

## Approaching a Time we can Prevent Pernicious Malignant Tumors?

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### Abstract

Mitotic divisions and regeneration were investigated on excised epidermal patches of developing and diapausing insect pupae. During normal development, periods of mitotic divisions coincide with peaks in endogenous concentration of polyhydroxylated 6-keto, 7-dehydrocholesterol (ecdysone), which was accidentally discovered in the search for an insect moulting hormone. We have found that these compounds cannot be considered as animal hormones. Their true biological status can be defined as essential vitamins supporting growth and mitotic cell divisions probably in all multicellular organisms. The established growth-promoting vitamins D2 and D3 are the purely lipid soluble compounds that do not occur in plants. They need to be activated by UV-light or additional hydroxylation in liver or kidneys (vitamin D3-triol). The polyhydroxylated derivatives of 6-keto, 7-dehydrocholesterol have been retrospectively identified as the previously neglected vitamin D1. This vitamin is partly soluble in both water and lipid solvents. It is biosynthesised and widely distributed in numerous species of lower and higher plants. In addition, vitamin D1 exhibits strong anabolic, growth promoting effects in insects, mice, birds, domestic animals and also in humans. It has been found that the presence of this vitamin D1 in insect haemolymph or human blood is essential for the construction of bipolar cytoplasmic membranes during the mitotic cell divisions. Deficiency of the dietary vitamin D1 leads to aberrant regeneration manifested by formation of defective cytoplasmic membranes, leading to formation of polynuclear syncytia of cells with endomitotically dividing nuclei. These features are characteristic in the occurrence of malignant tumors. The D1 avitaminosis theory of malignant growth has been proposed.

**Keywords:** *Vitamins D2; D3; Vitamin D1; Polyhydroxylated 6-keto, 7-dehydrocholesterol; Ecdysteroids; Insect Hormone; Anabolic Growth Effects; Mitotic Divisions; Polynuclear Syncytia; Tumor Growth*

### Introduction

Pernicious malignant tumors are not a regular human disease. They cannot be disseminated like infections and do not produce a fever response. Tumors occur in young or old people, occasionally even in athletes in outstanding physical condition. These symptoms point to the physiological disturbance of tissue growth and regeneration. In principle, animal growth mostly depends on increased cell numbers, not cell size. The mitotically dividing and differentiating animal cells depend on three essential conditions: a) Nutritional resources (carbohydrate, lipid and protein); b) Special products manufactured by biosynthetic capacity of animal's own body, and; c) Essential dietary constituents, which are not supplied by the metabolic capacity of the animal, because they can be obtained from food, known as essential vitamins.

The most important vitamin required for animal growth is the antirachitic (to cure or prevent rickets) vitamin D. Original investigations performed some 100 years ago revealed that the chemical nature of vitamin D might be somehow related to 7-dehydrocholesterol

[1]. At that time, organic chemists believed that cholesterol and its derivatives were purely lipid soluble compounds, occurring in animals (zoosterols), not in plants. Later, during the 1930<sup>ies</sup>, the pioneers of vitamin D research found the presence of special secosterol derivatives of 7-dehydrocholesterol in animal skin. They became known as vitamins D2 (ergocalciferol) and D3 (cholecalciferol) [1,2]. Unfortunately, the growth promoting, antirachitic biological activity of vitamins D2 and D3 was rather low. However, it could be enhanced by exposing animal skin to UV radiation. This cumbersome, 100-year-old dogmatic story of UV-potentiated provitamins D2 and D3 (awarded by Nobel prize to A. Windaus) has persisted in human pharmacology and medicine until this time [1].

Unlike vitamins D2 and D3, the chemical nature of the mysterious vitamin D1 remained unresolved. The D1 story begins in 1931, when Windaus and his co-workers [3] irradiated vitamin D3 in UV-light and obtained a product with substantially increased antirachitic biological activity. Their attention was concentrated mainly on the lipid soluble, nonpolar fractions expected to contain the derivatives of 7-dehydrocholesterol, but failed in identifying the biologically active component. The story of the UV-irradiated vitamin D1 has been frequently discussed in the literature [4], without elucidation of its chemical structure.

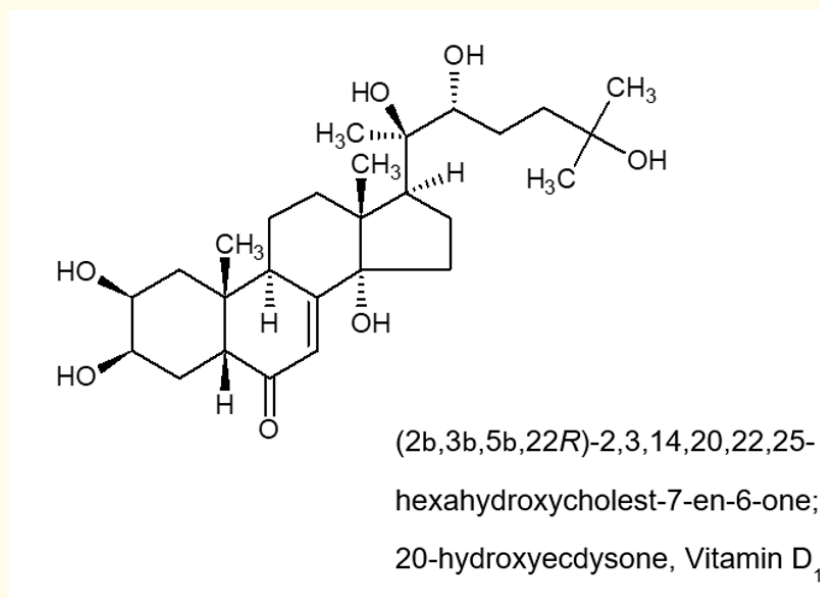
Based on our recent knowledge, it can be retrospectively assumed that the unidentified, biologically more active product of vitamin D3 UV-irradiation could be a more polar, partly water soluble, hydroxylated 7-dehydrocholesterol, which escaped from the pool of the purely lipid soluble sterols. The polyhydroxylated derivatives of 7-dehydrocholesterol were unknown to organic chemists until 1965 [5] (see the next section). I assume, retrospectively, that the mysterious vitamin D1 might be related to the more recently discovered, slightly polar and biologically more active, structural modification of vitamin D3 formed by its metabolic hydroxylation in the liver or kidneys (calcitriol triol) [6].

### Ecdysone

About three decades after the isolation of vitamin D3 [2], Karlson and his co-workers accidentally discovered a polyhydroxylated sterol in the search for an insect moulting hormone. They extracted 500 kg of silkworm pupae and obtained 25 mg of a crystalline compound, identified as a polyhydroxylated 6-keto, 7-dehydrocholesterol, named ecdysone [5] (Figure 1). The discovery of ecdysone represented a great achievement in organic chemistry. The finding of a partially water soluble "cholesterol sugar" suddenly opened the eyes of many phytochemists, who found similar compounds in plants, but were unable to elucidate their chemical structure. Curiously enough, almost immediately after Karlson disclosed the chemical structure of ecdysone, there emerged numerous reports claiming that diverse plants might produce enormous quantities of these "insect hormones" [7]. The statement can be illustrated, for instance, by a fern *Polypodium vulgare* which contained in just 1g of its rhizomes more ecdysone-related compounds than 500 kg of silkworm pupae [8].

The study of ecdysones ("arthropod moulting hormones or ecdysteroids") in plants became a great fashion for two generations of phytochemists. They described several hundreds of these "phytoecdysones", distinguished mainly by different positions of the hydroxyl groups, in various species of higher or lower plants [9,10]. Nobody asked a simple question: What would an insect hormone do in plants? There was an old theory of Sláma and Williams [7,11] which proposed that during the millions of years of insect-plant interactions, certain plants could evolve a sophisticated mechanism to be protected from insect herbivores by manufacturing their hormones. This possibility was later reinvestigated using insect populations dwelling on plants with a relatively high ecdysteroid content (*Leuzea carthamoides*). However, the results did not confirm the above mentioned theory, because larvae eating plants with high content of ecdysteroids turned out to be virtually resistant against hormonal derangements of their development. They rapidly excreted the surplus dietary supply of ecdysteroids out of the body [12].

The most common and widely distributed ecdysteroid in plants is 20-hydroxyecdysone with 6 hydroxylic groups, occasionally called ecdysterone (Figure 1) [9]. In insects, ecdysteroids with more than 3 hydroxylic groups are all biologically active, provided that they contain the conjugated, 6-keto,7-dehydro unsaturation in B-ring of the sterol nucleus [13]. Location of the hydroxyl groups across the sterolic molecule and structural modifications within the side chain determine changes in species-specificity [7,13]. Similarly to the case of fatty



**Figure 1:** Chemical structure of the polyhydroxylated 6-keto, 7-dehydrocholesterol (vitamin D<sub>1</sub>, 20-hydroxyecdysone) which is widely distributed in insects and plants.

acids, the composition of endogenous ecdysteroids in the insect body reflect pattern found in the food plant. Biological activity of ecdysteroids with 5 to 7 hydroxyl groups is almost the same (ponasterone, ecdysone, with hydroxyl function in the position of C-11, known as turkesterone, which was highly active on Diptera, being almost inactive on Lepidoptera (*Galleria*). In Cyasterone, containing the modified side chain, these structure-activity relationships were reversed [13]. It can be expected that similar structure-activity relationships would apply with respect to the growth-promoting, anabolic and other effects of ecdysteroids in vertebrate animals and also in the humans [14,15].

#### Insect hormone, plant growth factors, or human medicine?

This enigmatic question has been raised already in 1993 [28]. The association of the plant-borne, vitamin D-like ecdysteroids with the enhancement of somatic growth in various animals became evident, although the main-stream scientific community notoriously adhered to insect hormone version. Nevertheless, the original definition of ecdysone as the moulting hormone secreted by the prothoracic glands of insects [6] persisted and was strictly respected by the chemists [14]. The first provocative statement about the true nature of ecdysteroids as the reserve growth factors of plants [16], was merely considered as insane. It is obvious that this was not fair with regard to a great number of plant species, some containing more than 1000-fold higher concentrations of these compounds than insects [7,8,13,16].

We investigated the ecdysteroids of plants and insects for more than 50 years [7,12,13,17-19]. The results revealed serious conflicts with the established hormonal doctrine. The main arguments can be summarised as follows: 1. The secretion of ecdysone from various peripheral tissues in insects breaches the definition of an animal hormone [18,19]; 2. Ecdysone is not the prothoracic gland hormone [18-22] and 3. Ecdysone does not induce but inhibits the act of insect ecdysis [17-21].

While scientists of western countries were financially supported to investigate mainly the ecdysteroid nuclear receptors (Ecr, coming into the body massively from food), researchers in the former Soviet Union intensively worked on pharmacologically more important effects of ecdysteroids in vertebrate animals and humans. Their work was motivated by the reported increase of milk and meat production in cattle by the dietary addition of ecdysteroid-containing plants. They described a number of anabolic, growth-stimulating effects of ecdysteroids in various domestic animals. What is more important, they also described a plethora of beneficial, rejuvenating effects in humans [22]. In 1980, they registered the first ecdysteroid-based pharmacological preparation ECDISTEN, with outstanding anabolic, immunogenic, tonic, neurogenic, rejuvenating, muscular strength-supporting and other activities, practically used by athletes [23,24].

We have investigated the anabolic, vitamin D-like effects of ecdysteroids on insects [13], mice [25] and Japanese quails [26]. In addition, we found that the growth-stimulating effects of ecdysteroids in plants were not associated with regular functions of the so far known phytohormones [27]. The dietary source, not biosynthesis of the hormone in animal body, provided evidence that the mode of action of ecdysteroids are not directed to intracellular nuclear receptors, as it is generally believed [15]. Apparently, the most important physiological function of ecdysteroids in mammals and humans depends on the essential function of vitamin D [28-30].

With respect to the above mentioned, vitamin D-like growth effects of ecdysteroids in domestic animals and humans [23,24] and with regard to the recent results demonstrating that ecdysteroids are neither insect hormones nor inducers of insect ecdysis [19-21], it can be reasonably proposed [19] to abandon the old incorrect terminology of ecdysteroid and define the plant-borne, biologically important, polyhydroxylated derivatives of 6-keto, 7-dehydrocholesterol by the new generic name derived from the previously neglected vitamin D1 [19,29,30].

### The effect of vitamin D1 on mitotic divisions and regeneration

It is extremely difficult to investigate mitotic divisions in the human body, due to a closed vascular system of arteries, veins and capillaries. The difficulty can be restrained by using insects, which contain an open circulatory system with individual organs freely suspended in the haemocoelic cavity. In this respect, it appeared suitable to use the model of small, excised epidermal windows in larvae and pupae of the tobacco hornworm (*Manduca sexta*) [19]. The excised epidermal patches were covered by microscopic slides, the margins were sealed by beeswax melted with electrocautery.

The location and size of the excisions can be observed in figure 2. The results can be briefly summarized as follows:

- Intact cells of epidermal and other tissue and organs create a mutually integrated unit;
- Disturbance of the integrity by a natural cell death or artificial injury induces the regeneration process manifested by mitotic divisions among the disconnected marginal cells;
- After regeneration of the wounded area, the daughter cells unite again and re-establish the tissue integrity;
- The mitotic divisions become arrested;
- In presence of vitamin D1, the mitotically dividing cells construct physiologically compatible bipolar cytoplasmic membranes responsible for issuing the final start-stop signal;
- In the absence of vitamin D1, the regenerating cells produce defective cellular membranes unable to arrest further cell divisions;
- These cells cannot be integrated into the regenerated tissue, their nuclei continue to divide endomitotically, which results in formation of the polynuclear syncytia reminiscent of the malignant metaplasia [19,29,30].

As already mentioned, there exist piles of old papers claiming that vitamin D1 (ecdysteroids) were insect moulting hormones produced by the prothoracic glands [14-16]. However, the wide distribution of vitamin D1 in plants, including the common vegetables [7-10], raises a question of whether these vitamin-like derivatives of cholesterol can be synthesized by isoprenoid cyclization in the animal body.



**Figure 2:** Excisions of 3x3 mm patches of epidermal cells, made on 4th abdominal tergite of pupae of the tobacco hornworm (*Manduca sexta*). The excised epidermal windows were covered by microscopic cover slides cemented to pupal cuticle by melted beeswax. The preparations were used for microscopic observations of the induced mitotic divisions during regeneration of the wounds.

There are some indications that animals do not perform the triterpenoid cyclization of the backbone sterol nucleus [31], which can be easily obtained from vegetable food [32]. Moreover, insects can reutilize vitamin D1 obtained from food and incorporated into larval tissue and organs during the feeding period. In the course of nonfeeding periods, the old, disintegrating larval tissues are used to sequester the hydrolysed, hydroxylated and partly water soluble vitamin D1 (endogenous ecdysteroid) into the haemolymph for its reutilization by the proliferating, new pupal or adult tissues [21]. Similarly, plants also economize and reutilize the photosynthetically produced vitamin D1 of the shoot and store the vitamin for later use in winter roots or seeds [18].

The fate of the dietary vitamin D1 in the human body is unknown. So far, the relatively low available quantities of pure vitamin D1 have not permitted extensive clinical assays. In addition, clinical investigations were hampered by the notorious belief in the insect hormone. Certainly, there are some animals dwelling on a vitamin D1 deficient diet (Termites). However, they develop extensive symbiotic intestinal flora, which can provide the vitamin. Similar sources of vitamin D1 can be expected in vertebrate animals with the well developed intestinal symbiotic flora (ruminants), tentatively also people?

In insects it has been well established [7,14,21,28] that endogenous vitamin D1 can be stored and exhibits large variations in insect haemolymph (endogenous ecdysteroid peaks). The largest peaks occur in the nonfeeding pupal stages of Lepidoptera, with degenerated and nonexistent prothioracic glands [21]. In vertebrate animals (Japanese quails), the blood concentration of vitamin D1 was directly proportional to content of vitamin D1 in food [26]. The generally known vitamins belong to two main categories. One includes water soluble and the other lipid soluble compounds. In this respect, the vitamin D1 falls into a special category, because it is partly soluble both in water and lipids. Such amphoteric feature is physiologically very important. It may be responsible for construction of the bipolar cytoplasmic membranes during mitotic divisions of the regenerating tissues.

Various taxonomic groups of food plants show considerably different displacement of the hydroxylic groups across the sterol nucleus of vitamin D1, In addition, large structural changes are eventually combined with structural variations in the side chain [9,10,15]. No-

body knows whether these structural variations in molecule of the dietary vitamin D1 affect its biological activity in animal body. There is an old phytochemical problem, if cholesterol can be regarded as a plant sterol. The plant *Leuzea carthamoides* was found to contain 700-fold more 7-dehydrocholesterol in comparison with the true phytosterols, like ergosterol or  $\beta$ -sitosterol. It was concluded that cholesterol and its derivatives were preferentially hydroxylated and thus escaped from the pool of nonpolar, purely lipid soluble phytosterols [34]. Similarly unusual problems also arise from the existence of special bisacylhydrazine insecticides, generally considered to be ecdysteroid agonists [35]. These synthetic materials are effectively mimicking ecdysteroid action in insects, including inhibition of the locomotory activity, decreased overall metabolism and precocious stimulation of the new cuticle [36,37]. From pharmacological point of view, there arises important pharmacological question, namely, whether these synthetic ecdysteroid agonists could also mimic the growth-promoting, anabolic action of vitamin D1.

### The D1 avitaminosis theory of malignant tumor growth

The effect of vitamins on cell growth does not appear to be a focal issue of human health care. Neither is it a direct pharmacological issue, especially without knowledge of the responsible vitamin. For a long time, scientists did not make an effort to question the low effectiveness of vitamins D2 and D3. It may take some time before medical science elucidates the incorrect determination of the vitamins D2 and D3. I found that the polyhydroxylated 6-keto, 7-dehydrocholesterol (Ecdysone, Vitamin D1), which was discovered some 30-years later cannot represent an animal hormone. It turns out to be a very important biological material, perhaps the previously neglected vitamin D1. Obviously, it is not a phytohormone [27], but a secondary constituent of microorganisms and plants. During the long time of animal-plant coevolution, vitamin D1 evolved as an essential, growth-promoting material required probably for enhancement of growth in all multicellular organisms [16-19,28].

There exist newly synthesized drugs recommended for destruction of the existing malignant tumors [33]. The incompletely understood cause and origin of malignant tumors motivated to use at least cytotoxic agents for a temporary arrest of all mitotic divisions. The D1 avitaminosis theory stems from observations on insects [19,29,30]. Its application to other animals is completely legal, because the elementary physiological systems of insects and humans are very similar. They are conditioned by 37% of homologous genes [38]. I hope sincerely, that the absolutely nontoxic, growth-promoting properties of vitamin D1 might help people suffering from malignant tumors to substitute or avoid the chemotherapy.

According to our previous findings with Japanese quails [26], the dietary supplements of vitamin D1 can be stored in the blood. The concentrations were 3 ng to 300 ng/ml of blood, the concentrations were proportional and reflected well additions of vitamin D1 to the diet. In comparison with natural endogenous content of vitamin D1 in insect haemolymph (0.1  $\mu$ g to 5  $\mu$ g/ml) [19], the content of vitamin D1 in quails is very low. As a natural food constituent, the general toxicity of vitamin D1 is very low [14,23-25]. This has been confirmed by experiments on plants [27], insects [19], mice [25], quails [26], or domestic animals and humans [23].

In principle, the proposed avitaminosis theory of malignant tumor growth represents application of findings from insect science into the human health care. It is mainly based on the following facts:

- The mitotically dividing, regenerating tissue and cells need vitamin D1 for construction of the faultless, bipolar cytoplasmic membranes;
- Insects, other animals and humans do not synthesize the triterpenoid vitamin D1, which is obtained from food or intestinal symbiotic flora;
- Different species of plants may contain substantially different amounts of vitamin D1;
- Prolonged consumption of vitamin D1- deficient food may lead to depletion of vitamin D1 from the blood and avitaminosis;
- In presence of vitamin D1, an artificially injured tissue regenerates the wound by induction of mitotic divisions;



- The regenerated cells re-establish the integration unity with the intact cells and the mitotic divisions are arrested;
- In the absence of vitamin D1, mitotic divisions are retarded, become irregular and form aberrant syncytia with defective cytoplasmic membranes;
- The endomitotically dividing nuclei are unable to re-establish the integration unity with intact cells and continue to divide [18,19,28-30].

The D1 avitaminosis theory opens a lot of obvious questions that are difficult to answer. For instance, would it be possible to shrink the already existing tumors by exogenous addition of vitamin D1? What are the reliable sources of vitamin D1 in other vegetables than spinach, quinoa or *Leuzea*? How much of the vitamin D1 may be contained in the current food like fruits, potatoes, other vegetables or meat? What is the blood level of vitamin D1 in healthy people in comparison with the oncological patients? Answering to these questions was a long time hampered by the notorious belief in insect hormones, relatively high price and absence of sufficient amounts of vitamin D1 for clinical assays.

### Conclusion

Regenerating animal cells require essential vitamin D for construction of compatible, bipolar cytoplasmic membranes. This is needed for termination of regeneration and arrest of further mitotic divisions. The vitamins D2 and D3 were incorrectly assigned. The neglected vitamin D1 (polyhydroxylated 6-keto, 7-dehydrocholesterol; ecdysone) has been accidentally discovered 30 years later in a search for insect moulting hormone. Vitamin D1 exhibits strong anabolic, growth effects in animals and humans, It occurs in a number of lower and higher plants. Deficiency of vitamin D1 in the diet produces malformed mitotic divisions associated with formation of special syncytia of cells containing endomitotically dividing nuclei, characteristic for the malignant tumors.

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