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!

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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

can be produced in its absence. Its distinction in producing characteristic lesions, when given in great excess, is shared only by vitamin D. Obviously a short paper must be highly selective in the choice of material, and must omit mention of many interesting pathological findings. The quotation of references must be very incomplete.

The occurrence of avitaminosis and hypervitaminosis A. Vitamin A is present in substantial amounts in the livers of most human subjects, and of most animals living under natural conditions. An important point established in the famous Sheffield experiment on adult human volunteers (Hume & Krebs, 1949) was the confirmation of the ability of the reserves of vitamin A known to be stored in the liver (Moore, 1937) to prevent signs of deficiency for months, or even years.

The presence of these reserves in well-nourished subjects, however, must not give rise to feelings of false security. Diets which are largely based on cereal foods, other than yellow maize, and from which green vegetables and preformed vitamin A are excluded, are always a source of danger. Outbreaks of vitamin A deficiency occur only too readily in pigs, poultry and calves fed on conventional diets, unless adequate sources of vitamin A are provided. The old story seems to keep repeating itself in different modifications: thus the most recent outbreak of deficiency has been in young cattle raised intensively on barley (Abrams, Bridge, Palmer, Spratling & Sharman, 1961). Other cattle foods conducive to vitamin A deficiency are sugar-beet pulp and poor-quality straw. Parched pastures can cause deficiency: Blaxter (1951) drew attention to a biblical reference (Jeremiah, Ch. 14, v. 6) about wild asses which showed behaviour highly characteristic of vitamin A deficiency 'because there was no grass'. In human subjects deficiency can arise from the excessive consumption of farinaceous foods. The classical cases described by Bloch (1920–1) occurred mainly in infants fed upon oatmeal gruel. A diet of sweetened skim milk, however, can also cause deficiency (de Haas & Meulemans, 1938).

Poisoning by excess of vitamin A occurs in animals only under experimental conditions. Man, however, has sustained injuries in three ways. (1) Arctic explorers have unwisely eaten polar bear's liver, which is very rich in vitamin A (Rodahl & Moore, 1943); (2) young infants have been given more vitamin A than they can tolerate, purely as a protective measure (Josephs, 1944; Marie & Sée, 1954); (3) adult patients with certain rare skin diseases, such as ichthyosis and Darier's disease, have been treated by prolonged and heavy dosing for the purpose of ameliorating the skin lesions (Gerber, Raab & Sobel, 1954).

Hypervitaminosis A can only arise from excess of preformed vitamin A, and not from excess of provitamins. In man a minor abnormality called 'carotenaemia' or 'hypercarotenosis', involving yellow discoloration of the palms of the hands and soles of the feet, can result, *inter alia*, from an excessive liking for carrots (von Noorden, 1907; Moro, 1908). In itself it is harmless, but it may accompany diabetes or thyroid imbalance.

The lesions in avitaminosis A. The following list (Moore, 1957, 1960) includes abnormalities observed in one or other of several species, such as the rat, rabbit, dog, ox, hen, monkey and man:

(1) Defective dark adaptation.
(2) Failure of growth.
(3) Xerosis and keratinization of mucous membranes. Xerophthalmia. Secondary infections.
(4) Faulty bone modelling, with the production of thick, cancellous bones instead of thinner, more compact bones.
(5) Nerve lesions, often associated with bone lesions.
(6) Increased pressure of the cerebrospinal fluid, developed independently or in association with deformed skull bones. Hydrocephalus.
(7) Abnormalities of reproduction, including degeneration of the testes and abortion, or the production of malformed offspring.
(8) Certain forms of skin disease.
(9) Death.

The first effect of dietary deficiency of vitamin A, apart from congenital malformations, is usually defective dark adaptation. This abnormality was, indeed, the only functional defect observed in the Sheffield experiment. Work on rats, by Dowling & Wald (1960), has demonstrated an important difference between defective dark adaptation and most of the other characteristic lesions. The animals were given a diet deficient in vitamin A, but with supplements of vitamin A acid, a substance which first came to light as a step in the chemical synthesis of vitamin A. On this regime the animals grew well and survived for indefinite periods, but they became totally blind. This was not due to xerophthalmia, which was avoided, but to a failure of the retina to respond to light, and finally to degeneration of the retina. Apparently the visual function of vitamin A, for which vitamin A alcohol was necessary, could be divorced from its functions in the general system, for which vitamin A acid could replace vitamin A alcohol. Subsequently, however, Thompson, Howell & Pitt (1964) disturbed this simple picture by demonstrating that vitamin A alcohol is also required for the reproductive functions, which cannot be supported by vitamin A acid.

Defective dark adaptation can be conveniently regarded as a biochemical lesion, since in its early stages it can respond very rapidly to vitamin A therapy. The response of the other lesions to therapy will depend on the severity of the resulting structural damage, and to some extent on the growth period still available before maturity is reached.

Death in avitaminosis A. Death has been listed among the characteristic 'abnormalities' because avitaminosis A seems to be one of the most lethal of all dietary deficiencies. If weanling rats are given a diet deficient in vitamin A they will usually grow for 4 or 5 weeks, survive for 2 or 3 weeks more at steady or declining body-weight, and then promptly die. In contrast, young rats given a diet deficient in vitamin E usually show clear signs of deficiency, by the dialuric acid haemolysis test, within 1 or 2 weeks. Abnormalities in the testes or uterus will be demonstrable at 3-4 months, but the rat will still remain in good health, and continue to grow. Eventually, after 8-10 months, a long decline in body-weight will set in, accompanied by the development of severe muscular dystrophy. The animal may linger on, however, to the age of 15 or 18 months, before finally succumbing to intercurrent

disease. In my own experience rats may also continue to linger on for long periods, although obviously abnormal, in deficiencies of protein, calcium, copper, riboflavine and essential fatty acids.

Emphasis of the lethal aspect of vitamin A deficiency may possibly provoke criticism, particularly on the grounds that conclusions drawn from experiments on growing rats cannot safely be applied to other species, and indeed to other stages in the life cycle of the rat. Thus vitamin E deficiency is rapidly fatal to foetal rats (Evans & Burr, 1927), and even to weanlings if they are also deficient in protein (Schwarz, 1949; Lindan & Himsworth, 1950). Chicks also succumb rapidly to avitaminosis E (Adamstone, 1942). Moreover, lack of vitamin A is perhaps a less rapid cause of death in larger animals such as cattle (Guilbert & Hart, 1934; Madsen & Earle, 1947) and pigs (Hughes, Lienhardt & Aubel, 1929–30) than in rats. According to McLaren (1960), however, untreated vitamin A deficiency is notorious as a cause of death, no less than of eye lesions, in young children. If vitamin A deficiency were less dangerous to life, the incidence of permanent blindness, as the aftermath of xerophthalmia, would be much increased.

As McLaren has also pointed out, death may forestall the development of xerophthalmia in young rats. Similarly, the full development of all the other lesions, except presumably failure in dark adaptation, may depend upon the time of survival of the animal. Another important factor is the rate of growth, and the resulting stress imposed on the developing tissues. It is probably for such reasons that some of the lesions of avitaminosis A are more common in some species than in others. In particular, gross bone lesions are produced more readily in larger animals, such as dogs and cattle, than in rats. Presumably rats succumb to other lesions, such as the xerosis and infection of membranes, before the bone lesions have time to reach full development.

Delayed lesions after vitamin A deficiency. In young growing rats, and in human subjects who have suffered from the early stages of night-blindness, adequate vitamin A therapy should produce complete cures. Other lesions, such as perforation of the cornea as a sequel of xerophthalmia, may lead to permanent injury, even if the general health of the subject can be restored.

Another type of response is less familiar, but of considerable pathological interest. Thus instances are known, in at least two species, of the restoration of vitamin A to the diet first causing a cure, but with the subsequent development of lesions.

(1) In calves a period of vitamin A deficiency may cause retardation of growth and also malformation of the bones of the skull, but for a time may leave the optic nerve intact. The restoration of a diet rich in carotene, such as fresh green pasture, will restore growth, and improve the general condition of the animal. Expansion of the malformed bone, however, may distort or break the optic nerve, and so cause sudden and permanent blindness at a time when the diet is adequate in carotene (Blakemore, Ottaway, Sellers, Eden & Moore, 1957).

(2) In rats a prolonged period of partial vitamin A deficiency may be imposed by restriction to a deficient diet, supplemented either with daily small doses of vitamin A, or with larger doses given only occasionally. This treatment will at first allow

survival and slow growth. More liberal dosing will promote more rapid growth. Eventually, however, growth may cease, and the rat will decline in weight and die. Autopsy will show that death has been caused by cystitis, provoked by the accumulation of stones in the bladder. The culmination of this lesion can occur at a point when substantial reserves of vitamin A have accumulated in the liver. It can therefore be regarded as a 'hangover' from an earlier period of deficiency. A lesion then started has gone on developing, unchecked and for a time not lethal, until the point is reached when it becomes intolerable to the organism (Moore, 1957).

Diseases which precipitate vitamin A deficiency, or affect vitamin A metabolism. A comprehensive review of this wide field is impossible, but in view of its great importance it must not be completely overlooked. The relationships between vitamin A and disease can perhaps be divided into three types, although overlapping is inevitable.

(1) In human subjects receiving diets deficient in vitamin A the typical lesions of avitaminosis may be precipitated by intercurrent illness, or other forms of nutritional stress. Thus in the observations of Bloch (1920–1) xerophthalmia usually followed a period of general ill-health and diarrhoea in infants whose diet was usually deficient in protein.

(2) In subjects receiving a diet adequate in vitamin A, secondary deficiency may result from failure of absorption of the vitamin, or defects in its metabolism. Blegvad (1924), to cite a single example, reported on the occurrence of severe night-blindness in a man with cancer of the liver. The diet appeared to be adequate in vitamin A, and there was no response to oral therapy. Parenteral injections of a vitamin A concentrate, however, rapidly corrected the night-blindness.

(3) In many diseases, defects in the absorption or metabolism of vitamin A may be demonstrated by measurements of the level of vitamin A in the blood or liver, but without functional or pathological evidence of vitamin A deficiency. Thus the level of vitamin A in the blood falls during fever (Lindqvist, 1938) and excretion of the vitamin in the urine sometimes occurs (Boller & Brunner, 1936). In subjects who have died from certain diseases, the liver reserves found at autopsy are low. In chronic nephritis, for example, the reserves average only about one-tenth of those in cases of accidental death, and indeed often virtually disappear (Moore, 1937).

It would be rash to exclude the possible occurrence of undetected secondary deficiency of vitamin A, severe enough to increase the ill effects of the primary disease, in many common diseases. Up to the present, however, there has been no positive evidence that any beneficial results can be obtained by dosing with vitamin A. Further research on this topic is obviously desirable.

As already mentioned, there is considerable overlapping under the above three headings. Protein deficiency, for example, not only precipitates vitamin A deficiency when the diet is low in vitamin A (Bloch, 1920–1), but interferes with the carriage of the vitamin in the blood when the dietary intake of the vitamin is adequate (Friend, Heard, Platt, Stewart & Turner, 1961).

The lesions in hypervitaminosis A. The lesions caused by grossly excessive intakes of

vitamin A, which can also be induced by excess of vitamin A acid (Thompson & Pitt, 1960), have been reviewed by Moore (1957). They include:

(1) Cessation of growth (rat).
(2) Skin abnormalities (rat, man).
(3) Increased pressure of the cerebrospinal fluid (human infant). Nausea and vomiting (adult man).
(4) Bone abnormalities (man). Bone fractures (rat).
(5) Profuse, fatal internal haemorrhage (rat). Secondary vitamin K deficiency (rat).
(6) Congenital malformations (rat).

Some similarity is apparent between the lesions in deficiency and excess of vitamin A. Thus both these conditions can cause increased cerebrospinal fluid pressure, abnormalities in the bones, and congenital malformations. The lesions in the bones caused by deficiency, however, are very different from those caused by excess. Congenital malformations can occur in many other forms of nutritional deficiency, in infections, and in poisoning by drugs. The early and more recent workers who studied the effect of vitamin A deficiency (Hale, 1935; Andersen, 1941; Warkany & Schraffenberger, 1946) and excess (Cohlan, 1953; Giroud & Martinet, 1954) on the development of the foetus, can be honoured as pioneers in a field which extended far beyond their original interests.

In passing, I may mention some recent experiments which I have made on combined hypervitaminosis A and calcium deficiency in rats. It has long been known, of course, that hypervitaminosis A does not cause decalcification of the bones. Nevertheless, I have myself described the hypervitaminotic bones as soft, and have implied that this softness is the cause of their spontaneous fracturing. It occurred to me that if the bones were made soft by a diet deficient in calcium, they should become much more vulnerable to hypervitaminosis A. Rats were therefore fed on a diet of meat, either alone or with added calcium carbonate, and with or without toxic overdoses of vitamin A. To my surprise the absence of calcium from the diet had virtually no influence on the incidence, or speed of development, of skeletal fractures. Under the conditions of my experiment, softness depended on calcium deficiency, and fracturing on hypervitaminosis A.

A characteristic of the hypervitaminotic bones, seen best in the femurs, was growth in length without corresponding growth in thickness. Fracture was probably the final result of extreme attenuation of the bone. This thinning of the bones in hypervitaminosis A had already been noticed by Rodahl (1949). It may be contrasted with a thickening, or stubbiness of the bones, which has been reported by Wolbach (1954) as an effect of vitamin A deficiency. Irrespective of the details of the mechanisms involved, therefore, the changes observed are consistent with vitamin A having a single overall effect on development of the bone length, which is hindered in deficiency, and overemphasized in excess.

Summary

Vitamin A deficiency occurs readily in farm animals that are fed on diets based mainly upon cereals, or other foodstuffs low in carotene. Cereal diets, or diets based on skim

milk, can also cause deficiency in human subjects, particularly infants. The lesions in avitaminosis A in various species include defective dark adaptation, xerosis of membranes, skeletal abnormalities, raised cerebrospinal fluid pressure and congenital malformations. Infections frequently follow xerosis. Vitamin A deficiency is usually characterized by a high mortality rate, and by the short period of survival after the first signs of deficiency have appeared. Instances are known of the delayed appearance of injuries resulting from a past period of vitamin A deficiency. The functions of vitamin A in the retina and in reproduction differ from its general functions in failing to accept vitamin A acid as a substitute for vitamin A alcohol. Various forms of interaction are known to occur between vitamin A deficiency, disease, and other dietary deficiencies. Hypervitaminosis A has resulted in man from the consumption of polar bear's liver, or from grossly excessive vitamin A therapy. It can also be induced readily in experimental animals. The injuries sustained in various species include skeletal abnormalities, raised cerebrospinal fluid pressure, secondary vitamin K deficiency and congenital malformations.

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