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LABELING OF RECEPTOR LIGANDS AND OTHER COMPOUNDS WITH HALOGEN RADIONUCLIDES

PROGRESS REPORT

FOR PERIOD

APRIL 1, 1987 - AUGUST 31, 1989

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DISCLAIMER

NOTICE

Labeling of Receptor Ligands and Other Compounds with Halogen Radionuclides

Comprehensive report.

A. Major research accomplishments.

Three years ago in our continued application, the research goals were:

1. To continue our studies on the usefulness of fluorine-18 labeled 16α-fluoroestradiol-17β (^{18}FES) for detecting estrogen receptor containing tumors and metastases and to attempt to correlate the uptake of the radiolabeled estrogen with the receptor levels.
2. To attempt to develop new labeling techniques for synthesizing halogen labeled radiopharmaceuticals in high yield. Of particular importance is the synthesis of fluorine-18 labeled estrogens designed for reduced or directed metabolism.
3. To test the new fluorine-18-labeled estrogens using an in vivo rat model.
4. To attempt to label estrogens with iodine-123 for single photon studies.
5. To investigate the synthesis and biologically evaluation of fluorine-18-labeled ligands for the androgen and progesterone receptors.
6. To apply robotics for synthesizing potentially useful compounds.

In the last application, increased budget was requested in order to carry out aims 1 and 4. Since the budget was funded at a level that provided no increase, a NIH grant has been written and funded to carry out the clinically evaluation of the compounds developed under this grant. We have also been unable to fund area 4. The NIH grant funded April 1, 1989, supports a physician to carry out clinical studies as well as providing PET time for the extended clinical studies.

Major advances have been made in all the areas. Specifically in area 1, patient studies have been carried out (see publications 7,16,24,28). This work has shown that the uptake of fluorine-18 labeled 16α-fluoroestradiol-17β correlates well with receptor levels measured in vivo and also that the uptake of the tracer is blocked in humans by the administration of the antiestrogen tamoxifen. An image from this work (presented in publication 15) was designated Image of the Year by Dr. Wagner, Jr., following his summary of the 1987 Society of Nuclear Medicine Meeting. We have also evaluated the brain uptake of both estrogen and progesterone, (publication 19) and this work was awarded the Berson-Yalow Award from the Society of Nuclear Medicine in 1988. This publication represents a new application of radiolabeled sex hormones. Hines and coworkers (M. Hines, Psycol. Bulletin 92:56, 1982) have suggested that hormone levels in the brain are important for sexual differentiation of human behavior. We have shown that both 16α-[F-18]-fluoroestradiol-17β and 21-[F-18]-fluoro-16α-ethyl-19-norprogesterone (FENP) accumulate in the hypothalamus and pituitary tissues of primates and humans; and in primates this uptake can be blocked by administration of nonradioactive competing ligands. This presents an opportunity for studying sex hormone receptors in mammalian brain.

In research area 2, we have developed new labeling techniques, both for fluorination and (publications 1,3,17,23) and for radiolabeling with other halogens (publications 8,10,11,20,21). The labeled estrogens have been

evaluated in an in vivo model to compare their possible clinical potential with the 16α-[F-18]-fluoroestradiol-17β.

In area 5, we have investigated new ligands for the progesterone, androgen and glucocorticoid receptor systems (publications 6,12,22,25,27).

We have continued our work in the application of robotics to radiopharmaceutical production. A laboratory robot now prepares four cyclotron produced radiopharmaceuticals and is involved in the preparation of the majority of fluorine-18-labeled radiopharmaceuticals prepared at Washington University (publications 4,5).

B. Our plans for the continuation of this work are to proceed as discussed above. In particular, we will attempt to prepare a new series of labeled estrogens with very high in vitro binding affinities. Work carried out in the previous year suggests that simply evaluating uterus to blood ratios in rats is not sufficient to carefully evalute new agents and so a more complete evaluation will be carried out. We will also attempt to prepare labeled androgen and glucocorticoid receptor ligands with higher binding affinities than the ligands studied to date. In the evaluation of these new types of ligands, dosimetry and toxicity studies will be carried out in order to prepare the agents for human use.

C. Over the past funding period, Henry vanBrocklin has been supported on this project and should obtain his Ph.D. degree in early 1990. Dr. James Brodack has been supported on this project, he is still at Washington University holding the rank of Research Assistant Professor.

D. Publications.

1. DY Chi, MR Kilbourn, JA Katzenellenbogen, MJ Welch. A rapid and efficient method for the fluoroalkylation of amines and amides. Development of a method suitable for incorporation of the short-lived positron emitting radionuclide fluorine-18. J Org Chem 52:658-664, 1987.
2. CJ Mathias, MJ Welch, JA Katzenellenbogen, JW Brodack, MR Kilbourn, KE Carlson, DO Kiesewetter. Characterization of the uptake of 16α-([^{18}F]-fluoro)-17β-estradiol in DMBA-induced mammary tumors. Nucl Med Biol 14:15-25, 1987.
3. MJ Welch, JA Katzenellenbogen, CJ Mathias, JW Brodack, KE Carlson, DY Chi, CS Dence, MR Kilbourn, JS Perlmutter, ME Raichle, MM Ter-Pogossian. N-(3-[^{18}F]fluoropropyl)-spiperone: the preferred ^{18}F labeled spiperone analog for positron emission tomographic studies of the dopamine receptor. Nucl Med Biol 15:83-97, 1988.
4. JW Brodack, MR Kilbourn, MJ Welch. Automated production of several positron-emitting radiopharmaceuticals using a single laboratory robot. Appl Radiat Isot 39:689-698, 1988.
5. JW Brodack, CS Dence, MR Kilbourn, MJ Welch. Robotic production of 2-deoxy-2-[^{18}F]fluoro-D-glucose: a routine method of synthesis using tetrabutylammonium [^{18}F]fluoride. Appl Radiat Isot 39:699-703, 1988.
6. MG Pomper, JA Katzenellenbogen, MJ Welch, JW Brodack, CJ Mathias. 21-[^{18}F]fluoro-16α-ethyl-19-norprogesterone: synthesis and target tissue selective uptake of a progestin receptor based radiotracer for positron

emission tomography. J Med Chem, 31:1360-1363, 1988.
7. MA Mintun, MJ Welch, BA Siegel, CJ Mathias, JW Brodack, AH McGuire, JA Katzenellenbogen. Breast Cancer: PET imaging of estrogen receptors[1]. Radiology 169:45-48, 1988.
8. SM Moerlein, D-R Hwang, MJ Welch. No-carrier-added radiobromination via cuprous chloride-assisted nucleophilic aromatic bromodeiodination. Appl Radiat Isot 39:369-372, 1988.
9. MJ Welch, MR Kilbourn. Potential labeling of monoclonal antibodies with positron emitters. In Radiolabeled Monoclonal Antibodies for Imaging and Therapy, S.C. Srivastava, ed., Plenum Press, New York, 1988, pp 261.
10. SM Moerlein, D Parkinson, MJ Welch. Radiosynthesis of high effective specific-activity [^{123}I]SCH 23982 for dopamine D-1 receptor-based SPECT imaging. Appl Radiat Isot (in press).
11. SM Moerlein, GS Lannoye, MJ Welch. No-carrier-added radiosynthesis of [^{123}I]HIPDM: N,N,N'-Trimethyl-N'(2-hydroxy-3-methyl-5-[^{123}I]iodobenzyl)-1,3-propanediamine. Appl Radiat Isot, in press.
12. MC Pomper, KC Pinney, KE Carlson, CJ Mathias, HF vanBrocklin, MJ Welch, JA Katzenellenbogen. Target tissue uptake selectivity of three fluorine-substituted progestins: potential imaging agents for receptor-positive breast tumors. Nucl Med and Biol, in press.
13. MC Pomper, HF vanBrocklin, AM Thieme, RD Thomas, DO Kiesewetter, KE Carlson, CJ Mathias, MJ Welch, JA Katzenellenbogen. 11β-Methoxy-,11β-ethyl, and 17α-ethynyl-substituted 16α-fluoroestradiols: receptor based imaging agents with enchanced uptake efficiency and selectivity. J Med Chem, submitted.
14. PL Chesis, LK Griffeth, CJ Mathias, MJ Welch. Sex-dependent differences in N-(3-[^{18}F]fluoropropyl)-N-nordiprenorphine biodistribution and metabolism. J Nucl Med, in press.
15. HF VanBrocklin, JW Brodack, CJ Mathias, MJ Welch, JA Katzenellenbogen, JF Keenan, GJ Mizejewski. Affect on blood uptake by the serum binding of 16α-([^{18}F]-fluoro)-17β-estradiol to alphafetoprotein in sprague-dawley female rats: dependence on age and weight. (in preparation).

Abstracts

16. MA Mintun, MJ Welch, CJ Mathias, JA Brodack, BA Siegel, JA Katzenellenbogen. Application of 16α-[F-18]-fluoro-17β-estradiol (I) for the assessment of estrogen receptors in human breast carcinoma. J Nucl Med 28:561, 1987.
17. MC Pomper, JA Katzenellenbogen, RD Thomas, CJ Mathias, HF vanBrocklin, MJ Welch. Fluorine-18 labeled 11β-substituted estrogens: synthesis, receptor binding, and comparative target tissue uptake studies. J Lab Cmpd Radiopharm, 26:323-325, 1989.
18. MJ Welch, CJ Mathias, SM Moerlein, JM Connett, GW Philpott. Tyramine-cellobiose (TC) labeling of antibodies: a comparison with other labeling techniques. J Lab Cmpd Radiopharm, 26: 269-270, 1989.
19. MJ Welch, JS Perlmutter, AH McGuire, CJ Mathias, JW Brodack, MA Mintun, JA Katzenellenbogen. Uptake of fluorine-18-labeled sex hormones in mammalian brain; measured with F-18 ligands and PET. J Nucl Med 29: 795-796, 1988.
20. SM Moerlein, GS Lannoye, D-R Hwang, MJ Welch. No-carrier-added synthesis

of Br-77 bromospiperone via microwave-treated, CuCl-assisted nucleophilic bromodeiodination. J Nucl Med 29:776-777, 1988.
21. MJ Welch, CJ Mathias, SM Moerlein, JM Connett, GW Philpott. Radioiodinated tyramine-cellobiose (TC) labeling of monoclonal antibodies (MAbs): comparison with lactoperoxidase (LP) labeling. J Nucl Med 29:835, 1988.
22. MG Pomper, MJ Kochanny, AM Thieme, KE Carlson, CJ Mathias, HF vanBrocklin, MJ Welch, JA Katzenellenbogen. Fluorine-18 labeled corticosteroids for imaging hippocampal receptors. AHAF Conference on Alzheimer's Disease, Tucson AZ, Feb, 1989.
23. HF vanBrocklin, MG Pomper, CJ Mathias, JA Katzenellenbogen, MJ Welch. 17α-ethynyl-11β-substituted, fluorine-18 labeled estrogens improved PET imaging agent. J Nucl Med 30:753, 1989.
24. AH McGuire, AP Lyss, JW Brodack, MA Mintun, CJ Mathias, BA Siegel, JA Katzenellenboen, MJ Welch. Positron tomographic assessment of 16-α-[F-18]-fluoroestradiol (FES) uptake in metastatic breast carcinoma: effect of antiestrogen therapy. J Nucl Med 30:788, 1989.
25. MG Pomper, MJ Kochanny, AM Thieme, KE Carlson, HF vanBrocklin, CJ Mathias, MJ Welch, JA Katzenellenbogen. Imaging agents for brain corticosteroid receptors: synthesis and tissue distribution of fluorine-18 substituted corticosteroids. J Nucl Med 30:821-822, 1989.
26. MJ Welch, CJ Mathias, JW Brodack, HF vanBrocklin, JA Katzenellenbogen. Tamoxifen therapy and in vivo determination of estrogen receptor concentration with ^{18}F-16α-fluoroestradiol-17β (FES). J Nucl Med 30:910, 1989.
27. MG Pomper, KG Pinney, KE Carlson, HF vanBrocklin, MJ Welch, JA Katzenellenbogen. Uptake selectivity of fluorine-substituted progestins: analogs of R5020 and a novel retroprogestin. J Nucl Med 30:928, 1989.
28. MJ Welch, AH McGuire, D-R Hwang, JA Katzenellenbogen. Clinical prospects of new PET radiopharmaceuticals. To be presented at the 5th European Conference on Clinical Oncology.
All these publications are included in the progress report as appendixes.

E. The major applications of positron tomography to date have been in the areas of brain and cardiac imaging. The application of PET to oncology, could give important information about the status of tumors. The ability to determine the receptor status of tumors without surgery, should enable the oncologist to accurately determine the ideal course of therapy. Antiestrogen therapy is only effective if the tumor being treated has a high level of estrogen and/or progestin receptors. In a significant number of cases, if a primary tumor is receptor positive, metastases will be receptor negative. We have demonstrated uptake of 16α-(F-18)-fluoroestradiol-17β in metastases prior to antiestrogen therapy and, subsequently, quantitate the blockage of the estradiol uptake after the patient has begun antiestrogen therapy. This could be helpful diagnostically, since at this time patients must wait until demonstratable evidence of decreasing lesions is noted on x-rays; we hopefully will be able to assess the effectiveness of antiestrogen therapy in a much shorter time frame. By following the patients studied to date and accumulating more cases we will be able to correlate the clinical outcome after antiestrogen therapy with the original tracer uptake and the degree of uptake blockage after antiestrogen therapy. Although 16α-(F-18)-fluoro-

estradiol-17β gives good images, there is still a significant amount of nonspecific uptake, particularly observed in the posterior chest wall of patient images. Also the liver uptake of this tracer is such that the imaging of liver metastases is impossible; additionally the radiation dose to the liver and intestines limits the amount of material that can be administered to patients. By continuing to develop improved agents, we hope to be able to overcome these shortcomings of our current agent.

PROGRESS REPORT

Over the past funding period, progress has been made in five general areas. These areas are:

1. The development of new labeling techniques for use with halogen radionuclides.
2. The labeling of new receptor ligands, particularly ligands specific for the estrogen, progestin, androgen, and glucocorticoid receptor systems.
3. The application of robotic techniques for the preparation of positron emitting radiopharmaceuticals to reduce the radiation dose to the chemist.
4. Evaluation in animal models of receptor ligands in order to predict the preferred ligand for future studies.
5. The evaluation of receptor ligands in groups of patients.

The work in these areas is described in detail in the appendixes to this progress report. The development of new labeling technique has led to a general method of fluoroalkylation (Appendix 1) which has been specifically applied to the production of fluoroalkyl spiperone derivatives (Appendix 3). More recently we and others have used this technique to prepare a series of ligands for dopamine (1,2) and opiate receptors (Appendix 14).

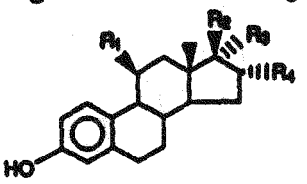
In the area of new receptor ligands, we have evaluated a series of ligands with substituents at the 11β and 17α position of fluoro-estradiol in a rat model (Appendices 13,17,23). The binding affinities of these ligands are shown in Figure 1 and the method of synthesis in Figure 2. We have radiolabeled 16α-fluoro-11β-ethylestradiol, 16α-fluoro-11β-methoxyestradiol, 16α-fluoro-17α-ethynylestradiol, 16α-fluoro-17α-ethynyl-11β-ethylestradiol, and 16α-fluoro-17α-ethynyl-11β-methoxyestradiol. The precursor for the 11β-methoxy and 11β-ethyl derivatives were prepared as described Appendix 13. Although all of these compounds can be synthesized as shown in Figure 2, the relative amounts of alpha and beta product vary depending upon the substituent. The ratios of 17β-hydroxy to 17α-hydroxy diastereoisomers are shown in Table 1 (last two compounds are derivatives of FEES). It is seen that when preparing the ethynyl derivative of 11β-ethylestradiol, the major product is in fact the alpha diastereoisomer. It is not at first obvious why substituents at the 11β position will effect the stereochemistry at the 17 position, but space filling models of estradiol show that the 11β substituents, in fact, alter the relative confirmation of the molecule.

Table 1

Ratio of 17β-OH to 17α-OH Diastereomers

Compound	β/α
FES	3.5
11βOMe	2
11βEt	0.7
FEES	1
11βOMe	1-1.2
11βEt	0.1-0.2

Relative Binding Affinities of Estrogen Ligands



Ligand	R_1	R_2	R_3	R_4	RBA[1]	BSI
Estradiol (ES)	H	OH	H	H	100	100
16αF ES	H	OH	H	F	60	86
16αF-11βEthyl ES	CH_2CH_3	OH	H	F	891	482
16αF-11βMethoxy ES	OCH_3	OH	H	F	28	128
16αF-17α Ethynyl ES	H	OH	C≡CH	F	61	66
16αF-17β Ethynyl ES	H	C≡CH	OH	F	5	5.4
16αF-17α Ethynyl-11βEthyl ES	CH_2CH_3	OH	C≡CH	F	--	--
16αF-17β Ethynyl-11βEthyl ES	CH_2CH_3	C≡CH	OH	F	159	84
16αF-17α Ethynyl-11βMethoxy ES	OCH_3	OH	C≡CH	F	--	--
16αF-17β Ethynyl-11βMethoxy ES	OCH_3	C≡CH	OH	F	--	--

[1] 25°C

Figure 1

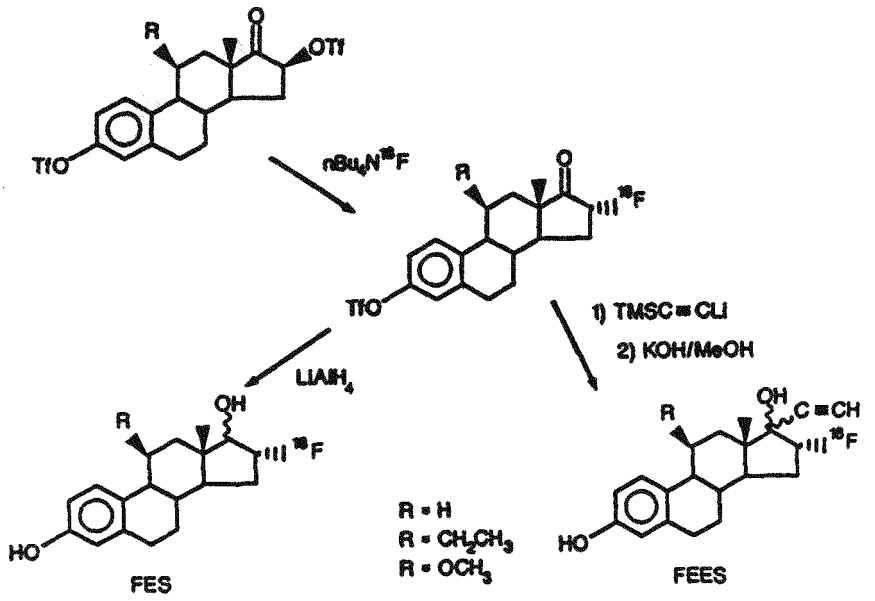


Figure 2: Schemes for the synthesis of the 11β substituted and 17α-ethynl labeled estradiols. The starting materials for the 11β derivatives were prepared as discussed in Appendix 13.

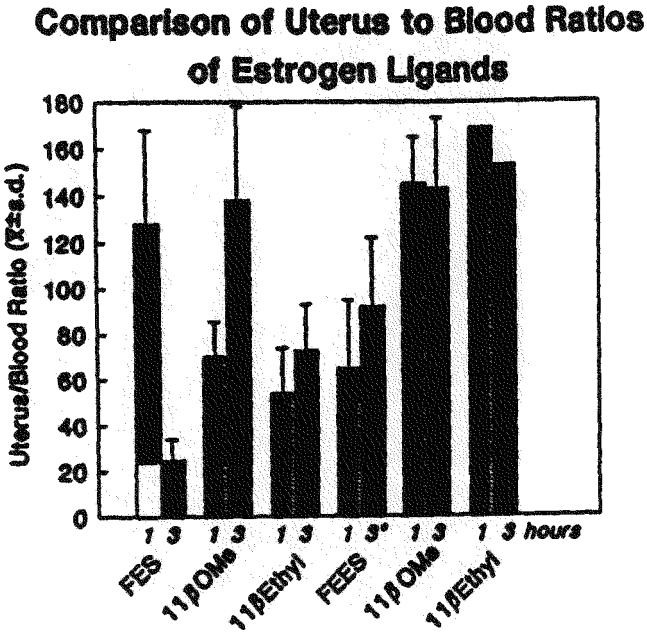


Figure 3

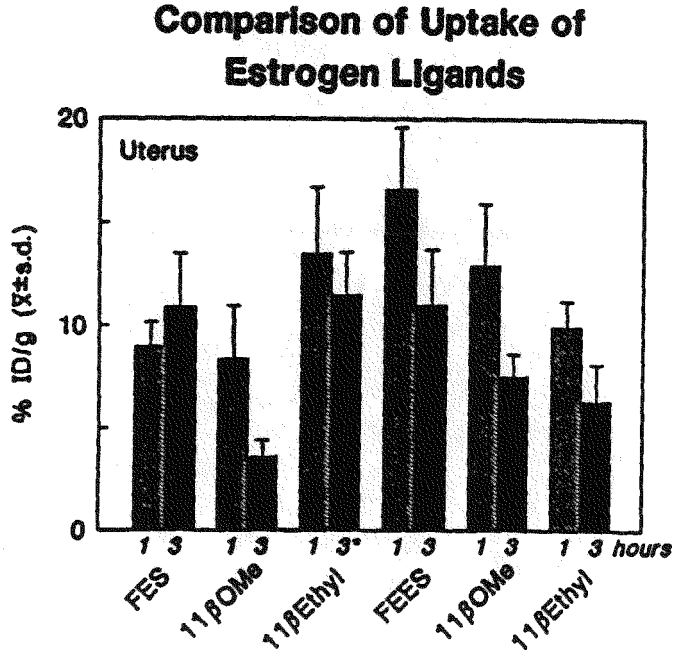


Figure 4

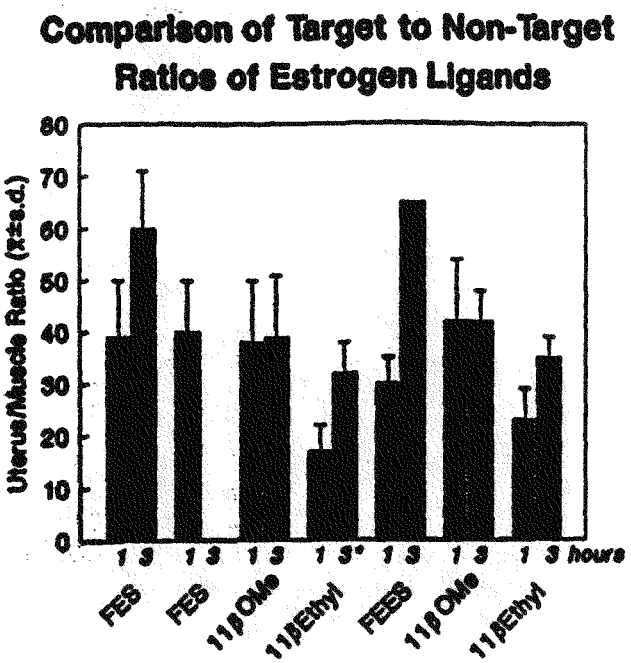


Figure 5

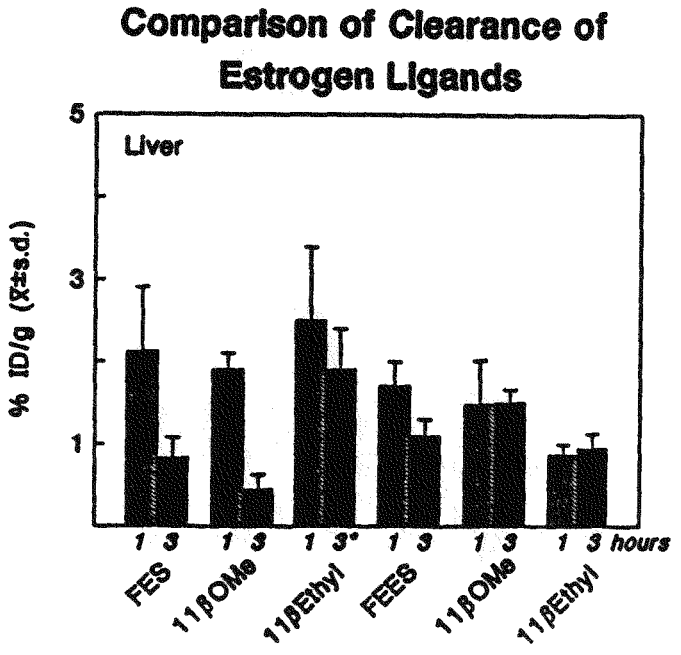


Figure 6

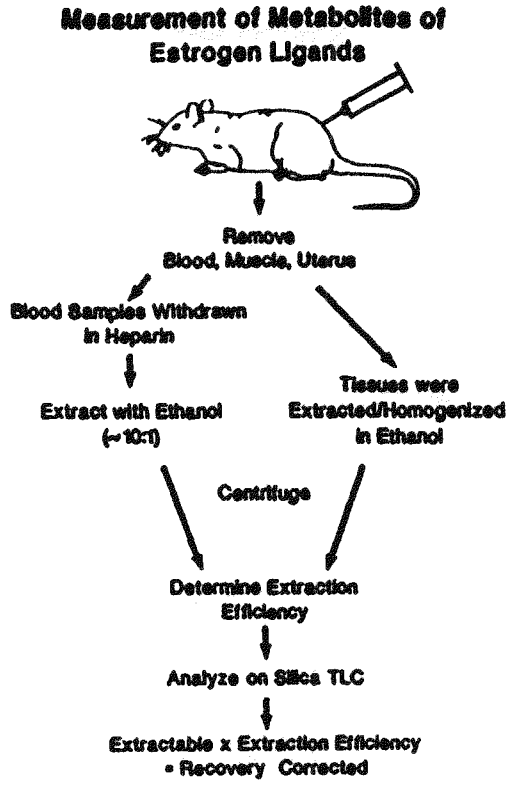


Figure 7

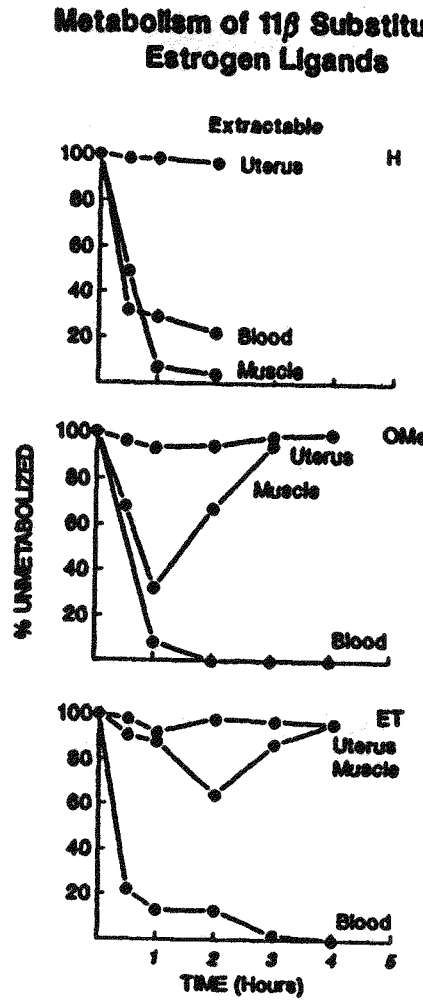


Figure 8

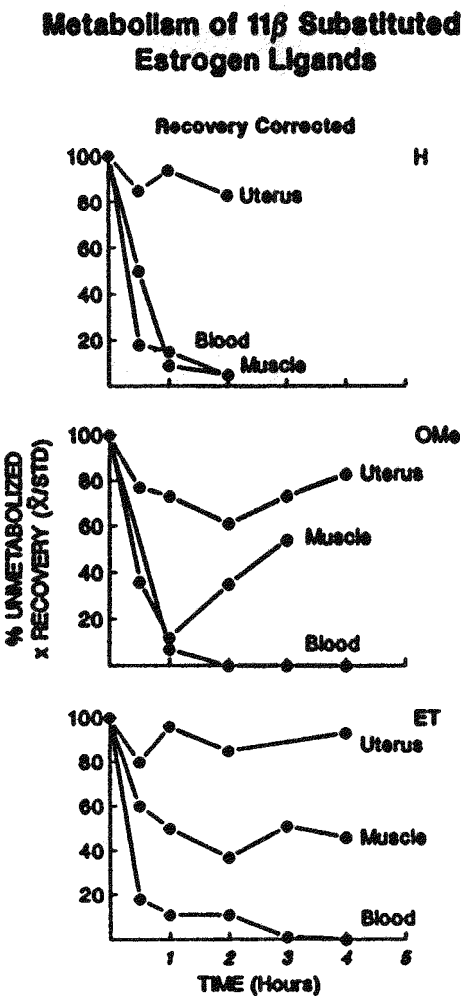


Figure 9

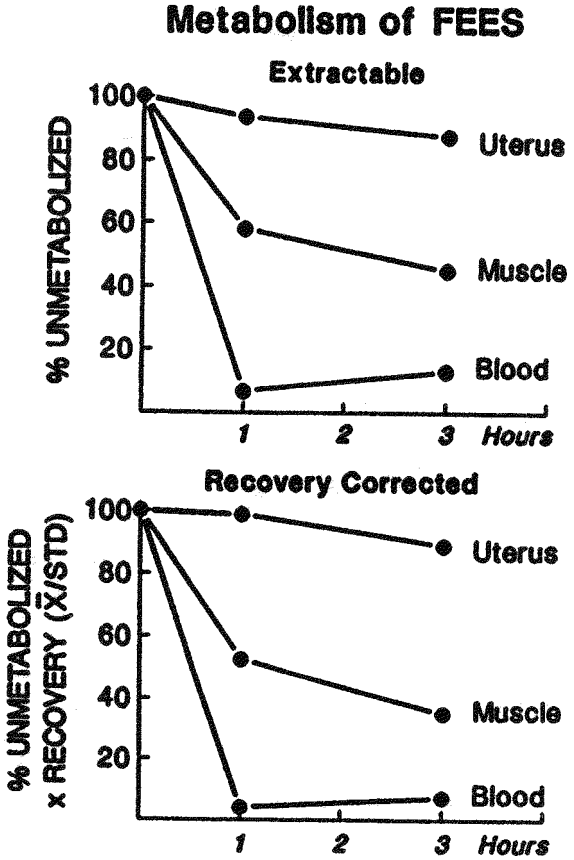


Figure 10

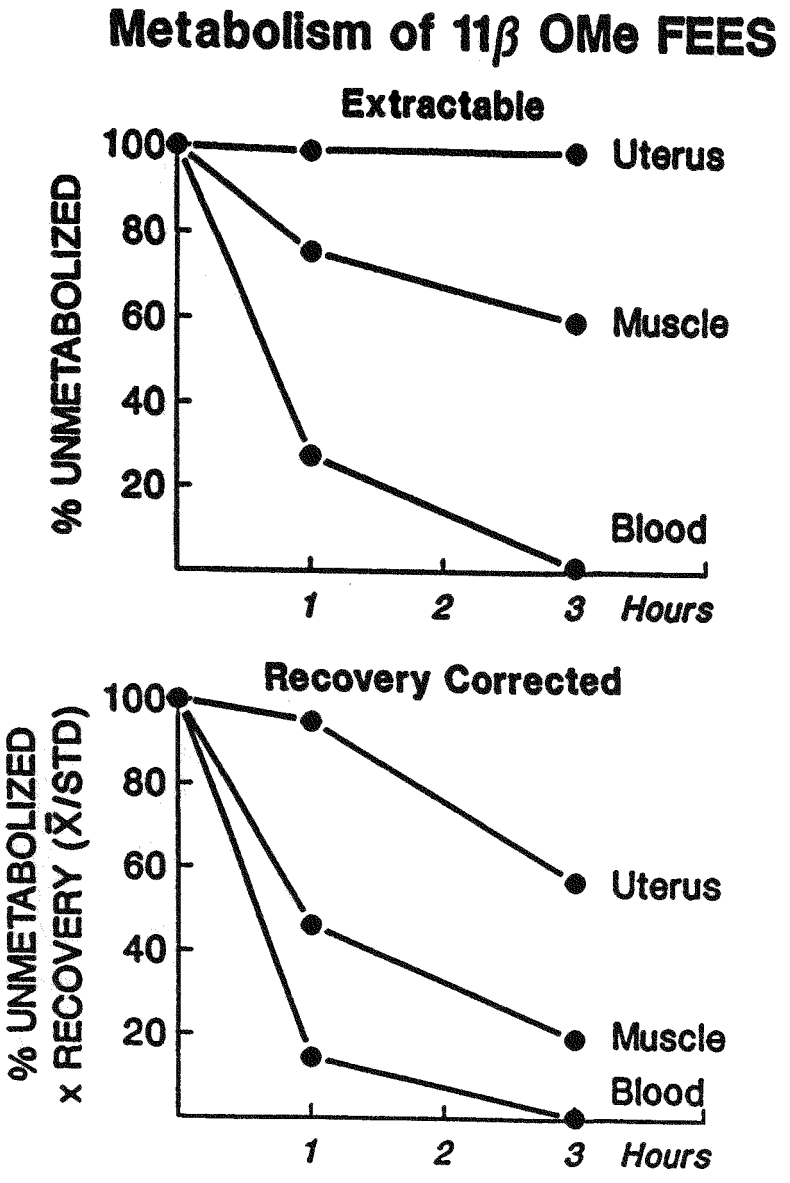

Metabolism of 11β OMe FEES
Extractable
% UNMETABOLIZED
100
80
60
40
20
Uterus
Muscle
Blood
1
2
3
Hours
Recovery Corrected
% UNMETABOLIZED
x RECOVERY (X̄/STD)
100
80
60
40
20
Uterus
Muscle
Blood
1
2
3
Hours

Figure 11

We have compared the behaviour of these ligands in several animal models. Initially we evaluated the absolute target uptake in the uterus, as well as, the uterus/blood and uterus/muscle ratio, this data is given in Figures 3-5. (In these figures the last two compounds on the right are the 11β-OME and 11β-ethyl derivatives of 16α-[F-18]-fluoro-17α-ethynylestradiol-17β). Since radiation dose to clearance organs is important, the levels of activity in the liver at 1h and 3h is shown in Figure 6. Adding substituents at the 11β position and the 17α position should reduce the metabolites; we have, therefore, measured the metabolites of these estrogen ligands by the technique shown in Figure 7. The data for metabolism of these compounds is shown in Figures 8-11. It is not easy to select the ideal compound for human studies, depending upon which characteristic one optimizes, any five of the compounds studied could be regarded as the best compound (Table 2).

Table 2

Desirable Characteristics for Optimal PET Imaging of Human Estrogen Target Sites

1) High Absolute Uptake (FEES)

2) High Target to Non Target Ratios (FES, FEES)

3) High Uterus to Blood Ratios (11βEt-FEES, 11βOMe-FEES)

4) Reduced Metabolism (11βEt-FES)

5) Rapid Clearance from Critical Organs (11βEt-FEES)

It is also interesting to note that the 17α-ethynyl-11β-ethyl compound cannot be prepared with high radiochemical yield. We have synthesized the alpha and beta derivatives of this compound and compared the biodistribution of the two isomers (Table 3). It is seen that the 17β-ethynyl-17α-hydroxy compound has significant uptake in the target tissue.

Table 3

Biodistribution of 11-β-Ethyl-17-α & β-Ethynyl-^{18}F-16-α-Fluoro-Estra-3,17-β & α-diol in 28 day old Sprague-Dawley Female Rats

% ID / gram

	17-β-Ethynyl, 17-α-OH n=4		17-α-Ethynyl, 17-β-OH n=4	
	1 hr	3 hr	1 hr	3 hr
Blood	0.2041 ± 0.0405	0.1225 ± 0.059	0.06 ± 0.0089	0.0409 ± 0.0071
Liver	2.2949 ± 0.366	1.2293 ± 0.3544	0.8779 ± 0.1391	0.9488 ± 0.1791
Muscle	0.5231 ± 0.0742	0.289 ± 0.1685	0.4367 ± 0.0615	0.1794 ± 0.0501
Bone	0.6165 ± 0.0768	0.4732 ± 0.0465	0.2992 ± 0.0478	0.1694 ± 0.0454
Uterus	8.3441 ± 0.6741	4.9332 ± 0.9681	9.8598 ± 1.2728	6.3047 ± 1.8385
Ovaries	2.2743 ± 0.4864	1.7426 ± 0.805	3.2645 ± 0.3046	2.4069 ± 0.8581
Ut/Blood	41.62 ± 5.23	44.25 ± 14.1	169.17 ± 45.87	153.05 ± 29.66
Ut/Musc	15.15 ± 2.16	19.54 ± 6.8	23.11 ± 6.18	35.14 ± 3.65

One of the major goals in our renewal application are to compare and contrast new agents in an animal model. In Figures 3 and 4, two sets of data are give for fluoroestradiol. In one set of data the compound was injected into immature female Sprague Dawley rats that were 21 days old and 26 days old in the other. Significant differences were obtained with these different age rats, presumably due to the concentration of alphafetoprotein present in the blood of younger rats. Alphafetoprotein binds only 16α-fluoroestradiol-17β of all of the derivatives studied. We have investigated the effect of the serum binding on the blood uptake, a preliminary draft of a manuscript is shown in Appendix 15. It is important to measure the alphafetoprotein level of the rats when evaluating new estrogen ligands and in the future will be routinely carried out.

We have extended our animal studies with 16α-fluoroestradiol-17β in order to determine the estrogen receptor capacity and affinity in the target and non-target tissue. This type of analysis may be needed in order to carefully compare the 11β and 17α substituted estradiols as discussed above. Since one of the major uses of radiolabeled estrogens will be to study the effects of tamoxifen; we have investigated the effects of tamoxifen upon the number of available receptor sites in the immature rat uterus. In this study increasing amounts of estradiol were added to the fluoroestradiol in order to estimate the binding sites responsible for target tissue uptake. This study was performed in untreated immature female rats and in two groups of animals that have been pretreated with either an intermediate dose or a high dose of tamoxifen. The effect of these doses of tamoxifen upon the biodistribution of no-carrier-added ^{18}FES are shown in Table 4.

Table 4

Effect of Tamoxifen Treatment on Target Uptake of ^{18}FES in Imature Rats (%ID/g)

	Control	Tamoxifen 0.57mg/kg*	Tamoxifen 1.7 mg/kg*
Blood	0.113 ± 0.046	0.123 ± 0.069	0.077 ± 0.034
Muscle	0.162 ± 0.042	0.088 ± 0.029	0.058 ± 0.010
Uterus	4.63 ± 0.574	1.66 ± 0.662	1.34 ± 0.37
Ovaries	1.23 ± 0.213	0.726 ± 0.32	0.722 ± 0.188
Ut/Blood	48.92 ± 24.94	17.65 ± 10.64	20.21 ± 9.37
Ut/Muscle	29.35 ± 4.54	18.80 ± 3.27	23.07 ± 3.57

* 2 days pretreatment

Tamoxifen not only depresses uptake in target organs but also in non-target (muscle). Target to non-target ratio is still high after tamoxifen treatment. Very large (~7mg/kg) doses of tamoxifen were required to depress uptake (target to non-target ratios ~3).

When the fluoroestradiol is diluted with estradiol the uptake in various organs behaves as shown in Figures 12 and 13. It is seen that at approximately 0.5ug ES added per animal the specific receptor binding is blocked. The extent to which estradiol displaces the F-18-fluoroestradiol uptake in the target can be better appreciated from the uptake plots (Figures 14-15). The uptake is plotted relative to the dose of fluoroestradiol and estradiol (administered in picamoles). Alternatively the uptake dose ratio is plotted relative to the uptake to give plots reminiscent to the Scatchard plot for equilibrium binding, these pseudo Scatchard plots are shown in Figures 16-18. In these figures "A" are the data for untreated animals, "B" for animals treated with an intermediate dose (.57 mg/kg) of tamoxifen and "C" for animals treated with a high dose (1.7 mg/kg) of tamoxifen. From these data uptake, capacities are determined (Table 5).

Table 5: Uptake capacity for ^{18}FES in tissues as determined by pseudo Scatchard plots.

	B_{max} (pmol/g)		
	Uterus	**Ovaries**	**Muscle**
Untreated	160	55	4
Intermediate Dose Tamoxifen	60	35	-0
High Dose Tamoxifen	~ 0	0-15	-0

It is seen that specific binding sites exist in the muscle that appear to be blocked even with low doses of tamoxifen, and that the available sites in the uterus are reduced by tamoxifen administration. In order to measure the K_D in vivo, one needs to make some assumption regarding nonspecific binding. In the tamoxifen treated animals, no specific binding occurs in muscle so therefore the muscle uptake is a measure of nonspecific binding. Making this assumption and accounting for metabolites in the muscle, a Scatchard plot can be generated (Figure 19) from which K_D can be calculated. In the example shown which is with the intermediate dose of tamoxifen B_{max} = 80 picamoles/-gram and K_D = 0.38 nmoles. This latter value is in good agreement with in vitro data (reference 3).

We have also evaluated several radiolabeled progestins in animal models (Appendices 6,12,27) and have carried out preclinical studies on FENP. FENP has recently been approved for human use and its distribution in one normal determined Figures 20-21. In this normal subject, the urine was analyzed for fluoride; very low levels were determined (as can be seen from the images,

Figure 12 Effect of Added Estradiol (ES) on Tissue Uptake of ^{18}FES in Untreated Rats

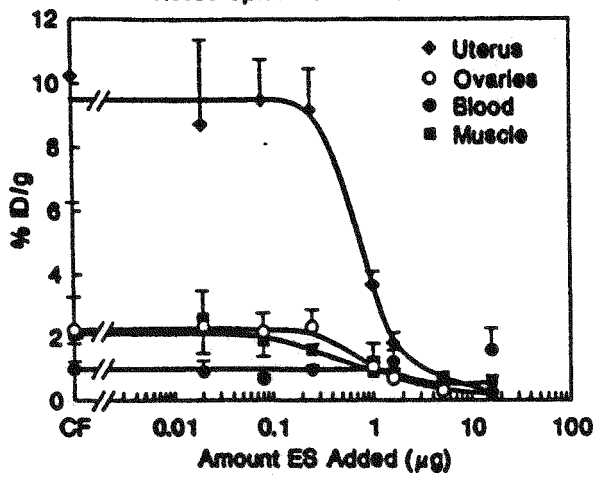
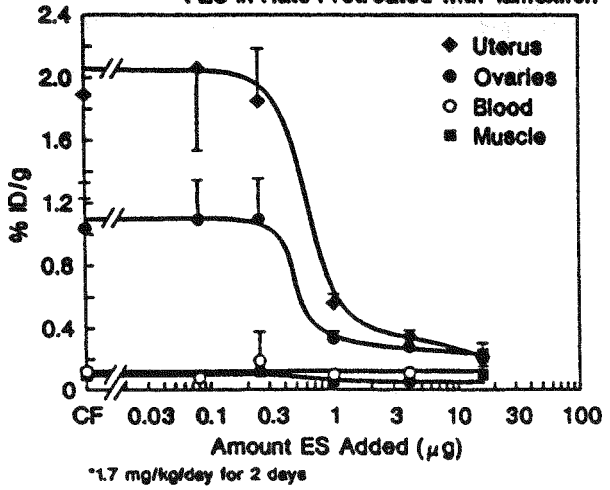


Figure 13 Effect of Added ES on Tissue Uptake of ^{18}FES in Rats Pretreated with Tamoxifen*



*1.7 mg/kg/day for 2 days

Figure 14. Uptake of ^{18}FES in 25d Old Female R

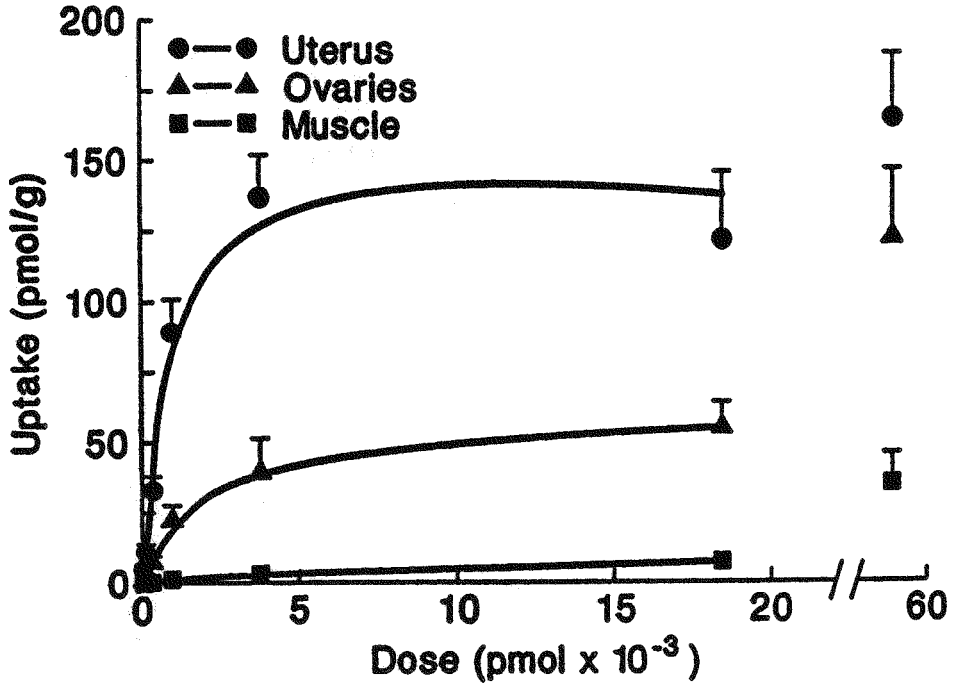
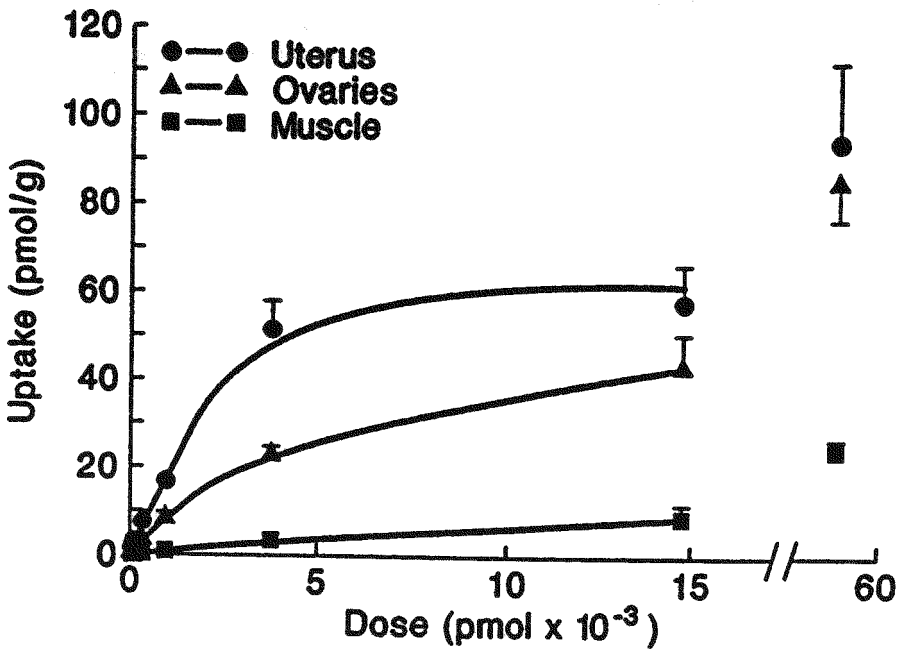
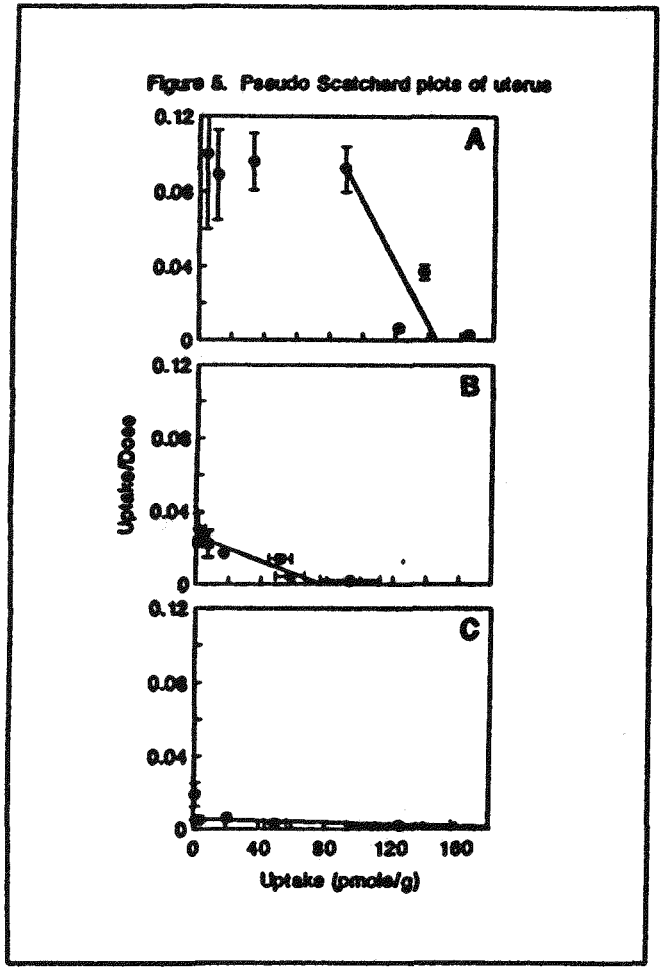
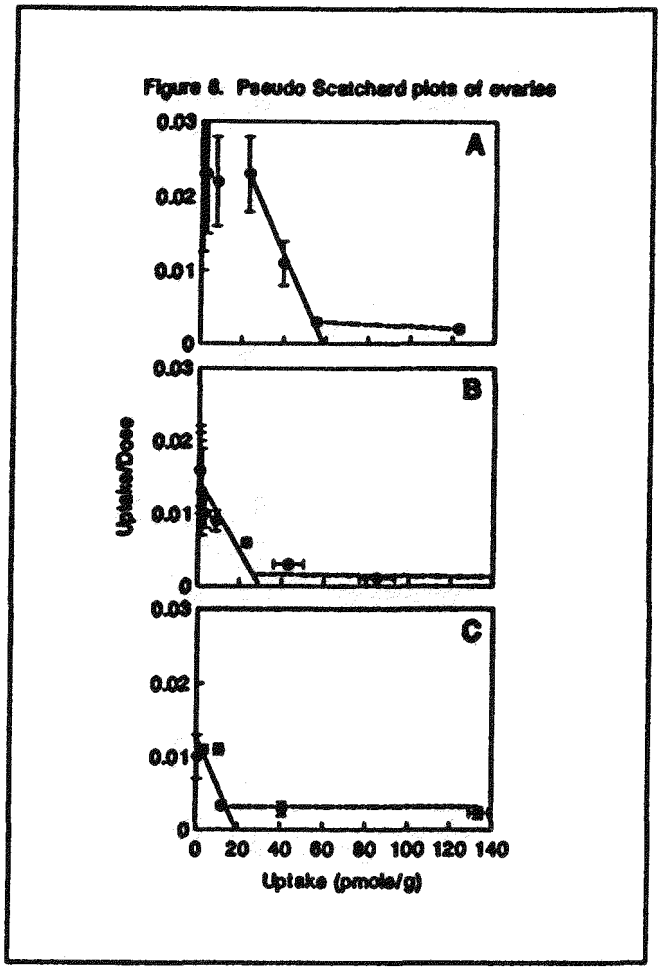


Figure 15. Uptake of ^{18}FES in 25d Old Female Rats Pretreated with Intermediate Dose of Tamoxifen







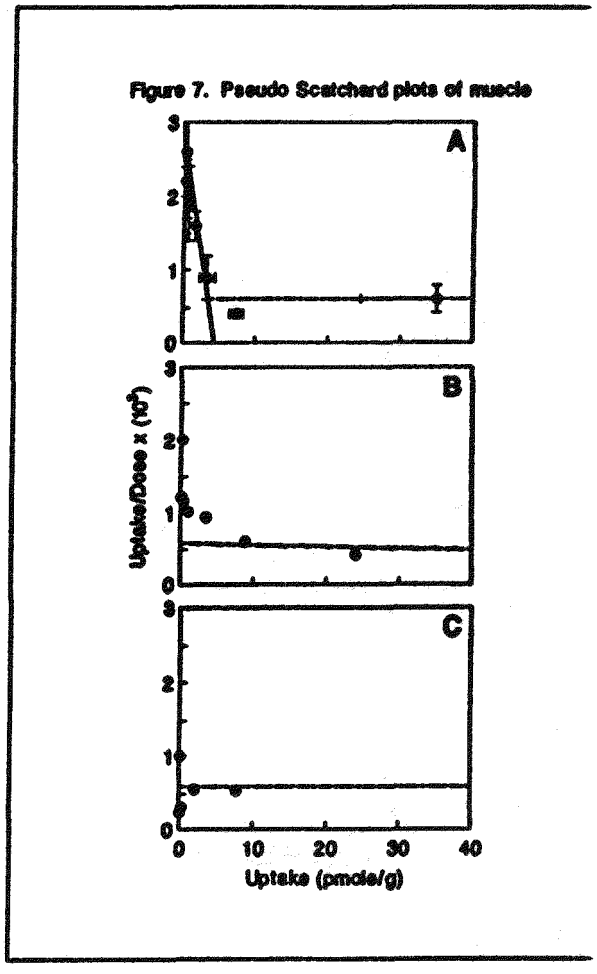
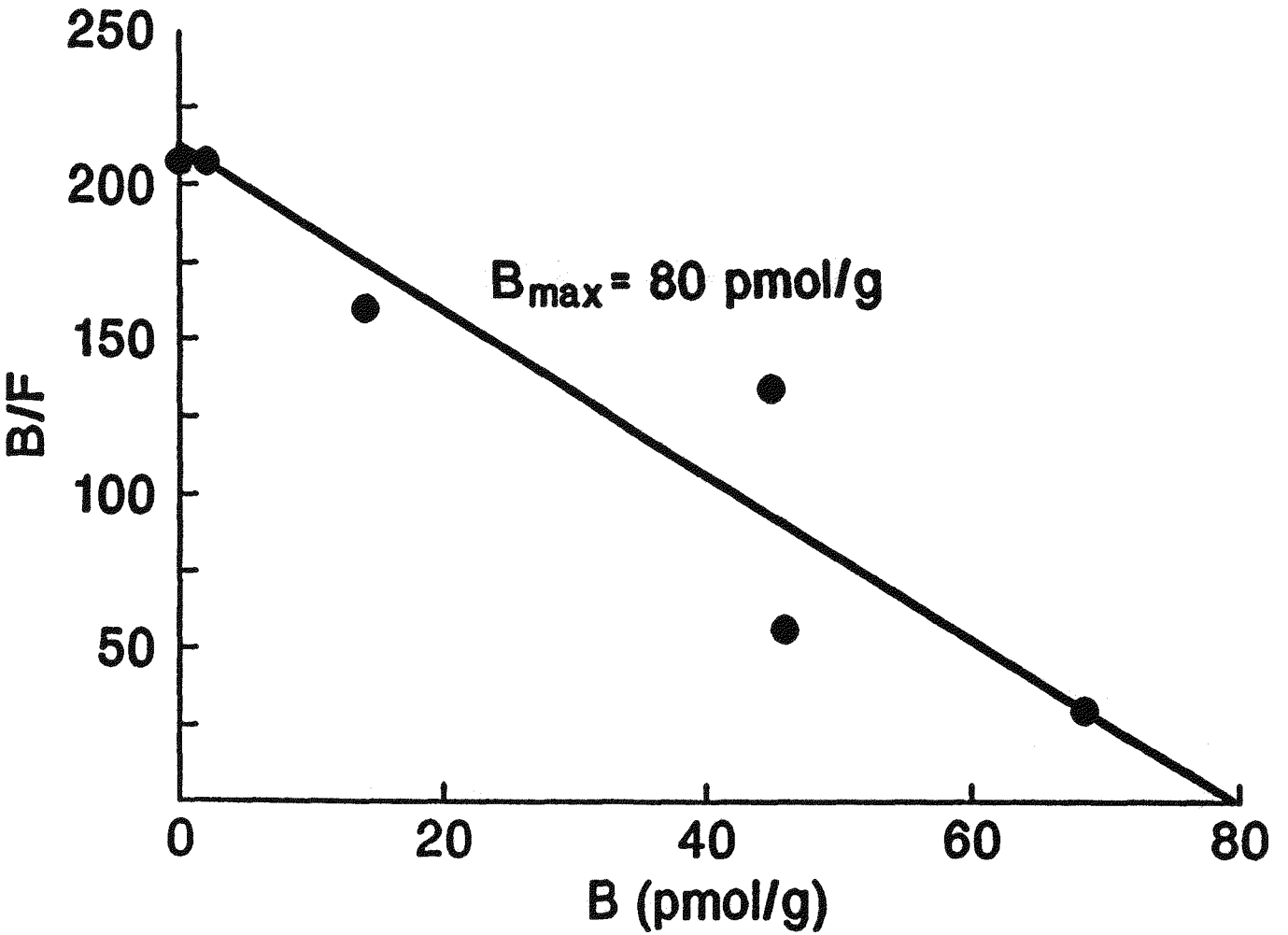


Figure 16

Figure 17

Figure 18

Figure 19. **Scatchard plot of bound/free vs bound for uterus uptake pretreated with intermediate dose of tamoxifen**



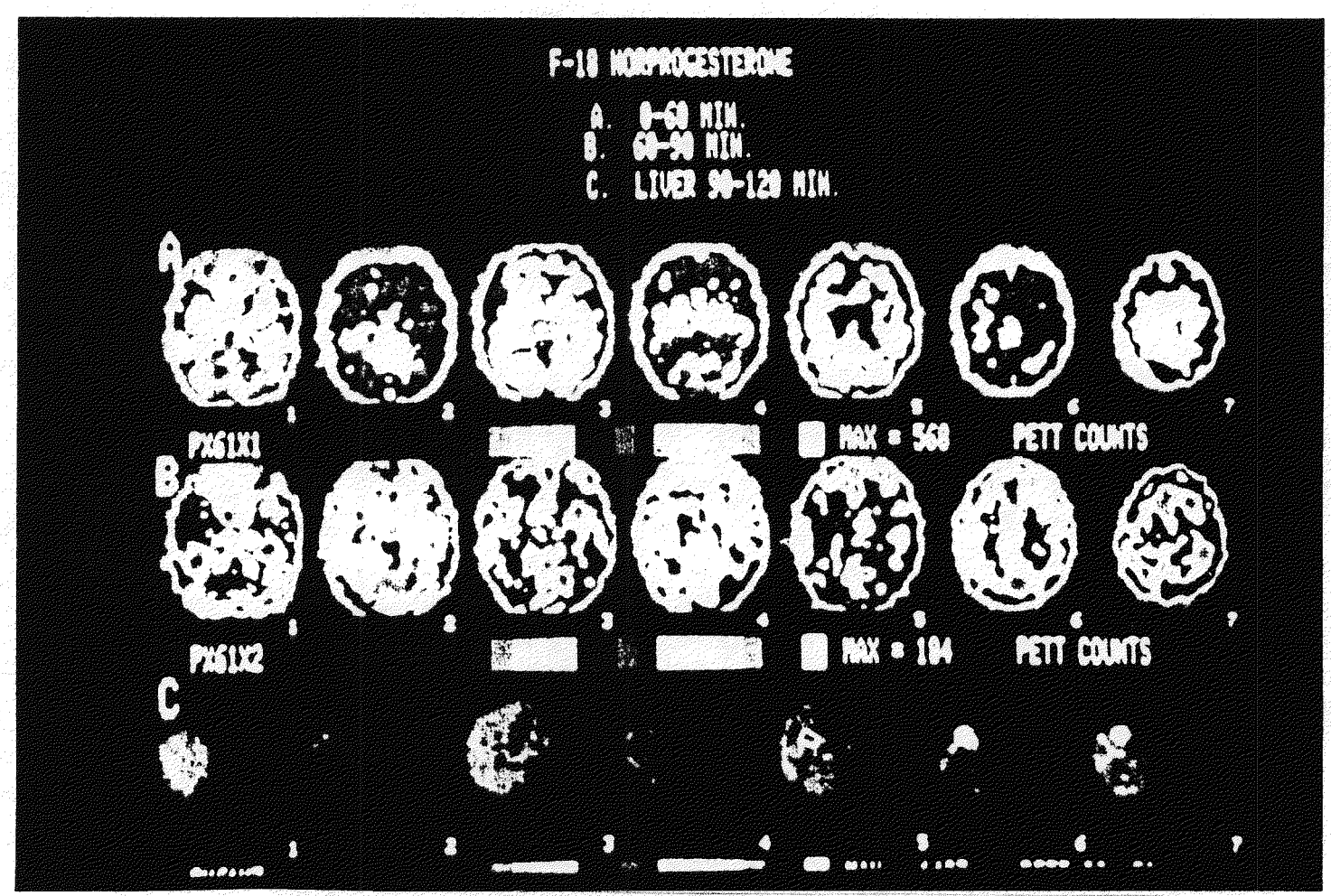


Figure 20: The biodistribution of FENP in a normal human subject. Slices A and B show the distribution of activity in the brain. Note the small amount of skull uptake. Slices C show the clearance of this compound through the liver and gallbladder.

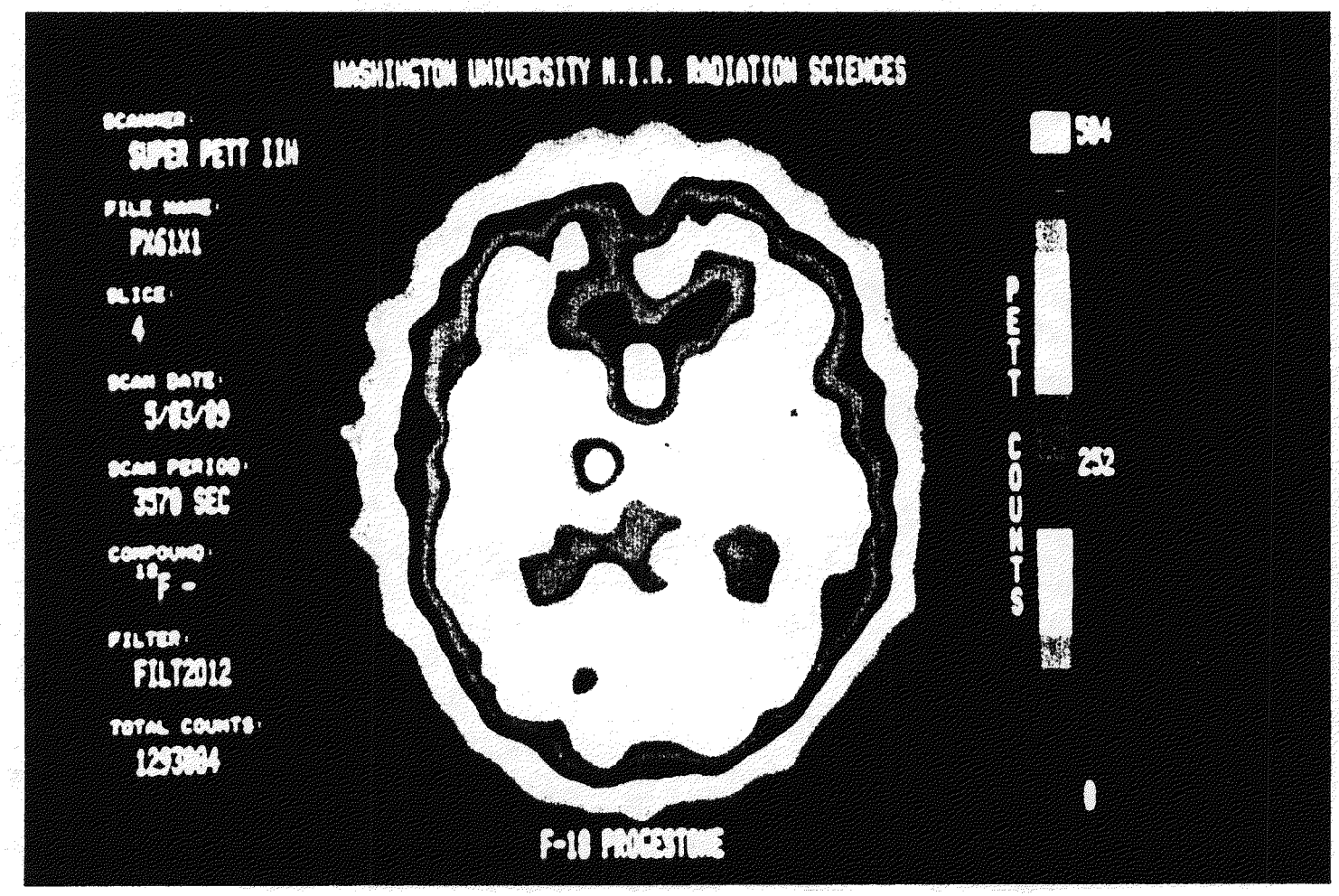


Figure 21: Distribution of FENP in brain of a normal human subject. The specific uptake is in the regions of the hypothalamus.

little or no uptake is observed in bone). This is in contrast to the rat studies where significant bone uptake is observed. In a different study (Appendix 14) utilizing a fluoroalkyl derivative of diprenorphine, we have observed very different biodistribution in male and female rats; extensive bone uptake occurs in male rats. When this diprenorphine analog was studied in primates, no bone uptake is observed. It appears that extrapolation of metabolism from rats to primates is difficult and one of our goals in the future work will be to attempt to evaluate the anticipated metabolites from higher mammals. The uptake of progesterone in human brain is very similar to the uptake of estradiol in both primate (Figure 22) and human brain (Figure 23). In primates the FENP uptake can be blocked (Figure 24) and these agents have potential for studying sex hormone receptor binding sites in the brain.

Largely supported by NIH, we are continuing to study ^{18}FES in human subjects. One of our initial human images (selected by Dr. H.N. Wagner as image of the year) is shown in Figure 25. Our current protocol involves patients with metastatic breast carcinoma studied before and during tamoxifen treatment. Images from a typical patient in this protocol are shown in Figure 26.

References

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3. Katzenellenbogen JA, Heima DF, Carlson KE. In Receptor Binding Radiotracers. (WC Eckelman Ed) C.R.C. Press (Boca Raton FL) 1982 p. 93.

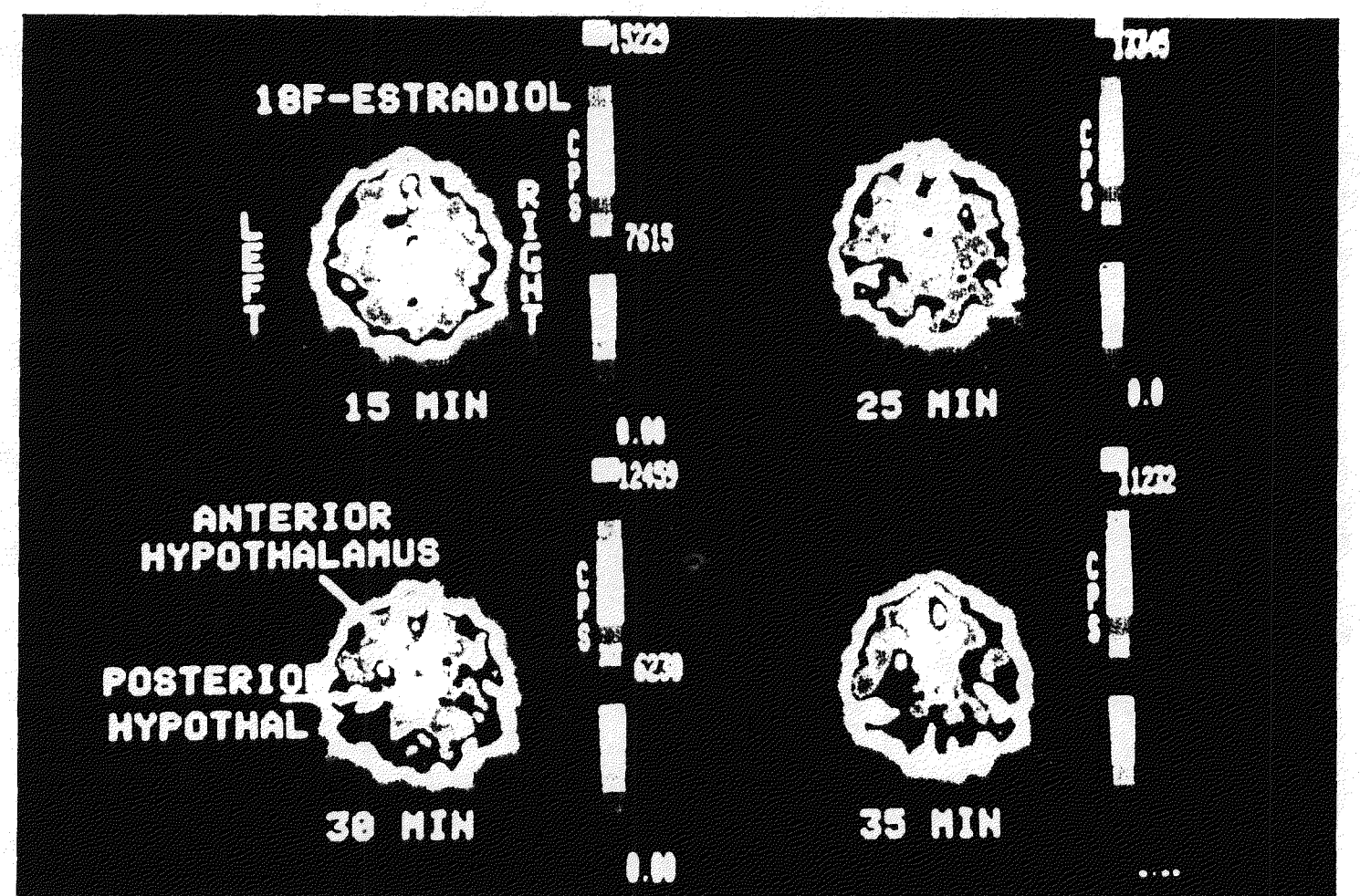

Figure 22: Uptake and washout of ^{18}F-fluoroestradiol in a baboon brain. There is specific accumulation in the hypothalamus.

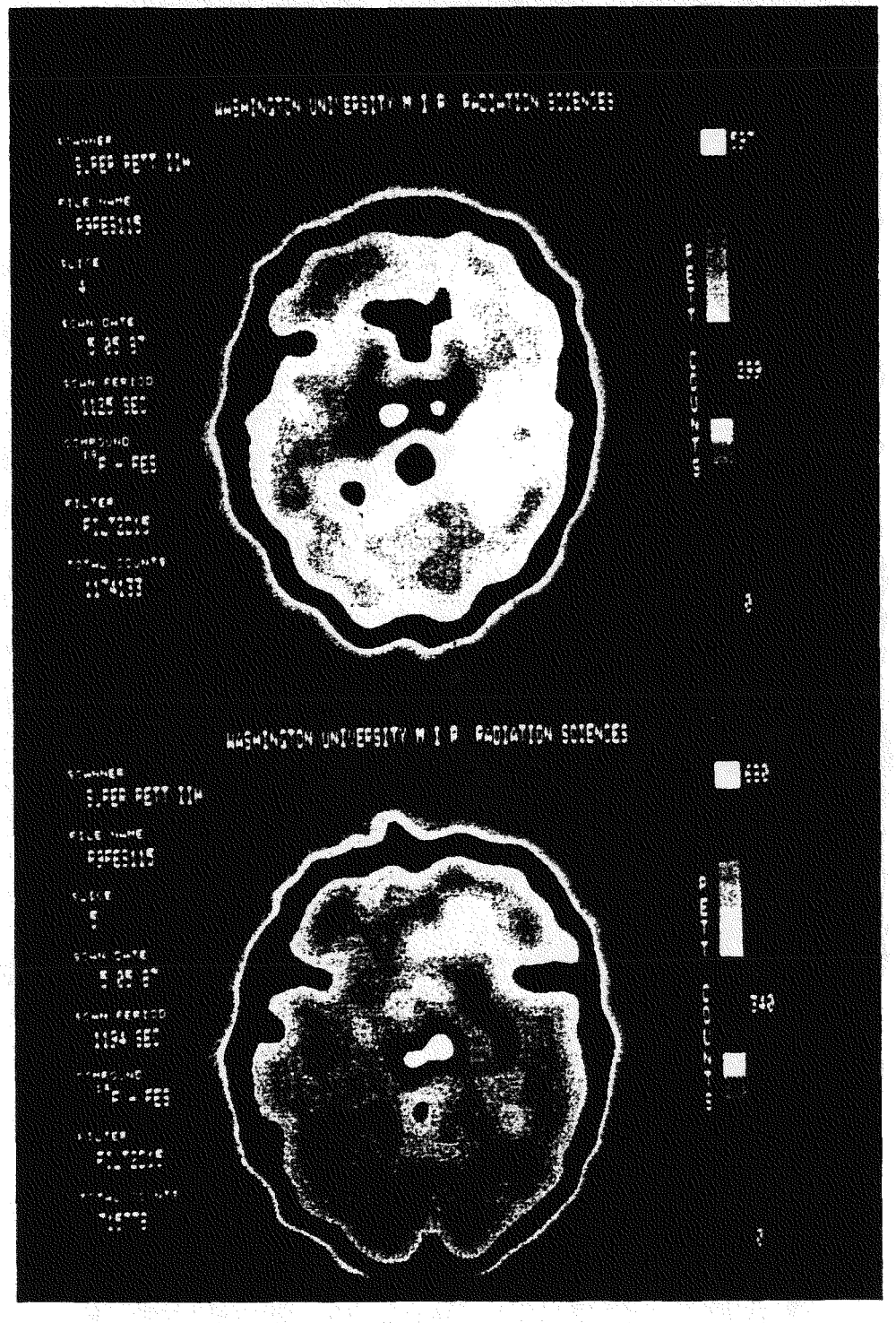

Figure 23: Distribution of ^{18}F-fluoroestradiol in a normal human brain, uptake occurs in the regions of the hypothalamus.

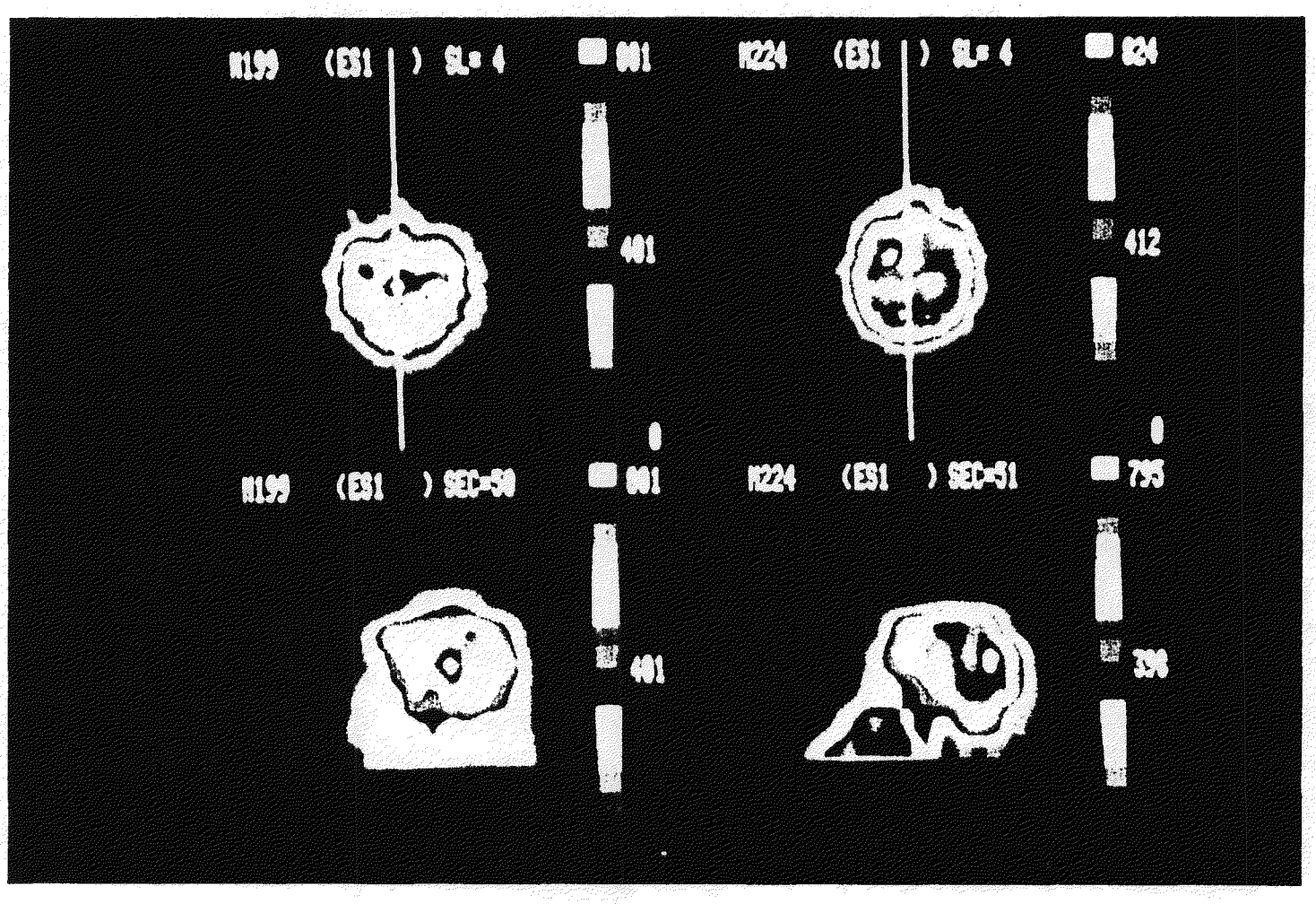

Figure 24. Uptake of FENP in a baboon brain at high (left) and low (right) specific actitivities. At low specific activities diffuse uptake is obtained. Significantly more activity was administered in the low specific acitivity case accounting for the similar PET numbers.

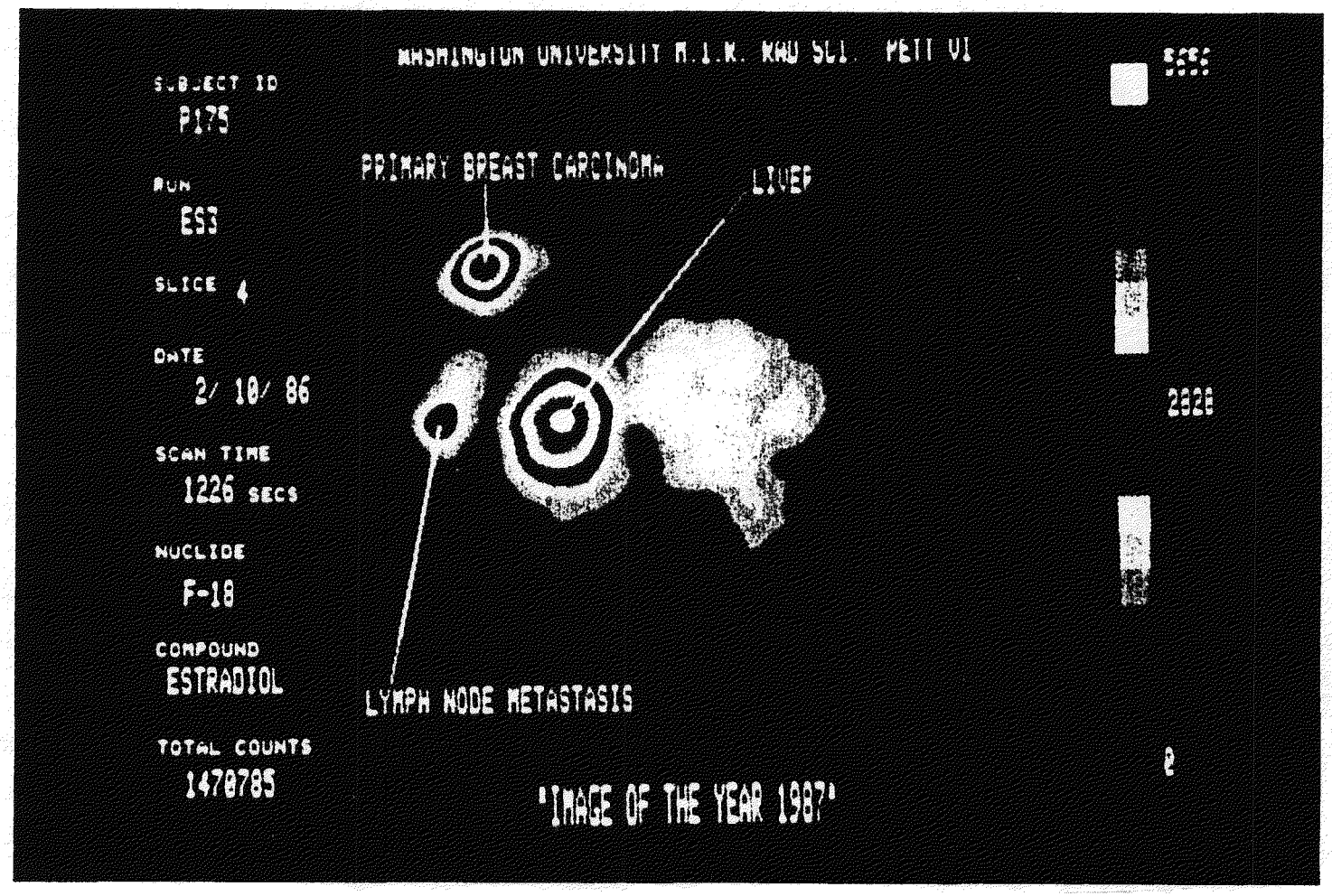

Figure 25: Distribution of ^{18}F-fluoroestradiol in a patient with a primary breast carcinoma, uptake in the breast tumor and lymph node metastasis as well as the clearance through the liver is observed.

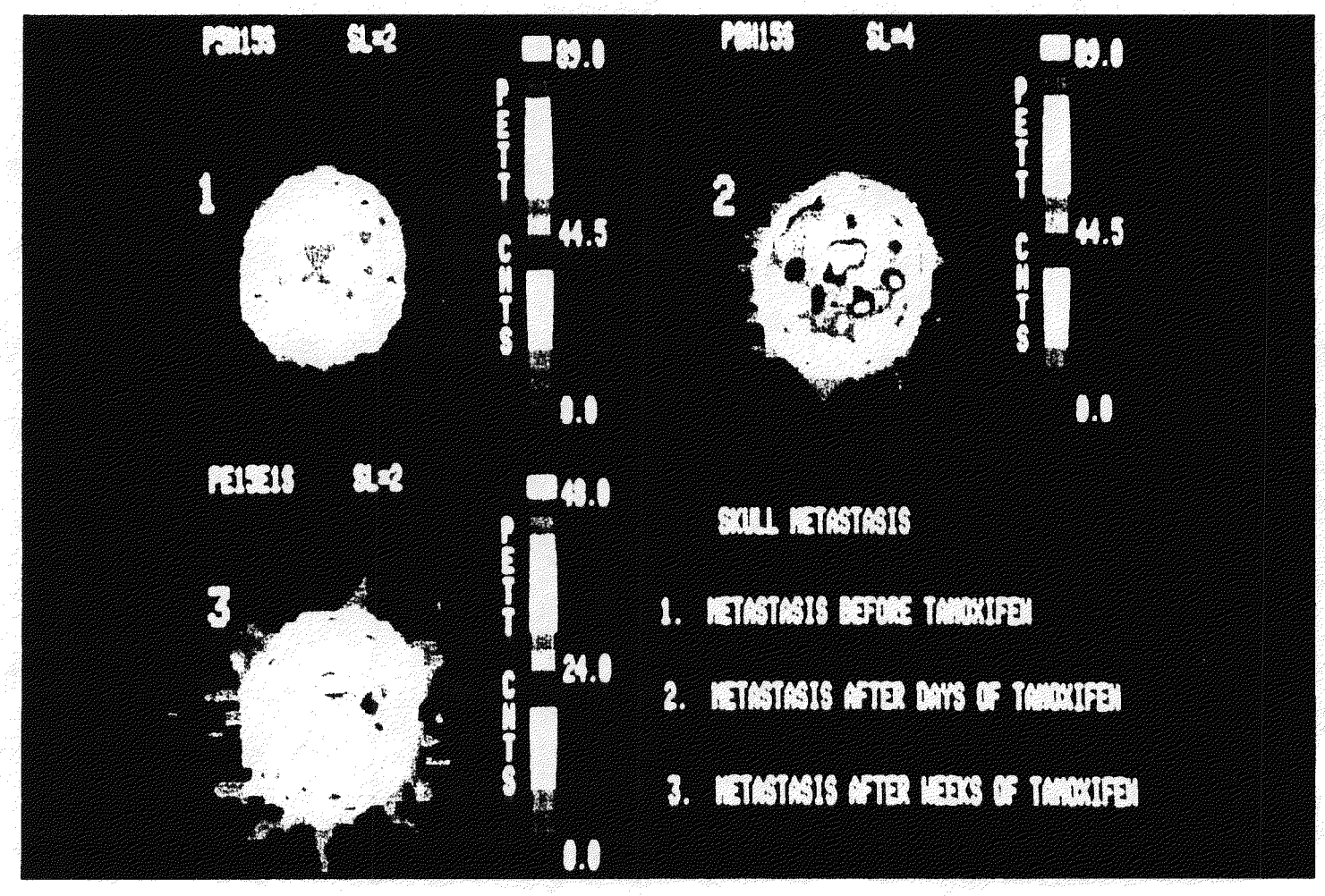

Figure 26: Uptake of ^{18}F-fluoroestradiol in a patient with skull metastasis of breast carcinoma. ^{18}F-FES administered before tamoxifen (1), 5 days after the initiation of tamoxifen (2), and 6 weeks after initiation of tamoxifen (3). The reduction in target to non-target ratios is clearly observed.

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