

**ADVANCES IN
PHARMACOLOGY AND THERAPEUTICS**
**Proceedings of the 7th International
Congress of Pharmacology**

General Editors: J. R. BOISSIER, P. LECHAT & J. FICHELE

Volume 8

**DRUG-ACTION MODIFICATIONS
COMPARATIVE PHARMACOLOGY**

Editor: G. OLIVE

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Introduction

The scientific contributions at the 7th International Congress of Pharmacology were of considerable merit. Apart from the sessions organised in advance, more than 2,200 papers were presented, either verbally or in the form of posters, and the abundance of the latter in the congress hall is a good indication that this particular medium of communication is becoming increasingly attractive to research workers, and offers scope for discussions which combine an elaborate, thorough approach with a certain informality.

It would have been preferable to have published the entire congress proceedings within the framework of the reports. That was, however, physically impossible, and the organisers had to adopt a realistic solution by publishing only the main lectures, symposia and methodological seminars. The amount of material presented necessitated the printing of ten volumes, each volume containing congress topics regrouped according to their relevant content and subject areas. This system of division may give rise to criticism on account of its artificiality, and we readily admit that certain texts could have been placed in more than one volume. We are asking the reader to excuse this arbitrariness, which is due to the editors' personal points of view.

I draw attention to the fact that most of the symposia finish with a commentary which the chairmen had the option of including, presenting their personal opinions on one or several points. We think that such an addition will facilitate reflection, discussion, indeed even controversy.

The launching of the scientific programme for this congress began in September 1975 on returning from the last meeting in Helsinki. Long and delicate discussions took place in the Scientific Programme Committee and with the International Advisory Board. Should it be a pioneer, 'avant-garde' congress? Or one laid out like a balance-sheet? Should we restrict the congress to the traditional bounds of pharmacology, or extend the range of papers to cover the finest discipline? The choice was difficult, and the result has been a blend of the two, which each participant will have appreciated in terms of his training, his tastes, and his own research.

A certain number of options, however, were taken deliberately: wide scope was given to toxicology, from different points of view, and to clinical pharmacology, a subject much discussed yet so badly practised; the founding of two symposia devoted

to chemotherapy of parasitic diseases which are still plagues and scourges in certain parts of the world; a modest but firm overture in the field of immunopharmacology, which up until now was something of a poor relation reserved only for clinical physicians; the extension of methodological seminars, in view of the fact that new techniques are indispensable to the development of a discipline.

We have been aware since the beginning that, out of over 4,000 participants who made the journey to Paris, not one could assimilate such a huge body of knowledge. Our wish is that the reading of these reports will allow all of them to become aware of the fantastic evolution of pharmacology in the course of these latter years. If one considers pharmacology as the study of the interactions between a "substance" and a living organism, then there is no other interpretation. Nevertheless, one must admit that there exists a period for describing and analysing a pharmacological effect, and that it is only afterwards that the working mechanism can be specified; a mechanism which will permit these "substances" to be used for the dismantling and breaking down of physiological mechanisms, a process which justifies Claude BERNARD'S term, "chemical scalpel".

The reader will be able to profit equally from more down-to-earth contributions, more applied to therapeutics, and less "noble", perhaps, for the research worker. He will realise then that his work, his research and his creative genius are first and foremost in the service of Man, and will remember this statement from Louis PASTEUR:

"Let us not share the opinion of these narrow minds who scorn everything in science which does not have an immediate application, but let us not neglect the practical consequences of discovery."

I would like to renew my thanks to my colleagues in the Scientific Programme Committee and also to the members of the International Advisory Board, whose advice has been invaluable. I owe a particular thought to J J BURNS, now the past-president of IUPHAR, who granted me a support which is never discussed, and a staunch, sincere friendship. The Chairmen have effected an admirable achievement in the organisation of their proceedings, and in making a difficult choice from the most qualified speakers. The latter equally deserve our gratitude for having presented papers of such high quality, and for having submitted their manuscripts in good time.

The publisher, Robert MAXWELL, has, as always, put his kindness and efficiency at our service in order to carry out the publication of these reports. But none of it would have been possible without the work and competence of Miss IVIMY, whom I would like to thank personally.

My thanks again to the editors of the volumes who, in the middle of the holiday period, did not hesitate to work on the manuscripts in order to keep to the completion date.

Finally, a big thank you to all my collaborators, research workers, technicians and secretaries who have put their whole hearts into the service of pharmacology. They have contributed to the realisation of our hopes for this 7th International Congress, the great festival of Pharmacology. Make an appointment for the next one, in 1981, in Tokyo.

Jacques R BOISSIER
Chairman
Scientific Programme Committee

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Influence of Nutritional Factors on Toxicity

An Overview of the Problem with Emphasis on the Use of Discreet Nutritional Manipulations in the Elucidation of Toxic Mechanisms

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The role of nutrition in health and disease has been appreciated for many years. That the diet may modify responses to therapeutic agents or the adverse effects of incidental chemicals is also well known. Additionally, special attention has been afforded to the topic of nutrition in cancer causation (1,2).

Nutritional manipulation of diets for the purposes of studying the alteration in response to exogenous agents centers on "macro" alterations (protein, lipid or carbohydrate) or "micro" alterations (involving discreet nutritional substances).

The present symposium seeks to explore the influence of various nutritional factors on toxicity and especially seeks to explore how nutritional manipulations may assist in the elucidation of toxic mechanisms.

An example of what may be learned by applying nutritional manipulation to the study of toxic mechanisms is illustrated by the work of Bus, Aust, Cagen and Gibson (3,4,5,6). Paraquat (1,1'-dimethyl-4,4'-bipyridilium) is a widely used herbicide which undergoes a one-electron reduction in vivo to form a free radical. Reduced paraquat is, however, readily re-oxidized in vivo in the presence of oxygen.

Paraquat causes lethality in man and animals due to pulmonary damage involving edema formation and eventually interstitial fibrosis. The pulmonary damage resembles that produced by oxygen, an observation which is of considerable interest since the lethality of oxygen is enhanced by acute or chronic paraquat exposure (7). Experimental evidence has been obtained which strongly supports the idea that paraquat induces tissue damage during its cyclic reduction-oxidation through the sequential generation of superoxide radicals and singlet oxygen, followed by the initiation of membrane lipid peroxidation (8). Evidence for this sequence of events has been provided by the fact that mouse lung microsomes incubated in vitro catalyze an NADPH-dependent paraquat reduction. Furthermore, the addition of paraquat to an enzymatic system which supports its oxidation and reduction, stimulates peroxidation of microsomal lipid, which is inhibited by the addition of a singlet oxygen trapping agent, or the enzyme superoxide dismutase.

In vivo, the toxicity of paraquat is increased by nutritional deficiencies of vitamin E, selenium or chemical depletion of reduced glutathione.

These observations support the hypothesis presented above that paraquat initiates lipid peroxidation and produces damage to cell membranes. The relationship between

lipid peroxidation and the observed potentiation of paraquat toxicity by nutritional manipulation is related in the following way. Biochemical defense systems which exist in mammalian organisms may prevent the propagation of free radical reactions that occur in the lipid peroxidation process. Among these are the antioxidants ascorbic acid, glutathione and the tocopherols. Antioxidants function by allowing a hydrogen to be abstracted from themselves rather than from the allylic hydrogen of an unsaturated lipid. Furthermore, it is known that the enzyme glutathione peroxidase detoxifies hydrogen peroxide and hydroperoxides which arise from lipid peroxidation reactions. Selenium is a necessary cofactor for glutathione peroxidase. Selenium deficiency reduces the activity of the enzyme and increases tissue susceptibility to peroxidation reactions.

The presentations of this symposium explore the role of several protective systems having a nutritional basis in a variety of drug or chemical toxicities. Clearly, the body of knowledge in nutrition is now sufficient to allow for the application of that knowledge in the study of toxic mechanisms.

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The Role of Retinoids in the Prevention of Carcinogenesis

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Cancer is a multifactorial disease characterized by a long development period preceding the final phase of invasive malignancy. In many systems, most notably mouse skin (reviewed in Ref. 1) and rat liver (2), carcinogenesis can be divided into two stages, initiation and progression or promotion. The initiation phase is rapid and irreversible, while the progression phase is of a variable time period and is reversible in its early stages. It is now established that retinoids, which play an essential role in maintaining the proper differentiated state in epithelia, can interfere with the progression phase of carcinogenesis in epithelial tissues, such as lung, skin, breast, and bladder (3,4). The retinoids, possibly acting as anti-promoters, are able to prevent the dedifferentiation characteristic of malignant transformation and are able to maintain the initiated cells in a normal differentiated state.

Early evidence for this effect of retinoids on the progression of carcinogenesis was provided by Lasnitzki (5) who showed that the precancerous dysplasia resulting from treatment of organ cultures of mouse prostate gland with the carcinogen 3-methylcholanthrene, could be reversed by the addition of retinyl acetate to the culture medium. More recently, Merriman and Bertram (6), also using 3-methylcholanthrene, have shown that retinyl acetate can prevent malignant transformation in cultured mouse fibroblasts. In both of these experiments, the retinoid was added after the initiation period and was in constant supply in the culture medium for the duration of the experiment. Direct evidence of the anti-promoter activity of retinoids has come from investigations by Verma and Boutwell (7) into the action of a tumor-promoting agent, 12-O-tetradecanoyl-phorbol-13-acetate (TPA), on mouse skin. The rapid induction of epidermal ornithine decarboxylase by TPA is inhibited by retinoids applied to the skin just prior to treatment with the promoting agent. The same sequential application to the skin of retinoid and promoter also leads to a reduction in the number of papillomas per mouse in animals that have been pretreated with 7,12-dimethylbenzanthracene.

Another example of the effective use of retinoids in the prevention of cancer in experimental animals is provided by a recent study by Moon *et al.* (8) of mammary cancer induced in rats by N-methyl-N-nitrosourea. Animals placed on a diet containing beadleted retinyl acetate (250 mg per kg of diet) were found to have a cancer incidence less than 50% that of a control group fed a placebo diet. Dietary supplementation of retinoids was begun 3 days after the last dose of carcinogen, again precluding the interference of retinoids in the initiation phase

of carcinogenesis. The efficacy of the retinoid increased as the dose of carcinogen was reduced towards a level that gave cancer incidence rates in the range of that seen in human populations. Retinoid administration not only decreased the incidence of the cancer, but also reduced the actual number of tumors, and perhaps most importantly, greatly increased the latent period for the development of the tumors. This last observation could be the most significant one with regard to the prevention of human cancers.

From the foregoing, it can be concluded that the chemopreventive role of retinoids now has a broad scientific base. The difficulty lies in its practical clinical application. The requirement that retinoids be given for very long periods of time, and the fact that the patient would be one at high risk for the development of cancer, but yet a seemingly healthy individual with no immediate life-threatening disease, necessitates a safe regimen without undesirable side effects. The natural retinoids, all-*trans*-retinol, retinal, retinyl esters, and retinoic acid, do not meet these criteria. The more reduced forms, retinol and retinal, are interconvertible and are sequestered by the liver in the form of long-chain retinyl esters. Release of the vitamin from the liver is highly regulated and is in the form of retinol bound to a specific carrier protein, retinol-binding protein (9). Because of this, plasma retinoid levels are not proportional to dose, and high effective target tissue concentrations cannot be reached. Furthermore, excessive liver deposition of retinyl esters can lead to toxic effects in that organ (10). Retinoic acid, on the other hand, is formed irreversibly from retinal, cannot be stored, and is rapidly metabolized and excreted (11). However, it, like retinol and retinal, is intrinsically toxic, causing a myriad of effects including the lysis of membranes (12) and bone fractures (13).

The clinical use of retinoids for the chemoprevention of cancer therefore involves the synthesis of new retinoids with improved pharmacokinetics, targeted tissue distribution, and lesser toxicity compared to the parent compounds retinol, retinal, and retinoic acid. Numerous synthetic analogs with an altered ring, side chain, or terminus (Fig. 1) have been made and have been tested by several criteria (14-16). Most recent work in this laboratory, however, has focused on two aspects of this problem which will form the basis of this report. One has been a study of the effects of endgroup modifications of the retinoids on their

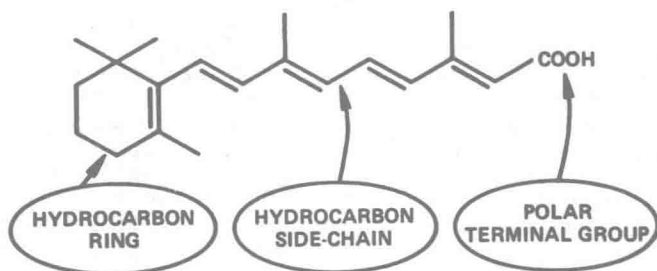


Fig. 1. Retinoid structure. The compound shown is all-*trans*-retinoic acid. Change of the terminus to the corresponding aldehyde or alcohol results in retinal, or retinol, respectively. Reprinted with permission from (4).

properties. Specific classes of compounds that will be discussed include ether derivatives of retinol, diketone adducts of retinal, and amide derivatives of retinoic acid. The other approach has been a systematic investigation of the metabolism of retinoic acid in an epithelial target tissue with the goal of identifying specific activating or deactivating sites on the molecule.

Testing of Synthetic Retinoids

Essential to any investigation into structure-function relationships are a series of reliable assays for the parameters of interest. Before any new retinoid is tested for efficacy in the prevention of carcinogenesis, it is first assayed for in vitro activity in the control of epithelial cell differentiation, for in vitro toxicity to cartilage, for in vivo toxicity as measured by growth inhibition, and for tissue distribution following dosing at pharmacological levels. The first criterion that must be met by any new retinoid is activity in the control of epithelial cell differentiation. This is conveniently tested by a tracheal organ culture (TOC) assay developed in our laboratory (17). In this assay, tracheas from vitamin A-deficient hamsters are cultured in a vitamin A-deficient medium for 3 days. At this time, 50-75% of the epithelium has undergone squamous metaplasia, with the additional marker of the presence of keratohyalin granules and keratin. The addition to the medium of a retinoid, usually at 10^{-8} to 10^{-10} M, can reverse the differentiation pattern to that of a normal ciliated and mucus-secreting epithelium within a week. Slides prepared from the trachea are then scored for the degree of reversal of the keratinizing metaplasia. Comparison of the activity of a series of analogs in this assay with their activity as chemopreventive agents has been made with regard to the anti-promoter effect of retinoids in the ornithine decarboxylase test system of Verma *et al.* (5,18) and with the effect of retinoids on the reversal of malignant transformation in mouse prostate organ culture (19). With a few exceptions, good qualitative agreement has been found, indicating that activity in the TOC assay is predictive of the effects of retinoids on preneoplasia. The intrinsic toxicity of retinoids is measured in TOC by quantitating the percentage of total cartilage proteoglycan released into the medium (14). The basic procedure was developed by Goodman *et al.* (20) for measuring the effects of retinoids on rabbit ear cartilage in organ culture. These two in vitro assays can be carried out with less than 1 mg retinoid and serve as screening devices for the selection of retinoids for further testing. In vivo tests of toxicity and measurements of tissue distribution require gram quantities of synthetic retinoids and are reserved for those analogs which appear to be particularly promising based on the results of the in vitro tests.

It is apparent from the results of our tests (14) and from the work of other researchers using diverse systems (19,20,21) that the toxic effects of retinoids are independent from their activity either in the TOC assay or in the reversal of preneoplasia. Therefore, an additional selection criterion for in vivo testing of a synthetic analog should be an improved therapeutic ratio over that of the parent retinoid, i.e. reduced in vitro toxicity relative to in vitro activity. As an example, retinyl methyl ether has a ten-fold greater therapeutic ratio than does retinoic acid, based on the in vitro assays described (14).

In addition to the above considerations, the oxidation state of a synthetic analog should perhaps be taken into account. Two types of intracellular binding proteins, one specific for retinol (22), and one specific for retinoic acid (23,24) have recently been discovered. Each of these binding proteins has been shown to bind retinoids with altered ring structures, but to have an absolute requirement for the hydroxyl or carboxyl terminus. The function of these proteins is presently unknown. However, the specificity for the endgroup oxidation state, together with the observation that certain tumors have been reported to contain

elevated concentrations of the retinoic acid binding protein (25) suggest that different cell types might require different oxidation states of the parent retinoid, as is already known to be the case for the various functions of the vitamin in reproduction (alcohol), vision (aldehyde), and growth (acid). Furthermore, if binding of a retinoid to these proteins is obligatory in the control of differentiation, endgroup derivatives would be active only after cleavage to the parent retinoid. At the present time, these two considerations remain highly conjectural, although indirect evidence will be presented for the latter, particularly for ether and amide derivatives.

Endgroup Modifications of Retinoids

Representative endgroup derivatives of retinol, retinal, and retinoic acid have been studied in our laboratory. At the alcohol level of oxidation, two ethers, retinyl methyl ether and retinyl butyl ether have been extensively tested (14). The TOC activity of retinyl methyl ether is only marginally less than that of retinol but is ten-fold greater than that of the butyl ether derivative. Masking of the alcohol function greatly reduces the intrinsic toxicity of the analogs. Tissue distribution is altered as well, due in part to the decreased polarity of the terminus. It was found that retinoid levels in the breast of rats were 9-fold higher following dosing with retinyl methyl ether than with retinyl acetate. All the test criteria having been met, retinyl methyl ether was tested for its effectiveness in the prevention of mammary carcinoma induced by 7,12-dimethylbenzanthracene. Correlating well with the increased levels of retinoid in the breast tissue, retinyl methyl ether was shown by Grubbs *et al.* (26) to be 3-4 times as effective as retinyl acetate both in increasing the latency period, and in reducing the numbers of palpable mammary tumors (Fig 2).

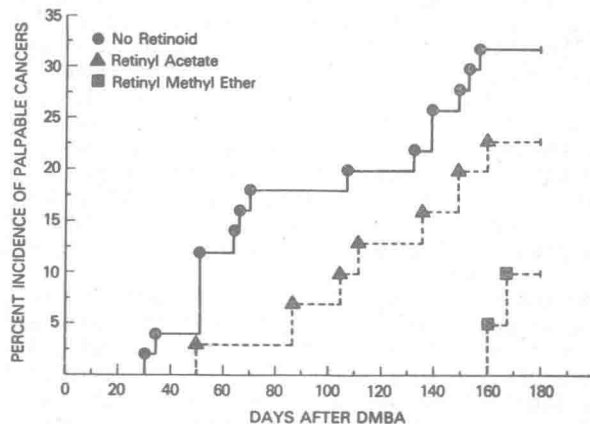


Fig. 2. The effect of retinyl acetate and retinyl methyl ether on the time of appearance of palpable mammary cancers that were confirmed histologically. Virgin female Sprague Dawley rats were placed on the various retinoid diets 1 week after the intragastric instillation of 5 mg of 7,12-dimethylbenzanthracene. The diets contained 760 μ moles retinoid per kg of diet. Modified with permission from (26).