Concluding remarks

By T. MOORE, 17 *Chesterton Hall Crescent, Cambridge CB4 1AW*

As one who started research on vitamin A 56 years ago, and who faded out from laboratory work 3 or 4 years ago, my attendance at this meeting has made me feel closely akin to the legendary Rip Van Winkle. Many of you may recall from childhood the story by Washington Irving about a frail old man who tottered back into civilization, after sleeping for several decades in a lonely mountain cave. Frankly, I had been getting rather out of touch with the very latest findings in vitamin A research and therefore feel deeply indebted to those speakers, today and yesterday, who have helped to bring me up-to-date. Perhaps I can most usefully respond to the honour of being allowed to make these concluding remarks by trying to bridge the gap, as far as is possible in the short time available, between periods of research that might conveniently be labelled as 'old-fashioned' and 'new-fashioned'.

One of the main differences between these periods lies in the contrast between the crude apparatus available in the early days, and the sophisticated, computerized, often radioisotopic automatons that have been developed during recent years. I suspect that modern investigators may sometimes distrust old findings that were based on quickly matching the transient blue colour that vitamin A produces when treated with arsenic trichloride (Rosenheim & Drummond, 1925), or antimony trichloride (Carr & Price, 1926), by means of tinted glasses that had been devised by the house of Lovibond for measuring the colours of wines, or of brews of tea (Rosenheim & Schuster, 1927). Equally, old-timers like me may sometimes wonder whether the elaborate modern methods are always beyond the range of human weaknesses, and can never go wrong.

Unfortunately old-fashioned methods often went wrong, or at least could be in grave danger of so doing. Thus in the early 1930s I undertook a survey of the vitamin A reserves of healthy and diseased British people (Moore, 1937), as indicated by the application of the antimony trichloride method to specimens of liver taken at autopsies. In Holland, the late Dr L. K. Wolff (1932) had the same idea, and in due course we got into correspondence for the purpose of comparing our results. To our surprise it seemed at first that the reserves in Britain were about ten times greater than in Holland. Wolff seemed quite prepared to accept this finding, and suggested that the dietary intakes of vitamin A were obviously much greater in our country than in his. But eventually it turned out that we were just calculating our 'blue units' in slightly different ways, and that really there was little difference between the vitamin A reserves of the two countries.

Twenty years later, when Dr Sharman, our good friend the late Dr Zoltan Leitner, and myself (Leitner *et al.* 1960) were engaged on a similar survey, but this time on the blood of live, healthy people rather than the livers of dead ones, we

came upon a snag that was less massive numerically than the contretemps just mentioned, but which proved even more puzzling to clarify. By this time we had discarded our old Lovibond tintometer in favour of a primitive photoelectric absorptiomer, as recommended by Dann & Evelyn (1938). Midway through our survey, which eventually lasted from 1948 to 1957, we observed a sudden increase of about 20% in the mean retinol levels for both men and women. We got in touch promptly with Dr Dorothy Hollingsworth, then of the Ministry of Agriculture, Fisheries and Food, to see whether changes in the national diet could explain the quite unexpected increase.

There certainly had been small increments in our vitamin A intake, although neither large enough or so timed as to account for the sharp 20% rise. Only after we had racked our brains for several weeks did it dawn on us that recently the officially accepted u.v. extinction coefficient at 328 nm for pure vitamin A had been changed from 1600 to 2000, and that we had not allowed for this change when calibrating a new absorptiometer. After attending to this point we were still left with small, gradual rises in blood vitamin A between 1952 and 1957, which fitted in well with the dietary information kindly supplied by Dr Hollingsworth. It seems highly probable, of course, that the change in the extinction coefficient did not only affect us, and that most vitamin A measurements made before 1950 should be increased by 20%, or more correctly 25%, to make them comparable with more recent findings.

At other times we ran into trouble through unduly long storage of blood specimens, or through the necessity of enlisting new technical assistants for the highly skilled procedures of extraction, the addition of reagent, and the rapid and consistent recording of galvanometer readings. Such tribulations forced us to conclude that statistical significance was not the only consideration necessary for valid comparisons between mean blood retinol reported from different laboratories, or indeed from the same laboratory at different times. But it is not for me to advise modern experts as to whether such extreme caution is still required for comparisons between findings obtained by the much more highly developed methods now commonly in use.

Turning now to the present Symposium, but still in reminiscent mood, I may remark that Professor Simpson's excellent paper, on carotenoids as vitamin A precursors, reminded me of my own first contribution to this field. The late Dr Stanley Willimott persuaded me to join him in the laborious task of isolating a specimen of xanthophyll from stinging nettles, and then testing its vitamin A activity on rats (Willimott & Moore, 1927). We only had two of these animals, and were lucky to get the correct result, and find the pigment inactive. I have often wondered since why animals make so little use of xanthophyll, apart from the colouring of egg yolks, feathers and other items mentioned and illustrated in Isler's (1971) admirable treatise. I have been puzzled even more by the absence of retinol from the vegetable kingdom. Why should the apparently simple change from carotene to retinol never take place in plants?

Professor Glover enlarged our knowledge of the intricate ways by which vitamin

A is carried to those parts of the body where its action is needed, and stored when in excess. Over the past 14 years retinol-binding protein (RBP), studied by Glover and others in this country (Glover & Walker, 1964), but eventually isolated by DeWitt Goodman and co-workers in America (Kanai *et al.* 1968), has added a new dimension to vitamin A research. Thus biochemical measurements of the vitamin can now be supplemented by immunological measurements of its carrier. This allows numerous interesting retinol–RBP interrelationships, as explored by Professor Glover in light exposure and egg laying, to be clearly established.

Dr Weber has brought us up-to-date about current theories, perhaps now more than theories, about how vitamin A exerts its action throughout the general system of the body. To me it has always seemed strange how much more we have known, in the past, about the detailed biochemistry of the minute amounts of vitamin that are present in the eye than about the presumably different biochemistry of the bulk of the vitamin throughout the rest of the body. With regard to the well-known role of retinol in mucopolysaccharide metabolism, observed 30 years ago by Fell & Mellanby (1952) with reference to the effects of toxic excess, Dr Weber reviewed his modern evidence of the importance of complexes such as guanosine-diphosphate–mannose and mannosyl–retinyl-phosphate as steps in the formation of mannosyl-glycoprotein.

His finding that vitamin A deficiency decreases the incorporation of mevalonic acid into cholesterol, with associated increases in squalene and ubiquinone, recalls a minor controversy, long ago, between myself and my good friend the late Professor Alan Morton. His team (Lowe *et al.* 1953) first thought that a metabolite showing absorption at 275 nm in liver extracts from vitamin A deficient rats, which was later named ubiquinone (Morton *et al.* 1957), was an abnormal product resulting from the deficiency. Earlier, however, Moore & Rajagopal (1940) had observed the same absorption band in liver extracts from rats that had received marginal, but adequate doses of the vitamin. Was ubiquinone formed only in vitamin A deficiency? Or was it present in normal liver, but masked in extracts by the stronger absorption of the vitamin? Probably Dr Weber will agree that his new evidence supports an intermediate conclusion that ubiquinone is indeed a constituent of normal liver, but that its concentration rises considerably in avitaminosis A (Moore & Sharman, 1960).

Let us hope that in the not too distant future Dr Weber's work will enable us to understand the mechanisms of all the interrelationships that have been found to pertain between vitamin A and other vitamins or hormones. Thus we may soon know exactly how vitamin E protects the body's stores of vitamin A (Davies & Moore, 1941), why female rats can sometimes be made to store more vitamin A in their livers than their male counterparts (Brenner *et al.* 1938), and why retinol regularly shows a higher average level, and carotene a slightly lower one, in men's blood than in women's (Kimble, 1938–39). It will become clear why, in various species and in various physiological and pathological circumstances, the level of retinol tends to run parallel to the level of zinc (Smith *et al.* 1973), or as first noticed inversely to the level of copper (Moore, 1969).

Dr George Pitt followed appropriately after the previous speaker with his well informed discussion of relationships between chemical structure and vitamin A activity. His finding that retinoic acid cannot replace retinol in the reproductive processes (Thompson *et al.* 1964) certainly gave a very unexpected twist to our ideas about the mode of action of the vitamin. Thus we are faced with a strange situation in which the acid can prevent or cure many of the effects of deficiency, including growth cessation and xerophthalmia, but cannot prevent two apparently unrelated lesions, defective dark adaptation and infertility. The relationships between detailed chemical structures as, for example, between α- and β-retinol, with their ability to combine with retinol-binding protein, present a fascinating challenge to Dr Pitt's perspicacity and ingenuity in experimentation.

With Dr Antoinette Pirie distinguished not only as a world authority on human xerophthalmia, but also as the Editress of a periodical 'Xerophthalmia Club Bulletin' devoted entirely to this distressing condition, I need hardly try to 'gild the lily' by commenting in detail on the specialized subject of her paper. I can only express my admiration of her classification of xerophthalmia, and of the excellent colour slides that she showed us in illustration of her points. We must hope that her propaganda for 'a handful of dark green leaves every day for every Indian child' will soon gain general support, and that the very high carotene content of Dr Norman Pirie's leaf protein preparations will come to be more widely appreciated.

But in regard to experimental xerophthalmia in rats I sometimes wonder whether it might be instructive to look back on old reports that dietary defects other than vitamin A deficiency can sometimes aggravate the eye lesions. Thus McCollum *et al.* (1922) once claimed that salt mixtures rich in chloride could be harmful to the eyes. Baumann & Steenbock (1934) reported that a powdery diet was more conducive to xerophthalmia than the same diet after it had been made into a moist paste. In my own work I used to go through periods when all my rats promptly developed xerophthalmia, but other periods when they just died off without showing serious eye lesions.

Dr Chris Bates deserves our thanks for having combed the literature on the transfer of vitamin A from mother to child so carefully, and for reporting so adequately his own recent investigation in West Africa. In that part of the world the conversion of carotene to retinol must be an even more important mechanism than it is in England and other developed countries. His suggestion that a 6:1 conversion value may be unduly high when applied to food containing red palm oil seems highly plausible. Looking back on the famous 'Sheffield Experiment' (Hume & Krebs, 1949), carried out during World War II, we may recall that carotene was much better absorbed from oily solution than from boiled, sliced carrots, although absorption from the latter could be greatly improved by homogenization. Perhaps the West Africans have good teeth, and chew their food well. In any case, it would have delighted the late Dr Frank Wokes, a prominent vegetarian member of the Nutrition Society, to know how well they got on in producing babies with hardly any animal food.

We now come to our two final papers, by Dr Peto and Dr Hicks, on relationships

between vitamin A and cancer. Here I must declare myself an interested party, since the thousand or so liver specimens that I examined in my early vitamin A survey, mentioned at the beginning of these remarks, included seventy-six from patients who had died from cancer. My comments on this important topic can conveniently be divided into two parts, according to whether we look upon vitamin A as a drug, or as a nutrient.

Used as a drug, vitamin A can be administered, in the form of retinol, in doses much larger than those contributed by an ordinary diet. The normal distribution pattern of the vitamin throughout the body may be overwhelmed, with its appearance in substantial concentrations in parts of the body, such as the kidneys, suprarenal capsules, lungs and adipose tissues, where usually only low concentrations are to be found. If very high doses are continued for periods long enough to outstrip the powers of the liver in 'mopping up' and storing the great excess of vitamin, then poisoning, known as hypervitaminosis A, will develop.

On this basis, we may speculate that 'drugging' with vitamin A could be beneficial against cancer in one or other of two different ways. As a first alternative, it might extend the beneficial action of the vitamin on normal tissues to sites that it does not normally reach in substantial concentrations. As a second alternative, the toxic action of local hypervitaminosis might prove even more harmful to neoplasms than to the surrounding normal tissues.

On this latter assumption hopes are kindled, as Dr Hicks so clearly explained to us, that retinol and other retinoids, some even departing widely in structure from retinol and having little or no activity as vitamins, should prove even more inhibitory to the growth of tumours. Irrespective of the mechanisms involved, however, the question whether retinol, or other retinoids, can usefully be employed against cancer seems entirely a matter for trial. In common language 'The proof of the pudding is in the eating'.

With vitamin A as a nutrient, as discussed by Dr Peto, we seem to be facing a somewhat different problem. Here a balanced approach is needed, with due reference to the mass of information about interrelationships between vitamin A and disease that has been accumulated during the past 50 years, and which includes the results of the survey of liver vitamin A reserves that I mentioned at the beginning of these remarks.

My general conclusions, admittedly reached 40 years ago, but substantially confirmed by recent workers in the same field (Hoppner *et al.* 1969; Huque, 1981), were first, that even in cases of accidental death the liver retinol reserves varied over a wide range. Second, with the notable exception of diabetes and possibly also thyroid diseases, the ranges of reserves in disease were always lower than in health. Third, in comparisons between different diseases the reduction below the range for accidental death was much greater for some diseases than for others. Cancer was not outstanding for its association with lowered vitamin A reserves.

Thus in forty cases of accidental death the median vitamin A reserve, recalculated in modern terms as pure retinol, was 66 μg/g, whereas in seventy-six cancer cases the median was halved, at 33 μg/g. But in certain other diseases the

reduction was much greater. For example, in forty-eight cases of chronic nephritis the median was only 8 μg/g, or 12·5% of the accident median, or 25% of the cancer median. Out of a list of twenty-nine disease groups, cancer came eleventh in order of magnitude of retinol reserves. In addition to chronic nephritis the groups for pneumonia, empyema, valvular heart disease, abscesses, prostate diseases and urinary infections also had outstandingly low retinol reserves.

We know now, of course, that chronic nephritis, and certain other diseases, including some cancer cases, involve the urinary excretion of retinol (Boller *et al.* 1937), although the amounts lost do not seem in themselves enough to account for the low liver reserves. The mutual relationships between vitamin A deficiency causing disease, and disease causing vitamin A deficiency, are still far from being fully understood. Their possible implications must not be overlooked in all attempts to link specific diseases with defective retinol levels.

Coming back from Rip Van Winkle's cavern into modern times, I must join in sincere congratulations to Dr Peto for his comprehensive and scholarly review of the mass of recent evidence through which the risk of cancer may be linked statistically with either low blood retinol or with low dietary carotene. Although in the past it has been notoriously difficult to make valid comparisons between retinol and carotene findings obtained in different laboratories, or even sometimes in the same laboratory, the almost unanimous agreement among modern workers about the importance of the vitamin A status in cancer is certainly impressive.

Nevertheless, if we regard the liver retinol reserve as the ultimate test of vitamin A status, then preoccupation with cancer as a foul, and often incurable disease must not blind us to the possible importance of the vitamin in other diseases. It would be childish, of course, to argue that because the retinol reserves in chronic nephritis are only 25% of those in cancer, then the former disease should be four times more responsive than the latter to appropriate vitamin A therapy. It seems eminently desirable, however, that the present surge of interest in the importance of vitamin A in cancer should be extended to cover all other common diseases, but particularly those in which abnormalities in the vitamin A status are already well recognized.

I must conclude by expressing my appreciation, doubtless shared by us all, of the trouble taken by all the contributors to this Symposium in the preparation of their excellent papers. To all those not living in Cambridge we must express our congratulations at their getting here in these uncertain times, and must wish them a safe and comfortable journey home.

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