EXCRETION AND STORAGE OF ^{131}I LABELLED IODO ANALOGUE OF CHLOROTRIANISENE*

By W. E. Funcke and J. P. Hummel

Department of Biochemistry, State University of Iowa, Iowa City

Received May 28, 1956

Introduction

In recent years the long-acting synthetic pro-oestrogen†, chlorotrianisene (tri-*p*-anisylchloroethylene, TACE) has been used successfully for anti-androgenic therapy of prostatic carcinoma[1], for suppression of postpartum lactation and for vasomotor symptoms of menopause[2]. Thompson and Werner[3] demonstrated that after administration of TACE to laboratory animals the oestrogenic activity from the faeces was greater than that administered whereas urine had only negligible activity. They attributed the long duration of oestrogenic activity of TACE to fat storage as revealed by oestrogen bioassay in fat depots of animals administered the compound. Greenblatt and Brown[5] and Thompson and Werner[6] observed considerable and prolonged oestrogenic activity in fat depots of women administered the pro-oestrogen.

The present study was undertaken to gain further information about the metabolism of TACE and other halotrianisenes. The iodo analogue iodotrianisene (hereafter abbreviated as TAIE) was chosen for this purpose because of the ease with which it could be labelled with a radioactive element. Since both compounds, and the corresponding bromo analogue all possess comparable potency and duration of oestrogenic response[7], labelled TAIE may be reasonably assumed to represent this group of halotrianisenes in metabolic studies. Its use offers the additional advantage over conventional bioassay methods in that non-oestrogenic as well as oestrogenic metabolites may be detected. This paper describes the preparation of this radioactive compound and its storage and excretion by animals.

Experimental

Materials and Methods

TAIE was recrystallised from ethanol to remove traces of dark coloured impurities. The purified material melted 117·5–119°, and had absorption maxima at 250 and 292 mμ. At 250 mμ the molar extinction was $2{\cdot}62 \times 10^4$.

Preliminary experiments showed that TAIE could be labelled with ^{131}I by an exchange with $Na^{131}I$. The optimum pH for exchange between iodide and organic iodine compounds is reported to be about 5[8,9]; this pH was also satisfactory for the present reaction. TAIE (20 mg.) was

*Supported by a grant from the Wm. S. Merrill, Co., Cincinnati and by a summer fellowship to W. E. Funcke from the College of Medicine Central Scientific Fund of the State University of Iowa.

†The terms oestrogen and pro-oestrogen are used in the sense defined by Emmens.[4]

dissolved in 10 ml. of warm methanol and the solution was acidified with about 3 drops of 10 per cent glacial acetic acid in methanol. To this solution was added 0·5 ml. of an aqueous carrier-free solution of sodium iodide ^{131}I containing about 5 mC. of radioactivity. The solution was refluxed gently on a heating mantle in a hood. Maximum exchange took place within 12 hours, but only about 70 per cent of the theoretical ratio of iodine incorporation was obtained. Possibly volatisation of the ^{131}I in the form of iodine, or its adsorption on the glassware contributed to the loss. After 24 hours, water was added to the point of turbidity, the solution clarified by briefly reheating and it was then placed in the cold for several hours. The resultant suspension was diluted to about 30 ml. with water and centrifuged. The precipitant was recrystallised from dilute methanol and dried *in vacuo*. The product (18 mg.) had an activity of $9{\cdot}5 \times 10^6$ c.p.m./mg. as measured with the counter used. It had an infra-red spectrum identical to that of the unlabelled TAIE. The radioactive preparation was administered as a 1 per cent corn oil solution.

For comparative excretion studies, the diiodo derivative of the true oestrogen, hexoestrol, was employed. This was prepared and made radioactive in the following manner. To 500 ml. of hot 6 N hydrochloric acid were added 5 g. of hexoestrol in 225 ml. of ethanol. The hot solution was treated with 10 ml. of iodine monochloride in 50 ml. of 6 N hydrochloric acid. After several mintues on the steam bath a heavy crystalline precipitate formed. The solution was heated for an additional 15 minutes, then was placed in the cold overnight. The tan crystals were filtered by suction, washed with water and dried. The product, composed of a mixture of various iodohexoestrols, was dissolved in 100 ml. of ethanol and hot water was added to the point of turbidity. After adding sufficient sodium sulphite to remove the colour, the solution was briefly heated and then allowed to cool slowly, finally in the cold. The crystals, after filtering and drying *in vacuo*, were recrystallised from hot Skellysolve E, giving 4·7 g. of a white product, m.p. 143–144°, which on elementary analysis gave C, 41·5; H, 4·38 and I, 52·3; calculated for $C_{18}H_{20}O_2I_2$, C, 41·4; H, 3·83 and I, 48·6. It has not been determined whether this compound is the 3:5- or 3:3′-diiodo derivative. The oestrogenic potency was found by Dr. Sheldon Segal of the Department of Urology to be approximately 1/20th that of hexoestrol. The exchange between iodide ^{131}I and diiodohexoestrol was carried out in the same manner as used for the preparation of TAIE-^{131}I. No comparable studies were made on the kinetics of this exchange although the extent of incorporation of ^{131}I into diiodohexoestrol was found to be of the same magnitude as that with TAIE.

For localisation studies, the animals were killed at a designated period after the injection. Weighed samples of tissue were digested in 10 volumes of 10 per cent caustic soda at 100°. In the case of the fatty tissues, addition of Bloors solution was necessary to dissolve the tissues completely. Triplicate 1 ml. aliquots were dried in cup planchets, and were counted to a 95 per cent level of confidence with an end window

geiger counter for all but the least active of the tissue samples. Certain of the determinations were made with a scintillation counter. Both methods of counting were standardised with ^{131}I samples from the National Bureau of Standards. For the excretion studies, the animals were placed in metabolism cages in which urine and faeces were readily separated. The urine was evaporated directly in planchets for counting. The faeces were suspended in water, and disintegrated in a blendor. Aliquots were dried in planchets. As a precautions against iodine loss during drying, 0·5 ml. of 2 per cent sodium sulphite was added to urine and faeces samples before drying. Triplicate values for the activity in the excreta were averaged and the values are expressed as the fraction of total administered activity. Because of the uncertainty about the chemical stability of the radioactive iodine on TAIE, a dilute ethanolic solution of TAIE and urine samples from animals administered TAIE were treated with carrier sodium iodide and silver nitrate so as to precipitate any iodine ^{131}I present. No radioactivity could be detected in the silver precipitate from the TAIE solution. Although the activity was too low to measure accurately, the 12 hour and 5 day urine samples contained only about 0·9 and 1·3 per cent respectively of their total radioactivity in the form of inorganic iodide, indicating that liberation of ^{131}I from TAIE was relatively minor.

Results and discussion

Preliminary experiments on tissue localisation of TAIE indicated that the subcutaneous route of administration was preferable; by intraperitoneal injection a high concentration of radioactivity was found at injection site and oral administration was undesirable since we wished to study the concentration of TAIE and its metabolites in the gastro-intestinal tract.

Four days after 70 mg. of TAIE-^{131}I in 2·5 ml. of corn oil (equivalent to $3{\cdot}9 \times 10^6$ c.p.m.) was injected subcutaneously into the leg of a 7 kg. male dog, the animal was killed and the radioactivity in the tissues was measured. As shown in Table I, the thyroid contained the highest localisation of radioactivity; intraperitoneal fat and adrenals were the only other tissues which had counts per minute appreciably above the background. It appears likely that the ^{131}I which was concentrated in the thyroid is the result of deiodination of TAIE and subsequent incorporation of the radio-iodide into thyroid metabolites. The radioactivity in the fat depots is consistent with the view that this type of pro-oestrogen is readily stored in body lipids by virtue of its lipid solubility. The concentration of radioactivity in the adrenals might be

TABLE I

Distribution of ^{131}I in tissues of a dog administered TAIE-^{131}I 4 days previously

Tissue*	Per cent administered ^{131}I/g. wet tissue
Thyroid	0·583
Fat, intraper.	0·050
Adrenals	0·009
Bone marrow	0·003
Spleen	0·001
Testis	0·001

*Other tissues examined and found to contain less than 0·001 per cent of administered ^{131}I were: liver, kidney, lung, skeletal muscle, brain, blood, pancreas and prostate.

explained on their high lipid content. The remaining tissues studied contained so little radioactivity that their sequential order is probably not significant.

To verify these findings with a larger number of animals, rats were used for subsequent studies. Adult male animals (with one exception) were used because of our interest in possible localisation of this material in the male sex organs. The animals were administered 2 mg. of TAIE-^{131}I in 0·2 ml. corn oil and a comparison group was given 0·2 ml. of a solution containing 15 μC. of ^{131}I iodide in 0·02 mg. of potassium

TABLE II

RECOVERY OF ^{131}I IN RAT TISSUES AFTER ADMINISTRATION OF TAIE-^{131}I OR Na^{131}I

Compound	TAIE-^{131}I		Na^{131}I	
Hours after administration	4	48	4*	48*
No. animials	2	4	3	3
Thyroid (total organ)	0·016	0·480	16·170	6·700
Stomach	0·120	0·098	1·180	0·016
Intestine	0·257	0·086	0·276	0·014
Mesenteric fat	0·022	0·064	0·097	0·009
Testicular fat	0·005	0·088	0·097	0·016
Adrenals (total organ)	0·004	0·001	0·017	0·001
Liver	0·035	0·030	0·110	0·047
Kidney	0·022	0·012	0·203	0·020
Lung	0·021	0·024	0·173	0·010
Pancreas	0·025	0·024	0·100	0·023
Testis	0·015	0·014	0·167	0·014
Skeletal muscle	0·007	0·007	0·053	0·006
Brain	0·003	0·001	0·026	0·001

Data expressed as per cent of administered ^{131}I/g. wet tissue (except for thyroid and adrenals) from mean values of the group.

*All tissues in this group were counted directly with a scintillation type counter.

iodide subcutaneously. The animals were killed after 4 hours or 48 hours and the distribution of ^{131}I in the tissues measured thus is summarised in Table II.

Slow absorption from the site of injection undoubtedly accounted for the relatively low ^{131}I content of tissues of rats given TAIE four hours previously as compared with the tissue radioactivity of comparable animals given radioactive iodide; even after two days a highly radioactive fluid pocket could be found at the site of TAIE injection. The variable rate of absorption of TAIE makes the absolute magnitude of the recovered tissue ^{131}I of little significance. On the other hand, the relative concentration in the different tissues is illuminating.

In the TAIE administered animals, the highest content of ^{131}I after 4 hours was found in intestine, stomach and liver; no appreciable concentration was observed in fat at this time. The content of ^{131}I in the thyroid was low, indicating that little deiodination of TAIE had occurred. This is marked contrast to the distribution in rats administered ^{131}I iodide in which the organs having most radioactivity were thyroid and stomach, particularly the pyloric portion, probably due to parietal secretion, as suggested by Goldsmith and others[10] from similar observations in humans.

After 48 hours, the tissue distribution of ^{131}I in TAIE treated animals was greatly different. The tissues having the highest content of ^{131}I were thyroid, stomach, fat and intestine. The content in the thyroid and in the pyloric portion of the stomach is taken as evidence that release of inorganic iodide from TAIE had become appreciable after two days. The intestinal content of ^{131}I probably reflects the continued excretion of TAIE metabolites as well as thyroid metabolites. The content of ^{131}I in fatty depots became appreciable after two days. Although the presence of radioactivity does not necessarily connote the presence of the administered compound, comparison of the distribution of tissue ^{131}I after administration of TAIE-^{131}I and Na^{131}I clearly shows that despite the activity in the thyroid, deiodination of TAIE was relatively small. Thus after 48 hours the thyroid had taken up 0·48 and 6·7 per cent, respectively, of the total TAIE-^{131}I and Na^{131}I administered; at the same time, mesenteric fat contained 0·064 and 0·009 per cent of the administered ^{131}I after giving TAIE-^{131}I and Na^{131}I respectively. Although these data must be interpreted with some reservations, especially regarding the amount of radioactivity remaining at the injection site, they appear to be consistent with the conclusions of Thompson and Werner[3] that extensive storage of TACE in depot fat accounts for its long duration of oestrogenic activity. Other tissues appeared to have no appreciable content of ^{131}I either after 4 or 48 hours. The chemical and metabolic instability of ^{131}I tagged compounds is well known. Most organic iodine compounds appear to participate in an exchange with Na^{131}I in hot ethanolic solution; however, at pH 7 at 37° in aqueous solution, the exchange appears to be sufficiently slow to justify metabolic studies of iodinated compounds.

Because of the well known differences in duration of oestrogenic effect between TACE and the true oestrogens, it was of interest to compare the rate and route of excretion of TAIE and iodohexoestrol as indicated by radioactivity in urine and faeces of rats which had been administered these compounds labelled with ^{131}I. TAIE (1 mg. $= 8 \times 10^6$ c.p.m.) was given intraperitonially in 1 ml. of ethanol; diiodohexoestrol (0·5 mg. $= 1{\cdot}2 \times 10^4$ c.p.m.) was given similarly in 1 ml. of propylene glycol to adult male rats weighing approximately 200 g. The intraperitoneal route was selected because rapid absorption was desired and tissue localisation was not of prime interest in this portion of the study.

In Figure 1 is illustrated excretion of radioactivity in faeces and urine over an eight-day interval. It will be noted that diiodohexoestrol and/or its metabolites were predominantly excreted in the faeces, probably via the biliary pathway. On the other hand, the excretion of TAIE and its metabolites was approximately equal in both urine and faeces.

Thompson and Werner[3] have shown that compared with hexoestrol, TACE possesses a long duration of oestrogenic activity, accompanied by a low rate of excretion of biologically active material. In agreement with these authors, the present data show that the TAIE or its metabolites, compared with hexoestrol, are slowly cleared from the body. Indeed, only about 37 per cent of the total administered radioactivity

was recovered from faeces and urine after eight days whereas 85 per cent of the hexoestrol radioactivity was recovered after a similar interval.

A comparison of the amount of radioactivity in faeces and urine shows that with iodohexoestrol the faecal route is predominant, in agreement with Thompson and Werner. With TAIE, however, the activity was found to be equally distributed between urine and faeces. This is in striking contrast to the finding of these authors that oestrogenically active material was present only in the faeces of animals administered TACE. When larger doses of TAIE were given subcutaneously the faecal route became more important but the urine still contained considerable activity. For example, eight days after a 10-mg. subcutaneous dose, 4·8 per cent of the total dose was recovered in the faeces whereas only 0·3 per cent was found in the urine. This further suggests that there is a limit to the renal clearance of TAIE metabolites, and that mobilisation and excretion from the subcutaneous route is slow. The discrepancy between these results and those of Thompson and Werner must therefore be due to the urinary excretion of a non-oestrogenic metabolite of the halotrianisene. In a preliminary attempt to determine whether this might be due to urinary non-oestrogenic glucuronic acid conjugate of an oestrogenic TACE or TAIE metabolite, 48-hour urine samples of adult male rats administered 1 mg. doses of TACE were bioassayed for oestrogenic activity after incubation with β-glucuronidase (200 units/ml. urine) but failed to show any oestrogenic activity.

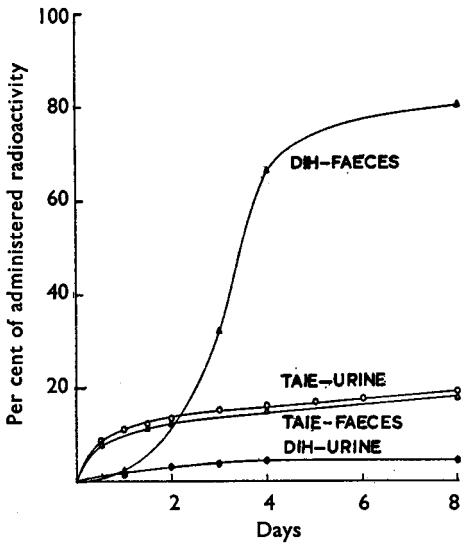

FIG. 1. Comparison of excretion of radioactivity in faeces and urine after administration of TAIE and diiodohexoestrol (DIH) to rats.

SUMMARY

1. The pro-oestrogen, iodotrianisene (TAIE) was labelled with ^{131}I by exchange between unlabelled TAIE and ^{131}I iodide.

2. Appreciable deposition of ^{131}I in depot fat was observed several days after subcutaneous administration of TAIE-^{131}I to a dog and to rats. The concentration of ^{131}I in the thyroid and stomach of rats, two days after the subcutaneous administration of TAIE-^{131}I, indicated that some release of the ^{131}I from TAIE-^{131}I had occurred. This was shown to be of small magnitude by comparative studies with rats given $Na^{131}I$. Other than in the above-mentioned tissues and in the gastrointestinal

tract, there was no significant localisation of radioactivity following TAIE-^{131}I administration.

3. Excretion of radioactivity in both faeces and urine was slow following administration of TAIE-^{131}I to rats and only traces of the radioactivity were excreted in the form of inorganic iodide. On the other hand, the excretion of diiodohexoestrol labelled with ^{131}I was rapid and occurred predominantly by the way of the faeces.

We are indebted to Mr. C. R. Thompson of Wm. S. Merrill and Co. for generous supplies of TAIE.

References

1. O'Conor and Sokol, *Quart. Bull. Northwestern Univ. Med. School*, 1952, **26**, 161.
2. Hendricks, *J. clin. Endocrin.*, 1954, **14**, 339.
3. Thompson and Werner, *Proc. Soc. exp. Biol. N.Y.*, 1951, **77**, 494.
4. Emmens, *J. Endrocrin.*, 1941, **2**, 444.
5. Greenblatt and Brown, *Amer. J. Obstet. Gynecol.*, 1952, **63**, 1361.
6. Thompson and Werner, *Proc. Soc. exp. Biol. N.Y.*, 1953, **84**, 491.
7. Shelton, Van Campen, Meisner, Paramenter, Andrews, Allen and Wyckoff, *J. Amer. chem. Soc.*, 1953, **75**, 5491.
8. Ruliffson, Lang and Hummel, *J. biol. Chem.*, 1953, **201**, 839.
9. Miller, Anderson, Madison and Salley, *Science*, 1944, **100**, 340.
10. Goldsmith, Stevens and Schiff, *J. Lab. clin. Med.*, 1950, **35**, 497.