My Years in Lipid Research

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Thanks to the American Oil Chemists' Society and the award sponsor, Supelco Co., for the award I had the honor to receive and for the opportunity afforded to me to talk "on a subject of my own choosing."

I look back on my presence in the arena and pick out some things not easily found in the literature, not even with the most advanced information retrieval techniques. It is a kind of "Recollection of a Travelling Chemist" (1).

Lipid-soluble vitamins constitute part of lipid chemistry. Therefore, I feel I can spend some time on the history of vitamin A synthesis. In close collaboration with Arens, not only vitamin A was synthesized, but also vitamin A acid (2), which proved to be as active as vitamin A in a growth test when injected intravenously as its sodium salt into rats (3). At that time, we were fully aware that the acid could not possibly replace vitamin A or its aldehyde in the visual cycle.

At the XIth International Congress of Pure and Applied Chemistry in London, in 1947, I presented our work in a talk entitled: "Relationship between Structure and Biological Activity in the Vitamin A group" and said that it would be interesting to investigate whether vitamin A acid is able to replace vitamin A in the visual cycle. Considering the outcome of investigations of Morton (4) into vitamin A aldehyde, this seemed to be highly improbable, the more so because we did not get any vitamin A from the organs of vitamin A deficient rats fed massive doses of this acid. This was our first evidence that vitamin A has multiple functions. In a survey in the journal "*Produits Pharmaceutiques*," we discussed this problem and a possible antagonism between vitamin A and the acid in the visual cycle (5). The idea that vitamin A acid could not prevent night blindness was put forward by Moore as his own idea (6, 7), which was taken up by Dowling and Wald, who indeed showed in a series of beautiful experiments that this was correct (8, 9). When discussing these experiments, they stated that it had permitted them to separate the mechanism of night blindness from the other actions of vitamin A in the tissues. It appears that this vitamin A and its aldehyde are needed by the rat to perform its visual function. For all other functions of vitamin A, the acid apparently does as well. I heard that statement before! However, I am gratified with the way in which Dowling put vitamin A acid to work. You will understand that I think the name "retenoic acid" does not make much sense, because it does not work in the retina.

Before leaving vitamin A research, just one remark about syntheses: although there are now technical syntheses galore, one possible or impossible project we left unfinished still intrigues me. I do hope someone will carry it through. I warn you, however, that it has no commercial benefits. Figure 1 shows the formula of a compound we could obtain in nearly 90% yield from β-ionone and a pyridine derivative. Ring-opening—and you see the possibilities. The compound resists, however, the most ferocious attacks (10; D. A. van Dorp, J. P. Ward, and C. M. Lok, unpublished results).

After spending 20 years in the pharmaceutical industry, I fired myself and was looking for another job. I was very fortunate indeed to find a position at the Unilever laboratory at Vlaardingen. The director of research of that laboratory, Jan Boldingh, had succeeded in creating an atmosphere such that applied and basic research formed an ideal mix. I was given the opportunity to set up a department in which organic, bio- and radiochemists could devote themselves to the task of unravelling many puzzles connected with biological studies in the field of nutrition. Head of the biological department was the late H. J. Thomasson (Fig. 2), who was greatly interested in the role of essential fatty acids and made important scientific contributions in this field. His reception was most cordial when I entered the service of the Unilever Research Laboratory. For his work on the structural requirements for EFA activity to take place, polyunsaturated fatty acids with all kinds of structures were needed. The total synthesis of various polyunsaturated fatty acids by Osbond (11) came at the right time, and by partly using his reaction schemes we were soon able to synthesize many compounds with structural variations, a game I had played earlier in vitamin A studies.

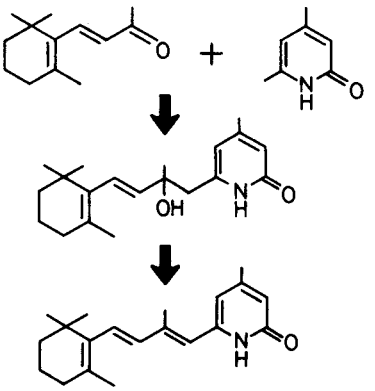

FIG. 1. Unfinished synthesis of vitamin A from β-ionone and a pyridine derivative.

During this period, I pondered about the possible biochemical significance of the U-shaped structure proposed by Holman in 1951 for arachidonic acid (12). His work and that of his colleagues of the Hormel Institute furthered the general insight into lipid nutrition immensely.

This model deserves a picture (Fig. 3) on which the "U" has the shape of a question mark. Is it a question of the mood of the investigator as to how a certain shape appeals to him?

One remark about the synthesis of various acids. Acetylenic acids are often intermediates and also suitable to make labelled compounds. I learned that hydroboration, often claimed to be the method of choice for the *cis*-unsaturated compounds, is not very suitable. Boron compounds, with which the products are always contaminated, are very hard to remove, and the use of these boron-contaminated compounds for biological studies is not advisable. During that same period, my department was also concerned with research related to oleochemicals. An American of Dutch descent, Robert Van Tuyle, had been able to develop the ozonolysis of oleic acid to a technical process.

FIG. 2. H.J. Thomasson.

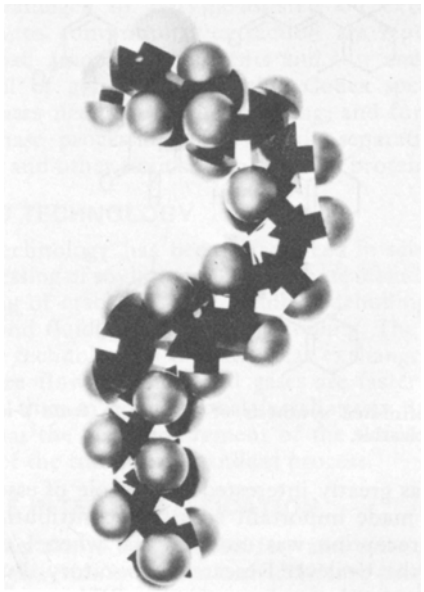

FIG. 3. Arachidonic acid poses a question!

Dutch technology with respect to ozone happened to be a little out of date. In 1785, van Marum of the Teyler Foundation (13) was able to produce it, as he observed that oxygen gas through which electrical sparks had passed developed a peculiar sharp smell, later identified as ozone. His equipment is shown in Figure 4.

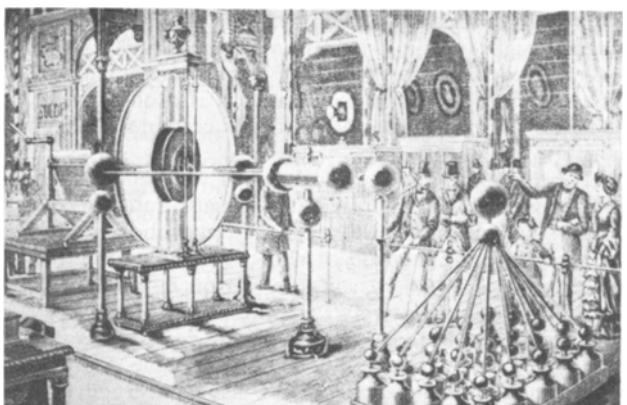

FIG. 4. Van Marum's electrification apparatus.

We looked at what actually happens during ozonolysis and were able to demonstrate that methyl oleate can give rise to six different ozonides, which you see in Figure 5. In fact, this work provided experimental proof of the theory of Criegee on the zwitterion mechanism (14). It taught *me* also that you can handle these very labile compounds quite well and this experience proved useful for the work on essential fatty acids and prostaglandins, because very active peroxides also occur in living animals. Besides, the knowledge that we could handle these ozonides encouraged us to look into the isolation of these natural peroxides also in the pure state.

Let us go back to unsaturated fatty acids. Plants are able to synthesize the unsaturated fatty acids they need, which are all incorporated in the phospholipids which, in their turn, are used as building units of membranes. The chain length and the degree of unsaturation of the fatty acids contribute to specific physical properties of these membranes. We could call these the functions of these acids.

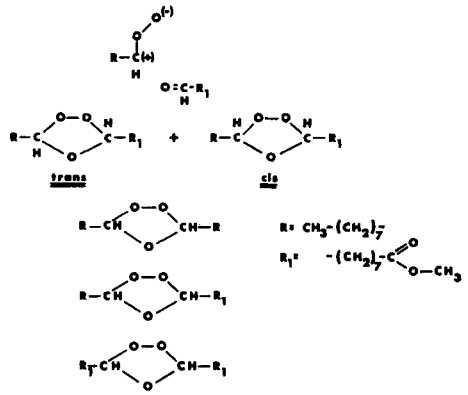

FIG. 5. Six different ozonides (three pairs *cis* and *trans*) (ref. 14).

Another function is, of course, that they can be used as a source of energy. It is not known whether unsaturated fatty acids in the plant have any other function.

In the animal kingdom, the situation is different. Linoleic acid as precursor of arachidonic acid is essential in mammals in the sense that it has to be supplied by the food. Also in the lower animals there are clear indications that specific fatty acids are indeed essential. For the cabbage interlooper (Trichoplusia ni), α-linolenate is an essential nutrient (15), which cannot be replaced by linoleate. It is necessary for the proper development of its wings. Might it be possible that α-linolenate does not act per se, but only after conversion into a biologically active compound (16)? In the animal kingdom, the polyunsaturated fatty acids have structural as well as other functions. Structurally, they are important because of their physical properties with respect to membranes. They also constitute part of certain enzymes with a lipoprotein character. This sounds very similar to what has been said about plants. But in animals the acids of the n-6 types, such as linoleic acid and the dihomo-γ-linolenic and arachidonic acids derived from it, take part in a number of fascinating reactions leading to prostaglandins, thromboxanes and prostacyclins.

(After this lecture was presented, Dr. Brady A. Vick, from the US Department of Agriculture, North Dakota, handed me a reprint of his very recent publication on "The biosynthesis of jasmonic acid: a physiological role for plant lipoxygenase", published in *Biochemical and Biophysical Research Communications* 3 (2):470-477 [1983]. In this article the authors describe very clearly how linoleic acid via 12-oxophytodienoic acid is converted into jasmonic acid, a plant growth regulator in *Vicia faba*. This proves that the difference in function of unsaturated fatty acids between the plant and animal kingdom is not as strict as presented in this lecture.)

On the 20th of August 1963 I wrote a memo on "Prostaglandins and Related Factors", which started as follows:

> "In the J. Am. Chem. Soc. 85: 1878 (1963), an article has appeared that had my utmost attention, because the biologically very active compounds described therein could, in my opinion, form the key to the mystery of the essential fatty acids."

I developed the idea that the structures of these compounds, called prostaglandins by their Swedish discoverer von Euler, provoke associations with dihomo-γ-linolenic, arachidonic and an ω3, C20 pentaenoic acid. The U-form of arachidonic acid helped me to conclude that an oxidative ring closure was involved. In other words: essential fatty acids could be the precursors of prostaglandins. How Bergström contacted me to get hold of labelled arachidonic acid

is a well known story.

The state of affairs around 1978 has been summarized in Figure 6. It should be pointed out that the compounds derived from the pentaenoic acid are far less active and, in my opinion, not very important. However, highly unsaturated fatty acids derived from α-linolenic acid with five and six double bonds are constituents of brain lipids—their function has not yet been elucidated. Two unstable endoperoxides, PGG_2 and H_2, are intermediates in the process towards prostaglandins. Their structures are shown in Figure 7. The final piece of evidence for the structure of the end product PGH_2 which we had isolated in a pure state, is shown in Figure 8. Using a 300 MHz NMR apparatus, we were able to get a perfect spectrum of this unstable compound by running it at low temperature and integrating 250 recordings (17). We needed only 120 μg of material. There was also a spectrum in the literature, but that must have been taken of a very impure product. The central role of the endoperoxides in the formation of a good many products is shown in Figure 9.

You can see that arachidonic acid is a jack-of-all-trades and is a multifunctional performer just like vitamin A. I am not going to discuss the enzymes in the many reactions that take place, nor the biological properties of the products. With one exception: Kloeze of the Unilever Research Laboratory established that prostaglandin E_1 influences ADP-induced platelet aggregation (18) and that PGE_2 has only a weak effect. The follow-up of this line of research has ardently taken place all over the world. As Kloeze is here and will talk about this subject, I would leave it at that.

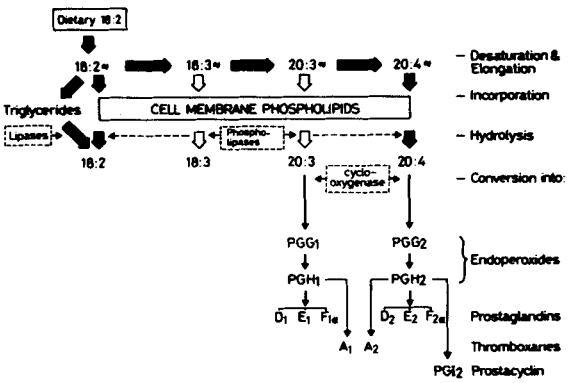

FIG. 6. What happens to linoleic acid in the body?

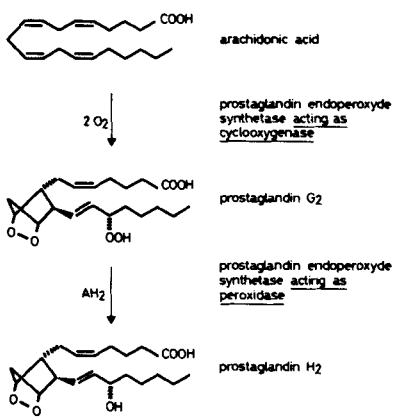

FIG. 7. Activity of pure prostaglandin endoperoxide synthetase.

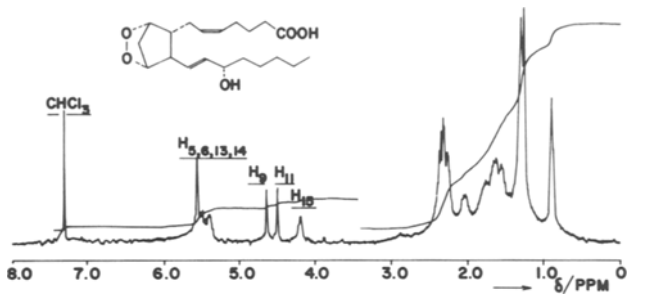

FIG. 8. The 300 MHz ^{1}H-NMR spectrum of 120 μg PGH_2 in $CDCl_3$ at -21 C. The sharp signals at δ 4.50 and 4.64 are due to the endoperoxide bridgehead protons H_9 and H_{11}. The double-bond protons absorb at δ 5.4-5.6 and H_{15} at δ 4.20. The upfield part shows a small alkane type contamination (δ = 1.24).

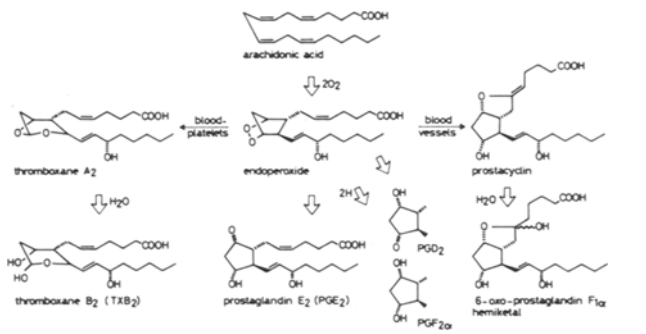

FIG. 9. Transformations of prostaglandin endoperoxides.

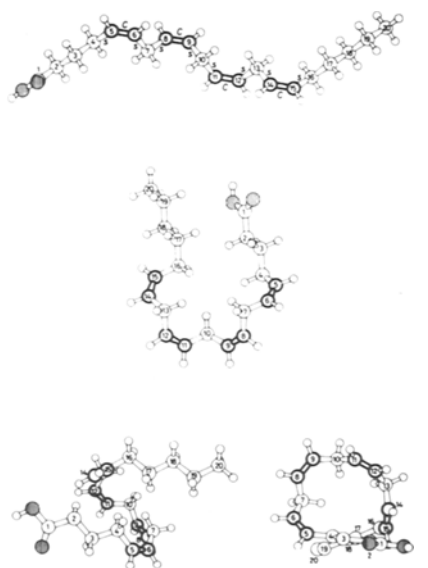

FIG. 10. Various conformations of arachidonic acid.

So far, I have discussed briefly the conversion of essential fatty acids into prostaglandins and related compounds. All are converted by various reactions and eventually excreted as inactive metabolites. The total production of these metabolites in man is about 1 mg/24 hr. An intake of linoleic acid of about 10 g/24 hr is normal. There is, therefore, a factor of at least 10,000 between the linoleic acid entering the body with the food and the prostaglandin metabolites leaving the body in the urine. There is an abundance of essential material. The part of dietary essential fatty acids used for prostaglandin-like compounds seems to be very low.

Once more I would like to come back to the conformation of arachidonic acid and its possible structure in the crystalline state. With respect to the shape of phospholipids in membranes, data on their conformation could be valuable, but when we started our investigations very little was known. The X-ray analysis of oleic acid had been performed and extrapolation from these data would lead to a kind of U-shape for arachidonic acid. However, other models with quite different conformations might also be possible (19). A structural investigation by means of a single crystal could not be made as we failed to obtain suitable crystals. But now single-crystal data are available for arachidonic acid (20). From these data it could be deduced that arachidonic acid has an extended geometry in the crystalline state. The conformation is: ttsCssCssCssCsttt (21). However, it has been pointed out that thinking in terms of a single conformation for flexible molecules like arachidonic acid is not correct (Fig. 10). The U-form is only one of many possible conformations. The X-ray analysis of arachidonic acid has been a long cherished wish of mine. Therefore I am grateful that Führhop and colleagues and de Jong of the Unilever Research Laboratory, have almost completed this work.

The years in lipid research have been exciting and I am very grateful that I have had the opportunity to work with a great many people in a good atmosphere. Figure 11 shows the whole team when we had a reunion a few years ago. I will not give you all their names for they can be found in relevant publications. However, I want to make an exception for two of them. Good fortune has brought me into contact with Nugteren, a man able to unravel the most complicated questions posed to us by Nature; and Beerthuis, with his fine insight into analytical problems and his gift for making people work in harmony.

FIG. 11. Dr. D.A. van Dorp and Mrs. A.W. van Dorp-Vuystingh together with a team of coworkers in 1978.

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