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VITAMIN A

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Vitamin A: Chairman's opening remarks

By R. A. MORTON, *Department of Biochemistry, University of Liverpool*

We have come a long way in vitamin A research and if the journey has often been rough the scenery has been fine! Destinations were chosen and duly reached, but scientific workers, like the performers in a travelling circus, dwell less on the triumph of yesterday than on the task of today and the challenge of tomorrow. This is as it should be, but younger workers in the field have to depend upon cursory and even off-hand accounts of what actually happened. In retrospect there were many intellectual sign-posts on the way; some were seen and acted upon but others were missed. As I must not poach on the preserves of other speakers, I propose to devote this introduction to some of the clues which proved most useful and to mention others which were less effective.

McCollum (1952) relates how Wisconsin farmers in 1908 maintained that 'yellow corn is a better hog feed than white corn' but failed to convince the experts in animal husbandry. Yet 20-odd years later carotene was shown to be a provitamin A. Even today it is necessary to emphasize the distinction between a carotenoid provitamin and the vitamin itself. When carotene was first isolated and proved to have the formula $C_{40}H_{56}$, 'xanthophyll', $C_{40}H_{56}O_2$, looked like a simple oxidation product but the idea of a redox system here turned out to be a false clue. As far back as 1922 contemporaries of mine at Liverpool were chromatographing the carotenoids from nettles and getting good separations, but the sign-post to α -, β - and γ -carotenes was ignored. Nobody was mentally prepared to perceive it; indeed, too little was known about carotenoid structures, or for that matter about vitamins.

Looking back, tribute must be paid to the work of the early exponents of biological assay procedures. At the stage of recognizing and identifying fat-soluble vitamins the biological assay was the alpha and omega of the 'tracking-down' process. Assay methods gained in precision as time went on and—perhaps even more important—investigators generally came to terms with the concept of fiducial limits.

The fact that vitamin A could be destroyed by ultraviolet irradiation was a clue which led to spectroscopic 'labelling'. The antimony trichloride colour test, itself fascinatingly empirical in its provenance, ran roughly parallel with the intensity of ultraviolet absorption in fish-liver oils, and both yardsticks were in harmony with

potencies determined biologically. The arguments, only moderately rigorous at first, were cumulatively convincing. The chemical and physical tests simplified the task of controlling isolation procedures. Eventually, almost pure vitamin A alcohol (retinol) was obtained and its structure determined by classical methods. The work of Karrer, Heilbron and others had its counterpart in that of Kuhn on the purification and characterization of individual carotenoids.

The decision to use carotene as an international reference standard ran into heavy weather when it was found that the preparation in use was a mixture of α - and β -carotene, the latter having about twice the provitamin activity of the former. Inevitably pure β -carotene became the later reference standard. There were awkward intellectual problems about this for, although the β -carotene standard was itself obviously serviceable in biological assays of carotene preparations, its usefulness as a yardstick for the chemically distinct vitamin A needed to be demonstrated. Yet it served its purpose well as did the 'standard' cod-liver oil samples used until synthetic vitamin A acetate became available in a stable form. This was a difficult phase in vitamin A research, made inescapably urgent because a great many fish-liver oils were becoming available commercially and in the public as well as commercial interest they had to be assayed as accurately as possible. Large amounts of fish-liver oils, including highly potent oils, came to England from America under lend-lease arrangements and permitted wartime margarine to be fortified.

It was realized fairly early in the train of events that vitamin A was too important a substance to be neglected by synthetic organic chemists. A good deal of elegant work was done by Heilbron, Milas and others, and the final achievement (e.g. by Isler's group) of the commercial synthesis of vitamin A, as well as of a whole range of carotenoids, is still a spectacular feat of pure and applied chemistry.

The discovery of vitamin A₂ arose quite simply from the spectroscopic scrutiny of the antimony trichloride colour test. A second absorption peak (at 693 nm as compared with 620 nm for vitamin A₁) provided a label which ran parallel in suitable preparations with a variant of the ultraviolet absorption—ultimately traced to the presence of an extra conjugated double bond in the terminal ring system of the molecule.

This vitamin A₂ fitted very well with the retinene₂ discovered by Wald. The proof that retinene₁ derived from a visual pigment, rhodopsin, was really vitamin A₁ aldehyde led naturally to the proof that retinene₂ was the aldehyde of vitamin A₂. The successful use of solid manganese dioxide for effecting the conversion of retinol into retinal at room temperature gave organic chemists a versatile new reaction procedure. This is not the place to go into the rather splendid story of the chemistry of vision, but we must pause to note the importance in it of *cis-trans* isomerism, the 11-*cis* isomer of vitamin A being known from the elegant work of Wald and Hubbard to be specially significant. The *cis-trans* isomers of vitamin A differ appreciably both in respect of their spectroscopic properties and of their biological potency as measured by growth tests on rats. It is today clear that the very nature of the problem only permitted assaying fish-liver oils and natural products containing mixtures of isomers to be somewhat approximate. The theoretical basis is quantitatively a little

dubious but in practice the results are very good—partly in fact because errors tend to be compensated! Correction procedures devised specially to meet difficulties over vitamin A assays are actually theoretically safer in most of the many other situations in which they have been used! I suppose there are lessons here as well as an element of irony.

The problem of the site and manner of conversion of β -carotene into vitamin A was another problem which had its ups and downs, but the importance of processes occurring in the lining of the gut is beyond doubt. There have been interesting quantitative aspects of the storage of esterified vitamin A in the liver and its release as alcohol into the blood. The human requirement for vitamin A has been another problem with its own chequered history of good work done under difficulties, and the Sheffield wartime experiment on volunteers has its niche in the history of the subject.

The deficiency syndrome was at first (perhaps necessarily) oversimplified, but as time has gone on appreciation of its complexities has grown. Hypervitaminosis A has in some respects been as revealing as hypovitaminosis, and experiments with tissue cultures have enriched notions based on experiments with intact animals. Links with the incidence of congenital abnormalities have shown that vitamin A can be teratogenic as well as indispensable. The variety of deficiency signs shown by different species has enlarged and complicated our outlook.

My colleagues, Howell, Pitt and Thompson, have studied vitamin A acid (retinoic acid) with results which have not lost any interest to me through familiarity. I am left with a feeling that despite the enormous—and in many ways deeply satisfying—body of knowledge about vitamin A we are still short of essential biochemical information. It is not my place in an introduction to speculate about the possibility of new light on the mode of action of vitamin A. I am here to listen. I am convinced, however, that the intellectual consummation of half a century of research has still to come. When it does we may be surprised at the sign-posts on the road which escaped our attention but were there all the time!

REFERENCE

McCollum, E. V. (1952). *Nutr. Rev.* **10**, 161.

Vitamin A deficiency and excess

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The pathological effects of deficiency of vitamin A (Wolbach, 1954; Moore, 1957, 1960) and of excess (Rodahl, 1949; Wolbach, 1954; Moore, 1957) have already been extensively reviewed. It is difficult, therefore, for me to contribute any new ideas as a start for this important Symposium. I can only recapitulate salient points in our knowledge, and so provide an introduction for various topics which will be discussed by subsequent authors.

Vitamin A seems rivalled only by vitamin E in the wide variety of lesions which