events that lead to the stimulation of mitosis, e.g., a derepression of the genome, remains to be elucidated.

In the present study the central lens epithelial cells cultured in KEI-4-insulin showed an increase in the size and number of mitochondria at seven hours of culture. In this respect, the central lens epithelial cells of the insulin-treated lens exhibited a morphology similar to that noted in cells residing in the germinative zone in the living animal. Increases in mitochondria have also been detected in the traumatized lens prior to the onset of cell division.

The epithelial cells of lenses exposed to insulin, dibutyryl cAMP, and theophylline were elongated in a plane parallel to the overlying lens capsule. Theophylline and cyclic AMP also engender a morphological change in cultured newt iris cells, which mimics that detected during Wollfian lens regeneration. Moreover, explanted chick lens epithelial cells become markedly elongated if exposed to insulin. Dibutyryl cAMP stimulates cell elongation in one-day-old rat lenses and simultaneously accelerates the appearance of lens-specific proteins.

Hormones are known to influence mitosis both in the mammalian and amphibian lens. Lenticular mitosis in larval amphibians can be initiated by thyroid hormones. In addition, thyroxin and growth hormone trigger a central mitotic activation in the epithelium of the adult frog lens. Studies from Rothstein’s laboratory have shown that the lens epithelium of the hypophysectomized frog become amitotic; such lenses fail to undergo mitosis even if cell-to-cell relationships are disturbed by a mechanical needle injury. Injury related cell division can be reinstated subsequent to the administration of somatotropin or whole pituitary extract. The effect, if any, of growth-promoting hormones on the mammalian lens in situ remains to be documented.

The isolation of insulin-like substances from rabbit serum and from the aqueous humor obtained from injured or inflamed eyes, which is known to contain a potent mitogen, is the focus of current studies. The presence of such factor(s) in the aqueous may be required for wound related mitogenesis and for the restoration of tissue transparency in the traumatized or cataractous lens.

The technical assistance of Carol Genyea is gratefully acknowledged.

Key words: lens epithelium, ultrastructure, rabbit, mitosis, migration, organ culture, defined medium, papaverine, cyclic AMP, ribosomes, cell surface, mitochondria.

REFERENCES

Xerophthalmia. Antoinette Pirie.

Some characteristics of children with xerophthalmia are described and the difference in age between those with serious corneal xerophthalmia and those showing milder conjunctival xerophthalmia is noted. The various public health measures
instituted to prevent xerophthalmia are examined. The particular importance of protein in food given to severely malnourished children suffering from xerophthalmia is stressed and studies on enzyme activities which are enhanced in the xerophthalmic cornea are briefly noted.

Xerophthalmia is caused by lack of vitamin A; the ocular signs start with night blindness and progress through xerosis of the conjunctiva to end with perforation of the cornea and irreversible blindness. The stages through which xerophthalmia progresses have recently been reclassified in a form suitable for use both in prevalence surveys and in hospital departments (Table I).

The victims of severe xerophthalmia involving the cornea are almost always infants or young children, the peak age being 2.5 years in Indonesian children brought to hospital (Figs. 1, A and B). The younger the age the greater the likelihood of serious xerophthalmia (Fig. 2). A similar relationship is shown in India and other parts of the world.

There is a puzzling discrepancy between the age relationships of children brought to hospital with the milder signs of xerophthalmia and of children showing the same clinical signs but living in the community. Figs. 1, A and B show that the peak age for children coming to hospital with XN or XI is also near 2.5 years, but when the prevalence of xerophthalmia in the general community has been surveyed, ophthalmologists have found in India and elsewhere a steady increase in the mild signs with age of children; in India it was 1.5 per cent in the age group 12 to 24 months and 16.0 per cent in the age group 48 to 60 months.

This marked difference in age between children coming to hospital with either mild or serious xerophthalmia and children found with the mild form in the community may be connected with the fact that the hospital children are not only deficient in retinol but are as well almost always severely malnourished and, have, or have recently had, recurrent infections. The children who are in the hospital are a separate group and, as Fig. 2 shows, are most highly at risk from severe blinding xerophthalmia at a very early age. It is impossible to follow severe cases in prevalence studies in the community. Here they appear few and far between but they congregate at hospitals.

Laboratory animals, deficient from weaning solely in vitamin A and its precursor β-carotene, develop the full sequence of the signs of xerophthalmia ending with perforation of the cornea and shriveling of the eye. Thus we can be as certain that xerophthalmia can be caused by vitamin A deficiency alone as we are for example, that beriberi is solely due to deficiency of thiamine. In the laboratory the eye signs are cured by dosage with vitamin A alone unless severe ulceration of the cornea has already set in.

<table>
<thead>
<tr>
<th>Table I. Classification of ocular signs (from Control of vitamin A deficiency: Priorities for research and action. W.H.O., U.S.A.I.D. Jakarta 1974, in preparation.)</th>
</tr>
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<tbody>
<tr>
<td>X1A Conjunctival xerosis</td>
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<tr>
<td>X1B Bitot spot with conjunctival xerosis</td>
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<tr>
<td>X2 Corneal xerosis</td>
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<tr>
<td>X3A Corneal ulceration with xerosis</td>
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<tr>
<td>X3B Keratomalacia</td>
</tr>
<tr>
<td>XN Night blindness</td>
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<tr>
<td>XF Xerophthalmia fundus</td>
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<tr>
<td>XS Corneal scars</td>
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Those lesions enclosed within the box are indicative of clinically active vitamin A deficiency. Those outside the box are either difficult to assess, less specific, or unrelated to active disease. (1) These signs are descriptive rather than diagnostic. All signs noted at the time of examination are recorded. (2) In general, a progression of severity is reflected in the classification of signs within the box. (3) The classification can be used in field surveys, in hospitals, and clinics. (4) When tabulating the frequency of these signs each child should be included only once, under his or her most severe sign. (5) Only those Bitot spots accompanied by conjunctival xerosis, usually in zero to five years old, are indicative of vitamin A deficiency. This xerosis may be hidden by the overlying foam of the Bitot spot, and only revealed when this is rubbed away. (6) Secondary signs, located outside the box, may often occur in association with, or result from vitamin A deficiency, and should be noted separately.

The connection between xerophthalmia in children in vitamin A was first described by Bloch in Denmark. During World War I there was insufficient butter to go round and the poor ate margarine. Severe xerophthalmia developed in epidemic form among infants in orphanages and in poor families. Bloch found that it could be cured by cod liver oil, 10 grams twice a day, without change of diet and suggested that the oil supplied the traces of “fat-soluble A bodies” needed for growth and well being. Curiously, xerophthalmia in Denmark vanished when the German U-boat blockade was stepped up so that ingredients for the manufacture of margarine could no longer be imported. Butter was then rationed and the price stabilized so that everyone got their share. The last case reported in London was in 1938; it is therefore not so long since xerophthalmia disappeared from western Europe.

But in India and Southeast Asia it is still rampant. The Indian Government estimates that 10,000 children become blind each year from xerophthalmia, whereas in Britain there are at any one time about 2,000 children blind from all causes (none from xerophthalmia). In Indonesia, ten Doesschate found that xerophthalmia was the prime cause of blindness in young children. Any natural or man-made disaster can precipitate an epidemic. It seems now to be a much more serious problem in Bangladesh than it was before the turmoil of war.

These are the parts of the world where serious xerophthalmia is most obviously a scourge but it is also endemic in Lebanon, Thailand, Brazil, El Salvador, and parts of Africa. Poverty is the basis of this global distribution. But poverty is
made worse by disease and lack of understanding of the finer points of nutrition (not confined to poverty-stricken people) and, in the worst hit countries by the use of rice as the staple food. Rice is food; if all else fails, rice will still fill the belly.

Many of the worst hit areas are not deserts but, for at least part of the year, lush green tropics or semitropics full of edible wild plants or easily cultivated green vegetables which could provide ample \( \beta \)-carotene quite cheaply. Unfortunately green vegetables are no longer considered valuable food either by the rich or poor. India, for example eats less of them than any other country listed by FAO. Indeed, green vegetables are often considered harmful for young children. This may have its basis in the fact that they are often grown under unsanitary conditions and need careful handling and cooking. But in season they are cheap, both wild and cultivated species used to be popular and should again be encouraged, particularly for young children. About one ounce of fresh, dark-green leafy vegetable a day will provide ample \( \beta \)-carotene for a young child.

Where poverty has become less grinding, and is accompanied by efficient health care and nutrition education in schools, xerophthalmia has vanished. It is no longer present in Singapore and has been much reduced in Sri Lanka and possibly also in North Vietnam.

The worldwide distribution of xerophthalmia and the terrible consequence of child blindness has stimulated the Governments of the countries concerned, the World Health Organization, and UNICEF, together with nongovernmental agencies in many countries to undertake preventive measures. The most ambitious schemes are those now in operation in parts of India, Bangladesh, and Indonesia where capsules containing 200,000 IU of retinol, as 110 mg. of retinol palmitate, are being distributed every six months to children between one and five years of age. The capsules are given orally and it is hoped that this dose will keep the level of vitamin A in the blood and liver well above deficiency level for six months. There has been a good deal of controversy about these schemes, both as to whether the serum vitamin A level stays up for as long as six months and also whether distribution is reliable and reaches those in real need. These points are not yet settled but pilot surveys in India, Indonesia, and Bangladesh have shown that the prevalence of mild signs of xerophthalmia, i.e., conjunctival xerophthalmia, diminish after distribution of the capsules. So far there is no evidence that serious xerophthalmia has diminished.

Another method, about to be put into practice in Guatemala is food fortification. Sugar, which is produced centrally, is to have vitamin A added, without increase in price to the consumer, just as, in Britain margarine is fortified with vitamin

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*Fig. 1. A. Xerophthalmia according to age. Boys.*

*Fig. 1. B. Xerophthalmia according to age. Girls. (From J. ten Doesschate, Causes of blindness in and around Surabaya. Thesis, Jakarta, Indonesia, 1968.)*

*Fig. 2. Percentage of each age group of xerophthalmia patients with serious xerophthalmia X2, X3, and X4. (J. ten Doesschate, 1968.)*
The problem in other countries has been to find a suitable, generally consumed food which is centrally produced.

Finally, there is the method of nutrition education which may be used separately or in conjunction with the other methods described. For prevention of xerophthalmia the message will vary according to the local foods and diet. In Indonesia red palm oil is abundant but not appreciated. There are also dark green leafy vegetables. In Southern India locally available dark green leafy vegetables and leaves free for the picking should be stressed. The basis of education is that the parent, who stays at the nutrition Center with the child, should participate in buying and cooking the necessary, nutritious food and then share the meal. All food recommended must be easily available locally and within the means of the family when the child returns home. All pots and pans, stoves etc. at the Center must be similar to those the parents are familiar with.

Such a Nutrition Rehabilitation Center to care for children showing signs of xerophthalmia has been set up at the Government Erskine Hospital Madurai, Southern India under the direction of Professor G. Venkataswamy. About 1,000 children have been cared for in 2.5 years. Among children admitted for treatment of xerophthalmia, 67 per cent of those most carefully followed-up and documented were less than 60 per cent of their normal weight for age on admission (Grade III malnutrition on the Gomez scale). Some had kwashiorkor and showed the edema of protein deficiency; more usually they were marasmic. Such malnourished children also had other diseases, the most usual being intestinal infections leading to diarrhea, which can cause decreased absorption of vitamin A and carotene. Or they might have fever caused by respiratory infection or viral disease which increases usage of vitamin A by the body and may precipitate severe xerophthalmia in a child teetering on the edge of deficiency.

The treatment at Madurai consists in (1) food rich in β-carotene from the dark green leafy vegetables and in protein from groundnut flour, which is given to all children and their parents—rice and oil supply the calories—(2) general medical treatment, and (3) initial dosage with vitamin A of those showing severe xerophthalmia. Change in weight was recorded as well as change in the ocular condition. Under this regimen, the children gained weight but their "catch up" growth was not as fast as if they had been given eggs and milk and other too expensive but excellent sources of protein and vitamin A. Those given vitamin A as well as the standard diet with ample β-carotene, protein, and calories grew no faster than the others.

Xerophthalmia X1A, X1B was arrested and almost invariably cleared either while the child was at the Center or on return home. Corneal xerosis (X2) cleared and initial vitamin A did not improve the curative effect of the food alone. Children with more serious xerophthalmia X3A, X3B were almost invariably given initial doses of vitamin A, so that no comparison of the effect of food alone with that of food plus vitamin A was possible for this group of children. The results seemed as good as those achieved by inpatient treatment but treatment was less costly.

There are, therefore, three public health methods in use for prevention and cure of xerophthalmia, two based on provision of extra vitamin A and one based on provision of food rich in β-carotene and protein, with addition of vitamin A where considered medically advisable. The first two methods reach hundreds of thousands of children, the third can, by its nature, reach only thousands directly. The first method—distribution of large doses of vitamin A has, in pilot studies, been shown to diminish the prevalence of conjunctival signs of xerophthalmia, but so far has not been shown to diminish the number of children brought to hospitals with severe corneal xerophthalmia. This group of children is manifestly different from those who develop mild signs. They are more severely malnourished and more likely to have intestinal or respiratory infections.

It is clear that we do not yet know all the factors that cause the rapid worsening of xerophthalmia with involvement of the cornea and its sudden perforation resulting in permanent blindness. Two approaches are being made. The first concerns the importance of protein for the conversion of β-carotene to retinol in the gut, and the absorption, storage, and ultimate use of retinol by the tissues.

The details of the relationship are now becoming clearer. Arroyave showed that vitamin A provoked no response in vitamin A-deficient children who were also deficient in protein unless milk were given as well. However, giving vitamin A-deficient children some skim milk, which is devoid of vitamin A, increased the level of vitamin A in their blood. This increase of serum vitamin A when the children were given protein suggested that the decreased vitamin A in their blood was secondary to a decrease in a postulated protein carrier.

The concept of retinol-binding protein (RBP) and of pre-albumin, the transport proteins for vitamin A in the serum, is now familiar from the fascinating work of De Witt Goodman and of Vahlquist and their associates. This has illuminated the relationships between protein and vitamin A and may well lead to changes in the treatment of xerophthalmia. In rats, deficient only in vitamin A, the RBP level in the blood falls and that in the liver rises. When vitamin A is given to such animals the RBP level in the blood...
starts to rise within 1.5 hours of the dose. There is a direct relation between the level of retinol, RBP, and pre-albumin in the blood. But if children who are deficient in vitamin A and also malnourished were given vitamin A parenterally the level of RBP in their blood did not increase for 24 hours. 5 In contrast, when protein alone without vitamin A was given to children with kwashiorkor or marasmus the levels of RBP, pre-albumin, and vitamin A all increased. 6 "These findings suggest that the low levels of vitamin A in kwashiorkor largely reflect a functional impairment in the hepatic release of vitamin A rather than vitamin A deficiency per se. Hepatic release of vitamin A is apparently impaired because of defective production of plasma transport proteins for retinol because of a limited supply of substrate for protein synthesis. When substrate is provided by dietary calories and proteins, the hepatic production of plasma proteins increases, plasma RBP and pre-albumin rise and hence plasma vitamin A concentration increases. 7"

The toxicity of excess vitamin A is well known, and even the dose of 200,000 IU by mouth distributed to preschool children caused vomiting, diarrhea, and malaise in 4 per cent of them. 8 This toxicity may be due to free retinol. Dingle, Fell, and Goodman 7 found that free retinol was toxic to tissue cultures but that the RBP-retinol compound was not, and recently, excess retinol palmitate given to normal rats has been found to depress the level of RBP in the blood, possibly by inhibiting its synthesis in the liver. 9 Thus it seems possible that although a large dose of vitamin A to a child who is not seriously protein deficient is a useful boost to the liver store; such a dose to a child who is seriously protein deficient may not be helpful.

One of the characteristics of serious xerophthalmia is the rapidity with which it goes from bad to worse, to end with perforation of the cornea. Brown, Slansky, and Berman 8 (whose work has recently been reviewed by Lemp 9) have shown, by culture of the tissue, that both animal and human corneas contain collagenase and develop a more active one after injury. It is possible that the same enzyme or enzymes are responsible for the rapid dissolution of the xerophthalmic cornea. This possibility has been examined in the rat. 10 Unfortunately, rat collagenase isolated from cultures of rat uterine or skin, digests collagen to multiple products, unlike the enzyme from other mammals which digests collagen to two characteristic products only. The small amount of tissue available from a rat cornea has made it impossible to attempt separation of collagenase from other enzymes so that it is only possible to speak of a collagenolytic system in the tissue. At least two enzyme activities have been found in cultures of normal rat cornea. Collagen is digested to multiple fragments of the same nature as those produced by collagenase isolated from other rat tissues and there is also neutral proteinase activity using azocasein as substrate. The activity in culture medium from normal corneas is less than the activity shown by culture medium of xerophthalmic corneas. 11

One of the characteristics of the ulcerating cornea of the xerophthalmic rat, shared by that of the xerophthalmic child, 11 is the massive influx of cells which precede the main capillary invasion. It is possible that these cells play a part in the destruction of the cornea. Local therapy of injured corneas with collagenase inhibitors has had some success. 9 If such local therapy of the xerophthalmic cornea is contemplated much more needs to be known of the enzymes present in the tissue.

They say there are more ways than one of skinning a cat; the work described in this paper suggest that there are more ways than one in which to endeavor to mitigate the ravages of xerophthalmia. After twenty years in Indonesia, Johanna ten Doesschate concluded "It cannot be repeated often enough that the treatment of xerophthalmia should take into account all of its component factors."


Key words: xerophthalmia, vitamin A deficiency, cornea, epidemiology.

REFERENCES

Lens growth in the Nakano mouse. HENRY N. FUKUI, H. OBAZAWA, AND J. H. KINOSHITA.

The growth curve of the lens of the Nakano mouse was compared to that of the normal mouse. There was no obvious difference for the first two weeks of age. After this period the growth of the normal lens continued while that of Nakano mouse lens slowed abruptly and eventually ceased. Studies with labeled leucine, however, showed that even after the appearance of the "pin-head" opacity the protein synthesis, although depressed, continued. These findings combined with previous histological observations suggest that fiber formation is probably unaffected in the early stages of the Nakano cataract. The apparent cessation of lens growth is probably associated with the extensive liquefaction that is observed to occur at the posterior nuclear region.

Changes in many parameters occur during the development of a cataract in the Nakano mouse strain. The first sign of a cataract in these mice is the appearance of a small nuclear opacity ("pin-head" opacity) observed in the lens about three weeks after birth. The opacity is accompanied by marked alteration in the lens in regard to morphology and many biochemical parameters. Most striking is the sudden increase in lens hydration which appears to be caused by an influx of sodium ions. A partial deficiency in the lens Na-K ATPase, possibly caused by the presence of an endogenous inhibitor of the enzyme, may be responsible for the defect in the cation pump mechanism that leads to the initial osmotic change.

This report concerns an observation made in the development of this type of cataract namely the apparent cessation of growth of the lens. Seeking an explanation of this phenomenon a study was made on the uptake and incorporation into lens proteins of the amino acid, leucine.

Material and methods. The hereditary cataractous strain of Nakano mice originated from an inbred strain from Japan. These mutants were crossed with the Charles River albino mice (Charles River Breeding Laboratory, Wilmington, Mass.) and a cataractous strain was developed as described previously. The normal mice used in the study were the Charles River albino mice.

The 14C-leucine uptake and incorporation studies were accomplished by incubating mouse lenses in 5.0 ml of medium, the composition of which was described by Epstein and Kinoshita. Isotope procedures used were similar to those previously described. The data are reported for an incubation period of three hours. Incubation periods for two and four hours were also run to show that the uptake and incorporation processes were linear. Trichloroacetic acid was used to precipitate the protein and the filtrate assayed for the amount of 14C-leucine taken up in the lens. The precipitate was washed with alcohol, dried with ether, weighed, dissolved in Hyamine, and counted.

Results. The effect of age on the changes in the dry weight and wet weight of the lenses of the Nakano and normal mice are shown in Fig. 1, A and B. The normal lens increases in size rather dramatically during the first two weeks of age. The rate of growth, as judged by the increase in dry weight, can be estimated as about 60 micrograms per lens per day. After two weeks of age the growth rate slowly tapers off but with age the lens continues to increase in size. In contrast to the normal lens the Nakano mouse lens presents a different growth curve. Up to two weeks the growth rate of the Nakano mouse lens is about that of the normal mouse lens. After this period, however, the growth rate of the Nakano mouse lens abruptly falls off, and beginning at about 21 days of age it reaches a plateau. Thus, at the time when the "pin-head" opacity appears the lens appears to cease growing.

The lens growth curves derived from the wet weight data resemble the curves from the dry weight results except for the subtle differences in the Nakano mouse lens. The growth curve of the wet weight of the normal lens more or less superimposes upon the growth curve of dry weights. This indicates that the dry weight to wet weight ratio does not appear to change appreciably throughout the age period studied.

In the Nakano mouse lens the curve for the dry weight reaches a plateau after three weeks of age. The growth curve from the wet weight data appears to parallel that of the dry weights except that a slow but progressive increase is observed after the three-week period. This means that an increase in hydration begins at three weeks and progresses slowly. This is consistent with the findings previously reported that the osmotic change occurs just about the time when the "pin-head" opacity appears in the Nakano mouse strain.