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INHIBITION OF CARCINOGENESIS BY RETINOIDS

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DISCLAIMER

The aim of this presentation is to summarize the essential information which was emerged from the last 10 years of "anticarcinogenesis" studies with retinoids (vitamin A and its analogues). Several reviews of this topic have appeared recently (1-4). For the purpose of this conference, I will limit this discussion to a review of experimental attempts to explore and demonstrate antineoplastic effects of retinoids. To this end I will select exemplary studies from the literature rather than attempt a comprehensive review. The chemistry, biochemistry and physiology of retinoids will not be discussed. Those interested in this subject may be referred to a recent review by De Luca (5).

Retinoids are needed for the proper development and maintenance of mucous membranes and surface epithelia. For close to 50 years evidence has suggested that the mucous membranes of vitamin A deficient rats might develop cancer, and indications that vitamin A might inhibit carcinogenesis have existed for over 30 years (for review see ref. 1). In the last 10 years efforts to firmly establish the anticarcinogenic effects of natural and synthetic retinoids have gained great momentum. These investigations can be categorized according to the principal experimental approaches used.

1) In vitro studies: prevention or reversal of transformation or carcinogen induced tissue changes; and inhibition of growth of neoplastic cell lines. 2) In vivo studies: inhibition of tumor induction, and growth inhibition of established tumors.

Before discussing some key examples of antineoplastic studies with retinoids, it should be mentioned that much of the most recent work has been conducted with synthetic retinoids, because it has become clear that the natural retinoids have too low a therapeutic index, i.e. relative to the

intended therapeutic effect their toxicity is too high. For this reason extensive studies have been launched, concerned with the search for structural modifications of the retinoid molecule which will yield retinoids with a higher therapeutic index than the natural retinoids. Modifications of the retinoid molecule are being attempted either in the ring portion, the side chain or the terminal end (see Figure 1). Examples of useful structural modifications of the retinoid molecule which have either a modified ring or a modified terminal group are depicted in Figure 2.

In vitro studies of the anticarcinogenic effects of retinoids. Over the last two decades a series of studies were conducted by Lasnitzki and collaborators using mouse prostate in organ culture (e.g. 6, 7). More recently Chopra and his colleagues have used the same system (e.g. 8, 9). These studies have shown that natural, as well as a variety of synthetic, retinoids with ring and polar group modifications have antimitotic and antihyperplastic activity in prostate cultures exposed to chemical carcinogens (3-methylcholanthrene and N-methyl-N-nitro-N-nitrosoguanidine). The retinoids were active in the culture medium either with the carcinogen present or after the carcinogen exposure. The methylketo cyclopentenyl and the 1-methoxyethyl cyclopentenyl analogues of β-retinoid acid were at least 50 times as effective as the retinoic acid itself in reversing the carcinogen induced hyperplasia. Recently it was reported (10) that a synthetic retinoid (Ro 11-1430, the 4-methoxy-2,3,6-trimethylphenyl analog of retinoic acid ethyl amide) inhibits oncogenic transformation in vitro of 10T½ cells exposed to γ-radiation and that the natural retinoids-retinal, retinal and retinylacetate inhibit at non-toxic concentrations the neoplastic transformation of 10T½ cells by the carcinogenic polycyclic hydrocarbon 3-methylcholanthrene (11).

Another type of *in vitro* study was carried out by Lotan and Nicolson (12) and by Dion et al. (13) measuring the effects of retinoids on various growth parameters of transformed and untransformed cell lines. Lotan and Nicolson (12) found that retinoic acid (all-trans-β-retinoic acid, at 10^{-5} M concentration) and to a lesser extent retinyl acetate inhibited the growth of many of the 31 cell lines that were tested. The tumor lines that were tested included lymphoma, myeloma, sarcoma, neuroblastoma, melanoma and carcinoma cell lines from a variety of species. The degree of growth inhibition varied considerably from cell line to cell line. In many of the tumor lines, including two mammary carcinomas, growth was severely inhibited (>75% growth inhibition). Whether the differences in responsiveness to retinoids can be attributed to the presence, absence, or relative concentration of the appropriate intracellular binding protein, as suggested by some (14, 15), is still an open question. The other group of investigators showed that retinoic acid increased density dependent growth control (16) and anchorage dependent growth (13), two biological markers commonly used to distinguish between transformed and untransformed cells, in some but not all transformed cells which were tested (Table 1). The concentration of retinoic acid which restored anchorage dependent growth (measured by a 50% inhibition of colony formation in methyl cellulose) of the mouse transformed fibroblast line L-929 was 2×10^{-9}M as compared to 1×10^{-5}M for retinyl acetate. These investigators also found that this effect was reversible when the retinoids were withdrawn from the culture medium. Recently, it was also shown that retinoids can block phenotypic cell transformation produced *in vitro* by sarcoma growth factors (17).

In summary, the *in vitro* studies have shown 1) that natural and synthetic retinoids can effectively inhibit carcinogen induced hyperplasia metaplasia and proliferation in prostate organ cultures. 2) That the transformation of

fibroblast cultures by chemical and physical transforming agents can be inhibited; 3) that the growth of some (but not of other) untransformed and transformed cell lines can be inhibited; 4) that the expression of some phenotypic markers which are characteristic of transformed cells can be inhibited and 5) that these effects can be much more readily achieved with some of the less toxic synthetic retinoids.

In vivo studies of the anticarcinogenic effects of retinoids. The most extensive work on the effects of retinoids on the induction of tumors has been carried out in the two-stage skin carcinogenesis model. (In this model an initiating dose of carcinogen, which by itself is nontumorigenic, is applied; this is followed by repeated applications of a promoting agent. The end result is development of papillomas and carcinomas. Neither the initiator nor the promoter alone cause skin tumor formation). Such studies have been carried out by Bollag and others (e.g. ref. 1). A typical example of the inhibition of skin carcinogenesis by synthetic retinoids, in this case by an aromatic retinoic acid analog, is illustrated in Figures 3-5 (18). After initiation with a carcinogenic polycyclic hydrocarbon the promoter is applied to the dorsal skin, twice weekly for the duration of the experiment. The retinoid feeding (30 mg/kg body weight per day) commenced with the start of promotion. The development of papillomas and carcinomas was markedly inhibited by the retinoid feeding. Bollag also showed (19) that established papillomas and carcinomas could be treated with the same retinoid. By oral administration of 5-40 mg/kg body weight for 2 weeks a 17-76% reduction in papilloma size was achieved, while the tumors in untreated controls increased in size by 23%. With administration of 400 mg/kg of body weight for 2 weeks, 9 out of 11 carcinomas were reduced in size by 14-97%. The carcinomas in control animals, in the meantime increased by 14-316%. The mechanism of this inhibi-

tion and induced regression is not clear. Trigg and Torhorst (20) in an autoradiographic study, failed to discover any effect on cell proliferation in retinoid induced regression of skin tumors. The prophylactic effects of retinoids in skin carcinogenesis have been further investigated by Boutwell and his collaborators in a series of studies. As reported in a recent communication (21) these investigators demonstrated that skin tumor formation can be inhibited by topical application of a variety of retinoids. The retinoid was applied to the skin 1 hour prior to each application of the promoter (Figure 6). It was further shown (22, 23) that this inhibitory effect closely correlates with the ability of the same drugs to inhibit the stimulation of orinthine decarboxylase (ODC) activity by the phorbol ester TPA, (Fig. 7 and Table 2). Retinoids which do not inhibit TPA-induced stimulation of ODC lack significant prophylactic effects in terms of papilloma formation. The authors also found taht the retinoids do not interfere with tumor initiation and do not inactivate initiated cells. Thus in this tumoigenesis assay the retinoid seems to inhibit only the action of the promoting agent.

Studies in other tumor induction systems are less plentiful, less systematic and generally less dramatic. Moon et al. (24) and Grubbs et al. (25) showed evidence for an inhibition of mammary tumor formation of rats (carcinogen DMBA) by retinylacetate and retinylmethyl ether (Figure 8). Subsequently the same group of investigators presented data suggesting that in the rat mammary tumor system retinoids inhibit the progression of early neoplastic lesions to carcinoma and that continuous retinoid intake is required to maintain the preventive effect (26). Inhibition of carcinogenesis in the bladder of rats and mice has been achieved with administration of 13-cis retinoic acid (27-29) (Table 3). In all of these experiments, since the retinoid administration was started after the carcinogen exposure it is most

likely that the retinoid interfered with whatever processes follow the initiating events. It must be noted that the endpoint measured in all of these experiments is tumor incidence (or number of tumors per animal) at a predetermined point in time, and not survival.

Attempts to inhibit the formation of intestinal tract carcinomas induced in rats by dimethylhydrazine (29) or by local application of N-methyl-N-nitrosourea to the colon have been less successful (30). Niether retinyl palmitate nor 13-cis-retinoic acid nor the trimethylmethoxy phenyl analog of retinoic acid ethylamine (Ro 11-1430) inhibited the development of intestinal tract cancers. The latter compound had been shown to inhibit the growth of some transplantable tumors (see below).

The inhibition of tumor induction in the respiratory tract of mice, rats or hamsters has also not been convincing. Despite repeated attempts, it has not been possible to consistently reproduce the earlier findings of Saffiotti and collaborators (31), who reported a reduction of respiratory tract tumor incidence from 32% in controls to 11% in hamsters treated with retinyl palinitate (10,000 TU per week). The tumors were induced by repeated intertracheal injections of benzo(a)pyrene adsorbed for ferric oxide particles. In 1975 Smith et al. (32, 33) published two independent studies, each with several hundreds of hamsters, which failed to demonstrate any inhibition of lung cancer induction by retinyl acetate (weekly doses ranging from 100 to 2400 up of retinyl acetate per week). The tumor incidence was between 60 and 80% in one study and between 40 and 50% in the other study. Similar to Saffiotti et al., 1967 (see above) these workers observed a significant reduction of stomach papillomas in the retinyl acetate treated hamsters. The third study conducted with the same lung tumor induction system was discussed by Sporn et al. in a review article (3). In over 130 control hamsters the

tumor incidence was 10%; in hamsters receiving 3 mg of 13-cis-retinoic acid the incidence was 1.3% (2 out of 152) and in 158 hamsters receiving 9 mg of 13-cis-retinoic acid no tumors were found in 158 hamsters. The combined lung tumor incidence in the two treated groups was 0.6%.

Studies in our laboratory have not unequivocally established the inhibition of lung tumor induction in rats (34-37) or in hamsters (38) by retinyl acetate, 13-cis-retinoic acid or Ro 11-1430 (trimethylmethoxy phenyl analog of retinoic acid ethylamide). Hamsters treated with the latter compound (Table 4) appeared to show a trend towards a somewhat reduced cancer risk in 4 of 4 groups receiving Ro 11-1430. In contrast 13-cis-retinoic acid, probably due to the toxicity encountered, resulted in an acceleration of death from cancer. We did find, however, that the incidence of preneoplastic lung nodules in rats as well as the incidence of lung cancers was significantly higher in rats maintained on a subnormal retinoid level than in rats receiving normal or above normal levels of retinoids (Table 5) (34-37). This suggests an increased susceptibility to the induction of cancer in the respiratory tracts of vitamin A deficient rats.

It is presently not clear why the data on the inhibition of lung cancer induction are so ambiguous or even contradictory, compared to the findings with mammary gland, urinary bladder and skin tumor induction. One possibility is that this is simply a reflection of the differences in reproducibility and sensitivity of the different experimental models. We believe, however, that the skin carcinogenesis studies may lead to a possible biological mechanism. The studies carried out in Boutwell's laboratory (21-23) suggest that one major mechanism of the inhibitory effects of retinoids on carcinogenesis is through inhibition of promotion. If this finding also has relevance

to the development of other tumors, then one would expect to find little or no inhibitory effect under those conditions where promotion is not a major factor. This is the case when "complete" carcinogens are applied at relatively high doses and/or when no promoter (internal or external) exists to "drive the initiated cells to the point of tumor development. The carcinogen doses used in some of the respiratory tract tumors induction studies might be "completely" carcinogenic and "natural" promoters which appear to exist in mammary tumorigenesis may be absent in respiratory tract carcinogenesis.

Finally, numerous attempts have been made to inhibit the growth of established tumors (or tumor lines) in vivo with administration of various types of retinoids. Earlier attempts were in many cases disappointing (for review see 1). However, more recently several encouraging results have been reported (see also 19) with retinyl palmitate, inhibiting the growth of a transplantable murine melanoma (Table 6) (39) and of a transplantable adenocarcinoma (40). 13-cis-retinoic acid and 3 synthetic aromatic retinoids (Ro 10-1670, Ro 10-9359, Ro 11-1430) were reported to inhibit growth and to cause regression of established chondrosarcomas in rats (Table 7) (41, 42). It has been suggested that the reason for the differences in the responsiveness of various tumors and tumor lines to retinoids may be related to the presence or absence of cellular retinal and/or retinoic acid binding proteins (14, 15, 43). It has been repeatedly suggested that this type of antitumor effect may be in part an immunological component since retinoids have been shown to enhance humoral and cellular immune responses (for discussion see 39). However, the inhibitory effects on neoplastic cell lines in vitro (see above) suggest that a direct inhibition of tumor growth, by a yet unknown mechanism, is likely to be a major factor in the inhibition of tumor growth in vivo by retinoids.

In summary, the in vivo studies indicate that 1) tumor induction can be effectively inhibited, at least in some tumor models, by natural and synthetic retinoids; 2) that the effect may result from inhibition of the promotion phase, i.e. the post initiation phase during which the progression of initiated cells to fully transform neoplastic cells occurs, due, at least in some tumor models, to exogenous or endogenous promoters; 3) that some established primary tumors as well as transplanted tumors will at least partially regress under intensive retinoid therapy; 4) that some of the synthetic retinoids have a much higher therapeutic index in prevention or therapy of cancers, than the natural vitamin A compounds.

SUMMARY AND CONCLUSION

With this brief and selective review, I have tried to highlight the progress made in recent years in the search for retinoids with anticarcinogenic activity. There are many studies to be found in the literature which show no substantial effect of retinoids on carcinogenesis or tumor growth (for review see 3). Some of these negative findings may be related to the carcinogen dose used, the type of retinoid used, the dose, dose schedule or mode of administration of the retinoid. Others may indicate that the particular type of tumor or tumor system is, indeed, refractory to retinoids in general or to those retinoids that were tested. A great gap still exists in our knowledge concerning the pharmakokinetics of most retinoids their availability to various normal and cancerous tissues, the role and existence of transport and binding proteins, etc. There are studies which indicate that under certain conditions, particularly conditions of topical application, some retinoids may even enhance carcinogenesis (44-46).

It seems, however, indisputable by now that some retinoids are effective inhibitors of carcinogenesis in some organ systems and can even inhibit the growth of some established tumors. While the mechanisms of these inhibitory effects are presently not understood, it does seem clear that they are not

mediated via the cytotoxic mechanisms typical of chemotherapeutic agents. The hope that retinoids might become an effective tool to halt the progression of some neoplastic diseases, seems to be justified.

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TABLE 1

Sensitivity of seven transformed cell lines to inhibition of clone formation (in methyl cellulose) by retinoid acid as expressed by the 50% clonal inhibitory dose (CID_{50}). (From Dion et al., Exp. Cell Res. 117, 17, 1978).

Cell line	Species	Tissue source	CID_{50} [a]
L929	Mouse	Connective	2.1×10^{-9}
B16C3	Mouse	Melanoma	1.2×10^{-8}
HeLa	Human	Cervical carcinoma	4.5×10^{-7}
AV_3	Human	Amnion	$>1.6 \times 10^{-5}$
SVA31	Mouse	Connective	$>1.6 \times 10^{-5}$
L132	Human	Lung	$>1.6 \times 10^{-5}$
CHO	Hamster	Ovary	$>1.6 \times 10^{-5}$

[a] CID_{50} is expressed as retinoic acid molarity.

TABLE 2

Doses of retinoids that when administered topically, inhibit 50% of TPA-induced mouse epidermal ODC activity

(Modified from Verma, A. K. et al.; Cancer Res. 38: 793, 1978)

Retinoid	Median inhibitory dose (nmoles)
DMECP analog of retinoic acid	0.09
β-Retinoic acid	0.12
13-cis-Retinal	0.14
α-Retinoic acid	0.20
8-Fluoro-TMMP analog of methyl retinoate	0.21
13-cis-Retinoic acid	0.24
5,6-Dihydroretinoic acid	0.43
DACP analog of retinoic acid	0.54
12-Fluoro-TMMP analog of ethyl retinoate	5.00
10-Fluoro-TMMP analog of methyl retinoate	8.90
TMMP analog of retinoic acid	12.8
TMMP analog of ethyl retinoate	14.0
Trimethylthiophene analog of retinoic acid	16.4
TMMP thio analog of retinoic acid	32.0
Lactone of retinoic acid	60.0
10-Fluoro-TMMP analog of 13-cis-methyl retinoate	139
Phenyl analog of ethyl retinoate	192
TMHP analog of ethyl retinoate	400
TMMP analog of N-ethylretinamide	400
9-cis-10-Fluoro-TMMP analog of methyl retinoate	540
N-(2-Hydroxyethyl)retinamide	540
Furyl analog of retinoic acid	NA[a]
13-Trifluoromethyl-TMMP analog of ethyl retinoate	NA

[a] NA, not active

[b] TPA, 12-O-tetradecanoylphorbol-13-acetate; ODC, ornithine decarboxylase; DMECP, dimethylmethoxyethylcyclopentenyl; DACP, dimethylacetylcyclopentenyl; TMMP, trimethylmethoxyphenyl; TMHP, trimethylhydroxyphenyl

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TABLE 3

Effect of 13-cis-retinoic acid: incidence of bladder neoplasms in each treatment group (Modified from Becci, P. J. et al.; Cancer Research 38: 4463, 1978)

No. of mice	Total dose of OH-BBN[e] (mg)	Dose of 13-cis-retinoic acid (mg/kg diet)	Total bladder neoplasms[a]		Transitional and squamous cell carcinoma		Squamous cell carcinoma	
			No. of mice	No. of bladder areas	No. of mice	No. of bladder areas	No. of mice	No. of bladder areas
24	90	None	9(38)[b]	11(23)	8(33)	10(21)	6(25)	8(17)
19	90	200	1(5)[c]	1(3)[d]	0(0)[d]	0(0)[d]	0(0)[c]	0(0)[c]
22	140	None	12(55)	16(36)	10(45)	14(32)	6(27)	9(20)
25	140	200	8(32)	9(18)[c]	7(28)	8(16)	5(20)	6(12)
10	None	None	0	0	0	0	0	0
10	None	200	0	0	0	0	0	0

[a]Bladder neoplasms included carcinomas and noninvasive transitional cell papillomas.

[b]Numbers in parentheses, percentage.

[c]Significantly different from respective control; $p<0.05$.

[d]Significantly different from respective control; $p<0.01$.

[e]OH-BBN = N-butyl-N-(4-hydroxy butyl)nitrosamine.

TABLE 4

Relative risk of dying from tracheal tumors in groups treated with retinoids[a]

(Modified from Yarita, T. et al.; J. Natl. Cancer Inst., submitted, 1979)

No. of NMU exposures	Retinoid concentration (per kg of diet)	Relative risk	P
18	150 mg Ro11-1430	0.67	0.097
	75 mg Ro11-1430	0.85	0.347
	128 mg 13-cis-retinoic acid	1.71	0.223
20	150 mg Ro11-1430	0.90	0.393
23	150 mg Ro11-1430	0.84	0.353
	172 mg 13-cis-retinoic acid	2.01	0.043

[a]The retinoid-treated groups were compared with their respective placebo control groups.

Hamsters were exposed 18-23 times 1% N-nitroso-N-methylurea using a tracheal exposure method. Ro 11-1430 is the 4-methoxy-2,3,6 trimethyl phenyl analog of retinoic acid ethyl amide.

TABLE 5

Effect of retinyl acetate (RA) on the incidence of squamous cell carcinomas in rats[a]

(Modified from Nettesheim, P. et al.; Environmental Health Perspectives, in press, 1979)

	Carcinogen (3 methylcholanthrene) dose (mg)								
	10		5		2.5		1.3		TBA
RA/week (μg)	TBA[b] (%)	MST[c] (week)	TBA (%)	MST (week)	TBA (%)	MST (week)	TBA (%)	MST (week)	% Combined[d]
1744.0	66	77	20	104	9	110	10	111	24
174.0	40	85	21	100	10	105	0	112	16
17.4	93	70	65	82	27	101	23	103	48

[a] Each of the 12 subgroups consists of 15–22 "effective" animals (233 rats total). 3-MCA was administered i.t.; the RA i.g.

[b] TBA, tumor-bearing animals. All tumors were invasive squamous carcinomas.

[c] MST = mean survival time.

[d] For each RA level % TBA of all carcinogen dose groups were combined, there were 77–79 rats per RA level.

TABLE 6

Comparison of the inhibition of tumor development by varying doses of intraperitoneal retinyl palmitate. The significance of the differences was measured by the chi-square test. For the saline (S) versus the A_2 group $P<.01$. For the saline versus the A_3 group, $P<.005$.

(Modified from Felix, E. L. et al.; Science 189: 886, 1975)

Group	Dose of retinyl palmitate (units/day x 5 days)	No. of mice with tumor/ No. of mice inoculated
S	0	20/25
A_1	2500	16/28
A_2	3500	9/22
A_3	5000	4/17

TABLE 7

Inhibition of Growth of a Chondrosarcoma in Fischer Rats by Various Retinoids

Two Week Studies

(Modified from Trown, P. W. et al.; Cancer Treatment Reports 60: 1647, 1976)

Daily Dose[1] mg/kg	% Inhibition[2] Ro 10-1670 ip	Ro 10-9359 ip	Ro 11-1430 ip
80	91.4	--	94.6
40	63.8	78.6	83.8
20	75.1	73.4	77.8
10	63.1	73.5	61.1
5	40.5	36.3	33.5
2.5	34.6	--	11.5

[1] Drugs were suspended in 0.1% carboxymethylcellulose in water containing a trace of Triton X-100 by sonication and administered daily, Mon.-Fri., each week. Ro 10-1670 = all-trans-9-(4-methoxy-2,3,6-trimethylphenyl)-3,7-dimethyl-2,4,6,8-nonatetraenoic acid; Ro 10-9359 is its ethyl ester and Ro 11-1430 is its ethylamide.

[2] Calculated using the formula $C-T \div C \times 100\%$ where C = the mean tumor weight for control animals and T = the mean tumor weight for treated animals. Groups of 8 animals were used for each experiment and the % inhibitions for each experiment were averaged.

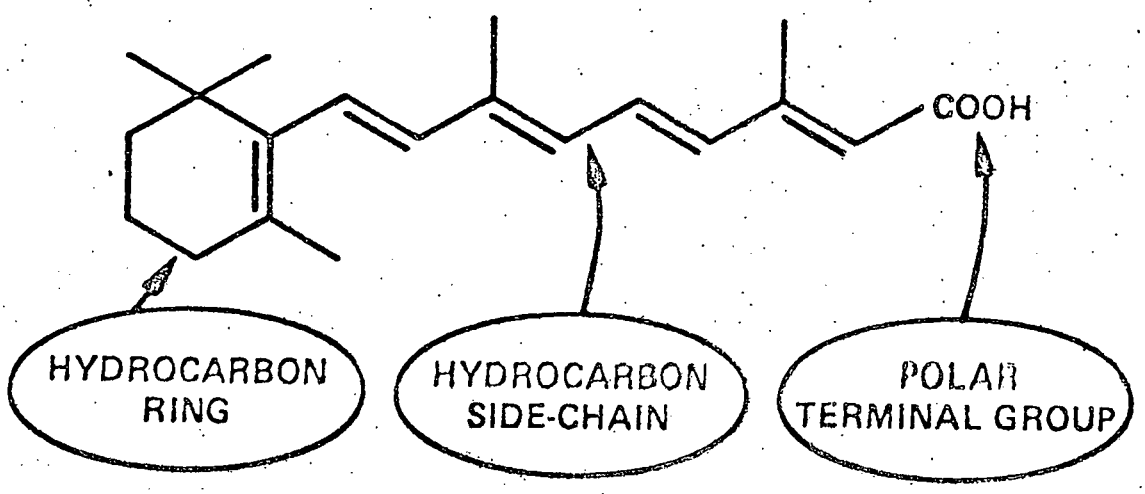

Figure 1

Components of the retinoid molecule. The structure shown is all-*trans*-β-retinoic acid. (Modified from Sporn, M. B. et al.; Federation Proceedings 35: 1332, 1976)

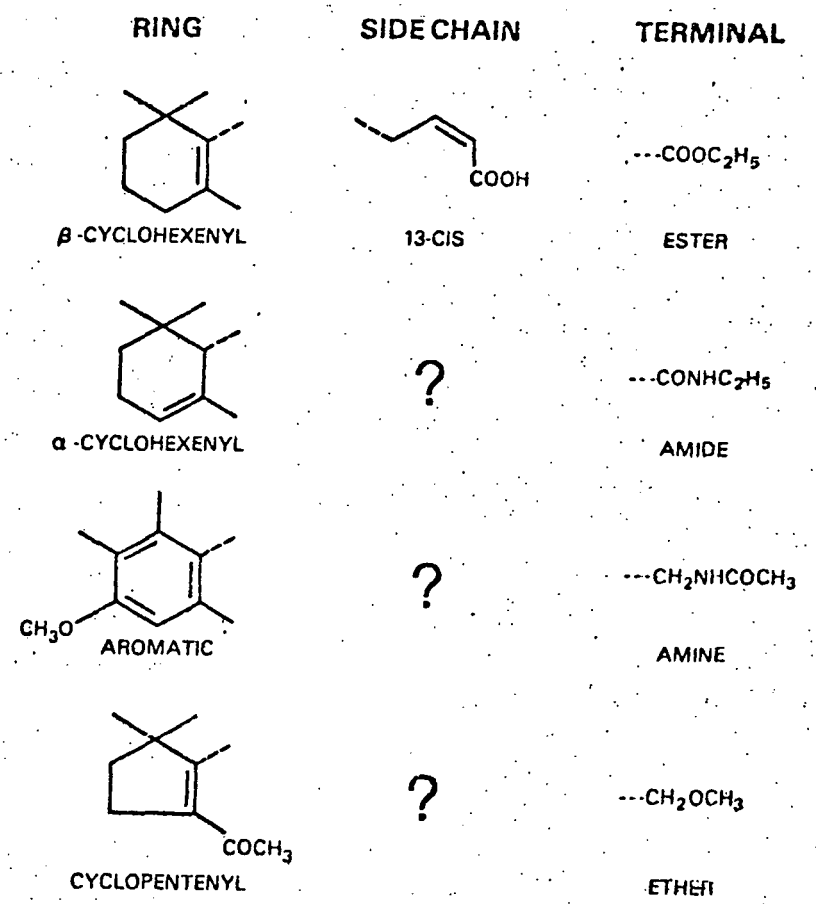

Figure 2

Modifications of the reinoid molecule which have significant biological activity. The question marks under SIDE CHAIN indicate the relative lack of progress in this area. (Modified from Sporn, M. B. et al.; Federation Proceedings 35: 1332, 1976)

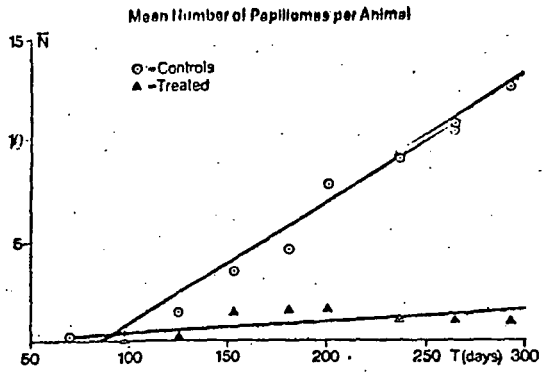

Figure 3

Ro 10-9359 is an aromatic retinoic acid analog (30 mg/kg body weight, per day); 66 mice per group. Mean number of papillomas per animal in controls [⊙] and Ro 10-9359 treated mice [△]. $\bar{N}$ = Mean number of papillomas per animal; T = Days after first application of carcinogen. (Modified from Bollag, W.; Europ. J. Cancer 11: 721, 1975)

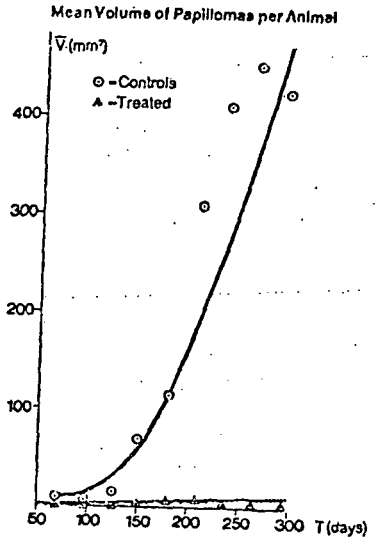

Figure 4

Ro 10-9359 is an aromatic retinoic acid analog (30 mg/kg body weight, per day); 66 mice per group. Mean volume of papillomas per animal in controls [⊙] and Ro 10-9359 treated mice [△]. $\bar{V}$ = Mean volume of papillomas per animal (mm^3); T = Days after first application of carcinogen. (Modified from Bollag, W.; Europ. J. Cancer 11: 721, 1975)

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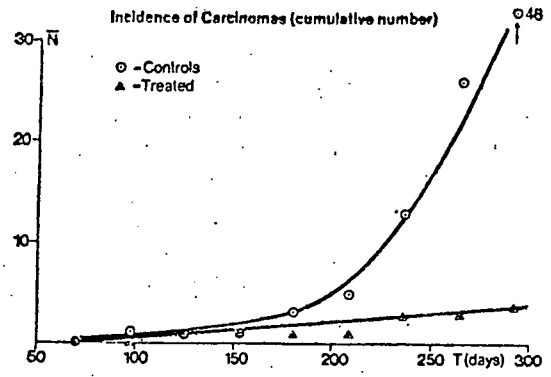

Figure 5

Ro 10-9359 is an aromatic retinoic acid analog (30 mg/kg body weight, per day); 66 mice per group. Incidence of carcinomas (cumulative number) in controls [⊙] and Ro 10-9359 treated mice [△]. $\overline{N}$ = Number of carcinomas; T = Days after first application of carcinogen. (Modified from Bollag, W.; Europ. J. Cancer 11: 721, 1975)

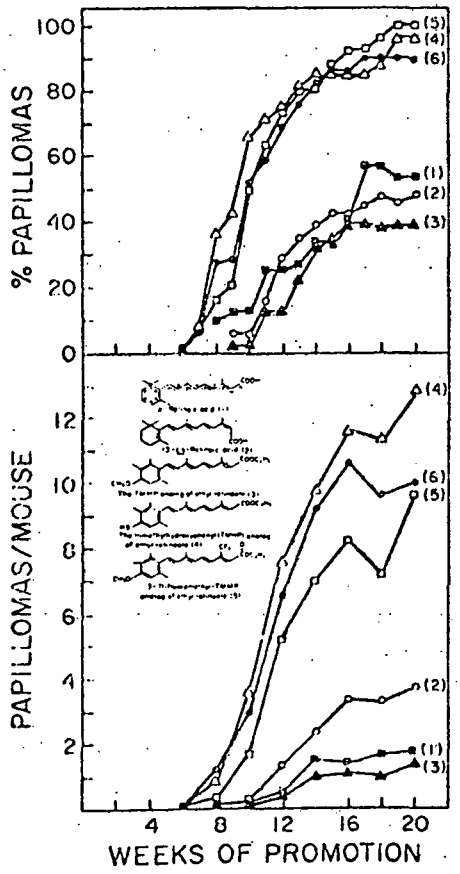

Figure 6

The effect of various retinoids on skin tumor promotion. All mice were initiated and promoted . Retinoids were applied 1 hr before each promotion with 8 nmol of TPA. Doses for retinoids were 34 nmol for retinoic acid and 13-cis-retinoic acid and 140 nmol for the TMMP analog of ethyl retinoate, the TMHP analog of ethyl retinoate, and the 13-trifluoromethyl-TMMP analog of ethyl retinoate. The control mice (curve 6) were pretreated with acetone only. TMMP = trimethylmethoxyphenyl; TMHP - trimethylhydroxyphenyl. (Modified from Verma, A. K. et al., Cancer Research 39: 419, 1979)

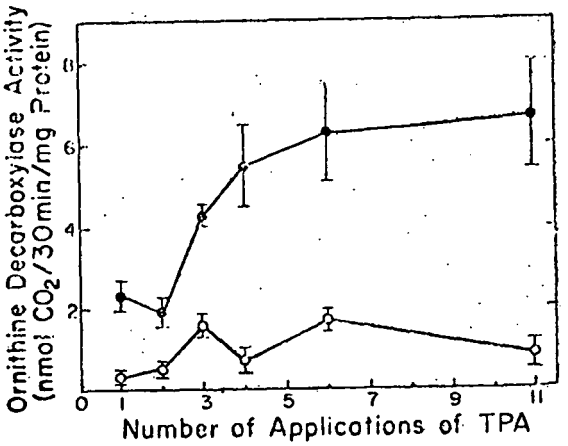

Figure 7

The effect of repeated applications of TPA on induction of epidermal ODC activity and its inhibition by retinoic acid pretreatments. Mice were initiated with 0.2 μmol of DMBA in 0.2 ml of acetone; 14 days later, mice were treated with either 0.2 ml of acetone (❂) or 1.7 nmol of retinoic acid (○) in 0.2 ml of acetone 1 hr before treatment with 17 nmol of TPA on Days 1 and 4 of each week. Mice were killed 4.5 hr after TPA treatment, and ODC activity in soluble epidermal homogenates was determined. Each point is the mean ± S.E. (bars) of the determinations carried out in 3 groups of 3 mice each. ODC activity was not determined beyond the 11th application of TPA because the mice started bearing papillomas. (Modified from Verma, A. K. et al., Cancer Research 39: 419, 1979)

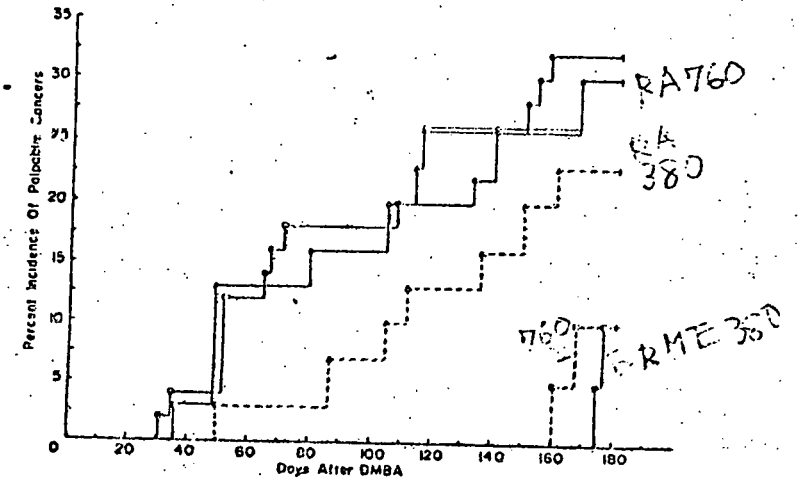

Figure 8

The effect of retinyl methyl ether and retinyl acetate on the time of appearance of palpable mammary cancers that were confirmed histologically. Animals were placed on the various retinoid diets 1 week after the intragastric instillation of 5 mg DMBA. The rats were palpated for mammary tumors twice weekly for the duration of the experiment. Diets fed (μmoles of retinoid per kg of diet) were: ● , placebo; ■——■ , retinyl methyl ether, 380; ■- - -■ , retinyl methyl ether, 760; ▲——▲ retinyl acetate, 380; ▲- - -▲ retinyl acetate, 760.

(Modified from Grubbs, C. J. et al.; Cancer Research 37: 599, 1977)