Wanted – a time machine

Life expectancy in 1900 was 45 years but in 2000 it was over 80. In 1951 there were fewer than 300 centenarians alive in the England and Wales: by 2035 it is estimated this number will be over 40,000. All this is, of course, a Good Thing. It came about because the diseases which killed off millions before their three score years and ten have largely been overcome. This success has allowed the so-called diseases of old age to enter the scene and now cancer, neurological problems, cardiovascular deterioration and various degenerations are the killers. And if people escape these, it seems they get senile and just wear out so they cannot resist a simple infection, like pneumonia, that a younger person would shake off.

Some people, noting that their motor cars work for longer and better if an effective program of preventative maintenance is carried out, have felt that it might be possible to take similar action with humans as they age. I am one of these people, but my expertise is in the limited area of vision. However, for the elderly, poor sight is an important disability and for the nation, a costly problem. It would be very worthwhile to come up with a life-time maintenance programme for vision and extend visual function to 100 years and more.

Table 1 shows how visually impaired numbers increase with age. The causes of visual impairment and blindness are many but they change with age as Table 2 shows. Below 65 years, diabetes and optic nerve atrophy are major causes, but for the over 65's, macular lesions and cataracts head the list. These days cataracts are, in the developed world, no longer a serious problem and are easily removed, but macular lesions are a different story. Figures 1 & 2 show the retina at the back of the eye and how the foveal pit is positioned in the centre of the macula. Also shown is the position of the macular pigment (MP) within the retina. This pigment is a yellow mixture of two carotenoids, lutein (L) and zeaxanthin (Z). The presence of L and Z poses several questions, some of which we flatter ourselves by believing that we know the answer.

John Mellerio

ONS population estimates		estimates for 1996 of visually impaired persons by age group 0 to 15	16 to 64	65 to 74	over 75	total
number	58,801,500	24,200	166,140	125,940	750,460	1,066,740
as % of population		0.04	0.28	0.21	1.28	1.81

Table 1 Estimates of visually impaired people by age group for 1996 in the UK – figures from RNIB. Visual impairment may be taken as the inability to read correctly the large top letter of an eye test chart at distance greater than six metres (a normal person would perform the same at a distance of 60 m).

condition	1969-76 0 – 64 yrs	1970 only over 65 yrs
glaucoma	7.1	14.6
cataract	7.6	21.1
choroidal atrophy	11.2	5.0
retinal detachment	2.9	0.3
retinitis pigmentosa	6.4	0.2
macular lesion	4.7	46,5
retinal diabetes	18.7	4.3
optic nerve atrophy	15.5	1.9
several miscellaneous causes	25.9	6.1

Table 2 Causes of blindness in the under 65's and over 65's

As Figure 2 shows, MP is situated in front of the light-sensitive outer segment portion of the photoreceptors which in the fovea are mostly cones. It is yellow which means that it absorbs blue light and this led to the first suggestion for the function of MP. The eye is not a perfect optical instrument, it suffers from chromatic aberration – the retinal image shows coloured fringes which will reduce the acuity of the eye. The yellow MP absorbs the blue light so the spread of the colour fringes will be reduced. Acting as a pre-receptor yellow filter, the MP would be expected to interfere with colour vision

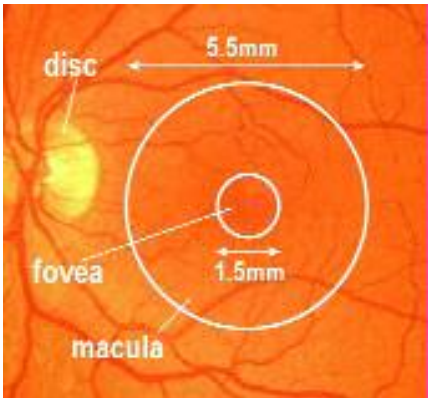

Figure 1 Photograph of the retina of a human eye with the extent of the fovea and macula overlaid. The macular pigment is distributed in a bell shaped curve with the peak in the centre of the fovea and trailing to insignificant concentrations well before the edge of the macula.

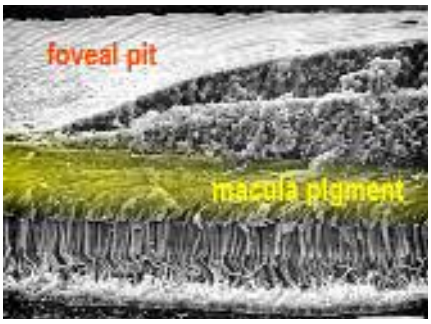

Figure 2 Scanning electron micrograph of a section through half of the foveal area clearly showing the foveal depression. The macular pigment is shown mainly concentrated in the fibre layer of Henle – the axons of the cones. *Courtesy of John Marshall.*

effective at this job (Hammond *et al*, 2001). On average, the optical density of MP is about 0.4 log units, i.e. it absorbs about 60% of the incident light, so some blue fringes would remain. Also, the centre of the fovea, the foveola where visual acuity is best, contains no blue sensitive or S cones which are responsible for absorbing blue short wavelength light to facilitate colour vision. It is as though the foveola is tritanopic, that is it behaves as though it was blue colour blind. The

Figure 3 The spectral absorption of the macular pigment and the spectral sensitivity curves of the three cone systems, S (short wavelength or blue absorbing), M (medium, green absorbing) and L (long, red absorbing). The curves have all been normalised.

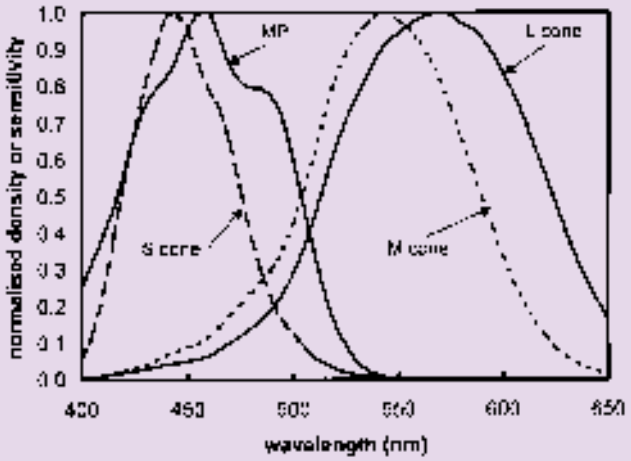

Figure 4 The two carotenoids that make up macular pigment.

– indeed, the first attempts to measure the absorption spectrum and the amount of pigment present in an eye were made by vision scientists investigating colour vision (REF to Pease). They wanted to know what the receptors would do without a yellow filter. Figure 3 shows that the spectral absorption curve for MP has a maximum absorption for blue light of 462 nm wavelength. Although the idea of MP as a chromatic aberration correcter is undoubtedly sound, it may not be very

MP will not, therefore, affect the blue channel of the trichromatic colour vision system at the very centre of gaze and its effect on the M and L cones (green and red absorbing, respectively) will be small, as Figure 3 shows. It remains to be shown how important for vision is the aberration correction function of MP.

MP, acting as a blue light absorber, has another role. It has been shown that short wavelength light, because of its relatively high photon energy, more readily damages the retina than yellow or red light which is less energetic. In animal experiments long exposures to levels of light such as are produced by fluorescent lamps or by a bright cloudy sky can lead to retinal damage and the short wavelength light is very effective in this. MP will absorb the energetic blue light and thus protect the photoreceptors of the macula. That MP is concentrated in the macula, around the optical axis of the eye, is an additional protective factor because the intensity of the retinal image is greatest in this on-axis area.

Recently, it has become apparent that MP has a much more important role in protecting the retina. Carotenoids in general are known for their antioxidant and free radical scavenging properties (Krinsky, 1989; Jacob & Burri, 1996). Lutein and zeaxanthin (Figure 4) are two very similar carotenoids that are not manufactured in the body but have to come directly from the diet. The best food sources of L and Z are not the same as for β-carotine which is perhaps the most famous carotenoid. Whilst dark green vegetables (spinach and so on) were recommended as sources of L and Z, it seems that maize and orange pepper are better respectively for L and Z (Sommerberg *et al* 1998).

When radiation such as UV and short wavelength (blue) light interact with tissues, especially with molecules called photo sensitisers, there is formed a range of excited singlet state molecules. These are very reactive and are short lived: they lose the energy they gained in the excitation process by forming photoproducts, by fluorescing or by generating so-called triplet state molecules (Mellerio, 1991). These excited triplet state molecules are longer lived and may react with the molecules found in the tissues, especially with oxygen, to produce free radicals such as the oxygen free radical superoxide and the hydroxyl radical, or give rise to the destructive singlet oxygen. These various radicals are not

good news as they can in turn oxidise tissue molecules. At especial risk are polyunsaturated fatty acids (PUFA) which enter a chain reaction called lipid peroxidation. Any cellular structure that is rich in PUFA's is at risk, and the photosensitive outer segments of rods and cones are formed of stacks of discs made from plasma membrane rich in PUFA's (Marshall, 1985). Figure 5 shows a rod outer segment in the first stages of light damage – the regularity of the discs is interrupted by centres of lipid peroxidation and the picture is reminiscent of a woolly sweater after a visitation by moths.

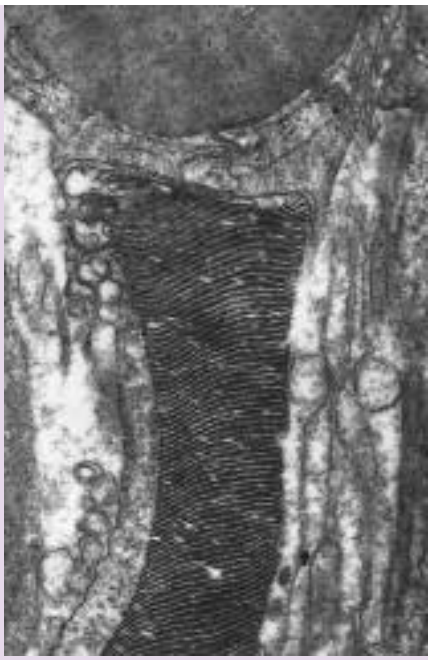

Figure 5 The outer segment of a retinal rod that was exposed to white fluorescent light for six hours. The first stages of light damage are visible where the ordered membranes of the discs in the rod outer segment (the photosensitive part of the rod) is broken down presumably by lipid peroxidation.

The two important protective properties of carotenoids, namely singlet oxygen quenching and the scavenging of reactive oxygen species, vary across the range of carotenoids and with the conditions used to measure these properties (Schalch *et al*, 1999). For example, *in vitro*, zeaxanthin has about twice the capacity to quench singlet oxygen than lutein and five times the ability to repair the α-tocopheryl radical cation. Whether these effectiveness ratios apply *in vivo* is not known, but it is interesting to see that L and Z are not equally distributed across the retina. In the fovea the ratio of L concentration to Z concentration is 0.7 and about 1.3 in the outer macular zone and higher still in the retinal periphery (Figure 6a). Indeed, L and Z occur in many retinal tissues but in much smaller amounts than in the fovea – free radicals are not only produced by the interaction of radiation and tissues, but are formed as by-products of normal cellular metabolism and all cells contain systems to mitigate against the destructive actions of these radicals.

The interest in the antioxidant properties of L and Z in MP arises because of laboratory experiments which show that prolonged exposure to light can induce damage to the retina and that the damage can resemble certain aspects of AMD. Epidemiological research has suggested many factors that might predispose a person to AMD (Evans, 2001) but there is no universal consensus on all of these. One factor that the lab light-exposure experiments point to is chronic exposure to light, especially blue light and UV radiation. Oxidative stress is high in the retina, especially so in the fovea where the metabolic rate and the oxygen tensions are high, and where the incident light is most intense and there is a plethora of PUFA'S awaiting peroxidation (Beatty *et al*, 2000; Marshall, 1985). A good supply of carotenoid pigment in the macula would be a useful sight-preserving component to have throughout life to inhibit the slow destruction of the retinal cells. The idea that macular pigment is protective to retinal function (Haegerstrom-Portnoy, 1988) and AMD (Snodderly, 1995) is now firmly established but not at all confirmed.

Although epidemiological studies (Taylor *et al* 1992; Cruickshanks, Klein & Klein, 1993) have shown that a history of exposure to strong sunlight

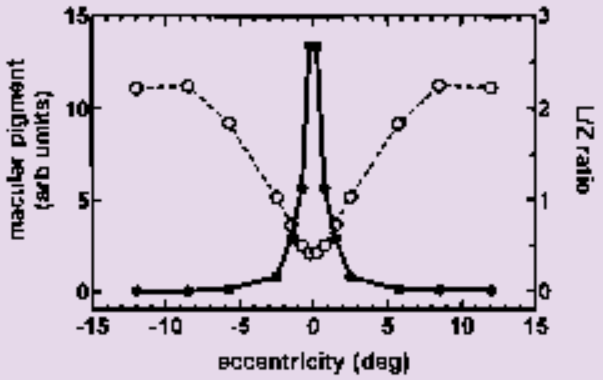

Figure 6a The optical density of macular pigment plotted against retinal eccenticity from the fovea outwards. Also shown is the ratio of lutein to zeaxanthin and how it varies with eccentricity. (from Landrum and Bone, 2001)

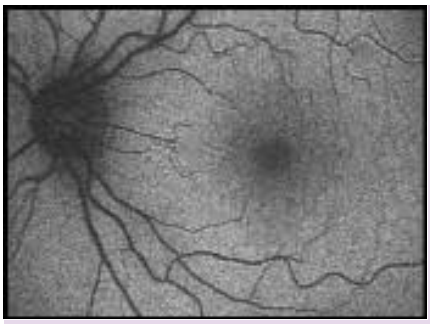

Figure 6b An autofluorescent photograph of the central retina of a man. This technique induces fluorescence of retinal components with blue light and, with appropriate barrier filters, records the green fluorescence. The presence of macular pigment is shown dark as fluorescence is less, the pigment absorbing the exciting blue light (courtesy of van Kruijk *et al*, 2001).

is associated with an early onset of AMD, the effect is smaller than might be supposed from the very definite laboratory results that show that too much light of normal daylight intensity given over hours or days is harmful. However, associations between visual impairment and the amount of solar radiation in the subject's locality may be seen even in the UK. Figure 7 plots, county by county, the percentage of visually impaired people (corrected for the size of the local cohort of elderly) against the latitude of the county town. The dashed line shows the how the solar constant varies with latitude. These data are crude and open to criticism (the visual impaired figures include all impairing conditions and not just

AMD), but they are suggestive and point up another good reason for living north of Potters Bar.

The situation for oxidative stress and light exposure in the eye is such that it might be sensible to err on the side of caution and, until it is proved otherwise, assume that there is a connection between light exposure history and AMD. Thus it would be worthwhile knowing if you have a high concentration of MP and, if not, to increase it. This would mean eating more L and Z in your diet – and, just as importantly, improving your life style by – yes, you guessed it – not smoking or drinking and keeping out of the sun, sun beds and tanning saloons. MP levels can be measured fairly easily with small portable instruments that are just emerging from development (Mellerio *et al*, 1998; Wooten *et al* 1999) so screening is now possible.

Dietary intake of L and Z can be increased by eating more of the appropriate vegetables or by taking supplements. There are important questions about which supplement to take and how much, and whether this is safe and effective. A number of studies (Landrum, Bone and Kilburn, 1996) have shown increases in MP when the diet is supplemented with L but as L is mainly found outside the foveal centre (Figure 6), would supplementation with Z be better? So far only one pilot trial with Z supplementation has been reported (as poster at a meeting), but there is some evidence from elsewhere that L might be converted into Z in the fovea (Landrum and Bone, 2001). It was known that the distribution of MP across the macula, although radially symmetrical, may have wide or a narrow spread and this has recently been demonstrated nicely (Figure 7) by van Kruijk *et al* (2001). It is an open question whether supplementation would widen the distribution or raise the peak value and what would be the best supplementation regime to achieve either or both effects.

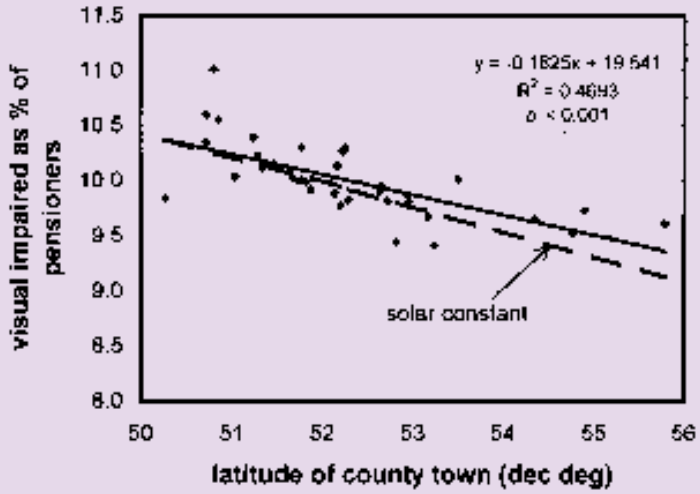

Figure 7 The number ofpeople with visual impairment, plotted as a percentage of the over 65s, county by county, versus the latitude of the county town. The fitted line shows a significant and large correlation. The dashed line is the solar constant versus latitude, scaled to fit the visual impairment data at latitude 50 degrees, 20 minutes.

Even though the 'protection of MP against AMD' theory is attractive, it needs to be proven. Beatty *et al* (2000) have measured MP values in AMD patients and shown they had less pigment than age matched normals, but the measurement is open to criticism. It was made using a psychophysical technique called heterochromartic flicker photometry which assumes that there are the same relative number of M and L cones in the fovea, where there is pigment, and outside at a comparison position where there is no pigment. In patients with AMD, this is not certain – indeed it is unlikely. And there is also the objection that it may not be low MP that leads to AMD, it may be that AMD causes so much retinal dysfunction that MP is lost and all that is measured is the progression of another facet of the disease. A similar relationship was found by Landrum *et al* (1999) when they measured MP levels in enucleated normal and AMD) eyes by HPLC. But, as these authors state, an association does not necessarily reflect a causative relationship. The situation is complex and there are several confounding factors, e.g. age and race (Mare-Perlman *et al* 2001), to name just two.

What is required is a properly controlled longitudinal study that runs for about thirty years. Some of us with grey hair can't wait that long, hence the need for a time machine to do in two years what nature would normally do in thirty. Do you know where I can get one?

John Mellerio

References

Beatty S. Koh HH, Henson D, Boulton ME. The role of oxidative stress in the pathogenesis of age-related macular degeneration. *Survey Ophthalm.* 2000, **45**:115-134.

Cruickshanks KJ, Klein R, Klein BE. Sunlight and age-related macular degeneration. The Beaver dam eye study. *Arch Ophthalmol*, 1993, **111**:514-518.

Evans JR. Risk factors for age-related macular degeneration. *Prog retinal Eye Res*, 2001, **20**:227-253.

Haegerstrom-Portnoy G. Short-wavelength-sensitive-cone sensitivity loss with aging: a protective role for macular pigment? *Jnl Opt Soc Amer*, 1988, **5**:2140-2144.

Hamond BR Jr, Wooten BR, Curran-Celentano J. Carotenoids in the retina and lens: possible acute and chronic effects on human visual performance. *Arch Biochem Biophys*, 2001, **385**:41-46.

Jacob RA, Burri BJ. Oxidative damage and defense. *Amer Jnl Clin Nutrition*, 1996, *63*: S985-S990.

Krinsky NI. Antioxidant functions of carotenoids. *Free Radical Biol Med*, 1989, **7**:617-635.

Landrum JT, Bone RA. Lutein, zeaxanthin and the macular pigment. *Arch Biochem Biophys*, 2001, **385**:28-40.

Landrum JT, Bone RA, Kilburn MD. The macular pigment: a possible role in protection from age-related macular degeneration. *Adv. Pharmacol*, 1996, **38**:537-556.

Landrum IT, Bone RA, Chen Y, Herrero C, Llerena CM, Twarowska E. Carotenoids in the human retina. *Pure Appl Chem*, 1999, **71**:2237-2244.

Mare-Perlman JA, Fisher M, Klein R, Palta M, Block G, Milieu AE, Wright JD. Lutein and zeaxanthin in the diet an serum and their relation to age-related maculopathy in the third national health and nutrition examination study. *Amer Jnl Epidemiol*, 2001, **153**:424-432.

Marshall J. Radiation and the aging eye. *Ophthalmic Physiol Opt*, 1985, **5**:241-263

Mellerio J. The interaction of light with biological tissues and the potential for damage. In, Ed; Marshall J. *The Susceptible Visual Apparatus*, 1991, London, MacMillan Press, chapter 3.

Mellerio J. Interaction of Light on the Retina. In: Albert DM, Jakobiec FA. *Principles and Practice of Ophthalmology: The Basis Sciences*. 1994, Philadelphia, WB Saunders, chapter 116.

Mellerio J, Palmer DA, Rayner M.J. Macular Pigment Measurement with a Novel Portable Instrument (Abstract). *Ophthalmic Res*. 1998, **30** (suppL),302

Schalch W, Dayhaw-Barker P, Barker FM. The carotenoids of the human retina. In: Taylor A. *Nutritional and Environmental Influences on the Eye*, 1999, Boca Raton, CRC Press, chapter 12.

Sommerberg O, Keunen JE, Bird AC, van Kuijk FJ. Fruits and vegetables that are sources for lutein and zeaxanthin: the macular pigment in human eyes. *Brit Jnl Ophthalmol*, 1998, **82**:907-910.

Snodderly DM. Evidence for protection against age-related macular degeneration by carotenoids and antioxidant vitamins. *Amer Jnl Clin Nut*. 1995, **62**:S1448-S1461.

Taylor HR, West S, Munoz B, Rosenthal FS, Bressler SB, Bressler NM. The long term effects of visible light on the eye. *Arch Ophthalmol*, 1992, **110**:99-104.

Wooten BR, Hammond BR, Land RI, Snodderly DM. A practical method for measuring macular pigment optical density. *Invest Ophthalmol Vis Sci*. 1999, **40**:2481-2489.

van Kruijk FJ, Pauleikhoff D, Fitzke F. Personal communication, 2001

COMMENTARY

The Clinical Academic – The Clinical Scientist

Those of you who think that life is hard being a scientist nowadays are unlikely to give much thought to clinicians who have academic aspirations (overpaid, in the way, uneducated and always in a hurry). Nevertheless, there has been a minor crisis in academic medicine in the past few years. A major reorganisation of postgraduate training for clinicians called Calmanisation after the then Chief Medical Officer of Health has been introduced. Effectively, this gives a tight structure for the training in all the medical specialities for the 5-6 years leading up to consultant grade (i.e. the specialist registrar). It has had the benefit of improving the standards of training for many clinicians but has had the unfortunate effect of inflexibility. Thus many clinicians who might have wanted to "dip their toe" into scientific research are often discouraged from doing so. Certainly if they spend more than a year out of their training scheme the perception is that they have great difficulty getting back in. This has resulted in poor recruitment into Academic Medicine in its broadest sense and there is great concern that it would lead to an erosion of the research base in clinical medicine and to a lack of scientific leadership in the next generation. The problem has been recognised by the powers that be and following several reports, most importantly, the Saville Report, a proposal has come to fruition whereby nationally there will exist 50 clinician scientist posts. Funding for the posts will come jointly from the MRC, the Wellcome Trust and the Department of Health. They will be held for 10 years by each individual and the idea is that it will give them some sort of security whereby they will obtain a PhD interdigitated with the training in their particular clinical discipline. After 5 or 6 years in training these individuals would be at senior lecturer level and would continue in their research area for a further 4 to 5 years in their institutions.

Fifty places is not a large number and the competition for these posts is likely to be fierce. Physiology is the most important subject which underlies the practice of Medicine (albeit that not many clinicians recognise it!) so there is an opportunity here for Physiology Departments to develop research projects and collaboration with this old species under a new guise – the academic clinician or clinical scientist.