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Metabolism of Ecdysteroids during the Vitellogenesis of the Tick *Ornithodoros moubata* (Ixodoidea, Argasidae): Accumulation of Apolar Metabolites in the Eggs

J.-L. CONNAT, P. A. DIEHL, AND M. MORICI

Institut de Zoologie, Université de Neuchâtel, Chantermerle 22, CH-2000 Neuchâtel 7, Switzerland

The fate of injected [^{3}H]ecdysone ([^{3}H]E) and 20-hydroxy-[^{3}H]ecdysone ([^{3}H]20E) has been investigated in the female tick *Ornithodoros moubata* (Murray, 1877; *sensu* Walton, 1962). When injected into fed mated vitellogenic females, [^{3}H]E is converted into [^{3}H]20E and two apolar classes of metabolites, AP1 and AP2. Injected [^{3}H]20E is directly converted into AP1 and AP2. AP2 is incorporated into the ovaries in a high proportion and at the end of the vitellogenic cycle represents about 25% of the total label recovered from the animal. The fate of labeled hormones injected into virgin females which perform an abortive vitellogenic cycle is quite similar. However, the ovaries incorporated less of the AP2 products. Ovaries of mated females cultured *in vitro* in the presence of [^{3}H]E are able to produce [^{3}H]20E and AP2. AP2 is incorporated, while [^{3}H]20E is mainly found in the medium. Ovaries of virgin females presented a slower rate of transformation and of incorporation of the label. Labeled AP2 is recovered in freshly laid eggs and AP1 in the females after oviposition. AP1 and AP2 can produce [^{3}H]20E, [^{3}H]E, and other minor polar peaks when submitted to hydrolysis by esterase. It is concluded that the female *O. moubata* possesses a special enzymatic mechanism for transformation of ecdysteroids into apolar products and for selective incorporation of AP2 into the ovaries. These products are present in the freshly laid eggs and could play a role during embryogenesis.

The presence of ecdysteroids (ES) in females and eggs of numerous species of insects (see review of Hoffmann *et al.*, 1980) or crustaceans (see review of Spindler *et al.*, 1980) is now well documented. ES are also present in the eggs of the Ixodid ticks: in *Amblyomma hebraeum* as determined by radioimmunoassay (RIA), gas chromatography of trimethylsilyl derivatives, and mass spectroscopy (Diehl *et al.*, 1982), and in *Ripicephalus appendiculatus* (Whitehead, Obenchain, and Mango, unpublished data). Our preliminary results using the RIA technique indicate that ES are also present in the ovaries, in the newly laid eggs, and in the hemolymph during vitellogenesis of the argasid tick *Ornithodoros moubata* (Murray, 1877; *sensu* Walton, 1962).

Only a few reports exist on the metabolism of injected ES during the gonotrophic cycle of the female arthropods. Hoffmann *et al.* (1974) indicate that the female *Locusta* metabolizes tritiated ecdysone ([^{3}H]E) to 20-hydroxyecdysone (20E), 3-dehydroecdysone, 3-dehydro-20-hydroxyecdysone, and polar products which are excreted in the feces, together with free unmetabolized [^{3}H]E. Briers and De Loof (1983) have studied the fate of injected [^{3}H]E in the female *Tenebrio molitor*. The major metabolites were highly polar products; however, most of the label (65%) was recovered in the feces as unmetabolized [^{3}H]E. In female *Sarcophaga bullata*, injected [^{3}H]E is metabolized to 20E, then to highly polar products which are conjugated forms of storage in the ovary. In addition, the ovary is the only tissue capable of storing considerable amounts of labeled ES (Briers *et al.*, 1983). The crab *Carcinus maenas* converts [^{3}H]E *in vivo* (as well as

in vitro) mainly to 20E, since less than 4% of the total label is recovered in other metabolites (Lachaise, 1981). The only study on the metabolism in ticks concerns the fifth nymph stage of the argasid *O. moubata*. [^{3}H]E is converted to [^{3}H]20E and to unidentified polar and apolar metabolites (Bouvier *et al.*, 1982). Here, we report on the fate of [^{3}H]E and [^{3}H]20E in the female during the gonotrophic cycle.

MATERIAL AND METHODS

Animals. Female *Ornithodoros moubata* (Murray, 1877; *sensu* Walton, 1962) were fed on defibrinated pig blood (at 37°) through a Parafilm membrane. They were kept in glass tubes loosely plugged with cotton at 27° and 30–40% relative humidity in the dark. Mated females accomplish their gonotrophic cycle within approximately 10 days, while virgin females have an abortive vitellogenesis and, in most cases, do not oviposit (see Aeschlimann, 1968; Germond and Aeschlimann, 1977).

Chemicals. All solvents were of analytical or chromatographical grade (Merck). [23,24-$^3H_2$(N)]-Ecdysone (sp act 53.6 Ci/mmol) was purchased from New England Nuclear Corporation and was purified by high-performance liquid chromatography (HPLC) immediately before use. 20-OH-[^{3}H]Ecdysone was kindly provided by Dr. Lafont (Paris ENS) and prepared from *Pieris* nymphs. Cold standard ecdysone (E), 20-OH-ecdysone, and makisterone A were purchased from Simes (Italy). Ponasterone A was a gift from Dr. Koolman, ecdysone acetates from Dr. Rees, 2-deoxyecdysone was kindly provided by Dr. Horn and Dr. Ohnishi, and 22,25-dideoxyecdysone by Dr. Thompson.

Injections. Labeled ecdysteroids were dried under a stream of N_2 and taken up in 0.9% NaCl or medium TC 199. About 10^5 cpm in 2 µl was injected with a fine capillary tube into the hemolymph of the tick through the articulation membrane of the leg.

Cultures. Ovaries were dissected in sterile TC 199 medium in which the osmotic pressure was adjusted to 370 mOsm with NaCl. They were rinsed three times in the sterile solution. Two ovaries were placed in each incubation dish (Nunclon®, Denmark) with 300 µl medium containing 3×10^6 cpm [^{3}H]ecdysone, 100 ng cold E/ml, and 20 µl penicillin/streptomycin (10,000 U and 10,000 µg/ml). The ovaries were incubated for 6 or 24 hr at 28°.

Extractions. Hemolymph of four or five females, depending on experimental groups, was collected with capillary tubes after puncturing the articulation membrane of several legs. It was then placed in methanol, vortexed, sonicated, and centrifuged (10 min, 10,000 rpm).

After the hemolymph was withdrawn, the females were dissected in TC 199. The ovaries were removed, placed in methanol, and homogenized in a Potter, and the suspension was sonicated and centrifuged (10 min, 10,000 rpm).

The carcasses which remained after hemolymph removal and ovary dissection were homogenized with the dissection solution in 10 ml of methanol in an homogenizer (Polytron). The suspension was centrifuged and the pellet was resuspended twice in methanol.

Ovaries cultured in the medium containing [^{3}H]ecdysone were rinsed three times in cold medium for 10 min, and then extracted in the same manner as the ovaries previously mentioned.

Some of the extracts were purified by the SEP-PAK method. The methanolic extract was dried, solubilized in 5 ml 25% methanol and deposited on the C18 SEP-PAK cartridge. Then 5 ml 60% methanol was used to elute the free ES fraction and 5 ml 100% methanol the apolar fraction (Lafont *et al.*, 1982).

High-performance liquid chromatography. The HPLC apparatus was a Perkin–Elmer Series 3 chromatograph with a LC 55 variable-wavelength spectrophotometer at 242 nm. Cold standards were coinjected with the different samples. The reverse-phase mode (Merck HIBAR Column RT, 25 cm, id 4 mm, packed with lichrosorb RP-18, 5 or 7 µm) was used with a gradient of methanol–Tris buffer (pH 7.5, 20 m*M;* 30 to 45% linear gradient within 10 min, 45% isocratic for 15 min; and 45–100% linear gradient within 20 min, followed by a 10-min purge). The flow was 0.8 ml/min and the column temperature was 27°. An HPLC silica column (Perkin–Elmer Silica A; 0.26 × 25 cm) was used under isocratic conditions (chloroform–isopropanol–water 100:20:1.25) with a flow of 1 ml/min.

Hydrolysis with esterase. Crude extracts or apolar products, purified by HPLC or by the SEP-PAK method, were placed in glass vials, dried under a flow of N_2, and hydrolyzed with 200 µl of esterase (Boehringer) in 1.8 ml 0.1 *M* borate buffer, pH 8. After an overnight incubation at 37°, the ecdysteroids were extracted with methanol.

Liquid scintillation counting. The HPLC fractions were mixed with a scintillation cocktail (Riatron). Radioactivity was measured with a Kontron MR 300 automatic liquid scintillation system. Results are expressed in cpm since only a small difference of quenching exists among the different HPLC fractions in our conditions.

Radioimmunoassay. The dried extracts were dissolved in 0.1 *M* citrate buffer at pH 6.2. The ecdysteroid concentration was determined by the radioimmunoassay method described by De Reggi *et al.* (1975). The concentration in biological samples is expressed as a 20-OH-ecdysone equivalent. The RIA is

about 2.5 times more sensitive to ecdysone than to 20-OH-ecdysone.

The antibodies were generously supplied by Dr. Delaage. Assay of the esterase suspension proved that no RIA-positive material was detectable in our enzymatic solution.

RESULTS

Metabolism of Ecdysteroids in Mated Female O. moubata

Mated females *O. moubata* begin to oviposit within about 10 days after their blood meal. [^{3}H]Ecdysone ([^{3}H]E) was injected 3, 8, or 9 days after feeding (3d, 8d, or 9d, respectively) and the fate of this labeled hormone in the hemolymph, in the ovaries, and in the remainder of the body (carcass) was investigated 6, 24, and 48 hr after injection. Particular attention was given to these stages because they represent two moments where the physiological level of ES is high in the animal. In addition, on the third day, vitellogenesis is just beginning (previtellogenesis), while at the eighth and ninth days, there is a very active incorporation of vitellus. The radiochromatograms of the extracts from the different experiments generally demonstrated four radioactive peaks, or group of peaks (Fig. 1). The first two peaks (1 and 2) had the same retention time on a RP 18 column as did cold 20-hydroxyecdysone (20E) and E standards, respectively. Each of these peaks was collected and afterward injected on a silica column. They again migrated like the cold standards. Several unidentified radioactive products eluted later than peaks 1 and 2. These apolar products could be separated into two distinct groups: group 3 (AP1), which eluted between 40 and 52 min, and group 4 (AP2), which eluted between 52 and 65 min. Occurrence and relative distribution of these four peaks were investigated in 3d and 8d females (Fig. 2).

The injected labeled hormone [^{3}H]E corresponded to the second peak. In the hemolymph, the percentage of the labeling in this peak decreased to a very low level 48 hr after injection. The half-life of the hormone was shorter in 8d females than in 3d females (approximately 3 hr versus 8 hr). [^{3}H]E was also recovered from the ovaries after 6 hr, but it had disappeared after 24 hr. Surprisingly, the unmetabolized hormone represented 20 to 25% of the labeling recovered from the carcass 48 hr after injection.

[^{3}H]20E, which corresponds to the first peak, was found in the hemolymph, ovaries, and carcass 6 hr after injection of [^{3}H]E. The percentage of the cpm represented by this metabolite decreased rapidly, and after 24 hr no more was found in the hemolymph and ovaries of both 3d and 8d females. This metabolite, however, was still present in the carcass 48 hr after injection though it represented only a small percentage of the labeling. Thus, female *O. moubata* appeared to be able to synthesize 20-hydroxyecdysone from ecdysone, but the rapid disappearance of this product indicates that it does not constitute a terminal product.

In contrast with the first two radioactive peaks, which were present in their lowest percentages of labeling 48 hr after injection, the apolar products began to increase from the beginning and reached 70 to 75% of the total labeling 48 hr after injection in 3d and 8d whole females. Thus, these products appear to be the major terminal metabolites of [^{3}H]E. However, the proportion between AP1 and AP2 in the whole tick was not the same in the two stages examined. The proportion of AP2 increased from 19.4% of the apolar products in 3d females to 40.7% in 8d females. The percentages represented by AP1 and AP2 in each of the three compartments were very different. In the hemolymph AP2 were the major metabolites after 48 hr, and a lower percentage was represented by AP1. In contrast, however, AP1 represented the highest percentage of the labeling in the carcass, and almost no AP2 remained in this compartment after 48 hr. In the ovaries 48 hr after injection, AP2

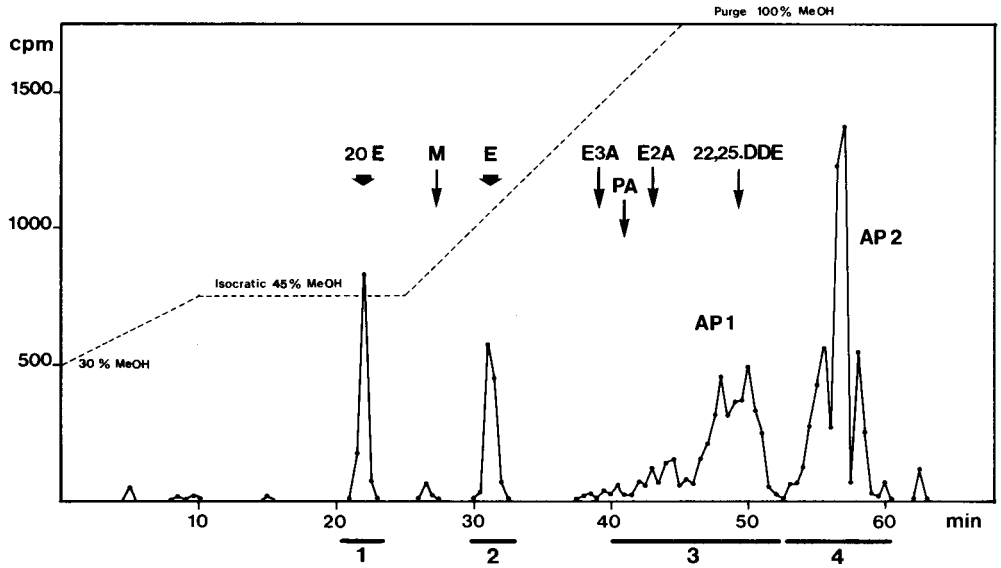

FIG. 1. Reverse-phase RP 18 HPLC radiochromatogram showing the four radioactive peaks or groups of peaks obtained after injection of [^{3}H]ecdysone into the hemocoel of female *Ornithodoros moubata*. (Radiochromatogram of an extract of carcass 24 hr after injection into females 3 days after feeding is shown here). 1 = 20-Hydroxyecdysone, 2 = ecdysone, 3 = AP1, and 4 = AP2. Retention times of different cold standards under our HPLC conditions are given for comparison: 20E = 20-hydroxyecdysone, M = makisterone A, E = ecdysone, E3A = ecdysone-3-acetate, PA = ponasterone A, E2A = ecdysone-2-acetate, 22,25-DDE = 22,25-dideoxyecdysone. Dotted line corresponds to the solvent gradient used for these experiments.

rapidly became the major product, reaching 80 to 85% of the radioactive products in this organ.

In another group of experiments, 3d and 8d females were injected with [^{3}H]20E, and the hemolymph, the ovaries, and the carcasses of these females were extracted 6 and 24 hr after injection. The half-life of this hormone was longer than that of [^{3}H]E. Radiochromatograms of the experiments demonstrated three radioactive peaks or group of peaks: [^{3}H]20E, AP1, and AP2. Thus, AP1 and AP2 were the only metabolites produced. These two products were both found in the hemolymph and the carcass but in inverse proportions. In the ovaries, after 24 hr AP1 represented less than 10% of the labeled products, and AP2 had reached approximately 50%. The remaining labeling in the ovaries corresponded to [^{3}H]20E.

Thus, after injection of [^{3}H]E or [^{3}H]20E, the ovaries contained a considerable quantity of labeling, principally in the form of AP2. This accumulation of radioactive compounds by the ovary was especially pronounced in 8d and 9d females (Fig. 3). In 3d females, at the beginning of vitellogenesis, the labeling of the ovaries represented a small percentage of the total labeling (2.5%), and this quantity did not increase with time. On the other hand, ovaries of 8d females, which are highly vitellogenic, actively incorporated the labeling. For injected [^{3}H]E, the amount of radioactivity in the ovary reached about 25% of the total radioactivity after 48 hr. The labeling appeared to accumulate more quickly in the ovaries after the injection of [^{3}H]20E than [^{3}H]E (20.6% versus 11.6% incorporated after 24 hr). In the ovaries of 9d females (approximately 1 day before the beginning of oviposition), the phenomenon was more pronounced since the ovaries

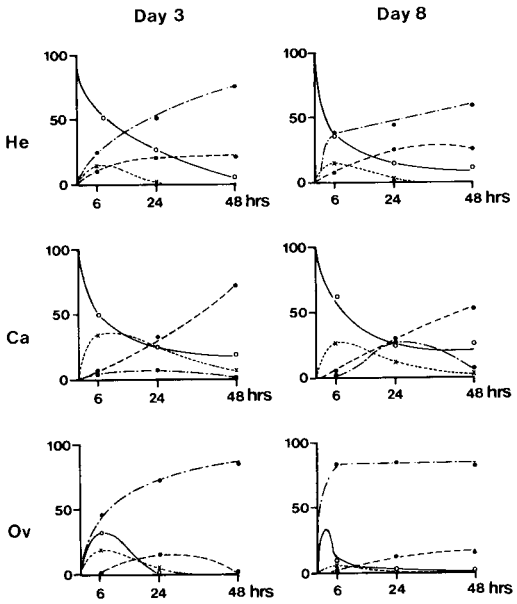

FIG. 2. Fate of injected [^{3}H]ecdysone in hemolymph (He), carcass (Ca), and ovaries (Ov) of female *Ornithodoros moubata* 3 and 8 days after feeding and 6, 24, and 48 hr after injection. Radioactivity of each metabolite is expressed as a percentage of the total label in each compartment. O—O, Ecdysone; X- - -X, 20-hydroxyecdysone; ●- - -●, AP1; ●— - —●, AP2.

contained about one third (31.4%) of the total labeling after only 6 hr.

We can conclude that AP2, which constitute the major part of the labeling in the ovaries, accumulate rapidly into these organs during the vitellogenic process.

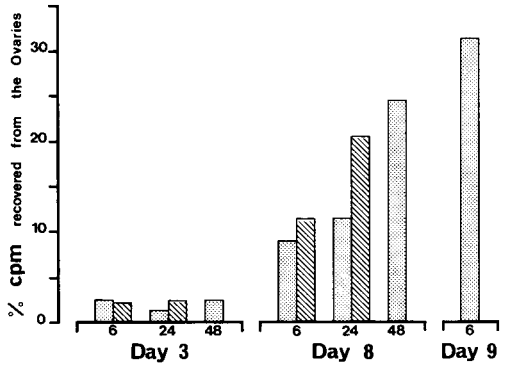

FIG. 3. Percentage of label recovered from the ovaries of females injected with [^{3}H]ecdysone, ▧, or 20-hydroxy-[^{3}H]ecdysone, ⊡, 3, 8, and 9 days after feeding (100% corresponds to the total label recovered).

Metabolism of Ecdysteroids in Virgin Females

In contrast to fed mated females, fed virgin females cannot normally complete the digestion, vitellogenesis is abortive, and oviposition does not occur. Vitellus is resorbed after 10 to 20 days. In order to compare the metabolism of ES in normal and abortive gonotrophic cycle, we injected virgin females 8 days after feeding with [^{3}H]E or [^{3}H]20E. The fates of the labeled injected hormones were similar to those in mated females. 20E, AP1, and AP2 were produced when [^{3}H]E was injected and only AP1 and AP2 when [^{3}H]20E was injected.

The half-life of these hormones was long. For [^{3}H]E it was 8 hr in the hemolymph, which corresponded to that of the 3d mated females. The half-life of [^{3}H]20E was 16–17 hr. After injection of [^{3}H]E or [^{3}H]20E only AP2 were present in the ovaries. However, the total amount of radioactivity incorporated into the ovaries at this time represented only 3.5% of the total labeling recovered in the females for [^{3}H]E and 1.5% for [^{3}H]20E. Thus, ovaries of virgin females presented a very low rate of incorporation of AP2 which was similar to the rate in 3d females where active vitellogenesis had not begun.

In Vitro Activity of the Ovaries from Mated Females

Ovaries from 3d and 8d females were cultured in the presence of [^{3}H]E. Table 1 summarizes the fate of the labeled hormone in the ovaries and in the medium after 6 and 24 hr of incubation. The radioactive peaks 1, 2, and 4 were present both in the ovaries and in the medium, whereas the products corresponding to peak 3 were not found in the ovary. AP2 always represented the major portion of the labeling in the ovary (93% in 8d ovaries after 24 hr). In the me-

TABLE 1

FATE OF [^{3}H]ECDYSONE AFTER 6 OR 24 HR OF INCUBATION WITH OVARIES FROM MATED *ORNITHODOROS MOUBATA* FEMALES 3 OR 8 DAYS AFTER FEEDING

Age of the ovary (days)	Incubation time (hr)	% of total cpm in the ovary			% of total cpm in the medium		
		E	20E	AP	E	20E	AP
3	6	30.2	14	56	93	1.2	3
	24	20	3.3	72	90.5	4.7	2.2
8	6	25	5.5	70	97.7	0.9	0.9
	24	5.3	1.6	93	92	5.1	1.8

Note. Unmetabolized Hormone (E), 20-hydroxyecdysone (20E), and apolar products (AP) are expressed as the percentages of label recovered either in ovary or in medium.

dium the largest part of the labeling corresponded to unmetabolized [^{3}H]E (more than 90%). The cpm remaining represented 20E and apolar products. Interestingly, 20E accumulated in the medium and reached about 5% of the labeling after 24 hr. A small percent of the apolar products (less than 3%) was also present in the medium.

Table 2 gives the percentages of the different metabolites among total labeling metabolized both in the ovaries and in the medium of the culture. It is thus clear that 20E did not remain in the ovary, but was secreted into the medium, while the apolar products stayed in the ovary. Thus, the ovaries of mated females seemed to be able to transform [^{3}H]E into 20E and AP2 and to incorporate apolar products rather than 20E.

Figure 4 (first two rows) gives the rates of incorporation of the ovaries at two different times during the gonotrophic cycle (remember that AP2 represented the major part of the labeling in the ovaries). Ovaries from 8d females incorporated more of the labeling and incorporated the labeling more quickly than did 3d ovaries.

TABLE 2

20-HYDROXYECDYSONE (20E) AND APOLAR PRODUCTS (AP) EXPRESSED AS PERCENTAGES OF ALL THE METABOLITES PRODUCED DURING 24 HR OF INCUBATION OF OVARIES FROM FEMALES 8 DAYS AFTER FEEDING

Product	% in ovary	% in medium
20E	1.25	23.47
AP	66.78	8.49

In Vitro Activity of Ovaries from Virgin Females

Figure 4 (last two rows) shows the difference between incorporation of the label by the ovaries of virgin females of different physiological conditions and by the ovaries of mated females. The ovaries of unfed virgin females, in which vitellogenesis was not initiated, presented a low level of incorporation. The products present in the ovaries after incubation were identified as 20E, E, and AP2. However, AP2 repre-

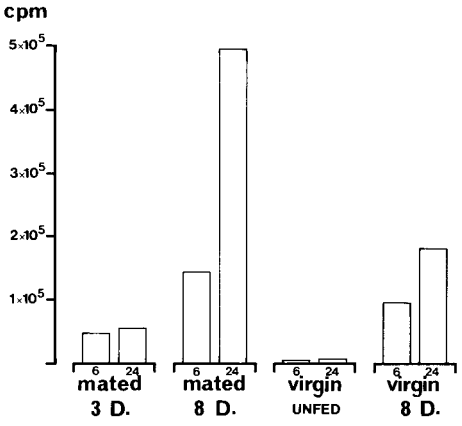

FIG. 4. *In vitro* activity of ovaries from females of different physiological states expressed as the cpm recovered from four ovaries in each experiment after 6 and 24 hr of incubation in medium containing [^{3}H]ecdysone.

sented 52% of the cpm in the ovaries after 24 hr.

The ovaries of 8d fed virgin females which performed an abortive vitellogenesis were much more active in view of the transformation and incorporation of [^{3}H]E metabolites. After 24 hr, AP2 represented 94% of the cpm in the ovaries, and the incorporation rate was significantly higher than that of unfed virgin female ovaries and also that of 3d fed mated females ovaries.

Metabolism in Eggs and in Females after Oviposition

Five mated females were injected with [^{3}H]E and five others with [^{3}H]20E 8 days after the blood meal. Freshly laid eggs were collected every day and extracted for HPLC analysis. Approximately 10 days after the end of oviposition, the two groups of females were also extracted. The nature and amount of labeling due to the injection of [^{3}H]E or [^{3}H]20E in freshly laid eggs and in females after the oviposition were investigated (Fig. 5). With [^{3}H]E, about 47% of the total label recovered (from eggs and females together) was transferred into the eggs. HPLC analysis revealed that AP2 was the only constituent. In the case of [^{3}H]20E injection, only 25.5% was transferred in the form of 20E and AP2. In contrast, AP1 products were present in whole body extracts of females, either alone after [^{3}H]E injection, or together with 20E after injection of [^{3}H]20E (Fig. 5).

Possible Chemical Nature of the Apolar Metabolites

The extracts of females and of eggs were hydrolyzed with esterase and then analyzed with HPLC (Fig. 5). Hydrolysis of the AP1 and AP2 with this enzyme liberated free [^{3}H]E and [^{3}H]20E, in the case of injected [^{3}H]E, or free [^{3}H]20E, in the case of injected [^{3}H]20E. These different peaks were collected after RP18 HPLC analysis and injected again on a silica column to ascertain their identity.

In addition to the free hormones, one or two peaks of polar products of less quantitative importance were liberated from the AP1 of both experimental groups. One of these peaks (the most polar) had the same retention time (approximately 15 min) and could represent free 20,26-dihydroxyecdysone. However, its exact nature has not yet been investigated.

DISCUSSION

As in many arthropods studied thus far, the argasid tick *Ornithodoros moubata* uses ecdysone and 20-hydroxyecdysone (20E) during its immature stages to control the molting processes (Germond *et al.*, 1982). Preliminary results with HPLC/RIA indicate that these hormones are also present in the adult female during the gonotrophic cycle. We have thus investigated the fate of labeled injected E and 20E in the females in order to compare it with that of fifth instar nymphs previously studied (Bouvier *et al.*, 1982). In addition, this study is of great interest since several species are able to incorporate ES conjugates into their eggs and possibly use them for the control of embryonic molting processes (Review, Hoffmann *et al.*, 1980).

Our results indicate that the female is able to convert [^{3}H]E into [^{3}H]20E, as is the case in the last instar nymphs (fifth nymphs) (Bouvier *et al.*, 1982).

One or two polar products (PP), more polar than 20E, were obtained after enzymatic hydrolysis. This indicates that the female can also produce them, although it is not possible to see them on the different radiochromatograms of the crude extracts. However, these products were not as polar as those produced by the fifth instar nymphs. Perhaps the female does not possess all the enzymes necessary for the last step of this metabolic pathway.

Like the fifth instar nymphs, the female *O. moubata* also produces apolar products (AP). These AP have the same retention times in both stages and can be separated

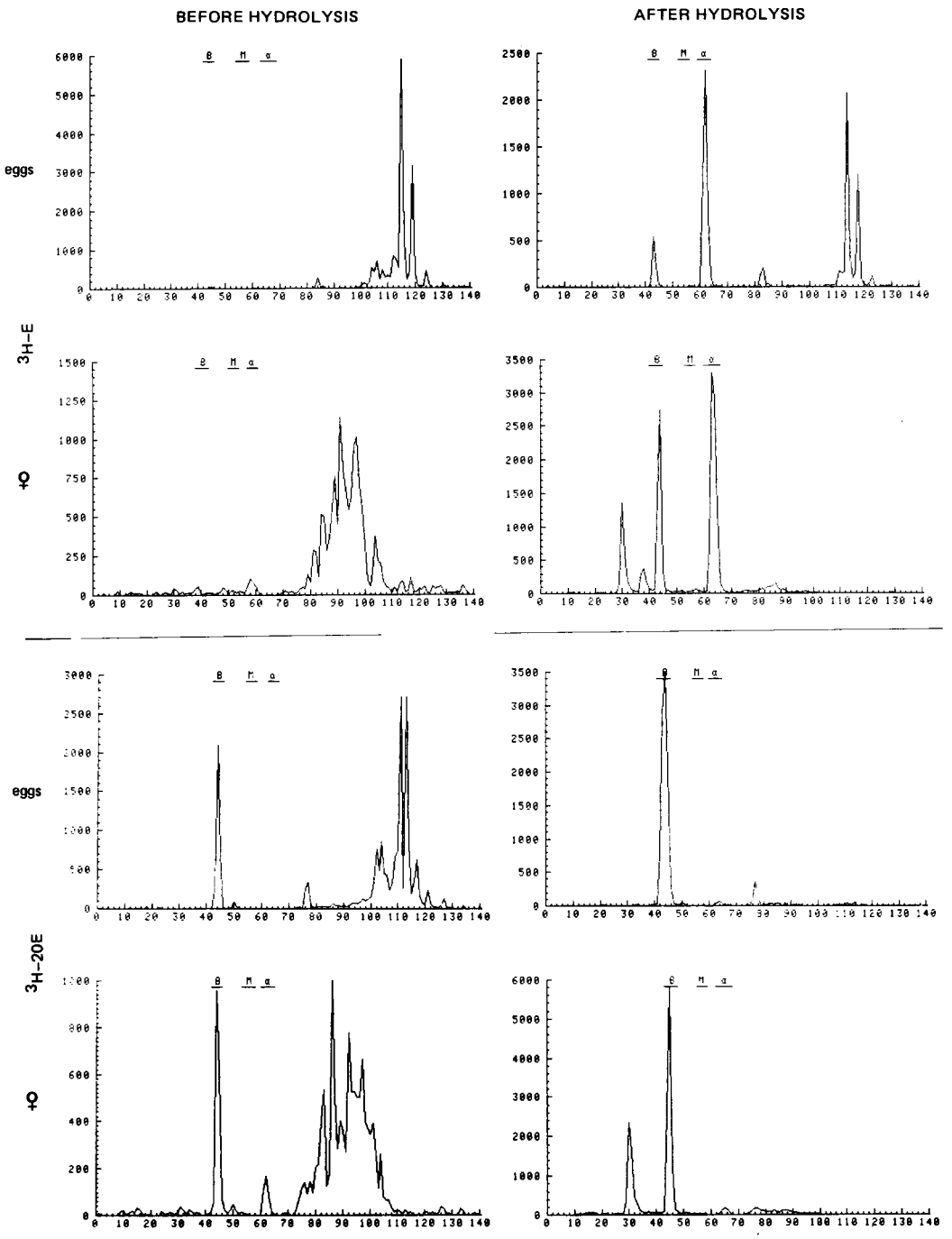

FIG. 5. HPLC radiochromatograms of extracts from freshly laid eggs and whole body females after the end of the oviposition, before and after hydrolysis by esterase. Females were injected with [^{3}H]ecdysone or 20-hydroxy-[^{3}H]ecdysone 8 days after feeding. The place where the cold standards migrated are marked α, for ecdysone; M, for makisterone A; and β, for 20-hydroxyecdysone.

into two distinct groups: AP1 and the less polar AP2. In fifth nymphs, AP2 rapidly appear during the first 24 hr after injection and thereafter are gradually converted into AP1. In females, the situation is similar. Both AP1 and AP2 are produced but 48 hr after injection only AP1 remain in the carcass. However, AP1 and AP2 circulate in the hemolymph. In contrast, AP2 dominate in ovaries, and thus, newly laid eggs contain large amounts of these products.

Our results indicate that the incorporation of AP2 into the ovary is linked to the vitellogenic process. The production of AP2 by the female is always possible; however, in virgin females or in 3d fed mated females, the incorporation into the ovary is low. In contrast, the phenomenon is particularly pronounced during the active incorporation of vitellus which occurs approximately 8 to 9 days after feeding. Our experiments allow us to hypothesize two different mechanisms for the incorporation of AP2 into the ovary. (1) From the *in vitro* experiment, we can see that the ovary is permeable to E from the environment and can itself produce and incorporate AP2, in the total absence of vitellogenins or proteins. (2) On the other hand, we can suppose that not only the ovary produces AP2, but that the AP2 produced by other organs and circulating in the hemolymph can also be trapped in the ovary during vitellogenesis.

The exact chemical nature of these AP is still unknown. However, according to their retention times on the RP-18 column, they represent compounds less polar than E-3-acetate, which has been reported in developing eggs of *Schistocerca gregaria* (Isaac *et al.*, 1981), or even E-2-acetate or 20E-2-acetate. The fact that these products liberate E and 20E after esterase hydrolysis suggests that they are ES esters, perhaps esters of fatty acids, which should be less polar than acetate esters.

Preliminary unpublished data show that compounds similar to AP2 may accumulate naturally in the eggs since hydrolysis of methanolic extracts of freshly laid eggs increase the quantity of RIA-positive material (up to 35 times). Thus, it seems that some conjugated forms of ES exist in the eggs of *O. moubata*. This situation is comparable to those in several arthropods: *S. gregaria* (Gande and Morgan, 1979; Dinan and Rees, 1981), *Locusta migratoria* (Hoffmann *et al.*, 1980), *Galleria mellonella* (Hsiao and Hsiao, 1979), *Bombyx mori* (Mizuno and Ohnishi, 1975). However, in these species, ES conjugates corresponded to highly polar products which were hydrolyzable by *Helix pomatia* enzymes.

The structure of some conjugates has been elucidated in detail in two orthopteran species: (1) *L. migratoria* (22-adenosine monophosphoric ester of 2-deoxy-E, 22-N^6-(isopentenyl)-adenosine monophosphoric ester of E, 3-phosphate of 3-epi-2-deoxy-E) (Tsoupras *et al.*, 1982a, b, 1983) and (2) *S. gregaria* (E-22-phosphate, 2-deoxy-E-22-phosphate) (Isaac *et al.*, 1982, 1983). In *Locusta,* the conjugates are metabolized during embryogenesis (Sall *et al.*, 1983) and the free ES liberated may fulfill several roles during embryogenesis, e.g., control embryonic molts (Hoffmann *et al.*, 1980). The conjugates present in the eggs of *O. moubata* may also represent a storage form of ES. This kind of apolar conjugate has not, however, yet been reported in the literature, but it could represent a normal fate of ecdysone in ticks since this type of conjugate has recently been described in a female of the family Ixodidae, *Boophilus microplus* (Wigglesworth and Rees, 1983). The authors also indicated that these products are probably esters of fatty acids since [^{3}H]ES and fatty acids are released upon alkaline treatment. These AP products are also produced *in vitro* by Malpighian tubules, ovaries, fat body, and gut. In addition, they are recovered in the freshly laid eggs.

Preliminary data on the metabolism of ES in the abdomen of female *Drosophila*

melanogaster also indicate the occurrence of comparable apolar metabolites (Dübendorfer and Maroy, 1983).

Thus, we have evidence of a class of ES metabolites which is new for arthropods. These apolar compounds are probably ES esters which represent a new storage form in eggs.

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