Comparison of the effects of vitamin A and its analogs upon rabbit ear cartilage in organ culture and upon growth of the vitamin A-deficient rat

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Abstract A study was conducted to explore the relationship between the effects of vitamin A upon cartilage and the biological role of vitamin A in maintaining growth and life. Retinol, retinoic acid, α-retinoic acid, and RO8-7699 (a cyclopentyl analog of retinoic acid) were highly effective in promoting the lysis of the extracellular matrix of cartilage grown in organ culture in vitro. Retinoic acid and its two analogs were quantitatively more active than was retinol in bringing about lysis of matrix and release of proteoglycan into the culture medium. A bioassay was then conducted to determine the ability of each compound to promote growth of vitamin A-deficient rats. In contrast to their effects upon cartilage, retinoic acid and its two analogs were considerably less active quantitatively than retinol in promoting growth of vitamin A-deficient rats. Moreover, the three acids tested showed graded biological activity in the growth bioassay, with α-retinoic acid showing reduced bioactivity (approx. one-fourth that of retinoic acid) and RO8-7699 being virtually inactive. The lysis of cartilage produced by these compounds was presumably caused by release of lysosomal enzymes as a result of the membrane-labilizing effects of the compounds. Thus, these membrane effects of the vitamin A-related compounds are poorly correlated with their biological growth-promoting activity.

The α-ionone analogs of retinol and retinoic acid were able to maintain good health and growth of vitamin A-deficient rats, although their quantitative activity was low. Rats fed α-retinyl acetate showed high liver stores of α-retinyl esters and low levels of serum retinol-binding protein (similar to the levels seen in retinoic acid-fed rats). The biological activity of the α-ionone analogs was apparently not due to contamination with or conversion to the normal β-ionone compounds.

Supplementary key words retinol · retinoic acid · α-retinoic acid · RO8-7699, a cyclopentyl analog of retinoic acid · retinol-binding protein · proteoglycan release

Vitamin A has a well-documented effect upon cartilage, both in vivo and in vitro. In 1947, Wolbach (1) reported that excess of vitamin A causes resorption of cartilage and of some bone; this process may, in fact, lead to bone fragility and fractures. In 1952, Fell and Mellanby (2) observed that a similar effect was produced by the direct action of retinol on embryonic limb bones grown in organ culture. Addition of vitamin A to the culture medium resulted in a drastic resorption of the intercellular material of both cartilage and fetal bone, although the cells remained viable. Subsequent studies demonstrated that the increased catabolism of cartilage proteoglycans seen in the presence of retinol was associated with an increased synthesis and release of lysosomal enzymes (3). These studies have been extended considerably during the past decade (4), and the role of lysosomal enzymes, released as a result of the addition of vitamin A, in effecting the resorption of the matrix of cartilage is now well established.

In addition to its effects on skeletal tissue and lysosomes, vitamin A is known to exert a number of biological effects. In general, the vitamin is necessary for the support of growth and life; in the absence of vitamin A higher animals cease to grow and in time die. Vitamin A is necessary for vision (5) and for reproduction (6), and it is involved in the maintenance of differentiated epithelia and of mucus secretion (7–11). Vitamin A can also counteract the effects of some carcinogenic agents, both in vivo and in vitro (12–15).

It is not known whether the effects of vitamin A on cartilage are related to the biological activity, for maintaining growth and life, of the vitamin. The study reported here was conducted in order to explore this question. The effects of two retinoic acid analogs, and of retinoic acid and retinol, upon cartilage grown in organ culture in vitro were examined. A bioassay was then conducted to determine the ability of each compound to promote growth of vitamin A-deficient rats. All four compounds were highly active in bringing about resorption of the intercellular proteoglycan of cartilage. In contrast, the compounds differed markedly in their growth-promoting biological activity.

Abbreviations: RBP, retinol-binding protein.

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MATERIALS AND METHODS

Compounds studied

The vitamin A-related compounds examined in the cartilage study were (see **Fig. 1**) all-*trans*-retinol and all-*trans*-retinoic acid (purchased from Eastman Organic Chemicals, Rochester, N.Y.); α-retinoic acid (a gift from Dr. W. E. Scott, Hoffmann-La Roche, Inc., Nutley, N.J.); and RO8-7699, a cyclopentyl analog of retinoic acid (a gift from Dr. M. B. Sporn, National Cancer Institute, Bethesda, Md., who had obtained it from Hoffmann-La Roche). In addition, the following compounds were tested in the growth bioassay: α-retinyl acetate (a gift from Hoffmann-La Roche); and all-*trans*-retinyl acetate, U.S.P. vitamin A reference standard, purchased from United States Pharmacopeia, Rockville, Md. The melting points, determined at the time of the bioassay study, were retinoic acid, 173°C; α-retinoic acid, 133–135°C and α-retinyl acetate, 44°C.[1] The α-retinoic acid was estimated by Dr. W. Scott to contain less than 1% all-*trans*-retinoic acid by NMR assays conducted at Hoffmann-La Roche.

Cartilage study

This study was conducted in Cambridge, England. Small pieces of cartilage were grown in organ culture in BGJ$_5$ medium (16) containing 5% heat-inactivated normal sheep serum and 100 units/ml nystatin (E. R. Squibb, Liverpool). White rabbits approximately 8 wk old were killed, the ears were removed and soaked in 70% ethanol, and the skin was removed with forceps and scalpel under nearly sterile conditions. From the resulting cartilage, pieces of about 5 mm diameter and 5 mg wet weight were obtained with a punch. These were washed three times in BGJ$_w$, which is BGJ$_5$ medium containing 6 mM $NaHCO_3$ and 10 times the normal concentration of antibiotics. Three pieces of cartilage were cultured together on a grid in a small plastic dish containing 1.5 ml of culture medium. Three dishes were used for each dose of each compound studied. Culture was carried out at 37°C in an atmosphere of 5% CO_2–20% O_2 for 8 days, with the medium being changed every 2 days.

Weighed amounts of each compound were dissolved in ethanol and stored under nitrogen in sealed glass ampules at −20°C. For each compound, immediately prior to each experiment a fresh ampule was opened, and a stable dispersion of the compound in sheep serum was prepared by the rapid addition of serum to a small measured volume of the ethanol solution of the compound, as described previously (17). BGJ$_5$ medium, 4.75 ml, was added to 0.25 ml of the serum to give a final concentration of serum of 5% by volume. Equivalent amounts of ethanol alone were added to a control medium for culture of tissue in the absence of vitamin A-related compounds. The final concentration of ethanol did not exceed 0.5% by volume in any of the experiments. The final concentrations of the vitamin A-related compounds ranged from 0.2 to 6 μg/ml of medium.

[1] The α-ionone analogs were said to be mixtures of the *R* and *S* isomers (Dr. W. Scott).

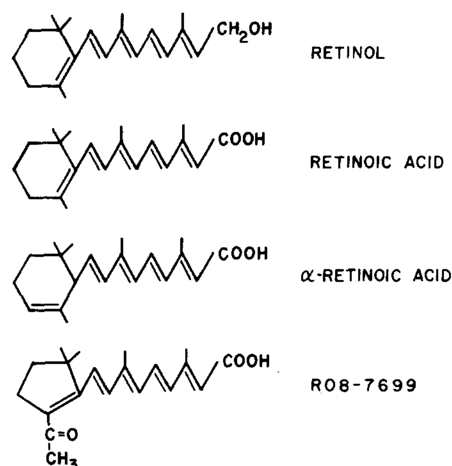

Fig. 1. Chemical structures of vitamin A and related compounds (retinol, retinoic acid, α-retinoic acid, and RO8-7699) employed in these studies.

Two experiments were conducted with cartilage. In the first experiment, the four vitamin A compounds were each studied at final concentrations of approximately 6, 2.5, and 1 μg/ml. In the second experiment, retinoic acid and its two analogs were each studied at final concentrations of 0.5 and 0.2 μg/ml. In both experiments, the culture medium removed from each dish after 2, 4, 6, and 8 days of culture was stored at −20°C and subsequently assayed for its content of proteoglycan. In the first experiment, one piece of cartilage from one of the three culture dishes for each dose of each compound was taken for histological study at the end of the experiment (8 days). In the second experiment, a fourth dish was cultured for each dose of each compound, and pieces of cartilage were removed from this dish for histological study after 2 and 4 days and at the end of the experiment (8 days).

For histological study the pieces of cartilage were fixed in Zenker's fluid containing 5% acetic acid, washed in tap water, dehydrated, and embedded in paraffin wax. Serial sections were cut and stained with toluidine blue or with hematoxylin–eosin.

The amount of cartilage proteoglycan released into the culture medium, and in a papain digest of the tissue, was measured by the Alcian blue assay, modified from the method of Whiteman (18).[2] Triplicate assays were made on each sample. Tissue assays were carried out on each group of three cartilage pieces in each dish. Upon completion of the assays, for each dish, the amount of proteoglycan found in the cartilage tissue at the end of the experi-

[2] Dingle, J. T., and P. Horsfield. Unpublished results.

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TABLE 1. Growth promoting biological activity of vitamin A-related compounds in vitamin A-deficient rats

Compound[a]	Intake	Weight Change in 4-wk Test Period[b]	Number Dead
	µg/day	*g*	
None		−108 ± 5.0	8
Retinyl acetate	0.37	−1.4 ± 11.8	0
Retinyl acetate	0.73	+40.8 ± 4.4	0
Retinyl acetate	1.46	+60.1 ± 3.6	0
Retinoic acid	9.2	+53.2 ± 4.0	0
Retinoic acid	18.4	+56.2 ± 5.9	0
α-Retinoic acid	45	+60.2 ± 6.3	0
α-Retinoic acid	90	+67.5 ± 4.6	0
RO8-7699	57	−33.2 ± 16.5	3
RO8-7699	114	−14.8 ± 12.3	1
α-Retinyl acetate	48	+36.3 ± 14.9	0
α-Retinyl acetate	96	+47.1 ± 9.3	0

[a] All rats were fed the vitamin A-deficient diet supplemented with the compound indicated. The group designated "none" received only the cottonseed oil vehicle.
[b] Mean ± SEM for each group of rats.

ment and the amounts of proteoglycan found in the samples of medium (days 2, 4, 6, and 8) from that dish were added together and the total sum was assigned a value of 100%. The amount of proteoglycan released into the culture medium was then expressed in terms of this total sum, as the percentage of the total proteoglycan (recovered in tissue plus medium) released during the experiment. This method of calculation corrects for differences in weight and proteoglycan content of different pieces of cartilage in different dishes. The means and standard errors of the means were then calculated for each group of three dishes representing each dose of each compound in each experiment.

Bioassay

The vitamin A bioactivity of the several compounds was tested in New York by a curative growth bioassay. The test compounds were each given dissolved in cottonseed oil containing 0.1% hydroquinone as an antioxidant. To prepare the cottonseed oil solutions, the all-*trans*-retinoic acid, α-retinoic acid, and α-retinyl acetate were each dissolved in 10 ml of peroxide-free diethyl ether, and the ether solutions were then added to an appropriate amount of cottonseed oil. The ether was removed under a stream of nitrogen. RO8-7699 was poorly soluble in ether and was dissolved in chloroform to prepare the cottonseed oil solution. The U.S.P. reference standard was supplied dissolved in cottonseed oil and was diluted to the appropriate concentration before use.

The procedures used in the bioassay were similar to those described by Embree et al. (19). To deplete their vitamin A stores, 148 weanling male Holtzman rats were fed a vitamin A-free diet (20). After 48 to 60 days the rats were judged to be vitamin A deficient by their failure to

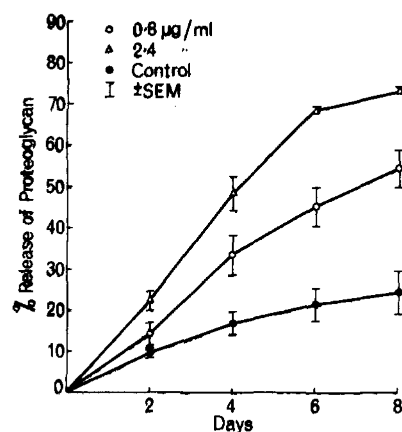

Fig. 2. Effects of retinol on the release of proteoglycan from cartilage in culture in vitro. Means ± SEM are shown.

grow on 5 successive days. As the rats individually became deficient, they were supplemented with 50 µg of retinoic acid per day to maintain them in good health until the start of the bioassay. After all of the rats had become vitamin A deficient, they were maintained on retinoic acid for one additional week and then given the vitamin A-deficient diet alone the following week, during which time their growth pleateaued and they again became deficient. 120 of these vitamin A-deficient rats were randomly divided into 12 groups of 10 rats each. The treatment assignments are shown in **Table 1.** The test compounds were given once each day with a calibrated dropping pipet. The growth of each rat in each of the 12 treatment groups was determined during a 4-wk test period. The conduct of the assay and the calculations of biopotency were carried out as described by Embree et al. (19), using the 0.73 and 1.46 µg/day retinyl acetate groups for controls.

At the completion of the study, serum samples were collected from the rats in the all-*trans*-retinoic acid, α-retinyl acetate, and all-*trans*-retinyl acetate groups by the technique of Phillips, Stafford, and Stuut (21). Five rats from each group that had received the highest levels of these

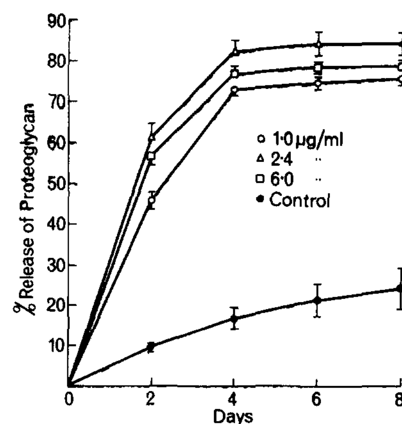

Fig. 3. Effects of retinoic acid on the release of proteoglycan from cartilage in culture in vitro. Means ± SEM are shown.

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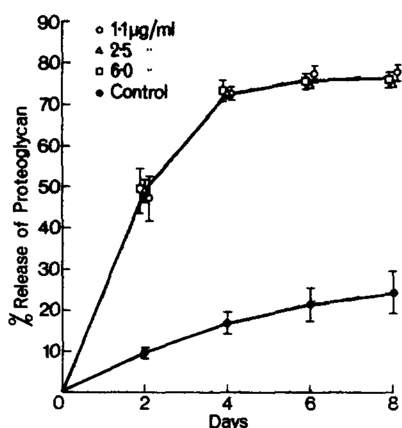

Fig. 4. Effects of α-retinoic acid on the release of proteoglycan from cartilage in culture in vitro. Means ± SEM are shown.

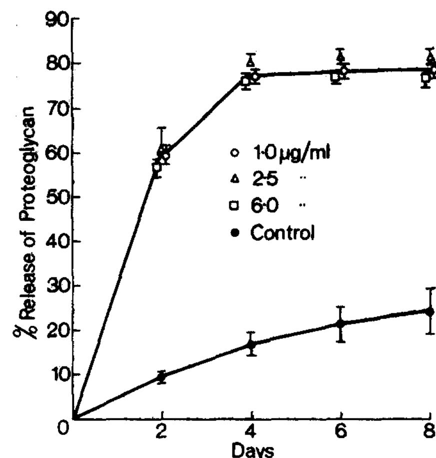

Fig. 5. Effects of RO8-7699, a cyclopentyl analog of retinoic acid, on the release of proteoglycan from cartilage in culture in vitro. Means ± SEM are shown.

three compounds were killed by a sharp blow on the head, and the livers were removed, frozen, and stored at −20°C. The serum and liver samples were analyzed for vitamin A by the fluorometric correction formula method of Thompson et al. (22). Since α-retinol is also fluorescent, it will interfere with the determination of retinol by this method. The fluorescence in the extracts, after saponification, from the livers of the rats given α-retinyl acetate was determined to be principally due to α-retinol, and not due to all-*trans*-retinol, by the shape of the fluorescence excitation (325 nm max, unc) and emission (425 nm max, unc) spectra. The levels of α-retinol in the extracts of saponified liver were estimated by fluorescence assay at the appropriate wavelengths, using the values of the extracts from the rats fed retinoic acid as a blank. An extract obtained from a saponified sample of pure α-retinyl acetate was used as the standard.

Retinol-binding protein concentrations were measured in serum and in liver homogenates by a modification of the previously described radioimmunoassay (20) in which polyethylene glycol (mol wt 6000–7500) was used to precipitate the antibody-bound RBP rather than a second antibody (23). The method of calculation was also changed to the logit–log method (24), and calculations were made with a Wang 700A programmable calculator (Wang Laboratories, Inc., Tewksbury, Mass.).

RESULTS

Cartilage study

In control incubations of cartilage cultured without the addition of a vitamin A-related compound, there was observed progressive release of a small amount of proteoglycan into the culture medium throughout the period of the experiment. In both experiments, after 8 days approximately 25% of the total proteoglycan had been released into the medium in the control incubations.

Figs. 2 through 7 describe the rate and extent of release of proteoglycan into the medium from cartilage cultured in the presence of different doses of one or another of the four vitamin A-related compounds. **Fig. 2** shows the effects of retinol at final concentrations of 0.8 and 2.4 µg/ml. Retinol is known from previous studies to produce a dose-related increase in proteoglycan turnover and release (17, 25). This effect was clearly demonstrated at the two doses studied (Fig. 2).

Retinoic acid and its two analogs were all highly effective in bringing about the release of cartilage proteoglycan into the culture medium. At concentrations between 1 and 6 µg/ml, all three compounds appeared to produce almost maximal release by about 4 days, with very little additional proteoglycan release being observed in the final 4 days of culture (**Figs. 3–5**). Within this dose range, each of the three acids caused release of 75–84% of the total proteoglycan during the 8-day experiment. This appears to represent the total amount of proteoglycan that can be released from the cartilage, even by direct enzymatic treatment.[3] All three acids were clearly more active than retinol in stimulating proteoglycan release.

Figs. 6 and 7 show the effects of retinoic acid, α-retinoic acid, and RO8-7699 at concentrations of 0.5 and 0.2 µg/ml. Of the three compounds, RO8-7699 was the most active and appeared to maximally stimulate proteoglycan release even at these lower concentrations. Within this lower dose range, retinoic and α-retinoic acids were less than maximally active, and their effects were dose-related.

Histological study of the sections of cartilage stained with toluidine blue showed a clear and striking correlation with results of the proteoglycan chemical assays. In control incubations, after 8 days of culture the cartilage matrix remained intensely metachromatic and stained strong-

[3] Dingle, J. T. Unpublished results.

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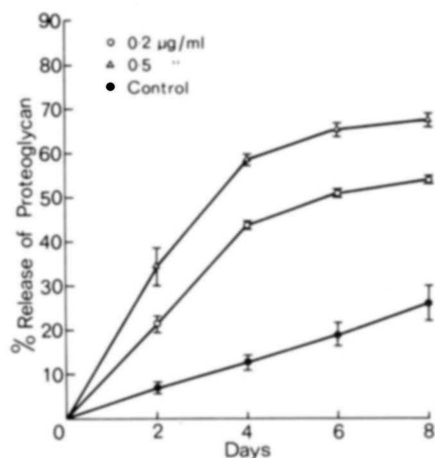

Fig. 6. Effects of lower concentrations of retinoic acid on the release of proteoglycan from cartilage in culture in vitro. Means ± SEM are shown.

ly with toluidine blue (**Figs. 8** and **9,** top panels). Cartilage that had been exposed to retinoic acid or either of its two analogs at concentrations of 1–6 µg/ml lost virtually all of its metachromasia during 8 days of incubation and showed essentially no metachromatic staining with toluidine blue. A representative example of the appearance of sections of these pieces of cartilage is shown in the bottom panel of Fig. 8. On handling, these pieces of cartilage were soft and floppy and did not show the firmness and texture characteristic of the control cartilage.

In samples where less than maximal release of proteoglycan was observed, the cartilage showed partial retention of its metachromatic staining with toluidine blue (Fig. 8, middle panel). The progressive loss of metachromatic staining, coincident with and quantitatively correlated with release of proteoglycan, was evident on examination of pieces of cartilage taken sequentially during the 8-day period of culture (Fig. 9).

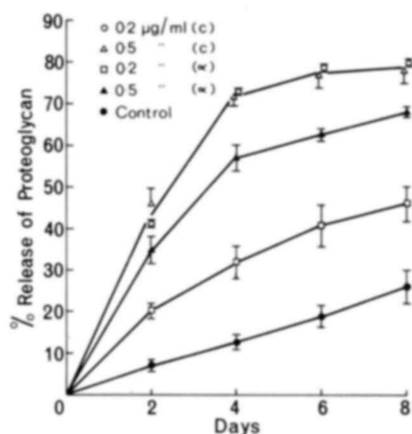

Fig. 7. Effects of lower concentrations of α-retinoic acid (α), and of RO8-7699 (C), on the release of proteoglycan from cartilage in culture in vitro. Means ± SEM are shown.

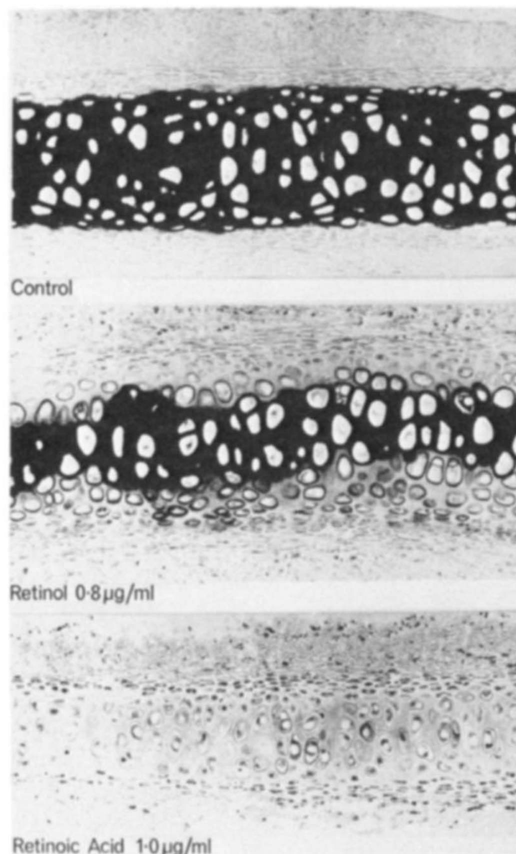

Fig. 8. Sections of cartilage grown in culture in vitro for 8 days without the addition of any vitamin A-related compound (control, top panel), in the presence of retinol, 0.8 µg/ml (middle panel), or in the presence of retinoic acid, 1.0 µg/ml (bottom panel). Sections were stained with toluidine blue; magnification × 88.

Bioassay

The growth data for the rats fed the vitamin A test compounds are presented in Table 1. All of the rats given the cottonseed oil alone lost weight, and 8 of the 10 died during the 4-wk test period. The rats given the cyclopentyl analog RO8-7699 also failed to grow, and 4 of the 20 rats given this compound died during the experiment. The rats receiving the lowest dose of retinyl acetate (0.37 µg/day) fared better than the rats receiving the higher dose of RO8-7699 (114 µg/day) (see Table 1). From these data it is apparent that the biopotency of RO8-7699 is considerably less than 1/300 that of retinyl acetate (see **Table 2**). Therefore, RO8-7699 can be assumed to be virtually biologically inactive.

Biopotencies for the other compounds were calculated on a molar basis relative to retinyl acetate taken as 100% and are listed in Table 2. The relative biological activity for retinoic acid of 14% is in good agreement with a biological activity of about 10% reported by Arens and Van Dorp (26) for free retinoic acid administered orally in peanut oil. The estimated relative biopotency of α-retinyl

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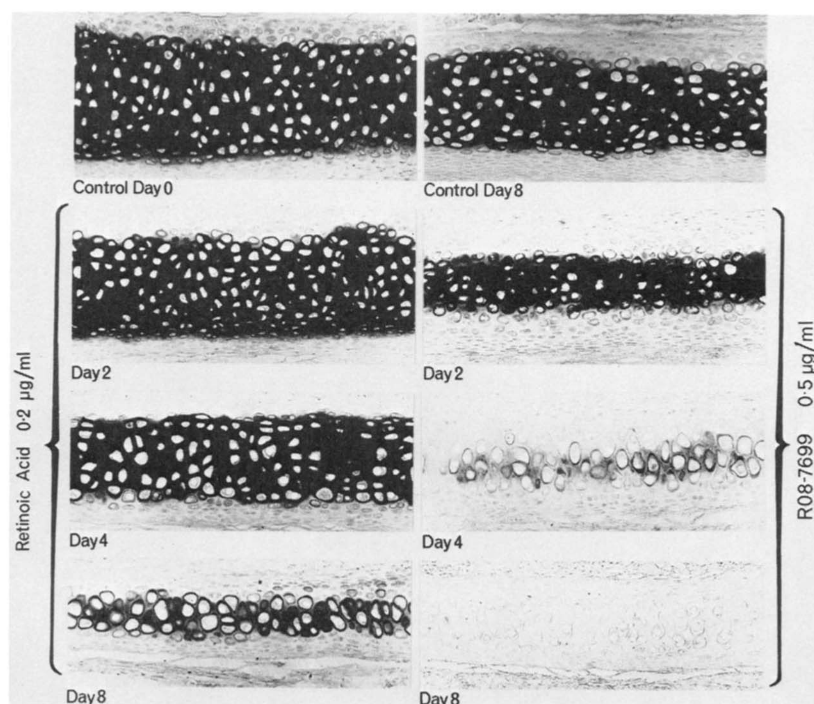

Fig. 9. Sections of cartilage grown in culture in vitro. The top two panels show the appearance of cartilage before culture (upper left panel) and after 8 days in culture without the addition of any vitamin A-related compound (upper right panel). The other panels show pieces of cartilage removed after 2, 4, or 8 days in culture in the presence of either retinoic acid, 0.2 μg/ml (left-hand panels), or RO8-7699, 0.5 μg/ml (right-hand panels). Sections were stained with toluidine blue; magnification × 44.

acetate (2.6%) is not very different from the relative biopotency of less than 2% reported by Ames, Swanson, and Harris (27) for α-retinaldehyde. The α-retinoic acid was almost twice as active as α-retinyl acetate. This is in contrast to all-*trans*-retinyl acetate, which was about seven times more active than retinoic acid. Although the doses given were relatively large, in general the rats receiving either of the α-ionone analogs grew fairly well and appeared in good general health.

Studies were carried out with sera and livers of rats given α-retinyl acetate in order to determine whether the biological activity of this compound might be due to contamination with or conversion to all-*trans*-retinol. The livers of the rats given α-retinyl acetate appeared to contain essentially no retinol, as estimated by the 360/330 nm wavelength ratio for fluorescence excitation at 475 nm emission (0.46 for retinol, 0.08 for α-retinol, and 0.09 for liver extracts from rats given α-retinyl acetate). The livers of the rats given α-retinyl acetate contained 727 ± 24 μg of α-retinol, or about one-third of the total compound administered, which is in accordance with the results of Ames et al. (27).

Table 3 shows the serum and liver vitamin A and RBP concentrations of rats fed retinyl acetate, α-retinyl acetate, or retinoic acid. The serum vitamin A levels of the rats receiving retinyl acetate showed a graded increase that was roughly proportional to the dose fed. The low plasma vitamin A level (normal > 20 μg/dl) and the lack of liver vitamin A accumulation in the rats fed the highest dose of retinyl acetate (1.46 μg/day) demonstrate that this dose was still (as anticipated) distinctly suboptimal. All three dose groups of rats given retinyl acetate showed a highly significant increase in serum RBP levels compared with the retinoic acid-fed groups (which do not differ from deficient controls [20]). In contrast, α-retinyl acetate supplementation had a minimal or no effect on serum RBP levels, and the rats given this compound had RBP levels that were much lower than those observed for the rats fed the lowest dose of retinyl acetate. Since the rats fed α-retinyl acetate grew much better than those fed the lowest dose of retinyl acetate, these data strongly suggest that the biologi-

TABLE 2. Relative biopotency, on a molar basis, of the vitamin A-related compounds assayed

Compound	Relative Biopotency[a]
Retinyl acetate	100
Retinoic acid	14
α-Retinoic acid	4.1
RO8–7699	<0.3
α-Retinyl acetate	2.6

[a] Relative to retinyl acetate, which was taken as 100% potency.

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TABLE 3. Serum and liver vitamin A and RBP concentrations in rats fed retinyl acetate, α-retinyl acetate, or retinoic acid (means ± SEM)

Compound	Intake	Serum RBP	Serum Vitamin A	Liver RBP	Liver Vitamin A
	μg/day	*μg/ml*	*μg/dl*	*μg/g wet wt of liver*	*μg/liver*
Retinoic acid	9.2	7.0 ± 0.5^{c}	1.6 ± 0.2^{h}	*a*	*a*
Retinoic acid	18.4	8.0 ± 1.0^{cd}	2.2 ± 0.3^{hl}	123 ± 15^{c}	1.5 ± 0.4^{f}
α-Retinyl acetate	48	9.2 ± 1.0^{cd}	2.9 ± 0.4^{il}	*a*	*a*
α-Retinyl acetate	96	10.6 ± 0.8^{d}	2.8 ± 0.3^{il}	138 ± 17^{c}	*b*
Retinyl acetate	0.37	17.4 ± 1.5^{e}	4.1 ± 0.5^{i}	*a*	*a*
Retinyl acetate	0.73	19.6 ± 1.6^{e}	5.9 ± 0.3^{j}	*a*	*a*
Retinyl acetate	1.46	24.6 ± 4.5^{e}	9.1 ± 0.6^{k}	152 ± 13^{c}	3.8 ± 0.6^{g}

[a] Not determined.
[b] Negligible retinol or retinyl esters. Livers contained a mean of 727 ± 24 μg of α-retinol per liver.
[cde] Means with different superscripts are significantly different from each other, $P < 0.005$; those with the same superscript are not different, $P > 0.05$.
[fg] Means significantly different, $P < 0.05$.
[hijkl] Means with different superscripts are significantly different from each other, $P < 0.025$; those with the same superscript are not different, $P > 0.05$.

cal activity of the α-retinyl acetate was not due to its contamination with or conversion to retinol.

DISCUSSION

The mechanism whereby retinol produces resorption of cartilage appears to be well characterized and involves the release of lysosomal enzymes that act hydrolytically on the intercellular matrix. Retinol is a highly surface-active compound (28) that is "membrane-seeking" and potentially membranolytic. When retinol is added nonspecifically to serum proteins in the culture medium, there apparently occurs sufficient uptake of the vitamin by the plasma and lysosomal membranes to alter their normal physical properties and probably to facilitate fusion of membranes (29). Lysosomal hydrolases are released more readily from the cells with altered, labilized membranes. Thus, lysosomal cathepsin D has been demonstrated extracellularly, after exposure of cells to retinol, by specific immunofluorescence localization studies (30), and the effect of retinol has been counteracted by specific immunoinhibition experiments directed against cathepsin D (4).

The molecular processes involved in the physiological functions of vitamin A are not known (except for its role in vision [5]). It has been suggested that vitamin A may normally play a role in regulating the stability and structure of biological membranes as one of its physiological functions (31). The effects of vitamin A on cell membranes have, however, mainly been studied under pathological conditions of hyper- or hypovitaminosis A and may not be relevant to the physiological role of the vitamin. This conclusion is supported by a recent study that demonstrated that retinol bound to its specific transport protein, retinol-binding protein, failed to influence the extracellular matrix of chick limb-bone rudiments in organ culture (17). Thus, the effects of retinol on biological membranes may represent largely nonspecific effects of the vitamin's surface-active properties.

Recent studies have demonstrated the biological formation of glycolipids containing retinol or its metabolites and have suggested that vitamin A may be involved in some glycosyltransferase reactions and in glycoprotein metabolism (32–35). These phenomena are under active investigation in several laboratories. It has also been reported that vitamin A deficiency affects RNA metabolism (36, 37). The significance of these observations is not yet clear.

The studies reported here were addressed to the question of the relationship between the effects of vitamin A upon cartilage and the biological role of vitamin A in maintaining growth and life. The results demonstrate that retinol, retinoic acid, α-retinoic acid, and RO8-7699 (a cyclopentyl analog of retinoic acid) were all highly effective in promoting the lysis of the extracellular matrix of cartilage in vitro. Retinoic acid and its two analogs were quantitatively more active than was retinol in bringing about lysis of matrix and release of proteoglycan into the culture medium. In contrast, the bioassay study demonstrated that retinoic acid and its two analogs were considerably less active quantitatively than retinol in promoting growth of vitamin A-deficient rats. Moreover, the three acids tested showed graded biological activity in the growth bioassay, with α-retinoic acid showing reduced bioactivity (approximately one-fourth that of retinoic acid) and RO8-7699 being virtually inactive. The observed lysis of cartilage matrix produced by these compounds was presumably caused by release of lysosomal enzymes as a result of the membrane-labilizing effects of the compounds. Thus, the surface-active properties of these vitamin A-related compounds are poorly correlated with their biological,

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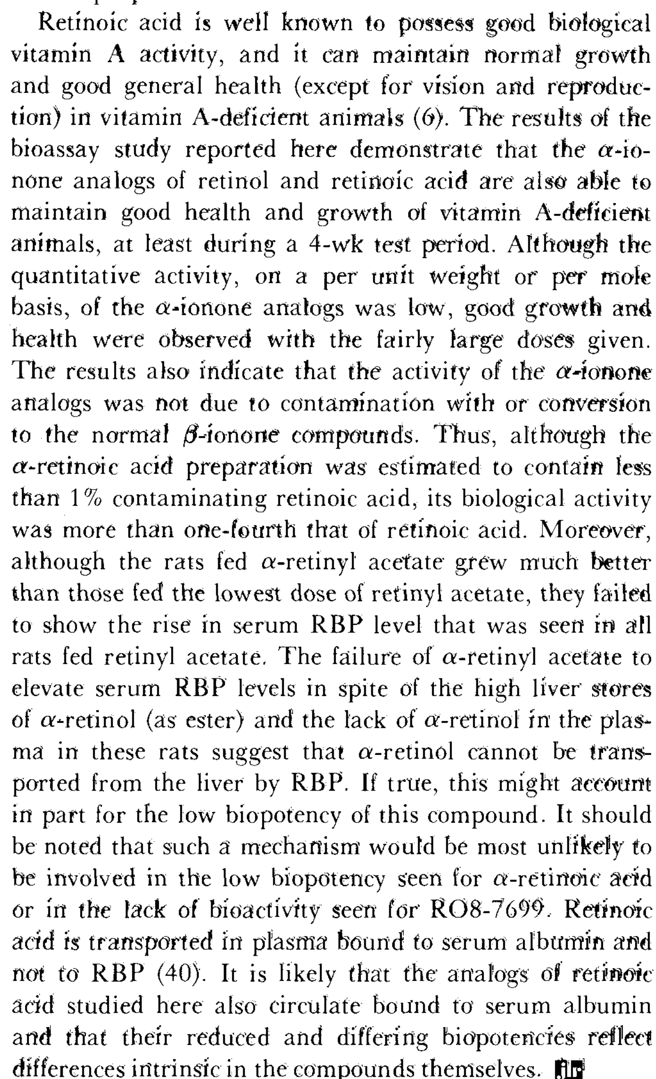
growth-promoting activity and presumably represent pharmacological effects of the compounds. In fact, the most active compound on cartilage in this study was the biologically inactive (for growth) RO8-7699. These findings are consistent with and extend the work of Pitt (38), who reported in preliminary form that α-retinol was able to produce the characteristics of hypervitaminosis A both in vitro and in vivo, yet had only 2.1% of the growth-promoting activity of retinol in rats.

A companion study carried out with the same four vitamin A-related compounds studied here with cartilage demonstrated that all four compounds were highly active in inhibiting the effects of methylcholanthrene on mouse prostatic epithelium in vitro (39). Hence, the anticarcinogenic activity of vitamin A is also not correlated with the growth-promoting biological activity of the vitamin. These studies suggest that it could be possible to dissociate some of the various physiological, pharmacological, and toxic properties of vitamin A-like compounds by using different structural analogs of vitamin A with different combinations of properties.



Retinoic acid is well known to possess good biological vitamin A activity, and it can maintain normal growth and good general health (except for vision and reproduction) in vitamin A-deficient animals (6). The results of the bioassay study reported here demonstrate that the α-ionone analogs of retinol and retinoic acid are also able to maintain good health and growth of vitamin A-deficient animals, at least during a 4-wk test period. Although the quantitative activity, on a per unit weight or per mole basis, of the α-ionone analogs was low, good growth and health were observed with the fairly large doses given. The results also indicate that the activity of the α-ionone analogs was not due to contamination with or conversion to the normal β-ionone compounds. Thus, although the α-retinoic acid preparation was estimated to contain less than 1% contaminating retinoic acid, its biological activity was more than one-fourth that of retinoic acid. Moreover, although the rats fed α-retinyl acetate grew much better than those fed the lowest dose of retinyl acetate, they failed to show the rise in serum RBP level that was seen in all rats fed retinyl acetate. The failure of α-retinyl acetate to elevate serum RBP levels in spite of the high liver stores of α-retinol (as ester) and the lack of α-retinol in the plasma in these rats suggest that α-retinol cannot be transported from the liver by RBP. If true, this might account in part for the low biopotency of this compound. It should be noted that such a mechanism would be most unlikely to be involved in the low biopotency seen for α-retinoic acid or in the lack of bioactivity seen for RO8-7699. Retinoic acid is transported in plasma bound to serum albumin and not to RBP (40). It is likely that the analogs of retinoic acid studied here also circulate bound to serum albumin and that their reduced and differing biopotencies reflect differences intrinsic in the compounds themselves. ■

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