Metabolic Fate of 11-Hydroxyandrostenedione-C^{14} in Human Subjects.* (23569)

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Introduction. It would appear that 11-oxygenated androgens are predominantly, if not entirely, of adrenocortical origin in human subjects. Romanoff, Hudson and Pincus isolated 11-hydroxyandrostenedione (Δ^4-androstene-11β-ol-3,17-dione, hereafter abbreviated Δ^4-11β-OH) from the adrenal vein blood of human subjects given ACTH(1). Salamon and Dobriner(2) isolated the same compound from the urine of a subject with myelogenous leukemia given ACTH. Twenty years ago Reichstein isolated adrenosterone and androstane-3β,11β-diol-17-one from adrenal cortical extracts(3). In addition, the 11-keto and 11β-hydroxy isomers of Δ^4-androstene-3,17-dione have been identified in blood perfused through beef adrenals(4). The latter isomer is probably also present in the adrenal venous blood of rats, sheep, and cats(5). All of these findings point to the probable adrenocortical origin of the 11-oxyandrogens. Produced in much lesser quantities (1-2 mg/day) than the 11-deoxyandrogens, the role of the 11-oxyandrogens in body metabolism, in both normal and abnormal conditions, remains obscure. The availability of C^{14}-labelled naturally occurring steroids has made it possible to study their metabolic fate in human subjects more accurately(6-15). Such studies have shown considerable differences among the various steroids in their rates of urinary, biliary and fecal excretions and in their physiological disposition. The biliary excretion plays a major role not only in the rapidity of urinary excretion of the metabolites of the steroids, but also in their ultimate disposition. The major aim of the present study was to investigate the metabolic fate of C^{14}-Δ^4-11β-OH and to compare the results with those of other steroids.

In this paper data are presented on the distribution of radioactivity in the bile, feces and urine following the intravenous injection of 4-C^{14}-Δ^4-11β-OH into 8 human subjects. In addition, the rates of clearance from the blood of the unconjugated steroids and their conjugated metabolites will also be presented. The identification of the various metabolites of the above steroid, as well as those of adrenosterone, is the subject of a separate communication.

Methods and materials. The 4-C^{14}-Δ^4-11β-OH was kindly supplied by the Endocrinology Study Section of the National Institutes of Health. The compound was chromatographed on paper in our laboratory and found to be more than 95% pure when the radioactivity was located with an Actigraph. (Nuclear-Chicago) and the Rf compared with that of the standard steroid. One to 2 μc (5 μc/1.03 mg) were injected intravenously into 8 subjects: 2 normal males, 2 female subjects with bile-fistulas (T-tube drainage of bile), 3 normal female subjects without cancer and 1 female subject with cancer of the breast. All of the subjects were in good clinical condition and none had evident renal or liver dysfunction. None of the subjects had more than a mild anemia (hemoglobin concentration not less than 11 g %). The C^{14}-Δ^4-11β-OH was dissolved in 1-2 ml of absolute alcohol, diluted with 30 ml of physiological saline, and injected intravenously (I.V.) over a period of 1-2 minutes. Subject E.J. received I.V. 50 mg of adrenosterone (Δ^4-androstene-3,11,17-trione) in propylene glycol and human serum albumin just prior to the injection of the radioactive steroid(15). The 2 female subjects with T-tube drainage of bile had undergone cholecystectomies for chronic cholecystitis. The liver function tests in these patients were normal pre-operatively. The steroid was injected after each patient's condition had become stabilized, without fever or other

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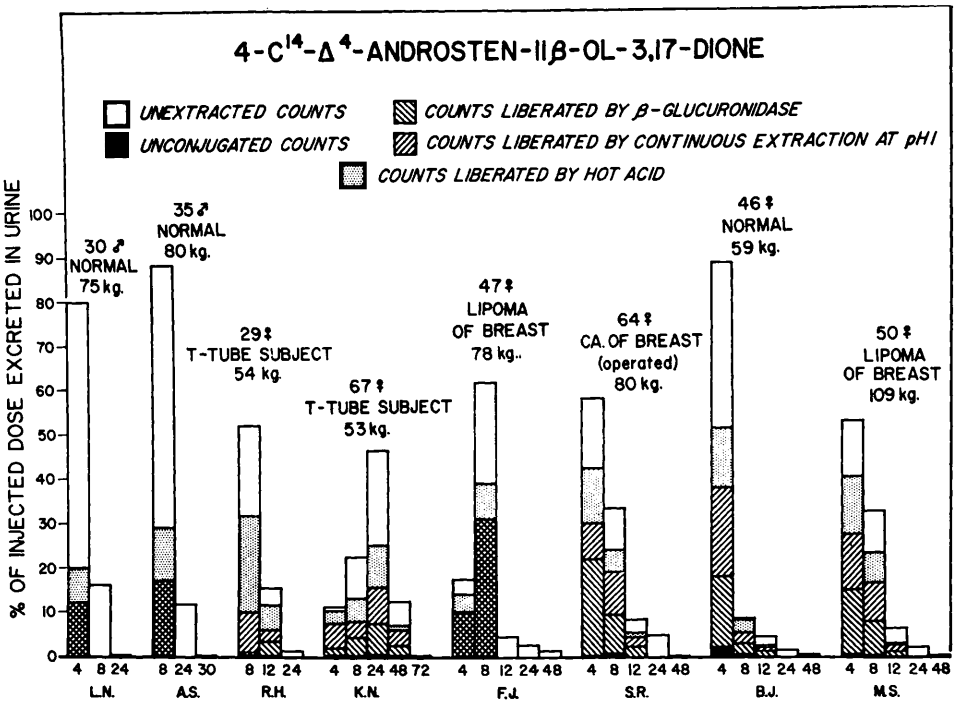

FIG. 1. Excretion of radioactivity in the urine following the inj. of C^{14}-Δ^4-11β-OH. Hydrolysis by β-glucuronidase of the urines of subjects L.N. and A.S. was incomplete due to low concentrations of the enzyme. Numbers on abscissa refer to the hour following inj. of the C^{14}-steroid when the collection was terminated. Totally blank columns indicate that no hydrolytic or extraction procedures were done on the urine specimens.

complications, after oral intake of fluids and food had become established, and 3 days or more post-operatively. The methods for collection and processing of urine, bile, feces and blood; the determination of radioactivity; the various hyrolytic and extraction procedures have been described in previous publications(12,15). Throughout this paper the word "unconjugated" will refer to steroid metabolites which are extractable with ether or chloroform before performance of any hydrolytic procedures; the "glucuronidates" to steroid metabolites which become extractable with ether or chloroform following β-glucuronidase hydrolysis; and the "sulfates" to steroid metabolites extracted continuously with ether for 48 hours at pH 1.

Results. The amount of radioactivity excreted in the urine following the I.V. injection of C^{14}-Δ^4-11β-OH averaged 95% for the group (range 79-106%), with the preponderant part of the radioactivity being excreted in the urine in the initial 8 hour period. In Fig. 1 is shown the urinary excretion of radioactivity during certain time periods following the injection of the radioactive steroid, as well as the amounts of steroid metabolites extractable following various hydrolytic procedures. Only negligible amounts of the injected steroid appear in the urine as unconjugated metabolites. Over 50% of the injected radioactivity was extractable following the various hydrolytic procedures (Fig. 1). When subject A.S. ingested 250 mg of adrenosterone and 2 μc of C^{14}-Δ^4-11β-OH, 61% of the radioactivity was recovered in the urine, of which 44% was extractable after β-glucuronidase hydrolysis and continuous extraction at pH 1, and 14% following hydrolysis by hot-acid.

Only small amounts of radioactivity were excreted in the bile (2.4 and 0.4%) of the two subjects with bile-fistulas and minute amounts were extractable following all hydrolytic procedures (0.3% and 0.08% of the total radioactivity).

TABLE I. Radioactivity Concentrations in Unconjugated (U), Glucuronidate (G) and Sulphate (S) Fractions of Plasma following the Injection of C^{14}-Δ^4-11β-OH. (Results expressed as % of inj. dose present in total plasma vol at various time intervals following administration).

	15 min.			30 min.			60 min.			120 min.			240 min.		
Subject	U	G	S	U	G	S	U	G	S	U	G	S	U	G	S
R.H.	3.2	23.5	.3	2.0	12.8	1.0	1.0	6.1	.1	.4	3.2	.1	.2	.9	.0
K.N.	5.4	11.6	.5	3.1	10.6	1.2	1.7	8.4	1.3	.6	5.5	.5	.2	2.9	.4
E.J.	4.4	14.1	1.5	2.4	16.7	.3	1.0	11.0	1.7	.9	6.1	.2	.4	2.9	.2
S.R.	3.1	10.8	2.4	2.8	12.1	.1	1.6	9.9	.7	1.0	6.5	.4	.8	4.3	.2
B.J.	3.1	19.8	.7	1.6	19.8	1.4	.8	13.6	.4	.4	7.1	.5	.1	2.6	.2
M.S.	4.1	22.1	.6	3.2	16.4	1.5	1.2	9.4	.5	.7	4.5	.4	.3	2.6	.3

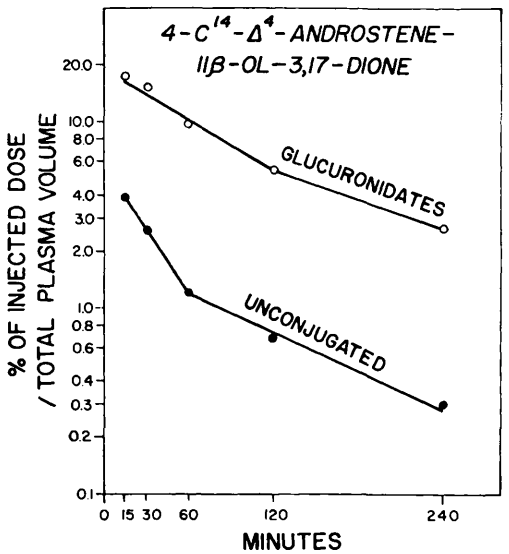

FIG. 2. Mean clearance of unconjugated and glucuronidated steroid radioactivity from plasma of 6 subjects following I.V. inj. of C^{14}-Δ^4-11β-OH. Note initial rapid rate and subsequent slower rate of clearance of the unconjugated radioactivity.

Negligible amounts of radioactivity were present in the stools of all the subjects.

The clearance of unconjugated radioactive steroids from the plasma of the injected subjects is shown in Fig. 2 and Table I. For the sake of comparison and uniformity, it was assumed that each subject's blood volume constituted 9% of the body weight (5% as plasma volume and 4% as the total red cell mass). It can be seen in Fig. 2 that the unconjugated steroids are apparently cleared at two separate rates with half-lives of approximately 30 and 80 minutes, respectively. Only negligible amounts (0.3%) of radioactivity were present in the unconjugated fraction of the plasma 4 hours following the injection. The glucuronidate fraction of the plasma contained 4 times as much radioactivity as the unconjugated fraction within 15 minutes following injection, and 10 times as much after 4 hours (Fig. 2 and Table I). The glucuronidate levels in the plasma declined rather rapidly during the initial 4 hours, decreasing from 17% at 15 minutes to less than 3% 4 hours after the injection. It is interesting to note that subject E.J., who received 50 mg of adrenosterone with the radioactive Δ^4-11β-OH, cleared the unconjugated and conjugated steroid metabolites at a similar rate as the other subjects.

Relatively small amounts of radioactivity were present in the sulfate fraction of the plasma (Table I). Negligible amounts of radioactivity were present in the plasma following hot acid hydrolysis or in the precipitated proteins. The amounts of radioactivity associated with the red blood cells are shown in Table II, and constituted, in general, a small fraction of the radioactivity injected when compared to that present in the plasma.

Discussion. From the data presented 2 findings concerning the metabolism of C^{14}-Δ^4-11β-OH are particularly of interest. First, more of the injected radioactivity is excreted in the urine following the injection of C^{14}-Δ^4-11β-OH than that seen following any other labeled steroid studies so far. In addition,

TABLE II. Total Radioactivity Levels Associated with the Erythrocytes following Injection of C^{14}-Δ^4-11β-OH.

Subject	% of dose present in total red cell mass				
	15 min.	30 min.	60 min.	120 min.	240 min.
R.H.	1.2	.7	.4	.1	.0
K.N.	.2	.5	.4	.4	.8
E.J.	1.2	.9	.8	.5	.5
S.R.	1.4	1.0	1.3	2.3	2.6
B.J.	1.6	.7	.5	.5	.4
M.S.	2.3	2.3	2.9	2.3	1.9

this radioactivity was excreted in the urine in a shorter period of time, essentially in the first 8 hours, than that required for any other C^{14}-steroid. This is in agreement with the findings of Bradlow, Hellman and Gallagher (13), who observed similar rapid excretion of radioactivity in the urine following the administration of C^{14}-Δ^4-11β-OH. Excretion of radioactivity in the bile or in the stools in the present study, was found to be the smallest when compared to that of any other steroid administered. These findings are particularly striking when compared to the rate of urinary excretion and the total radioactivity excreted in the urine, bile or feces following the injection of C^{14}-labeled estrone, estradiol, or progesterone(7,15,16). Even when compared to the urinary excretion of radioactive testosterone(6,12), cortisol or corticosterone(8,9,11), radioactive metabolites of Δ^4-11β-OH were excreted more quantitatively and more rapidly than was observed following the administration of any of the aforementioned steroids. These findings indicate rapid metabolism and excretion of metabolites of Δ^4-11β-OH. The lack of any substantial biliary excretion of radioactivity following administration of Δ^4-11β-OH probably plays an important role not only in the rapidity with which the radioactive metabolites are excreted in the urine, but also in the quantitative recovery of radioactivity in the urine. In previous studies it has been shown that substantial biliary excretion of steroid metabolites leads to slower excretion of radioactivity in the urine and/or lesser amounts of radioactivity in the urine due to involvement of the metabolites in a hepato-enteric circulation(12,16).

The authors have reported(14) the possible relationship between the binding of steroids to human plasma proteins and their biliary excretion. The most avidly bound steroids are excreted primarily in the bile (estrone, estradiol), whereas the least bound steroids are excreted largely in the urine (cortisol, cortisone). On the basis of the findings of the present study one would predict that Δ^4-11β-OH would be the least bound of the endogenous steroids studied, and, indeed, such is the case as reported previously (17).

The clearance of the unconjugated steroids from the plasma following the injection of Δ^4-11β-OH occurred at two different rates during the initial 4 hours. The rates were associated with metabolic pools whose half-lives were approximately 30 and 80 minutes respectively. Similar clearance rates have been observed following the administration of C^{14}-labeled progesterone(16), estrone(15), estradiol(15), testosterone(12) and corticosteroids(9,10). In the past, it has been postulated that the initial more rapid rate is related to the mixing of the radioactive steroid in the body pools, whereas the subsequent slower rate represents the true metabolic rate of the steroid(10). If this be true, it would appear that all these steroids have metabolic pools whose turn-over rates are not greatly different, although the pool sizes may be profoundly different.

Summary. 11-Hydroxyandrostenedione-C^{14} was administered I.V. to 8 human subjects: 2 normal males, 2 subjects with bile-fistulas, 3 females without and one female with cancer. The radioactivity was excreted in urine almost quantitatively within a very short period of time (8-12 hours). Only negligible amounts of radioactivity were found in the bile of the 2 subjects with bile-fistulas or in stools of all subjects. The unconjugated steroids were cleared from the plasma at 2 rates, a fast rate with a half-life of 30 minutes and a subsequent slower rate with a half-life of 80 minutes. The glucuronidate fraction of the plasma contained much higher levels of radioactivity than the unconjugated fraction within 15 minutes following the injection of the steroid and even more strikingly four hours following the administration of the radioactive steroid. The significance of these findings is discussed.

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A Method for Assay of Stuart Factor.* (23570)

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Stuart clotting defect was first described by Hougie, Barrow, and Graham in 1957(1). The name was applied after the surname of the patient in whom the deficiency was first identified, and who had previously been reported by Lewis, Fresh, and Ferguson(2) as a case of proconvertin deficiency. Although the patient showed the laboratory features of proconvertin deficiency, there was found to be cross correction of this patient with the original proconvertin deficient patient of Alexander, Goldstein, Landwehr, and Cook(3), indicating that the two patients were deficient in separate clotting entities. It was demonstrated, moreover, that this new factor was necessary not only for the action of blood thromboplastin but also for the action of tissue thromboplastin. It was present in serum, adsorbable by barium sulfate and Seitz filters, and relatively stable to heat and changes in pH. During early dicumarol therapy the concentration is relatively high, whereas later it is depressed. While in proconvertin deficiency the Russell viper venom time is normal(4), in Stuart deficiency the Russell viper venom time is prolonged.

The latter finding suggested that adsorbed beef plasma as prepared for the proconvertin test could be used as a substrate for the assay of Stuart factor if Russell viper venom was used as the thromboplastin. Consequently, the following experiments were performed using the proconvertin test as described by Owren and Aas(5) and modified by Adamis, Sise and Kimball(6).

Methods. The assay was performed in the same fashion as for proconvertin with the exception that Russell viper venom fortified with a lipoid extract of human brain (crude cephalin) was used as the thromboplastin. A chloroform extract of acetone dried human brain was prepared by the method of Bell and Alton(7). A dilution of one part of this crude cephalin preparation was made with 30 parts of veronal buffer pH 7.4, ionic strength 0.154.

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