^{80m}Br]Br-BHPE administered I.P. showed a high level of specific uptake in the uterus; by 2 hours very little uterine binding was seen in rats which received DES. The preferential uterine uptake via the I.P. route is especially evident when the data is presented as tissue to blood ratios (fig. 9). Under these conditions the uterus to blood ratios on I.P. administration are 30 to 70 while the ratios after subcutaneous or the I.V. administration are less than 10 at both time points. It is also of considerable interest for eventual therapeutic use, that an almost proportional increase in the uterus to blood ratio was seen with the higher dose of compound, which is still a minute dose because of the high specific activity.

Table 1 shows the tissue to blood ratios for all the tissues, comparing the I.P., sub Q and I.V. results, and the actual blood values for each group. It is evident from this data that blood levels are higher, especially at the early time points, following sub Q and I.V. administration compared with I.P. As expected, there is a more distinct decrease in the radioactivity in the

blood between 0.5 and 2 hr for I.V., compared with sub Q, administration of the bromoestrogen. The most striking differences seen, however, relate to the elevated tissue to blood ratios for the peritoneal target tissues (i.e. uterus, vagina and ovary) following I.P. administration of the Br-BHPE. This effect remains very pronounced at 2 hours for these tissues in animals administered the radiolabeled Br-BHPE alone, while in those animals administered Br-BHPE along with DES, by 2 hours there was no significant difference related to route of administration. One of the other important, significant differences related to route of administration was the relative localization of the labeled estrogen in the pituitary. Following I.P. injection the uterus to pituitary ratio (ie. uterus/blood to pituitary/blood ratio) varies from 3.6 to 5.9 for the 2 doses at the 2 time points, while after sub Q or I.V. administration this ratio is about 0.35 for both time points. A similar pattern is seen in the relative tissue to blood ratios for ovary compared to pituitary. While after sub Q or I.V. administration the ovary to blood ratio is only about 3, after I.P. administration consistent ratios of 12 to 17 are seen. That this ovarian uptake is receptor dependent is seen by the substantially lower ratios when DES was given. The kidney and adrenal levels of radioactivity appear to be generally higher after I.P. administration but the differences generally are not statistically significant. Neither are the differences in uptake by these tissues in animals receiving the [^{80m}Br]Br-BHPE alone compared with those also given DES. It would appear that the I.P. route is a particularly advantageous route to reach maximum uptake of radiolabeled estrogens in ER rich tissues in the peritoneal cavity.

DISCUSSION

The results presented in these studies indicate that the radiobromine-labeled, hydroxy substituted triphenylethylene, 1,1-bis(p-hydroxyphenyl)-2-bromo-2-phenylethylene would appear to be a good candidate for further study as an estrogen-receptor-directed therapeutic agent. It shows very good target tissue specific, diethylstilbesterol-inhibitable uptake by ER rich tissues, the uterus, pituitary, vagina and ovary, and much less significant and also non-DES inhibitable uptake by other tissues evaluated. It is evident that particularly for maximum localization of this radiolabeled estrogen in the uterus, vagina and ovary, there is a distinct advantage to I.P. administration where the compound apparently has greater direct access to the tissue prior to metabolism, which in all likelihood occurs extensively in the liver. While the target tissue localization of this compound following sub Q or I.V. administration is still appreciable, there is a clear advantage to the I.P. route to maximize uptake by the target tissues in the peritoneal cavity. The uptake of 17α-[^{80m}Br]bromovinyl estradiol, while also significant and DES inhibitable in the ER-rich tissues, nonetheless was consistently lower than that seen for the triphenyl bromoethylene. This would suggest that at least for treatment purposes it would appear that the nonsteroidal bromoestrogen may have an advantage over the steroidal Br-VE$_2$. When compared as tissue to blood ratios, the bromovinyl estradiol seems superior, so that for imaging use this steroidal bromoestrogen may have an advantage. This difference, as expected, relates to the concentration of radiolabel remaining in the blood as a function of time after administration, and the radiobromine concentration consistently remained significantly higher after the triphenyl ethylene. Whether this increased blood level of radioactivity actually consists of higher levels of unchanged radiolabeled

estrogen or merely circulating metabolites will have to be determined in subsequent studies. If the former were true, however, it would help explain the apparent longer retention or higher levels of radioactivity in target tissues at the latter time point following administration of the triphenylbromoethylene. Although, as seen in figure 6, there does appear to be some non-DES inhibitable binding of the Br-BHPE to a nonspecific, 4S component present in rat uterine cytosol, it comprises a small proportion of the total binding and thus appears to be less problematic than the high level of nonspecific binding which has been reported for 17α-[^{77}Br]bromoethynylestradiol (14).

Neither of these estrogens appear to show significant, DES inhibitable localization in the liver. Since the liver has been reported to contain low levels of estrogen receptor, one needs to be concerned about possible damage to this organ if sufficient radiotoxic estrogen were localized in the nucleus of the liver cells. Because the liver is probably the major site of metabolism of administered estrogens, and these enzymes are largely cytoplasmic, it is not clear what proportion of the radiolabeled estrogen which is found in the liver is present as cytoplasmic estrogen, as contrasted with nuclear localization via the receptor mechanism. Clearly both biochemical (15) and immunocytochemical (16) evidence strongly suggests that estrogen receptor is essentially entirely present in the nucleus even in the absence of estrogen. The distinct advantage of estrogen receptor directed therapy using Auger electrons is that the effectiveness of Auger emission is at least an order of magnitude greater for compounds near the DNA than those located in the cytoplasm and even greater when compared to the nuclide outside the cell. With the Auger-electron emitting isotope ^{125}I, the cytotoxicity of the isotope incorporated in DNA

(from [^{125}I]-IUdR) is about 300 times greater than the same dose localized at the membrane (^{125}I-Conconavalin A) (17). Our preliminary results (18) with ^{80m}Bromine confirm the importance of nuclear localization of the Auger emitter for effective radiotoxicity in that exposure of cells to similar concentrations ^{80m}Br labeled NaBr (which does not enter the cell) or bromoantipyrene (which distributes generally throughout the cell) showed little effect at concentrations in which [^{80m}Br]BrUdR incorporated in DNA was radiotoxic. This difference may in fact be larger with Auger electron emissions from ^{80m}Br than with ^{125}I in that the average Auger energy of the bromine isotope is about 1/3 that of iodine and therefore would be expected to have a shorter effective range[6].

The actual concentration of radiolabel in both liver and intestine obtained from assay of small portions of these tissues are probably not accurate reflections of the dynamic handling of the bromoestrogens by these tissues in vivo. Preliminary imaging studies carried out in rats using [^{80m}Br]Br-BHPE have shown very important time dependent differences in localization of ^{80m}Br in both these tissues. Clearly the route of administration has a significant impact on the time at which the amount of drug in the liver reaches a maximum level which is earlier for I.V. administration than I.P. or sub Q. Following the clearance of the bolus of radioactivity through the liver there is a distinctive movement of radiolabel through the intestine, clearly visualized by real time imaging. Since only a portion of the median lobe of liver and a 1 cm piece from the central portion of the small intestine were taken for radioassay, the actual concentration reported herein relates very much to the rate at which the material passed through these two organs and the position of the radioactive bolus in the intestine relative to

the position of tissue excised for sampling. Furthermore, preliminary data evaluating the radioactivity of the intestine by comparing the radioactivity of the intestinal contents with the tissue radioactivity have clearly shown that the intestinal contents comprise by far the greater concentration of radioactivity. While the total radioactivity in this organ can be appreciable, in particular because of the way in which the radioactive pulse dose is handled and passes through, if the cellular damage from the radiobromine depends upon nuclear localization of the label it is unlikely that any major radiotoxicity would occur in the intestine due to feces passing through.

It should be pointed out that although there are obvious disadvantages to working with a short half-life isotope like bromine-80m, the production and syntheses reported here are simple and rapid. The bromine-80m is obtained by dissolving the Se target in peroxide, phosphoric acid and separated by steam distillation. After neutralization and solvent evaporation the tributyltin precursor can be added and, following a 10 minute reaction, purified by HPLC for use, essentially from target to animal within 2 hours. The substantial advantage in the use of an isotope with a 4.4 hr half life, a similar half-life to that of the ER itself, is that there is a very good probability that the isotope will decay while still associated with the receptor at the DNA binding site.

Other potential candidates for bromine-80m labeled bromoestrogens for estrogen receptor directed therapy are 16α-bromoestradiol, 16α-BrE_2, (19) 16α-bromo-11β-methoxyestradiol, 16α-$BrME_2$ (20), and 17α-bromovinyl-11β-methoxyestradiol, 17α-$BrVME_2$. The two 16α-bromoestrogens have been previously synthesized in the radiobrominated form but not with

bromine-80m. Following I.V. administration of 16α-[^{77}Br]BrE$_2$ about 7% injected dose per gram was found at 1 hr in the uterus of the immature rat. Although the specific activity of that radiobromine-containing estrogen was not assayed, it was estimated to be about 1400 Ci/mmole (19). In a subsequent study with another preparation of the 16α-[^{77}Br]BrE$_2$, with an effective specific activity determined by receptor assay to be 389 Ci/mmole (21), the % injected dose per gram uterus was found to be 8.4%/g at one hour, in good agreement with the earlier study, and this uterine uptake was reduced to about 0.5%/g when co-injected with 18 μg unlabeled estradiol. 16α-[^{77}Br]BrME$_2$, prepared with an effective specific activity of 770-1450 Ci/mmole by Katzenellenbogen et al. (20), showed excellent uptake by uterus, over 12% injected dose/g at 1 hr. The improved uterus to blood ratio of this 11β methoxy bromoestrogen was attributed to its low binding to non-specific components. Such a characteristic would be expected to be especially important when used for imaging, to which purposes those studies were directed. Whether non-specific binding to serum is advantageous or a deterrent to potential radiotherapeutic use has yet to be shown. Although the tissue to blood ratios for the estrogen target tissues were generally higher for the BrVE$_2$ than for the Br-BHPE studied here, the actual concentration of radiolabel in the uterus was higher with Br-BHPE. It is possible that binding to serum components protects the compound from degradation and more rapid excretion. However more definitive conclusions will have to await direct analyses of radiolabeled components in the blood.

It is important to consider whether the current studies indicate anything about the feasibility of the suggested new therapeutic approach using estrogen receptor directed radiotherapy with an ^{80m}Br labeled estrogen. The 15% dose per gram tissue localization in the uterus following IP administration of the

high dose [^{80m}Br]Br-BHPE presented here corresponds to approximately 600 molecules of estrogen per cell. Calculations based upon the radiotoxicity of [^{80m}Br]Br-UdR for CHO cells (18) indicate that the D37 of about 77 fCi/cell for the ^{80m}Br incorporated into DNA corresponds to about 50 molecules per cell. This is reasonably close to the D37 of 130 fCi/cell reported for [^{77}Br]Br-UdR with V79 cells (22). If all the 600 molecules per cell of [^{80m}Br]Br-BHPE were radioactive, one would clearly expect to have a substantial radiotoxic dose delivered to the target organ. Since only about 1.2% of the bromine molecules of the radiolabeled BHPE in this experiment were radioactive (i.e., a specific activity of 8700 Ci/mmole compared to a theoretical specific activity of 710,000 Ci/mmole for the isotope), one would not expect the current conditions to deliver appreciable radiotoxic damage. It must be appreciated that the synthesis of [^{80m}Br]Br-BHPE using 5 mCi of radiobromide corresponds to only about 7 picomoles of bromide so even with moderate care natural bromine contamination of reagents or apparatus can cause a substantial reduction in the specific activity of the product. With a larger scale preparation of bromine-80m and special care taken to eliminate trace amounts of nonradioactive bromine it should be feasible to reach specific activities of radiotoxic potential in vivo. Furthermore even the high dose administered (7 pmole) is less than 1% of a dose which even begins to show saturation of uptake in the rat uterus by 2 hours (23) so that if enough [^{80m}Br]Br-BHPE were available one would expect to increase the injected dose by a substantial factor, possibly >50, and thus get substantially increased amounts of bromine-80m into target tissues. Hence while calculations indicate that we are not currently at levels sufficient for testing the radiotoxicity of estrogens labeled with this highly effective Auger-electron emitting bromine isotope, such a goal indeed seems

feasible.

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Figure Legends

Fig. 1. Specific uptake of [^{80m}Br]Br-BHPE in tissues of the immature female rat. The [^{80m}Br]Br-BHPE was administered I.P. alone (open bars) or with 1 μg DES (crosshatched bars). At the time indicated tissues were excised, weighed and assayed for radioactivity as described in the experimental section. Results presented are the mean % dose per gram wet weight of tissue. Error bars represent the standard error of the mean. Tissue codes: uterus, Ut; pituitary, Pit; vagina, V; ovary, O; fat, F; liver, L; adrenal, A; intestine, I; leg muscle, M; hemidiaphragm, D; brain, Br; blood, Bl.

Fig. 2. Tissue to blood ratios of radioactivity after I.P. administration of [^{80m}Br]Br-BHPE. Using the experimental protocol, described in fig. 1 and the experimental section, the DPM (corrected to time zero) per mg wet weight of each assayed tissue in each animal was divided by the DPM per μl of blood (also corrected to time zero) for each animal and the mean value for each group plotted along with the standard error of the mean. Crosshatched bars correspond to results from animals also given 1 μg DES.

Fig. 3. Specific uptake of 17α-[^{80m}Br]bromovinylestradiol administered I.P. alone (open bars) or in the presence of 1 μg DES (crosshatched bars). See legend to Figure 1 and experimental section for details.

Fig. 4. Tissue to blood ratios of radioactivity after I.P. administration of 17α-[^{80m}Br]bromovinylestradiol. See legend to fig. 2 and experimental section for details. Crosshatched bars represent results from animals also treated with 1 μg DES.

Fig. 5. Tissue to blood ratios of radioactivity after I.P.

administration of ^{80m}Br-sodium bromide. See legend to fig. 2 for description.

Fig. 6. Sedimentation analysis of the binding of [^{80m}Br]-Br-BHPE to low salt extractible estrogen receptor from immature rat uterus. Saturating concentrations of [^{3}H]E_2 (41 Ci/mmole) and [^{80m}Br]Br-BHPE, alone (solid lines) or in the presence of 1 μmolar DES (dashed lines), were incubated with the ER containing extract, excess unbound estrogen removed with DCC and the labeled extracts analyzed as described in the experimental section. The DPM were corrected for decay to time zero. Bot corresponds to the bottom of the gradient.

Fig. 7. Interaction of [^{80m}Br]Br-BHPE bound to rat uterine low salt extract with monoclonal antibody to the estrogen receptor. DCC-treated [^{80m}Br]Br-BHPE containing extract described for fig. 6 was incubated with buffer or 10 μg/ml monoclonal antibody H222 and analyzed by sedimentation analysis on high salt sucrose density gradients as described in the experimental section. Bot corresponds to the bottom of the gradient.

Fig. 8 Comparison of uterine content of radioactivity after administration of [^{80m}Br]Br-BHPE to immature female rats by 3 different routes. The [^{80m}Br]Br-BHPE, specific activity 8700 Ci/mmole, was administered, at the doses shown, alone or along with 1 μg DES, I.P., subcutaneously in the back or I.V. (tail vein) and the uteri assayed by the procedure indicated in fig. 1.

Fig. 9. Comparison of uterine tissue to blood radioactivity ratios after administration of [^{80m}Br]Br-BHPE, by 3 different routes. Details given in figs. 8 and 2.

Table 1. Tissue to Blood Ratios for ^{80m}Br-BHPE[a]

	High Dose I.P.				Low Dose I.P.			
	.5 Hr		2 Hr		.5 Hr		2 Hr	
	Alone	+DES	Alone	+DES	Alone	+DES	Alone	+DES
uterus	33.6±5.0	6.0±3.1	49.0±20.9	3.9±.98	32.7±6.7	6.6±.3	68.3±7.6	2.2±1.1
ovary	15.3±3.4	3.7±2.2	16.7±8.1	2.8±1.1	12.5±1.8	3.5±.05	13.5±.89	1.7±.40
vagina	3.5±.32	2.2±.93	6.1±2.9	2.5±.80	3.8±.36	2.3±.42	6.5±1.5	1.8±.60
kidney	2.2±.08	2.1±.38	1.9±.89	1.7±.16	2.3±.11	1.9±.01	1.9±.30	1.7±.43
adrenal	3.4±.48	2.3±.57	2.4±1.1	1.1±.35	2.5±.5	2.5±.7	3.0±1.2	3.4±4.3
pituitary	7.7±.85	5.0±.92	13.6±2.7	3.3±1.1	5.5±1.1	3.0±2.6	2.3±3.9	3.9±1.9
brain	.53±.03	.65±.23	.63±.56	.30±.02	.52±.02	.50±.10	.30±.20	.23±.14
fat	1.2±.35	1.4±.43	.74±.12	.80±.34	1.4±.46	1.3±.44	1.3±.35	1.1±1.0
muscle	.94±.27	.74±.15	.60±.10	.44±.10	1.0±.10	.80±.06	.55±.07	.36±.05
liver	3.6±.47	6.0±2.5	2.3±1.0	2.5±2.0	4.1±.07	4.3±.8	1.9±.32	1.5±.16
intestine	4.9±.52	10.5±1.2	9.8±7.2	18.8±9.1	5.9±.65	9.4±5.2	2.8±1.5	3.7±3.0
blood (%/g)	.46±.21	.40±.10	.27±.19	.42±.14	.70±.10	.43±.06	.42±.12	.40±.23

	Sub Q				I.V.			
	.5 Hr		2 Hr		.5 Hr		2 Hr	
	Alone	+DES	Alone	+DES	Alone	+DES	Alone	+DES
uterus	2.3±.14	1.1±.34	8.0±1.0	2.1±.5	3.6±.9	2.0±.59	6.6±2.7	2.4±.39
ovary	1.3±.17	1.0±.33	2.9±.25	1.4±.58	2.3±.92	1.8±.91	3.1±1.0	2.0±.33
vagina	1.3±.26	.90±.28	4.0±.07	1.6±.25	2.1±.5	1.3±.25	4.0±1.5	1.7±.19
kidney	1.9±1.0	1.8±.64	1.9±.21	1.5±.19	2.1±.36	1.8±.40	1.6±.55	1.5±.21
adrenal	1.7±.31	1.6±.53	1.1±.18	.65±.15	2.1±.25	1.8±.35	1.2±.58	.64±.49
pituitary	6.4±4.2	5.0±2.4	24.4±4.8	4.9±1.0	10.2±6.1	9.1±1.3	18.3±8.5	6.0±3.0
brain	.50±.02	.40±.21	.74±.26	.30±.08	.73±.28	.86±.07	.33±.31	.36±.05
fat	1.0±.14	.80±.52	1.1±.42	.74±.05	1.5±.27	1.1±.29	.99±.60	.71±.11
muscle	.60±.24	.42±.06	.65±.06	.74±.60	.87±.38	.90±.21	.67±.27	.51±.09
liver	2.9±1.1	2.1±.62	1.8±.20	1.7±.30	3.6±2.4	3.1±.80	1.5±.30	3.0±1.1
intestine	2.8±1.2	2.2±.28	12.5±18.5	2.4±.54	2.2±.07	2.4±.96	1.5±.21	2.45±.77
blood (%/g)	1.3±.10	1.1±.27	.61±.10	.73±.33	.94±.19	.86±.24	.50±.24	.46±.12

a DPM/mg tissue divided by DPM/µl blood for each animal

b 1 µg of DES/animal

c To allow comparisons actual % dose/g for bloods is given.

Table 1. Tissue to Blood Ratios for ^{80m}Br-BHPE[a]

	High Dose I.P.				Low Dose I.P.			
	.5 Hr		2 Hr		.5 Hr		2 Hr	
	Alone	+DES	Alone	+DES	Alone	+DES	Alone	+DES
uterus	33.6±5.0	6.0±3.1	49.0±20.9	3.9±.98	32.7±6.7	6.6±.3	68.3±7.6	2.2±1.1
ovary	15.3±3.4	3.7±2.2	16.7±8.1	2.8±1.1	12.5±1.8	3.5±.05	13.5±.89	1.7±.40
vagina	3.5±.32	2.2±.93	6.1±2.9	2.5±.80	3.8±.36	2.3±.42	6.5±1.5	1.8±.60
kidney	2.2±.08	2.1±.38	1.9±.89	1.7±.16	2.3±.11	1.9±.01	1.9±.30	1.7±.43
adrenal	3.4±.48	2.3±.57	2.4±1.1	1.1±.35	2.5±.5	2.5±.7	3.0±1.2	3.4±4.3
pituitary	7.7±.85	5.0±.92	13.6±2.7	3.3±1.1	5.5±1.1	3.0±2.6	2.3±3.9	3.9±1.9
brain	.53±.03	.65±.23	.63±.56	.30±.02	.52±.02	.50±.10	.30±.20	.23±.14
fat	1.2±.35	1.4±.43	.74±.12	.80±.34	1.4±.46	1.3±.44	1.3±.35	1.1±1.0
muscle	.94±.27	.74±.15	.60±.10	.44±.10	1.0±.10	.80±.06	.55±.07	.36±.05
liver	3.6±.47	6.0±2.5	2.3±1.0	2.5±2.0	4.1±.07	4.3±.8	1.9±.32	1.5±.16
intestine	4.9±.52	10.5±1.2	9.8±7.2	18.8±9.1	5.9±.65	9.4±5.2	2.8±1.5	3.7±3.0
blood (%/g)	.46±.21	.40±.10	.27±.19	.42±.14	.70±.10	.43±.06	.42±.12	.40±.23

	Sub Q				I.V.			
	.5 Hr		2 Hr		.5 Hr		2 Hr	
	Alone	+DES	Alone	+DES	Alone	+DES	Alone	+DES
uterus	2.3±.14	1.1±.34	8.0±1.0	2.1±.5	3.6±.9	2.0±.59	6.6±2.7	2.4±.39
ovary	1.3±.17	1.0±.33	2.9±.25	1.4±.58	2.3±.92	1.8±.91	3.1±1.0	2.0±.33
vagina	1.3±.26	.90±.28	4.0±.07	1.6±.25	2.1±.5	1.3±.25	4.0±1.5	1.7±.19
kidney	1.9±1.0	1.8±.64	1.9±.21	1.5±.19	2.1±.36	1.8±.40	1.6±.55	1.5±.21
adrenal	1.7±.31	1.6±.53	1.1±.18	.65±.15	2.1±.25	1.8±.35	1.2±.58	.64±.49
pituitary	6.4±4.2	5.0±2.4	24.4±4.8	4.9±1.0	10.2±6.1	9.1±1.3	18.3±8.5	6.0±3.0
brain	.50±.02	.40±.21	.74±.26	.30±.08	.73±.28	.86±.07	.33±.31	.36±.05
fat	1.0±.14	.80±.52	1.1±.42	.74±.05	1.5±.27	1.1±.29	.99±.60	.71±.11
muscle	.60±.24	.42±.06	.65±.06	.74±.60	.87±.38	.90±.21	.67±.27	.51±.09
liver	2.9±1.1	2.1±.62	1.8±.20	1.7±.30	3.6±2.4	3.1±.80	1.5±.30	3.0±1.1
intestine	2.8±1.2	2.2±.28	12.5±18.5	2.4±.54	2.2±.07	2.4±.96	1.5±.21	2.45±.77
blood (%/g)	1.3±.10	1.1±.27	.61±.10	.73±.33	.94±.19	.86±.24	.50±.24	.46±.12

a DPM/mg tissue divided by DPM/µl blood for each animal
b 1 µg of DES/animal
c To allow comparisons actual % dose/g for bloods is given.

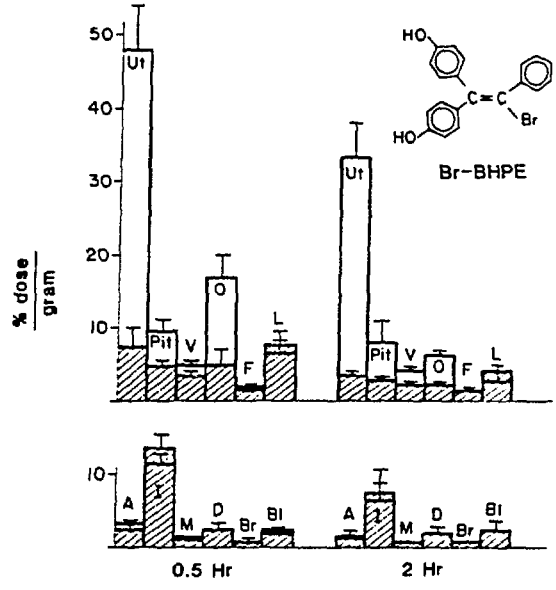
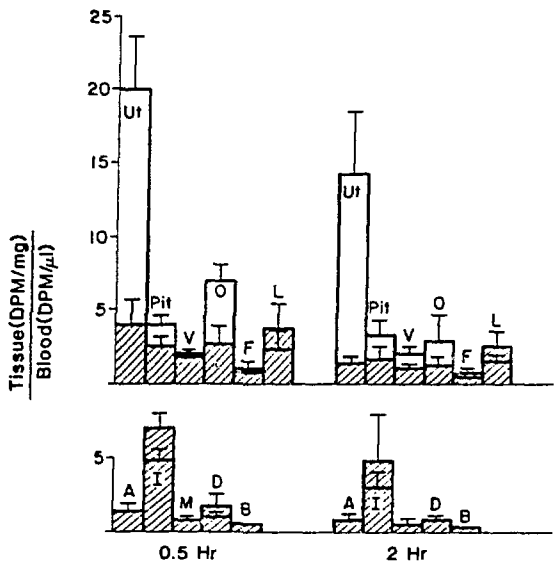


Fig. 1. Specific uptake of [^{80m}Br]Br-BHPE in tissues of the immature female rat. The [^{80m}Br]Br-BHPE was administered I.P. alone (open bars) or with 1 μg DES (crosshatched bars). At the time indicated tissues were excised, weighed and assayed for radioactivity as described in the experimental section. Results presented are the mean % dose per gram wet weight of tissue. Error bars represent the standard error of the mean. Tissue codes: uterus, Ut; pituitary, Pit; vagina, V; ovary, O; fat, F; liver, L; adrenal, A; intestine, I; leg muscle, M; hemidiaphragm, D; brain, Br; blood, Bl.



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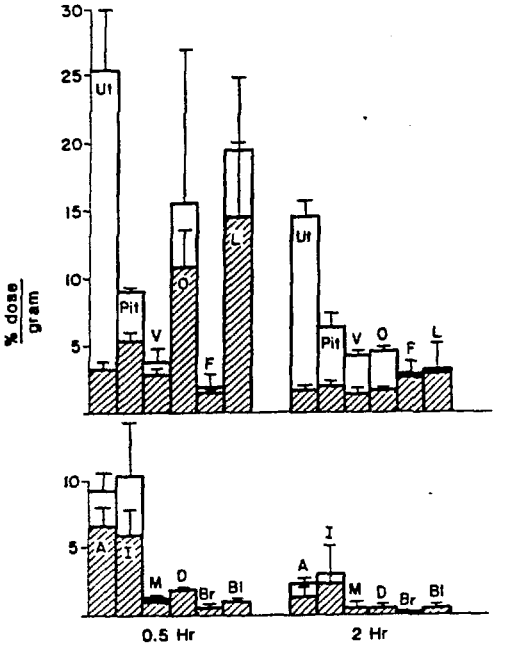

Fig. 3. Specific uptake of 17α-[^{80m}Br]bromovinylestradiol administered I.P. alone (open bars) or in the presence of 1 μg DES (crosshatched bars). See legend to Figure 1 and experimental section for details.

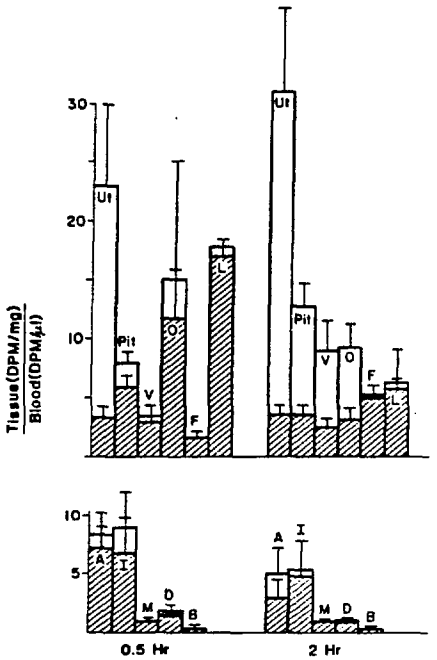

Fig. 4. Tissue to blood ratios of radioactivity after I.P. administration of 17α-[^{80m}Br]bromovinylestradiol. See legend to fig. 2 and experimental section for details. Crosshatched bars represent results from animals also treated with 1 µg DES.

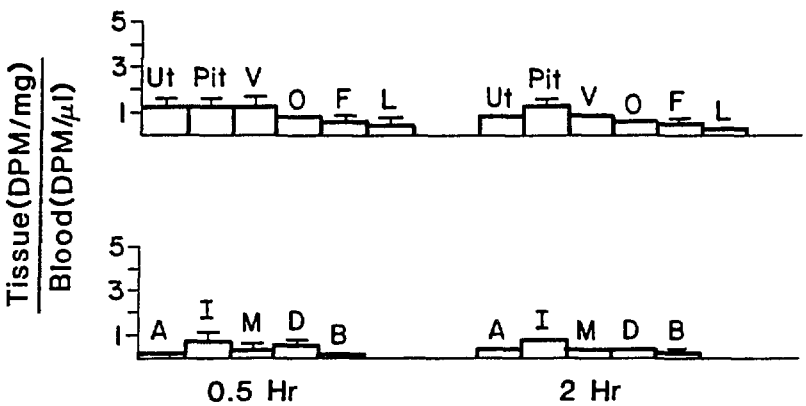

Fig. 5. Tissue to blood ratios of radioactivity after I.P. administration of ^{80m}Br-sodium bromide. See legend to fig. 2 for description.

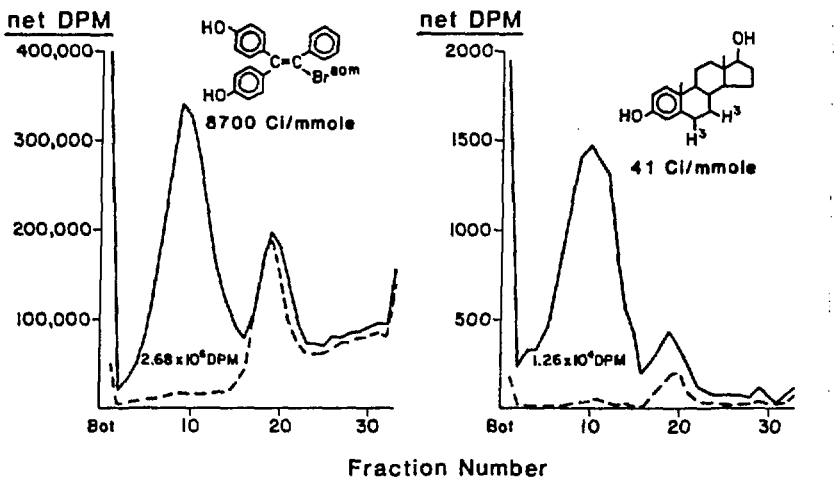

Fig. 6. Sedimentation analysis of the binding of [^{80m}Br]-Br-BHPE to low salt extractible estrogen receptor from immature rat uterus. Saturating concentrations of [^{3}H]E_2 (41 Ci/mmole) and [^{80m}Br]Br-BHPE, alone (solid lines) or in the presence of 1 μmolar DES (dashed lines), were incubated with the ER containing extract, excess unbound estrogen removed with DCC and the labeled extracts analyzed as described in the experimental section. The DPM were corrected for decay to time zero. Bot corresponds to the bottom of the gradient.

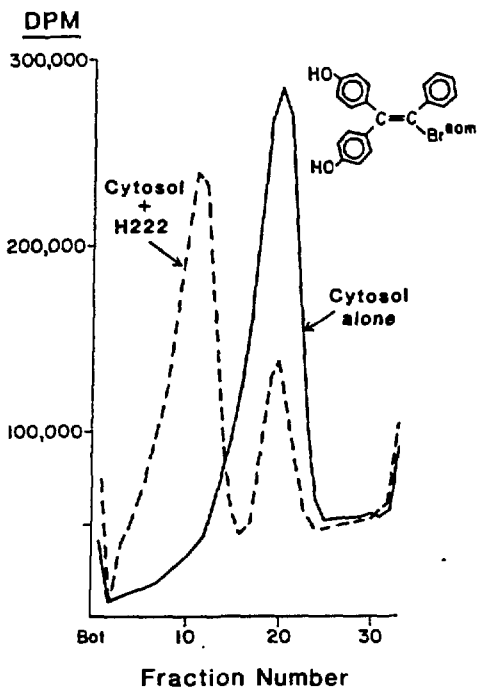

Fig. 7. Interaction of [^{80m}Br]Br-BHPE bound to rat uterine low salt extract with monoclonal antibody to the estrogen receptor. DCC-treated [^{80m}Br]Br-BHPE containing extract described for fig. 6 was incubated with buffer or 10 µg/ml monoclonal antibody H222 and analyzed by sedimentation analysis on high salt sucrose density gradients as described in the experimental section. Bot corresponds to the bottom of the gradient.

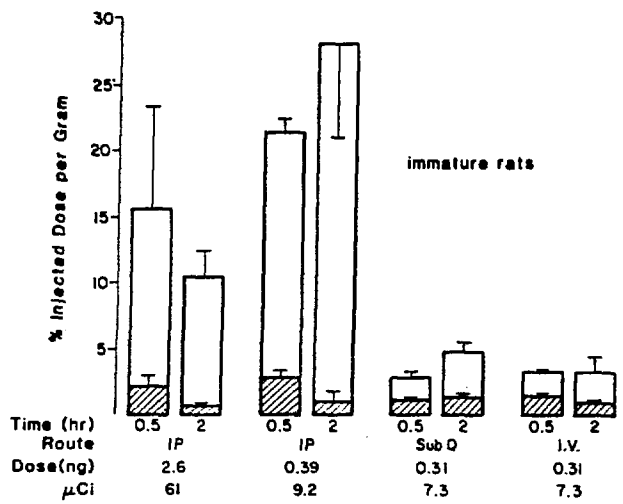

Fig. 8 Comparison of uterine content of radioactivity after administration of [^{80m}Br]Br-BHPE to immature female rats by 3 different routes. The [^{80m}Br]Br-BHPE, specific activity 8700 Ci/mmole, was administered, at the doses shown, alone or along with 1 μg DES, I.P., subcutaneously in the back or I.V. (tail vein) and the uteri assayed by the procedure indicated in fig. 1.

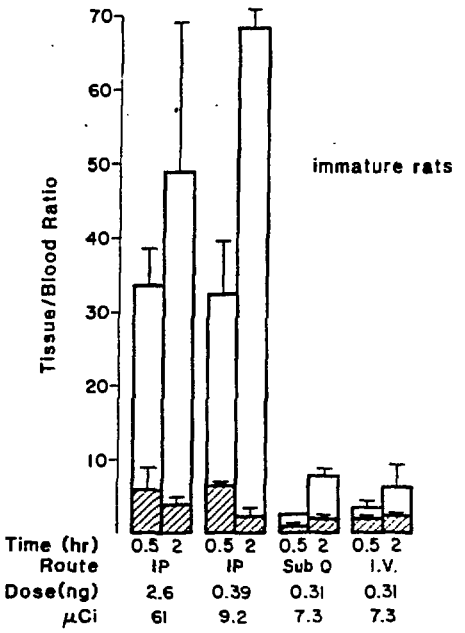

Fig. 9. Comparison of uterine tissue to blood radioactivity ratios after administration of [^{80m}Br]Br-BHPE, by 3 different routes. Details given in figs. 8 and 2.