J. steroid Biochem. Vol. 22, No. 2, pp. 155–160, 1985
Printed in Great Britain. All rights reserved

0022-4731/85 $3.00 + 0.00
Copyright © 1985 Pergamon Press Ltd

THE SYNTHESIS OF 16α-[^{131}I]IODO-OESTRADIOL AND EVALUATION OF ITS USE AS A RADIOTRACER FOR OESTROGEN RECEPTOR POSITIVE BREAST TUMOURS

ELIZABETH K. SYMES*, W. F. COULSON and D. N. L. RALPHS†
Courtauld Institute of Biochemistry, †Department of Surgical Studies, The Middlesex Hospital Medical School, Mortimer Street, London W1P 7PN, U.K.

(*Received* 13 *April* 1984)

Summary—16α-Iodo-oestradiol binds with high affinity to the oestrogen receptor and has been shown to accumulate in oestrogen sensitive tissues in many test systems. We have prepared the compound labelled with ^{131}I at four specific activities. Using these preparations we have attempted to image human primary and metastatic breast cancer deposits at various times from 15 min to 24 h post injection by external gamma scintigraphy. Clinical studies were conducted on 10 post-menopausal patients. The receptor status was determined in seven cases, four were positive and three negative. The imaging results were very poor, in only two cases were images obtained, these were very faint and only of the primary, never of the metastatic deposits. The oestrogen receptor status was only available in one of these cases, it was positive. Dynamic studies *in vivo* revealed that the compound is cleared rapidly from the circulation during the first 5 min and thereafter undergoes extensive enterohepatic recycling. Studies of the radiochemical identity of the circulating species revealed that the injected compound was extensively metabolised. Neither an increase in specific activity of injected radiotracer nor imaging at shorter times after injection improved the results.

INTRODUCTION

Early *in vivo* studies with [^{3}H]oestradiol [1] demonstrated the selective retention of this compound in oestrogen sensitive tissues. Subsequently a specific receptor protein for oestradiol was identified and shown to be present only in target tissues. It is now well established that the actions of oestradiol on target tissue growth and protein synthesis are mediated through the interaction of the oestradiol–receptor complex with nuclear components [2]. The growth of many breast tumours is oestrogen dependent and can be arrested by withdrawal of the oestrogenic stimulus [3, 4]. The oestrogen receptor status of the tumour has been shown to correlate well with remission after oestrogen ablation or anti-oestrogen therapy [5]. Thus a knowledge of the tumour receptor status has proved useful clinically.

Eckelman *et al.*[6] suggested that radiolabelled oestrogens with high receptor binding affinity should be accumulated by oestrogen receptor containing breast tumours and metastases and, therefore, permit the imaging of these tissues by external gamma scintigraphy. This technique is non-invasive and would provide information about both the spread of the disease and the receptor status of the tumour. However, it depends on the existence of suitable γ-emitting radiopharmaceuticals that are concentrated selectively in the target tissue. Best results are obtained when tissue to background ratios are high, i.e. 5:1 [19].

Hochberg in 1979 [7] described the synthesis of 16α-iodo-oestradiol. He demonstrated that its receptor binding affinity was equal to that of oestradiol and that it was oestrogenic *in vivo* [8]. Both the 16α-^{125}I and 16α-^{77}Br derivatives have also been shown to be accumulated by oestrogen sensitive tissues of the rat such as uterus and DMBA mammary tumours [9]. It therefore seemed possible that 16α-halogenated oestradiol derivatives would be suitable radiotracers for oestrogen receptor positive human breast tumour deposits. We have synthesized 16α-[^{131}I]iodo-oestradiol and attempted to image primary and secondary breast tumours using an external gamma camera. Subsequently receptor analyses were performed on the primary tumours removed at operation. We here report the outcome of these studies.

EXPERIMENTAL

In order to synthesize 16α-iodo-oestradiol labelled with a short lived isotope on a regular basis a stock of a stable immediate precursor is required. 16β-Bromo-oestradiol was, therefore, synthesised from which 16α-[^{131}I]iodo-oestradiol could be conveniently prepared as required by a one-step synthesis.

*To whom correspondence should be addressed.

Table 1. ^{1}H NMR analyses of (a) 16β-bromo-oestradiol-17β (b) 16α-iodo-oestradiol-17β

(a)	δ ppm		Int	J Hz	Assignment
	0.79	s	3H		18 methyl
	1.12–2.2	m	11H		7α, 7β, 8β, 9α, 11α, 11β, 12α, 12β, 14α, 15α, 15β
	2.79	m	2H		6α, 6β
	3.68	t	1H	$J_{17\alpha,16\alpha}$ 4.5 $J_{17\alpha,OH}$ 4.5	17α
	3.81	s	1H		3-OH (D_2O exchangeable slow)
	4.67	td	1H	$J_{16\alpha,15\alpha}$ 7.0 $J_{16\alpha,15\beta}$ 7.0 $J_{16\alpha,17\alpha}$ 4.5	16α
	4.75	d	1H	$J_{OH,17\alpha}$ 4.5	17β-OH (D_2O exchangeable fast)
	6.68	m	1H		4
	6.72	dm	1H		2
	7.13	d	1H	$J_{1,2}$ 8	1
(b)	δ ppm				
	1.08	s	3H		18 methyl
	1.22–2.5	m	11H		7α–15β
	2.81	m	2H		6α, 6β
	4.03	td	1H	$J_{16\beta,15\alpha}$ 8 $J_{16\beta,15\alpha}$ 8 $J_{16\beta,17\alpha}$ 1	16β
	4.29	bs	1H		17α (changes to 1 Hz doublet on D_2O exchanges)
	5.39	bs	1H		17β OH (D_2O exchangeable)
	6.64	m	1H		4
	6.70	dd	1H	$J_{2,1}$ $J_{2,4}$ 2.7	2
	7.15	d	1H	$J_{1,2}$ 8	1
	10.44	bs	1H		3-OH (D_2O exchangeable)

Preparation of 16β-bromo-oestradiol

This compound was prepared from oestrone by a modification of the published procedure [7]. The synthesis of 16α-bromo-oestrone by bromination of the enol di-acetate of oestrone was carried out as described. 16α-Bromo-oestrone was then converted to 16β-bromo-oestradiol by direct reduction instead of the two step procedure (epimerisation followed by reduction) published. This modification was introduced on the basis of the studies of Shoppee *et al.*[17] and Fajkos[18] on the stereo specific reductions of 16α-bromo-17-ketones of the androstane series.

16α-Bromo-oestrone (0.20 g) was dissolved in ethanol (15 ml) and cooled to 4°C. Sodium borohydride (40 mg) was added and the reaction left for 16 h at 4°C. Analysis of the mixture by silica gel thin layer chromatography (chloroform–ether, 9:1) revealed one major (50%) and 3 minor products. After dilution with water (2 vol) and chloroform extraction of the reaction mixture, the major product was purified by silica gel column chromatography using chloroform–ether (9:1, v/v) (1 × 30 cm). The product which eluted first from the column was recrystallized from methanol. Pure crystalline material was obtained in 25% yield, and characterised as 16β-bromo-oestradiol from its melting point (255–256° as published) and by ^{1}H NMR (see Table 1). The overall yield through the synthesis was 12%.

Preparation of 16α-iodo-oestradiol

This compound was prepared from 16β-bromo-oestradiol by prolonged refluxing with 10× molar excess of sodium iodide in acetone as described [7]. The product was purified by chromatography in chloroform–ether (9:1, v/v) on a 1 × 30 cm silica gel column. It was recrystallised from methanol and its structure was confirmed from the melting point (190–191°C as published) and by ^{1}H NMR (see Table 1). The yield was 48%. It was used subsequently to confirm the structure of radiolabelled derivatives.

Preparation of 16α-[^{131}I]iodo-oestradiol for administration to patients

All glassware was heat sterilised before use. Ten mCi of $Na^{131}I$ in 50 μl of sodium hydroxide solution (as supplied by Amersham International plc, Amersham, U.K.) was divided into 10 × 1 mCi aliquots and placed in micro reaction vessels (total volume 0.1 ml with screw top Teflon seal). To each was added 5 μl of a solution of 1 mM sodium thiosulphate* and 100 μl freshly distilled CH_3CN. The mixtures were taken to dryness under a stream of N_2. To each was added 20 μg of 16β-bromo-oestradiol in 2 μl of freshly distilled 2-butanone. After heating overnight at 75°C the cooled reaction mixtures were combined using 2-butanone and taken to dryness under a stream of N_2. The combined mixtures were transferred to a silica gel Sep-pak cartridge (Waters Associates, Milford, Massachusetts 01757) using the initial eluting medium. The column was eluted with 20 × 1 ml of 1.25% *iso*-propanol in *n*-hexane fol-

*In some preparations sodium iodide was also added at this point to yield a range of specific activities as detailed in Table 3.

lowed by 20 × 1 ml of 2.5% *iso*-propanol in *n*-hexane. Aliquots of fractions were analysed by silica gel thin layer chromatography in chloroform: ether, (9:1, v/v). 16α-[^{131}I]Iodo-oestradiol containing fractions were identified by radiochromatogram scanning and by comparison with the appropriate standard. Fractions containing 16α-[^{131}I]iodo-oestradiol were combined, taken to dryness under N_2 and reconstituted in 0.5 ml ethanol. Just prior to injection 1.0 ml of a sterile solution of Tween 80 in water (1% v/v) was added and the mixture was passed through a millipore filter (0.2 μ) which was washed with 1.0 ml of sterile normal saline. A total of 2.5 ml containing 0.25–0.5 mCi was injected i.v. to each patient.

Preparation of 16α-[^{125}I]iodo-oestradiol

Carrier free 16α-[^{125}I]iodo-oestradiol was prepared as described for the ^{131}I derivative except that Na^{125}I supplied in aqueous solution (New England Nuclear, Boston, Massachusetts 02118) was used in the reaction. The identity of the compound was established by silica gel chromatography, where it co-migrated as a single radioactive spot with authentic 16α-iodo-oestradiol. The radioactive compound was also tested *in vivo* in female rats and shown to accumulate in the uteri (Table 2).

Clinical studies

Ten post-menopausal patients with breast cancer deposits were studied. Some patients had primary disease without evidence of metastases, some had both primary and axillary node metastatic deposits and some only had metastatic deposits, the primary having been removed at an earlier date. 16α-[^{131}I]Iodo-oestradiol was prepared at four specific activities, two patients received the radiotracer at 30 Ci/mmol, four at 150 Ci/mmol, three at 1500 Ci/mmol and one at 15,000 Ci/mmol.

Each patient took Lugol's iodine (1 ml) prior to the intravenous injection of 16α-[^{131}I]iodo-oestradiol. Planar images were taken using an IGE 400AT/STAR large field of view gamma camera at various times after injection (15 min, 1, 2, 4 and 24 h).

Dynamic studies were performed on one patient (patient 2) who received 350 μCi of compound at 30 Ci/mmol. Using the STAR computer, curves were plotted of counts against time for areas of interest over heart, liver and intestine from images taken during the first hour.

Samples of heparinised blood (10 ml) were taken from three patients at various times after injection of 16α-[^{131}I]iodo-oestradiol. The blood was immediately separated and the plasma exhaustively extracted into chloroform. Combined chloroform extracts were taken to dryness under a stream of N_2 and analysed by silica gel thin layer chromatography in chloroform–ether (9:1, v/v). The region corresponding to 16α-iodo-oestradiol was identified by comparison with standard plates run simultaneously. Test plates were divided into 1 cm sections and each section taken for radioactive counting.

Table 2. Distribution of radioactivity after i.v. injection 16α-[^{125}I]iodo-oestradiol to female rats

Tissue	Time hours				
	½	1	2	3	4
Uterus	18,018	16,094	16,453	13,151	9,310
Blood	3,228	1,582	1,853	1,012	1,262
Liver	9,598	4,146	3,684	3,612	3,582
Kidney	4,056	1,651	1,715	932	836
Spleen	2,420	970	1,296	945	792
Lung	2,820	1,213	1,056	785	956

Wistar rats (200 g) received i.p. injection 0.2 μCi of the compound in ethanolic saline. At various times after injection animals were killed and the radioactivity (cpm/0.25 g tissue) determined by direct gamma counting of a number of tissues.

RESULTS

Unlabelled 16α-iodo-oestradiol was synthesised from 16β-bromo-oestradiol in good yield and its structure confirmed by ^{1}H NMR. The radioactive synthesis proved much more difficult and even using the optimised procedures described only a 5–10% yield of radioactively labelled steroid was obtained. The authenticity of both the ^{125}I- and ^{131}I-labelled compounds was established by comparing their chromatographic behaviour with that of the unlabelled compound. The ^{125}I-labelled compound was also administered to female rats and shown to accumulate in uteri (Table 2). These results were similar to those obtained after the injection of 16α-[^{125}I]iodo-oestradiol supplied by Amersham International, Amersham, U.K. and to published work [10, 11].

Clinical studies

All patients gave their informed consent to participating in this study which had the approval of the Middlesex Hospital ethical committee. The details of the study are given in Table 3. 16α-[^{131}I]Iodo-oestradiol was injected at doses of 250–470 μCi and at four different specific activities. In two cases an image of the primary lesion was obtained. However these images were very faint and only confirmed by comparison with subsequent pictures taken with an external radioactive source placed over the tumour mass. In one of those two cases the primary mass was removed and shown to be oestrogen receptor positive. In the remaining 7 patients we did not obtain an image of the primary deposit despite the presence of receptors in three of these tumours. Images were never obtained of any metastatic deposit.

No radioactivity was detected in the thyroid gland. The regions where there was greatest accumulation were the bile duct, liver and intestines which were still easily imaged 24 h after injection, indicating the extensive enterohepatic recycling of this compound.

The dynamic study of patient 2 revealed (Fig. 1) that the radioactivity was cleared rapidly from the circulation during the first 5 min. A small rise of radioactivity in the liver after 10 min and slow decline

Table 3. Imaging results in breast cancer patients with 16α-[^{131}I]iodo-oestradiol

Patient	Age yrs	Disease State	Specific activity Ci/mmol	Dose μCi	Receptor status E_2R cytosol	E_2R Nuclear	Prog R.	Image
1	61	1°	30	300	+ve (220)	+ve (119)	+ve (35)	−ve
2	51	1°	30	350	—	—	—	+ve, faint up to 4 h post injection
3	63	Bone + brain 2°; 1° removed 1982	150	400	—	—	—	−ve
4	57	1° + bone 2°	150	450	−ve	−ve	−ve	−ve
5	56	1° + axillary node 2°	150	300	−ve	−ve	−ve	−ve
6	42	1° + axillary node 2°	150	470	+ve (307)	+ve	+ve (100)	+ve faint, up to 4 h post injection 1° only
7	68	1°	1500	250	+ve (353)	+ve (281)	+ve (168)	−ve
8	55	1° Benign	1500	250	−ve	−ve	−ve	−ve
9	70	1°	1500	250	—	—	—	−ve
10	54	1°	Carrier free	250	+ve (202)	+ve (123)	−ve	−ve

Values of receptor concentration shown in brackets fmol/mg protein. Negative receptor value is < 5 fmol/mg protein.

Table 4. TLC analysis of plasma after injection 16α-[^{131}I]iodo-oestradiol, % of total radioactivity

Patient	Age yrs	Time post-injection ½ h		1 h		4 h	
		R_f 0.39	R_f 0.78	R_f 0.39	R_f 0.78	R_f 0.39	R_f 0.78
8	55			21	49		
9	70			41	25	12	35
10	54	25	28	17	39		

The % of radioactivity applied to the plates was determined for each 1 cm section of the plates. The % radioactivity in the two areas corresponding to the 16α-iodo-oestradiol (R_f 0.39) and the less polar metabolite (R_f 0.78) are tabulated.

thereafter demonstrated the non-colloidal nature of the material. Extensive accumulation of radioactivity occurred in the gut after 15 min (Fig. 1).

The analyses of blood samples taken from three patients are shown in Table 4. Two major areas of radioactivity were detected on the thin layer chromatograms. The first corresponded to the region of 16α-iodo-oestradiol which in this system had an R_f of 0.39, the second was a faster running spot, R_f 0.78. The remaining radioactivity was distributed evenly over the chromatogram. The amount of the faster running material, relative to 16α-iodo-oestradiol, increased with time after injection and was greater in the younger patient.

Dosimetry

Radiation doses were calculated from an estimate of the biological half-life of ^{131}I injected as 16α-[^{131}I]iodo-oestradiol and using values of absorbed dose per unit cumulated activity from MIRD pamphlet 11.

Oestrogens are cleared quite slowly in humans due to enterohepatic recycling. The half-life of clearance of oestradiol has been reported in the range 26–40 h [12]. Our imaging results show injected 16α-[^{131}I]iodo-oestradiol is also secreted through the bile duct into the gut. A direct measurement of the biological half life was not made, but published data [13] on the half-life of 16α-[^{77}Br]bromo-oestradiol confirms a value of 36 h. We therefore assumed a similar half life for the purpose of the calculation.

Thus if the injected material is uniformly distributed the whole body dose after a 250 μCi injection is 0.0011 Gray. If 50% of the activity is distributed through the intestine then the dose to the intestine is 0.076 Gray.

DISCUSSION

Our attempts to image breast tumours using 16α-[^{131}I]iodo-oestradiol have been discouraging. We were not able to image metastatic deposits at all and in only two cases were images of the primary mass obtained. These were weak and unsuitable for diagnostic purposes. The receptor status was only available in one of these cases, it was positive. However, we were unable to obtain images of three other receptor containing primary tumours.

Using a simple bi-molecular model based on the interaction of steroid and the receptor Eckelman *et al.*[6] predicted the target to background ratios which should be obtained under a variety of conditions. When the tissue receptor concentration was 10^{-9} M, the radiotracer had an affinity for the receptor of 10^{10} l/mol and the intial free steroid concentration was between 0.1 and 100 pM, a target to background

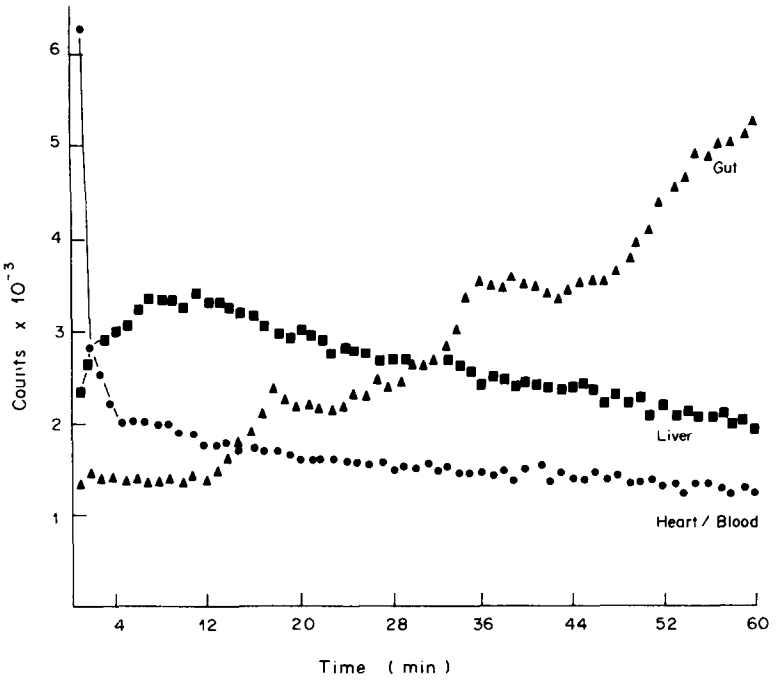

Fig. 1. Clearance of radioactivity during the first hour after injection of 350 μCi 16α-[^{131}I]iodo-oestradiol at 30 Ci/mmol (patient 2). Planar images were taken with an IGE 400AT/STAR large field of view gamma camera. Using the STAR computer curves were plotted of counts against time for regions of interest over heart, liver and intestine.

ratio of 10:1 was predicted. We injected 250 μCi of each of our four preparations of 16α-[^{131}I]iodo-oestradiol, hence if we assume a blood volume of 3 l, we should reach concentrations of 3 nM, 0.6 nM, 60 pM and 6 pM respectively. Our tumor receptor concentrations were about 10^{-9} M and the K_d of 16α-iodo-oestradiol for the receptor is 2.7×10^{10} l/mol [7]. Therefore the optimal initial concentrations were exceeded with the two lower specific activity preparations but were within appropriate limits with the other two preparations. We would, on this basis, have expected to image the primary tumour in patients 7 and 8, but could not do so.

The bi-molecular model does assume that the free steroid concentration in the tissue is equal to that in the blood. However this may not be a valid assumption if the tumour were not well vascularised, as diffusion through the tumour mass may be slow, and clearance of the compound from blood is fast.

Our imaging results using 16α-[^{131}I]iodo-oestradiol are less encouraging than those reported by McElvany *et al.*[13] who used 16α-[^{77}Br]bromo-oestradiol and were able to detect both receptor positive primary and some metastatic tumour tissue. These differences may be because a different compound was used, although animal studies indicate that the accumulation of the 16α-iodo and 16α-bromo compounds in target and non target tissues are very similar [10].

However the discrepancy could also be due to differences in the amount and specific activity of the injected radiotracer. McElvany *et al.*[13] injected 5 mCi of 16α-[^{77}Br]bromo-oestradiol at 1500 Ci/mmol which was equivalent in amount to our injection of ~500 μCi at 150 Ci/mmol of 16α-[^{131}I]iodo-oestradiol. With this preparation we did get a weak image. We might therefore expect to improve the imaging, with 16α-[^{131}I]iodo-oestradiol by injection of 5 mCi of a higher specific activity preparation. However, this would give an unacceptably high dose of radiation to the intestine because although the whole body dose after the administration of only 250 μCi is low, the dose to the intestine is close to the upper limit of acceptability. We also considered labelling 16α-iodo-oestradiol with ^{123}I to overcome the radiation dose problem but this proved to be impractical. The time taken for the labelling reaction is equivalent to two half-lives of ^{123}I and the yield is such that we would need to start with 200 mCi of Na^{123}I to obtain 5 mCi of 16α-[^{123}I]iodo-oestradiol for injection. This makes the procedure prohibitively expensive which is regrettable as the imaging characteristics of ^{123}I are superior to those of ^{77}Br and ^{131}I.

Another factor which might modify the accumulation of the radiotracer in target tissues would be the oestrogen status of the patients as selective accumulation in target tissues is greater if the endogenous hormone concentrations are low [14, 15]. However all our patients were post-menopausal so circulating oestrogens would be low.

The return to the circulation of metabolites which do not bind to receptors would also reduce the attainable target to background ratio. When we studied the radioactive species circulating in plasma we found that 16α-iodo-oestradiol is metabolised to

a less polar compound. Based on the known metabolism of oestradiol and the chromatographic properties of the metabolite we suggest that this compound may be 16α-iodo-oestrone. Since oestrone has a much lower receptor binding affinity than oestradiol and 16α-iodo-oestradiol has a similar receptor binding affinity to oestradiol, it might reasonably be expected that 16α-iodo-oestrone would have low receptor binding affinity. Hence the formation of this metabolite would reduce the relative tissue accumulation of ^{131}I-labelled steroid in receptor-rich tissues.

The development of 17α-substituted oestrogens such as ethynyl-oestradiol as pharmaceuticals was originally prompted by the need to inhibit the oxidative metabolism of oestradiol to oestrone and thus increase the potency of oestrogenic preparations. Iodinated derivatives of 17α-substituted oestrogens may, therefore, provide improved radiopharmaceuticals. 17α-Iodo-ethynyl-oestradiol has been prepared but de-iodination is rapid *in vivo* [16]. 17α-Iodo-vinyl-oestradiol has also been prepared, is stable *in vivo* and is accumulated by immature rat uteri against the blood gradient [11]. Clinical studies with this compound would be of interest.

In conclusion the satisfactory imaging of receptor positive breast tumours has not been achieved with 16α-[^{131}I]iodo-oestradiol despite the use of small doses of material of high specific activity. Indeed this compound appears to be inferior to the ^{77}Br derivative used by McElvany *et al.*[13]. This result is surprising in view of the compound's excellent receptor binding characteristics and rapid blood clearance. This failure may be attributed, in part to the low blood clearance rate of a major metabolite with low receptor binding affinity.

Acknowledgements—This work was supported by the Medical Research Council. Dr D. Barnes of the Department of Clinical Research, Christie Hospital and Holt Radium Institute, Manchester, U.K., kindly performed the receptor analyses. The co-operation and contribution of the Institute of Nuclear Medicine, The Middlesex Hospital and the technical aid of Mr R. Read are gratefully acknowledged.

REFERENCES

1. Jensen E. V. and Jacobson H. I.: Basic guides to the mechanism of estrogen action. *Recent Prog. Horm. Res.* **XVIII** (1962) 387–414.
2. Jensen E. V., Greene G. L., Closs L. E., DeSombre E. R. and Nadji M.: Receptors reconsidered: a 20-year perspective. *Recent Prog. Horm. Res.* **38** (1982) 1–40.
3. Beatson G. T.: On the treatment of inoperable cases of carcinoma of the mamma: suggestions for a new method of treatment, with illustrative cases. *Lancet* **ii** (1896) 104–107.
4. McGuire W. L.: Estrogen receptors in human breast cancer. *J. clin. Invest.* **52** (1973) 73–77.
5. Edwards D. P., Chamness G. C. and McGuire W. L.: Estrogen and progesterone receptor proteins in breast cancer. *Biochim. biophys. Acta* **560** (1979) 457–486.
6. Eckelman W. C., Reba R. C., Gibson R. E., Rzeszotarski W. J., Vieras F., Mazaitis J. K. and Francis B.: Receptor-binding radiotracers: a class of potential radiopharmaceuticals. *J. nucl. Med.* **20** (1979) 350–357.
7. Hochberg R. B.: Iodine-125-labelled estradiol: a gamma-emitting analog of estradiol that binds to the estrogen receptor. *Science* **205** (1979) 1138–1140.
8. Hochberg R. B. and Rosner W.: Interaction of 16α-[^{125}I]iodo-estradiol with estrogen receptor and other steroid-binding proteins. *Proc. natn. Acad. Sci., U.S.A.* **77** (1980) 328–332.
9. Katzenellenbogen J. A., Senderoff S. G., McElvany K. D., O'Brien H. A. and Welch M. J.: 16α-[^{77}Br]bromoestradiol-17β: a high specific-activity gamma-emitting tracer with uptake in rat uterus and induced mammary tumours. *J. nucl. Med.* **22** (1981) 42–47.
10. McElvany K. D., Carlson K. E., Welch M. J., Senderoff S. G., Katzenellenbogen J. A. and the Los Alamos Medical Radioisotope Group: *In vivo* comparison of 16α-[^{77}Br]bromoestradiol-17β and 16α-[^{125}I]iodoestradiol-17β. *J. nucl. Med.* **23** (1982) 420–424.
11. Hanson R. N., Seitz D. E. and Botarro J. C.: E-17α-[^{125}I]iodovinylestradiol: an estrogen-receptor-seeking radiopharmaceutical. *J. nucl. Med.* **23** (1982) 431–436.
12. Sandberg A. A. and Slaunwhite W. R.: Studies on phenolic steroids in human subjects. II The metabolic fate and hepato-biliary enteric circulation of C^{14}-estrone and C^{14}-estradiol in women. *J. clin. Invest.* **36** (1957) 1266–1278.
13. McElvany K. D., Katzenellenbogen J. A., Shafer K. E., Siegel B. A., Senderoff S. G., Welch M. J. and the Los Alamos Medical Radioisotope Group: 16α-[^{77}Br]-bromoestradiol: dosimetry and preliminary clinical studies. *J. nucl. Med.* **23** (1982) 425–430.
14. Symes E. K.: Uptake and retention of androgens by the rat ventral prostate and consideration of their use as site directing agents. *Biochem. Pharmac.* **31** (1982) 3231–3236.
15. McElvany K. D., Carlson K. E., Katzenellenbogen J. A. and Welch M. J.: Factors affecting the target site uptake selectivity of estrogen radiopharmaceuticals: serum binding and endogenous estrogens. *J. steroid Biochem.* **18** (1983) 6635–6641.
16. Mazaitis J. K., Gibson R. E., Komai T., Eckelman W. C., Francis B. and Reba R. C.: Radioiodinated estrogen derivatives. *J. nucl. Med.* **21** (1980) 142–146.
17. Shoppee C. W., Jenkins R. H. and Summers G. H. R.: Steroids and Walden Inversion Part XL. The configurations of the bromination products of androstan-17-one. *J. chem. Soc.* (1958) 3048–3058.
18. Fajkos J.: On Steroids. XV. The configuration of some 16-bromo derivatives of androstane. *Coll. Czech. chem. Com.* **20,** (1955) 312–355.
19. Ell P. J. and Khan O.: Emission computerised tomography: clinical applications. *Seminars in Nuclear Medicine* **XI** (1981) 50–60.