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STUDIES ON STEROID METABOLISM IN HUMAN PROSTATIC TISSUE

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SUMMARY

The metabolism of six neutral steroids and three steroid conjugates by human prostatic tissue has been investigated. A technique is described for the quantitative determination of radiometabolites from any number of substrates. The main products from progesterone were 5α-pregnanedione and 20α-dihydroprogesterone; and from 17α-hydroxyprogesterone, 17α,20α- and 17α,20β-dihydroxy-4-pregnen-3-one. There was no significant transformation of C_{21} to C_{19} steroids. Dehydroisoandrosterone was transformed in low yield to androstenedione and 5α-androstanedione. Androstenedione and testosterone were interconvertible. The main metabolites of the former were 5α-androstanedione and androsterone, and from the latter 5α-dihydrotestosterone, 5α-androstanedione and 5α-androstane-3α,17β-diol. Testosterone was isolated from incubations with testosterone sulphate and testosterone glucuroniside, and there was evidence for the formation of conjugated androstanolone from these precursors.

THE NORMAL growth and secretory activity of the human prostate has been shown to be dependent upon the biosynthesis and secretion of testicular hormones. Furthermore, many of the retrogressive changes in morphology and function which follow castration can be reversed by the administration of testosterone derivatives. However, there has not yet been a systematic study of the concentrations of steroid hormones in human prostatic tissue nor in effluent blood; and recent studies on steroid transformations *in vitro* have been limited to incubations with testosterone and 19-nortestosterone. The literature has been the subject of a recent review[1], and the principal findings were that testosterone (17β-hydroxy-4-androsten-3-one) and androstenedione (4-androstene-3,17-dione) are interconvertible[2–6]; and there is evidence for the further metabolism of testosterone to various products with a 5α-configuration[3]. In addition, the subcellular localisation of enzymes involved in the transformation of testosterone-4-^{14}C has been investigated[7] and it has been reported that preparations of human prostate can demethylate testosterone to compounds of the 19-nor series and form 2-methoxyoestrone[8, 9].

The present report extends this approach to an investigation of the formation of C_{19} steroids from C_{21} precursors, and the transformation of Δ^5-3β-hydroxysteroids to the corresponding Δ^4-3-ketonic steroids. This involved the identification and quantitative determination of radiometabolites from pregnenolone (3β-hydroxy-5-pregnen-20-one), progesterone (4-pregnene-3,20-dione), 17α-hydroxyprogesterone (17α-hydroxy-4-pregnene-3,20-dione) and dehydroisoandrosterone (3β-hydroxy-5-androsten-17-one) by minces of human prostate. In addition, the principal radiometabolites have been determined from incubations with androstenedione and testosterone, and further studies on the metabolism of testosterone have involved varying the time of incubation. This approach may provide further information on the sequence of reactions, and the production of both minor metabolites and the products of multiple enzyme activities. Finally,

studies have been performed on the transformation of C_{19}-steroids conjugated with either glucosiduronic or sulphuric acid.

MATERIALS AND METHODS

Solvents and reagents

All solvents were of analar grade and redistilled before use. Pyridine and acetic anhydride were redistilled under reduced pressure and stored over calcium chloride. Silica gel (Camag DSF-5) with binding agent and fluorescent indicator was used for thin-layer chromatography (TLC).

Steroids

Authentic steroid standards for use as chromatographic markers and for recrystallisation studied were obtained from Sigma Chemical Co., (St. Louis, Mo., U.S.A.).

Substrates

The following substrates were obtained from The Radiochemical Centre, Amersham, Bucks., England

4-^{14}C pregnenolone specific activity 24 mCi/mM,
4-^{14}C pregesterone specific activity 58·5 mCi/mM,
4-^{14}C 17α-hydroxyprogesterone specific activity 35·9 mCi/mM,
4-^{14}C dehydroisoandrosterone specific activity 57·1 mCi/mM,
4-^{14}C androstenedione specific activity 56·6 mCi/mM,
4-^{14}C testosterone specific activity 55·2 mCi/mM.

Testosterone-7α-^{3}H-β-D-glucuroniside specific activity 20 Ci/mM, testosterone-7α-^{3}H-sulphate (ammonium salt specific activity 10 Ci/mM), 4-^{14}C dehydroisoandrosterone sulphate (ammonium salt specific activity 58·8 mCi/mM) were obtained from New England Nuclear Corp., Boston, Mass., U.S.A. An aliquot from each batch was tested for radiochemical purity by thin-layer chromatography, followed by autoradiography and liquid scintillation counting.

Radioactivity measurements

A Nuclear Chicago Model 6860 (Mark 1) liquid scintillation counting (LSC) system was used for the determination of radioactivity. The scintillation fluid contained 3 g of 2,5-diphenyl oxazole (PPO) in 1 l. of toluene. The counting efficiency for each sample was determined by reference to a calibration curve which was plotted from a set of quenched standards using an external ^{133}Ba source and a channels ratio method.

Tissue

The prostate glands were removed at suprapubic prostatectomy, and in each case histological examination indicated benign hypertrophy.

Preparation of tissue and incubation conditions

The radioactive substrates (2 μg in 10 μl) were added to 25 ml incubation flasks and taken to dryness under a stream of nitrogen. Fresh tissue was used in all studies, the incubations starting 10–20 min after surgery. The tissue was

minced and divided into a number of portions which were weighed on water repellent paper, and washed into the incubation flasks with 3 ml of Krebs Ringer Bicarbonate Buffer at pH 7·4. 1 mg of benzyl penicillin was added to each flask. The tissues were incubated at 37°C for 4 hr under 5% carbon dioxide in oxygen. The reactions were terminated by the addition of 10 ml redistilled acetone, and the flasks stored overnight in a refrigerator at 4°C.

IDENTIFICATION AND DETERMINATION OF RADIOMETABOLITES

Extraction of neutral steroids

The incubation mixture was decanted through filter paper, and the residual tissue re-extracted twice with 4 vol. of hot acetone. The pooled acetone extracts were evaporated under reduced pressure in a flash evaporator until only the buffer remained. The volume was made up to 10 ml with distilled water and the pH adjusted to 10·0 with sodium hydroxide. The steroids were reextracted from the aqueous medium with 2×50 ml diethyl ether and the combined ether extracts washed twice with 20 ml distilled water. The ether extract was taken to dryness, and 5% was removed for liquid scintillation counting.

Analysis of conjugated steroids

After extraction with diethyl ether the aqueous fraction was hydrolysed with β-glucuronidase or solvolysed with ethyl acetate.

β-Glucuronidase hydrolysis

When testosterone-7α-^{3}H-β-D-Glucuronoside was substrate the aqueous fraction was heated on a water bath to remove any remaining ether. The pH was then adjusted to 4·5 with 0·1 *N* hydrochloric acid, and 2 ml of β-glucuronidase, 1 ml of acetate buffer (pH 4·5) and 5 mg of benzyl penicillin were added and incubated overnight at 37°C. The pH was re-adjusted to 10·5 and extracted with 3×50 ml diethyl ether. The ether extract was washed twice with distilled water, evaporated to dryness and 5% removed for liquid scintillation counting.

Solvolysis of sulphates

When testosterone-7α-^{3}H-sulphate and dehydroisoandrosterone-4-^{14}C-sulphate were substrates the aqueous fractions were solvolysed according to the method of Burstein and Lieberman[10]. The ethyl acetate extract was filtered, separated from the aqueous layer, and evaporated to dryness under vacuum in a rotatory evaporator. An aliquot (5%) was removed for liquid scintillation counting.

Thin-layer chromatography and formation of derivatives

The dried extracts were transferred to a glass plate coated with silica gel to a thickness of 250 μ. The chromatogram was developed in chloroform:methanol (99:1 v/v), and the spectrum of metabolites examined by autoradiography with overnight exposure of the plates to Kodirex non-screen X-ray film (Kodak Ltd., London, W.C.1.). The areas of radioactivity were aspirated on to a filter disc (No. 3 grade) and eluted with ethanol ($3 \times 0·5$ ml). The eluates were evaporated, redissolved to a known volume and 5% removed for liquid scintillation counting. The remaining silica gel along the path of the chromatogram was also aspirated onto a filter disc, eluted with ethanol and the radioactivity determined.

The dried steroid eluates were acetylated and further fractionated on silica

gel in the system toluene:ethyl acetate (9:1 v/v). The products were located by autoradiography and the radioactivity determined in areas corresponding to side and central standards, and in appropriate areas of residual silica gel, by liquid scintillating counting.

Recrystallisation to constant specific activity

Approximately 40 mg of the appropriate non-labelled steroid was added to each radiometabolite and after determination of the initial specific activity the mixtures were repeatedly recrystallised with appropriate solvents. All weighings were made on a Mettler M5 balance (E. Mettler Ltd., Zurich) until successive weights were within 0·5%. The radioactivity was determined by liquid scintillation counting to an accuracy of better than 2%, and the results corrected for quenching. A mathematical analysis of the errors inherent in this approach (Axelrod *et al.*[11]) indicates that if three successive recrystallisations yield values for the specific activity of the crystals which are within ±5% of the average of the three values, then the unknown radiometabolite is probably identical with the known non-radioactive substance.

Calculation of results

A generalised scheme for calculating the percentage of initial radioactivity in any metabolite from either neutral or conjugated fractions is illustrated in Fig. 1. In order to correct for losses during the procedure aliquots are taken for liquid scintillation counting at all stages. The stepwise calculation in terms of the radioactivity in various fractions and the residual activity after elution from the

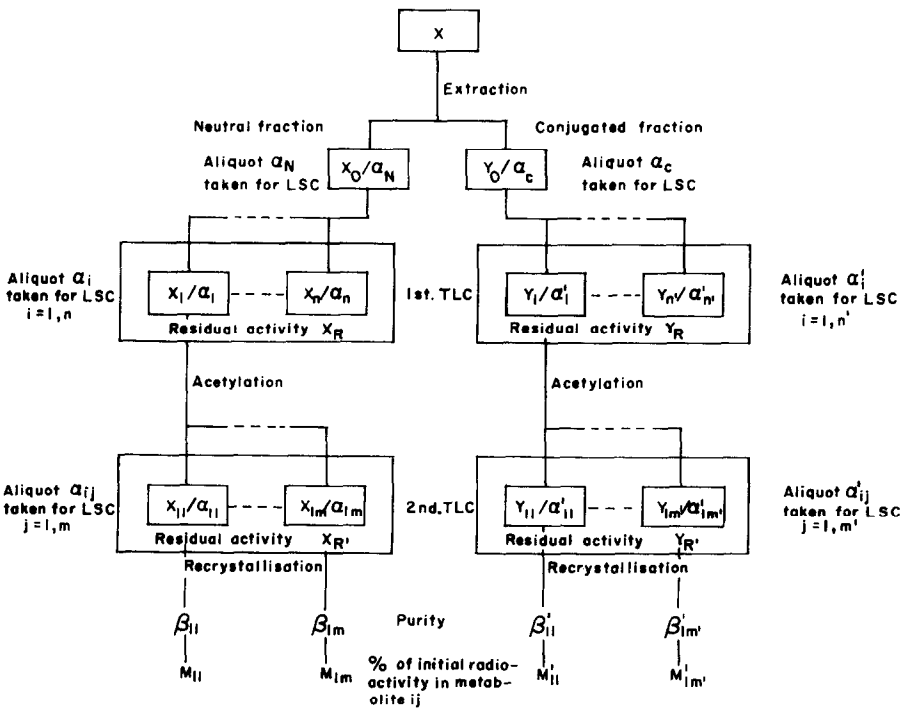

Fig. 1. Scheme for the identification and quantitative determination of radiometabolites.

plates is a tedious procedure, and for this reason the various corrections for losses have been expressed for calculation of the result by a bench-top computer. The symbols used for each fraction are illustrated in the figure:

X is the number of disintegrations/minute of substrate and X_0 and Y_0 the disintegrations/minute in the respective aliquots (α_N and α_C) taken after extraction and solvent partition into fractions containing principally neutral and conjugated steroids.

(1) *Quantitative determination of neutral radiometabolites*

X_R is the residual activity on the first thin-layer plate, and X_i the disintegrations/minute in the aliquots (α_i) taken from the areas removed for further purification. Similarly, $X_{R'}$ and X_{ij} refer to the second thin-layer plate, and after taking a further aliquot (α_{ij}) for liquid scintillation counting, the remainder of each extract is recrystallised to constant specific activity. This gives a factor β_{ij} which is the purity of metabolite M_{ij}. The formula for the calculation of the percentage of the initial radioactivity in metabolite M_{ij} is:

$$M_{ij} = \beta_{ij} \cdot \frac{X_{ij}}{\alpha_{ij}} \cdot \frac{X_i}{\alpha_i \left[X_{R'} + \sum_{j=1}^{m} \frac{X_{ij}}{\alpha_{ij}} \right]} \cdot \frac{100}{\left[X_R + \sum_{i=1}^{n} \frac{X_i}{\alpha_i} \right]} \cdot \frac{X_0}{[X_0 + Y_0]}. \quad (1)$$

(2) *Quantitative determination of conjugated neutral radiometabolites*

The procedure is the same as for the neutral steroids. Y_R is the residual activity on the first thin-layer plate, and Y_i the number of disintegrations/minute in the aliquots α'_i taken from the areas removed for further purification. Similarly $Y_{R'}$ and Y_{ij} refer to the second thin-layer plate after acetylation of the metabolites. After elution a further aliquot α'_{ij} is taken for liquid scintillation counting and the remainder of the extract is recrystallised to constant specific activity. The formula for calculating the percentage of the initial radioactivity in metabolite M'_{ij} is:

$$M'_{ij} = \beta'_{ij} \cdot \frac{Y_{ij}}{\alpha'_{ij}} \cdot \frac{Y_i}{\alpha'_i \left[Y_{R'} + \sum_{j=1}^{m'} \frac{Y_{ij}}{\alpha'_{ij}} \right]} \cdot \frac{100}{\left[Y_R + \sum_{i=1}^{n'} \frac{Y_i}{\alpha'_i} \right]} \cdot \frac{Y_0}{[X_0 + Y_0]}. \quad (2)$$

EVALUATION OF METHOD

The procedure for the quantitative determination of radiometabolites has been evaluated theoretically and in practice by replicate analyses of a known mixture of labelled steroids.

Theoretical assessment

In an attempt to determine the random error on the percentage of initial radioactivity in metabolite M_{ij} the random errors on each term in equation (1) were assessed. The overall percentage error on M_{ij} (ΔM_{ij} = (standard deviation × 100)/M_{ij}) was then determined from equation (3) below, derived from the usual laws for the combination of additive and multiplicative independent normal errors.

$$\Delta^2 M_{ij} = \Delta^2\beta_{ij} + \Delta^2 X_{ij} + \Delta^2 X_i + \Delta^2\alpha_{ij} + \Delta^2\alpha_i$$

$$+ \frac{\Delta^2 X_{R'} \cdot X_{R'}^2 + \sum_{i=1}^{m} \left(\frac{X_{ij}}{\alpha_{ij}}\right)^2 \cdot (\Delta^2 X_{ij} + \Delta^2\alpha_{ij})}{\left[X_{R'} + \sum_{j=1}^{m} \frac{X_{ij}}{\alpha_{ij}}\right]^2}$$

$$+ \frac{\Delta^2 X_R \cdot X_R^2 + \sum_{i=1}^{m} \left(\frac{X_i}{\alpha_i}\right)^2 \cdot (\Delta^2 X_i + \Delta^2\alpha_i)}{\left[X_R + \sum_{i=1}^{n} \frac{X_i}{\alpha_i}\right]^2}$$

$$+ \frac{\Delta^2 X_0 + \Delta^2 X_0 \cdot X_0^2 + \Delta^2 Y_0 \cdot Y_0^2}{(X_0 + Y_0)^2} \qquad (3)$$

where:

$\Delta\beta_{ij}$ = the overall percentage error on β_{ij}, and similarly for other terms.

The maximum error on each term in equation (1) was evaluated as follows:

α_{ij}, α_i. There are two errors in each term, one from diluting the extract to 1 ml and one from removing 50 μl for counting. These two errors were then combined to give:

$$\Delta = (\Delta_1^2 + \Delta_2^2)^{1/2} = (2{\cdot}0^2 + 1{\cdot}0^2)^{1/2} = 2{\cdot}2\%.$$

X_{ij}, X_i, $X_{R'}$, X_R. Similarly there are two errors in each of these terms. One from the counting error assuming Poisson statistics, and one from the determination of counting efficiency by an external standard channels ratio method. As 20,000 counts were recorded for each sample the counting error was about 0·7%, and the error on efficiency was estimated to be 3%. These combine to give an overall error of 3·1%.

X_0, Y_0. There are four errors associated with each of these terms – two in taking the aliquot and two in determining the corrected activity as evaluated above. These combine to give

$$\Delta = (3{\cdot}1^2 + 2{\cdot}2^2)^{1/2} = 3{\cdot}8\%.$$

β_{ij}. There are four errors contributing to this term. The weight of the aliquot of initial sample and crystals after the third crystallisation, and the determination of the number of disintegrations/minute in both samples. All samples were weighed to within 0·5% and the number of disintegrations/minute determined to within 3·1%. These values combine to give an overall error of 4·5%.

If these values are incorporated into equation (3) then the total random error on M_{ij} will be approximately 7%, and if the aliquots taken at each stage are the same and counted under identical conditions then the percentage error will be similar for all products.

Practical assessment

The theoretical evaluation of the procedure assumes equal extraction, complete separation into neutral and conjugated fractions and equal losses of steroids within the polarity range under investigation. To test these assumptions two series of experiments were undertaken. In the first, known amounts of carbon-14 labelled progesterone and testosterone and tritiated testosterone sulphate and testosterone glucuroniside (approximately 200,000 dis/min) were added to flasks containing 700 mg of prostatic tissue and 3 ml of buffer. The tissues were extracted with acetone and ether as described in the method and 5% of the ether extract and 5% of the aqueous fraction removed for liquid scintillation counting. The mean recovery of carbon-14 in the ether fraction was 94% and contained less than 2% of the added tritium. The mean recovery of tritium and carbon-14 in the aqueous fraction was 87% and less than 1% respectively.

In the second series of experiments a known mixture of carbon-14 labelled progesterone, testosterone, dehydroisoandrosterone, dehydroisoandrosterone sulphate and tritiated testosterone glucuroniside were added to 700 mg aliquots of prostatic tissue. The mixtures were analysed and the radioactivity associated with each component (corrected for quenching and experimental losses) was expressed as a percentage of the total radioactivity added. The results from six replicate determinations are shown in Table 1. The mean recovery of the five steroids was 85% and as the theoretical error was 7%, the mean practical recovery is within 2σ of the amount added.

RESULTS

The prostatic tissue mince used in this series of experiments was prepared from three complete prostate glands. Histological examination of all tissues indicated that benign hypertrophy had occurred. The tissues were incubated with six neutral steroid substrates and three steroid conjugates for four hours without the addition of co-factors. Approximately 1·40 g of tissue was incubated with the C_{21} substrates as preliminary experiments indicated that there was low transformation of these compounds. The C_{19} steroids which were more extensively transformed were incubated with approximately 700 mg of tissue.

Table 1. Replicate analyses of labelled steroids added to prostatic tissue

Mixture	Percentage added	Recovery from 6 replicate analyses mean % ± S.D.	% Recovery of mean
Progesterone	18·31	16·41 ± 0·69	89·6
Dehydroisoandrosterone	15·61	13·12 ± 0·72	84·0
Testosterone	17·08	14·89 ± 1·32	87·2
Dehydroisoandrosterone sulphate	20·13	17·41 ± 2·46	86·5
Testosterone sulphate	28·87	22·31 ± 3·61	77·3
Total	100	—	—

Control incubations

Seven incubation flasks were prepared with 700 mg of tissue, 3 ml of buffer and 10 ml of acetone. 2 μg of a different substrate were added to each flask, which were agitated by hand, and extracted after leaving overnight at 4°C. No identifiable radiometabolites were visible on the first autoradiogram and the percentage of each substrate recovered (corrected for losses and purity) ranged from 84 to 95%.

Incubation with C_{21}-steroids

When pregnenolone was incubated with prostatic tissue, 88% of the radioactivity was recovered as untransformed substrate, and insufficient progesterone or 17α-hydroxypregnenolone (3β,17α-dihydroxy-5-pregnen-20-one) had accumulated for positive identification. The principal product from progesterone was 5α-pregnane-3,20-dione (36%), with a lower yield (15%) of 20α-dihydroprogesterone (20α-hydroxy-4-pregnen-3-one); 30% of the initial radioactivity was recovered as untransformed substrate and a similar percentage was associated with unidentified products. When 17α-hydroxyprogesterone was used as substrate, 5% was recovered untransformed. However, there was no evidence for identifiable yields of androstenedione. The principal products were tentatively identified on the basis of their chromatographic properties as 17α,20α-dihydroxy-4-pregnene-3-one (31%) and 17α,20β-dihydroxy-4-pregnene-3-one (4·5%). In addition there was chromatographic evidence for the formation of a 5α-reduced metabolite of 17α-hydroxyprogesterone (15%) which resisted acetylation and formed 5α-androstanedione (5α-androstane-3,17-dione) after oxidation.

Incubation with C_{19}-steroids

The metabolites which were identified from dehydroisoandrosterone are shown in Table 2. Untransformed substrate accounted for 69% of the initial radioactivity, and only 0·7% was transformed to products which were readily identified. There was no significant yield of either 5-androstene-3β,17β-diol or testosterone. Androstenedione was converted in good yield to 5α-androstanedione (Table 3) and to smaller amounts of androsterone (3α-hydroxy-5α-androstan-17-one), 5α-dihydrotestosterone (17β-hydroxy-5α-androstan-3-one) and testosterone. 5α-Dihydrotestosterone was the principal metabolite from testoster-

Table 2. Percentage conversion *in vitro* of 4-^{14}C dehydroisoandrosterone into various metabolites by human prostatic tissue

Metabolites	Initial specific activity		Recrystallisation 1st	2nd	3rd	Purity	% Conversion
5α-androstanedione	42	C	57	54	52	100%	0·16
		ML	65	34	66		
Androstenedione	22	C	26	23	22	100%	0·54
		ML	30	31	27		
Untransformed dehydroisoandrosterone	2001	C	1955	2013	1971	99%	69·3
		ML	2286	2110	1989		
				Radioactivity accounted for			70·4

Table 3. Percentage conversion *in vitro* of 4-^{14}C androstenedione into various metabolites by human prostatic tissue

Metabolites	Initial specific activity		Recrystallisation 1st	2nd	3rd	Purity	% Conversion
5α-androstanedione	1630	C	1615	1589	1573	96%	39·7
		ML	1729	1618	1661		
5α-dihydrotestosterone	65	C	68	70	60	92%	1·70
		ML	72	69	64		
Testosterone	19	C	19	18	17	89%	0·41
		ML	56	24	17		
Androsterone	120	C	99	92	86	72%	5·39
		ML	249	185	119		
Untransformed androstenedione	1219	C	1239	1302	1297	100%	36·42
		ML	1264	1338	1448		
					Radioactivity accounted for		83·6

one (Table 4), with smaller yields of 5α-androstanedione, androstenedione and 5α-androstane-3α,17β-diol.

Kinetic study with 4-^{14}C testosterone

Seven gramme of tissue was minced and divided into five equal portions which were all incubated with 2 μg of testosterone. The reactions were terminated after 0·5, 1, 2, 3 and 4 hr. The transformation of substrate and percentage of the initial radioactivity incorporated into the principal metabolites is shown in Fig. 2.

Incubation with conjugated C_{19} steroids

Two aliquots of tissue mince, each weighing 1·40 g and removed from different glands, were incubated with 2 μg of dehydroisoandrosterone sulphate. From the

Table 4. Percentage conversion *in vitro* of 4-^{14}C testosterone into various metabolites by human prostatic tissue

Metabolites	Initial specific activity		Recrystallisation 1st	2nd	3rd	Purity	% Conversion
5α-androstanedione	347	C	357	351	347	100%	2·49
		ML	378	359	359		
Androstenedione	344	C	342	365	327	95%	2·68
		ML	350	320	362		
5α-dihydrotestosterone	1077	C	1038	994	978	91%	24·28
		ML	1182	1082	1092		
5α-androstane-3α, 17β-diol	390	C	392	389	385	99%	2·94
		ML	398	395	390		
Untransformed testosterone	2030	C	2075	2050	2008	99%	43·21
		ML	3259	2462	2264		
					Radioactivity accounted for		75·4

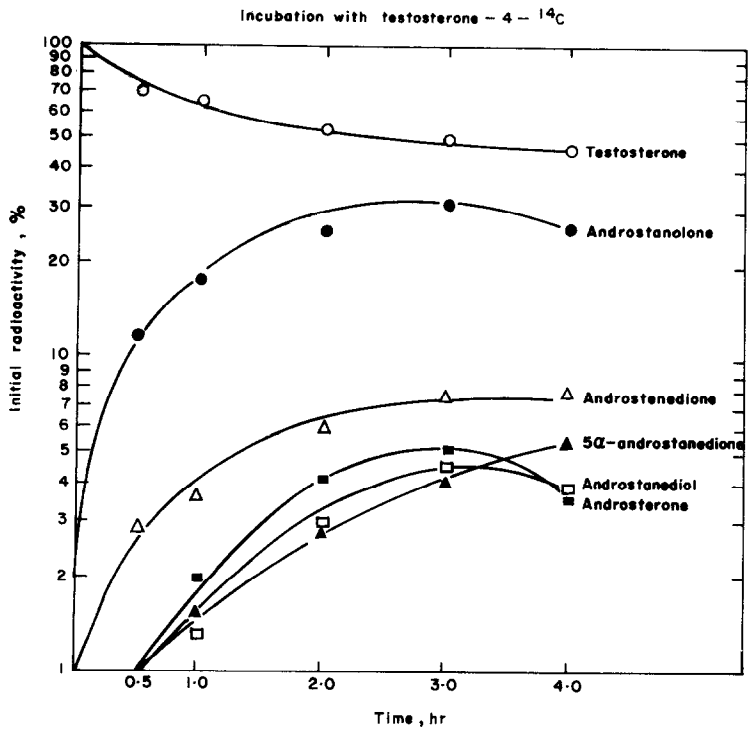

Fig. 2. The transformation of testosterone-4-^{14}C by human prostatic tissue with respect to time. (Androstanolone = 17β-hydroxy-5α-androstan-3-one; androstanediol = 3α,17β-dihydroxy-5α-androstan.)

two experiments 9 and 10% of the initial radioactivity was extracted with diethyl ether. Dehydroisoandrosterone was identified in this fraction by recrystallisation to constant specific activity and accounted for 8% and 9% of the initial radioactivity respectively. 48% and 31% of the initial radioactivity was recovered as untransformed substrate (Table 5). In another experiment two aliquots of tissue mince (1·4 g) from different glands were incubated with 5 ng of testosterone-7α-^{3}H-sulphate. 3·5 and 12·0% of the initial activity was extractable with diethyl ether and testosterone and 5α-dihydrotestosterone were identified in this fraction (Table 5) by recrystallisation to constant specific activity. 5α-Dihydrotestosterone was also identified and determined in the aqueous fraction after hydrolysis.

When a further two aliquots of tissue mince (1·4 g) from different glands were incubated with 5 ng of testosterone glucuronoside, 2·0 and 16·2% of the initial radioactivity was extractable with ether. Testosterone and 5α-dihydrotestosterone were isolated and determined after further purification of this fraction (Table 5). In addition, testosterone and 5α-dihydrotestosterone were recovered in the aqueous fraction after hydrolysis with β-glucuronidase.

DISCUSSION

The role of androgens and oestrogens upon the morphology and function of the prostate gland has been studied extensively since the pioneer investigations of Moore and Price in 1932[12]. However, there is limited information about the metabolic transformation of steroids in human prostatic tissue. The present

Table 5. Incubation of prostatic tissue with C_{19} steroid conjugates

	Initial specific activity		Recrystallisation 1st	2nd	3rd	Purity	% Initial radioactivity
*Dehydroisoandrosterone sulphate†	612	C	591	588	585	96%	49·21
		ML	673	654	613		
Dehydroisoandrosterone	481	C	485	478	480	100%	17·30
		ML	531	523	509		
*Testosterone sulphate†	56	C	43	43	42	75%	38·81
		ML	153	74	44		
5α-dihydrostosterone sulphate†	42	C	37	46	35	83%	2·73
		ML	155	189	84		
Testosterone	59	C	71	60	58	98%	0·19
		ML	240	109	61		
5α-dihydrotestosterone	49	C	48	33	34	70%	2·39
		ML	187	167	89		
*Testosterone glucuronoside†	241	C	172	166	174	72%	53·84
		ML	552	406	264		
5α-dihydrotestosterone glucuronoside†	29	C	36	22	26	90%	0·78
		ML	178	86	52		
Testosterone	55	C	61	58	56	100%	0·89
		ML	111	97	88		
5α-dihydrotestosterone	63	C	60	59	57	90%	0·22
		ML	116	131	61		

*Unmetabolised substrate.
†Recrystallised after hydrolysis.

investigation was primarily designed to isolate and identify the principal products derived from the incubation of human prostatic tissue with a wider spectrum of labelled steroid substrates without attempting to relate the transformations to prostatic morphology. This point is emphasised in view of the finding that certain areas of the gland respond differently to administered hormones [13] and the fact that carcinoma occurs mainly in the dorsal region [14]. With regard to the interpretation of the results, it must also be recognised that reactions other than those demonstrated under these experimental conditions may occur *in vivo* but it is generally accepted that the *in vitro* approach provides useful information about possible transformations and the probable sequence of metabolic events.

Previous studies *in vitro* have usually involved a similar approach to the isolation, identification and quantitative determination of radiometabolites. After the incubation has been terminated, the various products expected from a particular substrate have been added in known amounts to the incubation mixture. The extracts have then been taken through a procedure, devised on theoretical grounds to separate the particular steroids under investigation, and the amount of radioactivity in each product determined by liquid scintillation counting. The purity of the products are checked by recrystallisation with authentic steroids to constant specific activity, and the losses during the procedure are calculated for each product by estimating the decrease in mass of the steroids added at the end of the incubation.

This procedure has certain disadvantages. In the first instance only those steroids are examined which are expected to be found, or which the investigator

thinks are particularly important. Secondly, large amounts of the carrier steroids must be added so that dilution by endogenous steroids will not be too great. This is especially a problem in incubation flasks where the intermediaries may accumulate. Thirdly, the isolated steroids must be chemically as well as radiochemically pure; and finally it is sometimes difficult to find suitable physicochemical methods to determine the mass of certain products.

In view of these limitations the present procedure was developed which enables any metabolite from any number of sub-fractions to be determined with reasonable accuracy. The application to studies on steroid metabolites from human prostatic tissue has shown that there is minimal transformation of C_{21} substrates, with no significant $\Delta^5 \rightarrow \Delta^4$ isomerase, 17α-hydroxylase or lyase activity. Thus pregnenolone was not transformed to identifiable amounts of either 17α-hydroxypregnenolone or progesterone; progesterone was not transformed to significant yields of 17α-hydroxyprogesterone nor was the latter transformed to androstenedione. The principal products from progesterone were 5α-pregnanedione and 20α-dihydroprogesterone, and there was chromatographic evidence for the formation of 17α,20α-dihydroxy-4-pregnen-3-one (and a low yield of the 20β-isomer) from 17α-hydroxyprogesterone.

In general the C_{19} steroids were more extensively metabolised. A small proportion of dehydroisoandrosterone was transformed into radiochemically pure androstenedione, thus demonstrating a low 3β-hydroxy-dehydrogenase and $\Delta^5 \rightarrow \Delta^4$-isomerase activity with this substrate. However, there was no evidence for an accumulation of 5-androstene-3β,17β-diol or testosterone—suggesting low 17β-reductase activity. There was a small conversion of androstenedione to testosterone, and a low yield of androstenedione from testosterone in the same tissue, again indicating that under these basal conditions there was low 17β-reductase activity. With both substrates, there was high 5α-reductase activity. The principal product from testosterone was 5α-dihydrotestosterone, while the main products from androstenedione were 5α-androstanedione and androsterone. The time study with testosterone also demonstrated high 5α-reductase activity and relatively lower 17β-reductase activity.

The isolation of dehydroisoandrosterone from incubations with dehydroisoandrosterone sulphate indicated sulphatase activity in this tissue, as did the isolation of testosterone from the incubations with testosterone sulphate. In addition, the isolation of 5α-dihydrotestosterone in the conjugated fraction after hydrolysis from both testosterone sulphate and testosterone glucuroniside indicated that reduction at the 5α position may occur without prior removal of the sulphate or glucuronic acid group.

These studies suggest that there is a wide spectrum of metabolites from testicular hormones and their conjugates, which is especially interesting when correlated with the potential biological activity of the various precursors and products. Thus, Dorfman and Shipley[15] reported that dehydroepiandrosterone and androstenedione possessed only 20% of the activity of testosterone in promoting growth of the capon comb and in increasing the weight of the ventral prostate of the rat, while 5α-dihydrotestosterone has been shown to possess two to three times the activity of testosterone in promoting growth of the rate prostate [15] and seminal vesicles[16]. A recent study on the action of testosterone metabolites on rat prostatic tissue grown in culture medium suggested that 5α-dihydrotestosterone was especially active in promoting cell division[17].

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