THE FATE OF TESTOSTERONE INFUSED INTRAVENOUSLY IN MAN*

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This report describes the results obtained in two experiments with human subjects when small doses of testosterone dissolved in saline solution containing human serum albumin were given slowly by the intravenous route. The preceding paper (1) described the metabolism of testosterone after intramuscular injection. Since the rate of absorption of the steroid hormone from an oily depot can only be inferred, the intravenous experiments were a logical and necessary extension of the investigation. The physiological response as measured by nitrogen retention is reported to be different for the two routes of administration (2), and it was therefore desirable to determine whether the metabolic transformation and excretion of the steroid were altered by the manner of administration. Of perhaps still greater importance, it was possible to estimate the glandular production of testosterone by comparison of the metabolites normally excreted by a particular subject with the amount obtained from a small dose of the hormone slowly infused in excess of the glandular secretion. Finally, it was possible to demonstrate by means of testosterone labeled with both C^{14} and H^2 that the metabolic fate of both isotopes was identical and thus confirm the conclusions derived from experiments with one isotope.

EXPERIMENTAL

Testosterone-d (Subject A)—A sterile microcrystalline suspension of 23.1 mg. of testosterone-*d* (2.82 AD[1]) in 1 ml. of water was prepared by agitating the steroid with a trace of Tween 80. After a few days, approximately 10 ml. of 25 per cent of human serum albumin and 1 ml. of a solution of penicillin G were added and the mixture was agitated for 6 days; solution was complete at this time. The solution was passed through a Seitz filter and washed through with 200 ml. of sterile saline solution containing

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[1] The symbol AD signifies atom per cent excess deuterium.

200,000 units of penicillin G, with no attempt to effect quantitative transfer. The solution was diluted to approximately 2 liters with sterile physiological saline solution.

The subject was a normal adult male (Subject A of the preceding paper). A total of 1.552 kilos of the solution containing 11.3 mg. of testosterone (2.82 AD) was infused into the antecubital vein over the course of 8 hours; approximately 150 ml. were introduced during the first 2 hours, about 650 ml. during succeeding 3 hours, and approximately 750 ml. during the final 3 hours. The remainder of the infusion solution was reserved for the analysis of testosterone-*d* content.

Testosterone-d-4-C^{14} (*Subject F, Male, Age 51 Years, Aplastic Anemia*)—2.739 mg. of testosterone-4-C^{14} (679,000 c.p.m. per mg.) and 21.20 mg. of testosterone-*d* (2.82 AD) were mixed by solution in acetone and evaporation of the solvent. The hormone (77,900 c.p.m. mg.; 2.50 AD) was heated at 100° under diminished pressure for 25 minutes. With sterile precautions, 10 ml. of 25 per cent human serum albumin and a small amount of aureomycin were added. The mixture was shaken for 2 days. The solution was then filtered through a sterile filter and washed through with saline solution. The filtrate was diluted to 2 liters with saline solution and 200,000 units of penicillin G were added. The total weight of solution was 2.060 kilos. During a period of 4 hours, 1.687 kilos of this solution containing 16.6 mg. of testosterone based on the deuterium analysis, or 15.5 mg. of testosterone based on the C^{14} analysis, were infused intravenously.

Analysis of Infusion Solution for Isotopic Testosterone—A weighed portion of the infusion solution was extracted continuously with ether to which a known amount of carrier testosterone had been added. The ether extract was washed with water and dried over sodium sulfate. After removal of the solvent, the residue was sublimed and recrystallized from acetone-petroleum ether. The details of the analyses are given in Table I. The amount of testosterone in the infusion solution was calculated from the following equation.

$$V = \frac{(WX)100}{Y(Z - X)}$$

V = weight (mg.) of isotopic testosterone per 100 gm. of infusion solution
W = weight (mg.) of carrier testosterone added
X = AD or counts per minute per mg. of testosterone isolated
Y = weight (gm.) of solution analyzed
Z = AD or counts per minute per mg. of isotopic testosterone administered

Isolation of Urinary Steroids—The urine obtained from Subject A during the first 24 hours following the initiation of the intravenous injection was processed in three portions for the time intervals shown in Table II. The

urine from the second 24 hours following the initiation of the infusion was processed as a single collection.

TABLE I

Analysis of Testosterone-d and Testosterone-d-4-C^{14} in Infusion Solution

	Subject A		Subject F	
	Portion 1	Portion 2	Portion 1	Portion 2
Infusion solution (*Y*), *gm.*	222	315	146	227
Carrier testosterone (*W*), *mg.*	47.82	56.22	47.96	59.50
Diluted testosterone				
M.p., °*C.*	154–155	153.5–154.5	151–154	152–154.5
ϵ_{2410}			15,700	15,600
AD (*X*)	0.092	0.111	0.073	0.090
C.p.m. per mg. (*X*)			2,080	2,690
Isotopic testosterone in 100 gm. infusion solution (*V*), *mg.*	0.724	0.730	0.988* 0.901†	0.979* 0.937†

* Calculated from deuterium analysis.

† Calculated from C^{14} analysis.

TABLE II

*Urinary Excretion of Androsterone and Etiocholanolone after Intravenous Infusion of 11.3 Mg. of Testosterone-d in Subject A**

Period No. Time, *hrs.*	1 0–5	2 5–9	3 9–24	4 24–48	Total
Androsterone	0.55	1.21	0.45	0.07	2.28
Etiocholanolone	0.90	1.53	0.76	0.15	3.34
Total	1.45	2.74	1.21	0.22	5.62
Androsterone / Etiocholanolone	0.61	0.79	0.59	0.47	0.68

* Calculated from isotopic dilution; endogenous production disregarded. Periods 1 and 2 encompass the time of infusion. The results are in mg. of the product isolated.

The urine from Subject F during the first 24 hours was processed in four portions for the time intervals shown in Table III. That from the second 24 hours following the initiation of the infusion was processed as a single collection.

Both urines were hydrolyzed by Method B (3) (see the foot-note to Table III). Carrier androsterone and etiocholanolone in the amounts shown in Tables IV and V were added to the ether used for the Extract A. Since Extract B from Subject F had so little radioactivity, it was not further processed; Extracts A and C were combined and fractionated by the procedures described in the preceding report (1). The analytical samples of the urinary steroids were sublimed and recrystallized to constant

TABLE III

Distribution of Radioactivity Following Intravenous Infusion of 1,200,000 C.p.m. As Testosterone-d-C^{14} in Subject F

Period No. / Time, *hrs*	1 0–5	2 5–8.5	3 8.5–12	4 12–24	5 24–48
	c.p.m.	*c.p.m.*	*c.p.m.*	*c.p.m.*	*c.p.m.*
Neutral Extract A	178,000	72,500	12,300	14,000	25,000
" " C	187,000	46,000	32,000	48,000	61,000
" " B	4,200	3,000	1,000	1,000	4,100
Acidic	5,100	2,400	1,500	2,400	
Phenolic	2,000	500	100	0	0
Ketonic (A + C)	373,000	114,000	45,000	57,000	78,000
Saponified ketonic		97,000	42,000	58,000	74,000
Non-ketonic	20,000	22,000	9,200	11,000	10,000
α-Ketonic		77,000	33,000	54,000	69,000
β-Ketonic	3,400	3,000	1,900	2,200	3,700

Neutral Extract A was obtained by acidification of the urine to pH 1 and continuous extraction with ether for 48 hours; neutral Extract B was obtained by acidification of the urine after Extract A to 5 per cent sulfuric acid, volume for volume, heating under reflux for 30 minutes, cooling, and continuous extraction with ether for 48 hours; neutral Extract C was obtained from the acidic fraction from Extract A by the same acid hydrolysis procedure described for Extract B. In each extract the neutral, acidic, and phenolic fractions were obtained by methods previously described (3).

melting point; the physical constants and isotopic content are given in Tables IV and V.

Other α-Ketosteroids[2] *(Period 1, Subject F)*—From the early portion of the chromatogram of the α-ketonic fraction (Period 1) the eluates containing Δ^2-androstene-17-one, androstane-3,17-dione, etiocholane-3,17-dione, and 3β-hydroxyetiocholane-17-one were combined. Carrier steroids were

[2] The terms "α-ketonic fraction" and "α-ketosteroids" are used conventionally to indicate substances not precipitable by digitonin. Similarly, "β-ketonic fraction" and "β-ketosteroids" signify compounds that form insoluble digitonides. It is thus clear that such substances as Δ^2-androstene-17-one, etiocholane-3,17-dione, and androstane-3,17-dione, which do not have hydroxyl groups in the molecule, are, nevertheless, found in the "α-ketonic fraction."

added (Table VI) and the mixture was rechromatographed. Etiocholane-3,17-dione and 3β-hydroxyetiocholane-17-one were eluted together and were separated by precipitation with digitonin. Each steroid was purified by sublimation and recrystallization. The results are recorded in Table VI.

TABLE IV

Amount of Carrier Steroid Added and Physical Constants of Products Isolated in Subject A

Period No.	Androsterone			Etiocholanolone		
	Amount added to extract	M.p.	AD	Amount added to extract	M.p.	AD
	mg.	°C.		*mg.*	°C.	
1	27.28	185–186	0.052	50.87	152–154	0.046
2	27.28	185–186	0.112	50.87	152	0.077
3	27.28	185–186	0.043	50.87	152–153	0.039
4	25.58	185–186	0.007	24.68	152–153	0.016

TABLE V

Amount of Carrier Steroid Added and Physical Constants of Products Isolated in Subject F

Period No.	Androsterone				Etiocholanolone			
	Amount added to extract	M.p.	Specific activity	AD	Amount added to extract	M.p.	Specific activity	AD
	mg.	°C.	*c.p.m. per mg.* C^{14}		*mg.*	°C.	*c.p.m. per mg.* C^{14}	
1	40.76	184 –185	6890	0.185	44.10	151–152.5	3280	0.086
		184.5–185.5	6420			151–153	3080	
						151–153	3020	
2	25.34	187 –188	2720	0.079	26.39	151–152.5	880	0.023
3	20.27	184 –187	1380	0.039	22.00	151–152	550	0.016
4	20.27	184 –186	1600	0.045	22.00	151–152	1040	0.022
5	22.60	185 –187	1110	0.032	22.56	150–152	1730	0.049
		185 –186	1030			151–152.5	1710	

β-Ketosteroids (Subject F)—The β-ketonic fractions from Periods 1 to 4 (24 hours of the day of injection) were combined and recounted (9000 c.p.m.). 41.44 mg. of isoandrosterone and 44.89 mg. of dehydroisoandrosterone acetate were added. The mixture was acetylated with pyridine and acetic anhydride and was then treated with excess perbenzoic acid in benzene at room temperature overnight. The reaction product was chromatographed on silica gel. The eluates containing isoandrosterone ace-

tate were combined, sublimed, and recrystallized three times from methanol. Isoandrosterone acetate, m.p. 105–105.5°, was obtained with specific activity of 101 c.p.m. per mg. Saponification and recrystallization gave isoandrosterone, m.p. 171–172°, specific activity of 123 c.p.m. per mg., corresponding to 108 c.p.m. per mg. for the acetate (total activity 5100 c.p.m or 57 per cent of the total radioactivity found in the β fraction).

The eluates containing 3β-acetoxy-5α,6α-epoxyandrostane-17-one were combined and recrystallized from methanol and from acetone; the epoxide, m.p. 227.5–228.5°, was devoid of radioactivity.

TABLE VI

Urinary Excretion of Ketosteroids Other Than Androsterone and Etiocholanolone during Period 1 Following Intravenous Infusion of Testosterone-d-4-C^{14} in Subject F

Carrier steroid	Amount added to extract	Constants of product isolated			Amount recovered*	
		M.p.	Specific activity	Total		Per cent of dose
	mg.	°C.	*c.p.m. per mg.* C^{14}	*c.p.m.*	*mg.*	
Δ^2-Androstene-17-one	37.17	104–107	19	710	0.01	0.06
Androstane-3,17-dione	47.00	131–133	49	2300	0.03	0.19
Etiocholane-3,17-dione	47.66	132–134	12	570	0.01	0.06
3β-Hydroxyetiocholane-17-one†	42.07	150–153	1.7‡	70		

* From injected testosterone only; endogenous production disregarded.

† α-Ketonic fraction only.

‡ Gas count corrected to solid count.

The bulk of the remaining activity of the β fraction was eluted from the chromatogram after 3β-acetoxy-5α,6α-epoxyandrostane-17-one; total activity, 2900 c.p.m. or 32 per cent of the β fraction (0.2 per cent of the injected dose).

Fecal Steroids—The feces were collected from Subject F for 3 days after the infusion. Each day's collection was suspended in water, continuously extracted with ether for 96 hours, and the extract (A, Table VII) was counted. The residual fecal suspension was then acidified to pH 1, reextracted with ether, and the extract (B, Table VII) was counted. The suspension was acidified to 5 per cent by volume with sulfuric acid and then boiled for 30 minutes, reextracted, and the extract (C, Table VII) counted.

The ether Extracts B and C from the 3rd day were separately saponified with aqueous alcoholic base at room temperature for 1 hour. Each non-saponifiable fraction was extracted with ether, and the solvent was

removed. The residues were partitioned between 70 per cent ethanol and petroleum ether, and each fraction was counted. The petroleum ether extract from Extract B was devoid of radioactivity; that of Extract C had 3500 c.p.m. The 70 per cent ethanol-soluble material from Extract B had 9800 c.p.m., while there were 14,300 c.p.m. in the 70 per cent ethanol-soluble material of Extract C. The alcoholic solutions from Extracts B and C were combined, and 50.16 mg. of carrier androsterone and 46.49 mg. of carrier etiocholanolone were added. The mixture was recounted (23,900 c.p.m.) and chromatographed on silica gel.

The eluates containing androsterone were combined, sublimed, and the steroid was recrystallized twice from acetone-petroleum ether, m.p. 184–186°, 33 c.p.m. per mg. The specific activity was constant for three additional recrystallizations. Since 50.16 mg. of carrier were added, there

TABLE VII

Radioactivity in Fecal Extracts Following Intravenous Infusion of Testosterone-d-4-C^{14} in Subject F

The results are in counts per minute of C^{14}.

	Extract A	Extract B	Extract C
Day of injection	0	0	0
2nd day	5200	2500	3,400
3rd "	3200	7700	17,000

were 1700 total counts present in Extracts B and C as androsterone (equivalent to 0.021 mg. of injected testosterone).

The eluates containing etiocholanolone were combined, sublimed, and the steroid was recrystallized four times to constant melting point and specific activity of 40 c.p.m. per mg. Since 46.49 mg. of carrier were added, there were 1900 total counts in Extracts B and C as etiocholanolone (equivalent to 0.024 mg. of injected testosterone).

The eluates following etiocholanolone (9900 c.p.m.) were combined and separated by means of Girard's Reagent T into ketonic (2100 c.p.m.) and non-ketonic (7200 c.p.m) fractions. These were not further investigated.

Isotopic Analysis. Radioactivity—A known volume of a solution containing the extract or compound to be measured was evaporated on aluminum planchets. In most instances the amount of material was in the "infinitely thin" range; when larger amounts were used, the results were corrected to an infinitely thin layer by the data of Yankwich and Weigl (4). The samples were counted in internal flow gas counters, and sufficient counts were taken to obtain an accuracy of ±5 per cent. Samples of low specific activity were oxidized by the procedure of Van Slyke and Folch

(5), and the radioactivity of the carbon dioxide was measured by the procedure of Eidinoff (6).

Deuterium—The analytical procedure for this isotope is described in the preceding report (1).

DISCUSSION

Testosterone was rapidly metabolized and excreted after intravenous infusion. When testosterone was doubly labeled, the radioactivity during the first 24 hours (Table III, Periods 1 to 4) was 50 per cent of the administered dose, recovered from the urine as crude, ether-extractable, neutral metabolites. On the 2nd day of the experiment (Period 5), only an additional 7 per cent of neutral radioactive metabolites was obtained. The rapid excretion of the hormone metabolites is illustrated by the fact that of the total radioactivity eliminated on the 1st day 62 per cent was recovered during the period of infusion (Period 1). The experiment with singly labeled testosterone-*d* showed essentially the same rapid elimination of the hormone metabolites (Table II). Similar results were obtained after massive doses of testosterone by West *et al.* (2) who followed urinary 17-ketosteroid excretion by the Zimmermann color reaction. These authors reported a small secondary rise in the urinary 17-ketosteroids on the 4th to the 8th day after intravenous injection of testosterone. In our radioactive experiments with this hormone, the urinary excretion of testosterone metabolites had dropped to very low levels by the 3rd day and negligible amounts were obtained thereafter. There was no indication of a secondary rise in radioactivity. The difference may be a consequence of the large amount of hormone injected by West *et al.* in contrast with the small amount used in our studies. The neutral fraction accounted for nearly all of the radioactivity in the urine; the acidic and phenolic fraction had almost negligible radioactivity. The distribution of the radioactivity in the various subfractions of the urinary extracts is illustrated in Table III. The bulk of the activity was found in the α-ketonic fraction, and very little was present in the β-ketonic fraction; only a small amount was found in the non-ketonic fraction. These results are in good agreement with those of the long term experiments reported by Dobriner and Lieberman (7) and with those in the previous paper when testosterone-*d* was given intramuscularly.

Since the urine contained only about 60 per cent of the C^{14} administered, stools and respiratory carbon dioxide were examined for radioactivity. Only about 5 per cent of the dose administered was found in the feces over 3 days.[3] The radioactivity did not appear in the feces until the 2nd day,

[3] In other comparable experiments with radioactive testosterone, it has been found that, at most, 10 to 15 per cent of the total C^{14} administered is excreted in the feces during the course of 7 to 10 days.

and the major portion was found in the stools of the 3rd day (Table VII). Practically all of the activity in the feces of the 3rd day was found to be non-saponifiable material which could be partitioned into 70 per cent alcohol from petroleum ether; there was negligible radioactivity in the acidic fraction. There was relatively little androsterone and etiocholanolone in the feces as judged from isotopic dilution and isolation; a fairly large fraction was non-ketonic, and the remainder of the radioactivity was distributed over many fractions of the chromatogram.

Samples of expired air were collected from Subject F several times after administration of testosterone-4-C^{14}. There was no significant activity in any of the samples, a result to be expected from the very great dilution that would occur were even a large fraction of C-4 of testosterone completely oxidized to carbon dioxide.

It is now firmly established that androsterone and etiocholanolone are the chief metabolites of testosterone in man, all other products in the urine representing but a minor fraction of the total excreted. Thus the recovery as androsterone and etiocholanolone was 48 per cent of the administered dose in both intravenous experiments (Tables II and VIII), in excellent agreement with the recoveries demonstrated after intramuscular injection of the hormone. In the intravenous experiments, during the period of infusion, 68 to 70 per cent of the total 24 hour output of androsterone and etiocholanolone was eliminated and the radioactivity isolated in these two metabolites accounted for 91 per cent of the total present in the urine extracts.

Although the total recovery of androsterone and etiocholanolone was the same after intramuscular or intravenous administration of testosterone, the ratio of androsterone to etiocholanolone differs with the two modes of administration. Whereas this ratio during the first 24 hours was approximately 0.4 in the intramuscular experiment with Subject A (1) as well as in his control period, it was higher, 0.7, when a similar amount of hormone was given intravenously. More significant is the continually changing androsterone-etiocholanolone ratio in both intravenous experiments (Tables II and VIII). In Subject A during Period 1 (Table II), this ratio was higher than his control value, increased still further in Period 2, and declined nearly to the endogenous value by the 2nd day. A more marked change in androsterone-etiocholanolone ratio was observed with Subject F (Table VIII). During the infusion period the ratio was 2.08, increased to 3.4 in Period 2, and decreased until on the 2nd day there was more etiocholanolone excreted than androsterone (ratio, 0.65). These changes in the type of ring fusion effected, consequent to the introduction of hormone in excess of glandular production, may provide an index of hormone transformation by one or another organ or tissue. Experiments designed to test these possibilities are in progress.

Some other ketosteroid metabolites of testosterone were studied in Period 1 of Subject F, and the results are reported in Table VI. The four compounds accounted for only 3600 c.p.m. out of 373,000 c.p.m. recovered in Period 1 as ketonic metabolites, or less than 0.3 per cent of the total dose administered. In addition to these four substances, the β-ketosteroids of the combined periods of the 1st day were studied in Subject F. Less than 1 per cent of the dose administered was recovered in the β fraction (9000 c.p.m.). It was found that isoandrosterone contained more than half (5100

TABLE VIII

Recovery of Metabolites from Intravenously Infused Testosterone-d-4-C^{14} in Subject F

Period No.	Androsterone				Etiocholanolone				Androsterone Etiocholanolone
	Calculated from		Per cent dose injected calculated from		Calculated from		Per cent dose injected calculated from		Calculated from
	C^{14}	D	C^{14}	D	C^{14}	D	C^{14}	D	C^{14}	D
	mg.	*mg.*								
1	3.61 3.36*	3.50	23.3 21.7*	21.1	1.85 1.71*	1.68	11.9 11.0*	10.1	1.97	2.08
2	0.88	0.89	5.7	5.4	0.30	0.26	1.9	1.6	2.93	3.42
3	0.36	0.34	2.3	2.1	0.16	0.15	1.0	0.9	2.25	2.26
4	0.42	0.40	2.7	2.4	0.29	0.21	1.9	1.3	1.45	1.90
Total, 24 hrs.	5.02	5.13	32.4	31.0	2.46	2.30	15.8	13.9	2.04	2.23
5	0.32 0.29*	0.31	2.1 1.9*	1.9	0.50 0.50*	0.48	3.2 3.2*	2.9	0.58	0.65
Total, 48 hrs.	5.31	5.43	34.3	32.9	2.96	2.78	19.0	16.7	1.79	1.95

* Value used for total recovery.

c.p.m.) of this activity, and *no activity* was present in dehydroisoandrosterone.

From the results obtained in this and the preceding study, it is possible to estimate the normal endogenous production of testosterone by man. If it is presumed that the slow and intravenous infusion of a small dose of testosterone simulates the normal secretion of the hormone by the testis, and, in addition, that testosterone production is essentially at a constant level throughout the 24 hour day, the experiment with Subject A can be used to calculate approximately his daily endogenous production of hormone. In the experimental period, 11.3 mg. of testosterone were infused during 8 hours and, of this 1.7 mg. or 16 per cent of the administered hormone was isolated as androsterone from the urine excreted during the first

9 hours of the experiment. If, then, the testosterone infused behaved similarly to the hormone secreted by the testis, approximately 16 per cent of the endogenously produced hormone should appear as androsterone in the urine during any given interval of time. From three separate earlier control periods in this same subject, it was known that his daily excretion of androsterone averaged 2.7 mg., and, if this amount represented approximately 16 per cent of his glandular production, the latter should be very close to 17 mg. per day. Since it can be shown that part, at least, of the urinary androsterone is derived from steroids other than testosterone, it follows that the daily production of 17 mg. is a maximal estimate, and therefore the normal secretion is less than this figure by an unknown but probably small amount. The credibility of this estimate is confirmed by the fact that it corresponds closely to the minimal quantity of testosterone necessary to induce a detectable response in nitrogen metabolism (8, 9).

As anticipated, the calculation of daily testosterone production from the amount of etiocholanolone excreted yields a higher value than that obtained from androsterone. During the intravenous infusion, 2.43 mg., corresponding to approximately 22 per cent of the hormone, were excreted as etiocholanolone. The average daily excretion of this metabolite by Subject A was 8 mg., from which, on the same presumptions, it could be calculated that there was a glandular production of about 36 mg. per day. Since it is clear that a considerable portion of urinary etiocholanolone is derived from adrenal precursors, the estimate is not so reliable as that obtained from androsterone; reasonable agreement between the two estimates lends conviction to the postulates advanced for the calculations. The estimate from either metabolite may be somewhat in error as a result of the changing ratio of these two compounds during the experimental interval, as was dealt with earlier in the discussion. It is therefore desirable to confirm these preliminary calculations by experiments similarly designed with tracer doses of hormone. Both estimates are inherently credible and, in view of the fact that they are maximal estimates, provide some measure of the upper limits of testosterone production by a normal man.

SUMMARY

After slow intravenous infusion of testosterone-*d* in a normal man and testosterone-*d*-4-C^{14} in a man with aplastic anemia, essentially similar recovery of isotopic metabolites from the urine was obtained during several periods of the first 48 hours after injection. Androsterone and etiocholanolone were the principal metabolites. The ratio of these two products varied during and after the time of infusion. From the amount of exogenous hormone converted to either androsterone or etiocholanolone, it was calculated that the daily production of testosterone by a normal man does

not exceed 36 mg. and that less than 17 mg. per 24 hours is a more credible estimate.

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