Insect hormones – ecdysteroids: their presence and actions in vertebrates

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INTRODUCTION

At the early stages of comparative endocrinology, when only vertebrate hormones were known, there were extensive efforts to investigate the possible presence of pituitary and thyroid hormones in invertebrate animals. These efforts were unsuccessful as described in the review by Wense (1938).

Since that time, many vertebrate and invertebrate hormones have been identified and it became clear, at least for the peptide hormones, that certain structural homologies existed in hormones across the phyla (see Greenberg & Price, 1983; De Loof & Schoofs, 1990). This may explain some biological cross-reactivity of the immunoproteins in both groups, as for example among the peptides of the insulin family. That does not mean, however, that original structures and functions of the ancient animal hormones have been necessarily retained during animal evolution (Barrington, 1980).

The situation is equally complicated in the case of isoprenoid hormones, where “vertebrate-type” and “invertebrate-type” hormones have been described (for review see Bradbrook et al., 1990; Lafont, 1991; Swevers et al., 1991). The “vertebrate” sexual hormones and corticoids are present in insects, although, when present, they appear to have different functions. In addition to the more popular steroid hormones of vertebrates there are other isoprenoid molecules, the insect juvenile hormones and retinoids (see Sláma et al., 1974, for the actions of juvenile hormones in vertebrates).

Ecdysteroids are polyhydroxylated sterolic invertebrate growth hormones. They are also present in plants (phytoecdysones) and, as we shall see below, they are also claimed to cause a plethora of pharmacological effects in the vertebrates. We briefly review these studies here with some critical comments concerning methods and equivocal interpretation of the results. Our aim is to facilitate further research in this interdisciplinary field of science.

Studies on the effects of ecdysteroids in vertebrates were initiated by the pioneering work of Burdette (1960–1974). At the beginning he used only the extracts prepared from insect material. Later he carried out his experiments with synthetic ecdysone (E) when it became available (note that we are using common abbreviations E for ecdysone, 20E for the most common 20-hydroxyecdysone or ecysterone, etc., according to the terminology
recommended by Lafont et al., 1993) and, finally, he used several ecdysteroid compounds isolated from plants.

In some earlier papers by Burdette, the effects of ecdysteroids on vertebrate tissue and cells were referred to as the heterophylic effects, to stress out the action between different animal phyla. At the time this term was introduced, there was no notion that ecdysteroids could be present in most other phyla of invertebrates. More recently with the discovery of ecdysteroids in plants, invertebrates and also in the body of vertebrates, the action of ecdysteroids might be described by the term heterophylic (or more correctly heterophyletic) only with precautions. There are also reservations against using the term “hormonal” since all available data are supporting an earlier conclusion (Sláma et al., 1974) that the effects of ecdysteroids found in vertebrates should be considered as a nonspecific pharmacological actions rather than real hormonal effects.

**ECDYSTEROIDS AND VERTEBRATE CELL PROLIFERATION**

As early as in 1963, Burdette & Coda found that extracts prepared from silkworms, *Bombyx mori*, containing ecdysteroids, enhanced the rate of protein synthesis in mammalian tissue. Burdette (1962b, 1964a) performed assays in which various dosages of E were injected intraperitoneally into mice bearing transplanted tumors resulting in regression of 21% of the tumors, but the effect was not clearly dose-related. Working with the vertebrate tissue cultures, Burdette & Richards (1961) and Burdette (1962b, 1964a) cultured mammalian embryonic fibroblasts and Sarcoma 180 cells in hanging-drop cultures which were used for assaying E effects. The degree of growth was uniformly scored. It appeared that growth of the embryonic fibroblasts and neoplastic tissues was inhibited. In further experiments Burdette (1964b) achieved regression and inhibition of Sarcoma 180 tumors also with insect “brain hormone”, farnesol and E (for review see Burdette, 1972).

Hoffmeister & Lang (1969) investigated the effects of E (0.5–100 μg in 15 ml medium) on growth of the HeLa cells in culture. They also studied the effects of E on differentiation of chick embryos. Their conclusion was that E had no consistent effect on growth of the vertebrate cells in culture (see also Hirono et al., 1969). According to the most recent observations of Detmar et al. (1994), 20E influences the differentiation of human epidermis in vitro. It increases the number of cell layers and enhances the expression of markers of differentiation or maturation.

In addition to the assumptions listed above that ecdysteroids might have some beneficial effects on tumor growth, there were also reports which suggested that ecdysteroids might be carcinogenic for the vertebrates. Thus, El-Mofty et al. (1987) reported that ecdysteroids contained in bracken fern (*Pteris aquilina*) might be carcinogenic for adult toads (*Bufo regularis*). The toads were force-fed with 0.5 ml of amphibian physiological saline (containing 0.2 μg E/g body mass) three days per week for four months. The formation of neoplastic lesions were observed in 10 out of 58 experimental toads. These results are difficult to understand, because as we know today, many plants including common vegetables (spinach) contain equally high or even higher amounts of ecdysteroids than bracken fern and thus ought to be also carcinogenic. Plants usually contain 20-hydroxyecdysone (20E) as the major ecdysteroid, and it cannot be excluded that E and 20E might cause different effects. Quite recently, the same laboratory reports again on the
induction of carcinogenic effects in mice when fed twice a week with 3 ppm E for 22 months (El-Moffy et al., 1994). This statement requires also further confirmation, because the common laboratory food mixture for mice or rat usually contains 0.03–0.003% (= 300–30ppm) of endogenous 20E derived from plant material (Sláma, unpublished).

**ECDYSTEROIDS AND PROTEIN METABOLISM**

When the first ecdysteroids from plants became available (1965–1968), Japanese scientists Okui et al. (1968) and Otaka et al. (1968) studied their effects (20E, inokosterone, ponasterone A, cyasterone, pterosterone) on protein synthesis in mouse liver. In these experiments, ecdysteroids were administered intraperitoneally or orally in 0.9% saline in dosages from 0.5 to 5 μg per g body mass. The mice were decapitated, their livers were removed, homogenized, centrifuged, and the supernatant was used as an enzyme source for measurements of incorporation of the labeled amino acids. It appeared that all ecdysteroids, as well as 4-chlorotestosterone as a control, stimulated protein synthesis in mouse liver in both the male and female specimens. The time-course of changes induced by 20E and 4-chlorotestosterone was similar, with a maximum effect (200% of initial controls) observed after 5 hours and a return to initial level after 12 hours. It has to be stressed, however, that the untreated control animals were used only for zero time, so that it is not clear whether a similar time-course of the changes would not occur after saline injections alone (i.e. due to a circadian rhythm of metabolic activity, etc.). In similar studies (Otaka et al., 1969a,b), it was found that 20E was also able to exert its stimulatory effect on protein synthetic ability in microsomal and polysomal fractions. According to Otaka & Uchiyama (1969), the stimulatory effect of 20E on protein synthesis in rat liver can be explained in terms of the influence of 20E on messenger RNA synthesis, as the effect was abolished by addition of actinomycin D (see review by Uchiyama & Otaka, 1974). By contrast, more recent studies of Syrov (1984) and Syrov et al. (1978) show that the stimulatory effects of 20E on protein synthesis in mammals could not be abolished by actinomycin D, suggesting other than transcriptional levels of regulation.

In addition to liver, stimulation of protein synthesis by ecdysteroids has been found also in nervous tissue. Among these reports, Chaudhary et al. (1969) studied the effect of E on glutamic decarboxylase activity in the rat brain. They injected 2.5–50 μg.kg⁻¹ E intraperitoneally and after 4 to 24 h the animals were sacrificed, their brains were homogenized and used for enzyme assays. There was a marked increase (+ 25–30%) in the activity of glutamate decarboxylase in the brains of the E-treated animals, but without any clear cut time-course or dose-response relationship.

Catálan et al. (1984) investigated the effect of 20E and other steroids on acetylcholinesterase activity in tissue slices prepared from the rat cerebral cortex. In analogy with some earlier observations on insects, 20E produced an increase of acetylcholine esterase activity also in the mammalian brain, with a pattern similar to that produced by estradiol. In this case the effect did not involve a direct action on the enzyme molecule, because it required involvement of the protein synthesis (the effect was abolished by cycloheximide, an inhibitor of protein synthesis).
CARBOHYDRATE METABOLISM AND DIABETES

It is known that anabolic steroid hormones are effective in reducing the hyperglycaemic response to exogenous glucagon. Due to general metabolism stimulation and anabolic actions of 20E in vertebrates, Yoshida et al. (1971) inspected the existence of these biochemical relationships in the effects of 20E on carbohydrate metabolism. For these studies they used rats or mice with high glucose levels induced by administration of glucagon, alloxan or anti-insulin serum. 20E was injected intraperitoneally in the amounts of 0.5 μg per g body mass (mice 20 g, rats 200 g). The results revealed that 20E indeed had a suppressive effect on hyperglycaemia induced by the above hyperglycaemic agents. There was no effect on blood glucose levels in normal animals, although pre-treatment with 20E prior to hyperglycaemia was effective. After administration of 20E to alloxan-diabetic mice, the blood glucose level was reduced to about one half.

Incorporation of 14C-glucose into protein of normal mouse liver or into glycogen of normal and mildly diabetic mouse liver was also stimulated by 20E. These results by Yoshida et al. (1971), which suggested beneficial influence of 20E on the symptoms of diabetes mellitus, were later corroborated by further experimental evidence. Thus, 20E and turkesterone (11α, 20E) reduced the symptoms of artificially induced diabetes in the rat (Tashmukhamedova et al., 1985, 1986; Kosovsky et al., 1989). More recently, the use of ecdysteroids against diabetes has been again assayed by Syrov et al. (1992b) who had access to relatively large amounts of 20E (from the plant Leucaena leucocephala) and turkesterone (from Ajuga turkestanica). The isolation was conducted according to a method invented by Manatthanov et al. (1980). Oral administration of these compounds to male rats (180–220 g body mass) at a dose of 5 mg·kg⁻¹ (5 μg·g⁻¹) for 15 days caused reduction of pathophysiological symptoms after alloxane induced experimental diabetes. The content of glycogen, malonic dialdehyde, pyruvic acid and calcium transporting function of the liver mitochondria returned to normal conditions, which was also true for phospholipid spectrum of liver mitochondrial membranes that were pathologically changed owing to insulin deficiency. These findings led Syrov et al. (1992b) to conclude that the effects of ecdysteroids might depend on reparation of phospholipid fractions, which were playing a structural role in the mitochondrial membranes.

The antidiabetic properties of ecdysteroids have been a subject of patent application in Japan (Takahashi & Nishimoto, 1992). A single administration (1 mg) of 20E to rats rendered to be diabetic by streptozotocin has been claimed to evoke a large decrease of blood sugar levels after 4–6 hours.

EFFECTS ON LIPID METABOLISM

Lupien et al. (1969) analyzed the effects of E injections on cholesterol metabolism in rat liver and serum. They found considerably decreased hepatic cholesterol levels at all doses administered. In addition, high concentrations (10–50 μg·kg⁻¹) also inhibited de novo synthesis of cholesterol. It was concluded that the primary effect of ecdysone was to stimulate cholesterol excretion in the bile, thus causing increased bile cholesterol levels and decreased liver cholesterol levels.

Some further effects of ecdysteroids on lipid metabolism have been reviewed by Uchiyama & Yoshida (1974) and hypcholesterolemic effects of several ecdysteroids have been described by Mironova et al. (1982). Catalán et al. (1985) injected 20E...
intraperitoneally into rats in doses of 0.5 μg.g⁻¹ together with the labeled precursors of lipid metabolism. The animals were sacrificed 40 min. after injection, tissue samples were removed and the rate of lipid biosynthesis was determined. It appeared that 20E produced an increase in ¹⁴C-acetate and ³²P-orthophosphate incorporation into triglycerides. It also produced profound increases in the specific activity of phosphatidyl-ethanolamine and phosphatidyl-serine in liver. In adipose tissue, the most apparent effect of 20E was increased specific activity of phosphatidyl-choline.

As part of a more complex analysis of the hepatoprotective actions of ecdysteroids in mammals, Syrov et al. (1981b, 1986b) investigated the effects of ecdysteroids on some important functions of the rat liver, as are bile secretion, synthesis of bile acids, bilirubin metabolism or excretion of cholesterol. They found that 20E and cyasterone administered in a single dose of 5 and 50 mg.kg⁻¹, or in 7 daily doses of 5 mg.kg⁻¹ in food, markedly stimulated the bile secretion in normal rats. The chemical composition of bile was also improved due to increased levels of bile acids and bilirubin, while the content of cholesterol was decreased. These favourable effects of ecdysteroids were even more pronounced when applied to rats afflicted with toxic hepatitis induced by heliotrione.

ANABOLIC EFFECTS INDUCED BY ECDYSTEROIDS

In the early stages of ecdysteroid research (1968–1975), it became obvious that these compounds could stimulate protein synthesis and increase metabolic activity in some vertebrate organs. The amount of ecdysteroid available at that time was not sufficient for carrying out extensive feeding assays on mice or rats for determination of general anabolic effects. However, this situation changed when certain plants were discovered to contain enormously large amounts of ecdysteroids. Hikino et al. (1969) administered orally 20E and cyasterone to mice in dosages of 5–100 μg/animal/day for a period of 60 days. They found increased growth of the treated mice, enhanced protein synthesis in the liver and kidney and also somewhat altered morphological structure of the liver cells. These results provided some evidence that ecdysteroids might produce anabolic effects in the vertebrates. Later on, the clear anabolic effects were produced with ecdysteroids turkesterone, cyasterone and some other related compounds. In a series of papers, Syrov and his co-workers determined that these “phytoecdysones” could be effective as free molecules as well as in form of the corresponding acetates, which were assumed to be released slowly in the active free form by hydrolysis (Syrov & Kurnukov, 1975a,b, 1976a,b,c; Syrov et al., 1975a,b, 1981a; Aizikov et al., 1978).

The common design of these experiments, which were mostly carried out on territory of the former Soviet Union, was to use castrated immature rats which received intraperitoneal injections or intragastric administration of 0.5–50 mg.kg⁻¹.day⁻¹ of 20E or other ecdysteroids for 10 days or more. The effects were evaluated by measurements of the increased mass of specific muscles (i.e. m. levator ani and m. tibialis anterior) or the increased mass of the liver. Both the total protein and glycogen content of the organs were also increased. Turkesterone, bearing the important “corticoid” hydroxyl function at C-11, appeared to be the most potent phytocorticosteroid in these experiments (Syrov, 1984).

In general, the anabolic steroids increase animal body mass due to increased muscular tissue, which results in enhanced strength and longer performance of the skeletal muscles. Chernykh et al. (1988) investigated these effects in mice, making comparisons between
20E and the true vertebrate anabolic steroid, metandrosteno-
lone. The male mice (19–20 g body mass) received in- 
traperitoneally injections of 5 μg of either 20E or me-
tandrosteno-
lone during 7 days. The assays were made on two parallel experimental groups, one un-
trained and the other subjected to previous training in form of repeated swimming. They 
found that both metandrosteno-
lone as well as 20E, at dosages of 5 μg.kg⁻¹ caused anab-
olic increase of body mass only under the conditions of temporary training. Although 20E ge-
nerally stimulated physical capability for labour, metandrosteno-
lone could do the same only 
in association with temporary trainings. Metandrosteno-
lone stimulated biosynthesis of the 
myofibrilar proteins in musculus soleus but not in musculus extensor digitorum longus, 
while 20E increased the amount of myofibrilar proteins in both muscles.

Sergeev et al. (1991) investigated the thymolytic activity of anabolic steroids in mice 
(18–20 g) after administration of 50 μg.kg⁻¹ of the tested compounds for 10 days. Under 
these conditions testosterone and metandrosteno-
lone exhibited clear thymolytic effects, 
whereas 20E was ineffective. In contrast to anabolic vertebrate steroid hormones, the an-
bolic actions of 20E, or of the pharmacological preparation “ecdisten” (see below), were 
not associated with the adverse androgenic, antigonadotropic or thymolytic side effects 
(Syrov, 1984; Kuzmitsky et al., 1990; Sergeev et al., 1991). Provided that this is confirmed 
by further studies, it would represent an important pharmacological or medicinal factor.
Another specific prerequisite to the anabolic effects of ecdysteroids (which are far more 
polar and slightly water-soluble in contrast to the highly lipophilic anabolic androgens) is 
that these compounds cannot be readily detected by normal anti-doping assays. According 
to Mamakhanov (1994, pers. commun.) ecdysteroids have been occasionally used during 
training of athletes in the former Soviet Union for their anabolic effects.

PHARMACOLOGICAL AND PHYSIOLOGICAL EFFECTS

The complexity and the problems associated with pharmacological action of ecdyster-
oids in mammals have been discussed in the previous review articles by Syrov (1984) and 
Simon & Koolman (1989). A broad spectrum of pharmacological effects of various ecdy-
steroids, including description of some specific aspects of ecdysteroid action in the verte-
brates, have been described in a series of papers by Syrov and his co-workers (see Syrov 
beyond the scope of this brief review to comment all their findings. Some of the topics 
have been mentioned elsewhere in this article and the rest can be found in the review by 

When combining the data by Syrov (1984) with the list of pharmacological effects pre-
sentated by Simon & Koolman (1989) and, eventually, if we believed to all of the reports 
about the “ginseng-like” properties of drugs prepared from ecdysteroid containing plants, 
we might discover that ecdysteroids would form the best choice of pharmacological agents 
for the future “green medicine” (Sláma, 1993). Unfortunately, this should be treated with 
great caution, since some of the facts have not been based on regular statistical evaluation, 
they do not include reasonable control data, for example, or unrealistically small amounts 
of the material have been used.

Just for an illustration, in insects where ecdysteroids represent a true growth hormone, 
the standard in vivo ED-50 values are within the range of 1 to 100 mg.kg⁻¹ of insect body 
mass (Sláma et al., 1993). In pharmacological experiments with vertebrates (where
ecdysteroids have no hormonal status and where they are rapidly eliminated from the body, the reported dosages to be effective are often more than three orders of magnitude below the effective threshold in the invertebrates. Such very small dosages or concentrations (pmol) are still within the range of activity of the true vertebrate steroid or peptide hormones, but they are unrealistically small for most other biologically active compounds.

The pharmacological-ecdysteroid survey can be initiated by examining effects on the circulatory system. According to Kurmukov & Ermishina (1991) there are profound effects of 20E (10–20 μg·kg⁻¹) on heart functions in the acute or subacute experiments with cats and also in the experiments with artificially induced arrhythmia in rats (180–220 g, intraperitoneal injections of 5–20 μg of 20E per kg body mass, 5 min after introduction of arrhythmia). Although 1 μg·kg⁻¹ of 20E had no effect on heart functions in normal cats, there were profound effects obtained in the experimental preparations involving ligation of the descending branch of the left coronary artery. In this case, intravenous administration of 20E (10 μg·kg⁻¹) decreased the amount of extra-systolic contractions, and prevented the development of fibrillation.

In further experiments in which arrhythmia was artificially induced by aconitine or calcium chloride, 20E (20 μg·kg⁻¹) restrained arrhythmia, increased the number of surviving animals (by 75%), and in 50% of the specimens, the treatment restored a normal pulse within 51 min. The mechanism of such anti-arrhythmic action of 20E has been explained on basis of its cell membrane stabilizing properties, which lead to overall improvement of the hemodynamic conditions and heart contractility. These findings might have important implications in human medicine, especially for atherosclerosis (Matsuda et al., 1974; Khushbaktova et al., 1991). According to Khushbaktova et al. (1987, 1991), rabbits with experimentally induced atherosclerosis showed decreased Na/K ATPase activity of microsomes by 58.5%. After oral administration of 20E (10 mg·kg⁻¹·day⁻¹ for 28 days) the normal levels of activity of these microsomal enzymes were restored.

With respect to neuromuscular functions, Hikino & Takemoto (1972) observed slightly analgesic effects of ecdysteroids in the vertebrates. Babich et al. (1992) investigated the effects of 20E on tonic contractions of smooth musculature of the explanted rat urinary bladder, after stimulation of the contractions by carbachol. They found that 20E (2 × 10⁻³M) remarkably decreased the spastic contractions induced by carbachol in the urinary bladder. The authors concluded that 20E stimulated the Mg2+, ATP-dependent accumulation of Ca2+ within the smooth muscle cells of the bladder.

Recently Levitsky et al. (1993) inspected possible interactions between ecdysteroids and vitamins of the sterolic type (D3). They analysed the effects of 20E and vitamin D3 on liver chromatin in experimentally induced avitaminosis in rats (30–40 g body mass). The experimental animals were kept constantly on a diet inducing D-hypovitaminosis. They received 100 μg of 20E (or vitamin D3) every second day within a period of 20 days. It appeared that both 20E as well as vitamin D3 caused a partial corrective effect on the structural and functional changes (transcription and replication rates) of the nuclear chromatin in liver cells.

There are reports on antioxidative properties of ecdysteroids on isolated cell constituents. This information is related to the work of Osynska et al. (1992) who concluded that 20E (10⁻⁴–10⁻³M) had antiradical and antioxidative properties. They used a special model system containing liposomes of phosphatidyl-choline from avian eggs. The antioxidative
activity of 20E was as high as that of the known inhibitors of lipid peroxidation, e.g. diethylythroparaphenylene diamine or ethylenediamine tetraacetate (Osinska et al., 1992). Finally, there are reports on the delayed effects of ecdysteroids, which are difficult to be understood. For instance, Csaba et al. (1978) found that a single injection of E (100 μg/animal) administered subcutaneously to rats within the first 24 h of postnatal life increased the future mass of the adrenals and decreased that of the thymus gland in the adults, i.e. 3.5 months later.

**ECDYSTEROIDS AND THE IMMUNITY SYSTEM, INFLAMMATORY RESPONSES**

The reported anabolic, metabolism-stimulating and cell membrane protective properties of 20E motivated Sakhibov et al. (1989) and Kuzmitsky et al. (1990) to look for possible effects of ecdysteroids on cell immunity. They used mice which were immunized with intraperitoneal injection of sheep erythrocytes (5 x 10^7 cells per mouse). The 20E was administered in a single dose of 5–20 mg.kg⁻¹ into the gut. It appeared that 20E stimulated slightly the primary immune reactions and phagocytosis. Higher doses of 20E (50 mg.kg⁻¹) caused considerable reduction of the amount of antibody-producing spleen cells in the treated mouse. In the in vitro experiments with cultures of human lymphocytes, following their polyclonal stimulation with phytohaemagglutinin, the immuno-modulatory effect of ecdysteroids (10⁻²–10⁻⁸ M) on lymphocyte transformation could not be demonstrated (Sakhibov et al., 1989).

Subsequently, Pomovska et al. (1991) tested the effects of several ecdysteroids (E, 2deoxyE, 2deoxy20E, 20E and its 2,3,22-triacetate) on the model system of the lymphocytes. All of the compounds tested elicited similar effects: low or medium concentrations (10⁻¹²–10⁻⁸ M) stimulated DNA synthesis in the lymphocytes activated by concanavalin A (ConA), whereas concentrations above 10⁻⁸ M inhibited the activating effect of this mitogen. The stimulatory effect of 20E on DNA synthesis was less expressed on the splenocytes than on the thymocytes. Furthermore, additions of 20E inhibited DNA synthesis in the cultures of lymphocytes taken from the peripheral blood of healthy donors, activated by ConA. Clearly, these effects were strongly dependent on 20E concentrations. Chiang et al. (1992) observed stimulation of DNA synthesis in splenocytes activated by ConA already at 20E concentrations of 0.001 μg.ml⁻¹ (2 x 10⁻¹⁰ M).

Sergeev et al. (1991) compared the effects of testosterone, metandrostanolone and ecdistin (commercial ecdysteroid preparation, see below) on murine thymus glands in vivo. When administered at large doses (50 mg.kg⁻¹.day⁻¹) over 10 days, the two former compounds induced a decrease of thymus weight and of DNA synthesis in this gland, whereas ecdistin had no effect. The antiinflammatory properties of ecdysteroids were analysed by Kurmuukov & Syrov (1988) in mice and rats. They found that 20E, when given orally at the doses of 10–20 mg.kg⁻¹.day⁻¹ might be even more effective against inflammation than amidopyrine or as active as cortisone acetate. There is a possibility that these effects could be caused by stimulation of corticosteroid release from the adrenals. Takei et al. (1991) found that 20E (10⁻¹²–10⁻⁴ M) inhibited histamine release from the rat peritoneal mast cells induced by anti-IgE or ConA. According to these authors, 20E might alter the mobilization of intracellular Ca²⁺, leading thus to a reduction in the release of histamine from the mast cells.
Other potential uses of ecdysteroids include the immunization of mammals against ecdysteroids as a general strategy to control reproduction and/or development of blood-sucking invertebrates. Karr (1988) and Karr et al. (1990) demonstrated that such immunization results in a much reduced fecundity of ticks, and this also might open new perspectives for practical use of ecdysteroids in vertebrates.

**ECDYSTEROIDS AND MAMMALIAN REPRODUCTION**

Due to their steroidal nature, ecdysteroids were often suspected as being mimics of the vertebrate sexual hormones. According to Ogawa et al. (1974), however, ecdysteroids were absent in mammals and they induced no specific steroidal effect if administered to male adults, i.e. they had no effect on the mass of the prostate or seminal vesicles. No toxic responses in the adults to E could be demonstrated even at a dose of 9 g.kg⁻¹ body mass. Prabhul & Nayar (1974) assayed possible sex hormone activity of 20E in 4-day cycling, adult female mature white rats. The ecdysteroid was administered intravaginally (30 to 500 µg) and the effects were evaluated by examination of vaginal smears. No estrogenic or antiestrogenic effects (compared against 1 µg of estradiol dipropionate) were encountered.

Recently, Mirzaev & Syrov (1992) reported that oral administration of ecdysteroids (20E or turksterone) to male rats (5–10 mg.kg⁻¹ per day) enhanced their sexual activity when used for 1–5 days. Unfortunately, a prolonged treatment had the opposite effect. In contrast to all of the data described above on the stimulation of cell metabolism and protein synthesis, 20E was found to have spermicidal activity. A concentration of 20 µg.ml⁻¹ immobilized 100% of the spermatozoa in fresh human semen within 20 sec. (Bandara et al., 1989).

**ECDYSTEROID PRESENCE, METABOLISM AND TOXICITY**

The biological assays used in older studies were not sensitive enough to reveal the presence of ecdysteroids in the vertebrates. Burdette (1962a), using the standardized Calliphora assay for E, screened large quantities of liver, brain, skeletal muscle, small intestine and lungs of the vertebrates without obtaining any positive response. Ogawa et al. (1974) also concluded that E was absent in the mammals.

A different situation occurred with the development of more sensitive RIA methods, which revealed small amounts of the positively reacting materials (from 0.9 nmol.L⁻¹ in the dog, to 22.8 nmol.L⁻¹ in the rat) in blood serum of different mammals (Simon & Koolman, 1989). Among the sources of ecdysteroids in the body, dietary origins are of primary consideration. The plant and animal products used for human diet may contain up to 5 nmol.kg⁻¹ RIA positive E equivalents (Simon & Koolman, 1989). Recently it appears, however, that many plants including the common sources of food (e.g. spinach) may contain much higher amounts of ecdysteroids, i.e. 0.001–0.1% (10–1000 mg.kg⁻¹ and in certain cases the leaves or seeds of some plants may contain up to 30 g of 20E in one kg; Sláma, 1993). There were assumptions that increased amounts of 20E in vertebrate blood serum could be related to parasitization. Lansoud-Soukate et al. (1989, 1990) and Baswaid et al. (1989, 1990) have found that certain patients infested by *Loa loa, Mansonella persians* or *Schistosoma* (parasitic worms) showed increased amounts of ecdysteroid RIA immunoreactive materials in the urine. This finding, which was originally based on an
assumption that ecdysteroids might be sequestered into urine and blood of the patients by the parasites (for review see Guo, 1989), has been confirmed by further observations of Koolman et al. (1984) and Koolman & Moeller (1986). Nirde et al. (1984) concluded that excretion of ecdysteroids by Schistosomiasis might represent a good clinical marker for the identification of parasite infections.

Gharib et al. (1991, 1993) have reinvestigated the presence of ecdysteroid immunoreactive materials in human urine. They found that the phenomenon was not restricted to helminthiasis, but was widely spread among patients suffering from various diseases or injuries. There was an important suggestion that the presence of ecdysteroid in urine might in fact represent a good clinical marker for certain severe pathological conditions. The actual levels of the positive RIA materials in urine of healthy controls were from undetectable values to 46 nmol.L⁻¹, with the mean around 17 nmol.L⁻¹. The highest detected value was 518 nmol.L⁻¹ and the average value for patients suffering from various diseases was 40.3 nmol.L⁻¹.

Among the patients that often (but not always) showed increased ecdysteroid content in the urine were, for example, patients suffering by cranial trauma, CVD, meningioma, or advanced cirrhosis. These results clearly show that parasites need not to be necessarily involved, the importance of this phenomenon for clinical diagnostics being quite obvious. There were further suggestions that intestinal microflora could be a possible source of ecdysteroids in human body, but as we have already seen, food will always represent the principal source. With respect to the content of ecdysteroids in plants, which besides free steroids may include also several kinds of polar and/or apolar hydrolysable conjugates, it is impossible at the moment to make any precise estimate of the dietary intake of ecdysteroids by the vertebrates; the amounts could be severely underestimated when using only free ecdysteroids as a criterion.

According to Simon & Koolman (1989), the acute toxicity of ecdysteroids in mammals is extremely low. The results of Japanese workers Matsuda et al. (1970) and Ogawa et al. (1974) revealed LD-50 values of 6.4 g.kg⁻¹ in intraperitoneal injections in mice and more than 9 g.kg⁻¹ in oral application of 20E. For inokosterone these values were 7.8 g.kg⁻¹ and > 9 g.kg⁻¹, respectively. In rabbits, there were no detectable effects after intravenous application up to 0.1 g.kg⁻¹ and in the prolonged feeding experiments with rats there was no subacute toxicity up to 2 g.kg⁻¹.day⁻¹. Thus, according to Ogawa et al. (1974) we can conclude that ecdysteroids have no toxic response in mammals.

This conclusion can be perhaps best corroborated by the fact that rats or birds can feed and live on the seeds of Luehea, which contain incredibly large amounts of 20 g or more of 20E per kg, without any apparent syndrome of intoxication.

**DISTRIBUTION, METABOLISM AND EXCRETION**

The dynamics of distribution, excretion and metabolism of tritiated 20E in mice were first investigated by Hikino et al. (1972a,b). Following intraperitoneal administration, 20E was rapidly and easily resorbed into the blood stream, being quickly distributed into various organs (liver, gall bladder, intestine and kidney). The clearance of 20E from the organs, with exception of the excretory organs, was rather rapid, the radioactivity was gradually transported into the intestinal lumen via the gall bladder which showed the highest and continuous uptake. After oral administration of ³H-20E, the absorption from
the intestine was rather slow. On the other hand, elimination of the administered 20E from the body appears to be fast (4–10 min). The pattern of excretion indicates the main excretory pathway through the faeces, being mostly derived from biliary excretion. This is more important than urinary excretion after both intraperitoneal and oral administration. Close examination of the subcellular distribution of \(^3\)H-20E in the liver revealed that more than 80% of the radioactivity was present in the supernatant fraction whereas relatively small amount of radioactivity occurred in other fractions. Thin-layer chromatography of the radioactive substances present in the liver indicated that 20E was fairly rapidly metabolized.

The findings of Hikino et al. (1972a,b) described above were later confirmed by the experiments with 20E injections in rats (Dzukharova et al., 1987). From human body, 20E was eliminated within 9h after ingestion. The rates of elimination were considerably shorter in sheep, \(t_{1/2} = 0.4 \text{ h for oral, 0.2 h for intravenous and 2 h for intramuscular application. In mice or rats the half-time was only 8 min after injections (Dzukharova et al., 1987). These results are in good agreement with the results obtained in mice by Lafont et al. (1988), which equally reveal rapid excretion of E with the faeces in unchanged form together with some less polar metabolites.}

In extension of these studies, Girault et al. (1988) injected mice with \(^3\)H-E (10 Ci, 0.5 mg/animal) analysing metabolites in urine and faeces during the period of 6 days. They have isolated some E metabolites which had not been previously observed in invertebrates or plants. The metabolites could be characterized by three sequential reactions of E catalysis in mice: a) 14-dehydroxylation, b) complete reduction of the 7-ene-6-one and c) epimerization at C-3. These reactions were in good conformity with those known from metabolism of sterols, steroid hormones or bile acids in vertebrates. The metabolic fate of 20E, on the other hand, has been much less documented.

The main obstacle in these studies may be that there occurs possible cleavage of the side-chain between C20 and C22, which would preclude further analysis of the steroid metabolites, because the precursors available so far were just labeled in the side-chain. A combination of side-chain cleavage with the reactions described above would lead to molecules (see Fig. 1) showing considerable homology with certain progesterone derivatives (= neurosteroids) that are active on nerve cells (see Paul & Purdy, 1992).

It would be useful to investigate further metabolism of 20E labeled in the nucleus, and reinvestigate again the effects of ecdysteroids with the oxidized side-chain, e.g. poststerone and rubrosterone. In the early studies on stimulation of protein synthesis in mammalian liver, Otaka et al. (1968) found that rubrosterone was equally effective as 20E, but since that time neither rubrosterone nor poststerone have been tested again in the vertebrates.
EXTERNAL EFFECTS ON THE SKIN

Use of ecdysteroids as a cosmetic has probably found its origin in Kunming (People’s Republic of China), where ecdysteroids were extracted on large scale from plants for their use in sericulture (Guo, 1989). The first patent application (Lin & Lin, 1989) claims the use of 20E as a skin moisturizer. The recommended content of 20E in the preparation has been very high, 50–300 mg per 100 g of the cream base.

Further applications cover the use of ecdysteroid-containing liposomes for wound healing and skin regeneration (Meybeck & Bonte, 1990). Ecdysteroids extracted from plants Polypodium vulgare, Ajuga decumbens and Cyanotis arachnoidea are mixed with lecithin and sitosterol to prepare a liposome suspension which is further incorporated in a gel. This preparation has been shown to stimulate the wound healing in rats. So far, we were unable to find any records of possible commercialization of these inventions, which emphasize, nevertheless, the special effects of ecdysteroids in the vertebrates (Meybeck & Bonte, 1993). Most recently, some furthercosmetical properties of ecdysteroids have been described, for example, softening of the skin, healthier looking hair and also successful treatment of psoriasis (Meybeck et al., 1994). As we have already mentioned, 20E can influence differentiation of human keratinocytes in vitro (Detmar et al., 1994).

MODE OF ACTION OF ECDYSTEROIDS IN VERTEBRATES

It is generally assumed that steroid hormones exhibit two kinds of biological responses: a) nuclear responses through the receptors and transcription of specific genes and, b) rapid nontranscriptional responses by affecting membranes (cf. Schumacher, 1990; McEwen, 1991). This may be also applied to vitamin D and its metabolites (Barsony & Marx, 1988). Recently Kotsyuruba et al. (1992) investigated the slow and rapid responses of ecdysteroids in chickens which received 20E orally in doses of 0.1 μg per g of body mass (at 0.5, 1, 2, 24, 48 and 72 h before autopsy). They determined the dynamics of many biochemical changes in the blood serum and in certain tissues, mainly with respect to metabolism of nucleic acids, purine or pyrimidine nucleotides. According to these authors, administration of 20E was associated with extensive biochemical changes in the blood serum, in the composition of lipid, protein, nucleotide and nucleic acids in liver, spleen, intestine and pancreas. They found both the immediate changes elicited within 0–1 h as well as the slow effects lasting 24–72 h, which were considered as genomic. They concluded, therefore, that 20E might exhibit in chickens both the genomic and non-genomic actions characteristic for the vertebrate steroid hormones.

Catalan et al. (1979a) assumed that the heterophyletic action of 20E in mammals could be mediated through the cyclic AMP system (20E was injected intraperitoneally in doses of 10 μg per mouse; the mice were killed by decapitation, blood was analysed). They concluded (Catalan et al., 1979b) that these injections of 20E could produce a decrease in the cyclic AMP levels and also in the levels of cyclic AMP-binding protein. They determined (Catalan et al., 1980) that the cyclic AMP-protein kinase system may be also involved in the heterophyletic action of 20E. This conclusion was later confirmed by in vitro experiments (Catalan et al., 1982) which revealed that 20E (3.10⁻⁷M) could produce a decrease in the activity of protein kinase in slices of rat liver, rat fat pads and in slices from rat lungs. By contrast, Kotsyuruba et al. (1993a,b) found an early increase of cAMP content in various tissues of rats receiving 20E. This clearly shows that we still need more
experimental evidence for elucidation of the true nature (membrane ?, nucleus ?) of ecdys-
teroid action in the vertebrates.

It has been recently demonstrated by Christopherson et al. (1992) that a *Drosophila*
ecdysone receptor can function in cultured mammalian cells as an ecdysteroid-dependent
transcription factor. The activity of the ecdysone receptor was not induced by any of the
mammalian steroid hormones tested. The DNA-binding and transactivation activities of
viral, mammalian, or bacterial proteins were rendered ecdysteroid-dependent when fused
to the ligand-binding domain of the ecdysone receptor. The ecdysone receptor may prove
useful in selectively regulating the expression of endogenous or heterologous genes in
mammalian cells. These studies demonstrated the feasibility of using non-mammalian ec-
dysteroid hormone receptors to regulate genes in mammalian cells.

All these strategies assume that ecdysteroids have no direct genetical effects in mam-
mals, but this can hardly be assured in light of the data presented above. On the other
hand, the second alternative would pave the way to possible use of ecdysteroids in human
 genetic therapy strategies. These are based on transfection human cells with an ecdyster-
oid receptor, a chimaeric gene containing an ecdysone-responsive element (EcRE) and a
gene of interest to be expressed under ecdysteroid control.

**Dietary Effects of the Ecdysteroid-Containing Plants**

Some plants, which were long time known for their medicinal effects, turned out later to
be a rich source of ecdysteroids (Table 1). As a good example we can mention the Asiatic
plants *Achyranthes* or *Cyathula*, which were used as a tonic or diuretic drug ("Go-shitsu")
in old Chinese medicine. Both of them turned out to be a rich source of ecdysteroids
(Hikino & Takemoto, 1972).

Another such medicinal plant, which is playing important role in recent ecdysteroid re-
search, is an Asiatic medicinal plant, *Leucaea* (*Rhaponticum*) *carthamoides* Iljin ("root of
the deer Maralu") whose anabolic, metabolism stimulating, antidepressive and tonic prop-
erties were known long before it became recognized as one of the richest sources of ec-
dysteroids (for review see Abubakirov, 1984). In 1974, Veraskouski et al. reviewed the
stimulatory pharmacological effects of the drug prepared from *Leucaea* in several domestic
animals. The effects included, among others, stimulation of metabolism, increased growth
of the body, enhanced muscular functions, improved nerve activity, and pronounced ana-
abolic effects. They described a number of organic compounds isolated from the plant as
possible candidates for the above effects. They listed for instance catechins, flavonoids,
phenylcarboxylic acids, polysaccharides, lignins, etc., but still there was no notion about
ecdysteroids.

Three years later, however, 20E and other ecdysteroids were already judged responsible
for the "miraculous" rejuvenating effects of *Leucaea* by Syrov & Kurmukov (1976c, 1977).
In reference to some previous results on vertebrates, they concluded that 20E increased
growth of the body, stimulated protein and carbohydrate metabolism in various organs,
enhanced erythropoiesis under experimentally induced anemia, enforced uterine contrac-
tions, etc. Based on their own experience, they claimed 20E to be responsible for most, if
not all, properties of the drug prepared from *Leucaea*, i.e. they confirmed the tonic and
adaptogenic effects, interruption of artificial sleep induced in mice by hexenal or chloral
hydrate, stabilization of the conditioned reflexes in rats (doses 5 μg,kg⁻¹ to 50 μg,kg⁻¹).
Table 1. Examples of certain traditional medicinal plants which contain ecdysteroids (* indicates that ecdysteroids may be the active principles).

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Effects (use)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Achyranthes radix</td>
<td>Diuretic, tonic</td>
<td>Hikino &amp; Takemoto (1972)</td>
</tr>
<tr>
<td>Ajuga iva</td>
<td>Antidiabetic</td>
<td>Wessner et al. (1992)</td>
</tr>
<tr>
<td>Boerhaavia diffusa</td>
<td>Diuretic*</td>
<td>Suri et al. (1982)</td>
</tr>
<tr>
<td>Cyathula capitata</td>
<td>Diuretic, tonic</td>
<td>Hikino &amp; Takemoto (1972)</td>
</tr>
<tr>
<td>Diploclisia glaucescens</td>
<td>Bilioussness, venereal diseases, spermicidal*</td>
<td>Miller et al. (1985)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bandara et al. (1989)</td>
</tr>
<tr>
<td>Helleborus sp.</td>
<td>Stomachic, bittertonic, antialgic, antirheumatic</td>
<td>Colombo et al. (1990)</td>
</tr>
<tr>
<td>Ipomeea calonyction</td>
<td>Febrifuge, purgative, “kaladana”</td>
<td>Canonica et al. (1975)</td>
</tr>
<tr>
<td>Leuzea carthamoides</td>
<td>Tonic*, roborant*, adaptogenic*, antidepressive*</td>
<td>Syrov &amp; Kurnukov (1977)</td>
</tr>
<tr>
<td>Paris polyphylla</td>
<td>Hypotensive</td>
<td>Singh &amp; Thakur (1982)</td>
</tr>
<tr>
<td>Pfiella iresinoides</td>
<td>General stimulant, “Brasil ginseng”</td>
<td>Nishimoto et al. (1988)</td>
</tr>
<tr>
<td>Sida carpinifolia</td>
<td>Stomachic, tonic, antipyretic, antiinflammatory</td>
<td>Pandit et al. (1976)</td>
</tr>
<tr>
<td>Taxus cuspidata</td>
<td>Antidiabetic</td>
<td>Nakano et al. (1982)</td>
</tr>
<tr>
<td>Vitea glabrata</td>
<td>Astringent, antihelminthic, gastro-intestinal disorders</td>
<td>Werawatnametin et al. (1986)</td>
</tr>
</tbody>
</table>

The electroencephalographic records in rabbits (3–4 kg) showed apparent calmisation and decrease of the spike amplitudes after intravenous administration of 5–10 μg.kg⁻¹ of 20E. In further experiments with mice, 10–50 μg.kg⁻¹ doses of 20E considerably prolonged the moment of death after continuous swimming. Similarly in rats, equal doses of 20E increased the rate of survival at elevated temperature for more than 21%. These findings of Syrov & Kurnukov (1977) were very ambitious, they ascribed ecdysteroids a status of an ideal, plant-borne, natural drug (see also Kholodova, 1979).

The above enthusiasm, and imagination of the domestic animals that would grow more while eating less food, stimulated efforts on the territory of the former Soviet Union to use Leuzea as an ideal food additive or fodder plant in agriculture. Kudzinau et al. (1980) reported on large experiments in which pulverized green parts of Leuzea were fed to cattle, with pronounced anabolic effects.

Similar results were obtained when this material was added to food of experimental mice and rat. In spite of the pronounced growth-stimulating effects, there were no adverse effects on reproduction in the adult stage. Positive results were also obtained with the ethanolic extracts of the roots of Leuzea, which contain large amounts of 20E (0.4% of dry matter). In addition to cattle, anabolic effects caused by the dietary addition of the pulverized green parts of Leuzea have been found also in pigs (Šelepcová, 1990). In rats, addition of up to 20% of Leuzea produced remarkable anabolic effects, while more than 20% caused a decrease in growth of the body mass. The rats could grow and live on 50% Leuzea powder with somewhat reduced growth rate, but without any adverse effects on the internal organs, e.g. intestine, colon, liver, kidney and spleen (Šelepcová & Magič, 1992).
In cattle, the veterinary effects produced by ingestion of the food enriched with *Leuzea*
were not mediated by affecting the ruminal microflora (Šelepcová et al., 1993). In another
report from 1993, however, Purser & Baker claimed that E improved the productivity of
ruminants by elimination of protozoa from the digestive tract. The preferred dose was
around 0.02 μg·kg⁻¹·day⁻¹. Sheep on a low quality hay diet with E showed 180% and
8.5–10.5% greater weight gain and wool growth, respectively, than sheep without E.
Quite recently, Koudela et al. (1994) performed a large-scale experiment directed to
investigation of the anabolic effects of ecdysteroids in Japanese quails. The quails were fed
with a constant feed mixture supplemented with 0.2, 1.0 and 5.0% of the pulverized seeds
of *Leuzea* (containing 20–30 mg of 20E per g of dry matter). There were pronounced anabolic
effects manifested by profound increase of body mass in the groups receiving 1 and
5% addition of the drug. Determination of 20E content by RIA revealed that the amounts
of 20E circulating in the blood of the quails were directly proportional to the amount of
*Leuzea* seed in the diet. The quails, which received whole seeds of *Leuzea* ad libitum in
addition to the standard diet had extremely high levels of 20E in the blood (80 ng·ml⁻¹).
During preparation of this manuscript, Koudela et al. (1995) repeated the described large
copy test experiment in Japanese quails, for the first time with pure 20E (6g, 95.6% pure 20E),
without *Leuzea*. The results, which will be published in detail elsewhere, have clearly indi-
cated that pure 20E produces almost identical effects when compared to *Leuzea* seeds of
the same 20E content. This provides direct evidence to show that 20E has been indeed re-
sponsible for the anabolic effects of this ecdysteroid-containing medicinal plant.

**SOME COMMERCIAL PHARMACOLOGICAL PREPARATIONS**

The most important pharmacological preparation whose active principle is based on the
presence of ecdysteroids is “Ecdisten”, commercialized in the former Soviet Union by
Medexport, Moscow. It has been developed and invented in the Institute of Natural Com-
ounds, Uzbek Academy of Sciences in Tashkent (see Abubakirov et al., 1980). Originally
it was advertised as a natural compound with the tonic effects, obtained from the roots of
*Leuzea carthamoides*.

Among the indications of “Ecdisten” we find asthenic and astheno-depressive states,
weakening of the organism, long intoxications, somatic and infectious diseases, neuras-
thenia, neurosis, hypotension, fatigue and general tonic effect during mental and physical
tension. The recommended dosages are 0.005–0.01 g (1–2 tablets) orally, three times a day
before the meals. The recommended course of the treatment is 15–20 days, if necessary it
could be repeated in 1–2 weeks. One pill has been advertised to contain 5 mg of 20E
equivalent and, indeed, our analyses of the pill content revealed the presence of 4.3 mg of
an ecdysteroid mixture, where 20E was by far the major component (Fig. 2A). The prepa-
ration has been commercialized since 1982.

Another commercial preparation whose active ingredient is 20E is a green tea prepara-
tion “Maralan” (Kren et al., 1992) available in the Czech Republic. It consists of the dried
green parts of *Leuzea carthamoides* (Herba leuzeae, 0.08–0.22% 20E content). The adver-
sisements claim increased resistance of the organism, especially against stress, stimulation
of functions of the central nervous system, removal of the fatigue, improvement of psychi-
cal conditions, increased appetite and improved digestion.
Fig. 2. HPLC analysis of some commercial ecdysteroid preparations. Operating conditions: column Zorbax-Sil, 250 × 4.6 mm, solvent cyclohexane-isopropanol-water (100:40:3, v/v/v), flow-rate 1 mL min⁻¹, temperature +50°C, detection UV at 254 nm. A – analysis of "ecdistan" pills; B – analysis of Leuzea "elixir".

"Leuzea drops" (root extract) have been available in Czech or Slovak Republics as an alcoholic concentrate, containing also relatively high concentrations of ecdysteroids (Fig 2B). This "elixir" has been advertised as general stimulating, adaptogenic, antidepressive and rejuvenating medicine.

GENERAL CONSIDERATIONS

After examining all available informations related to the effects of ecdysteroids in vertebrates we have developed an impression that there hardly exists any biochemical, physiological or pharmacological process, which would not be affected by these compounds in one or other way. This shows a situation superficially similar to that in the invertebrate animals, but closer analysis of the effects reveals some basic differences.

In invertebrates, ecdysteroids by nature are growth hormones which directly cause tissue proliferation, regulate the progress of morphogenesis and control developmental cycles. In the vertebrates, by contrast, we have documented numerous cases of stimulation of some already existing biological processes, but in no case do ecdysteroids appear to be the rate-limiting factors of growth as they are in insects.

This confirms our earlier conclusions (Sláma et al., 1974) that ecdysteroid cannot be regarded as a hormone in the vertebrates. A similar situation has been recently found with respect to ecdysteroid status in the plant kingdom (Sláma, 1993; Macháčková et al., 1995), e.g. ecdysteroid is not a phytohormone.

The basic endocrinological question is, whether there exists any biological analogy between developmental processes regulated by ecdysteroids in invertebrates and similar processes in the vertebrates. We find that there are some analogies of this kind, especially in the regulation of morphogenesis. Unfortunately, this process is localized in the vertebrates almost exclusively within the embryonic period, i.e. within the egg stage or intrauterine period of the mammals. By contrast, all information pertaining to the ecdysteroid action in vertebrates, as seen from the present data, are limited to the postembryonic or postnatal periods.

Another drawback is that, in contrast to plants and arthropods, where synthesis of ecdysteroid from acetate or cholesterol has been well documented (see Lafont, 1991; Lafont & Horn, 1989), there has been hitherto no evidence for possible biosynthesis of
ecdysteroids by the vertebrate cells. Most of the findings of ecdysteroid in the vertebrates trace the sources to parasitic worms or to ecdysteroid ingested with food.

What, then, are ecdysteroids to the vertebrates, if they are not hormones, and why should they exhibit so many important chemical and biological actions? This problem is of general biological importance, because ecdysteroids and the related polyhydroxylated sterols are also essential for microorganisms, lower and higher plants, most invertebrates, vertebrates and, finally, they may be essential for humans as well (Sláma, 1993).

The hormonal action of ecdysteroids in insects is almost exclusively limited to the 6-keto, Δ7 conjugated system in the B ring of the steroid molecule and cis-fused A/B rings. By contrast, a cell of both the plant and animal kingdom seems to require a more general structural feature, which is characterized by the slightly polar, polyhydroxylated, "sugar-like" sterolic molecule. Existence of these, partly water soluble, polyhydroxylated sterols (and triterpenes) has been long time overlooked by chemists (until structure elucidation of ecdysone by Karlson and his co-workers), who did not expect to find a steroid in the polar fractions. Some of these compounds display, for instance, toxic effects on tumour cells or alteration of immune responses (Astruc et al., 1978; Kandutsch et al., 1978; Luu & Ourisson, 1989; Ji et al., 1990), i.e. similar properties to those reported above for ecdysteroids. Thus, we cannot exclude a possibility that the effects of ecdysteroids in the vertebrates may be only consequences resulting from general structure of a polyhydroxylated sterol. Without respect to these structural problems, however, the described pharmacological effects in vertebrates suggest great potential importance of these compounds in human medicine, which still remains neglected.

With these general aspects in mind, and realizing that a polyhydroxylated sterol might be essential for the lipo/hydrophilic relations on biological membranes of all living cells, we can conclude that the role of ecdysteroids in vertebrates would not be a hormone, but rather that of an essential vitamin. It is obvious that one can easily put forward such hypothetic biological generalization, but it may take a whole generation to confirm whether it is true.

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