

The lutein free diet, a treatment option for autism.
Review of lutein as it relates to autism.
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Introduction

In our previous work, we have described how the human immune system could have evolved to result in an immune system selection of lutein as a non-self pathogen. ^{113, 67}
In this article, we review the literature on lutein as it relates to autism and a lutein-free diet as a treatment option.

Visual impairment:

“Ten autistic children, 6 girls and 4 boys, underwent a complete ophthalmologic examination in the Department of Pediatric Ophthalmology at the Hospital La Timone, Marseilles, France. Their age ranged from 1 to 14 years (mean = 8.5 +/- 3.8).
RESULTS: Refraction showed:

- Hypermetropia (far-sighted) in 7 cases (70%);
- Astigmatism (**Unequal curvature of the refractive surfaces of the eye, hence a point source of light cannot be brought to a point focus on the retina, but is spread over a diffuse area**) in 6 cases (60%)
- Strabismus (deviation of the eye which the patient cannot overcome) was present in 6 cases (60%)

CONCLUSION: Ophthalmologic findings in autistic children appear to be mainly unilateral or bilateral astigmatism and binocular vision troubles. They can lead to **amblyopia** (chronic visual disorders) with the risk of functional loss of vision. **Early diagnosis of visual problems in autistic children is also essential** in order to be able to propose adequate psychological and educational care for the children and their family.” [Ophthalmologic signs in children with autism; Denis D et al; 1997]

“A comparison between the 15 blind children who had IQs over 70 and 10 sighted children group-matched for age and verbal ability revealed that a number of autistic-like features were more common in the blind. When the nine blind children who had IQs less than 70 were compared with nine group-matched autistic children, the picture that emerged was of substantial overlap in clinical presentation, despite subtle differences on clinical impression. Similar results were obtained when blind subgroups were reconstituted according to the children's non-autistic or autistic-like clinical presentation, rather than IQ.”¹⁷

These studies strongly point to a disturbance which could be anticipated in a metabolism with an abnormal immunogenetic (IoGc) relationship to lutein (xanthophyllic pigments) resulting in the ophthalmologic findings in the eyes of people with autism. Coupled with dietary information which strongly indicates many people and particularly young children with autism actively attempt to avoid dietary lutein sources, the theory that lutein is a potential causal factor in autism is strongly supported by the research. ⁶⁷

“Children with blindness due to retinopathy of prematurity (ROP)—who are at greatly increased risk of cerebral damage - have been noted to have a high rate of autistic symptoms, but systematic controlled studies have been lacking. A controlled population-based study was performed; one group was blind due to ROP (N=27) and the other was congenitally blind due to hereditary retinal disease (N=14). Fifteen of the 27 children with ROP had autistic disorder.”¹⁸

The controlled study clearly shows that a disease which is suspected to result from lack of lutein protection in the eye has a strong occurrence within the autism population. It is possible, if not probable, that the lutein disturbance goes further than just a lack of eye pigment protection for people with autism. ⁶⁷ Additional reports provide information such as incidents of ‘delayed onset of visual maturation’ - children born blind who later develop visual acuity with onset of autistic symptomology. ¹⁰³

Blindness

‘As early as 1964, Fraiberg and Freedman made the observation that "ego deviation" (as they termed this condition) was present in 25-30% of blind children. This of itself is a very alarming assertion which might have been expected to arouse considerable concern among parents, educators and doctors. Perhaps one reason that it did not give rise to widespread comment is the association between visual impairment and learning disability. It is, of course, hard to obtain accurate data on the frequency with which learning disability accompanies visual impairment, not least because visual impairment is greatly under-reported in children with severe and complex disabilities. However, given the relative rarity of conditions resulting in "uncomplicated" blindness, compared to the more frequent situation in which visual impairment is just one component of a more widespread disability (as can occur in prematurity, pre-natal infection, birth asphyxia, various syndromes, etc), one can give a conservative estimate that at least 50% of blind children might be expected to have learning disability. Looking at the relationship between learning disability and autism, Wing and Gould, in their large population-based study in Camberwell, found the triad of impairments which characterise autism in 56% of those with severe learning disability (IQ <50). It might therefore be surmised that since learning disability is a common accompaniment of visual impairment, and autism a common accompaniment of learning disability, the association between visual impairment and autism needs no further explanation or investigation.

... Perhaps a partial answer to this problem lies in the question of whether the behavioural disturbance observed in visually impaired children is the same phenomenon as autism in the sighted, or whether we are observing a superficially similar end-point arising from two quite different processes. At this point it is necessary to take a step back and consider a fundamental question; namely, what is autism? Accepting that our knowledge of normal social development in blind babies is very sketchy, the next question to address is which social development processes can go wrong. It has taken a long time to reach an understanding that sighted autistic children do not have a global impairment in their social interactions, and this is a widespread source of confusion amongst both parents and professionals. In fact, very specific processes are affected:

AUTISTIC CHILDREN ARE ABLE TO:

Show attachment behaviors

Show proximity seeking behaviors

Recognize self/non-self in the mirror and recognize faces of other people

AUTISTIC CHILDREN ARE POOR AT:

Sharing and directing attention

Imitation

Recognition of affect

To sum up the current state of knowledge, we have a poor grasp of how normal social development proceeds in blind children, and given the blind child's very different experience of the world, it is in some ways more remarkable that the process frequently succeeds than that it sometimes fails. Whilst we might make the assumption that the autistic-like picture observed in a proportion of blind children arises from the same core deficit as that in sighted autistic children, we do not yet have enough data on the range of normal social development in blind children to be able to prove this assertion, not to identify early markers of incipient disaster. The field of visual impairment and autism is extremely confusing and this paper has perhaps raised far more questions than it has answered. Nonetheless, although the "tidy-minded" doctor likes to take a cause-effect approach to developing treatment approaches, this is often not the way that medicine advances, with progress in appropriate treatment and remediation often preceding our understanding of causation. For this reason it is quite possible to attack the problem from both ends and hope that somewhere in the midst of the confusion we are able to advance our management of this difficult condition to the advantage of the children in our care.' ⁷⁸

Vision impairment as an established finding in autism

The 1996 paper by Dr. Cass was unfortunately published just a year before the Denis paper 'Ophthalmologic signs in children with autism', 1997. Had this study been available when Dr. Cass was presenting her information then she might have had greater confidence in the 1964, Fraiberg and Freedman observation that "ego deviation" (as they termed this condition) was present in 25-30% of blind children. The current research includes that many children have overlapping blindness and autism and that a significant percentage of the sighted children with autism have some severe ophthalmologic signs.

Embryogenesis

Evidence exists which suggests that human rhodopsin activation is developing within the embryonic kidney cells or as these are forming. Vitamin A actions which occur in the mammalian eye are being determined during embryogenesis. ¹⁹ Evidence correlates 'retinal histopathology with functional changes caused by the rhodopsin Q64ter mutation. ...Microscopic changes in the donor retinas correlated well with the abnormalities in visual function in the patient donor and other family members. Postreceptoral ERG defects may relate to the abnormal photoreceptor processes found in the inner retina.' ⁴² These types of findings correlate well to scientific investigation which alludes to changes in the human genome, evolution of the human immune system and changes taking place which take us one step farther from plant life. 'The number of pigments found in the different animals increases from man to fish.' ⁴⁵

Beta-carotene is not Vitamin A

The simple truth is vitamin A, not beta-carotene, is needed to prevent night blindness, to protect from vitamin A deficiency syndromes and protect from vaccine related injuries which are known to cause blindness and death. ^{47, 48, 49, 50, 51, 52}

‘Vitamin A deficiency is a major public health problem in the countries of the Sahel. It causes xerophthalmia and high rates of child mortality and it occurs mostly in underdeveloped regions. People of all ages may suffer from vitamin A deficiency but it is a particular problem in pre-school-age children. Each year, about 250,000 children throughout the world become blind due to vitamin A deficiency. Measles, pneumonia and diarrhea reduce the child's reserves of retinol and increase the dietary requirement for vitamin A. Improvement of social conditions is a radical approach to preventing vitamin A deficiency.’ ⁵⁸ The most readily available, cheap, locally grown foods in Africa include and are primarily rich sources of both beta-carotene and lutein including corn (mealie meal), spinach, many fruits and vegetables and eggs. More expensive and less readily available foods include fish and dairy the primary food sources of vitamin A. Many types of cheap vegetable oils and cheap margarine products are also available whereas butter and healthy oils are significantly more expensive. Many nuts are grown in Africa but often these foods are exported and when they are sold in Africa they are sold at world market prices keeping them out of the reach of most families many of whom are actively employed in production and harvesting of these exported foods. Often migrant farm workers are working for less than minimum wages which are now set at the equivalent of 45 cents (US\$) per hour (2003) in South Africa. Millions of people are living below the UN defined poverty level of \$1.00 per day.

Vitamin A deficiency

‘Vitamin A was first discovered in 1913. Its deficiency was soon associated in animal models and case reports with stunting, infection, and ocular changes (xerophthalmia) resulting in blindness. The ocular consequences dominated clinical interest through the early 1980s. A longitudinal prospective study of risk factors contributing to vitamin A deficiency and xerophthalmia revealed a close, dose-response relationship between the severity of mild preexisting vitamin A deficiency and the subsequent incidence of respiratory and diarrheal infection (relative risk [RR], 2.0-3.0) and, most dramatically, death (RR, 3.0-10.0). Subsequent community-based prophylaxis trials of varying design confirmed that vitamin A supplementation of deficient populations could reduce childhood (1-5 years old) mortality by an average of 35%. Concurrent hospital-based treatment trials with vitamin A in children with measles revealed a consistent reduction in measles-associated mortality in Africa of at least 50%. **It is now estimated that improving the vitamin A status of all deficient children worldwide would prevent 1-3 million childhood deaths annually.**’ ⁵⁹

The World Health Organization has made an error, nearly four decades ago, when they included recommendations in the food guide which were based on evidence that some people could produce vitamin A from beta-carotene precursor foods. ⁴⁶ Science has been trying to prove this for nearly four decades and the jury is still undecided. ^{1, 6, 8, 12, 16, 28, 29} The expert witnesses don't agree and science is often misused to sway the public interest. ⁴⁴ Having made such a heinous error, the World Health Organization, has not yet come forth to acknowledge the mistake.

‘Nutritional blindness is loss of useful vision resulting from vitamin deficiency. Vitamin A malnutrition Xerophthalmia refers to all of the ocular manifestations of

inadequate metabolism of vitamin A, nutritional blindness being the end result of the most severe cases. The estimated overall prevalence of nutritional blindness in Africa is very low, below the WHO levels of significance, although isolated clusters of locally high prevalence exist, usually in arid, sparsely-populated regions. The peak age group affected is 2-year olds, with most nutritional blindness having its effect before age 6 years. Xerophthalmia may be considered as a serious side effect of protein-energy malnutrition (PEM). When associated with corneal sequelae of xerophthalmia, PEM has an estimated overall mortality of 50%. Intervention programs, therefore, are more appropriately aimed at the broader condition of life-threatening PEM than at the specifically vision-threatening problem of xerophthalmia.’⁵⁸

‘Pupils attending 12 schools for the blind in Malawi, 3 schools in Kenya and 2 schools in Uganda were examined to determine the causes of severe visual impairment or blindness (visual acuity in the better eye of less than 6/60). **A total of 491 pupils aged 3-22 years was examined.** Visual acuity was measured in each eye using a Snellen E chart. ...Corneal pathology, attributed to vitamin A deficiency and measles infection in the majority, was responsible for proportionally more severely visually impaired /blind (SVI/BL) in students in Malawi than in Uganda or Kenya’⁶⁰

Vitamin A deficiency is not limited to the poorer nations:

‘Studies from Africa suggest that vitamin A supplementation may reduce morbidity and mortality rates associated with measles among poorly nourished children. We studied 20 children with measles in **Long Beach, Calif.**, and found that **50% (95% confidence interval; 28% to 72%) were vitamin A deficient.** This frequency among presumably well nourished American children supports evaluation of vitamin A status as a part of acute management of measles in the United States.’⁶¹ ‘Health conscious’ Americans with limited information on nutrition in public education have relied on the food guide pyramid for several decades. Fear of fats and cholesterol as well as inaccurate reporting of vitamin A bio-availability from precursor beta-carotene foods has likely contributed to decreased intake of natural vitamin A foods and use of truly supportive cod-liver oil supplementation. Fish liver oil supplementation is the first recorded supplement used in the human diet with records identifying this practice in Ancient Egypt (Olsen, J).

WHO: Edible vaccines, genetic modification of foods and beta-carotene

Instead WHO continues with efforts to produce even more potentially population damaging interventions; supporting research into edible vaccines⁶². ‘Vaccine-induced immunity against measles is less robust than natural immunity. Waning of immunity in vaccines may eventually require a revaccination of adults. **Measles antigens expressed in plants** have been shown to be antigenic and immunogenic both after invasive and **oral vaccination.** Strategies for the vaccination of adults, the potential of an **oral measles vaccine produced in edible plants** and the design of suitable antigens are discussed.’⁶⁴ WHO is actively involved in genetic modification of foods to increase carotenoid production in plant foods and allowing mega-dosing of infants, children and study participants with precursor beta-carotene all in the name of science.’⁵³

A deficiency of vitamin A can result in tragic errors when the dietary source of vitamin A is identified to be present but it has been calculated from the dietary precursor beta-carotene. How many studies have provided false information based on the findings where carotenoids were considered adequate sources of essential fat

soluble vitamin A? Research continues and it has been determined that supplementing vitamin A at the time of vaccination does not provide the hoped for change in vitamin A status. Research continues to find whole populations which cannot make vitamin A from carotenoids such as this study from Indonesia: 'The subgroup that was most in need of vitamin A could not obtain it from plant foods.' ⁷⁵

Marion Nestle PhD says it best:

'The suggestion that "golden" rice, bio-engineered to contain β -carotene, could have "a real impact on the health of children living in Southeast Asia" deserves critical scrutiny from nutrition professionals. This rice, although not yet available commercially, has become the "poster child" of the food biotechnology industry's extensive public relations campaign to convince the public that the benefits of genetically engineered agricultural products outweigh any safety, environmental, or social risks they might pose. National magazines promote golden rice as a means to prevent the more than one million annual deaths and cases of blindness that occur among children in developing countries as a result of vitamin A deficiency. The creation of golden rice appears to confirm the belief that biotechnology is the key to solving world food and nutrition problems.

. . . Consideration of basic principles of nutrition suggests that rice containing β -carotene is unlikely to alleviate vitamin A deficiency. To begin with, the bio-availability of β -carotene is quite low - 10% or less by some estimates. To be active, β -carotene - a pro-vitamin - must be split by an enzyme in the intestinal mucosa or liver into two molecules of vitamin A. Like vitamin A, the pro-vitamin is fat-soluble and requires dietary fat for absorption. Thus, digestion, absorption, and transport of β -carotene require a functional digestive tract, adequate protein and fat stores, and adequate energy, protein, and fat in the diet. Many children exhibiting symptoms of vitamin A deficiency, however, suffer from generalized protein-energy malnutrition and intestinal infections that interfere with the absorption of β -carotene or its conversion to vitamin A. In numerous countries where vitamin A deficiency is endemic, food sources of β -carotene are plentiful but are believed inappropriate for young children, are not cooked sufficiently to be digestible, or are not accompanied by enough dietary fat to permit absorption. In addition to doubts about cost and acceptability, biological, cultural, and dietary factors act as barriers to the use of β -carotene, which explains why injections or supplements of pre-formed vitamin A are preferred as interventions. The extent to which the β -carotene in golden rice can compensate for these barriers is limited.

Vitamin A deficiency is undeniably the single most important cause of blindness among children in developing countries and a substantial contributor to illness and death from infectious diseases. Mortality rates are higher among children with even mild vitamin A deficiency, but fall by as much as 54% when vitamin A - not β -carotene - is supplemented or injected. Because such intervention methods are expensive and difficult to accomplish in the field, and because so many children exhibit signs of generalized protein-energy malnutrition, food-based approaches to improving vitamin A status seem especially desirable. The addition of a one or two nutrients to an existing food does not constitute a food-based approach.

Furthermore, the use of β -carotene as a single-nutrient supplement itself raises questions. Although fruits and vegetables containing β -carotene are demonstrably protective against disease, the results of clinical trials of β -carotene supplements as a means to prevent cancer or cardiovascular disease have proved disappointing. Some laboratory studies support the idea that β -carotene produces biological effects that

might protect against cancer, but others suggest that it might be co-carcinogenic. Still others argue that β -carotene is a pro-oxidant that may be harmful or beneficial, depending on circumstances. What all this means is that the short- and long-term effects of supplementation of β -carotene as a single nutrient - distinct from the foods that contain it - are as yet uncertain. The complexity of the physiological, nutritional, and cultural factors that affect vitamin A status suggest that no single-nutrient added to food can be effective as a remedy for dietary deficiencies. Instead, a combination of supplementation, fortification, and dietary approaches is likely to be needed, along with a substantial commitment to improve socioeconomic status. Food biotechnology may yet lead to products that improve nutrition and health, but at the moment its benefits remain theoretical.’ ⁶³

Vitamin A vs. beta-carotene – what the research really says:

What we do know is that vitamin A retinol levels **may** rise when carotenoids are ingested or given as supplements, in some people. This does not prove that carotenoids are being converted to retinol. Carotenoids compete for retinol binding receptor sites. **‘The most likely explanations for carotenoid interactions appear to be competition for incorporation into micelles, carotenoid exchange between lipoproteins in the postprandial state, and inhibition of provitamin A (beta-carotene) cleavage.’** ³ Some populations studied have NO increase in vitamin A levels when given vitamin A precursor foods or supplements. ^{73, 74} Major studies on the effectiveness of beta-carotene supplementation for cancer prevention called the CARET studies failed miserably and had to be stopped early. ‘The analyses of two major lung cancer prevention trials, beta-Carotene and Retinol Efficacy Trial (CARET) and Alpha-Tocopherol Beta-Carotene (ATBC), were also published this past year. Both found an increased incidence of lung cancer in individuals receiving beta-carotene.’ ⁵⁴

‘CARET participants receiving the combination of beta-carotene and vitamin A had no chemopreventive benefit and had excess lung cancer incidence and mortality. The results are highly consistent with those found for beta-carotene in the Alpha-Tocopherol Beta-Carotene Cancer Prevention Study in 29,133 male smokers in Finland.’ ⁵⁵ ‘Supplementation with alpha-tocopherol or beta-carotene does not prevent lung cancer in older men who smoke. beta-Carotene supplementation at pharmacologic levels may modestly increase lung cancer incidence in cigarette smokers, and this effect may be associated with heavier smoking and higher alcohol intake. IMPLICATIONS: While the most direct way to reduce lung cancer risk is not to smoke tobacco, smokers should avoid high-dose beta-carotene supplementation.’ ⁵⁶

Evolution of color

R.A. Nicolaus in his review of the evolution of pigments in living species states that there is every indication that pigments are used less and less over time and that the most decreased levels of use are in humans. ⁴⁵ This trend in evolution towards limited use of pigments as well as science which still includes that carotenoids are NOT essential to the human diet are well hidden from public attention. The health food industry, pharmaceutical companies and even food suppliers have been allowed to replace the accurate labeling of beta-carotene with the vitamin A description without industry regulation. Beta-carotene has gradually and deliberately become synonymous with vitamin A in the public eye and indeed in some medical literature and product representation and through misrepresentation of the science some doctors rapidly assume or are erroneously taught that beta-carotene can replace essential vitamin A. ⁴⁴

'Physicians are accustomed to making decisions based on information regarding the prevalence of disease, symptoms, physical signs, laboratory test results, and the risks and benefits of alternative treatments. If nutritional assessment and therapeutics are to become more common components of medical practice, significant barriers in each of these areas must be overcome. Even rudimentary dietary assessment is often missing from physician education. Dietary assessment tools that are readily available and that have demonstrated usefulness are largely unknown. In addition, many nutritional interventions have not been formally investigated in randomized, controlled trials, and thus their cost-effectiveness remains unknown. We present one approach to these issues by discussing the construction of a decision model examining strategies for vitamin D and calcium screening. The application of medical decision making techniques to problems in clinical nutrition illustrates how findings from research studies may be used to determine the risks, benefits and costs of alternative population based health related nutrition policies which can then be applied by physicians in their daily interactions with patients.'

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Evolutionary stress

Not only is the human population showing signs of **evolutionary stress** but so are the animals in our food chain. 'Pale-bird syndrome (PBS), defined as the failure of birds to realize the color potential of their diet, has been demonstrated to be caused by malabsorption or by hyperexcretion of carotenoids' ⁶⁵ 'Birds hatched from breeders fed lutein had significantly lower relative liver weights than chicks of the other treatments, whereas birds hatched from the breeders fed beta-carotene and canthaxanthin had significantly lower spleen weights than the control. These experiments suggest that carotenoids may not be effective in increasing neonatal immune response when they supplement practical breeder diets.'

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Carotenoid toxicity ⁷²

Carotenoids can be toxic and even result in fatalities. Aplastic anemia and Canthaxanthin retinopathy have been reported. ^{70, 71} 'Tanning pills,' commonly available by mail order or sold in tanning salons are reported to have caused aplastic anemia, which led to death in a healthy young woman. A 20-year-old woman was hospitalized with headaches, fatigue, a general feeling of not being well, easy bruising, and weight loss four months after beginning the use of canthaxanthin-containing tanning pills. She had noticed the onset of fatigue and easy bruising about two weeks after beginning the pills. Canthaxanthin can remain in the body for long periods of time. It has been found in blood several months after use was discontinued. It may accumulate in the retina of the eye, and has been reported to cause hepatitis (inflammation of the liver) with itching and hives.'

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"As reported earlier, crystalline yellow-glistening deposits in the retinal inner layer were identified in 22 of 53 patients treated with PHENORO (containing 10 mg beta-carotene and 15 mg canthaxanthin per capsule) for various light sensitive dermatoses at cumulative doses of canthaxanthin of up to 178 g for up to 12 years. 14 of the 22 patients with deposits could now be investigated again, 5 years after discontinuation of treatment. There was an extensive reduction of the number of deposits, confirming other reports which have demonstrated that the formation of the crystalline deposits is reversible. Analysis of serum samples revealed significant concentrations of canthaxanthin, resulting from the intake of this carotenoid via food.'

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Something is wrong

'A north Wales-based supermarket chain has stopped selling eggs with artificially coloured yolks after its chairman began keeping free-range birds and realised the colour was false. Deeside-based Iceland - the first supermarket chain to ban genetically modified foods - has now removed chemical colourants from its fresh egg range. Chairman Malcolm Walker said he discovered the myth behind the bright yellow yolk while rearing his own chickens.

'Something was wrong'

"I keep eight or nine hens at home just as I grow my own vegetables," he explained. "I became worried about the eggs when I noticed the yolks were different colours. I had even thrown ones with lighter coloured yolks away as I thought something was wrong." Mr Walker consulted Iceland's egg buyer about the issue just eight weeks ago and discovered the difference in colour was a totally natural occurrence.

Damaged eye retinas

"I found, to my horror, we have been dyeing eggs for 25 years in Britain by putting commercial dye in hens' feed." The shade of yellow of an egg's yolk depends on what a hen has eaten in the 10 days before laying it. Commercial animals are fed on high protein pellets which can contain three artificial chemicals to create an even yellow yolk. Of the three artificial colours used Iceland claim some 30% contain Canthaxanthin (E161g). This substance was formerly used in artificial tanning pills, but withdrawn when it was suggested its use could damage the eye's retina.

Customer reaction not positive

Although eggs stamped with the egg industry's Lion code do not contain this, Iceland says another 30% of eggs on sale in Britain which are not Lion branded could contain the chemical. Iceland admits that initial customer reaction on testing panels to the paler yolks was not positive. But when shoppers heard the story behind the change in colour they wanted the more natural product.' ⁶⁸

European Commission

The additives: carotenoid sources of canthaxanthine and lutein from substances such as marigold flowers and sunflowers makes egg yolks appear more yellow

'The additive makes egg yolks appear more yellow. The European Union has limited permitted levels of a food additive given to salmon and poultry, which may damage the eye. The chemical, canthaxanthin, is added to feed. It makes salmon appear more reddish, and chicken skin and egg yolks appear more yellow. But research has suggested that a build up of pigments can damage the retina. The level of the chemical allowed in salmon feed is to be cut by over two thirds. Limits will be even stricter in the feed for laying hens. Officials say the restrictions will not have any impact on the quality of farmed salmon or supermarket eggs.

Assessments

The European Commission said levels of the chemical should be reduced from a maximum of 80mg per kilogram to 25mg per kilogram of feed for fish and poultry, and 8mg per kilogram for laying hens. All EU members will have to enforce new regulations by the end of the year. Commissioner David Byrne said: "Scientific assessments have shown that a high intake of canthaxanthins produces an accumulation of pigments in the retina, affecting the sight. The use of this feed additive is purely cosmetic."

Extreme consumption

A spokeswoman for the UK's Food Standards Agency (FSA) told BBC News Online: "What the EU has been looking at is the very extreme consumption of products

containing canthaxanthin.” But it's something that's related to long-term exposure rather than acute. “Eating products with low levels of canthaxanthin is not something that people need to be worried about.” She said the FSA wanted to see levels of canthaxanthin in fish flesh, rather than in the feed measured, and labelling of products so consumers could see how much canthaxanthin they contain.’ 69

Lutein in human milk

‘Approximately 5-7% of all infants are born prematurely, and birth before 37 weeks is the most common cause of neonatal mortality, morbidity and long-term disability. Premature infants are poorly equipped for life outside the womb, and oxidant stress has been implicated in the aetiology of visual impairment in these infants, who are often exposed to increased O₂ concentrations and high light intensity in neonatal units. ...Little information is available on blood lutein and zeaxanthin levels in neonates. **Levels of lutein in human milk are two to three times higher than those of beta-carotene**, whereas their concentrations in the mothers’ blood are approximately the same. **Human milk is the main dietary source of lutein and zeaxanthin for infants until weaning occurs. The biochemical mechanisms which mediate the transport of the macular carotenoids into the eye are not known**, but tubulin has been identified as the major carotenoid-binding protein, and may play a role in the physiology of the macula.’ 21

Kanner ...

Early research in autism began with two well-known doctors who followed similar paths. The results of their work have become known today as Autism/Asperger syndrome(s). Swiss psychiatrist Eugen Bleuler first introduced the term autism in 1911. Autism and autistic stem from the Greek word ‘autos’, meaning self. The term autism originally referred to a basic disturbance in schizophrenia, in short, an extreme withdrawal of oneself from the fabric of social life, but not excluding oneself. 76 If we were to concentrate on this aspect solely then we would likely agree that even a typical child will exhibit extreme withdrawal from the fabric of social life when they are not well. When we, as humans, fall injured or sick, then our symptoms - pain, weakness, nausea, delirium, stomach ache, muscle contractions, breathing difficulties and the like - force us to withdraw from social life. Kanner reported his findings in an article entitled ‘Autistic Disturbances of Affective Contact’. Out of the 11 cases he reported, eight mentioned an unusual relationship to food, or feeding difficulties in early childhood.

From another recent review from the Journal of Abnormal Child Psychology June, 2001 ‘Does DSM-IV Asperger's Disorder Exist?’, we can see that ‘dislike of certain foods’ has been set apart from the diagnostic criteria and is included as an associated feature.

From ‘Autistic Disturbances of Affective Contact’ Kanner states “Since 1938, there have come to our attention a number of children **whose condition differs so markedly and uniquely from anything reported so far**, that each case merits - and, I hope, will eventually receive - a detailed consideration of its fascinating peculiarities.

Kanner Case 1 - Donald T: He was breast fed, with supplementary feeding, until the end of the eighth month; there were frequent changes of formulas. ‘Eating,’ the report said, ‘has always been a problem with him. He has never shown a normal appetite.

Seeing children eating candy and ice cream has never been a temptation to him . . . At mealtime, repeating something that had obviously been said to him often, he said to his mother, "Say 'Eat it or I won't give you tomatoes, but if you don't eat it I will give you tomatoes,'" or "Say 'If you drink to there, I'll laugh and I'll smile,'"

Case 4 - Paul G: He vomited a great deal during his first year, and feeding formulas were changed frequently with little success. He ceased vomiting when he was started on solid food. He would express his desire for candy by saying, "You want candy."

Case 5 - Barbara K: was referred in February, 1942, at 8 years, 3 months of age. She nursed very poorly and was put on bottle after about a week. She quit taking any kind of nourishment at 3 months. She was tube-fed five times daily up to 1 year of age...She began to eat then, though there was much difficulty until she was about 18 months old. Since then she has been a good eater, likes to experiment with food, tasting, and now fond of cooking...In camp last summer she was well liked, learned to swim, is graceful in water (had always appeared awkward in her motility before), overcame fear of ponies, played best with children of 5 years of age. At camp she slid into avitaminosis and malnutrition but offered almost no verbal complaint.

Throughout all these procedures, in which, often after several repetitions of the question or command, she complied almost automatically, she scribbled words spontaneously: 'oranges'; 'lemons'; 'bananas'; 'grapes'; 'cherries'; 'apples'; 'apricots'; 'tangerine'; 'grapefruits'; 'watermelon juice'; the words sometimes ran into each other and were obviously not meant for others to read.

Case 6 - Virginia S: They remember that she loves candy so much and says 'Chocolate,' 'Marshmallow,' also 'Mama' and 'Baby.'

Case 7 - Herbert B: He vomited all food from birth through the third month. Then vomiting ceased almost abruptly and, except for occasional regurgitation, feeding proceeded satisfactorily...He persistently refused to take fluid in any but an all-glass container. Once, while at a hospital, he went three days without fluid because it was offered in tin cups ... He very frequently brought blocks and other objects to his lips.

Case 8 - Alfred L: For the first two months, the feeding formula caused considerable concern but then he gained rapidly and became an unusually large and vigorous baby. He did a good deal of 'worrying': He frets when the bread is put in the oven to be made into toast, and is afraid it will get burned and be hurt . . . When infantile thumb sucking was prevented by mechanical devices, he gave it up and instead put various objects into his mouth. On several occasions pebbles were found in his stools. Shortly before his second birthday, he swallowed cotton from an Easter rabbit, aspirating some of the cotton, so that tracheotomy became necessary. A few months later, he swallowed some kerosene 'with no ill effects'.

Case 10 - John F: The father said: 'The main thing that worries me is the difficulty in feeding. That is the essential thing, and secondly his slowness in development. During the first days of life he did not take the breast satisfactorily. After fifteen days he was changed from breast to bottle but did not take the bottle satisfactorily. There is a long story of trying to get food down. We have tried everything under the sun.' John was born September 19, 1937; his birth weight was 7½ pounds. There were frequent hospitalizations because of the feeding problem. No physical disorder was ever found, except that the anterior fontanelle did not close until he was 2½ years of age. He suffered from repeated colds and otitis media, which necessitated bilateral myringotomy. . . Mild obsessive trends were reported, such as pushing aside the first spoonful of every dish.

Discussion: Our patients . . . anxious to keep the outside world away, indicated this by the refusal of food. Donald, Paul ("vomited a great deal during the first year"),

Barbara (“had to be tube-fed until 1 year of age”), Herbert, Alfred, and John presented severe feeding difficulty from the beginning of life. **Most of them, after an unsuccessful struggle, constantly interfered with, finally gave up the struggle and all of a sudden began eating satisfactorily.**” 77

Biomedical intervention:

Novel noninvasive optical techniques being developed may facilitate studies of ocular carotenoids distribution in the human eye and be adapted for use in early screening to identify at risk infants. 20

Pigments, colors, vibrations – Autism a disturbance in energy metabolism

‘The pterins, neopterin and biopterin, occur naturally in body fluids including urine. It is well established that increased neopterin levels are associated with activation of the cellular immune system and that reduced biopterins are essential for neurotransmitter synthesis. It has been suggested that some autistic children may be suffering from an autoimmune disorder. To investigate this further we performed high performance liquid chromatography analyses of urinary pterins in a group of pre-school autistic children, their siblings and age-matched control children. Both urinary neopterin and biopterin were raised in the autistic children compared to controls and the siblings showed intermediate values. This supports the possible involvement of cell-mediated immunity in the etiology of autism.’ 105

Research continues and ‘Evidence for the presence of pteridines in iridophores, leucophores, and xanthophores in a wide variety of vertebrate chromatophores, and argument that the chemical and functional distinction between pterinosomes and reflecting platelets is not as clear-cut as previously believed. Observations indicate that: (1) Pteridines may, either alone or in conjunction with purines, form pigment granules that reflect light, (2) these pigment granules are highly variable ranging from fibrous pterinosomes to typical reflecting platelets and may be colored, reflect white light, or be iridescent, and (3) many “leucophores” probably contain typical pterinosomes and presumed associated colorless pteridines and are therefore more closely related to erythrophores and xanthophores than to iridophores with which they are usually classified. We propose that the classification of pigment cells should be modified to reflect these facts.’ 22

‘The fundamentally diverse vertebrate pigment cells, melanophores, xanthophores, and iridophores, contain pigmentary organelles known, respectively, as melanosomes, pterinosomes, and reflecting platelets. Their pigments are melanins, pteridines, and purines. Mosaic pigment cells containing more than one type of organelle have been observed and mosaic organelles containing more than one type of pigment have been discovered. It is proposed that the various pigment cells are derived from a stem cell that contains a primordial organelle of endoplasmic reticular origin. This primordial organelle can differentiate into any of the known pigmentary organelles.’ 23

Finding the truth

The health food industry has obviously already expanded its marketable products range to include lutein supplements based on some reports that there is a possibility that macular degeneration is directly related to levels of dietary lutein and no doubt is finding this to be profitable, and at the same time research scientists continue to spend a lot of research dollars trying to prove the theory. Some people, including medical

people, have been convinced by the early reports. The ongoing research continues and the difficulty of finding a truth that satisfies the demand for scientific proof and settle the dispute is still in the research stages:

Human eyes

‘Biochemical research has demonstrated that lutein and zeaxanthin, the two macular carotenoids, are bleachable pigments. Further, evidence suggests that exposure to UV light can degrade plasma carotenoid levels in vivo. The present study investigated the effects of exposure to normal levels of light on the levels of lutein and zeaxanthin in the retina. ...The results suggest that lutein and zeaxanthin levels in the eye are unaffected by light and oxidation throughout the day. This justifies current research methods in which Macular Pigment Optical Density (MPOD) measures are made regardless of the time of day. However, significant between-day variance indicates that multiple MPOD measures may be necessary to evaluate lutein and zeaxanthin levels in the retina accurately.’²⁹

‘Thus a final characterization of the macular pigment shows that it is composed of three carotenoids: lutein, zeaxanthin and meso-zeaxanthin. **Our identification of the non-dietary meso-zeaxanthin as a component of the macular pigment dramatically emphasizes the lack of understanding of xanthophyll metabolism by humans...**Despite the remarkable concentration of carotenoid present in the macula, the dimensions of this tissue are such that only nanogram amounts of carotenoids are present. The concentration in liver [10] and serum is three orders of magnitude lower. ...The difference in macular pigment density between AMDs (Age related macular degeneration) and controls tends to decrease from the inner to medial to outer regions, raising the possibility that the **disease itself may be partly responsible for a preferential loss of lutein and zeaxanthin in the macula.** ...From these data, we conclude that very low carotenoid levels in the retina are associated with an increased incidence of AMD. However, this association may not be causal. It could be, for example, that processes which lead to AMD are also responsible for the retina's sluggishness in accumulating carotenoids.’¹

‘**Prospects for future research in the study of macular pigment require new initiatives that will probe more accurately into the localization of these carotenoids in the retina, identify possible transport proteins and mechanisms, and prove the veracity of the photoprotection hypothesis for the macular pigments.**’³²

‘Xanthophyll epoxides such as neoxanthin, violaxanthin and lutein 5,6-epoxide, are more abundant than epoxy-hydrocarbon carotenes in a number of vegetables and fruits that humans consume. To determine whether xanthophyll epoxides are also absorbed by humans, lutein 5,6-epoxide (taraxanthin) and zeaxanthin 5,6,5'6'-diepoxide (violaxanthin) were chemically prepared, dissolved in corn oil and orally administered to three human subjects. **Analysis of plasma for carotenoids within 9 h after a single oral dose of either violaxanthin or taraxanthin failed to show any violaxanthin, taraxanthin or any of their metabolites.**’¹²

Sex, eye color and age are reported as significant factors in macular degeneration:

‘Sex differences in protection of the retina by MP (macular pigment) and in the relationship between the retina, blood and diet could be a factor in the incidence of retinal diseases, especially age-related macular degeneration.’¹³

‘Age-related macular degeneration (AMD) is a leading cause of irreversible vision loss in older individuals worldwide. The disease is characterized by abnormal extracellular deposits, known as drusen, that accumulate along the basal surface of the retinal pigmented epithelium. Although drusen deposition is common in older individuals, large numbers of drusen and/or extensive areas of confluent drusen represent a significant risk factor for AMD. Widespread drusen deposition is associated with retinal pigmented epithelial cell dysfunction and degeneration of the photoreceptor cells of the neural retina. **Recent studies have shown that drusen contain a variety of immunomodulatory molecules, suggesting that the process of drusen formation involves local inflammatory events, including activation of the complement cascade.**’²

It is safe to say that science just does not yet understand carotenoid metabolism in humans. This is not limited to beta-carotene or lutein but would include also sources of other carotenoid pigments as well as pterin and porphyrin substances and food dyes (pteridines) and as yet unidentified substances which get into the human food chain through animal and plant foods we ingest, environmental toxins and degradation products produced in our own bodies when these substances are stored or eliminated. There is a lot of research being carried out and a lot more to come. Science has limitations and funding is a major limitation. Curiosity, interest, intrigue and obsession drive some people to delve deeper into the mass of information and peek into microscopes looking for answers. Obstacles are overcome as scientists discover the laboratory is not always environmentally suited to studying how some bacteria produce pigmented substances in light or in darkness, in aerobic or anaerobic conditions. When a large funding organization has an interest in the findings then studies are funded. When no funding institution has an interest the scientist obsessed by his own curiosity and need to know seeks answers on his own. Some of the greatest scientific discoveries have been made by people with an obsessive need to find the truth. Lutein pigment may be one of the defining substances which elucidate the uniqueness of each individual, used in the immunogenetic development of the embryo to result in defining characteristics of eye color, pigmentation, circadian rhythm and brain function.

Research continues:

‘This is a biographical sketch of my research and its related personal episodes with respect to brightly colored pigmentation in lower vertebrates. It includes a brief story of the studies on; (a) pterinosomes as a specific site of pteridine deposition in xanthophores or erythrophores of fish and amphibians, (b) a mosaic phenotype of chromatophores occurring in the reptiles and its implication for their developmental origin and differentiation mechanisms, (c) erythrophoroma as a tumor of erythrophores in goldfish, (d) the pluripotentials of erythrophoroma cells for expression of neural crest-derived characters in vitro, (e) pigment disorders occurring in hatchery-raised flounders and (f) recognition of pigment cell types by murine tyrosinase genes transfected into an orange-colored variant of medaka fish. Some of the personal affairs associated with the history of the Japanese community

for pigment cell research were described to illustrate the background of these studies.' *Matsumoto* 30

Pterin metabolism

Equally as difficult as carotenoid metabolism is the understanding of pterin metabolism in humans. Conditions of unconjugated pterin metabolism which co-occur with autism include Tuberous Sclerosis, Phenylketonuria, Down's Syndrome, metal toxicity (lead and aluminum), confusional states, dystonia and depression, as well as conditions with some similarities to autism diagnosed in the adult and aging human population – Alzheimers and Parkinson's disease. 104 The co-occurrence of genetic conditions which involve unconjugated pterin metabolism and eye disorders including blindness co-occurring with autism encourage further study of lutein and pterin - pigment metabolism in the autism population. Substantial evidence exists to suggest that autism is an evolution which has occurred or been exacerbated through use of vaccination. 67

We have very little understanding of the causes of these disease states and even less understanding when they co-occur with autism.

'Three novel pteridines have been isolated from the urine of patients with a new variant of 6-(L-erythro-1',2'-dihydroxypropyl)-5,6,7,8-tetrahydropterin (tetrahydrobiopterin) deficiency, showing hyperphenylalaninemia. From the results of high performance liquid chromatography, oxidative degradation, and gas chromatography-electron impact mass spectrometry, their structures were identified as 7-(D-erythro-1',2',3'-trihydroxypropyl)-pterin (7-neopterin), 7-(L-erythro-1',2'-dihydroxypropyl)-pterin (7-biopterin), and 6-oxo-7-(L-erythro-1',2'-dihydroxypropyl)-pterin (6-oxo-7-biopterin). The ratio of biopterin to 7-biopterin in the patients' urines was 1:1, and after oral loading with tetrahydrobiopterin, 7-biopterin excretion rose parallel to biopterin. **This finding suggests that 7-substituted pterins may be formed endogenously by a yet unknown isomerization reaction.** The cause of hyperphenylalaninemia is still unclear. The activities of the enzymes involved in tetrahydrobiopterin biosynthesis and regeneration were found to be normal in the patients, and no effect of 7-biopterin on these enzymes was observed in vitro. However, compared with the normal cofactor, tetrahydrobiopterin, the Km values of tetrahydro-7-biopterin for phenylalanine hydroxylase and dihydropteridine reductase are 20 and 5 times higher, respectively.' 84

'A novel pterin intermediate, in addition to the expected 4a-hydroxytetrahydrobiopterin (4a-OH-BH4) and quinonoid dihydrobiopterin, was generated during catalytic turnover of tyrosine hydroxylase (TH) with tetrahydrobiopterin as the cofactor. Based on chromatographic, spectroscopic and stability properties its structure is proposed to be similar to the product formed by the non-enzymic conversion of synthetic 4a-OH-BH4 [Bailey, Rebrin, Boerth and Ayling (1995) J. Am. Chem. Soc. 117, 10203-10211]. This compound was tentatively described as a 4a-adduct of a side-chain hydroxy group, i.e. the O2', 4a-cyclic-tetrahydrobiopterin (4a-Cyc-BH4). The intermediate generated in the TH reaction has a UV spectrum which is similar to that of 4a-OH-BH4, but elutes with a longer retention time (tR = 1.69 min compared with 1.06 min) on reversed-phase chromatography. Its conversion into quinonoid dihydrobiopterin is catalysed by pterin-4a-carbinolamine dehydratase (EC 4.2.1.96), although 4a-OH-BH4 is the preferred substrate for that enzyme. A precursor-product relationship was demonstrated between 4a-OH-BH4 and the

putative 4a-Cyc-BH4 intermediate. The apparent stability of this compound is dependent on pH as well as on the nature of the buffer ions. At pH 8.0 a large amount was generated in HEPES and Tris, but little in phosphate buffer. At pH 7.0 in HEPES (standard assay conditions) and Tris buffer the putative 4a-Cyc-BH4, but no 4a-OH-BH4, was observed. None of the intermediates was observed at pH 6.0. The accumulation of these intermediates in the absence of dehydratase has important implications for the assay of TH and phenylalanine hydroxylase activities, and is also compatible with a possible physiological role of the dehydratase in the synthesis of catecholamines in vivo.’³⁴

Disturbance in pigment, pterin and carotenoid metabolism

To further contribute evidence of disturbance in pigment, pterin and carotenoid metabolism we can look at **Incontinentia pigmenti (IP)** (usually lethal prenatally in males) research for Hypomelanosis of Ito (HI) or incontinentia pigmenti achromians has a heavy co-occurrence rate with autism. ⁶⁷

‘Patients with the depigmentation disorder vitiligo lack the capacity to synthesize the melanins from L-tyrosine via the essential activity of tyrosinase. The aim of this study has been to examine both the supply of the substrate (L-tyrosine) and the regulation of tyrosinase in the epidermis of subjects with vitiligo. Patients with this depigmentation disorder have a 3- to 5-fold increase in GTP-cyclohydrolase I activity leading to an excessive de novo synthesis of (6R)5,6,7,8 tetrahydrobiopterin (6-BH4). Continuous production of 6-BH-4 leads to: (1) an accumulation of the non-enzymatic byproduct 7-tetrahydropterin (7-BH4) in the epidermis, and (2) increased synthesis of the catecholamines in keratinocytes, leading to an excess of norepinephrine in both the plasma and urine of these patients. In vitiligo, the time-dependent production of 7-BH4 is caused by decreased 4a-hydroxytetrahydrobiopterin dehydratase activity; the essential enzyme for recycling and maintaining normal levels of 6-BH-4. In the epidermis and in cultured melanocytes, 7-BH4 is a potent competitive inhibitor of phenylalanine hydroxylase ($K_i = 10^{-6}$ M) and its accumulation in the epidermis of patients with vitiligo blocks the supply of L-tyrosine from L-phenylalanine. 4a-hydroxytetrahydrobiopterin dehydratase has a dual function as the activator/dimerization catalyst for the transcription factor hepatocyte nuclear factor I (HNF-I). HNF-I binds to a 16-base inverted palindrome which seems to be present on the promoters of both the tyrosinase and phenylethanolamine-N-methyl transferase (PNMT) genes. Therefore, defective 4a-hydroxytetrahydrobiopterin dehydratase in vitiligo influences not only the supply of L-tyrosine but also the transcription of the tyrosinase gene in melanocytes. Furthermore, a similar transcriptional regulation of the PNMT gene in keratinocytes offers a possible explanation for the accumulation of norepinephrine in these patients.’³⁵ Vitiligo and its associated phenomena of abnormal tryptophan metabolism, C4Q-null allele, elevated Indole-acrolyl-glycine and pellagra-like photodermatotic conditions are found in autism.

Immune, pigment, pterin, tryptophan and brain activity come together

‘Sepiapterin reductase (SPR) is the enzyme that catalyzes the final step of the synthesis of tetrahydrobiopterin (BH4), the cofactor for phenylalanine hydroxylase, tyrosine hydroxylase (TH), tryptophan hydroxylase, and nitric oxide synthase (NOS). Although SPR is essential for synthesizing BH4, the distribution of SPR in the human brain has not yet been clarified. In the present study, we purified recombinant human SPR from cDNA, raised an antibody against human SPR (hSPR), and examined the

localization of SPR protein and SPR activity. Human brain homogenates from the substantia nigra (SN), caudate nucleus (CN), gray and white matters of the cerebral cortex (CTX), and dorsal and ventral parts of the medulla oblongata (MO) were subjected to Western blot analysis with anti-hSPR antibody or with anti-TH antibody. Whereas TH protein showed a restricted localization, being mainly detected in the SN and CN, SPR protein was detected in all brain regions examined. SPR activity was relatively high compared with the activity of GTP cyclohydrolase I (GCH), the rate-limiting biosynthetic enzyme of BH₄, and was more widely distributed than GCH activity. Immunohistochemistry revealed SPR immunoreactivity in pyramidal neurons in the cerebral CTX, in a small number of striatal neurons, and in neurons of the hypothalamic and brain stem monoaminergic fields and olivary nucleus. Double-staining immunohistochemistry showed that TH and SPR were colocalized in the SN dopamine neurons. Localization of SPR immunoreactive neurons corresponded to monoamine or NOS neuronal fields, and also to the areas where no monoamine or NOS neurons were located. The results indicate that there might be a BH₄ biosynthetic pathway where GCH is not involved and that SPR might have some yet unidentified function(s) in addition to BH₄ biosynthesis.’³⁶

Cell death, beautiful colors

Brightly colored Fall foliage excites the human traveler and many tourism dollars are to be obtained entertaining the naturalists and fun seekers. The beautiful coloration associated with Fall, Halloween parties and ending with Thanksgiving in the USA are the signs of cell death in the plant which is readying to drop the no longer needed dead and dying foliage. Unconjugated bilirubin (UCB), cell death and disease presentation are being studied. ‘Mitochondrial membrane, with release of intermembrane proteins, has been strongly implicated in cell death.

...The pathogenesis of bilirubin encephalopathy and Alzheimer's disease appears to result from accumulation of unconjugated bilirubin (UCB) and amyloid-beta (Abeta) peptide, respectively, which may cause apoptosis. ...In this study, we further characterize UCB- and Abeta-induced cytotoxicity in isolated neural cells, and investigate membrane perturbation during incubation of isolated mitochondria with both agents. ...Apoptosis was assessed by DNA fragmentation and nuclear morphological changes. ...Toxicity occurs through a mitochondrial-dependent pathway, which in part involves opening of the permeability transition pore. Furthermore, membrane permeabilization is required for cytochrome c release from mitochondria ...mitochondria is a pharmacological target for cytoprotection during unconjugated hyperbilirubinemia and neurodegenerative disorders...’⁴³

Developmental patterns in the fish:

‘In the zebrafish, the peripheral neurons and the pigment cells are derived from the neural crest and share the pteridine pathway, which leads either to the cofactor tetrahydrobiopterin or to xanthophore pigments. The components of the pteridine pattern were identified as tetrahydrobiopterin, sepiapterin, 7-oxobiopterin, isoxanthopterin, and 2,4,7-trioxopteridine. The expression of GTP cyclohydrolase I activity during the first 24-h postfertilization, followed by 6-pyruvoyl-5,6,7,8-tetrahydropterin synthase and sepiapterin reductase, suggest an early supply of tetrahydrobiopterin for neurotransmitter synthesis in the neurons and for tyrosine supply in the melanophores. At 48-h postfertilization, sepiapterin formation branches off the de novo pathway of tetrahydrobiopterin synthesis. Sepiapterin, via 7,8-dihydrobiopterin and biopterin, serves as a precursor for the formation of 7-

oxobiopterin, which may be further catabolized to isoxanthopterin and 2,4,7-trioxopteridine. **Neither 7, 8-dihydrobiopterin nor biopterin is a substrate for xanthine oxidoreductase. In contrast, both of these compounds are oxidized at C-7 by a xanthine oxidase variant form, which is inactivated by KCN, but is insensitive to allopurinol. The oxidase and the dehydrogenase form of xanthine oxidoreductase as well as the xanthine oxidase variant have specific developmental patterns. It follows that GTP cyclohydrolase I, the formation of sepiapterin, and the xanthine oxidoreductase family control the pteridine pathway in the zebrafish.**' ³⁷

'Carotenoid compositions of the flesh, skin, and ovaries were determined in sexually maturing and immature Arctic charr (*Salvelinus alpinus*) fed diets supplemented with astaxanthin (optical isomer ratio (3S,3'S):(3R,3'S; meso):(3R,3'R); 1:2:1). Astaxanthin comprised 64-79% of the flesh carotenoids, and the 3',4'-cis and 3',4'-trans glycolic isomers of idoxanthin, present in a 1:1 ratio, represented 20-35%. The flesh of the sexually maturing charr contained relatively more idoxanthin than that of sexually immature fish (20 vs 35% of total carotenoids), possibly being indicative of a higher metabolic turnover of astaxanthin in the latter. The relative proportions of flesh carotenoids were unaffected by sex. The relative carotenoid composition of ovaries was similar in sexually maturing and immature females. The 3',4'-cis and 3',4'-trans glycolic isomers of idoxanthin (ratio 0.7:1) were the major carotenoids (56% of total), followed by crustaxanthin (20%), and astaxanthin comprised less than 5% of ovarian carotenoids. Three glycolic isomers of crustaxanthin were detected (3,4,3',4'-di-cis-:3,4-cis-3',4'-trans-:3,4,3',4'-di-trans-glycolic isomer ratio 2.6:3.1:1) in the ovaries. Sex and maturity status had no apparent effect on the relative composition of skin carotenoids. The skin carotenoids consisted mainly of diesters (82-87% of total carotenoids) and monoesters (7-13% of total carotenoids). Saponification revealed that astaxanthin comprised 85% and idoxanthin 10% of total carotenoids, and minor amounts of tunaxanthin-, lutein-, and zeaxanthin-like metabolites were also present. Maturity status seems to be more important than sex in determining the relative carotenoid composition of the tissues of Arctic charr, with astaxanthin and its metabolites being selectively accumulated in different tissues.'

'Apparent astaxanthin (3,3'-dihydroxy-beta,beta-carotene-4,4'-dione) digestibility coefficients (ADC) and carotenoid compositions of the muscle, liver, whole kidney and plasma were compared in Atlantic salmon (*Salmo salar*) and Atlantic halibut (*Hippoglossus hippoglossus*) fed a diet supplemented with 66 mg astaxanthin kg(-1) dry matter for 112 days. The astaxanthin source consisted of 75% all-E-, 3% 9Z- and 22% 13Z-astaxanthin, of (3R,3'R)-, (3R,3'S; meso)-, and (3S,3'S)-astaxanthin in a 1:2:1 ratio. The ADC of astaxanthin was significantly higher in Atlantic halibut than in Atlantic salmon after 56 and 112 days of feeding ($P < 0.05$). The ADC of all-E-astaxanthin was significantly higher than ADC of 9Z-astaxanthin ($P < 0.05$).

Considerably more carotenoids were present in all plasma and tissue samples of salmon than in halibut. Retention of astaxanthin in salmon muscle was 3.9% in salmon and 0 in halibut. All-E-astaxanthin accumulated selectively in the muscle of salmon, and in plasma of salmon and halibut compared with diet. 13Z-astaxanthin accumulated selectively in liver and whole kidney of salmon and halibut, when compared with plasma. A reductive pathway for astaxanthin metabolism in halibut similar to that of salmon was shown by the presence of 3',4'-cis and trans glycolic isomers of idoxanthin (3,3',4'-trihydroxy-beta,beta-carotene-4'-one) in plasma, liver

and whole kidney. In conclusion, the higher ADC of astaxanthin in halibut than Atlantic salmon may be explained by lower feed intake in halibut, and the lower retention of astaxanthin by a higher capacity to transform astaxanthin metabolically.'

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Developmental patterns in birds:

'With the resurgence of interest in sexual selection in the last twenty years, bright and contrasting plumage has once again become a central topic of discussion (Baker & Parker, 1979; Burt, 1986; Butcher & Rohwer, 1989; Badyaev & Hill, 1999; Go'tmark, 1999; Senar, 1999) and has featured prominently in the discussion of various models for the evolution of sexual ornaments (Fisher, 1930; Zahavi, 1975; Hamilton & Zuk, 1982; Kirkpatrick & Ryan, 1991; Fitzpatrick, 1994; Hill, 1994a; Owens & Hartley, 1998; see Andersson, 1994 for a comprehensive review). Given such intense interest in the evolution and maintenance of brightly coloured plumage, it is curious that so little attention has been paid to the fact that colourful or highly contrasting plumage can result from several, very different mechanisms (Gray, 1996; Owens & Hartley, 1998). First, bright coloration can be produced by either the microstructure of the feather itself, which differentially absorbs and reflects various wavelengths of light, or it can result from pigments (biochromes) deposited in the feathers during development (Fox, 1976; Brush, 1978, 1990). The three primary classes of pigments that birds use to colour feathers are carotenoids, melanins, and porphyrins (Fox, 1976; Brush, 1978). In passerine birds, which will be the focus of this paper, pigment-based coloration comes exclusively from carotenoids and melanins.'

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Developmental patterns in the humans

'PURPOSE: To determine the stereochemistry of carotenoids in human ocular tissues in comparison with plasma and liver and to elucidate the possible transformations of dietary (3R,3'R,6'R)-lutein and (3R,3'R)-zeaxanthin in the eye. Similarly, to characterize the carotenoid profiles in the eye tissues, plasma, and liver of quails and frogs to determine whether these can serve as appropriate nonprimate animal models for metabolic studies. METHODS: Configurational isomers of carotenoids and their nondietary by-products from pooled human plasma, liver, retinal pigment epithelium (RPE-choroid), ciliary body, iris, and lens were characterized and quantified by high-performance liquid chromatography (HPLC) on a chiral column. Carotenoids and their nondietary by-products in pooled extracts from quail and frog plasma, liver, retina, RPE-choroid, iris, and lens were similarly characterized and quantified. RESULTS: (3R,3'R,6'R)-lutein, (3R,3'R)-zeaxanthin, (3R,3'S; meso)-zeaxanthin, (3R,3'S,6'R)-lutein (3'-epilutein), 3-hydroxy-beta, varepsilon -carotene-3'-one, and 5Z- and all-E-lycopene were detected in nearly all human ocular tissues examined. **(3R,3'S; meso)-zeaxanthin was not detected in the human plasma and liver but was present in human macula, retina, and RPE-choroid.** (3S,3'S)-zeaxanthin was detected in human macula in minute quantities. The carotenoid profiles in quail and frog ocular tissues were somewhat similar to those in humans, with the exception that lycopene was absent. Frog retina, plasma, and liver revealed the presence of (3S,3'S)-zeaxanthin. CONCLUSIONS: The most likely transformations of carotenoids in the human eye involve a series of oxidation-reduction and double-bond isomerization reactions. Quail and frog appear to possess the appropriate enzymes for conversion of dietary (3R,3'R,6'R)-lutein and (3R,3'R)-zeaxanthin to the same **nondietary by-**

products observed in humans and thus may serve as excellent nonprimate animal models for metabolic studies.’³⁹

Studies using human subjects are often comprised of very few study subjects.⁸⁶

Schemochromes

‘Blue iris color was associated with an increased risk of both late AMD and early ARM in this population. Abnormal skin sensitivity to sunlight was also associated with an increased risk of late AMD.’⁵

Schemochromes or pigments which do not exist

‘Several colourations of the animals are due to phenomena of scattering, interference and diffusion of light. The physical phenomenon which nature creates with an incredible perfection can be caused by a variety of optical objects: particles, microscopic prisms, lattices, stripes, thin strata or cavities. In general blue and green are schemochromes.

One of the most beautiful colours of physical origin which is seen in the animal kingdom is blue. The manifestation comes about because of particles, honey-comb cells or other optical heterogeneities, with particle diameters of ($\sim 0.6 \mu$), the same order as that of the wavelength of blue light, when their refractive index is different to that of the medium which surrounds them. This type of blue is only seen by reflection while with transmitted light reddish, brown or grey colourations appear. The relative size of the particles determines the intensity and the shade of the blue: the blue of the sky, due to the diffusion of solar light by gaseous molecules in the air increases in industrial areas because of the presence of very fine particles, while larger particles make the blue paler. After a storm the sky is bluer because the rain makes a selection of the particles and eliminates the larger ones. The blue of human eyes, the blue of the plumage of birds, of some reptiles, of the fish *Hypsurus caryi* and *Trachinotus Kennedyi* and the blue rings near the eye of the octopus *Octopus bimaculatus* are all due to a physical phenomenon which is commonly called Tyndall scattering. This type of colour, which is not modified by the angle of vision, unlike colours due to diffraction and interference, is due to particles or cavities of about 0.6μ in diameter, laid as a black or brown substrate, which are almost always made of melanin. Without melanin the blue disappears or reduces greatly: by absorbing the light reflected from other bodies the melanin prevents this light from masking the small fraction of light scattered by the particles or cavities.’⁴⁵

Melanin

‘The present study was designed to assess the relationship between iris color and macular pigment optical density. Both melanin and carotenoids (responsible for iris color and **macular pigment composition**, respectively) appear to protect the retina through similar mechanisms and higher concentrations may reduce the incidence of retinal degenerations. ...The covariation of iris color and macular pigment optical density (MP) indicates that past epidemiologic studies have not adequately determined the independent effects of either factor. **The relationship of MP and iris color may be the result of one or two factors: the evolution of a shared tendency to accumulate melanin and carotenoids due to similar environmental pressures (e.g. light and oxygen); and/or MP might be depleted due to the tendency for eyes with light irises to transmit more light than eyes with dark irises, thus causing increased oxidative stress.**’ (Schepens Eye Research Institute)²⁸

Immune activity, particularly inflammation and disease states are associated with macular degeneration. Some or many diseases, disorders and conditions have increased over the past century. Scientific research attributes some or most of the increase to changes in the immune and genetic make-up of the human population. Molecular biology attributes these changes directly to evolution which has been impacted and exacerbated by vaccination practice. ⁶⁷ The very people who may have the most susceptibility to further damage from carotenoid ingestion are the target market for products touting the benefits and denying, misinterpreting or ignoring the research findings.

Drusen

‘Recent studies implicate inflammation and complement mediated attack as early events in drusen biogenesis. The investigations described here sought to determine whether primary sites of complement activation could be identified within drusen substructure, and whether known inhibitors of the terminal pathway of complement are present in drusen and/or retinal pigmented epithelial (RPE) cells that lie in close proximity to drusen. Immunohistochemical examination shows two fluid phase regulators of the terminal pathway, vitronectin (Vn, S-protein) and clusterin (apolipoprotein J), to be present in drusen; Vn also accumulates in the cytoplasm of RPE cells that are closely associated with drusen. The membrane associated complement inhibitor, complement receptor 1, is also localized in drusen, but it is not detected in RPE cells immunohistochemically. In contrast, a second membrane associated complement inhibitor, membrane cofactor protein, is present in drusen associated RPE cells, as well as in **small, spherical substructural elements** within drusen. These previously unidentified elements also show strong immunoreactivity for proteolytic fragments of complement component C3 that are characteristically deposited at sites of complement activation. **It is proposed that these structures represent residual debris from degenerating RPE cells that are the targets of complement attack.** It is likely that RPE cell debris entrapped between the RPE monolayer and Bruch's membrane serves as a chronic inflammatory stimulus and a potential nucleation site for drusen formation. **Thus, the process of drusen biogenesis may be envisaged as a secondary manifestation of primary RPE pathology that is exacerbated by consequences of local inflammatory processes.’**

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‘The ocular fundi of many patients with membranoproliferative glomerulonephritis type II (MPGN-II) are characterised by the presence of deposits within Bruch's membrane that resemble drusen, hallmark lesions associated with age-related macular degeneration (AMD). Glomerulonephritis (GN)-associated drusen appear at a younger age, however, than do drusen in individuals with AMD. In light of recent evidence that immune-mediated events participate in drusen biogenesis and AMD, we examined the structure and composition of drusen in eyes obtained from human donors with two distinct glomerulopathies, both of which involve complement deposition within glomeruli. These features were compared with those of drusen from patients with clinically documented AMD. ...Subretinal pigment epithelial (RPE) deposits in both types of GN are numerous and indistinguishable, both structurally and compositionally, from drusen in donors with AMD. ...The composition and structure of ocular drusen associated with membranous and post-streptococcal/segmental glomerulonephritis (GN) are generally similar to those of drusen in individuals with AMD. In view of the recent data supporting the

involvement of **complement activation in drusen biogenesis** and the pathobiology of age-related macular degeneration (AMD), further studies of the biological relationships between AMD and diseases associated with complement activation are warranted.’³³

‘Center for the Study of Macular Degeneration, Neuroscience Research Institute, University of California, Santa Barbara, CA 93016, USA.

Age-related macular degeneration (AMD) is a leading cause of irreversible vision loss in older individuals worldwide. The disease is characterized by abnormal extracellular deposits, known as drusen, that accumulate along the basal surface of the retinal pigmented epithelium. Although drusen deposition is common in older individuals, large numbers of drusen and/or extensive areas of confluent drusen represent a significant risk factor for AMD. Widespread drusen deposition is associated with retinal pigmented epithelial cell dysfunction and degeneration of the photoreceptor cells of the neural retina. Recent studies have shown that drusen contain a variety of immunomodulatory molecules, suggesting that the process of drusen formation involves local inflammatory events, including activation of the complement cascade. Similar observations in Alzheimer's disease (AD) have led to the hypothesis that chronic localized inflammation is an important element of AD pathogenesis, with significant neurodegenerative consequences. Accordingly, the amyloid beta (A beta) peptide, a major constituent of neuritic plaques in AD, has been implicated as a primary activator of complement in AD. Here we show that A beta is associated with a substructural vesicular component within drusen. A beta colocalizes with activated complement components in these "**amyloid vesicles**," thereby identifying them as potential primary sites of complement activation. Thus, A beta deposition could be an important component of the local inflammatory events that contribute to atrophy of the retinal pigmented epithelium, drusen biogenesis, and the pathogenesis of AMD.’

38

Amyloid deposits

Amyloid deposits have been recently confirmed as having the potential for lutein accumulation.

‘Tissue deposition of soluble autologous proteins as insoluble amyloid fibrils is associated with a range of serious diseases including Alzheimer's disease, the transmissible spongiform encephalopathies (prion diseases), and systemic amyloidosis (Glenner, 1980; Kisilevsky, 1998). Although yellow or brownish skin amyloid plaques have been described in several different types of systemic amyloidosis (Touart & Sau, 1998; Asl et al.1999), the chromophore responsible for this yellowish hue has not been identified to date. We have used high-performance liquid chromatography (HPLC) to analyze for the first time such colored skin lesions *ex vivo*. We found a selective accumulation of the xanthophyll lutein in skin amyloid deposits of a patient with systemic amyloidosis suggesting the participation of a naturally occurring carotenoid in the pathogenesis of amyloidosis *in vivo*. ...

Consequently, an inescapable facet of linking the accumulation of lutein to amyloid pathology is the modulation of deleterious amyloid effects by the protective radical-scavenging potential of lutein. Further experimental elucidation of the role of lutein in amyloid deposits may provide new insight into amyloid-associated pathology with potentially important therapeutic implications.’⁹⁸

‘We report the neuropathological evaluation of a 24-year-old autistic woman suffering from a residual state of infantile autism and presenting with self-injury behavior since childhood. Her behavior included head-banging, eye-gouging and self-biting. All

intended therapeutic measures remained without effect, including high doses of psychotropic drugs. At autopsy, numerous neurofibrillary tangles were found in the perirhinal and entorhinal cortex where they were frequently grouped in nests or clusters. A few neurofibrillary tangles were also observed in the amygdala and in the prepiriform and orbito-frontal cortex. In the cortex, tangles were located in both layers II and III. **There were no neuritic plaques or amyloid deposits.**'⁹⁹

Whereas amyloid deposits have not been reported in autism these yellow pigment, potentially lutein containing structures have been found in Heller syndrome (CDD),¹⁰⁰ Down's syndrome¹⁰¹ and Tuberous Sclerosis.¹⁰² It is unlikely that people with autism would develop ARMD associated with amyloid deposition when every indication is that their eyes develop immunogenetically different as a result of an immune system selection of lutein as a pigment pathogen.⁶⁷ Macrophage engulfment of lutein would result in elevated levels of neopterin, and be associated with blindness and ophthalmologic signs. This is consistent with the evidence.

Vitamin A

'Large population studies have consistently shown that vitamin A, not beta-carotene and particularly fish oil derived omega-3 fatty acids, protect from oxidative damage associated with age related macular degeneration. **Higher intake of specific types of fat - including vegetable, monounsaturated, and polyunsaturated fats and linoleic acid--rather than total fat intake may be associated with a greater risk for advanced AMD.** Diets high in omega-3 fatty acids and fish were inversely associated with risk for AMD when intake of linoleic acid was low.'

¹⁰
'To assess whether dietary intake of fat or fish is associated with age-related maculopathy (ARM) prevalence. DESIGN: Cross-sectional, urban population-based study. PARTICIPANTS: People (N = 3654) aged 49 years or older. MAIN OUTCOME MEASURES: Subjects with ARM were identified from masked grading of retinal photographs. A 145-item self-administered, semiquantitative food frequency questionnaire was completed adequately by 88.8% of participants and was used to assess intakes of dietary fat and fish. RESULTS: A higher frequency of fish consumption was associated with decreased odds of late ARM (odds ratio for frequency of consumption more than once per week compared with less than once per month, 0.5). Subjects with higher energy-adjusted intakes of cholesterol were significantly more likely to have late ARM, with an increased risk for late ARM for the highest compared with the lowest quintile of intake (odds ratio, 2.7).

CONCLUSION: The amount and type of dietary fat intake may be associated with ARM.'

⁴
'Diets rich in fish oil-derived omega-3 fatty acids, by enhancing acid lipase, may reduce RPE lipofuscin accumulation, RPE oxidative damage, and the development of ARMD.'

⁹
'A higher frequency of fish consumption was associated with decreased odds of late ARM (odds ratio for frequency of consumption more than once per week compared with less than once per month, 0.5). Subjects with higher energy-adjusted intakes of cholesterol were significantly more likely to have late ARM, with an increased risk for late ARM for the highest compared with the lowest quintile of intake (odds ratio, 2.7). CONCLUSION: The amount and type of dietary fat intake may be associated with ARM.'

¹¹

DHA

‘Study of the metabolism, physiological importance, biological effects, and pathological role of omega-3-polyunsaturated fatty acids, particularly DHA, remains a relatively unexplored field. The notion that DHA in membranes such as those of photoreceptors has no function but to contribute to membrane fluidity is probably an oversimplification. More specific roles are envisaged in the structure and function of retinal and synaptic membranes. One such function may be to provide EPA by retroconversion which, in turn, will be oxygenated to biologically active metabolites that may affect other eicosanoids or directly elicit or induce other functions. A better understanding of the already described alterations in membrane properties of outer segments in inherited retinal degeneration may also lead to further elucidation of the fundamental mechanisms involved in senile macular degeneration and other retinal diseases. The fact that aging enhances the oxidative stress on cells and that the visual cells are enriched in DHA may result in functional impairments; DHA peroxidation may deplete crucial phospholipids from their sites in specific membrane domains. Also, DHA peroxidation generates toxic products that can damage the shedding of photoreceptor discs or their phagocytosis by the retinal pigment epithelium. Docosanoids, oxygenated derivatives of DHA that resemble eicosanoids, may well prove to be unique mediators of physiological processes in the central nervous system, including the retina, and may play a role in some ocular pathologies. More thorough knowledge of these compounds can be expected to lead to important new insights into ocular physiology and pathophysiology, just as research on the eicosanoid system, the primary subject of this volume, has achieved. However, it is of even more immediate importance that we bear in mind the potential contribution of docosanoids to retinal physiology and pathology and to other ocular processes when considering treatment modalities or when interpreting the results of research studies that involve manipulation of the cyclooxygenase and lipoxygenase pathways. The effects currently assigned to eicosanoids by virtue of their inhibition of the cyclooxygenase or lipoxygenase systems may, in part, be consequences of concomitant alterations in the production of docosanoids, especially in the eye, where the retina is an especially rich source of DHA, the endogenous precursor of this recently discovered family of metabolites.’¹⁴

Additional research includes that macro and micro nutrients are equally important for healthy eyes:

‘The nucleus of the lens is particularly sensitive to nutrient deficiencies. **Protein, vitamin A, niacin, thiamin, and riboflavin protected against nuclear cataract in this study.**’⁴¹

‘Large differences in individual susceptibility to vitamin neurotoxicity probably exist, and ordinary vitamin doses may harm occasional patients with genetic disorders.’¹⁰⁶ Foods which are naturally high in carotenoids are also generally good sources of vitamin C. Providing Vitamin C in excess of the RDA or RDI may not be a good idea for people with autism.^{87, 67} ‘The therapeutic use of such compounds (megavitamin intake) is based on the spectacular effect of vitamins on deficiency diseases; however, evidence that the ingestion of large amounts of vitamins beyond the "Recommended Daily Allowances" (RDA) is beneficial is not within the basic concept of nutrition. Vitamins, like many substances, may be toxic when taken in large quantities, especially the fat-soluble vitamins, and the concept of "more is better" is a common misconception.’¹⁰⁷ ‘The lack of controlled trials prevents us from defining the lowest human neurotoxic dose of any vitamin. Large differences in individual susceptibility

to vitamin neurotoxicity probably exist, and ordinary vitamin doses may harm occasional patients with genetic disorders.’¹⁰⁸ ‘These findings illustrate the complexity of the interactions of vit C in biological systems and indicate that with different concentrations vit C can cause or prevent genetic toxicity.’¹¹⁰ ‘Normal healthy volunteers were studied after they ingested various beta-carotene doses. Daily administration of 15 or 45 mg beta-carotene resulted in significant increase in plasma beta-carotene levels. The extent of increase and the pattern of plasma beta-carotene levels showed substantial interindividual variation. Absorption of beta-carotene was affected by dietary fat concentration. Individuals placed on a high-fat diet showed significant increases in plasma beta-carotene as compared with those placed on a low-fat diet. Pharmacological doses of beta-carotene (45 and 90 mg) were used in intermittent schedules (5-6 d intervals) without altering the steady state of beta-carotene plasma levels. Yellowing of the skin occasionally occurred during daily dosing with 45 mg beta-carotene without evidence of toxicity. The observed individual variation in bioavailability of beta-carotene raises questions regarding clinical use of this micronutrient. It appears that determination of target plasma beta-carotene concentrations is essential for effective use of this compound in prevention or treatment.’¹⁰⁹

‘For carnivorous and omnivorous species the main sources of vitamin A are the retinyl esters from prey tissues. For herbivorous species the main source is carotenoids. **Many carnivores, including several species of canines, bears and mustelids, do not absorb dietary carotenoids as such or only at levels below the limits of detection.**’ (Slifka et al. 1999).⁶

‘In several commonly studied species and under normal conditions, the blood level of RBP-bound vitamin A is rather independent of the dietary supply of vitamin A. In the rat and in man, a notable level of lipoprotein-carried retinyl esters in the blood is assumed to be an indication of vitamin A intoxication (Mallia et al. 1975, Smith and Goodman 1976). Normally, in human blood, extremely small amounts of retinyl esters are carried by very low-density lipoproteins (in addition to those in postprandial chylomicrons) (Wilson et al. 1983). However, in many carnivorous mammals a high percentage of vitamin A is carried in plasma lipoproteins as retinyl esters, which in this case is not a sign of vitamin A intoxication (Schweigert et al. 1990). In canines, a large amount of retinyl esters has been found to be distributed in all three classes of lipoproteins (very low-density, low-density and high-density lipoproteins) (Wilson et al. 1987). Furthermore, a recent study on dogs has shown that in blood plasma the level of retinyl esters is affected by dietary level of vitamin A much more sensitively than the level of alcoholic retinol is (Schweigert and Bok 2000).’⁷

Lutein and zeaxanthin and non-dietary meso-zeaxanthin

‘Lutein and zeaxanthin are the predominant carotenoids found in the human retina. The concentration of these carotenoids is greatest in the macula where they form the macular pigment. Within this region, R,S-zeaxanthin is found in nearly equal abundance to the more commonly occurring, dietary form, R,R-zeaxanthin. ... The macula lutea [1,2] of the human retina was first recognized to be a carotenoid by George Wald [3] in 1945, nearly 200 years following its discovery [4]. In the early 1960s, Brown and Wald [5] attributed the identity to ‘... lutein and its cis-isomers’. As late as 1983, Britton [6] stated the ‘The probable function of [the macula lutea] is to absorb ... blue light around 450 nm ... from its absorption spectrum the human

macular pigment appears to be a carotenoid but it has not been characterized.' A modern characterization of the components of the macula lutea, hereafter macular pigment, awaited the advent and ready availability of high performance liquid chromatography (HPLC), well suited to the detection and separation of the nanogram quantities of the carotenoids present.

...In subsequent investigations, we were able to characterize the stereochemical structure of the macular lutein and zeaxanthin utilizing a chiral chromatographic stationary phase after the methods of Maoka et al. [13]. The dibenzoate derivative of macular lutein was shown to elute as a single peak which co-eluted with an authentic standard of the 3R,30R,60R-stereoisomer of lutein. The dibenzoate derivative of macular zeaxanthin, however, was found to elute from the chiral column as two components of near equal abundance. Using authentic 3R,3'R-zeaxanthin and a racemic mixture of zeaxanthin stereomers produced by reduction of rhodoxanthin, the two macular zeaxanthin stereomers were shown to be indistinguishable from all-E 3R,3'R-zeaxanthin and all-E 3R,3'S-(meso)-zeaxanthin. Thus a final characterization of the macular pigment shows that it is composed of three carotenoids: lutein (3R,3'R,6'R-beta,epsilon-caroten-3,3'-diol); zeaxanthin (3R,3'R-beta,beta-caroten-3,3'-diol); and meso-zeaxanthin (3R,3'S-beta,beta-caroten-3,3'-diol). **Our identification of the non-dietary meso-zeaxanthin as a component of the macular pigment dramatically emphasizes the lack of understanding of xanthophyll metabolism by humans.**

...**The distribution of these carotenoids within the retina is not uniform. HPLC of extracts of tissue taken from the central fovea shows that the zeaxanthin isomers dominate over lutein and a zeaxanthin to lutein ratio of 2.4 : 1 is observed.** This ratio varies rapidly as a function of eccentricity, shifting to a 1 : 1.9 ratio over a 3mm spacing and eventually reaches the limiting ratio of 1 : 2.2 at an eccentricity of 6mm distance from the center of the fovea. The ratio of zeaxanthin to lutein in the serum is between 1 : 3 and 1 : 4, somewhat different from the peripheral retina, but consistent with the dominance of lutein in the diet. An active retinal metabolism of the xanthophylls is implicated by this variability in the distribution. An early suggestion for this was that there might be a preferential uptake by foveal cones for zeaxanthin in preference to lutein. We believe recent data suggest a more likely cause. The meso-zeaxanthin isomer described earlier can be mapped as a function of eccentricity in a manner similar to that used to characterize the relative abundances of lutein and zeaxanthin [17,18]. The mesozeaxanthin isomer is found to parallel the relative abundance of zeaxanthin as a component in the macular pigment. Therefore the percentage of lutein in the macula reaches its lowest value in the same place (the central fovea) where meso-zeaxanthin is at its greatest.

A Mechanism For meso-Zeaxanthin Formation

These data suggest a relationship between the two isomeric carotenoids, possibly a conversion of lutein into meso-zeaxanthin. This hypothesis would apparently be supported by a comparison of the absolute stereochemical configurations of lutein and meso-zeaxanthin. The 3' C absolute configuration is identical in these two compounds, consistent with a conversion hypothesis. More careful investigation of this idea shows that, while lutein to zeaxanthin conversion may be occurring, conservation of the stereochemical configuration at the 3' C may be chemically naive.

Khachik et al. [19] recently published results from a study of human and primate retinas in which minor carotenoid components were studied. A significant number of carotenoids were detected, which were characterized on the basis of

retention times, UV/visible and mass spectra. Their structures are readily explainable as metabolites of lutein and zeaxanthin via one or more oxidation-reduction steps. It is suggested that meso-zeaxanthin is the result of oxidation at the 3' C of lutein (or zeaxanthin), producing 3-hydroxy-beta,epsilon-caroten-3'-one, followed by reduction. If reduction is accompanied by concomitant migration of the double bond, both R,R-zeaxanthin and R,S-meso-zeaxanthin result. If the reduction process occurs without double bond migration, epi-lutein results. Khachik et al. report both epi-lutein and 3-hydroxy-beta,epsilon-caroten-3'-one to be present in the retina. An oxidation-reduction pathway is a reasonable pathway between lutein and meso-zeaxanthin which does not involve a conservation of the absolute configuration of the stereochemical structure at the 3' C. The reason zeaxanthin accumulates over lutein may be a result of the more readily oxidized allylic hydroxyl group in lutein and a slight preference in the thermodynamic stability of zeaxanthin as a product relative to lutein. The study of canthaxanthin accumulation within the primate retina [20,21] and its metabolism is consistent with the oxidation-reduction conversion hypothesis.

Canthaxanthin is dramatically accumulated by primate and human retinas.

Canthaxanthin, or 'gold dust', retinopathy is a well documented result of high canthaxanthin consumption and is characterized by the presence of high levels of canthaxanthin, including crystalline inclusions observable in the peripheral retina.' ⁸

'...Our knowledge of the function of the macular pigment has improved over Britton's 1983 evaluation. The macular pigment indeed may have as its primary function the absorption of blue light, but now we are able to point to possible consequences for the absence or presence of only low levels of macular pigment in human retinas. We now know that the carotenoids can function as antioxidants, singlet oxygen quenchers, and/or as triplet excited state deactivators [23]. If such a function occurs within the retina, it would mitigate the potentially harmful effects of radicals, generated by the presence of light and oxygen, on the high levels of polyunsaturated fatty acids found in the retinal membranes.'

ARE CAROTENOIDS ANTIOXIDANT?

'An hypothesis is presented that is opposed to the conventional viewpoint that beta-carotene is an in vivo free-radical scavenger. It is suggested that there are biochemical reasons why beta-carotene, other carotenoids, and especially their metabolites may be harmful to mammalian systems. Finally, the hypothesis that the macular pigment carotenoids, lutein and zeaxanthin, are free-radical scavengers is challenged.' (Schepens Eye Research Institute, Harvard Medical School) ¹⁶

Possibly both theories are correct, carotenoids can be both anti-oxidant and pro-oxidant. Plant research forming around study of cryptochrome (CRY1 and CRY2) activity have discovered a pterin mechanism of activation in plant circadian rhythm, reaction to light (moves towards or away from). ¹¹² Research on human eyes has also found the cryptochromes and associated this to circadian rhythm activity. Does the cryptochrome in the human eye also require a pterin trigger? Where would this come from? Macrophage engulfment of pathogens or debris results in release of neopterin. ⁸³ In a healthy human the release of neopterin as macrophages engulf the pigment waste products in the human eye might very well result in the release of neopterin triggering cryptochrome activity? Protection by xanthophylls substances i.e. luteins

or lutein structures could be protective during this critical activity. The macrophages are known to engulf pigment wastes. Could this sensitive immune reaction be disturbed in autism? Could the pigments themselves be identified as a pathogen instead of a pigment waste product? Would this lead to the disease presentation characteristics seen in autism:

- **Fear of food and feeding disorders**
- **Blindness and ophthalmologic signs**
- **Co-occurring diseases, disorders and conditions of pigment and/or pterin metabolism**
- **Disordered behavior**
- **Disturbed sleep**

Phosphatidylcholines

‘This paper reports the research on the effect of two main carotenoid pigments present in the membranes of macula lutea of the vision apparatus of primates, including humans, lutein and zeaxanthin, on the structure of model membranes formed with dimyristoylphosphatidylcholine (DMPC). The effects observed in DMPC are compared to the effects observed in the membranes formed with other phosphatidylcholines (PC): egg yolk PC (EYPC), and dipalmitoyl-PC (DPPC). The analysis has been focused, in particular, on the following aspects of the organization of lipid-carotenoid membranes: aggregation state of pigments, an effect on a thickness of the bilayer and pigment orientation within the membranes. These problems have been addressed with the application of UV-Vis absorption spectroscopy, linear dichroism measurements and the diffractometric technique. (1) Both lutein and zeaxanthin appear in a partially aggregated form in the oriented DMPC multibilayers, even at molar fractions as low as 2 mol.% with respect to lipid. (2) Orientation of the transition dipole of both xanthophylls with respect to the axis normal to the plane of DMPC membrane is different in the case of a monomeric form (34 \pm 3 degrees in the case of lutein and 26 \pm 3 degrees in the case of zeaxanthin) but essentially the same in the case of aggregated forms of both pigments (42 \pm 3 degrees in the case of lutein and 40 \pm 5 degrees in the case of zeaxanthin). **It was found that only lutein has an effect on the increase in the thickness of the DMPC membranes** (by about 3 Å at 25 degrees C). A similar effect was observed also in the case of DPPC at the same temperatures despite the differences in the physical state of both membrane systems. The differences between the effects of lutein and zeaxanthin observed are interpreted in terms of differences of stereochemical structure of both xanthophylls leading to the different localization in the lipid phase. **The results demonstrate significant differences in the behavior of lutein and zeaxanthin in model membranes, which may contribute to their different physiological functions and different efficacy as membrane antioxidants.**’²⁴

‘¹H-NMR technique was applied to study liposomes formed with egg-yolk phosphatidylcholine containing as an additional component two carotenoid pigments: beta-carotene or zeaxanthin (dihydroxy-beta-carotene). A strong rigidifying effect of zeaxanthin but not of beta-carotene with respect to hydrophobic core of lipid bilayer was concluded from the carotenoid-dependent broadening of the NMR lines assigned to -CH₂- groups and terminal -CH₃ groups of lipid alkyl chains. A similar effect of zeaxanthin with respect to polar headgroups was concluded on the basis of the effect of the pigment on the shape of NMR lines attributed to -N⁺(CH₃)₃ groups. In contrast, beta-carotene increases motional freedom of lipid polar headgroups. The

inclusion of both carotenoids to liposomes resulted in the enhanced penetration of Pr³⁺ ions to the polar zone of the external layer of a membrane monitored by the splitting of the -N+(CH₃)₃ signal, the effect of beta-carotene being much more pronounced. Differences in the effect on membrane structure and molecular dynamics observed for beta-carotene and its polar derivative are discussed in terms of organization of a carotenoid-containing lipid membrane.’ 31

- **Hypotonia**
- **Disturbance of steriogenesis**
- **Disturbance of acetylcholine metabolism potentially resulting in decreased ability to break down the dairy protein – casein and the wheat protein – gluten, reduced sialic acid production resulting in or contributing to candida overgrowth and gut dysbiosis.**

‘Carotenoids are the effective modulators of physical properties of model and natural membranes. To demonstrate the relationship between the structure of carotenoids and their effect on the molecular dynamics of membranes, we have investigated the influence of five structurally different carotenoids: beta-carotene, lycopene, lutein, violaxanthin, zeaxanthin and additionally carotane--a fully saturated derivative of beta-carotene, on thermotropic phase behaviour of dipalmitoylphosphatidylcholine (DPPC) multilamellar vesicles by means of differential scanning calorimetry (DSC). The results obtained indicate that the carotenoids used modulated the thermotropic properties of multibilayers to various extents, broadening the pretransition and the main phase transition peaks and shifting them to lower temperatures. Pronounced decrease of pretransition enthalpy ($\Delta H(p)$) proves that carotenoids very strongly alter the membrane properties in its gel phase. Comparison of the influence of several carotenoids shows that a rigid, polyisoprenoid chain plays a basic role in altering the thermotropic properties of such membranes and the presence of rings without oxygen-containing groups has a minor significance for the observed interactions. **Carotenoids containing epoxy and/or hydroxy groups attached to their rings modify the thermotropic phase behaviour of dipalmitoylphosphatidylcholine (DPPC) multilamellar vesicles stronger than carotenes--a result of their orientation in the DPPC bilayer.**’ 25

- **Thermotropic dysregulation**
- **Pigmentation disturbance in eye color, hair color, skin color**
- **Dark circles under the eyes, allergic shiners – a sign of immune activity**

Of mice and men

In the laboratory the mouse model has been used in research relating to luteinizing hormone (LH) activity and also carotenoid metabolism. 90 Xanthophyllic pigment ‘lutein’ has finally been confirmed as the yellow pigment of the corpus luteum. For many years there was confusion as to whether dietary lutein and xanthophyllic pigment found in chicken eggs, corpus luteum and plant foods was indeed the same substance. With the elucidation has quickly come recommendation for increased consumption of lutein containing foods in the human diet. Just as the World Health Organization quickly made recommendation to replace vitamin A with beta-carotene the media and health food industry is forcibly recommending increased consumption of lutein containing foods and or dietary supplements. No long term research is available. It may be that those who will be recommended to have an increased intake

of lutein from diet or supplements are those most likely to be adversely affected by the increased exposure.

In the human 'Restriction enzyme analysis verifies that both LHR genes are present in the human genome, and **gene dosing reveals four copies of the human LHR** in contrast to **a single copy in the rat genome**. Chromosomal mapping localizes all copies of the human LHR to the chromosome 2p16-21 loci. **These studies suggest that tissue-specific LHR promoter utilization and LHR gene expression may be correlated with gene diversity.'**⁹⁶

Much research on hormones, reproduction and genetic mutation has relied on research using mouse models and still does.⁹³ The research on human LHR and suggested correlation to gene diversity provides reason for tremendous reservations in accepting earlier research findings and conclusions which did not include knowledge of this significant finding. Human research is likely to find that lutein and luteinizing hormone related activity is one of the most challenging areas of study and information gathered for any disease, disorder, or condition will likely have additional diversity factors related not only to sex but ancestry and immunogenetics.^{91, 92} 'Human male sexual development is regulated by chorionic gonadotropin (CG) and luteinizing hormone (LH). Aberrant sexual development caused by both activating and inactivating mutations of the human luteinizing hormone receptor (LHR) have been described. All known activating mutations of the LHR are missense mutations caused by single base substitution. The most common activating mutation is the replacement of Asp-578 by Gly due to the substitution of A by G at nucleotide position 1733. All activating mutations are present in exon 11 which encodes the transmembrane domain of the receptor. Constitutive activity of the LHR causes LH releasing hormone-independent precocious puberty in boys and the autosomal dominant disorder familial male-limited precocious puberty (FMPP). Both germline and somatic activating mutations of the LHR have been found in patients with testicular tumors. Activating mutations have no effect on females.'⁹⁴ 'The different occurrence of activating mutations of the LHR compared to the FSHR is surprising, since the two genes are adjacently located on chromosome 2 and should therefore be affected by a similar mutation rate.'⁹⁵

Evolution of the immune system: GOD (Generation of Diversity)

The phylogenetic old immune system referred to by Docke: 'Heat shock proteins (HSP) or stress proteins are produced by prokaryotic and eukaryotic cells in response to a variety of environmental stressors. The heat shock response is one of the most universal reactions known and heat shock proteins are among the most conserved molecules in phylogeny. Recent findings concerning the immune response to heat shock proteins are discussed especially with respect to the role of HSPs postulated in septic disease and inflammation, in anti-pathogenic immunity and in the induction of autoimmune diseases. Results and speculations considering a relationship between HSPs and gamma/delta T cells or polyreactive antibodies, possibly as part of a phylogenetic old immune system...'⁸⁰ may provide an area for molecular biology research to unravel the mysteries of autism. New, albeit expensive, variable number tandem repeats 'VNTRs' and restriction fragment length polymorphisms 'RFLPs' techniques could be employed. Concentrating on differences in the genetic variables relating to eye development in the autism population may present with more similarities with each other than with the rest of the human population.

We are humans, higher mammals and therefore we 'do NOT produce carotenoids' but carotenoids are converted inside the human body. Whether all of this conversion is due to molecular or biologic activity, attributed to gut flora or is the result of carotenoid assembly has not been clearly elucidated:

[As Moore explained, their objective is being realized through investigation of the most significant early events of photosynthesis in plant and bacterial systems; specific studies include light absorption and excitation transfer in photosynthetic antennas, structure and assembly of photosynthetic complexes, pigment-protein interactions, and the mechanisms of biological electron transfer reactions. In particular, Moore's group is exploring artificial, biomimetic photosynthetic systems to learn more about the natural process and to find applications of the clever photochemistry of natural photosynthesis in artificial systems. As she put it, "Nature is not in a hurry, but humanity is." ...**Her group has experimented with porphyrin-carotenoid-quinone systems like those found in plant photosynthetic reaction centers, and most recently they have explored carotenoporphyrin-fullerene triads, some of which can produce ATP better than nature's own chloroplasts. Such complexes can be incorporated into a variety of devices now being developed under the rubric of "nanotechnology"--small energy-capture and transfer systems expected to have wide application in medicine, electronics, and other areas.] 81**

Today

'Carotenoids form structured self-assembly upon aqueous dilution of their organic solutions. In order to test the proposal predicting carotenoid aggregates to be organized in closely packed H-type (card-pack) manner by intermolecular hydrogen bonds, the trihydroxy derivative of capsanthin (1), (6'R)-capsanthol (2) ((all-E,3R,3'S,5'R,6'R)-beta,kappa-carotene-3,3',6'-triol) was acetylated to obtain all varieties of mono-, di- and triacetates and the corresponding supramolecules were studied by UV/Vis- and CD spectroscopy. It was verified that derivatives lacking hydroxyl functions at either of the end-groups form the loosely organized J-type (head-to-tail) aggregates. A model for the structure of the J-type self-assembly is proposed. Evidence was also obtained suggesting that close contacts of carotenoid molecules are not confined to hydrogen bonding.' 82

Tomorrow

Without full understanding of how carotenoids work in the human body science and the need for profit work hand in hand. The development of synthetic carotenoids which can be used in patented products is being actively investigated. At the same time re-discovery of natural substances can be included in patented products and rendered unavailable to the food industry and health product market:

'All-E-(3R,6'R)-3-hydroxy-3',4'-didehydro-beta,gamma-carotene (anhydrolutein I) and all-E-(3R,6'R)-3-hydroxy-2',3'-didehydro-beta,epsilon-carotene (2',3'-anhydrolutein II) have been isolated and characterized from extracts of human plasma using semipreparative high-performance liquid chromatography (HPLC) on a C18 reversed-phase column. The identification of anhydroluteins was accomplished by comparison of the UV-Vis absorption and mass spectral data as well as HPLC-UV-Vis-mass spectrometry (MS) spiking experiments using fully characterized synthetic compounds. Partial synthesis of anhydroluteins from the reaction of lutein with 2% H2SO4 in acetone, in addition to anhydrolutein I (54%) and 2',3'-anhydrolutein II (19%), also gave (3'R)-3'-hydroxy-3,4'-dehydro-beta-carotene (3',4'-anhydrolutein III,

19%). While anhydrolutein I has been shown to be usually accompanied by minute quantities of 2',3'-anhydrolutein II (ca. 7-10%) in human plasma, 3',4'-anhydrolutein III has not been detected. The presence of anhydrolutein I and II in human plasma is postulated to be due to acid catalyzed dehydration of the dietary lutein as it passes through the stomach. These anhydroluteins have also been prepared by conversion of lutein diacetate to the corresponding anhydrolutein acetates followed by alkaline hydrolysis. However, under identical acidic conditions, loss of acetic acid from lutein diacetate proceeded at a much slower rate than dehydration of lutein. The structures of the synthetic anhydroluteins, including their absolute configuration at C(3) and C(6') have been unambiguously established by ¹H NMR and in part by ¹³C NMR, and circular dichroism.' ²⁷

Aquasearch and Albany Molecular Research complete first drug discovery library U.S. National Cancer Institute to screen astaxanthin library for anti-cancer activity Kailua-Kona, Hawaii (August 2, 2000) - Aquasearch, Inc. (Nasdaq-BB: AQSE.OB) and Albany Molecular Research Inc. (Nasdaq: AMRI) today announced the completion of their first collaborative compound library for drug discovery. This library consists of a novel set of compounds derived from astaxanthin, a natural molecule found in microalgae.' ⁷⁹

Autism treatment options – evolution of the human immune system

Dietary intervention has come to the media and public attention as a treatment option for people with autism. Current research includes gluten and casein restricted diets (Reichelt, Shattock, Lewis). Recommendations for fish liver oil supplementation (Johnson, Desorgher, Megson). Lutein and carotenoid restricted diets with full recommendation for essential nutrient composition in the individual diets. (Desorgher). Advances in research are bringing these approaches closer together as the lutein content of some grains and dairy products i.e. durum wheat, spelt and skimmed or whole milk products are identified. Information coming from researchers contribute substantial useable information as with Cocchi's revelation that a strict gluten and casein free diet could contribute to the development of phenol (benzoate) sensitivity. The lutein content of many foods varies seasonally, among species and according to stress factors (light, temperature, pesticide use, soil conditions) and new research identifies the carotenoid composition of foods with greater accuracy. ⁸⁸

Improved research techniques and plant foods which contain pigment complexes of pterin and carotenoid(s) may be able to yield further information. ⁸⁹ Until research is forthcoming parents and care givers will rely on word of mouth, books and literature, anecdotal accounts and experimentation when trying to help a child. NIH spokesperson Volkmar's recommendation in 2002 to the parents of autistic children 'Don't change a thing in your autistic child's diet' will fall on deaf ears. Parents want to be told what 'to do' NOT what 'not to do'. Famous quotes in the autism community ending the twentieth century: 'Autism is not a ritalin deficiency' (Bernard Rimland, Autism Research Institute). 'You must treat the whole child' (Ivar Lovaas).

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