Journal of Steroid Biochemistry, 1976, Vol. 7, pp. 65–70. Pergamon Press. Printed in Great Britain

THE METABOLIC FATE OF MEDROXYPROGESTERONE ACETATE IN THE BABOON

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SUMMARY

The metabolic fate of medroxyprogesterone acetate (6α-methyl-17α-acetoxyprogesterone, MAP) was studied in intact baboons and in those with bile fistulas. The steroid moiety was labeled with ^{3}H at positions 1 and 2 and the 17α-acetate with ^{14}C, thus affording the opportunity to ascertain the loss of the 17-acetoxy group and the fate of both labels. Following the i.v. administration of labeled MAP only a small percentage (less than 15%) of the administered dose was recovered in the urine in 7 h in intact baboons, as well as in the urine of baboons with biliary fistulas. Higher amounts of radioactivity were excreted in the bile (approx. 25%), amounting to almost double the percentage excreted in the urine. The similarity in the urinary excretion of radioactivity in intact and biliary fistula animals indicates that, even though a substantial biliary excretion of the labeled MAP occurred, the amount involved in an enterohepatic circulation is probably small. Glucosiduronates were the predominent conjugates, both in the urine and bile. The loss of the 17α-acetate group appeared to be rather extensive, ranging from 30 to 70% among different conjugated and unconjugated metabolites of MAP. The degree of *in vivo* hydrolysis of the axial 17α-acetate of MAP, though extensive, appeared to be of a significantly lesser magnitude than that exhibited toward the equatorial 3β and 17β acetate groups of labeled ethynodiol diacetate injected into baboons. The deacetylation of the 17α-acetate in MAP was similar to that observed in humans given the drug. Oxygenation of MAP at positions 1 and/or 2 appeared to be rather minimal (< 5%).

INTRODUCTION

Medroxyprogesterone acetate (6α-methyl-17α-acetoxyprogesterone, MAP) is a derivative of 17α-acetoxyprogesterone with strong gestagenic and antigonadotropic effects. Since its introduction in 1960, MAP has been used extensively in the treatment of endometriosis, threatened and habitual abortions and contraception [1–4]. Clinical studies have shown this long-acting agent for intramuscular administration to be relatively safe [1–4]. Despite its widespread use, however, little is known about the metabolism of this compound in the human. Particularly, no *in vivo* studies of MAP in subhuman primates have been reported.

When administered to humans in the form of [4-^{14}C]-MAP, the labeled compound was recovered to the extent of 20–42% of the administered dose in urine after i.v. injection, compared to a recovery of 58% for 17α-hydroxyprogesterone [5]. Fotherby *et al.* [6] reported similar results using [^{3}H]-MAP. In general, the recovery of the dose in urine is much less, when compared to that of 19-nortestosterone derivatives, progesterone, and naturally occurring neutral steroids.

A report on the fate of "orally" administered MAP, labelled with ^{3}H in the 7α-position, in normal and pregnant women indicates that the preponderant amount of the radioactivity (approx. 85%) appeared in the stools and minimal amounts (< 5%) in the urine [7]. The normal women studied excreted less than 1% and the pregnant subjects 3·1–4·34% of the administered dose in the urine in 24 h. These contrast with the much higher urinary excretion of the radioactivity of MAP reported following its i.v. administration and points to the possibility that either the label at the 7α-position had been lost (e.g., through hydroxylation) and/or little absorption of the compound had occurred in the intestinal tract of the subjects reported. However, Helmreich and Huseby [8] have indicated that the form in which MAP is administered may play an important role in its intestinal absorption and it is possible that the difference in urinary excretion may be related to this facet of MAP administration. Certainly, the data of Helmreich and Huseby indicated a higher absorption of MAP from the intestinal tract than reported by Besch *et al.* [7].

In another study, Helmreich and Huseby [9] reported that a major metabolite of MAP was 6α-methyl-6β, 17α,21-trihydroxy-4-pregnen-3,20-dione 17-acetate, i.e., the 6β, 21-dihydroxylated derivative of the parent compound. The major portion of the metabolite was excreted as a conjugate, almost certainly a glucosiduronate, and probably conjugated via the 21-hydroxy group. Conjugation at the C-6 hydroxy group is very unlikely, since it is a tertiary alcohol. Formation of an enol-glucosiduronate at C-3 is theoretically possible, but not a 3,5-dienol, since both hydrogens at C-6 are substituted.

The most important considerations about the structural changes of administered MAP are related to the

This study has been carried out under contract N01HD-2-2724 and supported in part by grants AM-01240 and AM-11754 from the National Institutes of Health.

acetylation at C-21 and to hydroxylation at C-6 and C-21. Thus, it has been shown by other workers that the main metabolite in the urine was 6α-methyl-6β,17α,21-trihydroxy-4-pregnene-3,20-dione 21-acetate in humans [10]. Since a 17-aceylated compound was administered, the isolation of 21-acetylated metabolites was unexpected. These authors [10] assumed that after C-21 hydroxylation, a transesterification had occurred, but no final proof of this could be given. Hence, some divergence exists as to whether the 17-acetoxy group remains intact or is somehow transacetylated to the 21-position [8–11].

A related compound, megestrol acetate (17α-acetoxy-6-methyl-pregna-4,6-diene-3,20-dione) undergoes hydroxylation at the C-2, 6-methyl and C-21 positions [11]. It should be noted that all metabolites of compounds related to 17α-acetoxyprogesterone retain the 4-en-3-one group. Smith *et al.* [12] reported an increase in urinary 17-hydroxysteroids, but no change in 17-ketosteroids, in one subject treated with large doses of MAP. Oral administration favored urinary excretion of free and conjugated metabolites [13], most of which gave a Proter–Silber reaction. These studies indicate the presence of a dihydroxy keto side-chain in the MAP metabolites with apparently little of the side chain being lost. However, the quantitative aspects of these observations have been very difficult to evaluate. Apparently, following oral administration MAP accumulates in the liver and is excreted through the biliary system, though a marked uptake in the kidneys has been documented [14].

We have utilized the baboon as a surrogate for the human, since we have shown that this animal metabolizes a number of steroids similarly to the human [15–17]. Furthermore, no studies on the metabolism of MAP in subhuman primates have been published. In addition, because of the possible significance of the biliary route of excretion of MAP and the limitations for such studies in man, observations in the baboon, an animal which appears to have a biliary excretory pattern for steroids very much like that of the human [15–17], afforded another parameter possibly applicable for extrapolation of the results to the human.

In the following report we wish to present results of urinary and biliary excretion data in *Papio anubis* baboons injected with singly or doubly labeled medroxyprogesterone acetate. This compound was labeled with ^{3}H in the steroid nucleus and with ^{14}C in the 17α-acetoxy side chain. One of the objectives of the double labeled study was the determination of the metabolic integrity of the 17-acetoxy group.

MATERIALS AND METHODS

Steroids. [1,2-^{3}H]-MAP (58Ci/mol), kindly supplied to us by Dr. C. Wayne Bardin of the Milton S. Hershey Medical Center, Pennsylvania State University, was shown to be radiochemically pure by paper chromatography. Carrier MAP was purchased from Steraloids. The singly labeled compound with the ^{14}C label in the 17-acetate moiety was synthesized by previously described methods [18]. ^{3}H- and ^{14}C-labeled materials were mixed to give a S.A. for $^{3}H = 38{\cdot}4$ mCi/mmol and for $^{14}C = 1{\cdot}82$ mCi/mmol and a $^{3}H/^{14}C$ ratio of 21·2.

Animals. Six *Papio anubis* baboons were used in this study (Table 1), identified as U-5, W-1, W-4, R-8, T-3 and T-10. The first four baboons were used both intact and after surgical modification (biliary fistula). Baboons used in that manner were not injected for a second time until at least 2 months have elapsed after the first injection.

Administration of radioactivity. Each animal received labeled MAP (according to the schedule in Table 1) in 10 ml of 20% aqueous ethanol *via* an i.v. injection into the saphenous vein after being anesthetized with Diazepam (Valium, Roche) and phencyclidine hydrochloride (Sernylan, Parke–Davis). The syringes and containers used for the injection were washed with methanol and aliquots of the washings were counted in order to determine the exact amount of radioactivity injected.

Collection of urine and bile. After the injection of the radioactive steroid, urine was collected using a previously inserted catheter. Urine and bile collection times were as follows: 5, 10, 15, 30, 45 min; 1, 2, 3, 4, 5, 6 and 7 h after injection. The samples of excreta were immediately adjusted to pH 9 with concentrated ammonium hydroxide and kept at 0–4°C until processed.

Radioassay of samples. Alliquots of each urine sample were transferred to plastic counting vials containing 10 ml of Instagel (Packard Instruments) diluted with toluene (1 part toluene, 2 parts Instagel) for economy. The vol. of each bile collection was noted and brought up to 10 ml with water. Aliquots of each solution were absorbed on filter paper and oxidized in a Packard Tri-Carb Sample Oxidizer model 306. The urinary and biliary radioactivity were determined in Packard Instruments Tri-Carb Spectrometers models 3375 or 2450 to a relative standard error of 2% and corrected for background and quenching.

Chromatography. An Amberlite XAD-2 resin (Rohm & Haas, Philadelphia, Pa.) column of 50 ml capacity (15 mm i.d.) was used to process bile and urine according to the method of Osawa and Slaunwhite [19]. Usually, the total urine or bile sample was applied to the column and the latter was washed with water and eluted with methanol. The XAD column invariably retained more than 95% of the ^{3}H in the urine and less than 5% of the applied radioactivity was found in the water washes. The methanol eluent of the XAD column contained at least 80% of the radioactivity applied to the column. When the doubly labeled MAP standard was applied to the XAD column it was almost quantitatively retained by the column (>97%) and then almost quantitatively (>95%) recovered in the methanol eluent.

Table 1. Protocol of urinary and biliary metabolic studies in baboons following the i.v. administration of singly or doubly labeled medroxyprogesterone acetate

Baboon #	Conditions	Isotope Label	INJECTED DOSES– ^3H (μCi)	^{14}C (μCi)	μg	$^3H/^{14}C$ Ratio
U-5 female	Intact	[1,2-^{3}H and 17-acetoxyl-^{14}C]	40	1.7	400	21.1
W-1 female	"	[1,2-^{3}H]	*40	-	400	-
W-4 female	"	[1,2-^{3}H]	*40	-	400	-
R-8 male	"	[1,2-^{3}H]	*40	-	400	-
T-3 male	Biliary Fistula	[1,2-^{3}H and 17-acetoxyl-^{14}C]	40	1.7	400	21.1
T-10 male	"	[1,2-^{3}H and 17-acetoxyl-^{14}C]	40	1.7	400	21.1
W-1 female	"	[1,2-^{3}H]	*40	-	400	-
R-8 male	"	[1,2-^{3}H]	*40	-	400	-

* Diluted with carrier

The residues remaining after evaporation of the methanol were dissolved in 10–15 ml of 20% aqueous methanol and applied to DEAE-Sephadex A-25 columns (0·9 × 60 cm. K9/60 Sephadex columns; Pharmacia Fine Chemicals, Inc., Piscataway, N.J.). The columns were eluted with linear gradient obtained by mixing 400 ml of 0·06 M NaCl with 400 ml water and then with 2·0 M NaCl. A flow rate of approx. 80 ml/h was maintained. Fractions of 10 ml were collected and radioassayed as described above. The elution patterns from these columns were such that the early fractions contained non-charged compounds, while the later fractions contained charged compounds. Enzymatic hydrolyses were performed on these fractions with Ketodase (Warner–Chilcott, Morris Plains, N.J., 500 units β-glucuronidase/ml of eluent) at 37°C for 38 h followed by three ethyl acetate extractions.

RESULTS

Table 2 presents the excretion data from all four baboons studied. The urinary excretion of the injected radioactivity in the intact animals in the 7 h period was rather low, ranging from 6·6% to 12·0% of the administered dose. The excretion of radioactivity in the urine of the biliary fistula animals indicates that it was similar to that found in intact control animals (Table 2), with the exception of the urine from R-8 in which the rate was about double that of the others. The excretion of radioactivity in the bile from W-1, T-10 and R-8 was relatively high, when compared to that in the urine (22·6 to 33·8%; Table 2), but T-3 had a low level of biliary excretion (7·7 to 10·6% for ^{14}C and ^3H, respectively). There appeared to be no marked differences in the relative rates of excretion of the two isotopes, in the urines and biles from U-5 and T-10, in contrast to the drastic differences ob-

Table 2. Excretion of radioactivity in urine and bile following the i.v. injection of singly or doubly labeled medroxyprogesterone acetate

Conditions	Specimen	Baboon #	Isotope Label	----- PERCENT OF THE INJECTED DOSE ----- 0–1 Hr ^{14}C	0–1 Hr ^3H	1–3 Hr ^{14}C	1–3 Hr ^3H	3–7 Hr ^{14}C	3–7 Hr ^3H	Total ^{14}C	Total ^3H
Intact	Urine	U-5	Double	2.0	1.4	5.0	5.2	5.2	5.1	12.2	11.8
"	"	W-1	Single	-	1.9	-	4.1	-	4.1	-	10.1
"	"	W-4	"	-	1.4	-	2.5	-	2.7	-	6.6
"	"	R-8	"	-	1.6	-	4.1	-	6.3	-	12.0
Biliary Fistula	Urine	T-3	Double	2.7	2.4	7.2	4.4	6.1	3.9	16.0	10.7
"	"	T-10	"	1.7	1.6	5.9	6.2	7.7	7.1	15.3	14.9
"	"	W-1	Single	-	2.3	-	3.7	-	3.6	-	9.6
"	"	R-8	"	-	4.3	-	17.9	-	7.5	-	29.7
"	Bile	T-3	Double	1.7	1.7	2.9	4.1	3.1	4.8	7.7	10.6
"	"	T-10	"	3.2	3.2	13.9	16.2	8.1	9.5	25.2	28.9
"	"	W-1	Single	-	6.9	-	9.6	-	6.1	-	22.6
"	"	R-8	"	-	8.9	-	17.8	-	7.1	-	33.8

served for ethynodiol diacetate in the same animals [17]. The $^{3}H/^{14}C$ ratios in the urine of the biliary fistula T-3 baboon decreased with time. This pointed to the possibility of loss of ^{3}H from the 1 and/or 2 positions in some of the MAP metabolites excreted in the urine of that animal. However, since the nature of the ^{3}H and ^{14}C labels present in the unprocessed urine or bile was unknown, further studies, based on chromatographic techniques, were aimed at elucidating the $^{3}H/^{14}C$ ratios persisting in the metabolites of MAP. Such an approach should yield reliable data on the loss of ^{3}H and/or ^{14}C from the MAP molecule.

All urines and biles collected in the present study were chromatographed first on XAD-2 and then on DEAE-Sephadex columns, as described in previously reported work [16–19]. The XAD-2 column is a purification step, whereas Sephadex chromatography is a separation of compounds in the fluid into groups of conjugates or free steroids by virtue of differences in size and charge of the molecules. The following figures (Figs. 1–5) are examples of such chromatographies. Figure 1 is the elution pattern after fractionation of the 30–60 min urine collection from an intact baboon (U-5) injected with doubly labeled MAP. Peak I consists of the so-called "uncharged" compounds, containing possible free steroids and/or noncharged conjugates. Peaks II and III contain glucosiduronates. No sulfates or polyconjugates were detected in this urine collection upon chromatography. This was also ascertained by duplicate chromatography using a steeper gradient. Recovery of chromatographed radioactivity from these columns was >90%. It can be seen that the ^{14}C label is present in all peaks. The changes in the $^{3}H/^{14}C$ ratios in Peaks I–III, i.e., most of the ratios exceeding the injected value of 21·2, point to a substantial loss of the ^{14}C label of the 17α-acetate.

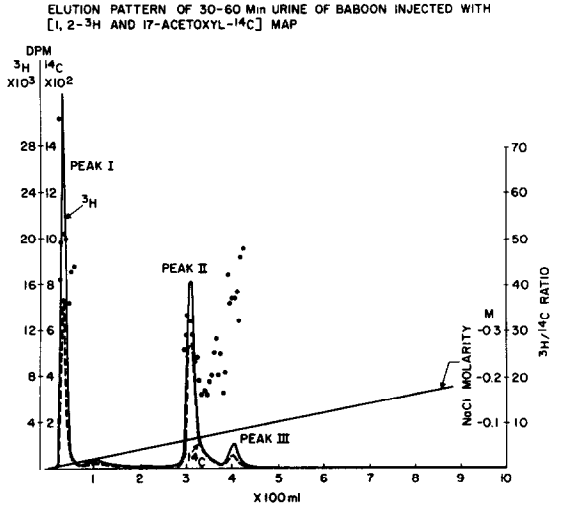

Fig. 1. Elution pattern obtained upon DEAE-Sephadex A-25 chromatography of the 30–60 min urine after the injection of doubly labeled MAP (1,2-^{3}H and 17-acetoxyl-^{14}C) into an intact female baboon. From the increased $^{3}H/^{14}C$ ratios in the three major peaks (Peaks I–III) it is apparent that considerable ^{14}C had been lost, indicating deacetylation of the MAP. The free dots indicate values of the $^{3}H/^{14}C$ ratio.

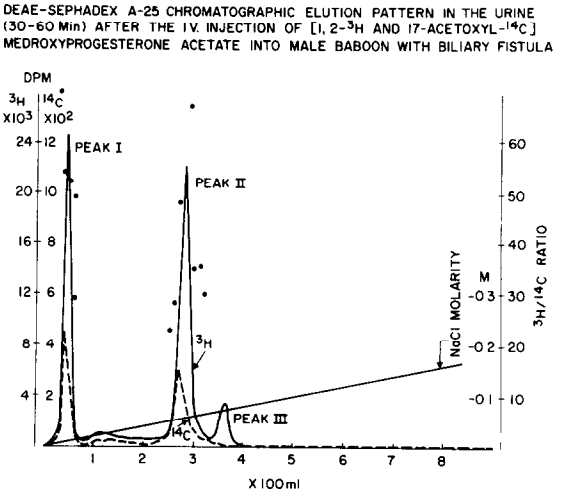

Fig. 2. Elution pattern obtained upon DEAE-Sephadex A-25 chromatography of 30–60 min urine after the injection of doubly labeled MAP into a male baboon with a biliary fistula. The three major peaks (Peak I–III) have $^{3}H/^{14}C$ ratios much higher than the injected one, indicative of considerable loss of ^{14}C and, hence, the 17α-acetate moiety of MAP. Peak III is almost totally free of ^{14}C. The free dots indicate values of the $^{3}H/^{14}C$ ratio.

Figure 2 is the elution pattern upon chromatography of the 30–60 min. urine of a bilary fistula baboon (T-10) injected with doubly labeled MAP. In Peaks I and II the $^{3}H/^{14}C$ ratios all exceeded the injected one, indicating considerable loss of the ^{14}C label of the 17α-acetate group. Indeed, Peak III lacks ^{14}C almost totally, indicating loss of the ^{14}C labeled acetate moiety. Figure 3 is the elution pattern after chromatography of the 30–60 min bile from the same baboon. Compared to the urine, the bile contains much less uncharged compounds, and more complicated glucosiduronate peaks (Peaks II, III; Fig. 3), with the ^{14}C label being present in all the major peaks. However, most of the $^{3}H/^{14}C$ ratios were significantly higher than the injected one and point to substantial loss of the ^{14}C of the 17α-acetate group.

Figure 4 is the DEAE-Sephadex elution pattern of the combined 5–7 h urine collections from baboon T-3. There is a multiplicity of peaks (designated I–VII) with a great variety of $^{3}H/^{14}C$ ratios. Peaks III, IV, VII and VIII contain little ^{3}H, indicating removal of that label from positions 1 and/or 2. These oxidations would account for the low $^{3}H/^{14}C$ ratio observed in the total urine. Peak VII was hydrolyzed with β-glucuronidase and the resulting aglycones were chromatographed on Sephadex LH-20 with benzene–methanol (85:15 v/v). The elution pattern is shown in Fig. 5. The presence of a complex mixture of aglycones is indicated; however, no elucidation of the structures of the aglycones was undertaken. All these aglycones are labeled with ^{14}C. However, in the two major Peaks I and II (Fig. 4) the $^{3}H/^{14}C$ ratios greatly exceeded the injected one (21·2) and, again,

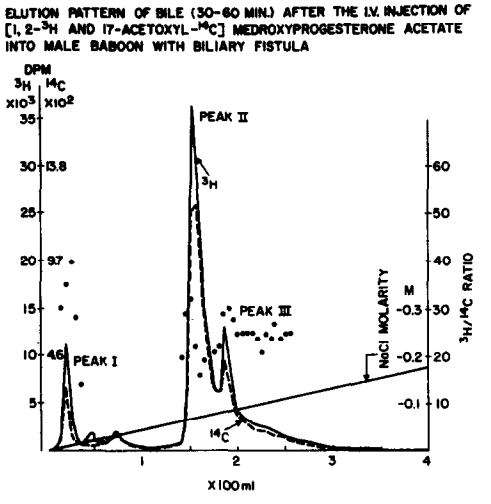

Fig. 3. Elution pattern of the 30–60 min bile from baboon T-10 and performed under identical conditions as described in Fig. 2. Again, as in the urine, the $^3H/^{14}C$ ratios are elevated above the injected value, pointing to loss of the 17α-acetate group. The free dots indicate values of the $^3H/^{14}C$ ratio.

point to considerable loss of ^{14}C of the 17α-acetate moiety. By comparison, the loss of ^{14}C greatly exceeded that of ^3H in positions 1 and 2 of the MAP molecule.

Table 3 is a compilation of the relative amounts of radioactivity present in each major metabolite fraction, as analyzed by DEAE-Sephadex column chromatography.

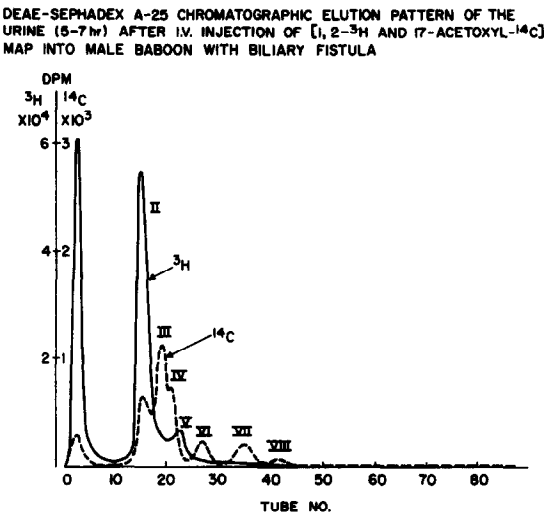

Fig. 4. Elution pattern (DEAE-Sephadex A-25 chromatography) of 5–7 h urine sample of baboon with biliary fistula injected with doubly labeled MAP. Peaks III, IV, VI–VIII have more ^{14}C than ^3H, i.e., a reverse of the injected ratio, pointing to oxygenation at positions 1 and 2 of MAP resulting in loss of ^3H. However, quantitatively more of the ^{14}C was lost, as indicated by the greatly increased $^3H/^{14}C$ ratios in the large Peaks I and II.

DISCUSSION

The results obtained with labeled MAP in the baboon resemble in many respects those reported for the human [5–12]: 1. The total recovery of radioactivity in the urine following the injection of labeled MAP tended to be rather low in both species, compared to that, for example, following the injection of progesterone or 17α-hydroxyprogesterone, 2. The excretions in the bile were similar, and both species appeared to lack an extensive enterohepatic circulation of MAP and its conjugated metabolites, and 3. In the urine of both species the glucosiduronates made up the bulk of the conjugates. In the present study, however, we were able to ascertain the extent of loss of the axial 17α-acetate group, which appeared to be substantial, varying from 30–70% among the different conjugated and unconjugated metabolites of MAP. This definitely contrasts with the nearly quantitative hydrolysis of the equatorial acetate at position 3β and more than 90% hydrolysis of that in position 17β observed following the injection of ethynodiol diacetate into baboons [17]. The data on the human, which can be extrapolated from published reports on the basis of various metabolites excreted following the administration of MAP [1–13], indicate a degree of deacetylation of MAP probably similar to that observed in baboons.

The present study has demonstrated that MAP is excreted in the baboon at a slower rate than ethynodiol diacetate or progesterone [15–17]. In the urine of baboons with biliary fistulas, except for one case, the excretion rate was almost the same as that of intact baboons. In contrast, the biliary excretion of radioactivity was high compared to that of urine. Since the urinary excretion of radioactivity in intact and biliary fistula animals was very similar, we can

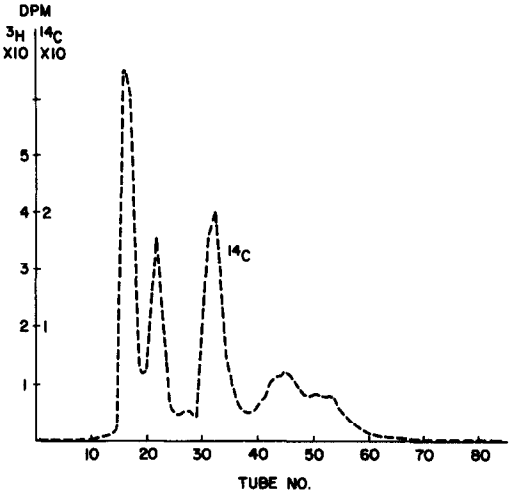

Fig. 5. Elution pattern upon Sephadex-LH20 chromatography of hydrolyzed (with β-glucuronidase) Peak VII shown in Fig. 4 and consisting almost exclusively of ^3H labeled MAP metabolites. It is apparent that a number of different aglycones was present in Peak VII. These peaks were not further identified.

Table 3. Radioactivity in fractions eluted from deae-sephadex columns as per cent of the amount fractionated

Baboon #			"Noncharged" * ^3H	"Noncharged" * ^{14}C	Gluc I ** ^3H	Gluc I ** ^{14}C	Gluc II *** ^3H	Gluc II *** ^{14}C
U-5 female	Intact	Urine	51	36	44	59	6	6
W-1 female	"	"	45	--	55	--	5	-
W-4 female	"	"	61	--	34	--	5	-
R-8 male	"	"	41	--	57	--	10	-
T-3 male	Bile Fistula	"	29	19	57	64	23	28
T-10 male	"	"	36	37	55	56	9	7.3
R-8 male	"	"	35	--	50	--	16	-
W-1 female	"	"	46	--	31	--	22	-
T-3 male	"	Bile	17	15	50	47	28	35
T-10 male	"	"	8	6	62	67	30	27
R-8 male	"	"	13	--	61	--	27	-
W-1 female	"	"	16	--	57	--	27	-

* = Peak I in the eluted material from the DEAE-Sephadex A-25 chromatography column (See Figs. 1-3).

** = Peak II as above.

*** = Peak III as above.

conclude that very little or no enterohepatic circulation of MAP takes place in the baboon.

"Uncharged" fractions from urine contained relatively more radioactivity after injection of MAP than in the case of progesterone or ethynodiol diacetate [16,17]. Glucosiduronates were the predominant conjugates and no significant amounts of sulfates or di-conjugates were detected. The glucosiduronates were identified not only by their location in the elution pattern of the DEAE column, but also by hydrolysis with β-glucuronidase (see Materials and Methods). Similar criteria were used for the identification of sulfates (hydrolysis with mylase-P) and diconjugates.

The metabolic fate of the doubly-labeled MAP in baboons indicates a degree of *in vivo* hydrolysis of its 17α-axial acetate of much lesser magnitude than that exhibited towards the equatorial 3β and 17β acetates of ethynodiol diacetate previously studied in baboons under similar conditions [17]. In that study [17] almost no 3-acetate carrying metabolite was found in the urine or bile of the baboon and only a low percentage of the metabolites separated by column chromatography indicated the presence of the 17β-acetate group in ethynodiol diacetate metabolites. In contrast, drastic changes in the relative excretion of both labels and the $^3H/^{14}C$ ratios were not ascertained in "unprocessed" urines and biles during the time course of the studies with labeled MAP. However, after separation of the metabolites of MAP into groups by DEAE-Sephadex column chromatography most of the peaks (particularly Peaks I and II in Figs. 1–3) show higher $^3H/^{14}C$ ratios (approx. 30–60), compared to the injected ratio of 21:2. Thus, it is estimated that about 30–70% of the separated peaks contained metabolites devoid of the 17α-acetate. There was found one minor peak (Peak III in Fig. 2) in which essentially only ^3H-labeled compounds were present, indicating that this constituted a conjugate of a metabolite of MAP almost entirely devoid of the 17α-acetate. The loss of ^3H, indicative of oxygenation or dehydrogenation at positions 1 and/or 2 of MAP, was small (<5%), as indicated by the minor peaks of Fig. 4 in which loss of the ^3H is evident.

The rather low recovery (<40%) of the radioactivity injected during the 7 h of urine and bile collections in baboons contrasts with that of labeled progesterone, estriol, and ethynodiol diacetate and other steroids in this animal [15–17]. Had the collection periods been extended, undoubtedly more of the radioactivity would have been recovered. However, the very slow rate of excretion of such radioactivity during the latter hours of collection indicates that the MAP had been sequested in body compartments from which egress and/or metabolism were very slow and probably requiring prolonged periods of time for its full recovery.

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