THE EXCRETION OF METABOLITES OF TESTOSTERONE AND OF ESTRADIOL IN MALE PATIENTS WITH CHRONIC RENAL FAILURE

E. van Kammen, J.H.H. Thijssen, G.H. Donker and F. Schwarz
Department of Clinical Endocrinology, University Hospital,
Utrecht, The Netherlands.

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ABSTRACT

Intravenous infusions of ^{14}C-testosterone (Te) either alone or in combination with ^{3}H-estradiol (E_2) were given to five normal male subjects, twelve male patients on haemodialysis (HD) treatment and to one patient with a very restricted renal function. The elimination of radioactivity was measured in urine, HD fluid and faeces.

Urinary excretion diminished with renal function. It was negligible at a creatinine clearance of less than one ml per minute. A quarter of both isotopes was eliminated by six HD treatments within three weeks. No difference was found in this respect between nephrectomized patients and those who were still in possession of their kidneys.

The main excretion occurred in the stools. E_2 metabolites, and to a lesser extent Te metabolites, appeared in the faeces within 24 hours, which might be explained by biliary excretion only. More ^{3}H (E_2 metabolites) than ^{14}C (Te metabolites) was found in the faeces; more ^{14}C than ^{3}H was found in HD fluid.

INTRODUCTION

The main route for the excretion of steroid hormone metabolites is the urine. In 1951 West et al. (1) demonstrated that the urinary excretion of testosterone (Te) metabolites (17-ketosteroids) decreased with diminishing renal function. No data are available on the excretion of Te or estradiol (E_2) metabolites in patients with severe chronic renal failure. As part of a study on the production and metabolism of sex hormones in uraemic males we (2) investigated the excretion of radioactivity in urine, faeces and haemodialysis (HD) fluid after intravenous infusion of ^{14}C-Te and/or ^{3}H-E_2. Subjects of the study were male patients on chronic intermittent HD treatment.

SUBJECTS

Eight male patients, age 20 to 50 years, who had been on HD treatment (during eight hours twice weekly) for at least three months, received an infusion of ^{14}C-Te. Four patients, age 28 to 51 years, who were also on HD treatment for at least three months, received a combined infusion of ^{14}C-Te and ^{3}H-E_2. ^{14}C-Te was given to one male patient (age 38 years) with a creatinine clearance of 12 ml per minute, who was not on HD, and to three normal volunteers, age 31 to 49 years. Two other normal males received ^{3}H-E_2 in combination with ^{14}C-Te.

Liver function tests in all subjects were within normal limits.

All subjects participated in this investigation after informed consent.

MATERIALS AND METHODS

Testosterone-4-^{14}C (specific activity 59 mCi/mmol) (10) and estradiol-6,7-^{3}H (specific activity 38.5 Ci/mmol) (10) were used from Radio Chemical Centre, Amersham (Great Britain). Purity was checked by thin layer chromatography.

All infusions were given between 1:30 and 4:30 P.M. The administered dose of radioactivity was on the average 15 microCurie for ^{3}H and 10 microCurie for ^{14}C.

Radioactivity was measured in a Packard Tricarb 3380 liquid scintillation counter with automatic quench correction (Packard Tricarb AAA 325). One ml of urine or 5.0 ml samples of HD fluid were counted with 10.0 ml Instagel^{R} (Packard). After homogenization of the faeces 0.5 g samples were burned in a Packard Sample Oxydizer Tricarb 306 and radioactivity of $^{14}CO_2$ and of $^{3}H_2O$ was measured. All samples were assayed in triplicate. Urine and faeces collections were started at the end of the infusion procedure with labeled steroids, in portions of 24 hours.

HD fluid was sampled at the arterial side of the artificial kidney at 10, 240 minutes, and shortly before the end of the HD treatment. From the mean values of these three samples the radioactivity was calculated on a total volume of 360 liters of HD fluid. Artificial kidney types used were Gambro Lundia Nova 17 micron and Rhône Poulenc R.P. 5 type.

RESULTS

The excretion of radioactive metabolites of ^{14}C-Te and of ^{3}H-E_2 in urine of males with normal kidney function is given in table 1 and table 2. The first columns of table 1 and table 2 show that in normal subjects the excretion of Te metabolites occurs faster than that of E_2 metabolites. In the patients with renal insufficiency the total excretion measured during four days decreases with diminishing renal

function. In one patient with an endogenous creatinine clearance of 2 to 3 ml per minute the excretion was followed during 13 days, after which period 24.9% of the ^{14}C-radioactivity was excreted.

A combined infusion of Te and E_2 was given to four patients with severe renal failure. As far as can be judged from the low activity found in their urine, there was no difference in their rate of excretion of tritium and carbon-14 (see table 2).

Table 1. Urinary excretion of radioactivity after administration of a tracer dose of ^{14}C-Te intravenously, in normal subjects and in patients with renal insufficiency.

	normals	creatinine clearance ml/min: 12	2-3	≤1
number:	5	1	1+)	7+)
day 1	69.0% (54.8-77.5)	30.2%	4.1%	0.7% (0.1-1.9)
day 2	8.4% (4.3-11.8)	20.1%	3.9%	0.9% (0.0-2.3)
day 3	2.3% (1.5- 3.4)	9.3%	5.0%')	0.7% (0.0-2.1)
day 4	1.3% (1.0- 1.6)	4.0%	2.0%	0.8% (0.0-2.3)
total	81.0% (66.8-90.5)	63.5%	15.0%	3.1% (0.1-8.6)

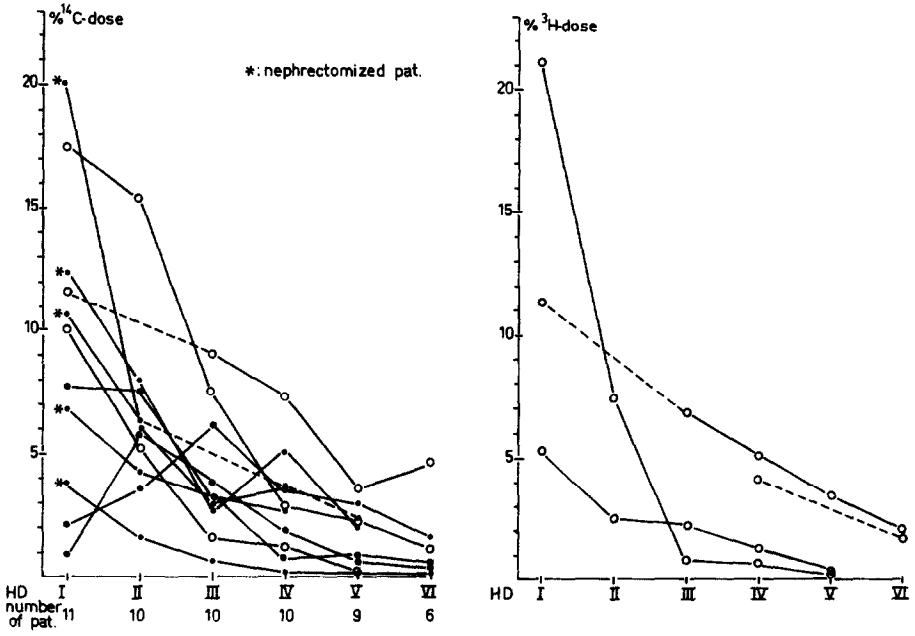
+) = patients on HD treatment
') = day of HD treatment

Table 2. Urinary excretion of radioactivity after administration of a tracer dose ^{14}C-Te combined with ^{3}H-E_2 intravenously, in normal subjects and in patients. Data on ^{14}C-Te excretion have also been included in table 1.

	^{14}C-Te metabolites		^{3}H-E_2 metabolites	
	normals	creat.clear. ≤1 ml/min	normals	creat.clear. ≤1 ml/min
number:	2	4	2	4
day 1	54.8%-72.3%	0.2% (0.1-0.5)	54.3%-49.7%	0.4% (0.1-1.0)
day 2	6.9%-11.8%	0.4% (0.0-0.9)	19.6%-23.1%	0.4% (0.0-1.2)
day 3	3.4%- 1.8%	0.3% (0.0-0.6)	9.0%- 3.3%	0.3% (0.0-0.9)
day 4	? - 1.6%	0.3% (0.0-0.5)	? - 1.6%	0.3% (0.1-1.0)
total	65.1%-87.5% 3-4 days	1.2% (0.1-2.5) 4 days	82.9%-77.7% 3-4 days	1.4% (0.3-4.1) 4 days

Radioactivity in HD fluid was measured in eleven patients for ^{14}C only and in four patients for ^{14}C and ^{3}H (figure 1, A and B). The total excretion in different patients varied considerably: from 6.4% to 46.6% (average 25.8%) for Te metabolites and from 11.9% to 30.2% (average 26.4%) for E_2 metabolites. Detectable radioactivity in HD fluid was present in three patients for ^{14}C and in two patients for ^{3}H as long as three weeks after the administration of the tracer (figure 1,A and B). No difference in the excretion of radioactivity could be found between nephrectomized patients and those who were not nephrectomized.

Figure 1. Radioactivity dose eliminated in HD fluid (360 L) after administration of ^{14}C-Te and ^{3}H-E_2.
Open cicles represent patients, who were given ^{14}C-Te and ^{3}H-E_2 simultaneously.



Faecal loss of radioactivity was measured in four patients with a creatinine clearance of less than one ml per minute. as shown in table 2, urinary excretion of radioactivity in them was negligible. Measurable

amounts of feacal radioactivity could be found in three of them even on the fourteenth day of collection (table 3). In all cases elimination of ^{3}H by the faecal route was quicker and more complete than of ^{14}C. After four days ^{3}H exceeds ^{14}C in each case by more than 50 per cent. Patients B and D showed a remarkably quick feacal excretion of ^{3}H. After four days they had already eliminated respectively 72 and 79 per cent of the given dose. From the two patients in whom complete data were obtained during two weeks, haemodialysis and faecal losses of radioactive metabolites are shown cumulatively in figure 2, A and B.

Table 3. Faecal excretion of radioactivity after administration of ^{14}C-Te and ^{3}H-E_2 simultaneously in four patients on HD treatment (the urinary excretion of these patients is given in table 2).

day	patient A		patient B		patient C		patient D	
	^{14}C %	^{3}H %	^{14}C %	^{3}H %	^{14}C %	^{3}H %	^{14}C %	^{3}H %
1	x	x	13.7	37.1')	4.5	24.1')	7.1	29.1
2	0.1	0.2	13.0	22.7	2.9	9.2	9.2	22.6')
3	12.1	20.7	7.7	8.4	x	x	9.6	12.4
4	12.0	15.3')	5.8	3.9	6.9	15.0')	10.2	14.6
5	8.8	6.4	6.8	2.2')	2.5	4.7	4.8	8.7
6	10.3	7.4	6.1	1.6	x	x	0.7	1.2')
7	5.3	4.9')	3.1	0.9	-	- ')	1.8	1.9
8	2.0	1.3	1.6	0.4')	-	-	-	-
9	2.9	2.5	2.9	0.3	-	-	-	- ')
10	1.9	1.5	1.9	0.2	-	-	-	-
11	1.8	1.6')	2.2	0.1	-	- ')	-	-
12	1.4	1.6	1.3	0.0')	-	-	-	-
13	0.8	2.1	1.4	0.0	-	-	-	- ')
14	0.6	0.6')	0.7	0.0	1.3	2.3')	0.4	0.3
total	60.0	66.1	68.2	77.8	18.1	55.3	43.8	90.8

x = no defaecation
\- = no faeces collection
') = day of HD treatment

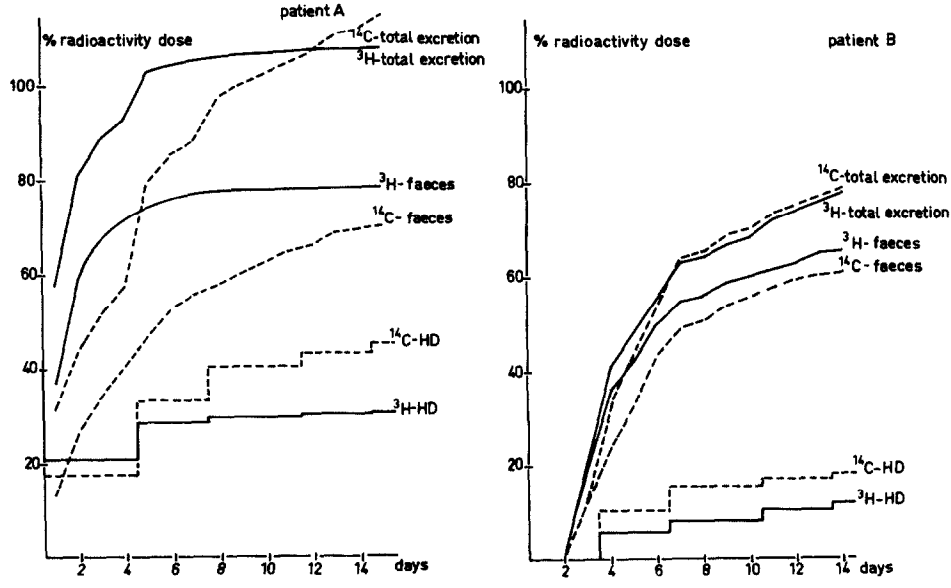
DISCUSSION

The findings in our normal subjects (table 1 and 2) are in good agreement with those of Sandberg and Slaunwhite (3, 4). Metabolites of Te as well as of E_2 are excreted in the urine to the extent of ca. 80 per cent within four days. The initial rate of excretion for Te is

greater than for E_2. This difference is probably due to the enterohepatic circulation which is of greater magnitude for E_2 than for Te.

In the patients with a creatinine clearance of less than one ml per minute urinary excretion of metabolite is negligible. HD accounted for about 25 per cent of the radioactivity eliminated in three weeks (six HD treatments). The greater part of the radioactivity was eliminated by the feacal route. In the two patients in whom complete collection could be done during two weeks, 60 to 80 per cent was found in the faeces. The excretion data are plotted together with the recovery from HD fluid cumulatively in figure 2. In patient A elimination appeared to be completed after two weeks; in patient B it was still continuing. In both cases more ^3H (E_2 metabolites) than ^{14}C (Te metabolites) was found in the faeces; more ^{14}C than ^3H in HD fluid. This difference in distribution might be explained by the metabolic fate of the steroids. Te metabolites circulate mainly as glucuronisidates (3), E_2 is presum ably converted for the greater part to E_1 sulfate , which is preferentially excreted into the bile and is probably less dialysable on account of protein binding (5, 6).

Figure 2. Cumulative excretion data of patients A and B, followed during 14 days after intravenous infusion of ^{14}C-Te and ^3H-E_2 simultaneously.



In three of our patients more than 24 per cent of E_2 metabolites appeared in the stools within the first 24 hours. These patients showed no clinical signs of accelerated transit through the bowels. The question arises whether it is possible to explain this fast excretion by biliary excretion only. In normal subjects Inoue et al.(7) demonstrated a direct elimination of estriol (10) (entero-enteric circulation). Direct passage to the bowels might be present in severe renal failure as was found by Van Waes (8) for urography contrast material. From our data it is not possible to prove direct elimination into the bowels as the fast excretion may be due to biliary excretion only. Preliminary experiments with ^{131}I-Rose-bengal in two of our patients (C and D) also showed excretion of radioactivity in the faeces within 24 hours.

In the patient with a creatinine clearance of 12 ml per minute, the pattern of urinary excretion of Te metabolites was preserved to some extent, be it greatly reduced. It was lost in the subject with a creatinine clearance clearance of 2 to 3 ml per minute. No faecal studies could be done in them. Figure 1 shows that there is no difference in HD fluid recovery between nephrectomized patients and those still in possession of their kidneys.

Finally it must be stated that our results are at variance with those of Raith et al.(9) who found in two nephrectomized patients only 7.5 and 8.0 per cent faecal excretion of Te metabolites during four days and only 3.4 per cent excretion into three HD fluid collections (one patient). Raith et al. used an extraction procedure to determine the radioactive metabolites in fluids and faeces. In our samples we measured all radioactivity without any extraction procedure. It may be assumed that the method used by our group, direct measurement of radioactivity, yields a better recovery.

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10. chemical names:
testosterone (Te) = 17β-hydroxy-4-androsten-3-one
estrone (E_1) = 3-hydroxy-1,3,5(10)-estratrien-17-one
estradiol (E_2) = 1,3,5(10)-estratrien-3,17β-diol
estriol (E_3) = 1,3,5(10)-estratrien-3,16α,17β-triol