

# Apolar Conjugates of Ecdysteroids Are Not Used as a Storage Form of Molting Hormone in the Argasid Tick *Ornithodoros moubata*

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Fifth (last) instar nymphs of the tick *Ornithodoros moubata* convert ingested 20-hydroxyecdysone (20E) to apolar conjugates AP2, which are then converted to the more polar conjugates AP1. Only small quantities of free hormone were transferred to the hemolymph and the carcass within the first 2 days after the blood meal. The proportion of radiolabel in these two compartments was highest at the time of the endogenous ecdysteroid peak; however, no traces of free [<sup>3</sup>H]20E were detected. The conversion probably occurs principally in the intestinal cells. Eleven days after ingestion, 84% of the radiolabel is located in the digestive tract, mainly in the form of AP1 conjugates.

AP1 obtained in second instar nymphs fed with [<sup>3</sup>H]ecdysone ([<sup>3</sup>H]E) remain stable throughout the following nymphal instars. The ecdysteroid moiety of AP1 remained unchanged. The hydrolysis, although not complete, always yielded a peak comigrating with the reference E but never 20E or any other clearly distinct peaks that may have corresponded to metabolites of 20E. Less label per individual was present in adults, but its nature remained the same, viz., AP1 mainly located in the digestive tract. In females, 2.5% of the label was transferred to the progeny during the first ovipositional cycle.

Apolar products (mainly AP2) that accumulated in eggs of females injected with [<sup>3</sup>H]E or [<sup>3</sup>H]20E during vitellogenesis remained unchanged during the whole embryonic development. During the molting cycle of larvae, there was only a slight conversion of AP2 to AP1, but esterase hydrolysis of these products released the same percentages of E and 20E as in the freshly laid eggs.

We conclude that in this tick species apolar conjugates of ecdysteroids are inactivation metabolites that are not reutilized during the development of the animal. These metabolites are mainly retained in the tick, probably because of its peculiar blocked midgut. Several studies have shown that in other arthropod species (ticks, spiders, and insects), these apolar metabolites are excreted in the feces.

**Key words:** metabolism of ecdysteroids, development

**Acknowledgments:** We are grateful for the generous financial support of the Swiss National Science Foundation (request #2.662.84). Ellen Dotson benefitted during this work from a 2-year scholarship from the Swiss Confederation; work on the embryos and larvae is a part of her doctoral thesis. We thank Mr. M. Morici and Miss M. Zweilin for their technical assistance.

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## INTRODUCTION

As in other arthropods, the molting cycle of ticks is triggered by ecdysteroids [1]. Fifth instar nymphs of the argasid tick *Ornithodoros moubata* molt 10 days after a blood meal, with an ecdysteroid peak composed essentially of 20-hydroxyecdysone occurring concomitantly with the beginning of cuticle synthesis (day 5–6) [2]. The injection of [<sup>3</sup>H]ecdysone into nymphs at various time after feeding, indicated that two different metabolic pathways exist, depending upon the endogenous ecdysteroid titer [3]. When endogenous ecdysteroid levels are low, ecdysone is converted principally to apolar conjugates, AP\*, while a pathway leading to the formation of polar products is prevalent during the ecdysteroid peak. Metabolic studies with radiotracers have demonstrated that when ecdysteroids are either injected or ingested, AP accumulate in intestinal cells and in the midgut lumen (Diehl, unpublished results; [4]). In the case of 20E ingestion, the first apolar products to appear (AP2) were identified as long chain fatty acid esters of 20E at the C-22 position [4]. These products were then converted to more polar conjugates, AP1, the structure of which is unknown. Both AP1 and AP2 conjugates are, however, hydrolyzable with esterase, liberating a mixture of free E and 20E. Because ecdysteroid fatty acid esters are less immunoreactive than their corresponding free ecdysteroids, it is possible to estimate these conjugates by performing RIA analysis on samples both before and after enzymatic hydrolysis. Thus we observed an increase in RIA-positive material after hydrolysis of tick extracts collected after the endogenous ecdysteroid peak. This amount, however, remained constant throughout the last part of the instar, indicating that conjugated ecdysteroids are stored in the animals. [5].

Additional studies have demonstrated that other tick life stages are capable of producing AP, as these products accumulate in the eggs of ticks that were injected with radiolabeled ecdysteroids as vitellogenic females [5–8]. The compounds that accumulate in eggs of the hard tick *Boophilus microplus* have been identified as the same long chain fatty acid esters of E (at C-22) as found in *O. moubata* [9].

Since the metabolic pathways leading to the production of E and 20E are unknown in ticks, we have investigated if AP conjugates in nymphs or in the eggs could serve as a source of molting hormone during the molting cycle or during embryonic development, respectively.

\*Abbreviations: AP = apolar products (with reference to compounds that are appreciably less polar than ecdysone) previously described [7,11] and specified as AP1 and AP2 according to their retention times in reverse-phase HPLC; E = ecdysone; 20E = 20-hydroxyecdysone; RP = reverse phase.

## MATERIALS AND METHODS

### Animals

Animals were obtained from a colony of the soft tick *O. moubata* (Murray, 1877; sensu Walton, 1962) (Ixodoidea; Argasidae), maintained in the laboratory for 5 years, and fed through a parafilm membrane with defibrinated pig blood. Adults molting from fourth instar nymphs are generally males, and those molting from fifth instar nymphs are females. Nymphs and adults are fed 1-2 months after ecdysis.

### Chemicals

Ecdysone, ( $2\beta,3\beta,14\alpha,22R,25$ -pentahydroxy- $5\beta$ -cholest-7-en-6-one) and 20-hydroxyecdysone ( $2\beta,3\beta,14\alpha,20R,22R,25$ -hexahydroxy- $5\beta$ -cholest-7-en-6-one) were purchased from SIMES (Italy). Tritiated [ $23,24$ - $^3H(N)$ ]ecdysone (53.6 Ci/mmol) was obtained from New England Nuclear (Boston, MA). Tritiated 20E was obtained by incubation of *Locusta* Malpighian tubules with [ $^3H$ ]E and purification with HPLC.

### Nutrition

Ten microliters of ethanol containing one of the two tritiated hormones were added to 5 ml fresh defibrinated blood and vortexed. The nymphs were then allowed to feed through a parafilm membrane. In argasid ticks, much of the water and salts from the ingested vertebrate blood are discharged from the body during feeding as a fluid excreted from a pair of slitlike apertures located between coxae I and II. This coxal fluid was collected during the blood meal with a Pasteur pipette, and the coxal fluid excreted the first 24 h following the blood meal was absorbed on filter paper. Second instar nymphs ingested approximately 20  $\mu$ l blood, and fifth instar 100  $\mu$ l.

### Loading of Eggs With Apolar Conjugates

Freshly laid eggs from vitellogenic females injected with [ $^3H$ ]E or [ $^3H$ ]20E contained apolar conjugates [7]. One of these hormones (500,000 cpm) dissolved in 5  $\mu$ l of TC 199 Seromed medium (Biochrom, Berlin, FRG) was injected with a glass needle into the hemocoel of females through the third coxal membrane 8 days after engorgement. Females were kept separate in cotton-plugged glass vials, and eggs were collected every day by removing the female. In order to obtain enough radiolabel in a sample for an RP-HPLC run, between 10 and 20 eggs were extracted for each day of development investigated.

### Dissections

The cuticle of each tick was cut circumferentially and the dorsum lifted away. The ventral half was covered with TC-199 medium, and the intestine (and in the case of female the ovary) was (were) removed. The intestine was torn open and washed in new medium until the contents had been completely expelled. The ovary and the carcass were also rinsed several times in

new medium. These rinses were combined with the medium containing the intestine contents.

Hemolymph was collected with calibrated capillary tubes after puncturing the articulation membrane of several legs.

### **Extractions**

Ticks or tissues were homogenized in pure methanol and centrifuged (15 min 10,000 rpm). The supernatant was collected, and adjusted to a known volume of methanol, and an aliquot of each extract was counted to estimate the radiolabel content. A suitable amount of methanolic extract was evaporated to dryness under nitrogen, and then resuspended in 300  $\mu$ l of 30% methanol for injection in HPLC.

### **HPLC Analysis**

HPLC analyses were performed with a Perkin-Elmer series 3 chromatograph with an LC 55 variable-wavelength spectrophotometer set at 242 nm. E and 20E standards were coinjected with the different samples. For analysis of the metabolites, the reverse phase mode (Merck HIBAR column RT, 25 cm, i.d. 4 mm, packed with Lichrosorb RP-18, 7  $\mu$ m) was used with a gradient of methanol-Tris buffer (pH 7.5, 20 mM), as previously described [7,10].

### **Hydrolysis With Esterase**

Hydrolysis of apolar conjugates was accomplished with 50 IU of pig liver esterase (E.C. 3.1.1.1) (Boehringer) in 600  $\mu$ l 0.1 M borate buffer, pH 8. After an overnight incubation at 37°C, 300  $\mu$ l of methanol were added. The sample was vortexed, centrifuged (10,000 rpm, 10 min), and directly injected into the HPLC.

### **Liquid Scintillation Counting**

We used an automatic liquid scintillation counter (KONTRON MR300 DPM) and Riatron scintillation cocktail (Kontron, Zürich, Switzerland). In the case of HPLC fractions, results were expressed as uncorrected cpm because only a small difference in quenching existed between the different HPLC fractions. However, to have a correct estimation of cpm in each organ after dissections, a quench curve was established. A linear correlation existed between the standard channel ratio and the counting yield estimated by addition of internal standards to the biological samples.

## **RESULTS**

### **Fate of Ingested 20-Hydroxyecdysone in Fifth Instar Nymphs**

Because of their larger size, fifth (last) instar tick nymphs were used to investigate the fate and distribution of ingested hormone in intestinal lumen, intestinal cells, hemolymph, and carcass (the remainder of the body), during the molting cycle. The animals were fed with blood supplemented with 10  $\mu$ g/ml 20E (and 10<sup>6</sup> cpm/ml [<sup>3</sup>H]20E).

As in a previous experiment performed with adult females [11], the ingested hormone was rapidly converted to AP2 corresponding to 20E esterified at C-22 with the common long chain fatty acids C16:0, C18:0, C18:1, and C18:2 [4]. The radiolabel in the lumen of the midgut slowly diminished within 6 days after the ingestion of the tritiated hormone (Fig. 1), and 20E was progressively replaced by AP2, and then AP1 (Fig. 2). Simultaneously, the radiolabel in the intestinal cells and carcass increased (Fig. 1). One hour after ingestion, 20E represented a high proportion of the radiolabel present in these tissues (Fig. 2), indicating a transfer of this hormone from the intestinal lumen to the carcass through the intestinal cells. 20E is rapidly conjugated to AP2, which are progressively converted to AP1.

In the hemolymph we observed that the radiolabel gradually increased to reach a maximum 6 days after ingestion (Table 1), coinciding with the endogenous ecdysteroid peak [2]. The amount of radiolabel in the hemolymph during the first day was insufficient for HPLC analysis. However, analysis of the samples from days 2 and 6 revealed only the presence of AP2. Thus no free [ $^3\text{H}$ ]20E was circulating in the hemolymph during the endogenous ecdysteroid peak.

Eleven days after ingestion (viz., 2 days after ecdysis) the label in the hemolymph and in the carcass decreased (Fig. 1), while that in the intestinal cells and the intestinal lumen increased. At this time, the major part of the radiolabel (84.3%) is located in the digestive tract under the form of AP1 (92.2%) and AP2 (7.8%).

Hydrolysis of these AP principally yielded 20E, but also liberated an unidentified polar product having a retention time of 12 min, which repre-

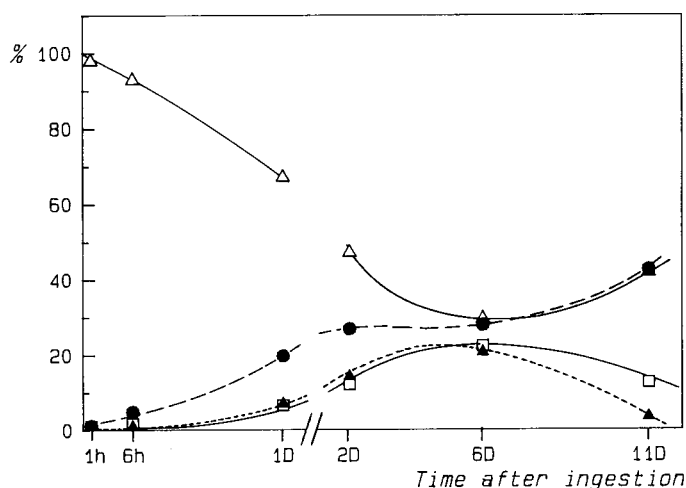


Fig. 1. Distribution of the radiolabel in *O. moubata* fifth instar nymphs after ingestion of blood containing 10  $\mu\text{g}$  20 E and 1,000,000 cpm [ $^3\text{H}$ ]20E per ml. Percentage of radiolabel from the whole animals contained in the intestinal lumen ( $\Delta$ ), in the intestinal cells ( $\bullet$ ), in the carcass ( $\square$ ), and in the hemolymph ( $\blacktriangle$ ). Hemolymphatic volume was approximated to 25  $\mu\text{l}$  per nymph, and label content of the intestinal lumen was calculated by subtraction of remaining contaminant hemolymph. One hundred percent always corresponded to approximately 190,000 cpm.

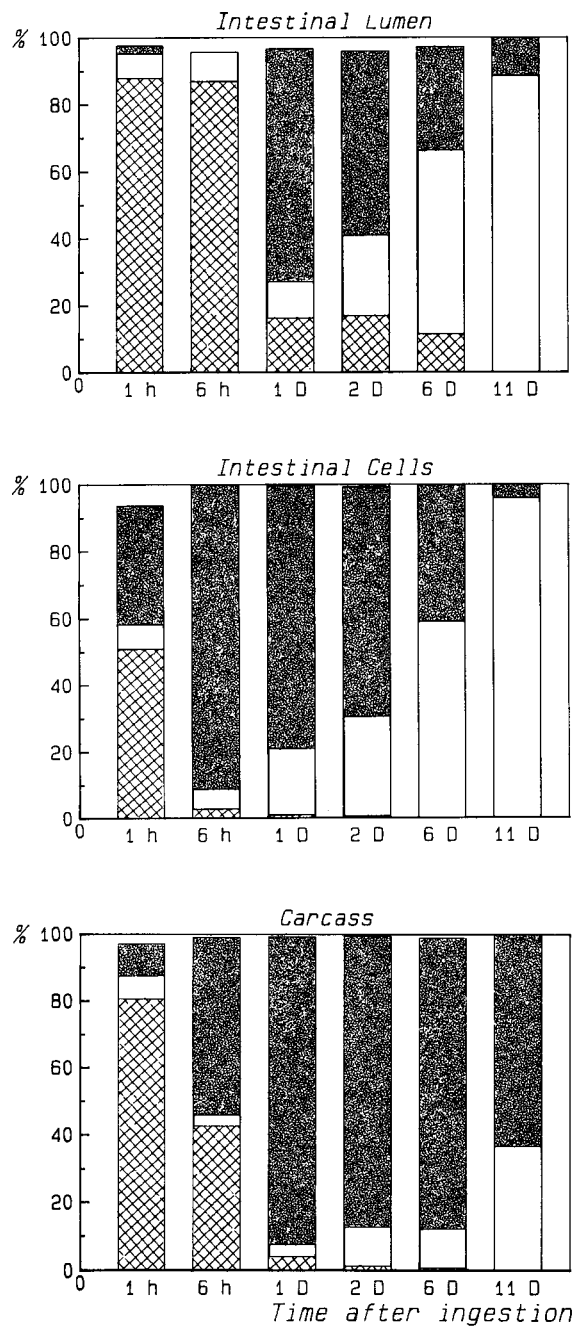


Figure 2

sented approximately 25% of the radiolabel (data not shown). In the hydrolysis experiments of carcass extracts, this peak was present in lower proportions (6.8%). This same peak has also been observed in adult *O. moubata* females [11].

#### Fate of Apolar Conjugates During Successive Molting Cycles

Several batches of second instar nymphs of *O. moubata* were fed blood containing  $10^5$  cpm of [ $^3\text{H}$ ]E per ml. The nymphs were then washed with water and 70% ethanol in order to remove any label that possibly remained on the body surface. As previously described for fifth instar nymphs or adult females [11], within the first few hours after the blood meal, the ingested hormone was converted to the apolar esterase-labile conjugates AP2. In time, these AP2 are converted to the conjugates AP1 (Fig. 3A). Following ecdysis to the third instar, only these latter compounds are recovered (Fig. 3B). Although the AP1 are composed of several peaks, they are all esters of [ $^3\text{H}$ ]E hydrolyzable with esterase (data not shown). We note that in this case no peak eluting at 12 min was present.

The third instar nymphs containing the radiolabeled apolar conjugates were then fed on blood without the added hormone, and ten nymphs were homogenized each day after feeding throughout the molting cycle. The radiolabel content of each group was analyzed by RP-HPLC. In a similar way, the remaining radiolabeled fourth instar nymphs were fed on blood without hormone, and the radiolabel pattern of each day of the molting cycle was monitored. In both cases, we observed that the radiolabel content of the nymphs did not change during the molting cycle. The pattern of four major peaks of AP1 followed by a minor one found in unfed third instar nymphs was observed throughout the two molting cycles (Fig. 3B and C). In order to ascertain that the apparent stability of AP1 did not hide rapid changes in their composition (e.g., liberation of E, conversion to 20E [for example], and then rapid reconjugation of this hormone), we analyzed extracts of fifth instar nymphs from each day of the next molting cycle, both by a direct RP-HPLC

**TABLE 1. Evolution of the Concentration of Radiolabel in the Hemolymph of *O. moubata* Fifth Instar Nymphs After Ingestion of Blood Containing  $10\ \mu\text{g}$  Cold 20E and  $10^6$  cpm [ $^3\text{H}$ ]20E per ml (Hemolymph from two nymphs was pooled for each time)**

	Time					
	1 h	6 h	1 day	2 days	6 days	11 days
Hemolymph withdrawn ( $\mu\text{l}$ )	12.0	10.0	7.7	6.5	6.2	8.8
Radiolabel (cpm/ $\mu\text{l}$ )	11.3	61.5	533.8	858.6	2049.6	258.2

Fig. 2. Nature of the radiolabel content investigated with RP-HPLC, in the intestinal lumen, intestinal cells, and carcass of *O. moubata* fifth instar nymphs that had ingested blood containing  $10\ \mu\text{g}$  20E and 1,000,000 cpm [ $^3\text{H}$ ]20E per ml. (dark spotted columns), AP2; (open columns), AP1; (cross-hatched columns), 20E. The difference between the total of the bars and 100% corresponds to products different from these compounds.

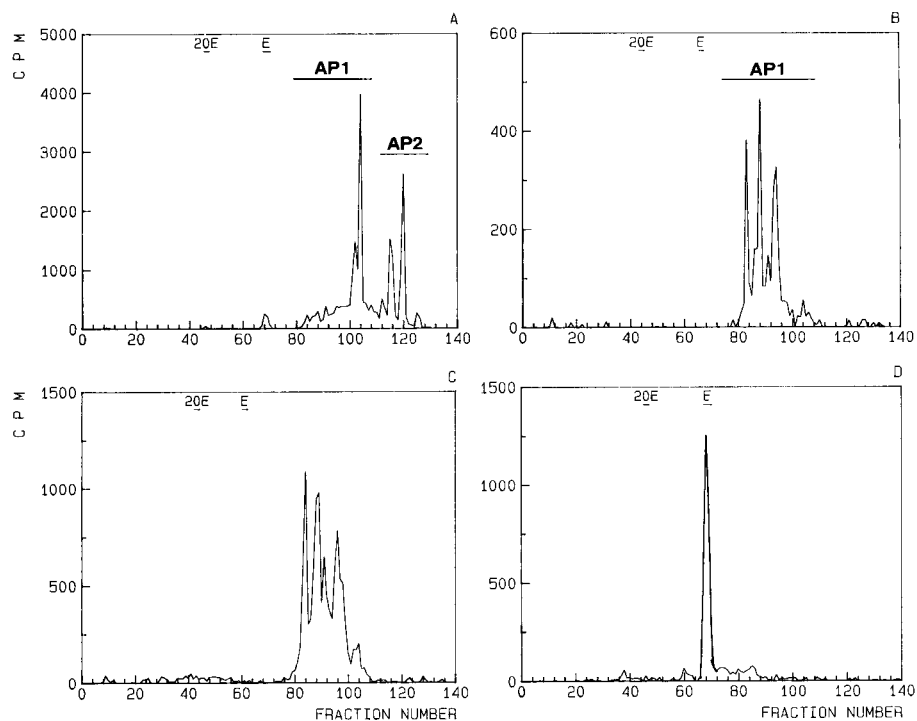


Fig. 3. Fate of [ $^3\text{H}$ ]E ingested by *O. moubata* second instar nymphs. **A:** RP-HPLC radiochromatographic pattern 24 h after ingestion. **B:** Pattern in freshly ecdysed third instar nymphs and at each day of the third instar nymphs' molting cycle. **C:** Pattern during the fourth instar nymphs' molting cycle. **D:** Pattern after hydrolysis with pig liver esterase of the extract from freshly ecdysed fourth instar. E and 20E indicate the retention time of cold standards, coinjected with the sample.

analysis and by analysis after esterase hydrolysis of the AP1 (Fig. 4). The pattern after hydrolysis was always similar. The more polar component of AP1, which represented about 20% of the total radiolabel in the extracts, was partially esterase-resistant. After hydrolysis, this peak comprised 17.1% (SD = 4.4) of the radiolabel. Most of the remaining label (57.2%, SD = 8.4) corresponded to a peak that comigrated with E. No other significant radioactive peaks were liberated by the hydrolysis. However, radiolabel was found in the fractions having shorter retention times than E. The proportion of this diffusely dispersed polar material increased slightly during the molting cycle of fifth instar nymphs, from 2% at the beginning of this instar to 15% after the molt.

#### Fate of the Apolar Conjugates in Adults

The resulting adult males and females contained less radiolabel than was originally ingested by them as second instar nymphs, indicating that the radiolabel was lost during the molting cycles. Males appear to have lost more than females, as they contained 26–37% of the label ingested as second instar nymphs, while the females contained from 46% to 60%. The different exuviae

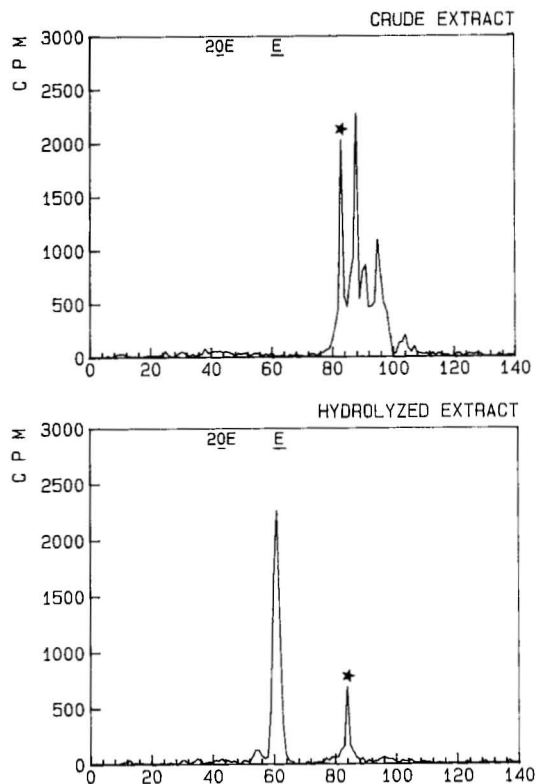


Fig. 4. HPLC pattern of labeled metabolites in fifth instar *O. moubata* fed [ $^3\text{H}$ ]E as second instar nymphs. The pattern is similar to that of third and fourth instar nymphs and is similar at each day of the molting cycle. Hydrolysis with esterase revealed that the AP1 conjugates contained only E. ★, unhydrolysed AP1.

from the nymphs contained less than 1% of the total label in the animals. Neither the coxal fluids collected during each blood meal nor the wash of the vials in which the animals were kept, which contained the excreted guanine, had significant amount of radiolabel. We suspect that the loss of radioactivity could be due to the regurgitation of some of the intestinal content during the successive blood meals.

In males the radiolabel was principally located in the intestinal lumen (65.5%). The remaining radioactivity was in the carcass (13.3%) and the hemolymph (14.6%). Females were fed and mated, and their progeny collected. The extracts of all the first instar nymphs from three females contained only 69 cpm, corresponding to approximately 2.5% of the label present in the three females.

#### Fate of the Apolar Conjugates in Embryos and Larvae

Freshly laid eggs from females injected with [ $^3\text{H}$ ]E contained apolar conjugates, mainly AP2 (74.7–84.8%) and smaller amounts of AP1 (12–24%) (Fig. 5A). Esterase hydrolysis of these AP, although incomplete, yielded compounds that co-eluted from the RP-18 column with cold 20E (6.1%) and E

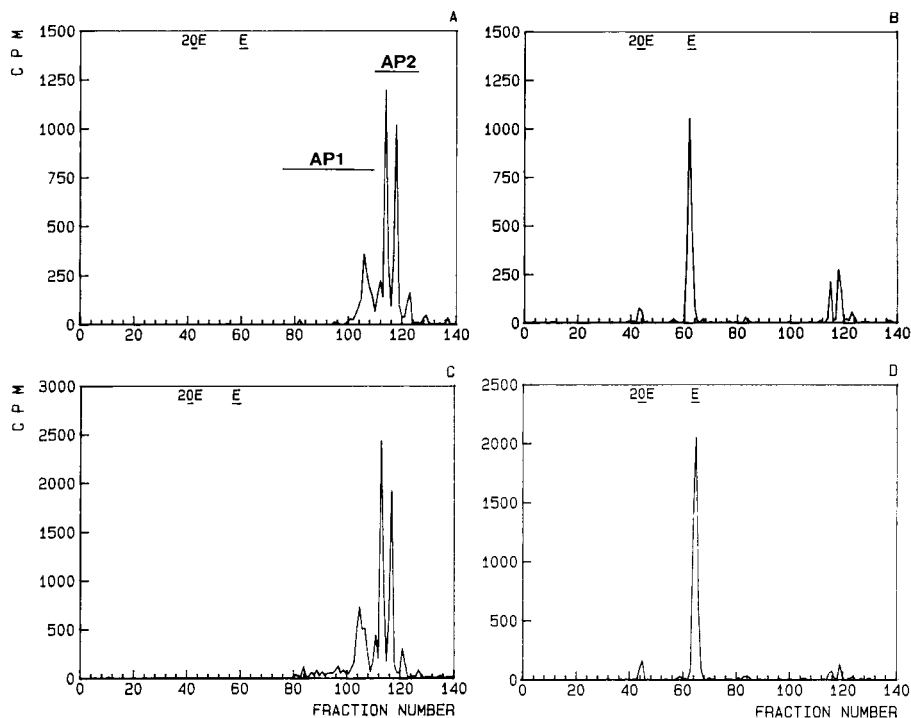


Fig. 5. Labeled apolar products in the freshly laid eggs and freshly ecdysed nymphs originating from *O. moubata* females injected with [ $^3\text{H}$ ]E during vitellogenesis. **A:** RP-HPLC radiochromatogram of crude methanol extract of freshly laid eggs. **B:** RP-HPLC radiochromatogram of esterase hydrolysis products of crude methanol extract of freshly laid eggs. **C:** RP-HPLC radiochromatogram of crude methanol extract of freshly ecdysed first instar nymphs. **D:** RP-HPLC radiochromatogram of esterase hydrolysis products of crude methanol extract of freshly ecdysed first instar nymphs. E and 20E indicate retention times of cold standards, coinjected with the samples.

(84.2%) standards (Fig. 5B). No other products were liberated. HPLC analysis of crude methanol extracts and esterase-treated extracts of several embryonic stages revealed that these products remained unchanged throughout embryonic development until the eclosion of the larvae 9–10 days after oviposition (Fig. 6).

The larval stage of *O. moubata* is a nonfeeding stage living on the remainder of the vitellus [12]. After eclosion, it continues developing, forming a new

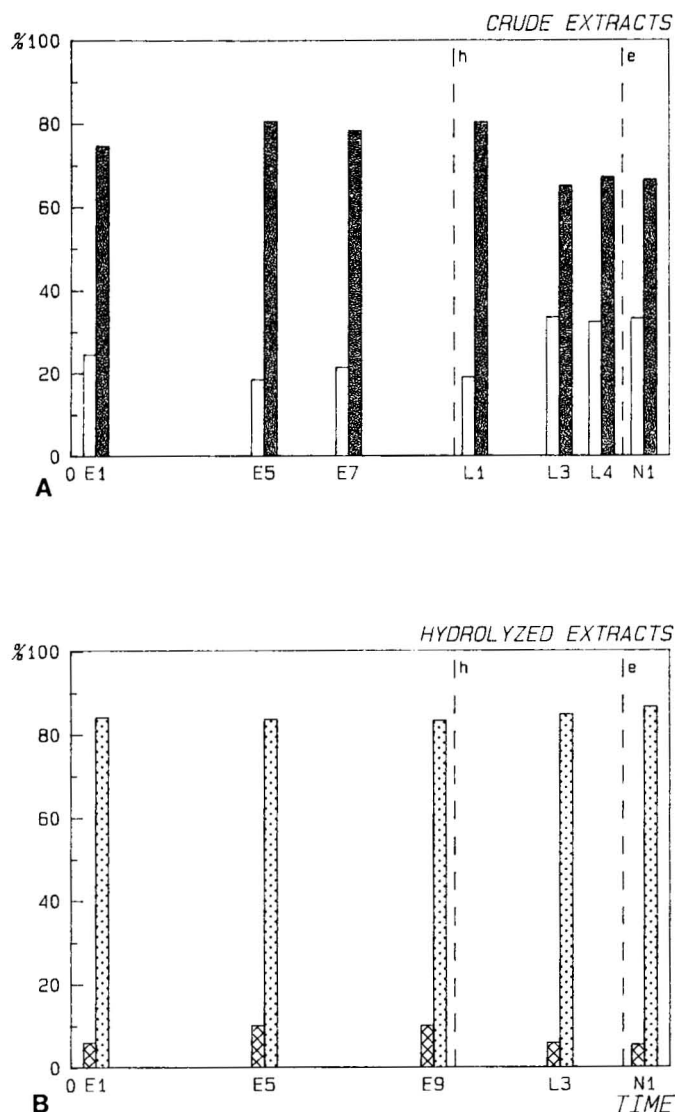


Fig. 6. Fate of the labeled apolar products contained in the freshly laid eggs of *O. moubata* females injected with  $[^3\text{H}]\text{E}$  during vitellogenesis. **A:** Crude extracts. Percentages of AP1 and AP2 in extracts of the 1st, 5th, and 7th day of embryonic development (E1, E5 and E7), 1st, 3rd, and 4th day of larval development (L1, L3, and L4), and freshly ecdysed first instar nymphs (N). (open columns), AP1; (heavy spotted columns), AP2. **B:** Hydrolyzed extracts. Percentages of radiolabel comigrating with 20E and E after esterase hydrolysis of extracts of the 1st, 5th, and 9th day of embryonic development (E1, E5 and E9), third day of larval development (L3), and freshly ecdysed first instar nymphs (N). (cross-hatched columns), 20E; (light spotted columns), E. Broken vertical line represents the hatching (h) of the larvae from the eggs or the ecdysis (e) of the first instar nymphs from the larvae.

nymphal cuticle, and molts 4–5 days later. During larval development there was a conversion of AP2 to AP1, and the percent of radiolabel attributed to AP1 increased to approximately 33%, while the percent of AP2 decreased to 65%. These percentages remained unchanged in the freshly molted first instar nymphs (Figs. 5C and 6). Esterase hydrolysis of apolar conjugates of larval and nymphal stages yielded approximately the same percentages of E and 20E as freshly laid eggs (Figs. 5D and 6).

Freshly laid eggs from three females injected with [ $^3\text{H}$ ]20E contained AP1 (20.7%), AP2 (74.0%), and small amounts of unmetabolized 20E (2.6%). As with the eggs coming from females injected with [ $^3\text{H}$ ]E, there was no apparent change of the metabolite pattern during embryonic development. Again, there appeared to be an increase of AP1 (33.4%) at the expense of AP2 (60.2%) during larval development. Esterase hydrolysis of these products yielded only 20E (data not shown).

## DISCUSSION

Our results show that, in the different nymphal instars of *O. moubata*, ingested molting hormones, E or 20E, are converted to apolar conjugates AP1 via the formation of AP2. Although the intestinal cells are most probably responsible for the major conversion of the hormone to the conjugates, other tissues are capable of this conjugation, as demonstrated by *in vitro* incubation of Malpighian tubules, dorsal integument, or ventral carcass with [ $^3\text{H}$ ]E (Vuillème, unpublished results, see in [6]). The possible pathways for 20E are shown in Figure 7. At the end of the first molting cycle after the ingestion, the hormones are principally located in the intestinal tract as AP1. The metabolite pattern remains unchanged during the successive molting cycles. No change in the composition of the ecdysteroid moiety of the apolar conjugates was found. If, for example, we had noted, in the case of E ingestion by second instar nymphs, the occurrence of free 20E or the release of 20E after esterase hydrolysis of the AP1 in the following instars, then we could hypothesize the reutilization of the apolar products during the molting cycle. In view of the specific activity of the tritiated E used, a detectable peak of 50 cpm is equivalent to 0.45 pg. Thus the contribution of the radiolabeled AP would only need to correspond to approximately 1/22,000 of the ecdysteroid peak in the fifth instar nymphs [2]. Since no apparent change in the AP1 occurs, it seems that the AP are inactivation products. These products are probably retained in the gut during the whole life cycle of this tick, as are most of the waste products of digestion, because the midgut does not communicate with the hindgut [13]. Although some regurgitation may take place, no excretion of the digestive waste products by the rectum occurs. In fact, in a closely related species, *Ornithodoros parkeri*, a part (25–76%) of the AP1 produced after ingestion of the tritiated hormones is excreted [10]. The exact chemical nature of these AP1 is not known, but, as demonstrated by hydrolysis with esterase, they remain esters. They are derived from the more apolar AP2 conjugates, which have been identified in the fifth instar nymphs of *O. moubata* by mass spectrometry as esters of 20E conjugated at the C-22 position with long chain fatty acids (C16:0, C18:0, C18:1 and C18:2) [4]. The

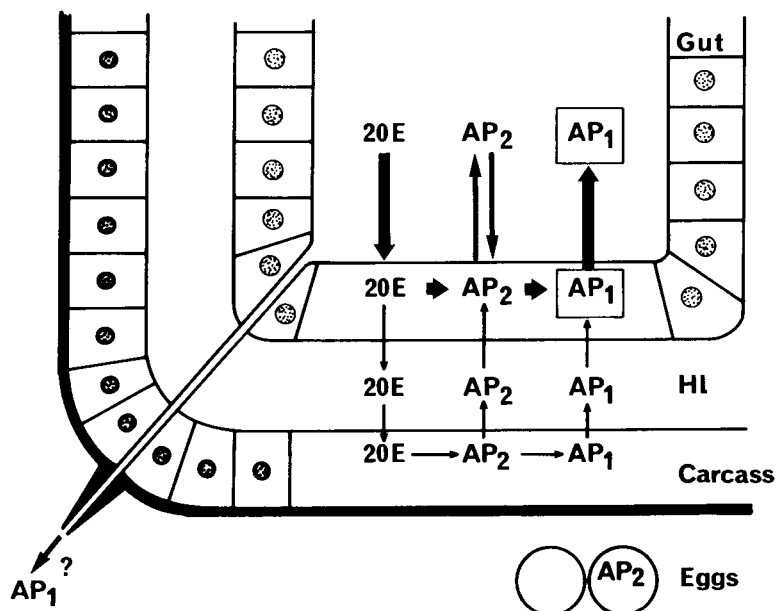


Fig. 7. Interpretative diagram of the fate of 20E ingested by nymphs of *O. moubata*. Within the first few hours after ingestion, part of the ingested 20E crosses the intestinal barrier and impregnates the peripheral tissues. These tissues can conjugate the hormone to AP<sub>2</sub>, and then to AP<sub>1</sub>. Afterwards, the conjugated hormone is slowly released and reabsorbed by the gut. The relative importance of the different pathway is represented by the width of the arrows. HL, hemolymph.

same products have recently been identified in feces of larval *Heliothis virescens* (Lepidoptera) [14] or *H. armigera* [15] after the animals had ingested large quantities of 20E. Although the exact chemical nature of the products is not known, apolar conjugates with retention times similar to the AP<sub>1</sub> of *O. moubata* are produced by spiders ingesting ecdysteroids, and these apolar products are excreted in the feces [16]. We thus think that the normal fate of these apolar products is their elimination in the feces. *O. moubata* probably represents an exception, because of the peculiar morphological formation of its gut [13].

The other part of our study concerns the possible reutilization of the AP by the embryos during their development. Apolar products probably occur naturally in the eggs and embryos, since it is possible to increase the quantity of RIA-positive material by hydrolysis with esterase in ticks [5; Dotson et al., unpublished results] and in insects, e.g., *Periplaneta americana* [17] or *Drosophila melanogaster* [18]. However, the percent of increase by hydrolysis in ticks is variable. The nutritional history of the female may contribute to this variability. When second instar nymphs ingest [<sup>3</sup>H]E, 2.5% of the ingested radiolabel is recovered in the eggs of the "resulting" females. If the adult females ingest the hormone, this proportion increases to 10.2% [11]. Thus the frequency at which the females encounter ecdysteroids and the concentration of these hormones in the blood meals throughout their life may influence the proportion of AP accumulating in the eggs. In the present

study, the [ $^3\text{H}$ ]E was injected into the hemocoel to obtain a higher percentage of radiolabel in the eggs than that found after ingestion [7]. No peaks of radiolabeled free 20E or its metabolites were found during embryonic development. Special attention was paid to the 7th day of development, which corresponded to an endogenous peak (20 pg/embryo) of 20E [Dotson et al., unpublished results]. Since a detectable peak of 50 cpm is equivalent to 0.45 pg, the contribution of the radiolabeled AP in this case would only need to correspond to 1/444 of the ecdysteroid peak of 200 pg per ten embryos (minimum number of eggs used for extraction). Contrary to what occurs to the polar conjugates found in the eggs of *Locusta migratoria* [19,20], or to what has been suggested by the in vitro hydrolysis of ecdysteroid esters by homogenates of 15-day-old *B. microplus* embryos [8], the AP of *O. moubata* are not hydrolyzed during embryonic development. Their incorporation into the eggs may be artifactual. The AP circulating in the hemolymph during vitellogenesis may be bound to the vitellogenins in a nonspecific manner and could thus be incorporated into the oocytes. In the early development of *O. moubata*, AP2 are converted to AP1 only after hatching, when the intestine has fully developed and begins actively digesting the vitellus, demonstrating that this transformation requires enzymes not present in the yolk but produced later by the intestinal cells.

The intestinal cells appear to play an important role in the conversion of ecdysteroids to AP. An analogous conversion occurs in the gastric caecae of *L. migratoria*, where ingested ecdysteroids are acetylated at the C-3 position. This acetylation has been interpreted as an efficient inactivation mechanism [21]. We conclude that the tick AP conjugates are also metabolites of an inactivation reaction, occurring mainly in the intestinal cells, and not a source of conjugated hormone to be liberated during the molting cycle or embryonic development.

## LITERATURE CITED

1. Diehl PA, Connat JL, Dotson E: Chemistry, function and metabolism of tick ecdysteroids. In: Morphology, Physiology and Behavioral Biology of Ticks. Sauer JR and Hair JA, eds. Ellis Horwood, Chichester, pp 165-193 (1986).
2. Germond JE, Diehl PA, Morici M: Correlations between integument structure and ecdysteroid titers in fifth-stage nymphs of the tick, *Ornithodoros moubata* (Murray, 1877; *sensu* Walton, 1962). *Gen Comp Endocrinol* 46,255 (1982).
3. Bouvier J, Diehl PA, Morici M: Ecdysone metabolism in the tick *Ornithodoros moubata* (Argasidae, Ixodidae). *Revue Suisse Zool* 89,967 (1982).
4. Diehl PA, Connat JL, Girault JP, Lafont R: A new class of apolar ecdysteroid conjugates: Esters of 20-hydroxyecdysone with long chain fatty acids in ticks. *Int J Invertebr Reprod Dev* 8,1 (1985).
5. Connat JL: Aspects endocrinologiques de la physiologie du développement et de la reproduction chez les tiques. Thèse de doctorat d'Etat, Dijon, France, 372 pp dont 14 annexes (1987).
6. Connat JL, Dotson EM: Comparative investigation of the egg ecdysteroids of ticks using radioimmunoassay and metabolic studies. *J Insect Physiol* 34,639 (1988).
7. Connat JL, Diehl PA, Morici M: Metabolism of ecdysteroids during the vitellogenesis of the tick *Ornithodoros moubata* (Ixodidae, Argasidae): Accumulation of apolar metabolites in the eggs. *Gen Comp Endocrinol* 56,100 (1984).

8. Wigglesworth KP, Lewis D, Rees HH: Ecdysteroid titre and metabolism to novel apolar derivatives in adult female *Boophilus microplus* (Ixodidae). Arch Insect Biochem Physiol 2,39 (1985).
9. Crosby T, Evershed RP, Lewis D, Wigglesworth KP, Rees HH: Identification of ecdysone 22-long-chain fatty acyl esters in newly laid eggs of the cattle tick, *Boophilus microplus*. Biochem J 240,131 (1986).
10. Connat JL, Diehl PA: Probable occurrence of ecdysteroid fatty acid esters in different classes of arthropods. Insect Biochem 16,91 (1986).
11. Connat JL, Diehl PA, Thompson MJ: Possible inactivation of ingested ecdysteroids by conjugation with long-chain fatty acids in the female tick *Ornithodoros moubata* (Acarina: Argasidae). Arch Insect Biochem Physiol 3,235 (1986).
12. Chinzei Y, Yano I: Vitellin is the nutrient reserve during starvation in the nymphal stage of a tick. Experientia 21,948 (1985).
13. Enigk von K, Grittner I: Die Exkretion der Zecken. Z Tropenmed Parasit 4,77 (1952).
14. Kubo I, Komatsu S, Asaka Y, De Boer G: Isolation and identification of apolar metabolites of ingested 20-hydroxyecdysone in frass of *Heliothios virescens* larvae. J Chem Ecol 13,785 (1987).
15. Robinson PD, Morgan ED, Wilson ID, Lafont R. The metabolism of ingested and injected [<sup>3</sup>H]ecdysone by final instar larvae of *Heliothis armigera*. Physiol Entomol 12,321 (1987).
16. Connat JL, Fürst PA, Zweilin M: Detoxification of injected and ingested ecdysteroids in spiders. Comp Biochem Physiol [B]. 91,257 (1988).
17. Slinger AJ, Dinan LN, Isaac RE: Isolation of apolar ecdysteroid conjugates from newly laid oothecae of *Periplaneta americana*. Insect Biochem 16,115 (1986).
18. Bownes M, Shirras A, Blair M, Collins J, Coulson A: Evidence that insect embryogenesis is regulated by ecdysteroids released from yolk proteins. Proc Natl Acad Sci USA 85,1554 (1988).
19. Lagueux M, Sall C, Hoffmann JA: Ecdysteroids during embryogenesis in *Locusta migratoria*. Am Zool 21,715 (1981).
20. Sall C, Tsoupras G, Kappler C, Lagueux M, Zachary D, Luu B, Hoffmann JA: Fate of maternal conjugated ecdysteroids during embryonic development in *Locusta migratoria*. J Insect Physiol 29,491 (1983).
21. Modde JF, Lafont R, Hoffmann JA: Ecdysone metabolism in *Locusta migratoria* larvae and adults. Int J Invertebr Reprod Dev 7,161 (1984).