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METABOLISM OF 4-C¹⁴-TESTOSTERONE IN HUMAN SUBJECTS. I. DISTRIBUTION IN BILE, BLOOD, FECES AND URINE

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The metabolism of testosterone administered to human subjects was studied soon after the isolation and synthesis of the testicular steroid hormone. In 1939, Dorfman, Cook, and Hamilton (1) and Callow (2) almost simultaneously reported the isolation of androsterone and etiocholanolone in the urine of men receiving testosterone propionate. During the 1940's, Lieberman, Dobriner and their co-workers (3-5) published extensive studies on steroids in human urine related to the metabolism of testosterone. West and his colleagues (6, 7) not only studied the urinary metabolites following the intravenous administration of testosterone (in human serum albumin), but in addition reported studies on the clearance from blood of testosterone and its metabolites (17-ketosteroids). All of the investigations cited were performed following the administration of very large, and hence unphysiological, doses of testosterone.

It was not until methods were developed for the synthesis of labelled testosterone that it became possible to study the metabolism of the hormone administered in physiological doses. Gallagher, Fukushima, Dobriner and their associates were the first to report observations on the metabolism of C¹⁴ and deuterium labelled testosterone (8-11). The amounts of testosterone administered by these investigators approximated, in most cases, the quantity of hormone calculated to be excreted by the testes. Fukushima, Dobriner, Gallagher, and Bradlow (10, 11) reported that the preponderant part of the administered labelled testosterone appeared in the urine with small amounts in the feces. The authors did not report on the radioactivity in blood.

The biliary excretion of steroids in human subjects has not been studied extensively. Such studies are of importance in the elucidation of testosterone metabolism in man, since it has been

demonstrated that following the administration of non-labelled or C¹⁴-testosterone to animals (dogs, rats, mice) most of the androgenic material or radioactivity appears in the bile or feces (12-14). In guinea pigs, however, most of the radioactivity following the administration of C¹⁴-testosterone has been found in the urine (15). A study of biliary excretion of steroids in human subjects following the oral administration of unlabelled testosterone propionate was reported by Rubin, Dorfman, and Miller (16). No increase in biliary excretion of either 17-ketosteroids or androgens was found. On the other hand, following the administration of estradiol dipropionate they found definite evidence for biliary excretion of its metabolites.

The present paper presents the results of studies on the metabolism of 4-C¹⁴-testosterone in 20 adult human subjects of both sexes. The group includes five patients with bile-fistulas, three patients with carcinoma of the prostate, four patients with cancer of the breast, one patient with malignant melanoma, and seven normal subjects. In addition to the urinary and fecal excretion of radioactivity following the administration of 4-C¹⁴-testosterone, data are presented on biliary excretion in five patients with bile-fistulas and on the clearance from plasma of free and conjugated radioactive metabolites of 4-C¹⁴-testosterone in 17 subjects. In following reports characterization of the metabolites in plasma, bile and urine will be presented.

MATERIALS AND METHODS

4-C¹⁴-testosterone (specific activity, 5 μ C per mgm.), synthesized according to the method of Fujimoto (17), was obtained from two sources.² When the preparations were chromatographed in our laboratory, over 99 per cent of the radioactivity, determined in an Actigraph

² We wish to thank Dr. George I. Fujimoto for the 4-C¹⁴-testosterone used in the original part of the study. The remainder of the 4-C¹⁴-testosterone was secured from Raychem Laboratories, Elmsford, New York.

¹ Scholar in Cancer Research of the American Cancer Society (1953-56).

(Nuclear Chicago), was located in the area corresponding to that of standard testosterone.

The 4-C¹⁴-testosterone was dissolved in absolute ethanol (1 to 2 ml.) and diluted with saline (25 ml.) before intravenous (I.V.) injection. The I.V. administration (1 to 5 μ C) usually lasted 1 to 2 minutes and the middle of the injection was taken as "zero" time. Blood samples of 30 ml. were drawn 15, 30, 60, 120 and 240 minutes after injection. Heparin was used as an anticoagulant (1 to 2 mgm. per 30 ml. of blood).

Immediately following the withdrawal of blood, the plasma was separated from the red blood cells. The plasma was extracted 3 times with equal volumes of freshly redistilled chloroform and this fraction was labelled as "free radioactive steroids." The residues (I) following chloroform extraction were then extracted twice with large volumes (100 to 150 ml.) of ethanol and discarded. The alcohol was evaporated by an air-stream (at 45° C) and the residue (II) dissolved in water (30 to 50 ml.). The solution was then adjusted to pH 5 \pm 0.1 (acetate buffer) and β -glucuronidase added (300 units per ml.). Following incubation for 48 hrs. at 37° C, it was extracted 3 times with freshly redistilled chloroform. The chloroform was evaporated by a stream of air and the residue comprised the "glucuronide fraction." The remaining solution (III) was adjusted to pH 0.8 to 1.0 with 65 per cent sulfuric acid and extracted continuously with ether for 48 hours. This ether extract was called the "sulfate fraction." The aqueous solution (IV) was made 5 per cent with respect to sulfuric acid, refluxed for 30 to 45 minutes, cooled and extracted in a separatory funnel with ether (3 times).

Bile was collected from patients who had undergone cholecystectomy with insertion of a T-tube into the common bile duct. Complete bile collections were assured by a suction apparatus attached to the T-tube, and by the

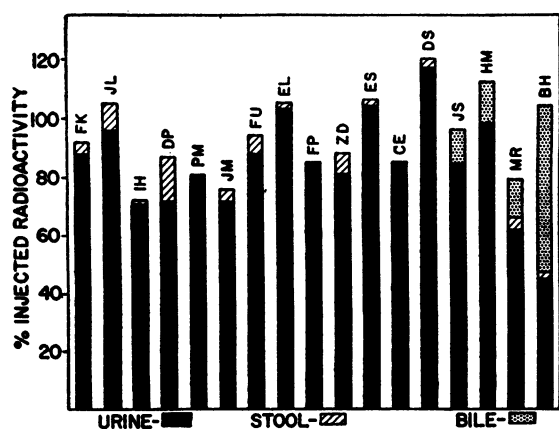


FIG. 1. THE PERCENTAGE OF RADIOACTIVITY EXCRETED IN THE URINE, STOOL AND BILE FOLLOWING 4-C¹⁴-TESTOSTERONE ADMINISTRATION

No stool collections were secured from subjects P. M., F. P., and C. E. Bile-fistula patients J. S. and H. M. had negligible radioactivity in the stools.

appearance of acholic stools during the period of investigation. The patients had normal liver functions preoperatively and the C¹⁴-testosterone was injected at least 3 days postoperatively.

The bile and urine samples were processed in essentially the same manner as the plasma samples except for the following. The free radioactive steroids were extracted with ether instead of chloroform; following the incubation with β -glucuronidase the steroids were extracted continuously for 48 hours with ether.

The feces were collected for at least 5 days following the administration of the radioactive testosterone and were mixed with large volumes of ethanol in a Waring blender and centrifuged. The extraction was performed three times. The alcohol was evaporated and the samples handled similarly to the urine samples.

Radioactivity was measured in a windowless gas flow-counter. A correction for self-absorption was made using suitable calibration curves for inorganic (urine) and organic residues.

The calculation of the total counts in the urine was based on a self-absorption correction curve obtained by the addition of the radioactive steroid to varying amounts of non-radioactive urine. The standard error of these counts did not exceed 5 per cent. The unextracted counts represent the difference between the total counts in the urine and the extractable counts. In those specimens in which the unextractable counts constituted a significant part of the radioactivity (for example, the 4 and 8-hr. specimens of subject J. L. in Figure 2; the 4, 8 and 12-hr. specimens in subject F. K.; the 4-hr. specimen in sub-

TABLE I

Patient	Sex	Age	Diagnosis
D. S.	F	39	Normal
F. K.	F	27	Normal
I. H.	F	38	Normal
S. A.	M	27	Normal
C. P.	M	24	Normal
P. M.	M	25	Normal
D. P.	F	29	Normal
J. L.	F	40	Normal
J. M.	M	73	Carcinoma of prostate with metastases
F. U.	M	66	Carcinoma of prostate, orchidectomy
E. L.	M	75	Carcinoma of prostate
Z. D.	F	46	Carcinoma of breast
E. S.	F	35	Carcinoma of breast
C. E.	F	50	Carcinoma of breast, NPN 69 to 101 mgm. per cent, albuminuria, patient receiving 100 mgm. testosterone propionate 3 times weekly I.M., generalized metastases
F. P.	M	24	Malignant melanoma
H. M.	M	50	T-tube drainage, cholecystectomy for cholecystitis
J. J.	M	38	T-tube drainage, cholecystectomy for cholecystitis
B. H.	F	52	T-tube drainage, cholecystectomy for cholecystitis
W. F.	F	69	T-tube drainage, cholecystectomy for cholecystitis
M. R.	F	60	T-tube drainage, cholecystectomy for cholecystitis, cancer of breast

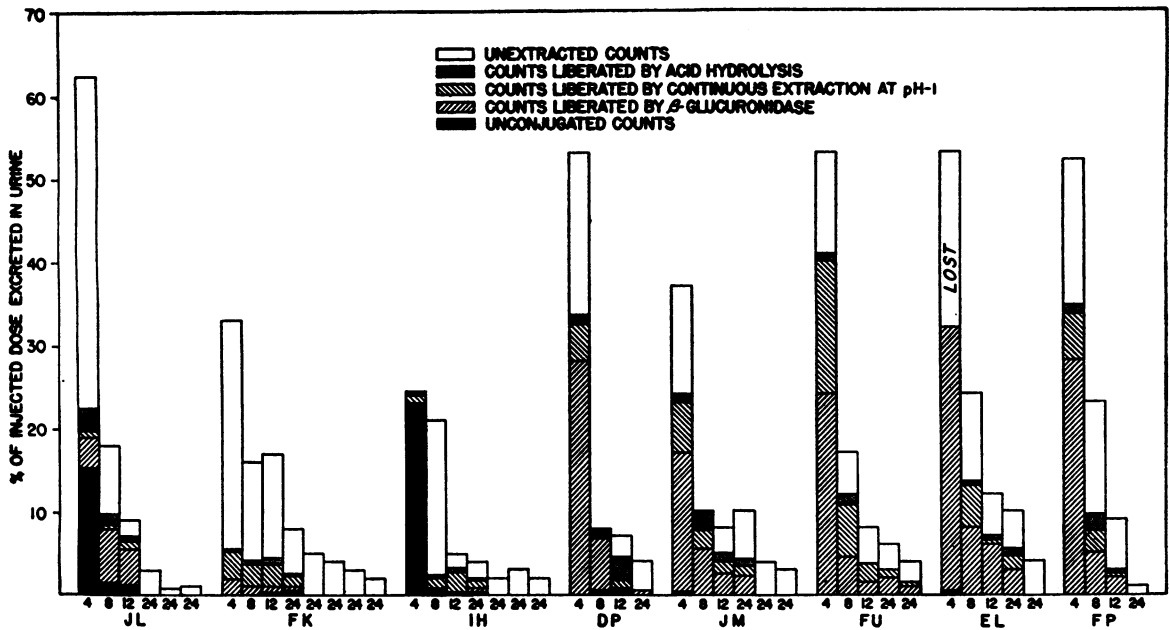


FIG. 2. EXCRETION OF RADIOACTIVITY IN THE URINE FOLLOWING THE INJECTION OF C¹⁴-TESTOSTERONE

The numbers refer to the number of hours covered by the period of collection.

ject D. P.; *etc.*) the urine residue, following the various hydrolytic and extraction procedures, was counted. In all cases 80 to 85 per cent of the calculated counts was present in the residues.

RESULTS

In Table I are shown some of the clinical data on the subjects given I.V. 4-C¹⁴-testosterone.

In Figure 1 is graphed the percentage of the injected radioactivity excreted in the urine, feces, and bile. The amount excreted in the urine in 48 hours ranged (exclusive of the bile-fistula patients) from 71 to 117 per cent (average 89 per cent). The total radioactivity excreted in the urine of normal subjects did not differ significantly from that of patients with cancer.

In Figures 2 and 3 are shown the percentages of the injected C¹⁴-testosterone excreted in the urine during certain time periods following the injection of the steroid. In addition, the amounts of steroid metabolites extractable following various hydrolytic procedures are shown. It is apparent that a preponderant part of the injected testosterone is excreted in conjugated form, primarily as the glucuronide. Except in a few urine samples where, for some reason as yet unknown, large amounts of free metabolites were present, sulfate-conjugated metabolites of testosterone con-

stituted an unimportant part of the radioactivity excreted. A significant part of the radioactivity could not be extracted from the urine even after strong acid hydrolysis.

It is apparent from Figures 2 and 3 that most of the radioactivity following C¹⁴-testosterone administration was excreted in the first 4 hours. An exception was noted in C. E., a patient with carcinoma of the breast, with impaired renal and liver functions, and receiving large amounts of testosterone propionate intramuscularly, who excreted the major part of administered radioactive steroid during the second 12-hour period after administration.

The clearance of free radioactive steroids (Figure 4 and Table II) from the plasma of normal subjects and of patients with cancer was rapid. The disappearance curves can be interpreted as indicating two possible rates. The first rate is related to a pool with a half-time of approximately 11 minutes, and the second, slower rate, is related to a pool with a half-time of approximately 100 minutes.

In Figure 5 and Table III are shown the steroid glucuronide plasma levels. Within 15 minutes following the injection, the counts in the plasma glucuronide fraction markedly exceeded those found

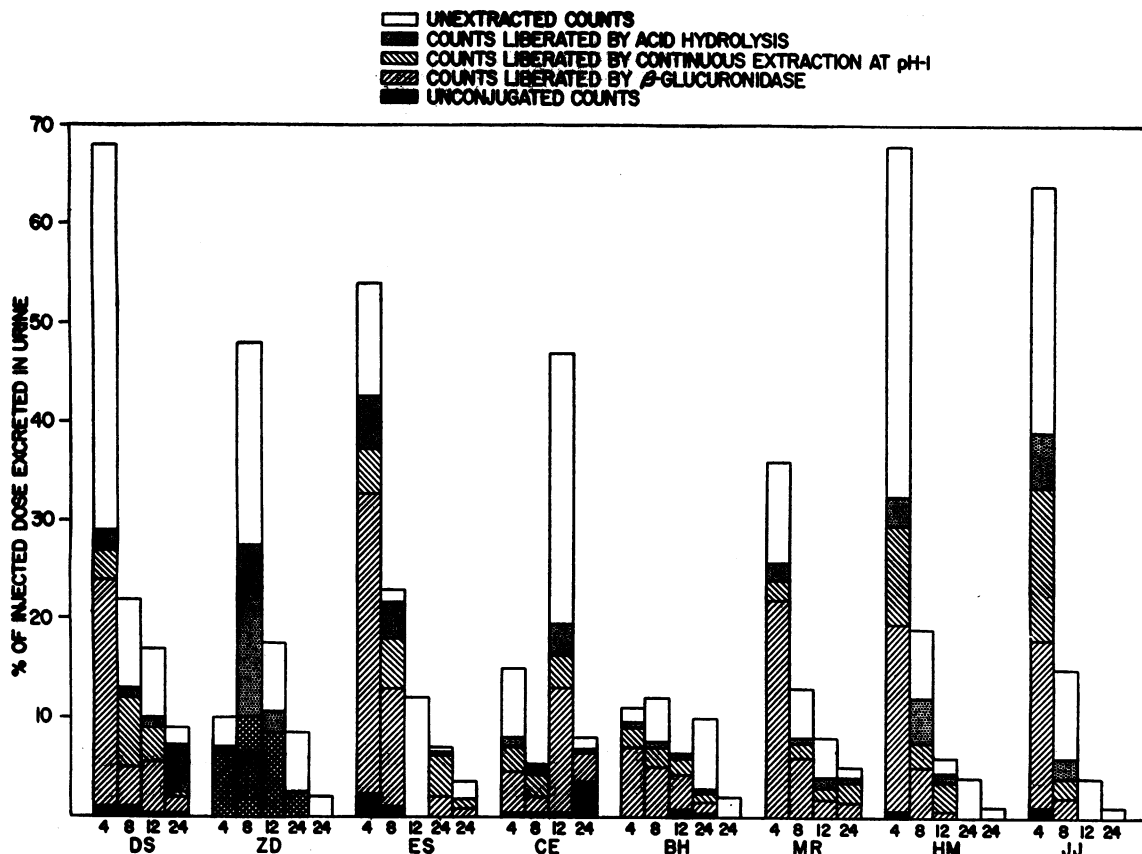


FIG. 3. EXCRETION OF RADIOACTIVITY IN THE URINE FOLLOWING THE INJECTION OF C^{14} -TESTOSTERONE

The numbers along the abscissa indicate the duration of each successive period of collection and are not cumulative.

with the unconjugated steroids. Activity declined slowly thereafter. Since the major identifiable conjugates in the urine are the glucuronides, it is interesting to note that patient C. E., with impaired renal function and receiving testosterone, maintained very high levels of radioactivity in the free steroid and glucuronide fractions during the time of study.

The counts in the sulfate conjugated steroid fraction were about 1/20 of the levels of the glucuronides and essentially parallel to them. Normal subject J. L. had strikingly higher sulfate levels than the rest of the group at 2 and 4 hours. We have no satisfactory explanation for this observation.

The radioactivity extractable from plasma following strong acid and heat hydrolysis was negligible. Similarly, negligible activity was found when the red blood cells were extracted with ab-

solute alcohol following lysis of the cells with water.

Patients with carcinoma of the breast cleared the free steroid radioactivity from the plasma at a somewhat slower rate than the remainder of the group. Since the number of cases studied was rather small, the possible significance of this finding must await confirmation of the observation in a larger group of similar patients.

The amount of radioactivity excreted in the bile is shown in Figure 6. Four of the patients with T-tube drainage excreted 11 to 14 per cent of the radioactivity of the injected C^{14} -testosterone in the bile, with most of the excretion occurring in the first 4 hours. Patient B. H. excreted 56 per cent of the injected radioactivity in the bile, mostly in the first 4 hours. In Figure 3 it can be seen that this patient (B. H.) excreted much less of the radioactivity in the urine during the first 12

TABLE II

Radioactivity levels in free steroid fraction of plasma following injection of 4-C¹⁴-testosterone*

Subject	15 min.	30 min.	60 min.	120 min.	240 min.
	% of injected dose per 100 ml. plasma per Kg. body weight $\times 10^4$				
D. S.	41.0	10.0	5.9	0.8	0.3
F. K.	34.0	23.0	11.0	3.9	1.9
I. H.	56.0	26.0	11.0	2.9	1.7
S. A.	11.0	5.1	2.3	1.9	0.4
C. P.	55.0	9.6	4.4	1.7	0.4
D. P.	38.0	15.0	4.3	1.1	0.3
J. L.	74.0	32.0	12.0	3.7	0.8
J. M.	59.0	33.0	13.0	4.3	0.3
F. U.	39.0	23.0	8.0	3.0	0.4
E. L.	45.0	26.0	9.6	5.5	1.6
Z. D.	93.0	47.0	15.0	6.6	3.8
E. S.	47.0	26.0	9.8	4.3	2.3
C. E.	70.0	34.0	25.0	13.0	8.8
H. M.	35.0	17.0	6.4	1.9	0.4
J. J.	27.0	20.0	8.8	3.8	1.4
B. H.	76.0	34.0	17.0	9.3	3.3
M. R.	64.0	23.0	15.0	7.5	3.2

* In Table II the number of counts present in the 120-minute samples was of such magnitude that the counting did not involve a standard error from the mean greater than 5 per cent; in the 240-minute specimens the counting did not involve a standard error from the mean greater than 10 per cent.

hours than any other subject in the whole group. It should be pointed out that although this patient had normal liver function tests preoperatively she developed a gram-negative bacterial septicemia with an ascending cholangitis postoperatively. Even though the C¹⁴-testosterone study was performed when the patient seemingly had made a recovery from the infection, it is possible that the infection may have influenced the amount of radioactivity excreted in her bile.

The excretion of free steroids in the bile was negligible, with most of the extractable steroids being glucuronides or sulfates. The bulk of the radioactivity could not be extracted following the hydrolytic procedures used. The amount not extractable from bile was even greater than that from the urine.

The radioactivity excreted in the stools (Figure 7) of non-fistula subjects varied from 2 to 15 per cent (average: approximately 6 per cent). Counts in the free steroid fraction comprised the largest amount. In the patients with T-tube drainage the fecal radioactivity excreted ranged from less than 0.1 per cent to 2 per cent.

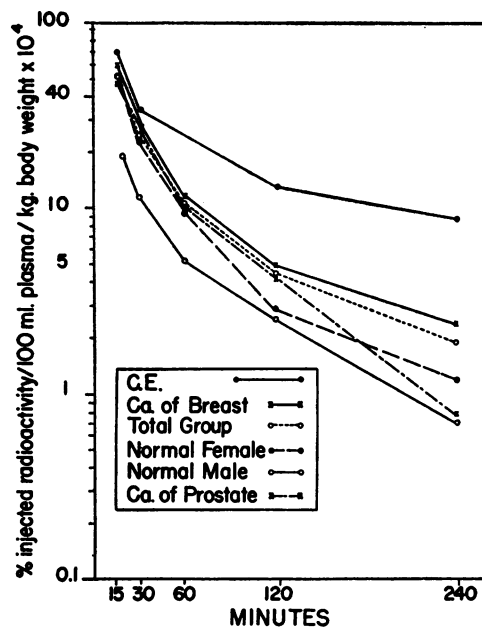


FIG. 4. THE CLEARANCE OF "FREE STEROID" RADIOACTIVITY FROM THE PLASMA FOLLOWING THE I.V. INJECTION OF C¹⁴-TESTOSTERONE

DISCUSSION

The nearly quantitative excretion of the radioactivity in the urine following the intravenous injection of 4-C¹⁴-testosterone is at variance with results obtained by others when excretion of non-labelled steroid products was studied. This is not surprising, since even with labelled testosterone only about 50 per cent of the radioactivity could be released and extracted following various hydrolytic procedures. In addition, the estimation of the urinary excretion of metabolites following non-radioactive administration depended on biological assay or the determination of 17-ketosteroids. It is known that neither procedure would estimate all the metabolites of testosterone in the urine. Our findings are similar to those of Fukushima, Dobriner, Gallagher, and Bradlow (10, 11), who were able to recover 50 per cent of the administered radioactivity from the urine (as crude, ether-extractable, and neutral metabolites) during the first 24 hours.

Gallagher, Fukushima, Dobriner and their associates (9-11) were able to extract from the urines of their published cases from 50 to 77 per cent of radioactivity in the neutral fractions following intravenous testosterone administration,

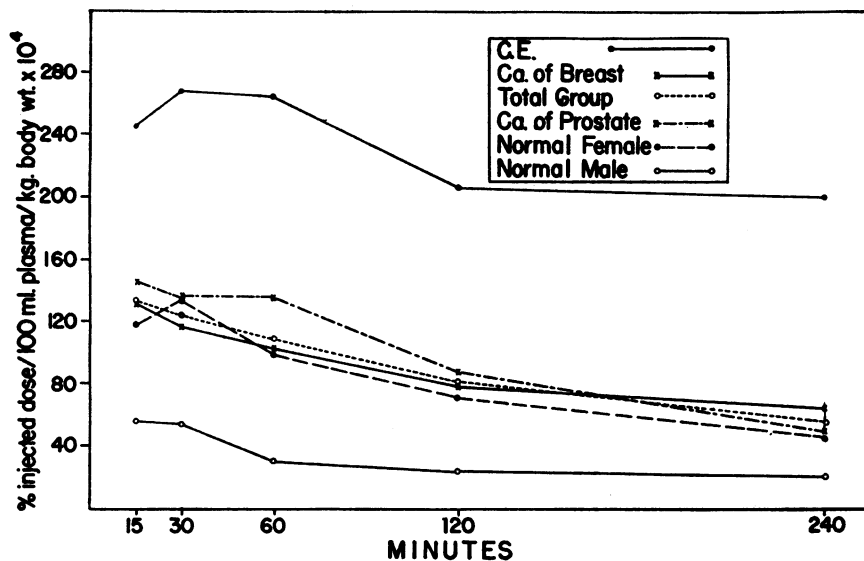


FIG. 5. RADIOACTIVE GLUCURONIDATE STEROID LEVELS IN THE PLASMA FOLLOWING THE ADMINISTRATION OF RADIOACTIVE TESTOSTERONE INTRAVENOUSLY

the amount extractable being dependent on the exact hydrolytic and extractive procedures used (9). It is interesting to note that their highest extractable percentages occurred in those urines on which a repeat β -glucuronidase hydrolysis was performed following an initial incubation with the

enzyme and extraction at pH 1. Even though in more than half of our cases 50 to 78 per cent of the administered radioactivity was extracted, it is possible that further incubation with β -glucuronidase would have released additional extractable radioactivity. Furthermore, it is possible that in-

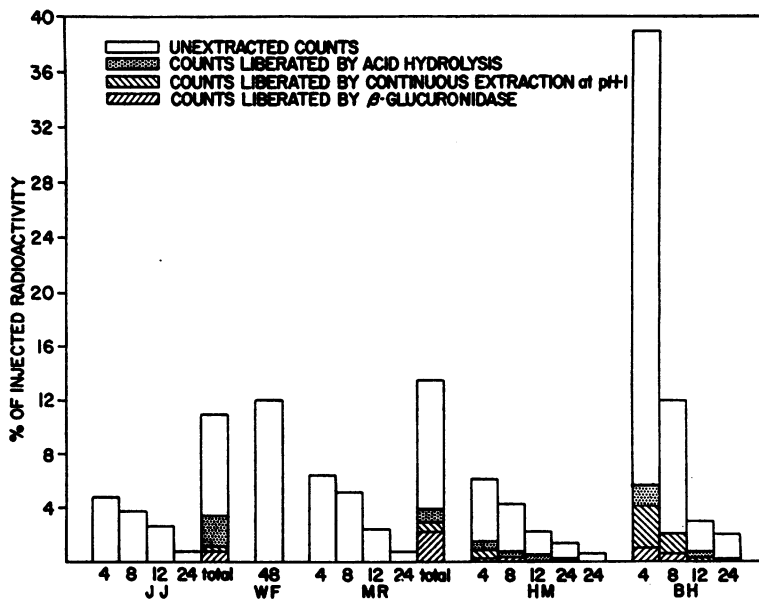


FIG. 6. EXCRETION OF RADIOACTIVITY IN THE BILE OF FIVE PATIENTS
The numbers refer to the number of hours covered by the collection.
Note absence of free steroids in the bile.

incubation with β -glucuronidase for longer periods of time, such as employed by Gallagher and associates (120 hours) (9), than used in our experiments (48 hours) would have resulted in a greater percentage of extractable counts in our cases. In addition, the minor discrepancies between our results and those of Gallagher, Fukushima, Dobriner, *et al.* (8-11) may possibly be due to the larger amounts of carrier used (8.4 to 16.0 mgm.) by the above authors and differences in the methods and rates of infusion of the C^{14} -testosterone.

Radioactive testosterone was cleared from the plasma rapidly when compared to the clearance of 4- C^{14} -cortisol (18-20). Within 15 minutes following the injection of the 4- C^{14} -testosterone the radioactivity in the glucuronide fraction reached its peak and was about threefold the level in the "free" steroid fraction. This contrasts with the metabolism of C^{14} -cortisol where the counts in the glucuronide fraction achieve their peak and equal the level in the free steroid fraction in about 120 minutes (19). The glucuronide fraction radioactivity following C^{14} -testosterone reached its peak early and declined slowly thereafter. Since the excretion of radioactive metabolites in the urine during the first 4-hour period was at least 50 per cent of the administered dose, it indicates that testosterone had been taken up by tissues and its glucuronide-conjugated metabolites released slowly over that period. Even though the differences in the methods used make absolute comparison difficult, the clearances of C^{14} -testosterone from plasma and of radioactivity of the conjugates in our studies give distributional findings similar to those of West, Tyler, Brown, and Samuels (7) following the administration of large doses of non-radioactive testosterone.

The free steroids disappeared from the plasma with at least two different rates, an initial fast rate and a subsequent much slower rate. This may indicate that at least two different metabolic pools exist or that the initial rate is that of 4- C^{14} -testosterone clearance from the plasma and the subsequent slower rate that of a "free" steroid metabolite of testosterone. Patient C. E. cleared the C^{14} -testosterone from her plasma slower than any other patient in the group. We believe this was due to impaired liver function in the patient, since it has been shown that the liver plays an important

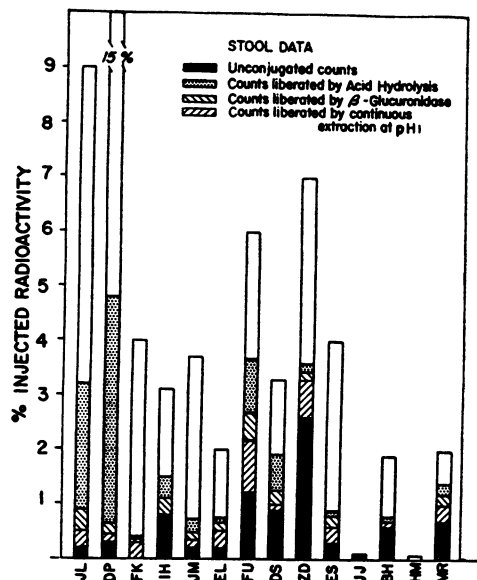


FIG. 7. EXCRETION OF RADIOACTIVITY IN THE STOOLS OF 14 SUBJECTS FOLLOWING THE INJECTION OF C^{14} -TESTOSTERONE

role in the metabolism of free steroids, although other factors in this lady may also have played a part.

The urinary excretion of metabolites following 4- C^{14} -testosterone injection, even though it varied somewhat from subject to subject with respect to the amounts of radioactivity released by the various hydrolytic procedures, showed an essentially con-

TABLE III

Radioactivity levels in steroid glucuronide fraction of plasma following injection of 4- C^{14} -testosterone

Subject	% of injected dose per 100 ml. plasma per Kg. body weight $\times 10^4$				
	15 min.	30 min.	60 min.	120 min.	240 min.
D. S.	52	53	38	31	23
F. K.	61	111	98	85	38
I. H.	165	165	126	88	78
S. A.	29	21	26	17	11
C. P.	39	36	29	21	18
D. P.	143	157	109	70	44
J. L.	99	98	63	47	21
J. M.	284	231	244	155	
F. U.		79		50	21
E. L.	75	101	85		42
Z. D.	241	200	168	124	103
E. S.	112	108	98	79	76
C. E.	245	268	263	206	200
H. M.	189	144	129	81	37
J. J.	99	101	34	32	27
B. H.	151	128	130	114	85
M. R.	124	111	103	82	44

TABLE IV

Radioactivity levels in steroid sulfate fraction of plasma following injection of 4-C¹⁴-testosterone

Subject	15 min.	30 min.	60 min.	120 min.	240 min.
	% of injected dose per 100 ml. plasma per Kg. body weight $\times 10^4$				
D. S.	5.9	11.0	3.1	5.2	8.0
F. K.	5.8	5.6	5.4	6.8	5.7
I. H.	6.9	6.7	5.0	7.2	5.9
S. A.	5.9	6.1	6.2	6.7	4.1
C. P.	1.9	7.7	8.6	6.7	8.0
D. P.	13.0	12.0	9.5	2.8	2.8
J. L.	5.4	7.2	7.7	18.0	34.0
J. M.	4.6	3.2	5.5	2.0	
F. U.		4.7		2.9	0.8
E. L.	1.0	2.5	4.1		3.3
Z. D.	14.0	6.3	4.7	10.5	4.7
E. S.	4.8	7.1	5.5	9.9	3.6
C. E.	13.0	12.0	8.1	11.0	5.1
H. M.	6.2	6.1	7.6	6.3	3.8
J. J.	7.1	5.5	5.4	6.4	3.5
B. H.	7.2	8.4	4.7	3.7	3.5
M. R.	5.1	4.1	4.5	4.6	2.0

sistent pattern. The preponderant part of the radioactivity was excreted in the first 4 hours following injection, except for patient C. E. who had impaired renal function and was receiving large doses of intramuscular testosterone propionate. It is likely that impaired renal function in patient C. E. played a part in the abnormal excretion. The role of large doses of non-radioactive testosterone is difficult to evaluate.

About 50 per cent of the radioactivity was ether or chloroform extractable following the various hydrolytic procedures, with the bulk being in the form of glucuronides. The amount of free steroid excreted in the urine, except for a few specimens in normal subjects I. H. and J. L., was very small when compared to the amount excreted in conjugated forms.

The urinary excretion of radioactivity during the first 4 to 8 hours following C¹⁴-testosterone injection was almost double that following C¹⁴-cortisol administration and may be related to the faster metabolism of the former steroid (18-20).

The amount of radioactivity excreted in the bile averaged 12 per cent of the injected dose, exclusive of patient B. H. who excreted 56 per cent. This patient had recently had ascending cholangitis and it is possible that her substantial biliary excretion was related to that. The findings in the present study regarding the biliary route of excretion of C¹⁴-testosterone and its metabolites dif-

fer from those observed in animals. As contrasted to human subjects, animals (rats, mice, dogs) excrete most of the administered testosterone in the bile and very little in the urine (12-14). These differences make comparisons regarding metabolism of testosterone among various species unreliable. Even though Burstein, Ungar, Gut, and Dorfman (15) have reported that most of the radioactivity following administration of C¹⁴-testosterone to guinea pigs appeared in the urine, the possibility of substantial biliary excretion of radioactivity with subsequent intestinal reabsorption cannot be excluded.

It has been shown that 1 to 5 per cent of radioactivity following intravenous C¹⁴-cortisol administration is excreted in the bile of human subjects (19, 20). On the other hand, at least 30 to 60 per cent of radioactivity following C¹⁴-progesterone (21), C¹⁴-estrone or C¹⁴-estradiol administration is excreted in the bile of man (22).

The fecal excretion of radioactivity in patients without T-tube drainage averaged about 6 per cent. It is apparent that some reabsorption of testosterone metabolites must occur in the gastrointestinal tract. The small amounts (1 per cent) of radioactivity present in the stools of patients with T-tubes may be due either to excretion of radioactivity in gastrointestinal juices or to the escape of small amounts of bile. The fecal excretion of radioactivity in our series is comparable to the results of Fukushima, Bradlow, Dobriner, and Gallagher (11).

Essentially, no unconjugated steroids were present in the bile and the percentage extractable after the various hydrolytic procedures fell far short of the percentages in the urine. On the other hand, the unconjugated (free) steroids constituted a major part of the steroids in the stools. Since it has been demonstrated that human stools contain β -glucuronidase and phenolsulfatase activities (20, 23), it is possible that some of the steroid conjugates originating from the bile are cleaved by the fecal enzymes.

SUMMARY

4-C¹⁴-testosterone has been administered intravenously to 20 human subjects. The radioactivity was excreted almost quantitatively in the urine in 48 hours with over 50 per cent in the first 4 hours. About 50 per cent of the radioactivity could be

extracted from urine following different hydrolytic procedures. C^{14} -testosterone was cleared from the plasma rapidly with at least 2 separate rates. These 2 pools had half-lives of 11 and 100 minutes. The conjugated metabolites of C^{14} -testosterone reached their peak in the plasma within 15 minutes following the injection of the steroid. From 12 to 14 per cent of the injected radioactivity was excreted in the bile of subjects with T-tube drainage and only 6 per cent in the stools of patients having no T-tube, which probably indicates reabsorption of steroids from the gastrointestinal tract. The metabolites in bile were in conjugated form, whereas a great part of the radioactivity in the stools was in the free form.

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