The identification of dehydroretinol (vitamin A_2) in human skin

A. Vahlquist[1]

Department of Dermatology, University Hospital, S-750 14 Uppsala (Sweden), 18 June 1979

Summary. Vitamin A_2 has for the first time been identified in a human tissue and, in contrast to vitamin A_1, its concentration in the epidermis is markedly increased in a hyperkeratotic condition *(Psoriasis vulgaris).*

Vitamin A is an essential requirement for proper differentiation in mammalian epithelial cells (see Olson[2] for review). In the absence of compounds with vitamin A-activity (retinoids) keratinizing metaplasia and hyperkeratosis occur. The molecular mechanisms involved have not been established. Retinoids entering the cells are probably further metabolized, and the existence of activated metabolites has been suggested[3-5]. The present report describes the detection of nonacidic retinoids in human epidermis, one of which preponderates in hyperkeratotic psoriatic lesions.

Skin samples consisting of epidermis and a thin layer of dermis were obtained from the backs of 10 healthy subjects and 5 patients with *Psoriasis vulgaris.* After the addition of an internal retinoid standard (Ro 12-0586) the skin was hydrolyzed in KOH-ethanol and extracted with light petroleum without prior neutralization. The extract was evaporated to dryness, dissolved in methanol, and subjected to high-pressure liquid chromatography (HPLC)[6]. Figure 1 shows typical chromatograms from a normal-appearing skin and from a psoriatic lesion. The retention time (R_t) of the internal standard is 7 min. The last peak in both traces appears at the position of all-trans retinol ($R_t = 11.5$ min). The fluorescence spectrum of this fraction was also identical to that of retinol. At a R_t of approximately 9 min a peak of variable height may be observed. This peak does not correspond to any of the commonly encountered nonacidic retinoids, such as the cis- and trans-isomers of retinol and retinal. A preparative scale extraction of psoriatic skin was performed in order to establish its identity.

5 g of psoriatic scales were prepared and extracted essentially as described above. The HPLC peak corresponding to the unidentified material was collected and rechromatographed. The fractions containing the unknown peak were collected and extracted with light petroleum followed by evaporation. Figure 2 shows the absorption spectrum in hexane of this material. 2 distinct maxima can be seen about 350 and 290 nm, respectively. The direct inlet mass spectrum of this material suggested a molecular weight of 284 daltons. Although the isolated compound is not completely pure, the results are in accordance with the data available for 3,4-dehydroretinol (vitamin A_2)[7]. On reaction with trifluoroacetic acid[8] the isolated skin material and pure vitamin A_2 produced identical absorption spectra (maximum 685 nm). Their chromatographic properties were also indistinguishable and it is suggested that the HPLC peak ($R_t = 9$ min) of figure 1 in fact represents vitamin A_2. Assuming an extinction coefficient for vitamin A_2 of 1460 ($E^{1\%}_{352}$)[9] its concentration in psoriatic skin lesions is about 200 ng/g wet tissue, which is close to the retinol (vitamin A_1) concentration in both normal and psoriatic skin[6]. Increased amounts of epidermal vitamin A_2 has also been found in several other hyperkeratotic conditions (unpublished observation).

Although vitamin A_2 may occur in food its presence in

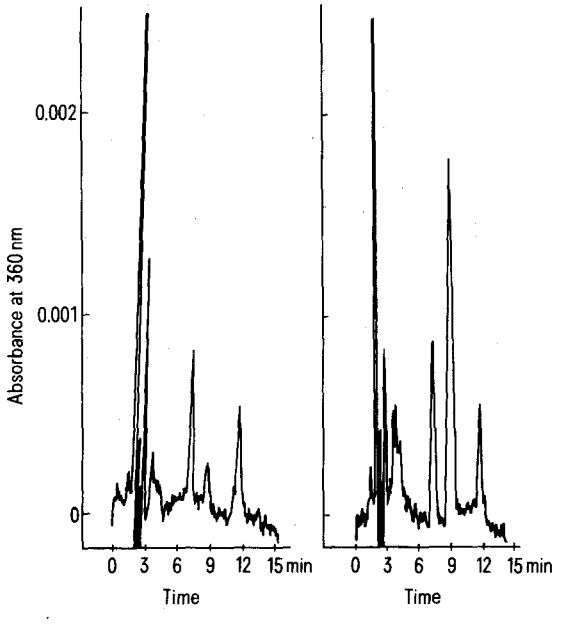

Fig. 1. Reversed-phase chromatography (HPLC) of lipid extracts from normal skin (left) and psoriatic lesion (right). The hydrolyzed skin (30 mg) was prepared as described in the text. A Rheodyne injector (50 µl) was used to apply the sample to a Nukleosil 5 µm PEAB-ODS column (5 mm inner diameter × 200 mm) isocratically eluted with 0.01 M acetate buffer pH 3.6 in acetonitrile (15:85). A flow rate of 1.2 ml/min was achieved with an Altex 110 pump equipped with a pulse damper. The effluent was monitored at 360 nm with a LDC 1203 apparatous.

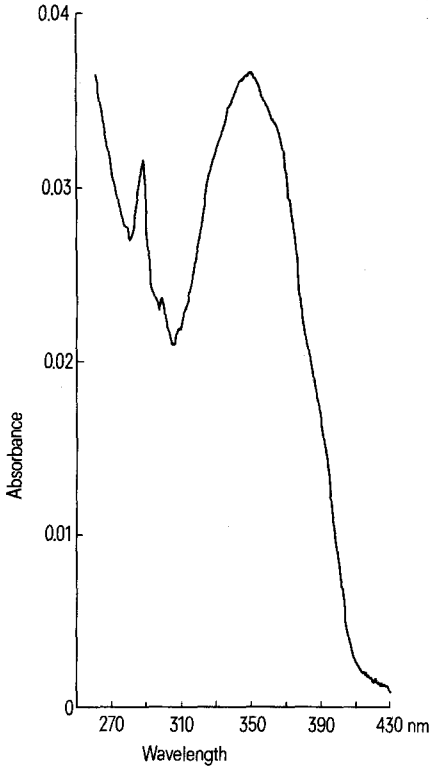

Fig. 2. Absorption spectrum in hexane of the isolated material from psoriatic skin purified by repeated chromatography. A Cary 219 spectrophotometer equipped with a microcell (250 µl) was used.

mammalian tissues has previously been reported only in animals which have been heavily dosed with this compound[10]. Vitamin A_2 is, however, the preponderant retinoid in fresh-water vertebrates[7], and its biological activity is considered to be about half that of retinol[11].

It is conceivable that dehydroretinol, like retinol, is a normally occurring retinoid in human epidermis, although its exact nature in unhydrolyzed skin has not yet been determined. Under certain conditions of hyperkeratosis changes in the retinoid metabolism may possibly lead to an accumulation of dehydroretinol. In this context reference should be made to the work of Wolfe et al.[12] demonstrating the accumulation of retinoyl complexes in the brains of patients with inherited Batten disease. Also, anhydroretinol (i.e. retinol lacking the terminal hydroxyl group) has recently been reported in transformed, but not in normal mouse fibroblasts incubated in vitro with retinol[13]. Thus, several independent observations have been made of a disturbed retinoid metabolism in relation to genetically determined cellular diseases. Whether such variations in retinoid metabolism may actually cause symptoms of a disease remains to be established.

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Effect of crustacean eyestalk extracts on carbohydrate levels in the South Indian scorpion *Heterometrus fulvipes* (Koch)

P. Sreenivasula Reddy and R. Ramamurthi

Department of Zoology, Sri Venkateswara University, Tirupati (India), 1 June 1979

Summary. Injection of eyestalk extracts of freshwater crab and marine prawn caused elevation of haemolymph sugar level, and decrease in free sugar and glycogen levels, in the hepatopancreas of the scorpion.

The chemical nature, mode and site of action of crustacean hyperglycemic hormone are well known[1–4]. A hyperglycemic principle has been identified in the cephalothoracic ganglionic mass of the scorpion *Heterometrus fulvipes*[5–7]. Some information is available on the interspecific action of crustacean hormones[8–10]. The present report examines the effects of eyestalk principles of the freshwater crab *Oziotelphusa (Paratelphusa) Senex senex* and the marine prawn *Penaeus monodon* on carbohydrate levels in the haemolymph and the hepatopancreas of the South Indian scorpion *Heterometrus fulvipes*.

Material and methods. Collection and maintenance of scorpions have been described earlier[11]. Scorpions normally fed daily with cockroaches were starved for 24 h prior to experimentation. Eyestalks from intermoult crabs and prawns were used. Prawn eyestalks were collected from the Kakinada area of Andhra Pradesh. Hyperglycemic principle was extracted from the eyestalks into 80% ethanol in the

Effect of injection of crustacean eyestalk extracts on free sugar and glycogen levels of hepatopancreas, and total sugar level of haemolymph, in the scorpion *Heterometrus fulvipes*

Tissue/component	Normal	Scorpion Ringer injected	Eyestalk extract injected	
			O. Senex senex	*P. monodon*
Haemolymph				
Total sugars	37.67 ± 8.41	37.20 ± 7.03	120.29 ± 10.18	85.86 ± 4.44
		−1.25%	+219.33%	+127.93%
		NS	$p < 0.001$	$p < 0.001$
Hepatopancreas				
Glycogen	0.9053 ± 0.0727	0.8757 ± 0.1059	0.6220 ± 0.1155	0.6170 ± 0.0604
		−3.27%	−31.29%	−31.85%
		NS	$p < 0.001$	$p < 0.001$
Free sugars	12.04 ± 1.74	11.96 ± 2.47	5.99 ± 0.81	7.17 ± 1.22
		−1.66%	−50.25%	−40.45%
		NS	$p < 0.001$	$p < 0.001$

Values are means (mg of glucose, g wet weight of tissue^{-1}, mg of glucose/100 ml of haemolymph) ± SD of 6 estimations. % change, 'p' calculated for normal-injected.