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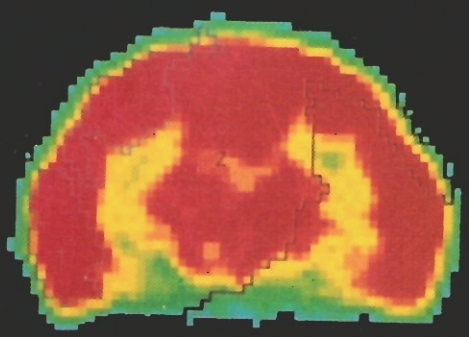

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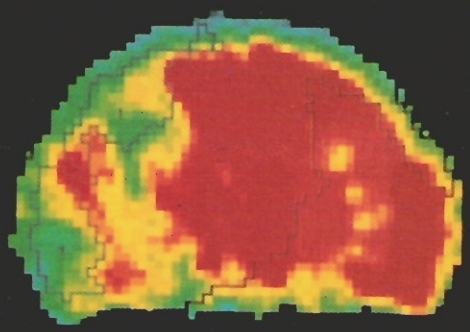

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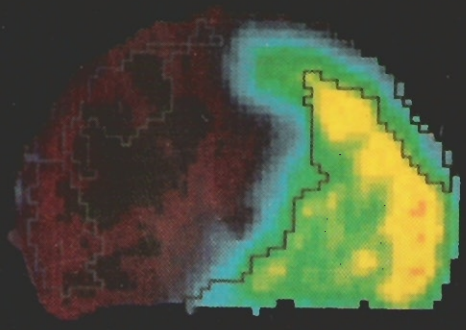

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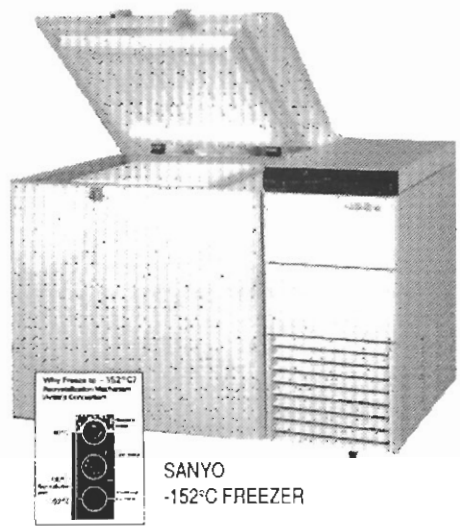

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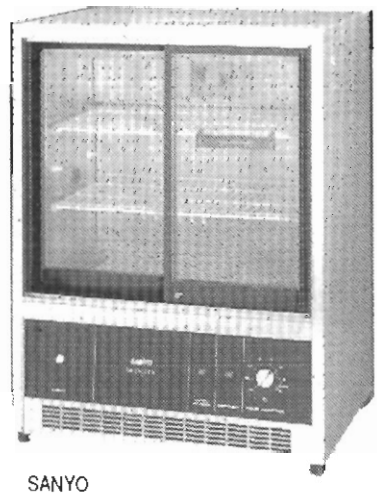

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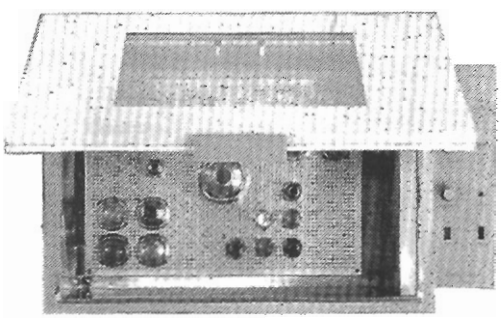

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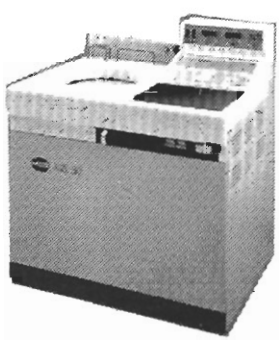

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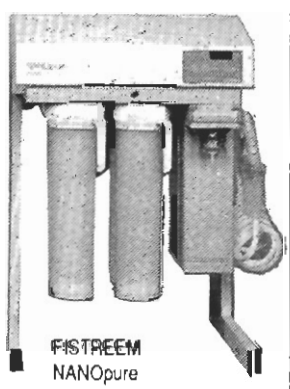

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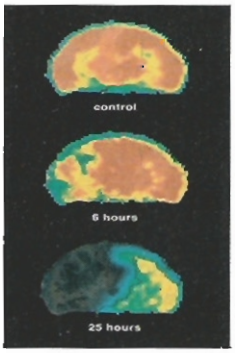

Cover Picture:
Progressive impairment of glucose consumption in focal cerebral ischaemia. Photograph supplied by Dr. R. Graf. Details on page 8.

Malta Chamber of Scientists

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Editorial

Waste management - what a load of rubbish!

EC Directive 75/442/EEC defines the term waste as "any substance or object .... which the holder discards or intends or is required to discard".

Waste is a by-product of our way of life. Its volume is growing all the time not to mention the problems to which it gives rise to - problems that are both specific and relatively complex. Today's average European produces about 1 kg of waste per day. This seemingly insignificant datum gives rise to some 4 billion tons of waste in Europe per year. We, Maltese, are responsible for over 900,000 tons!

Waste is not only a potential source of pollution - it can also constitute secondary raw material. Thus, we can also define waste as a misplaced resource, a resource in the wrong place at the wrong time. In fact, in the UK and in other countries, the authorities are commissioning the experimental 'mining' from closed landfills of discarded materials that a few years ago would have been considered without value.

In Malta in 1985 we produced approximately 239 kg of domestic waste per person per annum. In 1995 this rose to 346 kg and the trend is still towards an increase in this value. In the EU, in 1995, 420 kg of waste was generated per person per year.

Waste is a growing problem that we have repeatedly mismanaged to the point where it has reached problematic proportions - just have a look at Maghtab! Inconsistencies such as the failure to sustain the Household Waste Source Separation Programme, and the failure to identify sites for the building of further composting and sewage-treatment plants, have all contributed to our waste management problem. This current upward trend in waste must not only be halted but also reversed in terms of both volume and environmental hazard and damage.

The Maghtab Landfill has lately become a much reported upon and visited site, and rightly so. A succession of ministers and high officials from various departments have made solemn pronouncements about how the problem is going to be tackled. We even had the Prime Minister in the last Labour administration landing on it by helicopter to emphasise government's commitment to tackle the problem effectively. Yet this foul smelling, smouldering mass continues to grow. Although waste management in most countries continues to be dominated by the cheapest available option - landfilling - in Malta we need to look for other options due to the limited available land mass. It is now increasingly recognised that waste prevention and minimisation are more environmentally desirable solutions for waste management. All waste streams would benefit from the application of cleaner technologies and waste prevention measures.

The traditional waste hierarchy is dump and forget, dilute and disperse, treat and destroy. However, the emphasis today should be on waste minimisation at source.

A credible strategy includes a hierarchy of waste management options in which primary emphasis is laid on waste prevention, followed by promotion of recycling-and-reuse and then by optimisation of final disposal methods for waste which is not reused.

The principles of such a strategy could take the form of:

A) Prevention of waste production
- By technologies - clean technologies/lean production techniques
- By products - Eco-label and product criteria
- By avoidance - reuse
- By behavioural changes - producer and consumer

B) Recovery of the waste produced
- Segregation and sorting
- Separate collection
- Material recycling
- Energy recovery

C) Safe disposal
- Reduction of disposal
- Strict standards

This hierarchical strategy requires a concerted effort from both the domestic and the industrial worlds.

The domestic domain could be mobilised by the promotion of consumer information and education to influence consumption patterns. Support for private initiative in the segregation and recycling of waste streams is definitely an incentive but must be backed up by establishing and maintaining a reliable system of waste collection. A parallel waste data-collection system must be introduced to facilitate the formulation of adequate waste management legislation.

This reliable system must be extended to include the industrial domain. It would be wise to offer support for more widespread use of Eco-Audit schemes, as these act as a tool for industry to gauge its waste production and the effects it has on its economic viability and environmental impact.

In the long term, the gradual introduction of economic instruments to facilitate reuse, recycling and recovery schemes, will allow a transition to the higher rungs of the hierarchical ladder and, effectively, less waste production.

Although in Europe a variety of uncoordinated approaches are being used in an effort to tackle a waste generation problem that is running out of hand, in Malta it is not satisfactory for us to just follow *en suite* as we have now reached the Crisis point. The time for generating more reports by foreign consultants has been expended. Local expertise is available, even among members of the Malta Chamber of Scientists, who are able and willing to help the Authorities to prioritise the actions that need to be taken to lessen the impact that waste generation is having on our environment. Whether we like it or not, the challenge posed to our health and well-being by the ever-increasing mountain of waste needs to be faced today - not when we join the European Union - not in a year's time - but now!

P.S.: The problems mentioned above concern primarily solid wastes. There exists a whole range of liquid effluents and gaseous emissions that pose their own set of complex problems relating to waste management and environmental hazard.

Anton Pizzuto
Cleaner Technology Centre,
Malta University Services.

This was an invited editorial. It reflects the views of the author and not necessarily those of the Malta Chamber of Scientists.

Review Article

Emerging Insights into the Genesis of Cerebral Ischaemia and Stroke.

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Summary. *Stroke is a global problem with increasing significance because of the ageing population. Except for age, hypertension is by far the most important risk factor for stroke. Hypertension predisposes to a number of intracerebral and extracerebral vascular lesions which may cause cerebrovascular events by different mechanisms. The high metabolic need and low energy reserve make the brain very vulnerable to ischaemia. During the last decade a number of experimental studies - supported by PET studies in man - suggest the presence of a therapeutic window, i.e. the time during which the neurons can be saved. The penumbra is the zone surrounding the core of the infarct where the flow is decreased and the neurons are lethargic and may be electrically silent but still viable. The presumed role of calcium, excitatory amino acids, free radicals, platelet-activating factor, acidosis and brain temperature in the process of neuronal death is briefly reviewed.*

Keywords : Ischaemia, Stroke, Hypertension, Atherosclerosis, Positron Emission Tomography (PET), Middle Cerebral Artery Occlusion (MCA), Reperfusion, Free radicals, Nitric Oxide (NO).

Introduction

As a consequence of the high rate of oxygen metabolism and the lack of tissue oxygen stores, interruption of oxygen delivery to the brain causes immediate cell dysfunction and rapidly leads to cell death. Oxygen delivery to the brain is defined as the product of the oxygen content of arterial blood and the cerebral blood flow. Inadequate oxygen delivery (hypoxia) can result from inadequate cerebral blood flow (Ischaemic hypoxia), inadequate partial pressure of oxygen in arterial blood (hypoxic hypoxia), or inadequate oxygen-carrying capacity of arterial blood (anaemic hypoxia). The most common cause of brain hypoxia is ischaemia or inadequate cerebral blood flow. The level of cerebral blood flow at which the brain begins to exhibit energy failure is fairly well defined. Reduction of cerebral blood flow below 15ml/min/100g of tissue results in failure of electrical activity, and a reduction to less than 10ml/min/100g of tissue results in loss of the transmembrane ionic gradient. Cellular energy depletion appears to be a triggering event for many of the damaging biochemical processes occurring during ischaemia.

There are many causes of cerebral ischaemia in humans, including head trauma, stroke and cardiac arrest. Cerebral ischaemia may be further divided into focal and global categories. In global ischaemia, blood supply to the entire brain is interrupted, e.g., cardiac arrest. In focal ischaemia, blood supply to a particular region of the brain is interrupted, usually representing the area supplied by a particular vasculature. Cerebral ischaemia may also be described as complete or incomplete. Complete ischaemia is defined as total absence of blood flow to the entire brain or region of the brain. Incomplete ischaemia, on the other hand is defined as a severe reduction of cerebral blood flow in a focal or global pattern. Ischaemia may result in reversible cell injury or may be sufficient to cause tissue death (infarction) depending on the duration and severity of ischaemia. Not all regions of the brain are affected in the same manner during global ischaemia. The most sensitive, i.e., selectively vulnerable, neurons are located in the CA1, CA3 and CA4 regions of the hippocampus, portions of the caudate and cerebellum and layers 3, 5 and 6 of the neocortex. The mechanisms responsible for this selective vulnerability are not clear. In focal ischaemia, the anatomical location and extent of ischaemic damage depend on the distribution of the blood vessels whose flow is limited and on the presence of collateral circulation. Damage resulting from focal ischaemia commonly occurs in a graded fashion because collateral circulation partially perfuses the area surrounding the ischaemic core. This ischaemic 'penumbra' may receive blood flow that is inadequate to preserve normal cellular function but adequate enough to allow recovery. The concept of the ischaemic penumbra is important because the compromised status of these areas may be improved if effective early intervention is achieved. The purpose of our study which is being conducted at Max Planck Institute for Neurological Research, Cologne, in conjunction with the Department of Biomedical Science, University of Malta, in part, is to find early indicators for many of the damaging biochemical processes occurring during ischaemia.

Types and origins of stroke

Stroke or apoplexy is defined as an abrupt onset of neurological symptoms and signs indicating a disturbed cerebral circulation and lasting for at least 24 h or leading to death earlier. If lasting for less than 24 h, the episode is called a transient ischaemic attack (TIA) and is not included in the stroke definition. In Europe and North America about 80 % of strokes are caused by cerebral haemorrhages, the figures for haemorrhages being somewhat higher in East Asia.

Infarction can be caused by occlusion of large extracranial or small intracranial arteries. Occlusions can be caused by locally formed thrombi, by emboli coming from the heart or from the carotid and vertebral arteries. Arterial dissection, induced by trauma or occurring spontaneously, may be an underdiagnosed cause of brain infarction, particularly in young patients.

Arterial dissection is often associated with localized headache or facial pain at or before the onset of neurological signs. Cerebral infarcts can also occur in connection with hypotensive episodes.

Cardiac emboli have been reported to account for between 15 and 50 percent of cases; the wide range illustrates that the diagnosis often is uncertain. Atrial fibrillation is a well recognized risk factor for cerebral embolism and the most common causes are rheumatic and ischaemic heart disease. The frequency of stroke increases with the duration of fibrillation. Cardiac embolism as a cause of stroke increases with age and may constitute about half of the cases in patients > 75 years of age.

As an intrinsic intracranial source of strokes, lacunae reflect arterial disease of the small penetrating arteries supplying the internal capsule, basal ganglia, thalamus and paramedian regions of the brain stem. They are thought to account for about 15 to 20 percent of strokes. It is currently debated whether some lacunae are of embolic origin.

Most intracerebral haemorrhages occur in the supratentorial compartment, mostly involving the basal ganglia and the thalamus. The second most common location is the subcortical white matter of the cerebral lobes. Less than 15 percent of the haemorrhages are located in the cerebellum or pons. The symptoms will depend on the location and the size of the haematoma. Although the onset is usually abrupt, both the focal deficit and the level of consciousness usually undergo a gradual worsening due to further bleeding and /or secondary swelling. Haematomas of moderate or large size are accompanied by decreased levels of alertness.

Diagnosis

The neurological symptoms and signs will depend on the vascular territory involved. Most infarcts are supratentorial with the vascular territory of the middle cerebral artery being most often affected. The clinical features sometimes suffice to differentiate acute haemorrhage from infarction. Classification of the ischaemic stroke into subtypes can, to some extent, be performed on clinical grounds. However, it is not possible, clinically, to definitely separate haemorrhages from infarction, as revealed by brain computed tomography (CT), which is now routinely used in most stroke centres. Small haemorrhages can give comparatively minor symptoms or even transient symptoms. Whereas haemorrhages are seen on CT scan immediately after onset, brain infarcts may not be visible during the first days and, if small, may not be detected at all. A lumbar puncture, previously the investigation of choice to separate haemorrhage from brain infarction, is now less frequently performed in centres where CT is available, but might be of value in selected cases.

Ancillary Methods

1) Doppler techniques and ultrasound imaging techniques

The Doppler techniques depend upon the reflection of a beam of sound of very high frequency by moving red blood cells. Doppler techniques are widely used for the diagnosis of cerebrovascular occlusive disease. The most commonly used technique is that of plotting the Doppler frequency shift against time. When stenosis is present, blood flow velocity increases in the stenotic portion of the vessel and is detected by an increase in the frequency of the Doppler shift signal. Turbulence distal to the stenosis produces a characteristic visual pattern. To visualize the carotid arteries, a two-dimensional ultrasound imaging technique is used. Currently, the two techniques (Doppler flow and imaging) are combined in real time, defining structure and flow. This technique is called Echo-Doppler.

Transcranial Doppler ultrasonography is a non-invasive procedure for the assessment of intracranial cerebral circulation, allowing measurement of blood velocity in cerebral arteries at the base of the brain. However, since the diameter of the arterial lumen is unknown the blood flow cannot be determined. Despite this limitation, the method can be useful in answering specific questions such as detection of haemodynamically significant intracranial arterial stenosis. It is particularly useful in following changes in patients with subarachnoid haemorrhage who develop spasm, monitoring of brain-injured patients and intraoperative and postoperative monitoring of neurosurgical patients (Petty et al., 1990).

2) Magnetic Resonance Imaging (MRI)

The underlying principle of MRI is that many nuclei respond to the application of strong magnetic fields by absorbing and re-emitting radio waves, that can be detected and analysed and thus used to generate spectra indicating the concentration of various chemical species of these nuclei. Protons are among the most sensitive and abundant nuclei in biological tissues and have been widely used for MRI. Bone is not visualized and areas normally obscured by bone on CT scans are easily imaged. The resolution of grey and white matter is superior to that of CT scanning and cerebral infarction is evident much earlier, usually within 2 to 6 hours. However, CT is superior in early identification of brain haemorrhages and will probably continue to be the screening method at admittance of stroke patients. The recent development in MR angiography, MR diffusion weighted imaging, MR spectroscopy and functional MRI together with the higher time resolution and more widely accessible MRI than PET have made these techniques powerful tools in experimental and clinical stroke research (Baron, 1993; Neil, 1993).

3) Cerebral angiography

Cerebral angiography involves the selective introduction of a water soluble contrast medium into the carotid or vertebral arteries. It is used predominantly to detect and evaluate extracranial anomalies, particularly carotid stenosis, to diagnose arterial dissection and arterial and arteriovenous aneurysms.

4) Single photon emission computerized tomography (SPECT) and positron emission tomography (PET)

In acute stroke, the normal coupling between cerebral

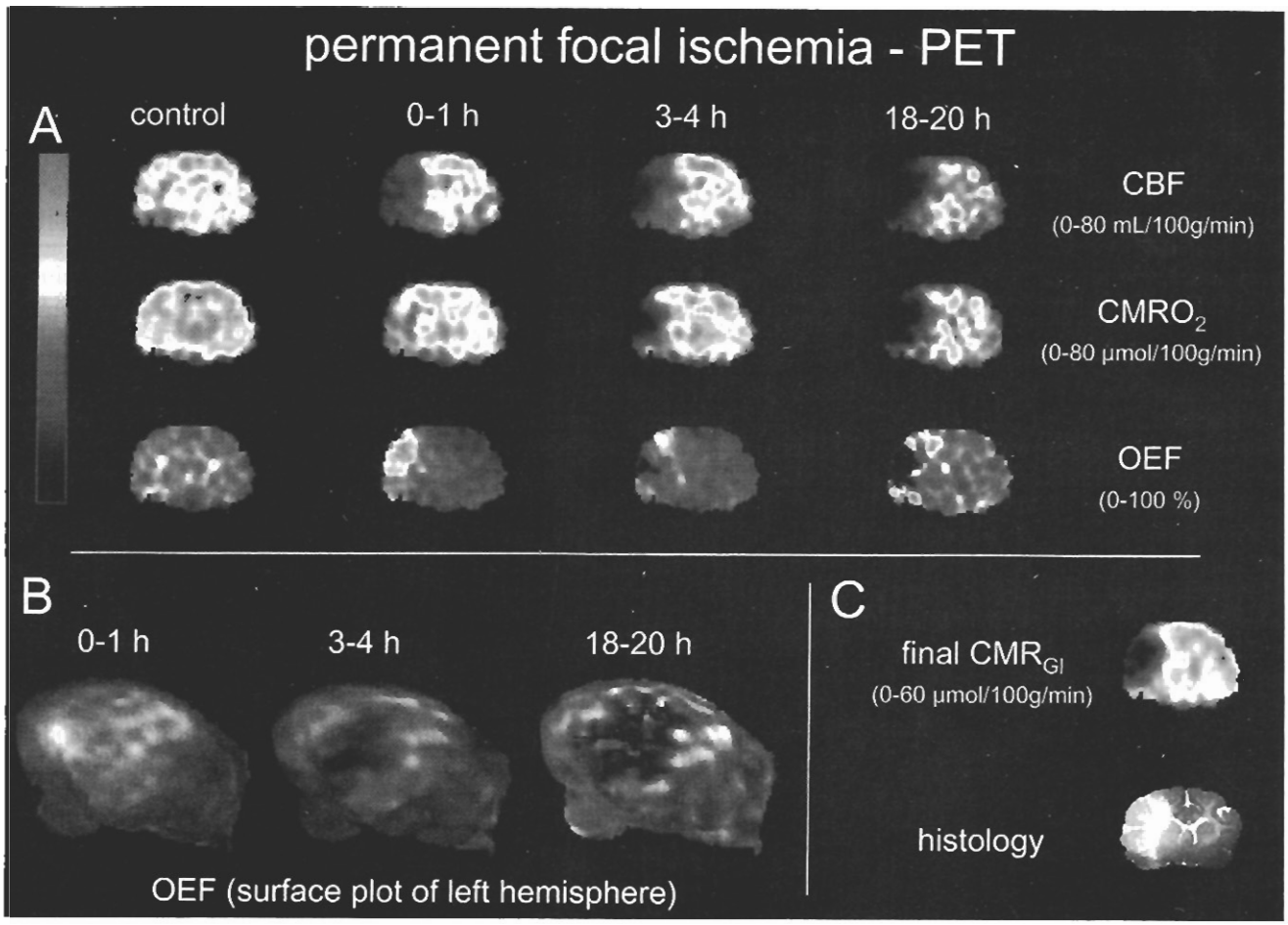

Fig 1.
A: Sequential quantitative PET images of an individual cat representing cerebral blood flow (CBF), cerebral oxygen consumption ($CMRO_2$) and oxygen extraction (OEF) before (control) and at 3 time points after permanent left middle cerebral artery (MCA) occlusion (0-1h, 3-4 h, 18-20 h). Progressive deterioration of oxygen consumption in the MCA territory corresponds with the spreading of the area with increased OEF and finally leads to hemodynamic and metabolic derangement.
B: Reconstructed OEF surface views of the left hemisphere (masked by control CBF) at the same 3 time points showing dynamic penumbra with progressive metabolic derangement.
C: PET image of glucose consumption (CMR_{Gl}) representing the area of final CMR_{Gl} suppression at 18-20 h after MCA occlusion and corresponding histological cross section showing the area of infarction at the same time point.
Adapted from Graf at al (1994) with permission.

blood flow and metabolism is not upheld. Without a concomitant determination of the oxygen extraction or cerebral metabolism, it is not possible to determine the state of the tissue by cerebral blood flow studies alone. This has been illustrated in studies using PET alone (Wise et al., 1983; Brooks, 1991; Heiss et al 1992). In the early stage, the blood flow and the metabolism might be low but oxygen extraction increased, suggesting that the tissue is still viable (Fig1). Later, particularly if the blood flow is restored, hyperaemia occurs while the oxygen extraction is low or nil indicating that the tissue is severely damaged. The rate of spontaneous reperfusion increases gradually with time and occurs within the first two weeks after stroke onset in 77 per cent of patients with cortical infarcts (Jorgensen et al., 1994).

Examples of remote metabolic depression after focal stroke have been observed with PET in man. Reduced metabolic activity can be seen in the cerebral hemisphere contralateral to cerebral infarcts, ipsilateral to thalamic and lenticulo-capsular lesions, the thalamus ipsilateral to a cortical infarct, visual cortex distal to lesions of the optic radiation and the cerebellar cortex contralateral to supratentorial infarcts (crossed cerebellar diaschisis). The exact mechanism underlying these metabolic changes that usaually are accompanied by changes in CT or MRI is not completely understood (Pappata et al., 1987; Ginsberg, 1990).

Risk Factors
The stroke incidence increases markedly with age. In a Swedish unselected population only 20 per cent of first-ever stroke patients were below the age of 65 and half of the patients were more than 75 years of age (Johansson et al., 1992). With the current ageing of the world population, stroke is likely to remain a major medical problem.

Hypertension
Hypertension is the most important risk factor for stroke. Hypertension predisposes to different types of intracerebral and extracerebral arterial lesions which may cause cerebrovascular events by different

mechanisms (Johansson, 1992). Hypertension leads to three main types of vascular changes: compensatory structural adaptation, degenerative vascular changes and, in the presence of other risk factors, to atherosclerosis.

Structural Adaptations to hypertension
When the blood pressure is increased abruptly to high levels in a previously normotensive individual, the autoregulatory capacity of the cerebral resistance vessels might be overcome and the blood flow increases. A stepwise increase in blood pressure will be tolerated. In chronic hypertension the vascular bed will adjust functionally and structurally to the increased load. The smooth muscle hypertrophy/hyperplasia will help to sustain wall tension and maintain adequate contractile function. In the cerebrovascular bed, an increased media thickness and lumen reduction has been observed over a large range of arterial sizes in vivo and in vitro, starting at rather large arteries. These changes will shift the autoregulatory curve to the right and protect the blood-brain barrier (Strandgaard,1978; Johansson, 1989). Although the structural adaptations basically are beneficial in protecting the vessels and preventing haemorrhage and permeability changes, they constitute a risk for ischaemia distal to any stenosis or occlusion or when the blood pressure is rapidly decreased because of the increased peripheral resistance and reduced collateral capacity. In connection with an abrupt decrease in blood pressure in hypertensive individuals, infarction may occur in the border zones between the territories of the main cerebral arteries. These 'water-shed' infarcts constitute approximately 10 percent of all human brain infarcts (Torvik, 1984).

Degenerative changes in hypertension
Degenerative changes in the small intracerebral blood vessels will occur when the compensatory mechanisms are not sufficient to protect the smaller intracerebral vessels. When the vessels yield to the high pressure, extravasation of plasma constituents seems to be the first step in hypertensive degenerative lesions in the vascular wall and in the development of brain lesions (Johansson,1992). Degenerative changes in the intracerebral arteries can lead to focal brain oedema, small lacunar infarctions or intracerebral haemorrhages due to intracerebral microaneurysms (Johansson,1992).

Hypertension, atherosclerosis and other risk factors
Hypertension is a risk factor for atherosclerosis. Vascular changes related to atherosclerosis or ageing are more pronounced in hypertensive individuals. The predominant sites, and usually earliest localization of atherosclerotic changes, are in the extracranial arteries. The second most common site is in the circle of Willis and with time, the changes may also occur in the smaller intracerebral arteries. This is reported to occur earlier in hypertensive than in normotensive individuals.

Other risk factors include diabetes, cigarette smoking, impaired cardiac function and high hematocrit and plasma fibrinogen levels. About 10 percent of strokes are preceded by transitory ischaemic attacks. There are substantial race differences with higher incidence in Japan and China, and in the US in the black population than in the white. There is an association between stroke and alcohol intake in men, which partly but not completely can be attributed to coexistent hypertension. Although epidemiological studies are lacking, drug abuse, particularly amphetamine, heroin and 'crack', a cocaine preparation that is inhaled, have repeatedly been reported to be associated with stroke in young individuals.

Several studies suggest that family occurrence of stroke is an independent risk factor. Homocysteinaemia, long known to be a risk factor in homozygotes, has in several recent studies been reported to be a risk factor in heterozygotes also.

Animal models of cerebral ischaemia
Animal models are required for detailed study of the pathophysiology of cerebral ischaemia. An 'ideal' animal model would have the following features:

- The animal model should closely mimic the development of the clinical insult in humans.
- The experimental insult should elicit similar responses in each individual animal tested; i.e., the insult should cause a reproducible injury.
- The model should be closely related physiologically; e.g., temperature, blood glucose, and blood pressure should be tightly controlled.
- The experimental animal pathology should be similar to that in humans.

Obviously, there are no 'ideal' animal models and it must be clearly understood that no animal model exactly reproduces cerebral ischaemia in man. Animals do not readily develop the cardiovascular disease that commonly underlies stroke, nor do they duplicate the age, nutritional status, or drug history of patients who suffer from stroke or cardiac arrest. Furthermore, animals are usually anaesthetized, are paralyzed, and have undergone surgery, particularly craniotomy, all of which may profoundly alter tissue response. Reproducible models are not easily achieved because of anatomical and physiological variability, both within and among species. In addition, lack of strict control of physiological variables, e.g., brain temperature, can lead to misleading results. In spite of these limitations, numerous animal models (rats, rabbits, gerbils, cats, dogs, goats, pigs and monkeys) have been developed and provide meaningful data that can be applied to the understanding of cerebral ischaemia in humans.
Experimental ischaemia is induced in animals by occluding vessels that perfuse the brain. Focal ischaemia is modeled by middle cerebral artery occlusion (MCA); in some models this is accompanied by occlusion of the ipsilateral common carotid artery. Models of ischaemia can be permanent (no reperfusion) or transient (allowing reperfusion). Models of global ischaemia are utilized to simulate ischaemic injury resulting from cardiac arrest. In large animals, global ischaemia has been modeled in numerous ways, including inducing cardiac arrest, using a neck tourniquet, cross-clamping the proximal aorta, and raising the intracranial pressure to levels greater than mean arterial blood pressure. Our experimental approach, for induction of focal cerebral ischaemia in cat

models involves occlusion of the proximal middle cerebral artery. Briefly, after enucleation, a small burr hole of 3-mm diameter is drilled transorbitally above the optic foramen in the posterior wall of the left orbit. The trunk of the left MCA is prepared just above the optic nerve by cutting dura and arachnoid membranes under microscopic control, and an occluding device (Graf et al., 1986) is implanted around the MCA trunk. This device consists of an outer cannula whose tip forms a hook to be put around the MCA, and an inner occluder, which can slide into the hook through the cannula. By pushing the inner occluder toward the silicon-coated wall of the hook, the MCA is compressed gently and firmly between the occluder and the hook wall, yielding total arterial occlusion, and by pulling back the occluder, the MCA occlusion is easily relieved.

Animal models are necessary to study cerebral ischaemia, and despite significant shortcomings, animal models have provided and will continue to provide insights into the pathophysiology of cerebral ischaemia which may lead to the development of effective pharmacological interventions.

Effects on brain tissue, mechanisms of neuronal death, post-ischaemic reperfusion

As already mentioned earlier, the high metabolic need and low energy reserve make the brain very vulnerable during ischaemia. When the perfusion pressure falls below critical levels, ischaemia develops and will progress to infarction if the flow is sufficiently reduced and the reduction persists long enough. In experimental studies on focal brain ischaemia, surprisingly extensive restoration of metabolism and function has been observed after ischaemic periods as long as 60 min or more. The length of time tolerated varies with factors such as the degree of reflow after ischaemia, and plasma concentrations of glucose, corticosteroids and catecholamines (see Figure 2).

Currently the penumbra is looked upon as a dynamic process with impaired and unstable perfusion and metabolism which is the target for possible therapeutic intervention (Heiss and Graf, 1994). There is some disagreement as to the time during which the tissue may be rescued but this is likely to be related to the degree of perfusion deficit and may be up to a couple of hours. However, with long recovery periods, permanent selective neuronal damage is seen already in ischaemia of 5-15min duration (Ito et al., 1975). This 'maturation' of neuronal damage following transient global ischaemia, which has been verified in a number of studies, indicates an on-going process in the post-ischaemic period.

Clot resolution or recanalization of an occluded artery may occur spontaneously but the frequency is unknown. Animal as well as clinical studies have demonstrated that this will often lead to postischaemic hyperaemia. Wheras hyperaemia has been reported to be essential for the functional recovery in global ischemia, hyperaemia has been associated with pronounced oedema and severe brain damage after focal ischaemia. Prevention of hyperaemia reduces the vasogenic oedema as well as the ischaemic brain damage in focal ischaemia in

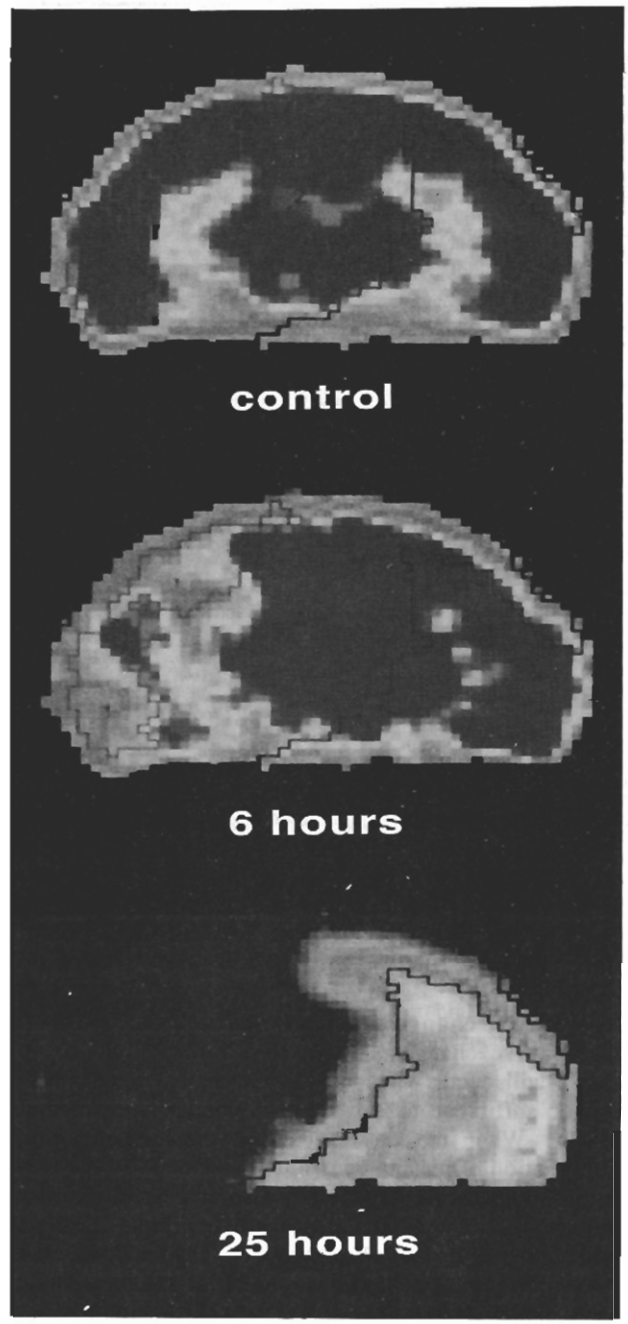

Fig 2. Sequential PET images of an individual cat representing CMR_{Gl} before (control) and at 2 time points after left middle cerebral artery occlusion (6 hours and 25 hours). Progressive deterioration includes in this case the hemisphere contralateral to the ischemic focus, probably due to malignant edema formation and rise of intracranial pressure.
[By courtesy of Dr. R. Graf. Max Plank Institute for Neurological Research, Cologne, Germany.]

experimental animals. If confirmed in man, these observations could be of importance in clinical trials with fibrinolytic agents and during operations on severely stenotic vessels where gradual rather than abrupt opening of an occluded vessel should be aimed at. However, in the clinical situation it would be a difficult task to decide if and when attempts to reduce the blood flow should be tried. An aggravating effect of postischaemic hyperemia could be related to any of the currently discussed mechanisms for nerve cell death following ischaemia such as free radicals, lactacidosis,

excitatory amino acids and possibly to some additional intrinsic or extrinsic factors. However, so far clinical studies have not confirmed that hyperaemia aggravates the lesions, rather hyperaemia has been proposed to be a prognostically good sign (Jorgensen et al., 1994; Marchal et al., 1993). Preliminary reports from studies on thrombolytic therapy in stroke indicate that it might be a smaller problem than expected. One possible explanation could be that the reflow does not occur so rapidly as under experimental conditions. Further studies are needed to clarify the possible adverse and beneficial effects of hyperaemia in stroke.

One other problem that must be anticipated in ischaemia is the underlying neuronal damage. Since energy is required to uphold the ion gradients across nerve and glial cell membranes, energy failure will lead to a shift with efflux of K^+ from cells and influx of Na^+, Cl^- and Ca^{2+} . This ion shift will lead to an accumulation of water within the cells, an intracellular oedema. Accumulation of metabolites within the cell will add to this oedema, which in the early stage is completely reversible. Ischaemia leads to a diffuse transmitter release since energy is needed to keep transmitters stored in their granules. The electrical activity of the neurons stops when the blood flow is decreased to about one-third of the normal values (under normal temperature and blood glucose levels) but some basic cell function is still present and the cells can regain their function if the blood supply is restored. There are various hypotheses as to the triggering mechanisms for neuronal death and some will be presented below. Current research indicates that these mechanisms combine in the process that finally kills neurons. The neuronal damage can be of two types: selective neuronal vulnerability affecting groups of neurons with a characteristic distribution within the brain, and infarction, affecting not only neurons but also blood vessels and glial cells.

The calcium hypothesis

That cell death may be a specific consequence of disturbance in intracellular Ca^{2+} homeostasis has long been discussed (Schanne et al., 1979). The concentration of calcium is 10,000 times higher in the extracellular fluid than in the cells and influx of calcium into the cells together with release of calcium from intracellular sources leads to uncontrolled activation of a number of calcium-dependent reactions (Siesjo, 1992; Morley et al., 1994). The combination of energy failure and calcium influx/release can lead to an extensive breakdown of phospholipids and proteins, to proteolytic degradation of cytoskeleton components and to free radical formation.

The excitotoxic hypothesis

Glutamate and some other excitatory amino acids have a transmitter function in the brain but can also be toxic to the neurons. First, exposure of neurons to glutamate may cause an acute neuronal swelling resulting from the depolarization-mediated influx of Na^+ Cl^-, and water. The degree to which this event contributes to neuronal injury is unclear, but it has been suggested that water entry causes osmotic lysis, which may disrupt neuronal function.

In order to prevent a high extracellular concentration of glutamate and other excitatory amino acids, these are taken up by efficient re-uptake mechanisms after release. The uptake mechanisms are energy dependent and during ischaemia the excitatory amino acids may accumulate in high concentrations. Current evidence indicates that ischaemic neuronal damage may be caused by enhanced release or diminshed uptake of glutamate or other excitatory amino acids enabling an enhanced calcium influx through channels gated by excitatory amino acid receptors (Rothman and Olney, 1986; Hossmann, 1994). The elevation of intracellular Ca^{2+} is known to activate lipases, phospholipases, proteases, and protein kinases, each of which, if not properly regulated, can easily be envisaged to produce considerable cellular damage. Antagonists which block N-methyl-d-aspartate (NMDA) and alpha-amino-3-hydroxy-5-methyl-isoxazoleproprionic acid (AMPA) glutamate receptors have been shown to ameliorate neuronal damage in experimental ischaemia (Choi, 1990; Graham et al., 1993).

Free radicals

The role of free radicals in the ischaemic nerve cell pathology has been debated for over a decade (Siesjo,1992; Chan, 1994). It has been suggested that free radicals are important particularly in the reperfusion period with good access to oxygen in an already damaged tissue. However, recent studies suggest a role also in permament ischaemia and free radical scavengers have been shown to reduce the infarct size in experimental brain infarction. Likewise, studies have shown that transgenic mice, overexpressing the enzyme Cu-Zn-superoxide dismutase, develop smaller infarcts than control animals (Chan et al., 1991). Calcium will enhance lipolysis and accumulation of arachidonic acid and interact with the free radical mechanisms in the degradation of lipids, proteins and DNA. Metals, including iron, increase the rate of lipid peroxidation and it has been suggested that free radical damage may also be related to alterations in iron-binding.

Another factor thought to be of importance for ischaemic injury is platelet-activating factor which may act at least in part by generating free radicals (Lindsberg et al., 1991). Since the metabolic needs of the brian increase with temperature, lowering of the brain temperature will lead to better survival of neurons during ischaemia. Free radicals also stimulate the release of glutamate in rat hippocampal slices and neuronal cultures. This suggests that free radical formation and glutamate release are mutually related and cooperate in a series of molecular events that link ischaemic injury to neuronal cell death.

The role of nitric oxide

Nitric oxide (NO) has been regarded as one of the mediators playing a key role in the pathophysiologic mechanism of focal cerebral ischaemia (Dalkara and Moskowitz, 1994; Dawson, 1994). As expected from the complex and diverse actions of NO, studies attempting to modify NO production in focal cerebral ischaemia report conflicting results (Dalkara and Moskowitz, 1994; Dawson, 1994). Nitric oxide acts as an intercellular messenger molecule in the brain; it simply diffuses out of a cell where it has been

synthesized into the neighbouring cells where its targets exist (Dawson and Snyder, 1994). Like authentic neurotransmitters, NO must diffuse across the extracellular space to exert its biological effects, and thus extracellular NO concentration probably is a good indicator of physiologic NO activity. With the invention of NO-sensitive electrodes, real time measurements of extracellular NO concentration has become feasible. The first attempts to apply them to focal cerebral ischaemia have reported an increase in tissue NO concentration (Malinski et al., 1993; Ohta et al., 1996) and NO is thus implicated as a mediator of tissue injury. Within 3 to 24 minutes after MCA occlusion, NO increases dramatically from approximately 10nM to 2.2 (M within cortex as detected by a porphyrinic microsensor (see Figure 3). Brain nitrite, cGMP levels and brain NOS activity increase as well (Malinski et al., 1993). Nitrite, NO and nitric oxide synthase (NOS) activity return to baseline levels within an hour. One speculation holds that NOS activity increases due to a rise in intracellular Ca^{2+}/calmodulin complex (Dawson and Snyder, 1994). Brain NO is synthesized from L-arginine and oxygen by NOS requiring NADPH, flavins and tetrahydrobiopterin. Among some isoforms, constitutive NOS is habitually present in neurons and endothelial cells in brain tissue, and is calcium/calmodulin dependent, ready to be activated by a small increase in cytosolic Ca^{2+} concentration (Dawson and Snyder, 1994). In ischaemic tissue, cytosolic Ca^{2+} can be raised by Ca^{2+} influx through NMDA receptor-mediated Ca^{2+} channels and voltage-sensitive Ca^{2+} channels (Siesjo, 1992). These Ca^{2+} channels require depolarization of the plasma membrane to let Ca^{2+} in, as in the case in ischaemic tissue. Intracellular Ca^{2+} stores, such as endoplasmic reticulum, must also be quoted as possible sources of a possible rise in cytosolic Ca^{2+} in the ischaemic tissue (Silver and Erecinska, 1990; Mitani et al, 1993).

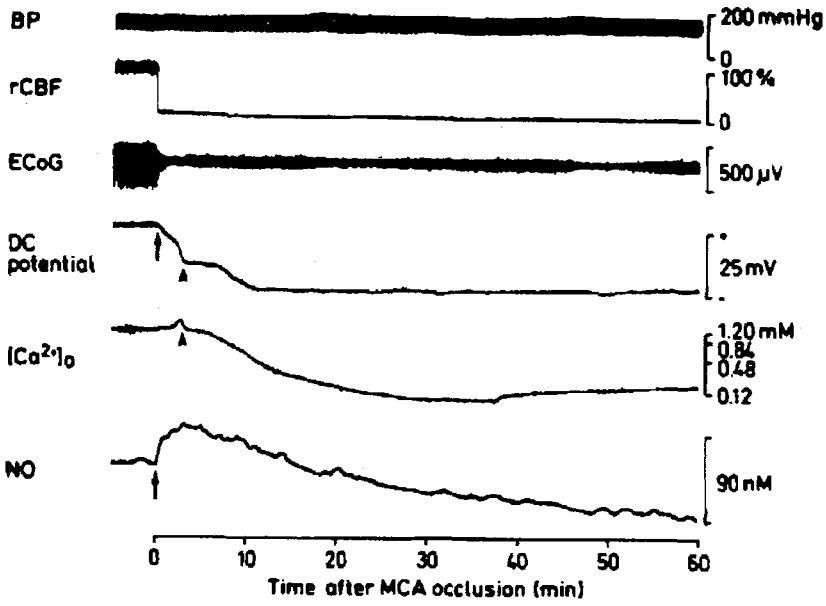

Fig 3. Actual recording of systemic arterial blood pressure (BP), regional cerebral blood flow (rCBF), electrocorticogram (EcoG), direct current (DC) potential, extracellular Ca^{2+} concentration ($[Ca^{2+}]_o$), and nitric oxide concentration (NO) in the ectosylvian gyrus. Middle cerebral artery (MCA) occlusion caused an immediated drop in rCBF accompanied by a steep negative shift of the DC potential and a sudden increase in NO (see arrows). Note that$[Ca^{2+}]_o$ showed a small elevation in the initial phase when NO increased, and that the steep fall $[Ca^{2+}]_o$ occurred much later at 160s after MCA occlusion. *Adapted from Ohta et al (1997) with permission.*

Although in normal brain NO seems to be a nontoxic mediator of cerebral vasodilation, recent data suggest that NO may have neurotoxic effects if present in abnormally high concentrations (Dawson et al., 1991). In endothelium and brain, ionized calcium is the intracellular messenger initiating the reduced nicotinamide-adenine dinucleotide-dependent oxidation of arginine to produce NO. It is well known that cerebral ischaemia causes an increase in extracellular concentration of glutamate and aspartate, and these excitatory amino acids bind to and stimulate NMDA receptors in brain (Beneviste et al ., 1984). Excessive activation of the NMDA receptors allows influx of Ca^{2+} into neurons, which may stimulate production of superoxide anion via a prostaglandin pathway and NO via stimulation of NOS. Stimulation of non-NMDA glutamate receptors is also thought to generate NO (Southam et al, 1991). Moreover, inhibition of NOS in neuronal culture ameliorates glutamate toxicity (Dawson et al., 1991). One postulated mechanism of NO toxicity is that at high concentrations, superoxide anion and NO may react to form the peroxynitrite anion, which decomposes at acidic pH into strong oxidants. Thus excessive NO generation might act as an agent of cell death in stroke by reacting with superoxide.

In conclusion, the discovery of NO has provided a further opportunity to explore the pathophysiology and treatment of cerebral ischaemia. Controversy abounds, which may reflect the importance of NO to the diversity of factors (both known as well as unknown) which impact cerebral ischaemia. However, it would be of interest to determine whether drugs that alter NO synthesis and metabolism will be of use in the treatment of ischaemia. More to the point, one still needs to demonstrate whether NO is relevant to the pathophysiology of global ischaemia. Nevertheless, answers should be forthcoming from a number of developments, namley;

- selective inhibitors for the neuronal or vascular isoforms of NOS
- transgenic mice in which neuronal or endothelial NOS are selectively knocked-out
- discovery of new methods for both directly measuring NO and assessing NOS activity routinely.

Models of ischaemia and reperfusion

It is evident from evaluating different animal models of ischaemia and reperfusion that a number of factors are important in determining the extent of neurological damage that occurs as a direct result of ischaemia. These include but are not limited to the amount of blood flow reduction, the duration of time that flow is reduced, the regional location of the flow reduction, and the metabolic state of the brain before the ischaemic period. Multiple mechanisms may contribute to the injury, and the relative contribution of each mechanism depends on the specific situation. For example, calcium-induced damage from the release of excitatory neurotransmitters is generally most prominent with moderate reductions of blood flow in regions with high NMDA receptors (Monaghan et al., 1985) and high excitatory amino acid innervation. With severe or complete reduction of blood flow, calcium entry through non-NMDA receptor-operated channels and voltage-sensitive channels becomes prominent (Siesjo et al., 1985). With reduced blood flow over prolonged periods, continued anaerobic glycolysis enhances the contribution of acidosis to ischaemic injury (Rehncrona et al., 1981). Therefore, when different potential mechanisms of ischaemia and reperfusion injury in brain are evaluated, it is important to recognize that the mechanism of injury will depend on the degree, duration, and localization of reduced blood flow and other aspects of the experimental model. Furthermore, the issue of radical-mediated injury is complicated because the various potential mechanisms are interrelated with radical injury. Acidosis can potentiate lipid peroxidation, calcium accumulation stimulates phospholipase activity, which may generate radicals via arachidonic acid metabolism; and NMDA receptor activation generates NO which in turn is capable of reacting with superoxide to form the cytotoxic oxidants as exemplified before.

Thus it is an oversimplification to consider an array of parallel pathways that can be independently blocked for assessing the individual contribution of these injury mechanisms in the brain.

Pharmacological intervention in ischaemia

Despite major advances in deciphering the biochemical events involved in the pathophysiology of cerebral ischaemia, little has been done to arrest, prevent, or reverse ischaemic injury. It has been realized that the extent of irreversible damage is governed by both the duration of ischaemia and the severity of ischaemia (complete versus incomplete). A number of pharmacological agents have been utilized for cerebral ischaemia in both animal models and humans, but to date, no agent has been shown to be of unequivocal value; i.e., there is no effective treatment for cerebral ischaemia, presently. However, a brief outline of the pharmacological agents together with their proposed pharmacological strategies for the treatment of cerebral ischaemia are listed below.

1) Calcium channel blockers

These drugs block the entry of calcium ions into the ischaemic neurons. These drugs do not themselves antagonize the effects of calcium ions; instead they prevent this ion from gaining access to its intracellular site of action. By blocking the entry of calcium ions, they may inhibit the essential role of this cation in the activation of lipolytic enzymes, protein kinases, and phosphatases during ischaemia. Improved cerebral blood flow, improved or no change in neurological outcome, decreased brain lactic acidosis, and decreased infarct size all have been reported following calcium channel blocker treatment in various studies (Wong et al., 1990).

2) Vitamin E

α-Tocopherol, a well known antioxidant, has beneficial effects on brain oedema and ischaemia. It inhibits the activities of phospholipase A_2 and lipoxygenase and plays a fundamental role in the stabilization of polyunsaturated fatty acid chains in membrane phospholipids. Vitamin E interacts with cellular membranes and prevents lipid peroxide formation by acting as a hydrogen donor (Traystman et al., 1991).

3) CDP-amines

CDP-amines are key intermediates in the biosynthesis of phosphatidylcholine and phosphtidylethanolamine. The therapeutic actions of CDP-amines are thought to result from restorative effects on phospholipid synthesis in the ischeamic brain. CDP-amines attenuate the fatty acid increases and counteract the disruption of cerebral mitochondrial lipid metabolism induced by hypnoxia. They have been reported to inhibit the activities of phospholipases A1 and A2. CDP-amines have also been reported to increase oxygen consumption and glucose incorporation into amino acids and phospholipids followed by a decrease in lactate production (Murphy et al., 1990).

4) Glutamate antagonist MK-801

The use of MK-801 for the treatment of ischaemia is controversial. It has been used successfully for the treatment of cerebral ischaemia in experimental models. MK-801 may exert its antagonistic effects via a site related to the ion channel. The onset of NMDA receptor blockage with MK-801 is more rapid in the presence of glutamate. These two effects may be relevant to the efficacy of MK-801 in cerebral ischaemia, which provokes a marked elevation in extracellular concentrations of glutamate. In addition to direct receptor blockade, MK-801, may protect neurons with severe, but not complete, energy failure by preserving ionic gradients across the plasma membrane and enhancing amino acid uptake (Buchan, 1990).

5) Superoxide dismutase

SOD has been proposed as a therapeutic agent for reperfusion injury because of its ability to scavenge superoxide anion. However, Cu-ZnSOD, is a large water soluble molecule (32 kDa) and therefore cannot penetrate the blood-brain barrier in significant quantities. In addition, SOD has a circulatatory half-life of only 8 mins in rats. In an effort to overcome these problems, Traystman (1991) conjugated this enzyme and administered it intravenously in rats as liposome-entrapped SOD and polyethylene glycol-conjugated SOD.

SOD delivered in liposomes has been shown both to increase brain SOD activity and to reduce infarct volume in a rat model of focal cerebral ischaemia. In contrast, polyethylene glycol-conjugated SOD does not appear to increase brain SOD activity but has been shown to reduce infarct volume in animal models of focal cerebral ischaemia.

6) Platelet-Activating factor antagonist

Large amounts of platelet activating factor (PAF) are produced by brain tissue and endothelium cells in response to ischaemia and reperfusion. PAF is a powerful vasoconstrictor and has many cytotoxic properties. PAF antagonists that are present in an extract of Ginkgo biloba leaves (ginkgolide B) appear to reduce edoema and neuronal damage in several mammalian species (Braquet et al., 1989).

7) U74006F, 21-Aminosteroids (Lazaroids)

U74006F, a nonglucocorticoid 21-aminosteroid, is a potent inhibitor of lipid peroxidation. It has a beneficial effect in animal models of severe head injury, posttraumatic spinal cord ischaemia, and cerebral ischaemias. The mode of action of 21-aminosteroids is not known, but it may act by inhibiting iron-dependent lipid peroxidation. The 21-aminosteroids significantly reduce Na^{2+} accumulation, K^{+} loss, and water entry into ischaemic brain. The effect was found to be most consistent and prominent in tissues surrounding the infarct site (Hall et al., 1990).

8) Cholesterol-lowering agents and oestrogen

Two agents that are in routine clinical use - inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase or 'statins' (Cholesterol-lowering agents), and oestrogen have been shown to reduce brain vulnerability to focal ischaemic insults (Endres, 1998). It is thought that the former may work largely by upregulating the activity of endothelial NOS; the mechanism of oestrogen-induced neuroprotection is unknown, but it may involve antioxidant actions or amplification of trophic mechanisms.

Future therapeutic directions

Advances in methods of brain imaging, such as magnetic resonance imaging and positron emission tomography, should allow more accurate delineation of tissue regions at risk because of impairment of blood supply, but not irreversibly damaged, and so will enhance the targeting of countermeasures in time and space. One can anticipate continued refinement in thrombolytic strategies, aiming to limit side effects at locations remote from the offending thrombus and thereby reducing bleeding complications, as well as more powerful approaches aimed at reducing brain oedema, or favourably altering the distribution of blood flow in cerebral arteries.

With regard to neuroprotection, it is predicted that several paths of research will gain momentum, aimed at blocking excitotoxicity in ways superior to that achievable with unselective NMDA antagonists, or moving away from central preoccupation with excitotoxicity and neuronal Ca^{2+} overload to target other processes and other ionic imbalances.

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Research Article

Field Identification of Calcified Red Algae

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Calcified red algae are important components of several marine communities where they are often responsible for bioconstructions on which depend a variety of other organisms. The vast majority of calcified red algae are the **coralline algae** which belong to the Order **Corallinales**. Most authors consider this order as comprising the single family **Corallinaceae** although the genus *Sporolithon* is sometimes placed in a separate family: the Sporolithaceae (Verheij, 1993). A few other algae, particularly in the genera *Peyssonnelia* (Order Gigartinales, Family Peyssonneliaceae) and *Galaxaura* (Order: Nemalionales, Family Chaetangiaceae) are sufficiently heavily calcified to be mistaken for corallines.

In the Central Mediterranean, the most important communities dominated by calcified red algae are:

- **Pavements** or *Trottoirs* in the lower Mediolittoral dominated by *Neogoniolithon notarisii*, often accompanied by the sessile vermetid gastropod *Dendropoma petraeum*.
- **Belts** and rims in the lower Mediolittoral / upper Infralittoral, typically on vertical rock faces, dominated by *Corallina elongata*, often accompanied by *Lithophyllum incrustans* and *Phymatolithon lenormandii*.
- **Rims** or Cornices in the middle Mediolittoral dominated by *Lithophyllum lichenoides*.
- Lower Infralittoral / upper Circalittoral **Coralligene** or Coralgal communities involving a variety of species such as *Neogoniolithon brassica-florida, Lithophyllum frondosum, Lithophyllum incrustans, Mesophyllum lichenoides* and a variety of sessile animals such as gorgonians and bryozoans.
- Lower infralittoral and circalittoral **Maerl** communities dominated by unattached rhodolith-forming species such as *Lithothamnion minervae, Lithothamnion corallioides, Phymatolithon calcareum, Lithophyllum racemus, Mesophyllum alternans* and *Peyssonnelia rosa-marina* (Lanfranco, 1993; Lanfranco et al. 1999).

Morphologically, coralline algae fall into two groups. The **geniculate** forms have a segmented branched thallus where calcification is weak at the joints between segments, thus conferring some degree of flexibility. The **non-geniculate** forms, on the other hand, are completely calcified and are therefore inflexible and with a stony texture and appearance. The latter may be either attached to a substrate or may be unattached thus forming part of the bottom sediments. In the latter case, such free-living corallines may occur in large populations giving rise to maerl communities, the individual plants being called rhodoliths. Some species occur in both attached and unattached forms, though most species occur predominantly in one or the other state. Of course, any species may occur accidentally in an unattached state due to fragmentation or displacement caused by hydrodynamism or bioturbation.

Due to their importance it is essential for the ecologist to be able to identify the species accurately. However their identification is notoriously difficult. This is largely due to the great plasticity of their gross morphology which is largely dependent on ecological factors. Accurate identification generally requires microscopic examination of sections of the thallus and, in many cases, requires fertile material, making this essentially a job for the specialist. In fact the majority of available keys are based on such microscopic characters.

The aim of this contribution is to provide a key which enables the field worker to identify calcified algae with some degree of confidence, using only gross morphological features and requiring no more equipment than a hand lens and ruler. Construction of the key involved the examination of a large number of specimens by means of which the most diagnostic gross characters could be chosen; ecological characters have also been widely used. The user should however beware that the key works best at a population level, where numerous and well developed specimens of known provenance are available. Attempting to identify individual, immature or fragmentary specimens gives much less reliable results. The key includes all non-parasitic corallines and other heavily calcified red algae known from the Maltese Islands. It is adapted and augmented from a previously published simplified version (Lanfranco, 1998). In the Maltese islands, the family Corallinaceae also includes the microscopic *Choreonema thuretii* (Bornet) Schmitz which parasitises genicluate corallines. The common green alga *Halimeda tuna* (Ellis & Solander) Lamouroux is heavily calcified but cannot be mistaken for a coralline. Several species of *Peyssonnelia* have some degree of calcification; only those which are competely calcified and inflexible are included here. Specimens of nearly all species are deposited in the reference collection of the Department of Biology museum.

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The Key

1. Geniculate: thallus jointed, profusely branched and flexible.....2

1a. Non-geniculate: thallus solid, inflexible 12

2. All branching pinnate throughout, thallus clearly flattened and in one plane .....***Corallina elongata***

Most Mediterranean records for *Corallina officinalis*, which has much sparser branching and clearly cylindrical axes, are probably misidentifications of this species.

2a. Branching dichotomous with or without pinnate terminal branches.....3

3. Branches normally less than 0.5mm in diameter.....4

3a. Branches normally more than 0.5mm in diameter or, if slightly less, regularly dichotomous with open branching 7

4. Basal axes up to 0.5mm in diameter. Apparently rare species from the lower infralittoral/ circalittoral .....***Jania longifurca***

4a. Basal axes up to c. 0.2mm in diameter 5

5. Ultimate branches with some segments bearing horn-like appendages at the apex which may give rise to segmented pinnate branches.....***Jania corniculata***

5a. No horn-like appendages or pinnate branches present.....6

6. Branches forking at less than 45°, plants often longer than 2cm, usually very densely branched, growing profusely on other seaweeds, often covering them completely; very common.....***Jania rubens***

6a. Branches forking at over 60°, very delicate, bases creeping and attached to host plant by sucker-like haustoria; very common.....***Jania adhaerens***

7. Most dichotomies covered by pinnate branches. Usually epiphytic but sometimes on rock; base creeping; frequent. Easily confused with *Corallina elongata* which has no dichotomous branching, and with *Jania corniculata* where any pinnate branches start off as a projection from the apex of a segment ..... ***Haliptilon virgatum***

The larger *Haliptilon squamatum*, with a creeping basal system, may also be present.

7a. No pinnate branches present 8

8. Branching irregularly dichotomous; on rock; common .....***Amphiroa rigida***

8a. Branching regularly dichotomous 9

9. Very flexible at the joints, tips of branches appearing tubular; rather uncommon. Easily mistaken for species of *Amphiroa* .....***Galaxaura oblongata***

9a. Less flexible at the joints, not hollow tipped 10

10. Small plants with branches < 0.4mm diameter; epiphytic on *Lithophyllum frondosum* .....***Amphiroa verruculosa***

10a. Plants usually more than 2cm long 11

11. Joints thin (c. 0.4mm diameter), usually on rock .....***Amphiroa cryptarthrodia***

11a. Joints thicker (c. 1mm).....***Amphiroa beauvoisii***

12. Free living on bottom sediments 13

12a. Attached 18

13. Profusely branched in all directions 14

13a. With few or no branches 17

14. Branching very dense, branches generally more than 3mm diameter.....15

14a. Branching open 16

15. Branches constricted at the base ...***Lithophyllum racemus***

15a. Branches not constricted at the base. Easily confused with *Phymatolithon calcareum* which is generally smaller and has more open branching. Rhodoliths often reach several centimetres in diameter. Together with *Lithothamnion corallioides*, it is the most abundant component of Maltese maerl beds.....***Lithothamnion minervae***

16. Branches less than 1.5 mm diameter, very open .....***Lithothamnion corallioides***

16a. Branches 2-3mm diameter, open to slightly crowded; rather uncommon. Specimens with crowded branching are easily confused with *Lithothamnion minervae*; small specimens can be confused with *Lithothamnion corallioides*.....***Phymatolithon calcareum***

16b. Branches usually more than 4mm diameter, often longer than 5mm and branched. Rhodoliths may be several centimetres in diameter. Not yet recorded with certainty from the Maltese Islands but is likely to occur .....***Lithothamnion valens***

17. Well developed specimens in the form of a series of overlapping lamellae, bright purple red, looking somewhat like a tight-petalled rose .....***Peyssonnelia rosa-marina***

17a. Well developed specimens usually longer than broad, made up of overlapping pink lamellae ..... ***Mesophyllum alternans***

17b. Stone-like, more or less featureless, often with a pustulate surface..... ***Neogoniolithon brassica-florida***

18. Thalli generally epiphytic 19

18a. Thalli generally epilithic 22

19. Thalli very thin, translucent 20

19a. Thalli not translucent 21

20. Thalli often more than 1cm wide ..... ***Melobesia membranacea***

20a. Thalli usually less than 1cm wide ..... genera ***Hydrolithon*** and ***Pneophyllum***

Species of these genera can only be separated by microscopic characters. Some species, particularly *Hydrolithon farinosum*, are extremely common epiphytes being particularly abundant on the leaves of the seagrass *Posidonia oceanica*. Other species known to occur are *H. cruciatum* and *Pneophyllum fragile*, but more are likely to be present.

21, Thallus minute (2 - 6mm), epiphytic on *Corallina* and *Haliptilon* ..... ***Lithophyllum corallinae***

21a. Thallus usually around 1cm diameter (up to 3cm), with prominent conceptacles. Very common on a variety of algae ..... ***Lithophyllum pustulatum***

21b. Thallus thick and irregular. Epiphytic on a variety of larger algae, particularly species of *Cystoseira* and *Sargassum* ..... ***Lithophyllum cystoseirae***

21c. Thallus consisting of relatively large (over 2cm) flat lamellae, easily separable from their substrate ..... ***Mesophyllum lichenoides***

22. Thallus thick provided with various crests, branches or other appendages perpendicular to it's plane 23

22a. Thallus flat with few or no excrescenses 24

23. Thallus having a spongy appearance, made up of numerous anastomosing cylindrical branches of about 1mm thickness; rare. ..... ***Lithophyllum byssoides***

23a. Thallus with cylindrical branches thicker than 1mm.... ***Sporolithon ptychoides*** and ***Spongites fruticulosa***

Most plants with this feature are probably *Sporolithon*. Most Mediterranean records of *Spongites fruticulosa* require comfirmation while most records for unattached plants refer to *Lithothamnion minervae* (Basso, 1995). Some variants of *Lithophyllum incrustans* and *Neogoniolithon brassica-florida* may also key here.

23b. Thallus consisting of large smooth crests; rare ..... ***Lithophyllum dentatum***

Also known in the unattached state.

23c. Thallus with a spongy appearance, being made up of a dense mass of small crests. Always in the Mediolittoral; rare ..... ***Lithophyllum lichenoides***

24. Thallus made up of wide, usually smooth lamellae, easily separable from the substrate 25

24a. Thallus strongly adherent to the substrate 26

25. Thallus rather thick but brittle, usually without a whitish edge..... ***Lithophyllum frondosum***

25a Thallus as above but having a whitish border, usually thinner, smaller and with a glossy surface ..... ***Mesophyllum lichenoides***

The two species above are often quite difficult to separate on gross morphological grounds

25b. Thallus usually adherent, though loosely, over a larger area of its undersurface, irregular ..... ***Lithothamnion philippii***

This species has not yet been recorded with certainty from the Maltese islands but is most likely to occur. There is much taxonomic confusion with *Mesophyllum lichenoides* (Basso, 1995a, 1995b)

25c. Thallus deep purplish red, usually more adherent to the substrate but with clearly raised edges ..... ***Peyssonnelia polymorpha***

26. Thallus thin with prominent dome-shaped conceptacles, circular, usually larger than 2cm diameter. Very common in the upper infralittoral and lower mediolittoral, often associated with *Corallina elongata* ..... ***Phymatolithon lenormandii***

26a. Thallus rather thick with prominent raised ridges where adjacent thalli meet. Sometimes with short branches. Very common in the upper infralittoral and also deeper ..... ***Lithophyllum incrustans***

Extremely variable. Branched specimens easily confused with *Sporolithon ptychoides*. Also occurs in the free living state in maerl beds where it can easily be confused with *Neogoniolithon brassica-florida*.

26b. Thallus rather thick and featureless though often with short protuberances. Very common ..... ***Neogoniolithon notarisii*** and ***N. brassica-florida***

The two species are often held to be conspecific but *N. notarisii* is particularly common in the mediolittoral as the chief algal component of extensive pavement-like formations (trottoirs), usually accompanied by the vermetid gastropod *Dendropoma petraeum*. *N. brassica-florida* tends to occur in deeper waters and often forms rhodoliths in mearl beds.

Taxonomic List of Species

Classification of non-geniculate genera is based on Woelkerling (1988). Recently/ commonly used synonyms are included.

Order: NEMALIONALES
Family: CHAETANGIACEAE

Galaxaura oblongata (Ellis & Solander) Lamouroux
= *Galaxaura adriatica* Zanardini

Order: GIGARTINALES
Family: PEYSSONNELIACEAE

Peyssonnelia rosa-marina Boudouresque & Denizot
Peyssonnelia polymorpha (Zanardini) Schmitz

Order: CORALLINALES
Family: CORALLINACEAE
sub-family: CORALLINOIDEAE

Amphiroa beauvoisii Lamouroux
= *Amphiroa exilis* Harvey
Amphiroa cryptarthrodia Zanardini
Amphiroa rigida Lamouroux
Amphiroa verruculosa Kützing
Corallina elongata Ellis & Solander
= *Corallina mediterranea* Areschoug
Corallina officinalis L.
Haliptilon squamatum (L.) Johansen, L. Irvine & Webster
= *Corallina squamata* Ellis
Haliptilon virgatum (Zanardini) Garbary & Johansen
= *Corallina granifera* Ellis & Solander
Jania adhaerens Lamouroux
Jania corniculata (L.) Lamouroux
= *Jania rubens* (L.) Lamouroux var. *corniculata* (L.) Yendo
Jania longifurca Zanardini
Jania rubens (L.) Lamouroux
= *Corallina rubens* L.

sub-family: LITHOPHYLLOIDEAE

Lithophyllum byssoides (Lamarck) Foslie
= *Titanoderma byssoides* (Philippi) Chamberlain & Woelkerling
= *Goniolithon byssoides.* (Lamarck) Foslie
Lithophyllum corallinae (P.L. & H.M. Crouan) Heydrich
= *Dermatolithon corallinae* (P.L. Crouan & H.M. Crouan) Foslie
= *Titanoderma corallinae* (P.L. Crouan & H.M.Crouan) Woelkerling, Chamberlain & Silva
Lithophyllum cystoseirae (Hauck) Woelkerling
= *Dermatolithon cystoseirae.* (Hauck) Huvé
= *Dermatolithon papillosum* (Zanardini) Foslie
= *Goniolithon papillosum* (Zanardini) Foslie
= *Lithophyllum papillosum* (Zanardini) Foslie
= *Lithothamnion papillosum* Zanardini
= *Titanoderma cystoseirae* (Hauck) Woelkerling, Chamberlain & Silva
Lithophyllum dentatum (Kützing) Foslie
Lithophyllum frondosum (Dufour) Furnari. Cormaci & Alongi
= *Lithophyllum expansum* Philippi
= *Lithophyllum grandiusculum* (Montagne) Woelkerling, Penrose & Chamberlain
= *Pseudolithophyllum cabiochae* Boudouresque & Verlaque
= *Pseudolithophyllum expansum* (Phlippi) Lemoine
Lithophyllum incrustans Philippi
Lithophyllum lichenoides Philippi
Lithophyllum pustulatum (Lamouroux) Foslie
= *Dermatolithon pustulatum* (Lamouroux) Foslie
= *Melobesia pustulata* Lamouroux
= *Titanoderma pustulata* (Lamouroux) Nägeli
Lithophyllum racemus (Lamarck) Foslie

sub-family: MASTOPHOROIDEAE

Hydrolithon cruciatum (Bressan) Chamberlain
= *Fosliella cruciata* Bressan
Hydrolithon farinosum (Lamouroux) Penrose & Chamberlain
= *Fosliella farinosa* (Lamouroux) Howe
= *Meelobesia farinosa* Lamouroux
Neogoniolithon brassica-florida (Harvey) Setchell & Mason
= *Goniolithon brassica-florida* (Harvey) Foslie
= *Neogoniolithon mamillosum* (Hauck) Setchell & Mason
Neogoniolithon notarisii (Dufour) Setchell & Mason
= *Goniolithon notarisii* (Dufour) Foslie
Pneophyllum fragile Kützing
= *Fosliella lejolisii* (Rosanoff) Howe
= *Pneophyllum lejolisii* (Rosanoff) Chamberlain
Spongites fruticulosa Kützing
= *Lithothamnion fruticulosum* (Kützing) Foslie

sub-family: MELOBESIOIDEAE

Lithothamnion corallioides (P.L. Crouan & H.M. Crouan) P.L. Crouan & H.M. Crouan
= *Lithophyllum solutum* (Foslie) Lemoine
= *Lithothamnium solutum* Foslie)
= *Mesophyllum corallioides* (P.L. Crouan & H.M. Crouan) Foslie
= *Spongites corallioides* P.L. Crouan & H.M. Crouan
Lithothamnion minervae Basso
Lithothamnion philippii Foslie
Lithothamnion valens Foslie
Melobesia membranacea (Esper) Lamouroux
= *Epilithon membranaceum* (Esper) Heydrich
Mesophyllum alternans (Foslie) Cabioch & Mendoza
= *Lithothamnion philippii* Foslie forma *alternans* Foslie

Mesophyllum lichenoides (Ellis) Lemoine
Phymatolithon calcareum (Pallas) Adey & McKibbin
= *Lithothamnion calcareum* (Pallas) Areschoug in J. Agardh
Phymatolithon lenormandii (Areschoug in J. Agardh) Adey
= *Lithothamnion lenormandii* (Areschoug in J. Agardh) Foslie
Sporolithon ptychoides Heydrich
= *Archaeolithothamnion mediterraneum* (Heydrich) Foslie
= *Sporolithon mediterraneum* Heydrich

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Research Article

Electric Vehicles: Potential for Pollution Reduction

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Summary: *The energy consumption of an electric vehicle (EV) is compared with that of the same model with a gasoline internal combustion engine (ICE) under local road and traffic conditions. It is found that as far as on-board energy is concerned the EV consumes about one tenth of the energy of its ICE counterpart. When overall (EV + power station) efficiency is concerned the EV still has an energy advantage of 2.5. As a result, the EV and power station combination allows strong reductions in almost all the major pollutants produced by road traffic, the one exception being SO2. As far as CO2 is concerned the EV emits under half that from ICE cars, because of its residual energy advantage. All these reductions can be significantly enhanced by photo-voltaic battery charging.*

Keywords: Electric vehicles, pollution, gas emission

Introduction

In the European Union (EU) transport currently accounts for around 25% of the total energy-related emissions of CO_2, as well as for smaller proportions of other greenhouse gases like N_2O and CH_4. Road traffic produces 80% of total transport emissions of CO_2 and passenger cars are responsible for 45% of that. So with a 1992 total of 8070 million tonnes(Mt) of CO_2 emissions, passenger cars are responsible for some 800 Mt. From another angle, passenger cars emit the largest amount of CO_2 of any land people mover: some 133 - 200 g/pass.km as against, for instance, 35 - 62 g/pass.km for a bus (Stanners & Bourdeau, 1995).

There are other major pollutants connected with road traffic. Vehicle diesel engines account for close to 3% of total SO_2 emissions, admittedly a rather small quantity compared to SO2 from electricity generation. However, the situation for CO, NO_x, volatile organic compounds (VOC) and particulates is very different. Road vehicles account for well over 50% of total CO, especially in urban settings; in the US 50% of urban CO comes from traffic (Mackenzie,1994). As for NOx and VOC, EU motor vehicles produce 45% of the first and 31% of the second.

Diesel produces almost the whole of transport SO_2 and close to 70% of particulates, especially of the sub-10 micron variety. NOx comes from both types of fuel but CO, lead and VOC come predominantly from petrol engines. The VOC, together with NO_x and strong sunlight are the ingredients of photo-chemical smog and tropospheric ozone.

The local situation

The local balance between pollution from electricity generation and from road traffic, which is of interest here, is not too easy to establish, mainly because of a serious lack of reliable information. We have attempted to arrive at total quantities of pollutants by a careful determination of fuel consumption (Mallia & Fsadni, 1999) with use of widely accepted emission factors. For CO we assumed different emission factors for power stations (where excess oxygen is present in the flue gases) and for car engines. For quantities of VOC we have simply kept proportionality to petrol consumed thus tacitly using the emission factor used by Buttigieg (1998) for the 1990 CORINAIR inventory.

Table 1. Emissions from Electricity Generation and from Road Traffic

Year		SO_2	NO_x	VOC	CO	CO_2
1990	V	2230	2652	4530	24661	309467
	E	18977	7590	---	2491	500100
1994	V	2667	3214	5362	28870	362267
	E	23987	7796	---	281	1695867
1997	V	3127	3325	5339	30994	388285
	E	29000	5036	---	251	1514333

Table 1 gives the quantities in metric tonnes (t) of five pollutants produced in electricity generation (E) and by road vehicles (V). It shows quite clearly that electric vehicles can be expected to make little impact on SO_2 emissions, where local road transport contributes only some 10% of the SO_2 from electricity generation. Some impact on CO_2, and a very marked impact on NO_x and especially on VOC and CO, can be expected. These last three pollutants have known negative health effects, while VOC and NO_x contribute to the formation of low-level ozone.

At present, there is no basis for comparing power station and vehicle particle emissions. The EU limit value of 50 mg Nm^{-3} is not observed at Marsa, where the electrostatic precipitators are inoperative. Recent efforts by Enemalta to curtail particle emissions at Marsa by use of fuel additives (Pace, 1999, pers comm) have provided information of particle densities in flue gas: minimum values of around 150mg Nm-3 have been reported, but these cannot be translated into reliable estimates of concentrations at ground level. As no flow rates have been published, even an estimate of total mass of emitted particles is difficult to arrive at. Deposition rate at any one place is in any case highly variable, depending on weather conditions and the timing of soot-blowing episodes, when particle density in the flue gas may increase by several orders of magnitude for periods up to 15 minutes. In the presence of an atmospheric

temperature inversion ground level particle densities could be very high indeed.

The work of Pulis (1996) on airborne particle density at roadsides, with collection times of 15m, established a strong correlation with traffic volume. Maximum densities of 95μg m^{-3} were recorded at Msida, with average values for low (500 vehicles an hour) and high (900 vehicles an hour) traffic volumes being 20μg m^{-3} and 63μg m^{-3} respectively.

Lead emissions from petrol amounted to 24t in 1997 (Mallia & Fsadni, 1999), in which year 75% of petrol sold was leaded. In urban areas at peak traffic times the density of lead particles has been estimated at 1.6μg m^{-3} (Savona Ventura, 1998).

Road concentrations of benzene and toluene from petrol have recently been reported by Vella and Gaerty (1998).

Electric Vehicle (EV) Energy Consumption

The vehicle used was a small four-seat passenger car with its 704cm^3 internal combustion engine (ICE) replaced by a 6kW DC series motor operating at 60V. The two rear seats had to be sacrificed to create space for the five 12V, 110Ah lead-acid batteries, with a nominal energy content of 6.6kWh and a total weight of 240kg. The overall weight gain of the car was 150kg from its standard 640kg. The original gear train and stick shift were retained.

Motor voltage and current, together with motor temperature and battery voltage, were sampled once a second and 10s-averages stored in an on-board computer. At the end of each run, energy consumption and other parameters of interest were displayed. Distance covered was read off to the nearest tenth of a mile from the car odometer.

The EV energy consumption was compared with that of an ICE version of the same car with an established fuel consumption of 7.0l/100km (40 mile/gallon) under local conditions. Petrol was rated at 33.4MJ/l or 9.28kWh/l (Goodgere, 1982), giving the ICE car an average energy consumption of 0.65 kWh km^{-1}.

About twenty unmonitored runs were made to determine parameters like range with full battery charge, top speed on the flat, acceleration at various speeds and hill climbing ability. Energy consumption was determined from two 50km runs separated by a few days (runs 1 & 2); a series of short commuter journeys over a total distance of 106km (run 3); and a shorter series of longer journeys over 125km (run 4). Table 2 carries the results for all four runs. The energy ratio is obtained by dividing the petrol equivalent for the ICE by that of the EV.

The average energy consumption for each of the four runs was 0.061 kWh km-1, 0.057 kWh km-1, 0.058 kWh km-1, and 0.060 kWh hr-1 respectively.

The energy ratios refer to electrical energy taken from the batteries. As such, they need to be adjusted for overall efficiency of the charging, distribution and generating system. For generation and distribution,

Table 2. Energy Consumption

Run no.	Distance (km)	Energy (kWh)	Petrol Equivalent (l)		Energy Ratio
			EV	ICE	
1	52	3.18	0.34	3.64	10.70
2	48.6	2.75	0.30	3.40	11.34
3	106	6.08	0.66	7.42	11.24
4	125	7.35	0.79	8.72	10.99

present efficiency can be taken as 25%; from a comparison of the energy meter attached to the mains and the energy actually going into the batteries, the charger efficiency was determined to be 90%. Incorporating these efficiencies, one obtains net energy advantages ranging 2.41 to 2.55 in favour of the EV.

So from the point of view of curtailment of CO_2 from road transport, use of electric vehicles as passenger cars can make a significant decrease (by a factor of 2.5). For the 1990 local vehicle fleet, passenger cars were reckoned to produce 79% of total road transport CO_2 emissions (Buttigieg, 1998, pers comm). For all other pollutants except SO_2, Table 1 shows that electric vehicles will produce marked reductions, the most significant of which would be for CO and VOC and consequently in photochemical smog and low-level ozone.

A marked reduction of airborne carbon particles, with their adsorbed polycyclic aromatic hydrocarbons (Hemminki & Pershagen, 1994) should also come about through the substitution of diesel engined passenger cars by EVs. As for power station sources of soot, until Marsa flue gases are properly filtered, EVs will not display their full advantage in this respect.

Two comparisons of electric and ICE vehicle emissions are shown below (Lestienne & Vergels, 1998). For CO_2, with the standard European driving cycle, the EV produces an average of 2.7 times less CO_2 km^{-1} as do petrol and diesel ICE cars. This value is close to those for overall energy advantage of the EV.

Local gains are likely to be greater than those shown in Figs. 1(a) and 1(b) because of the low average speeds of local traffic. The effects of speed might be seen in the results for the 1982 Swiss car fleet, for instance. The average emission of CO at 80km h^{-1} was 6g $km.^{-1}$ (Lamure, 1990), already significantly higher than that shown in Fig. 1(a), which deals with a more modern fleet. The CO emission went up to 14g km-1 for a speed of 36km h-1, which is close to average speeds in the heavily-built up area south of Mosta.

Conclusion

In our situation of limited distances and severe traffic congestion, electric vehicles can play a part in reduction of CO_2 as well as in strong abatements of most of the worst pollutants associated with road transport. Electric drives have motors with efficiencies at least three times the thermodynamic efficiency of a petrol engine; a low inertia rotor against pistons and crankshafts; zero energy consumption going downhill and stopped at lights or in

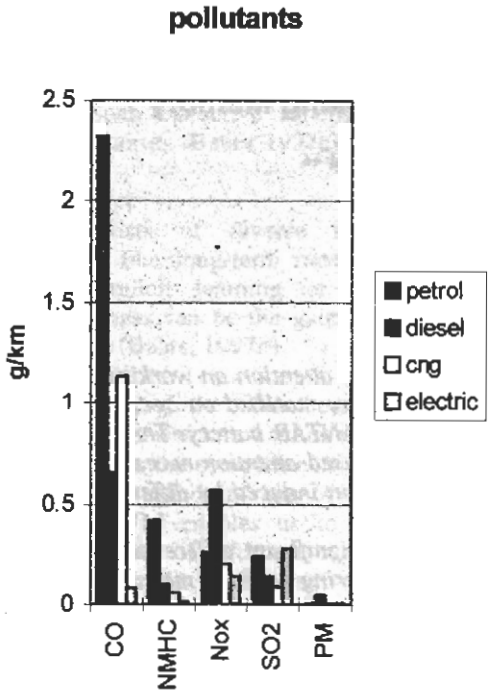

Figure 1(a).

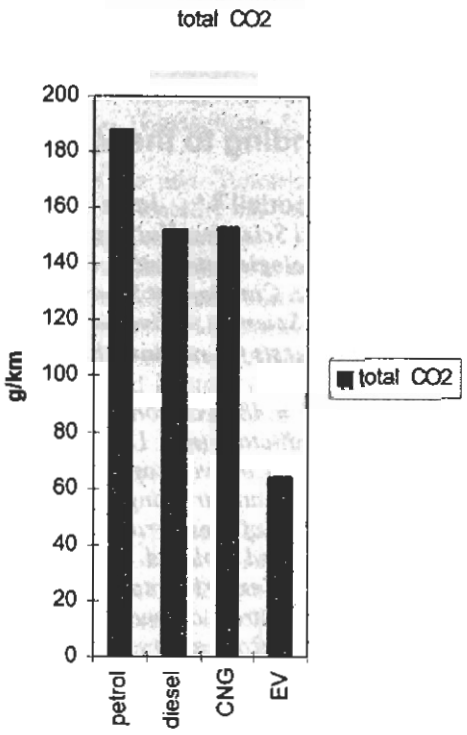

Figure 1(b).

N.B. CNG = compressed natural gas.

traffic jams; use less energy going low speeds. They are then ideally suited to local conditions (other than the state of road surfaces), while the properties of the ICE demand a different type of environment if the engine is to function at its best. Of course, even that best is far more polluting than an electric drive.

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Research Article

Staged Present: Attending to the Mystical on the Stage of Working Memory

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Summary: *An experiment (N = 48) was conducted to investigate the effect of focused attention on working memory. Two experimental groups, meditators (n = 12) and contemporary actors (n = 12), were matched on age, gender and level of education and measured on working memory and attention tests from the CANTAB battery. The practice of meditation and contemporary theatre training satisfied the criteria of maintaining focused attention necessary for this study. Through the performance of these groups the possible effect of focused attention induced by different training procedures could be compared and explored.*
Although on most measures the experimental groups did not differ significantly, a significant difference was found between meditators and their controls on one test (Paired Associates Learning) measuring working memory capacity. Since these two groups differed from actors and their controls in age (Mean age of meditators and actors = 37.00, 23.75 respectively), it was suggested this might account for the apparent non-significant difference between the two experimental groups. CANTAB norms establish different thresholds for age. The results also suggest that exercises of focused attention might contribute in delaying the normal degeneration of higher cognitive functions in old age.

Keywords: Working memory, attention, consciousness, actors, meditators, CANTAB

Introduction

In recent years, psychological research has advanced in a field where previously it feared to tread - consciousness. Research into the structural basis and process nature of consciousness has generated an impressive amount of literature and yet the nature of consciousness continues to elude and intrigue.

In this research, consciousness is examined from the perspective of working memory and attention. This relationship hinges upon Baars' (1988) Global Workspace (GW) theory of consciousness, in which Baars compares everyday human consciousness to a theatre. Baars describes the theatre of consciousness as having a stage of working memory over which a spotlight of attention roams.

Attention and the periphery of awareness are the two salient points in Baars' theory of consciousness (Baars, 1988). In Baars' model, focal consciousness acts as a 'bright spot' on the stage, directed by the selective 'spotlight' of attention. The bright spot is surrounded by a 'fringe' of vital but vaguely conscious events on a 'stage' of working memory. Information from the bright spot is globally distributed through the theatre to two classes of complex unconscious processors: those in the darkened theatre 'audience,' who receive information from the bright spot; and the 'behind the scenes', unconscious contextual systems, which shape events in the bright spot (Baars, 1997b).

The stage of working memory (WM)
Although WM can store up to seven plus or minus two elements, we are only conscious of a single element at any point in time. WM contents are mostly in the dark, but its active elements can come into awareness (Baars, 1997b). This core aspect of Baars' (1988) theory has been echoed by other theorists who view consciousness as the awareness of what is in WM (LeDoux, 1998). Kosslyn and Koenig (1992) argue that to be aware of something, it must be in WM, and Johnson-Laird (1988) notes that the contents of WM are what we can be conscious of at any moment. WM stores relevant information only temporarily, and its main feature is its ever-changing content. Thus the object in awareness can similarly change continuously.

The spotlight of attention
Only events in the bright spotlight are strictly conscious at any point in time. However the contents of WM can become conscious as the attention spotlight roams onto them.

The actors trying to get in the bright spot
Elements in WM compete to gain the spotlight of attention. The more an 'actor' requires being conscious, the more it will compete against the others. For example, our daily worries come into consciousness even when we are trying to concentrate on the task at hand.

Context is set behind the scenes
Often enough attentional selection is spontaneous and unconscious, as if commands from behind the scenes influence the direction of the spotlight. For instance, all perceptual systems are shaped by unconscious factors: for example, our visual perception of depth is shaped by the unconscious assumption that light comes from above. Similarly, conceptual assumptions can act as unconscious contexts.

The director
Working memory is guided by an executive system that

makes decisions guided by goals. But the goals themselves may not be entirely conscious. The intention of automatic actions is often beyond awareness. Thus it seems that the theatre director works invisibly behind the scenes. Such executive functions are located in the prefrontal cortex (Baars, 1997c).

The audience
This consists of diverse specialized unconscious capacities, like long-term memory, and operators that induce implicit learning or procedural knowledge. Consciousness can be the gateway to vast unconscious knowledge (Baars, 1997b).

The strength of the GW theory lies in its ability to describe what we know intuitively. In normal everyday consciousness the complex network system in our brains generates thousands of bits of information per second. However, WM enables us to focus and attend to a limited amount of information relevant for that task at hand. This produces a stream of consciousness, which contains the most relevant pieces of information from one moment to the next and enables the mind to continuously change the contents of working memory producing a myriad of thoughts, emotions and perceptions. Our normal everyday consciousness can be likened to a continuous divided attention task: we drive, whilst listening to the radio; listen to a lecture, whilst thinking about yesterday's party. We seldom focus on the same object in the environment for more than a few minutes.

This is contrary to the issue raised by Crook (1980) where he showed that when subjectively aware a person is completely focused on the environment or the task at hand. This implies that the object of attention remains fixed for a lengthy period of time. Forman (1998) showed that this is precisely the technique used by mystics to empty their mind and reach altered states. Through disciplines like meditation, where there is a focusing of attention on a single repetitive stimulus like breathing, the stream of consciousness is reduced to a single element over time. Forman (1998) raises the issue that the mystical experience is the simplest possible consciousness, and consequently should be studied to enlighten us on the more complex forms of everyday consciousness.

The central role of attentional processes in working memory (WM) has been further explored by Engle, Kane and Tuholski (in press) who have described WM as a system consisting of:

- a store in the form of long-term memory traces active above threshold;
- processes for achieving and maintaining that activation;
- controlled attention.

In this regard, WM capacity, refers to the capacity of just one element of the system: controlled attention. Therefore Engle, Kane and Tuholski (in press) do not focus on the entire WM system, but rather on the capabilities of the limited-capacity attention mechanism described by Baddeley and Hitch (1974) as the central executive. Thus, WM capacity is not really about storage or memory per se, but about the capacity for controlled, sustained attention in the face of interference or distraction (Engle, Kane & Tuholski, in press).

Engle, Kane and Tuholski (in press) argue that this attention capability is domain free and therefore individual differences in this capability reveal themselves in a wide variety of tasks. Indeed, Conway and Engle (1996) emphasize that the correlation between measures of WM capacity and higher-order cognitive tasks is not a result of skill in the specific tasks - as Ericsson and Kintsch (1995) propose with their studies on expertise - but rather of the underlying critical feature of controlled attention which is inherently different in each individual.

Consequently, the study of attention and working memory provides a singular means of understanding consciousness. Moreover, questions are raised as to whether engaging in activities which require maintaining focused attention over long periods of time will produce measurable differences in the cognitive elements of Baars' model of consciousness. More specifically, could the attention spotlight be undergoing particular changes that might affect the working memory stage or any other cognitive processes in Baars' Global Workspace theory of consciousness?

Methodology

Subjects
Four paired samples (N = 48) participated in the experiment. Two experimental groups, actors and meditators and their matched controls were identified for this study. The variables of age, gender and level of education were taken as matching criteria.
From a total sample of 48 participants (Mean age = 30.3; range 19.0 to 45.0), 50% of the participants were male and 50% female. 12.5% of the total sample had a secondary level of education, and 8.33% had a post-secondary level of education (all were employed in skilled labour.) 37.5% of the sample were students at a tertiary level, and 45.83% were graduates in professional employment.

The actors were selected for their specific training techniques. The training which these actors engage in entails intense physical exercise whilst being fully attentive to their creative process, thus satisfying the criteria for attention exercises which was adopted for this research.

Within the actors group (n = 12), 50% were male and 50% were female. Their mean age was 23.7 and ranged from 19.0 to 30.0. 8.33% had a post-secondary education, 25.0% were graduate professionals and 66.67% were University students.

Matching of the actors to controls resulted in the following characteristics: Mean age was 24.0 and ranged from 20.0 to 30.0. 8.33% had a post-secondary education, whilst 25.0% were graduate professionals and 66.67% were University students.

The meditators were selected from the Ananda Marga (AM) yoga and meditation school. Selection criteria for the meditators involved the ability to be fully concentrated on a single object or thought whilst the body is not engaged in movement.

In the meditators group (n = 12), 50% were males and 50% females. Their mean age was 37.0 and ranged from 20.0 to 45.0. 25.0% of the meditators group had a secondary level of education, 8.33% had a post-secondary level of education, 8.33% were enrolled in a university degree and 58.33% were graduates in professional employment.

The same characteristics were evident in the meditators control group except for a mean age of 36.33.

Both the actors and meditators had to have a minimum of one year regular training in their respective domains to be selected for the study. One year of training for the actors group was equivalent to 312 hours, or three weekly 2-hour sessions (six hours per week). For the meditators the minimum requirement was of 365 hours, or a twice daily 30-minute meditation session (seven hours per week).

In addition to matching criteria, naivete to the experimental conditions was a necessary prerequisite for the two control groups. The upper and lower age limits were set at 49 and 18 years respectively and all participants had to have a secondary level of education. Equal numbers of males and females were selected. Exclusion of participants who did not meet these inclusion criteria resulted in a total sample of 48 participants.

Measurement

The Cambridge Neuropsychological Test Automated Battery (CANTAB), developed by CeNeS Cognition (1987), is a computerised battery of neuropsychological tests. Twelve tests form its 'Attention Battery', 'Visual Memory Battery' and 'Working Memory and Planning Battery.' It provides for the assessment of a variety of cognitive functions, including working memory, attention, learning and problem solving, as well as tests of executive function and vigilance.

Specific tests were selected from this battery for this study: Intra/Extra-Dimensional Shift (IED), and Rapid Visual Information Processing (RVP) from the Attention Battery; Paired-Associates Learning (PAL) from the Visual Memory Battery; and Spatial Working Memory (SWM) from the Working Memory and Planning Battery.

Each of the following tests was selected for their accuracy in measuring the particular cognitive functions relevant to this research.

Intra/Extra-Dimensional Shift

This test measures the subject's ability to attend to the specific attributes of compound stimuli, and to shift that attention when required. The actors' and meditators' training in attention was expected to differentiate their performance from that of their respective controls. Moreover, performance on this test was expected to differentiate actors and meditators since actor training entails a multi-tasking component which necessitates a shifting of attention absent in meditator's training.

Rapid Visual Information Processing

The RVP is a test of sustained attention with a small working memory component. The actors and meditators' performance was expected to be significantly better than that of their controls as a result of their training.

Paired Associates Learning

The test is a form of delayed response procedure, which tests two different aspects of the ability to form visuo-spatial associations. First, the number of patterns placed correctly on the first presentation of each trial gives an index of 'list memory', which can also be described as WM capacity. Second, the number repeat-reminder presentations needed for the subject to learn all the associations provides a measure of list learning. This measure explores the relationship between focusing of attention and WM capacity. It was expected that the actors and meditators groups perform significantly better on this test than their control groups.

Spatial Working Memory

This test of spatial working memory, includes a planning and attention component. The actors, as a result of their training using the body as a medium in space, were expected to do overall significantly better in this test. Nevertheless, with their training in attention, the meditators were also expected to do significantly better than their controls.

In summary, a positive effect on all tasks was predicted as a result of training.

Procedure

Prior to the experimental phase, checklists were distributed amongst the actors and meditators populations. Information about gender, age, level of education, occupation, length of training in the respective disciplines and the frequency and duration of each training session was gathered. Similar checklists were distributed amongst prospective control participants. The two control groups were matched according to the demographic information gathered. Any prospective participant who had any experience of the experimental conditions was excluded.

A pilot test was conducted prior to the experimental phase. Completion time for the test was determined to be between 30 to 40 minutes. The participants, who still conformed to the inclusion criteria, experienced no difficulties in performing the test.

The experiment was conducted in a constant environment, and any extraneous variables were accounted for. Testing was carried out on an individual basis with a random allocation of participants to time of testing. The tests were administered in the following order; with tests from the Attention battery being administered first, followed by a test each from the Visual Memory Battery and Working Memory and Planning Battery:

1 Intra/Extra-Dimensional Shift
2 Rapid Visual Information Processing - Training
3 Rapid Visual Information Processing - Test
4 Paired Associates Learning,
5 Spatial Working Memory.

Standardized instructions were followed in accordance to the CANTAB manuals (CeNeS, 1998a, 1998b).

In the CANTAB battery, data is automatically scored and analyzed according to the variables of gender and age for each individual test. This data, together with information collected from the checklists which included age, gender, level of education, training history and total amount of training was explored to investigate the research hypothesis.

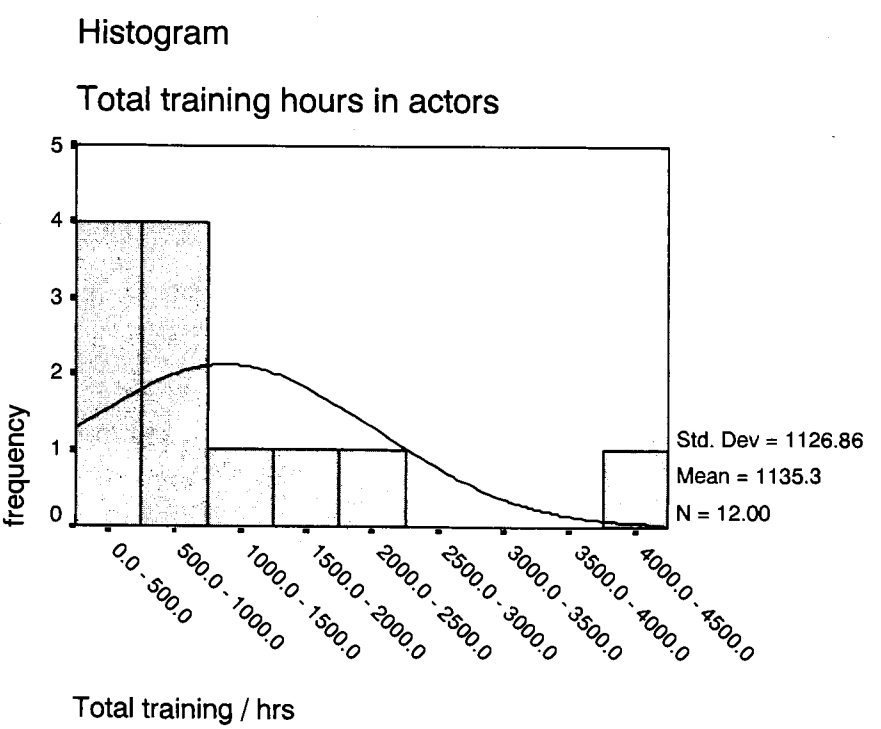

Figure 1. Histogram showing the frequency distribution of total training hours in actors.

Analysis

Multivariate Analysis of variance (MANCOVA) was performed to analyze main and interaction effects in a between-subjects design. Age was selected as a covariate. T-tests explored differences as a result of training, type of training and age.

Results

Descriptive statistics

Gender and age characteristics of the sample are illustrated in Table 1.

	Gender		Age		
	Males	**Females**	**M**	**SD**	**Range**
Actors	50%	50%	23.75	4.04	11.00
Actors - Controls	50%	50%	24.00	3.43	10.00
Meditators	50%	50%	37.00	7.37	25.00
Meditators - Controls	50%	50%	36.33	7.27	25.00

Table 1. Gender and age characteristics of sample

Amount of training in hours

The mean total hours of training in the actors and meditators population (n = 24) was 3605.531 hours (SD = 4035.78). The mean total training hours for the sample of actors (n = 12) was 1135.33 hours (SD = 1126.86). The range of total training hours in actors varied from a minimum of 312 hours to a maximum of 4160 hours.

A frequency distribution of the total training hours shows that 66.7% of the actors trained between 312 and 1000 hours. 16.6% trained between 1000 and 2000 hours, whilst 16.6% trained between 2000 and 4160 hours.

The mean total training hours for the meditators group (n = 12) was 6075.72 hours (SD = 4412.79). The range of meditation hours varied between a minimum of 730 hours and a maximum of 16060 hours. A frequency distribution of the total meditation hours shows that 66.7% of the meditators practised meditation between 730 and 6000 hours. 16.6% practised between 6000 and 10500 hours, whilst 16.6% have meditated between 10500 and 16500 hours.

Comparisons between Samples

Multivariate analysis of variance exploring main and interaction effects of training and type of training yielded the following results. Age was selected as a covariate as a result of the difference in mean age between actors and controls (M = 23.75, 24.00) and meditators and controls (M = 37.00, 36.33).

Intra/Extra-Dimensional Shift (IED)

The mean stage reached by the total population (n = 48) was 7.79 (SD = 1.93), with a minimum score of 1 and a maximum of 9. Table 2 illustrates a comparison of the mean stage reached by the four groups.

Group	**n**	**M**	**SD**
Actors	12	7.91	2.31
Actors Controls	12	7.83	1.03
Meditators	12	8.00	1.53
Meditators Controls	12	7.41	2.64

Table 2. Comparison among groups on stage reached in IED.

No significant main or interaction effects were obtained $F(5, 43)=0.72$, $p>0.05$. Age did not covary significantly.

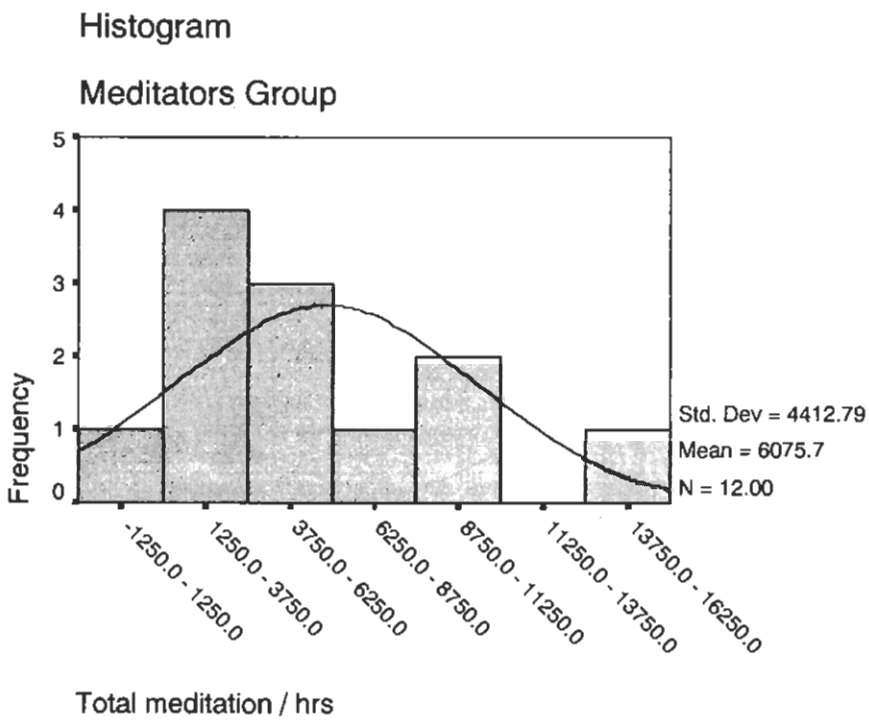

Figure 2. Histogram showing the frequency distribution of total meditation hours in meditators.

The population (n = 48) had a mean of 24.98 (SD = 11.63) on total errors made in the IED, with a minimum of 7 errors and a maximum of 54. Table 3 illustrates a comparison of the mean total errors among the four groups.

Group	n	M	SD
Actors	12	23.58	14.89
Actors Controls	12	25.25	10.63
Meditators	12	25.91	12.64
Meditators Controls	12	25.16	8.97

Table 3. Comparison among groups on total errors in IED

A MANCOVA showed no significant differences (F (5, 43) = .056, $p > .05$) among the four groups on the total errors in the IED.

Table 4 illustrates a comparison among groups on ID-ED errors at ED shift.

Group	n	M	SD
Actors	12	11.83	9.67
Actors Controls	12	16.83	12.86
Meditators	12	11.58	9.99
Meditators Controls	12	10.25	11.33

Table 4. Comparison of groups on ID-ED errors at ED shift

A MANCOVA yielded no significant main and interaction effects (F (5, 43) = 1.66, $p > 0.05$) on errors at ED shift.

Table 5 compares the groups on the ID-ED errors up to ED shift.

Group	n	M	SD
Actors	12	5.00	2.41
Actors Controls	12	7.75	4.52
Meditators	12	9.33	7.24
Meditators Controls	12	7.83	6.39

Table 5. Comparison of groups on ID-ED errors up to ED shift

A MANCOVA yielded a significant main effect, F (5, 43) = 8.48, $p = 0.01$. Age covaried significantly, (F (43, 1) = 13.50, p = 0.001).

Rapid Visual Information Processing (RVP)

The most significant data items for the RVP were the probability of hit, and the probability of false alarm.

The mean probability of hit of the total population (n = 48) was .687 (SD = .187), with a minimum score of .3 and a maximum of 1. Table 6.0. illustrates a comparison of the probability of hit of the four groups.

Table 6. Comparison among groups on probability of hit in RVP.

Group	n	M	SD
Actors	12	.67	.19
Actors Controls	12	.72	.13
Meditators	12	.69	.19
Meditators Controls	12	.65	.22

A MANCOVA showed no significant differences (F (5, 43) = .75, $p > .05$) among the four groups on probability of hit in the RVP.

The mean of the whole population (n = 48) on probability of false alarm in RVP was 3.333E-03 (SD = 5.19E-03), ranging from a minimum of .00 to a maximum of .02.

Table 7. illustrates a comparison of the probability of false alarm among the four groups.

Group	n	M	SD
Actors	12	8.33E-04	2.88E-03
Actors Controls	12	3.33E-03	4.92E-03
Meditators	12	5.00E-03	5.22E-03
Meditators Controls	12	4.16E-03	6.68E-03

Table 7. Comparison among groups on probability of false alarm in RVP.

A MANCOVA showed no significant main and interaction effects (F (5, 43) = .807, $p > .05$) among the four groups on probability of false alarm in RVP. However age covaried significantly F (1, 45) = 5.64, p = 0.02.

Paired Associates Learning (PAL)
The most significant data items for the PAL were the total trials and mean errors made.

The total sample (n = 48) had a mean of 10.93 (SD = 2.51), which ranged from a minimum score of 8 and a maximum of 20, on total trials in the PAL. Table 8 illustrates a comparison of total trials amongst the four groups.

Group	n	M	SD
Actors	12	10.58	2.87
Actors Controls	12	10.16	2.08
Meditators	12	10.50	1.38
Meditators Controls	12	12.50	2.97

Table 8. Comparison among groups on total trials in PAL.

A MANCOVA showed no significant differences (F (5, 43) = 1.67, $p > .05$) among the four groups on total trials in the PAL.

The mean of the whole population (n = 48) on mean errors in the PAL was 1.03 (SD = 1.04), ranging from a minimum of .00 to a maximum of 5.40. Table 9 compares the scores obtained on mean errors in PAL.

Group	**n**	**M**	**SD**
Actors	12	.85	1.10
Actors Controls	12	.68	.71
Meditators	12	.80	.57
Meditators Controls	12	1.80	1.32

Table 9. Comparison among groups on mean errors in PAL.

A MANCOVA yielded no significant results on mean errors in PAL (F (5,43) = 2.57, $p > 0.05$).

Spatial Working Memory (SWM)
The most significant data items for the SWM test were the between errors, and the strategy score.

The mean between errors in SWM of the total population (n = 48) was 17.58 (SD = 18.21), with a minimum score of 0 and a maximum of 68. Table 10 illustrates a comparison among the four groups on between errors in SWM.

Group	**n**	**M**	**SD**
Actors	12	14.33	19.82
Actors Controls	12	15.58	18.50
Meditators	12	22.08	18.09
Meditators Controls	12	18.33	17.75

Table 10. Comparison among groups on between errors in SWM.

A MANCOVA showed no significant differences (F (5, 43) = .09, $p > .05$) among the four groups on between errors in SWM.

The mean of the whole population (n = 48) on strategy score in SWM was 32.18 (SD = 4.42), ranging from a minimum of 21 to a maximum of 39.

A MANCOVA showed no significant differences (F (5, 43) = 0.39, $p > .05$) among the four groups on strategy score in SWM.

Effect of Training
Independent-samples t-tests explored differences in performance on the CANTAB sub-tests as a result of training. Interestingly, a training effect was found only on the ID-ED errors at the ED shift (t (46) = -.57, p = .007).

Type of Training
T-tests for independent samples (two-tailed) compared performance on the CANTAB tests of actors and meditators to establish differences as a result of the type of training undergone. A significant difference was found between actors and meditators on the probability of false alarms in RVP (t (22) = -2.42, $p = 0.0001$).
However, as Figures 1.0 and 2.0 illustrate, the range and total amount of training of actors and meditators vary considerably. Independent-samples t-test was performed between actors and meditators with comparable training experience (< 4000 hours). This also yielded a significant result for the ID-ED score up to ED shift (t (14) = 2.25, p = 0.001).

The effects of meditation and actor training were further explored using paired - samples t-test. Actors and meditators were compared to their respective controls. Surprisingly, actors did not differ significantly from their controls on all measures of the CANTAB.

Meditators differed from their controls on total trials in PAL (t (11) = -2.23, $p < .05$) and mean errors in PAL (t (11) = -2.70, $p < .05$).
Therefore, meditators and their matched controls differed significantly on both measures of the Paired Associates Learning test.

Amount of training
An analysis of the relationship between the total amount of practice for actors and meditators, and the performance on the CANTAB tests was performed. Surprisingly, no relationship was found between the total amount of training in actors and their performance on the IED, PAL and SWM. However, there was a strong positive correlation between the total hours training in actors and their probability of false alarms in the RVP (r = .833, $p < .01$). No significant correlations were found between the total hours practising meditation and the meditators' performance on the IED, RVP, PAL and SWM tests. Tables 11 and 12 illustrate the results obtained using Pearson's two-tailed correlations for actors and meditators.

Test	Data Items	*n*	*r*	sig (2-tailed)
IED	Stage reached	12	.15	.62
	Total errors	12	.04	.90
	Errors at ED-shift	12	.20	.52
	Errors up to ED-shift	12	.05	.87
RVP	Probability of hit	12	.07	.81
	Probability of false alarm	12	.83**	.001
PAL	Total trials	12	-.029	.93
	Mean errors	12	-.10	.73
SWM	Between errors	12	.02	.93
	Strategy score	12	.18	.56

** $p < .01$

Table 11. Pearson's correlations between total hours training in actors and performance on CANTAB tests.

Test	Data Items	*n*	*r*	sig (2-tailed)
IED	Stage reached	12	-.17	.58
	Total errors	12	-.02	.95
	Errors at ED-shift	12	.25	.42
	Errors up to ED-shift	12	-.16	.59
RVP	Probability of hit	12	.04	.88
	Probability of false alarm	12	-.03	.91
PAL	Total trials	12	.18	.56
	Mean errors	12	.16	.59
SWM	Between errors	12	.38	.21
	Strategy score	12	.20	.52

Table 12. Pearson's correlations between total hours training in meditators and performance on CANTAB tests.

Age Differences

Performance on the CANTAB tests varies significantly with age. In view of the mean age difference between actors and controls compared to meditators and controls, independent samples t-tests were performed to determine whether performance varied as a function of age. It was expected that actors perform better than meditator controls both as a function of training and age. The two groups differed significantly on the RVP probability of false alarm (t (22) = -1.58, p = 0.03) and SWM strategy score (t (22) = .78, p = 0.02.

Meditators were compared to actor controls using an independent - samples t-test (two-tailed). Although meditators are older than the actor controls, their performance on the CANTAB measures is better with the differences being significant for the ID-ED errors at ED shift (t (20) = 1.16, p = 0.02) and RVP probability of hit (t (19) = .51, p = 0.04).

The actor controls and meditator controls differ only as a function of age. Independent-samples t-test yielded a significant difference on the SWM strategy score (t (18) = .21, p = .03).

Discussion

This study has focused on the relationship between attention, working memory and consciousness by studying focused attention from the perspective of two disciplines, meditation and contemporary theatre. The effect of training in meditation and contemporary theatre was then explored through standardised tests that measure higher cognitive functions, notably working memory.

There were few significant differences on the performance of specific CANTAB tests among the actors, meditators and their controls.

In the Intra/Extra - Dimensional Shift (IED) test, which is a measure of shifting of attention no significant differences were obtained on stage reached in IED, total errors in IED and ID-ED errors at ED shift. However, a significant main effect was obtained on errors up to ED shift. Age covaried significantly. This implies that any differences between the groups are not necessarily a result of training but may be a result of an interaction with age. This is interesting in view of the fact that although, not statistically significant, meditators reached a higher stage on the IED than their controls and performed better than actors who did not differ from their matched controls. This result is surprising in view of the age difference between the meditators and actors. Meditators and their controls had a mean age approximately ten years older than that of the actors and control, therefore it is interesting that meditators performed better in this test than the actors and controls, especially when their age counterparts performed worse than the younger groups as expected.

Consequently, it might be suggested that whilst meditators' controls scored lower than the younger groups because of the normal weakening of cognitive functions due to older age, in meditators, this cognitive deterioration seems to be less marked. This claim is supported by the fact that when meditators were compared to the actors controls they differed significantly on the RVP probability of false alarm and SWM strategy score. Stoltzfus, Hasher and Zacks' (1996) findings that older adults find it more difficult to inhibit irrelevant thoughts and distractions could also support this pattern. In the IED, which is an exercise of attention and disattention, meditators' controls were probably more distracted as predicted by Stoltzfus, Hasher and Zacks (1996).

Actors made fewer errors in the ED shift, when the task was to shift their attention between two similar stimuli. Interestingly the meditators made most errors in these preliminary stages of the test. Perhaps the meditators' training in keeping fixed attention on a single object hindered them from shifting their attention at first, but gradually they learnt to attend and disattend according to the task at hand, improving their performance, ultimately reaching the highest stages in the IED.

It is interesting to note that all participants irrespective of condition performed lower than that expected from the CANTAB norms. This finding is interesting because the CANTAB is supposedly culture-fair, however, there are some indications that this might not be so. (Fray, 1999, personal communication).

Once again there were no significant differences amongst the groups' performances on RVP and the same pattern of results was obtained as in the ID/ED shift. Moreover, age covaried significantly on probability of false alarm. The suggestion regarding the possible beneficial effects of meditation in old age remains pertinent in this case.

Actors also train in attentional exercises. However, they did not perform any better than their controls. It is suggested that contemporary theatre training does not produce cognitive effects as marked as meditation. Moreover, the essence of theatre training is its motoric component, an aspect which is not captured by the CANTAB tests. It may be the case that these tests are not sufficiently sensitive or they do not tap the relevant cognitive functions, thus masking any differences between the two experimental groups.

The Paired Associates Learning (PAL) Test explores the cognitive functions of attention and working memory capacity. A significant difference between the performance of meditators and their controls was shown. Whilst actors and their controls scored within the average norm for their age, meditators scored significantly higher than their controls and higher than the average norm for their age. This finding strengthens the suggestion that attention exercises like meditation produce beneficial effects on higher cognitive functions, which become more labile due to old age.
Similairly, meditators and their controls differed significantly in the amount of errors made. The fact that significant results were found on the PAL, rather than the other tests is also highly interesting, since the PAL tests for working memory capacity. Consequently, it is suggested that attention exercises like meditation have an effect specifically on working memory capacity. This is significant in view of Baars' Global Workspace theory of consciousness. Indeed from this finding it is hypothesised that training this attention mechanism, should strengthen working memory capacity especially in old age, when it would otherwise deteriorate.
The Spatial Working Memory (SWM) test explores the capacity of spatial working memory and planning. No significant results were obtained from this test.
When the two control groups were compared, a significant age difference was obtained on the SWM strategy score. This fits in with Stoltzfus, Hasher and Zacks' (1996) suggestion that older adults compensate for their lowered working memory capacity by planning their actions more accurately. This is a rational, and consequently, a left-brain task. The practice of meditation, however, has been shown to sharpen more intuition (Wulff, 1997). Perhaps, meditators were relying more on their intuitive functions during this task, thus failing to create an adequate strategy leading to a lot of errors.

Actors however, who also had a poor strategy, still managed to make few errors. This might arise as a result of their training, which is focused on the movements which the body creates in space, which sharpens their

capacity to note spatial relations, which is another right-brain function (Cytowic, 1995).

It is surprising that no interaction effects were exhibited. However, as has been discussed age may have had a masking effect on any relationships between training and type of training.

Consequently, the results from this study can be summarised accordingly;
Attention exercises appear to produce a positive effect on working memory capacity and its attention mechanism as hypothesised by Engle, Kane and Tuholski (in press), possibly by limiting the deterioration of cognitive functions in old age. This effect was shown by meditators, but it is not clear whether contemporary theatre could have produced a similar effect had the actors been older and more experienced.

Amount and Quality of Training

Although no correlations were found between the total amount of hours of actors' training and meditation with the performance on any of the CANTAB tests, it is important to highlight particular differences between the two forms of attention exercises:

The actors' training is mostly physical and consequently the form of attention employed involves being completely involved with the task at hand. Since the task is continuously changing, attention is consequently continually changing, although the change is limited to the particular task. In meditation, however the object of attention is fixed throughout, since all stimuli, including movement and sensations are excluded from consciousness.

Mean total training hours in meditators was 6075.729, whilst that of the actors was just 1135.333. This wide difference is due to the fact that meditators, on average, had been practising for more than eight years, whilst most of the actors had only trained for two years. Moreover, meditators practice for seven hours weekly, whilst most of the actors only train six hours a week. However, meditators have a more consistent pattern of meditation with two daily thirty minute slots, whilst actors train three times weekly for two hours each time. This quantitative difference could have produced the patterns described above. Interestingly, when actors and meditators with similair amounts of training were compared, a significant effect of RVP probability of false alarm and ID-ED score up to ED shift resulted.

It could be argued that meditators tended to do better than actors because they have been in training for a number of years and their training is more consistent. Ericsson, Krampe and Tesch-Romer (1993) suggest that the most effective activity for skill acquisition is consistent and sustained training. It could also be argued that, since meditators have been in training for a longer duration than the actors, they have had more opportunity to experience mystical states of consciousness, which could produce the positive effects on the cognitive functions of the meditators.
Nevertheless, as has been already noted, meditators were, on average, ten years older than the actor cohort. This fact should have contributed to the actors performing better than the meditators on most tests. Therefore, it does seem important that, although the results were not significant, meditators still managed to perform as well - and sometimes even better - than the actors and their controls. This pattern may be worth pursuing in view of its potential practical implications in old people who experience a decreased efficiency in working memory. Regular meditation from early adulthood could perhaps prove beneficial in slowing down this cognitive deficit. Since meditation is practised sitting down, it can be maintained even if the elderly become less physically mobile.
In conclusion it appears that although there were no consistent significant results on a number of the CANTAB tests, meditation seems to be a better attention exercise than contemporary theatre that can effect higher cognitive functions, especially working memory.

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Research Article

Teaching Primary Science and Technology
Shower Gel Manufacture

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During the production of a science INSET programme for primary teachers, at the Open University, UK, the 'Shower gel' project was developed with staff at Unilever Research, Port Sunlight Laboratory, UK. Since then, the project has been used extensively with primary teachers on science Continuing Professional Development (CPD) courses, as well as students ranging in age and abilities from junior children to adult learners. All participants agree the project is stimulating, interesting and fun. Here, the project is described, including some background science and the technical details are included. If you want to try it, perhaps you might contact science colleagues at a nearby High School for materials and support(!!), or the Department of Primary Education at the University of Malta.

Project brief - the problem
You are in the research and development laboratory of a medium-sized company that sells personal washing products (soaps, shampoos, etc.). The company's marketing department has recently received information that one of your main competitors is test-marketing a new shower gel in a small area of the UK. They are concerned that your sales of soap will drop when the competitor's product is launched nationally. They have obtained a sample of the competitor's product and want you to develop a shower gel that is similar in appearance and properties so that you can rapidly respond with a 'counter-attack', by selling a new gel product alongside your soap. Your current customers may then stay loyal to your brand, and buy your gel for the shower as well as soap for the bathroom. Your company has a basic mixture, or formulation, for a gel product that was developed in this way - a basic formulation is produced but is not marketed at the time. They are often covered by patents, and hence become useful starting-points for new products in the future. The new product should be based on this basic formulation.

Whilst you are developing your new gel product, the marketing department will conduct trials to determine the perfume and colour for the new product. You will need to consult them before finalising your product.

While this investigation could be part of a broader topic exploring the science of soaps and detergents, this activity alone provides a stimulating and interesting session that could also be used to explore school-industry links or properties of materials within the classroom or as part of a CPD session for either primary or secondary teachers. Start where the individual is, and offer different levels of input to meet individual needs. Children can identify with the issue as can teachers on a CPD course. Work can be completed at novice or expert level. What is important is that individuals are given the opportunity to learn a great deal from the experience - both in terms of scientific problem solving and experimentation, coming to appreciate concepts important in chemistry as well as issues important to the chemical industry.

(CAUTION - Eye protection and rubber gloves must be worn when handling the shower gel ingredients and when testing the final product.)

Making a shower gel
The basic mixture of a shower gel comprises:
(i) surfactant and co-surfactant - these are the cleaning agents: the surfactant causes the lather, and the co-surfactant prevents too much lather forming
(ii) pH adjuster (citric acid solution) - body products need to be adjusted to give a pH of between 6.5 and 7
(iii) salt (sodium chloride) - this is used as a thickening agent in detergents
(iv) preservative (usually formaldehyde)
(v) water (deionized) to dilute the basic mixture.

The tasks are: (a) to develop a method of making and testing gels from a given formulation, optimising the amount of salt required to produce the required viscosity; (b) to finalise the details of the gel product, which includes deciding on its colour and perfume; and (c) to research and develop a marketing campaign for the product.

At this point encourage planning. Think about equipment, method and recording results. The best way of making a batch of gel is in a container or glass beaker, using a simple stirrer, such as a lollipop stick. For initial experiments, samples can be made either in a test-tube or in a small beaker, provided the weighing is accurate. From this, the most promising formulations can be found for manufacture on a larger scale.

A basic gel formulation for testing can be made simply by using surfactant, co-surfactant, water and salt. At this stage there is no need to add pH adjuster or preservative. An appropriate amount of basic formulation made initially can be say 100 cm^3. This formulation should consist of 13% surfactant, 2% co-surfactant, with the remainder being made up of deionized water. To this is added a small amount of salt to obtain a gel of the required viscosity. The task is to use this basic formulation to achieve the gel with the necessary properties.

At this stage, input can be at different levels depending on the starting point of the students. Advice can be given relating to appropriate quantities of salt, experimental technique, collaborative work, type of equipment available, and so on depending on the planned outcomes to be achieved. Questions can be asked relating to the stability of the viscosity, its measurement and even the viscosity of competitor products. However, students should be alerted to, and be able to provide a response for the question

"As they continued to add salt to the mixture, what did they notice about its viscosity?"

They should have noticed that the increase in viscosity can be quite sudden (it may vary slightly depending on the composition of the basic formulation): a little less than 4% salt by mass should give about the right viscosity - above this level the viscosity will start to reduce again. For many students, this is unexpected. Their predictions support the idea that the mixture will become increasingly viscous - and this often leads them to check and question their experimental technique.

Once the optimum amount of salt for the gel has been established, two other factors need to be considered. Commercial shower gels often contain a colouring agent and usually some perfume. To finalise the product, various food colourings and perfumed oils can be used to colour and perfume the gel. However, the marketing department needs to be consulted before making the final decision on colour and perfume.

The viscosity of the gel needs to be checked after adding colour and perfume and the amount of salt adjusted if necessary. The pH of the product can also be checked at this point.

The research department of a company has the responsibility of developing the shower gel. However, it cannot work in isolation. Since the product needs to be a commercial success it has to meet customer demands, and the research and development section of the company needs to know these requirements before finalising the formulation of the product. Input from other departments can be considerable in terms of: colour and perfume of product; design and packaging for the product; advertising campaign; and cost of final product. The scientists and chemical engineers need to be aware of these different aspects to development.

There are many different opportunities for students to get involved in thinking about the 'human and commercial' side of science and working in a collaborative way gives meaning and purpose to their project.

Why the viscosity increase?

The surfactant molecule consists of two different parts: a water-loving, or polar part, and a water-hating, or non-polar part. These parts absorb strongly at water/oil interfaces since the polar part can be surrounded by water while the non-polar part resides in the oil. Solutions of highly active surface molecules (that is, detergent solutions) exhibit unusual physical properties. At some concentrations, surfactant molecules aggregate to form structures known as micelles. In these aggregates the (water-hating) hydrocarbon tails lie towards the centre, while the water-soluble polar ends are at the surface of the micelle.

Most surfactants for small micelles of approximately spherical or ellipsoidal shape that contain roughly 40 to 200 molecules. However, if the solution's conditions, such as pH, temperature or electrolyte concentration, change, then the size and shape of the micelles are altered.

Thus surfactants can cluster into a variety of structures in aqueous solutions - and these can transform from one to another as the solution's conditions change. Adding salt to the basic shower gel formulation changes the structure of the micelles. The higher the viscosity, the larger is the structure. As salt continues to be added, the structure becomes unstable and breaks down. This has the visible effect of reducing the viscosity of the solution.

Notes

1. The surfactant should be diluted from 70% paste to a 25% w/w solution in water for ease of handling.
2. The co-surfactant should be available as a 30% w/w solution in water.
3. If citric acid is used, use saturated citric acid solution so that the concentration is the same each time.
4. A basic formulation for testing can be made simply by using surfactant, co-surfactant and salt.
5. When an approximate sale quantity has been established, it is better to add it as a solution since it mixes better. Remember that the water quantity must be adjusted accordingly.
6. If EMGS is used it must be molten to disperse effectively in the surfactant system.
7. To calculate the amount of water to add, the amount of water already in the product as a result of the other raw materials needs to be

Technical details - shower gel formulation

Surfactant (sodium lauryl ether sulphate, 3 EO) EMPICOL 0251/7	% by weight as pure material
Co-surfactant (amine oxide) EMPIGEN OB	Up to 6%
pH adjuster (citric acid solution)	To give pH 6.5-7.
Opacifier either ethylene glycol monostearate (EMPILAN EGM)	at 2%
or euperlan PK771	at 4%
Salt (sodium chloride)	As required for thickening
Preservative (formaldehyde)	0.1%
Water (deionised)	Balance to 100%

calculated and this quantity deducted from the total water required.

If required, students can obtain technical brochures and safety data sheets from the suppliers of the raw materials, along with the current cost of raw materials.

Handling and safety

1. Eye protection, gloves and laboratory coat are advised when handling the shower gel ingredients.
2. The final product should only be tested when wearing rubber gloves.
3. All products should be disposed safely.

Research Communication

Dating Archaeological Bone Specimens Using Natural Gamma Emitter Radionuclides

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Summary: *Gamma ray emitter elements in local cave-soils and buried bone are assessed to attempt to establish a rôle for the use of gamma ray spectrometry for dating of buried bones found in an archaeological context. Thorium[234] : Potassium[40] ratio measurements are suggested to be possible non-destructive means of dating archaeological skeletal remains.*

Keywords: radionuclides, gamma-emitters, archaeology, skeletal

Introduction

The first, and in some ways the most important, step in much archaeological research involves ordering things into chronological sequences. These sequences can then be used for relative dating. Careful attention to stratigraphy during archaeological excavations is essential for establishing the chronological sequence of the deposits. Problems with stratigraphical chronology may however occur in cases where there may have been physical movement of deposits or in burial deposits. A useful method of assessing whether several bones found in association are in fact of the same relative age is chemical and radiometric dating by studying nitrogen, fluorine and uranium content (Renfrew and Bahn, 1994).

The concentration of nitrogen in buried bone progressively decreases with time, while the concentration of fluorine and uranium increase with antiquity (Bowen, 1958). The loss of nitrogen in buried bone is dependent on the temperature and the water, chemical and bacteriological content of the environment in which the bone is buried. Similarly, the uptake of uranium and fluorine by buried organic remains is related to chemical factors in the soil deposit (Leute, 1987). The described methods to assay these elements in buried bones require either the partial destruction of the specimen or the use of an alpha-particle spectrometer. The present study attempts to elucidate the Maltese phreatic cave system dynamics pertaining to Uranium[238] daughter elements, and to correlate C14-dated bone with the gamma emitter daughter radionuclides of uranium using a simple gamma-ray spectrometer without the need of destroying the specimens.

Material and Methods

^{234}Thorium and ^{226}Radium, daughter elements of ^{238}Uranium present in soil, are gamma ray emitters. ^{40}Potassium is another gamma ray emitter present in soil and rock (IAEA, 1989). A high resolution gamma ray spectrometry system can be used to identify gamma-emitting radionuclides with energies ranging from 60 keV to 2 MeV, depending on the type of detector, in a large variety of sample matrices (IAEA, 1989). The simultaneous detection of several gamma radionuclide emitters in the sample material was carried out with a high resolution detector (coaxial germanium diode) connected to a multichannel analyser. Automatic processing of the collected spectral data was controlled by a computer system.

To assess the chemical dynamics of a phreatic limestone cave (Ghar Hasan) in Malta, gamma ray spectrometry was performed on cave rock, percolating water, recent cave-floor soil, and Pleistocene roof-soil. The results of these specimens were compared with soil obtained from the cliffs above the cave. Mammalian bone, also found buried in the cave, was similarly assayed. This bone was considered to be of very recent origin (probably less than a decade) in view of the good anatomical preservation of the individual bone components and the consistency of the soil in which the animal had been buried. The assays of the cave-floor earth samples obtained from Ghar Hasan were compared to further specimens obtained from other phreatic caves in Malta (Ghar il-Friefet and Ghar Dalam both at Birzebbugia).

Bones of varying antiquity obtained from various sites were further analysed for gamma-emitting radionuclides. The archaeological human bone specimens obtained from Fleur des Lys and BurMghez burials were radiocarbon dated at Oxford University and the British Museum by one of the authors (AM). The Fleur des Lys specimen [BM-3015] gave a date of 2500 years BP [Mifsud and Mifsud, 1997]. The BurMghez specimen [OxA-8165], presently conserved at the British Museum, yielded a date of 5300 years BP (Mifsud 1999). Pleistocene undated cervine fossil remains from Ghar Dalam from a private collection [AM/Vb:1-2] originating from the Carnivora Stage layer of the floor deposit and a hippopotamus fragment [AM/VII:1] originating from the Gliridae Stage layer were also similarly assayed (Zammit-Maempel, 1989; Savona-Ventura and Mifsud, 1998).

For the purposes of the present investigation, this first daughter radionuclide ^{234}Th was considered to be more closely representing the levels of ^{238}U than the other

daughter radionuclides. This assumption was based on the very short half-life of this radionuclide (24 days in contrast to the half-life of ^{226}Ra 1600 years). Since absolute values vary according to the type of specimen, the results obtained for the various samples were standardised as a ratio to ^{40}K. It was assumed that the high solubility of potassium salts results in equilibrium values of that radionuclide in all samples.

Results

The Ghar Hasan cave system yielded a series of $^{234}Th/^{40}K$ and $^{234}Th/^{226}Ra$ ratio results (Table 1). The $^{234}Th/^{40}K$ ratio of the percolating water (0.94) was higher than the ratio obtained for the cave-floor soil (~0.45) and even higher than the ratio obtained for the cave-roof soil (0.39) and the superficial cliff soil (0.26). Radium salts in limestone deposits appear to be markedly more soluble than Thorium salts, so that the soil samples had higher $^{234}Th/^{226}Ra$ ratios (1.22-1.79) than the cave rock (0.42). The recently buried bone samples appear to have equilibrated with the percolating water showing approximately similar values of $^{234}Th/^{226}Ra$ ratios (0.79 and 0.73 respectively). There appears to be an active absorption of Thorium by bone resulting in an elevation of $^{234}Th/^{40}K$ ratio (1.61 vs 0.94 in water).

Ghar Hasan SPECIMENS	^{40}Potassium Bq/g	^{234}Thorium Bq/g	^{226}Radium Bq/g	^{234}Thorium / ^{40}Potassium	^{234}Thorium / ^{226}Radium
Superficial Cliff soil	5.90E-01	1.54E-01	1.26E-01	0.26	1.22
Cave Rock	1.57E+01	9.99E+00	2.39E+01	0.63	0.42
Percolating Water	3.35E+00	3.20E+00	4.43E+00	0.94	0.73
Recent Cave-floor soil (2 sites)	2.57E-01 3.03E-01	1.13E-01 1.36E-01	6.97E-02 7.60E-02	0.44 0.45	1.61 1.79
Pleistocene Cave-roof soil	9.69E+01	3.78E+01	3.03E+01	0.39	1.25
Recent buried mammalian bones	1.22E-01	1.96E-01	2.47E-01	1.61	0.79

Table 1: Ghar Hasan system

The cave-floor soil samples (Table 2) obtained from the three sites in Malta show that similar factors are operative in various phreatic cave systems, with the $^{234}Th/^{40}K$ ratios being approximately equal in soil samples from the three caves; this gave a mean of 0.422 + 0.052sd (range 0.32 - 0.47). The mean $^{234}Th/^{226}Ra$ ratio showed a wider variation in result with a range of 0.72 - 1.79 (mean = 1.19 + 0.396sd).

The ratios obtained for the various buried bony remains suggest that bones initially equilibrate with the

Cave soil SPECIMENS	^{40}Potassium Bq/g	^{234}Thorium Bq/g	^{226}Radium Bq/g	^{234}Thorium / ^{40}Potassium	^{234}Thorium / ^{226}Radium
Ghar Hasan Cave-floor soil (2 sites)	2.57E-01 3.03E-01	1.13E-01 1.36E-01	6.97E-02 7.60E-02	0.44 0.45	1.61 1.79
Ghar Hasan Pleistocene Cave-roof soil	9.69E+01	3.78E+01	3.03E+01	0.39	1.25
Ghar il-Friefet Cave-floor (2 sites)	2.84E-01 4.29E-01	9.15E-02 2.01E-01	1.28E-01 2.36E-01	0.32 0.47	0.72 0.85
Ghar Dalam Pleistocene Cave-floor soil	4.46E-01	2.04E-01	2.18E-01	0.46	0.94
Mean + sd				*0.422+ 0.052*	*1.190 + 0.396*

Table 2: Cave-floor soils

Buried Bone! SPECIMENS	^{40}Potassium Bq/g	^{234}Thorium Bq/g	^{226}Radium Bq/g	^{234}Thorium / ^{40}Potassium	^{234}Thorium / ^{226}Radium
Percolating Water				*0.94*	*0.73*
Cave Soil Mean + s.d.				*0.422 + 0.052*	*1.190 + 0.396*
Mgarr Cave (surface find: recent)	5.93E-02	0	6.02E-02	0	0
Ghar Hasan (buried: recent)	1.22E-01	1.96E-01	2.47E-01	1.61	0.79
Fleur de Lys burial (C^{14}: 2500 yrs BP)	1.26E-01	5.81E-01	3.64E-01	4.61	1.6
BurMghez (C^{14}: 5300 yrs BP) *Mean + sd*	1.21E-01 1.29E-01	8.39E-01 1.11E+00	5.59E-01 5.05E-01	6.93 8.61 *7.77 + 1.19*	1.5 2.19 *1.85 + 0.49*
Ghar Dalam *Cervus* fossil LAYER Vb *Mean + sd*	2.60E-01 1.80E-01	1.96E+00 1.42E+00	1.12E+00 7.59E-01	7.54 7.89 *7.72 + 0.18*	1.75 1.87 *1.81 + 0.06*
Ghar Dalam Hippopotamus fossil LAYER VII	2.12E-01	2.07E+00	1.04E+00	9.76	1.99

Table 3: Bone samples

percolating water, so that the 234Th/226Ra ratio of the bone approximates that of percolating water. With increasing antiquity including that of Pleistocene fossils, the bone 234Th/226Ra ratio appears to approximate the value of the cave-floor soil. Recently buried bone appears to absorb Thorium actively, thus increasing the 234Th/40K ratio, from 0.94 in percolating water to a 1.61 value in recently buried bone (Table 1). With increasing antiquity, the 234Th/40K ratio of buried bone appears to increase progressively to give a 234Th/40K ratio of 4.61 after 2500 years, and a mean of 7.77 + 1.19sd after 5300 years (Table 3). The 234Th/40K ratio results obtained for the Pleistocene remains appeared similar to those of the Neolithic remains, with the younger cervine bones having a mean value of 7.72 + 0.16sd, and the older hippopotamus bone a value of 9.76.

Discussion

Buried bone equilibrates with its environment and undergoes active chemical changes, which reflect its antiquity. Thus with increasing age, the nitrogen content of buried bone progressively decreases at a rate dependent on the temperature and the water, and the chemical and bacteriological content of the environment in which the bone is buried (Protsch, 1986). At the same time, percolating ground water has significant effects on the composition of bone. Elements present in solution in the ground water - fluorine, uranium and iron - are absorbed gradually by the bone, their levels increasing with increasing antiquity. The rate of increase in fluorine, uranium and iron depend on the local concentrations of the elements in the percolating water and the rate of water flow (Protsch, 1986). Modern bone has only traces of these elements, with the level of uranium oxide being practically nil, fluorine being less than 0.1%, while iron amounts to about 0.007% (Leute, 1987; Diem and Lentner, 1975). The uptake of uranium by buried organic remains is related to chemical factors in the soil deposit, the rate of water flow and the concentration of the elements in the percolating water. Because local chemical factors can vary, the chemical tests cannot be used for the basis of

an absolute dating test. However on an individual site or in sites shown to have similar chemical characteristics, chemical dating can distinguish bones of different age found in apparent stratigraphical association (Protsch, 1986).

The three major naturally occurring primordial radionuclides usually present in soil and rock include the isotopes of uranium [^{238}U] and thorium [^{232}Th] plus their daughters, and an isotope of Potassium [^{40}K]. Native uranium contains three isotopes: 99.28% ^{238}U, 0.7% ^{235}U, and 0.006% ^{234}U. Natural isotopes of uranium are alpha emitters with very long half-life (4.5 x 109 years for ^{238}U). The ^{238}U series produces a number of gamma emitter radionuclides, notably ^{234}Thorium, ^{226}Radium, ^{214}Lead and ^{214}Bismuth. The Radium - Uranium ratio in natural compounds is 3.4 x 10-7. ^{234}Thorium and ^{226}Radium are beta and alpha emitters respectively, with gamma ray emission as a by-product of the surplus excitation energy. The amount of radionuclides varies according to the type of rock or soil formation found in the locality, being generally higher in volcanic regions. In a region where limestone predominates, such as the Maltese Islands, the radionuclide levels are low but of sufficient levels to allow the use of radionuclide levels to be used for archaeological relative and absolute dating (IAEA, 1989; Songina, 1970).

Buried bones and teeth are exposed to the action of the percolating ground water containing uranium salts in solution. The uranium irreversibly substitutes the phosphate content of bone, mainly the hydroxy-apatite. Fossil bones had been shown to contain uranium by Lord Rayleigh in 1908, but the technique was only revived and adapted in 1955 by Davidson and Bowie at the Atomic Energy Division of the Geological Society. It was established that although the uranium oxide concentration varied in different localities, the amount increased proportionately with its antiquity. Specimens in the same environment varied directly in proportion with the length of time that they were buried (Davidson and Bowie, 1955). Levels of uranium oxide in modern bone is practically nil, but in ancient buried bone these may rise to levels as high as 1000 ppm, depending on the concentration of uranium oxide in the percolating water. The range in fossil bone has been reported as lying between 1 and 1000 ppm (Leute, 1987; Aitken, 1990), the level increasing proportionately with its antiquity (Davidson and Bowie, 1955).

The level of uranium oxide and its daughter elements have thus been noted to vary in different localities depending on the surrounding rock strata and leaching effects (Bowen, 1958; Oakley, 1970). In spite of these expected differences in different geological formations, it is expected that the uranium oxide and daughter element concentrations should be similar in matching closed system environments. Karstic limestone cave environments are such situations, where the formative and erosive processes are approximately similar in their low levels of uranium salts (Bowen, 1958; Oakley, 1970). It would be expected that the concentration of uranium oxide and daughter elements in cave floor soil would equilibrate in a similar fashion, provided that the cave floor soil is of a similar age.

The different cave soils studied showed a relatively stable ^{234}Th/^{40}K ratio, with a mean value of 0.422 + 0.052sd. 40Potassium is an unrelated gamma emitter which, because of the high solubility of potassium salts, may be assumed to be in a steady state equilibrium in all samples. The stable ^{234}Th/^{40}K ratio in Maltese karstic caves, including Ghar Hasan, Ghar Dalam and Ghar il-Friefet, reflects the steady state relationship between these two gamma emitter elements in cave floor soil. The similarity of the results obtained from the different sites suggests that bony remains buried in karstic limestone cave systems in Malta may be compared since they are exposed to near identical chemical and physical conditions. The ^{234}Th/^{226}Ra ratios in the different cave soils appeared to show a wider variation in results around a mean value of 1.19 + 0.396sd, with a range of 0.72-1.79. The reasons for this wide difference range in the daughter elements of uranium have not been elucidated, but may reflect the possible age of the soil sample with older soils showing a lower ratio. The difference in the half-lives of the two radionuclides - ^{234}Th: 24 days; ^{226}Ra 1600 years - would result in higher Radium levels and thus a lower ^{234}Th/^{226}Ra ratio in older specimens. Further studies in this regard are required.

A dynamic interplay of factors would be expected to exist in any cave system, such as the one exemplified by Ghar Hasan. The cave-soil samples originated from the cave rock, and should have had an original value equivalent to the ^{234}Th/^{40}K ratio of the rock (0.63). The ratios in the various cave-soil samples have been apparently modified over time through the action of percolating rainwater. Rainwater is acidic in nature and has a near zero ^{234}Th/^{40}K ratio. This falls onto the superficial soil/rock dissolving and leaching away the various elements at variable rates depending on the solubility. With prolonged exposure, the acid soluble elements are leached away, leaving the terrarossa soil typical of the superficial cliff in which Ghar Hasan is found (Schembri and Baldacchino, 1992). As a result of this prolonged leaching, the ^{234}Th/^{40}K ratio of the superficial soil (0.26) was very much lower than the ^{234}Th/^{40}K ratio of the derivative cliff rock (0.63). The minerals are thus continuously leached into the percolating water, which showed a higher ^{234}Th/^{40}K ratio (0.94). This water with a relatively high ^{234}Th/^{40}K ratio leaches the cave-floor and cave-roof soils, which also originated from the same derivative rock (234Th/ 40K ratio 0.63), and reduces the ^{234}Th/^{40}K ratio of these soils to a lesser extent (0.44-0.46) than the superficial cliff soil. The presumably recent bony remains buried in the cave-floor soil equilibrate with the percolating water and concentrate uranium and its daughter elements by an active process, whereby the phosphate content of hydroxy-apatite in bone is replaced. This results in a high ^{234}Th/^{40}K ratio (1.61) [Figure 1].

The ^{234}Th/^{40}K of the bones appear to progressively increase with increasing antiquity, thus reflecting the active binding process of the bone with uranium series elements. The BurMghez specimens, deposited in the British Museum after their excavation in the early

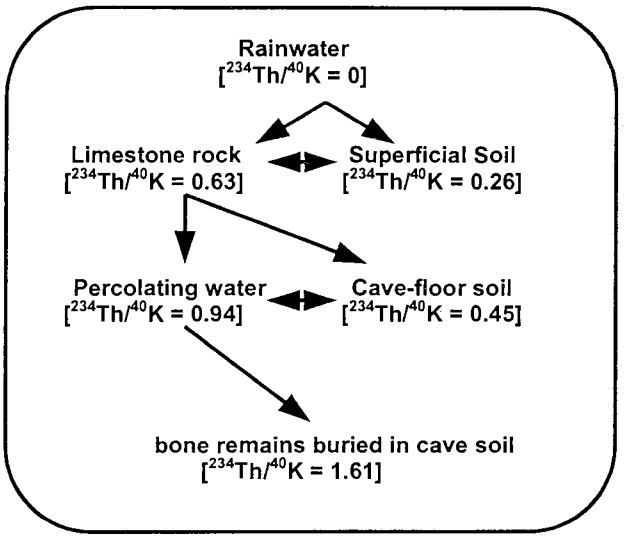

Figure 1: Ghar Hasan Cave system dynamics

twentieth century, had been dated, on the basis of pottery shards correlation, to the Ggantija Phase, circa 3600-3000 BC, or circa 5600-5000 BP (Evans, 1972). Carbon dating carried out at Oxford has confirmed a date of 5300 years BP for these bony remains. The $^{234}Th/^{40}K$ ratio for the BurMghez bones (mean 7.77 + 1.19sd) correlated very well with the established artifactual and carbon-14 dating, thus giving the highest reading. The Fleur des Lys specimens had been previously assayed for nitrogen percentage by the Natural History Museum, using the Weiler and Strauss unwashed technique. The nitrogen percentage for repeated tests varied from 0.43-2.58% [Bone Analysis Malta Samples Ma.27]. This very wide range for the same specimens reflects the unsuitability of relying solely on nitrogen assay as an index of antiquity (Mifsud and Mifsud, 1997). Carbon dating of these remains [BM-3015] gave a date of 2500 years BP. This date correlated with the $^{234}Th/^{40}K$ ratio obtained for these specimens (4.61), which gave a value approximately midway between the BurMghez and recent Ghar Hasan remains. The results suggests that the increase of $^{234}Th/^{40}K$ ratio in archaeological bones appears to be directly proportional to age, increasing at a rate approximating 1.5 per 1000 years of burial [Figure 2]. The process appears to eventually become saturated so that the $^{234}Th/^{40}K$ ratios of Pleistocene fossils remained approximately the same, or increased minimally, from the value obtained for the Neolithic remains. Definite dating of the Pleistocene layers at Ghar Dalam has yet to be undertaken. The only date is that obtained for a hippopotamus fossil dated by electron spin resonance and uranium series disequilibria to 130,000-110,000 years BP (Bouchez et al, 1988). Uranium oxide levels of cervine and hippopotamus bones have been previously carried out by the Natural History Museum, London. These gave values of 7.67 + 4.04sd for cervine bones and 6.50 + 3.54sd for hippopotamus bones (Mifsud and Mifsud, 1997; Savona-Ventura and Mifsud, 1998). The exact stratigraphical context of these tested fossils had not been defined.

The $^{234}Th/^{226}Ra$ ratios are more difficult to interpret, but appear to reflect the age of the specimens. ^{234}Th has a very short half-life (24 days) contrasting with the half-life of ^{226}Ra (1600 years). This would result in a gradual increase in the radium concentration with time. With increasing age however, the radium would also significantly break down to other radionucleides, further altering the $^{234}Th/^{226}Ra$ ratios of very old deposits. The $^{234}Th/^{226}Ra$ ratio for water approximated 0.73, while the cave-floor soil samples approximated 1.7. The cave-roof soil sample previously described as Pleistocene (Shaw, 1950; Shaw, 1953) gave a median $^{234}Th/^{226}Ra$ figure of 0.94. The recent bone samples appeared to be in equilibrium with the percolating water, while with increasing antiquity the $^{234}Th/^{226}Ra$ ratio of buried bone (1.5-2.19) approximates the ratio of cave-floor soil.

The present study suggests that the $^{234}Th/^{40}K$ ratios of various bone specimens collected from various caves in Malta show a definite trend in values, the ratio increasing with increasing antiquity of the bone sample. The measurement of $^{234}Th/^{40}K$ ratio may thus be useful in correlating the bone specimen to stratigraphy, and can be used for relative dating in an archaeological context. It does not however appear to be a useful dating method for fossil specimens. The present study can be considered only as a pilot study in view of the small number of carbon-dated bone specimens. Further analysis of carbon-14 dated bones is necessary to confirm the suitability and reliability of this technique. Further sources of error in the technique need to be investigated. Errors may be caused by improper spectral identities, changes in background, errors in calibration of various geometries, and lack of homogeneity in samples (IAEA, 1989). Since the samples tested in this study were not in a standardised geometry, some variation in the readings could be expected due to the difference in sample homogeneity. This technical drawback was partly overcome, where possible, by using samples of approximately equal weight and size. If confirmed as useful, the use of gamma ray spectrometry for dating of archaeological skeletal remains has the advantage of being a non-destructive technique which preserves the integrity of the specimen.

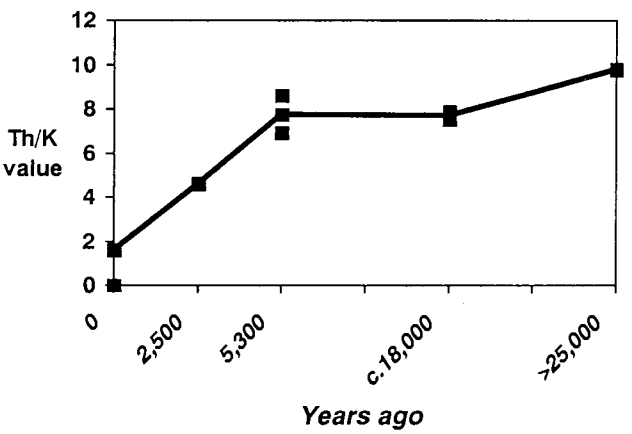

Figure 2: Bone Th/K ratio with increasing antiquity

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Research Communication

A Survey on Radon Levels in Local Dwellings.

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Summary. *This survey extends a previous study in which it was not possible to perform long term measurements. Passive detectors were used to determine annual average radon concentrations. International guidelines and standards on recommended intervention levels for radon are expressed as yearly averages. The survey results are within the safety limits stipulated by the World Health Organisation and expressed as a yearly average.*

Keywords: radon, etched-track detectors, extended monitoring, local dwellings.

Introduction

A pilot survey to determine the magnitude of radon levels in local dwellings was carried out between June 1994 and November 1995. The results of this survey were published in a previous issue of Xjenza (Mifsud et al., 1997). During the survey air sampling was carried out over 24 hour periods by means of portable electronic radon monitor. A second survey was carried out between May 1997 and April 1998 in order to determine yearly average radon concentrations.

Materials and Methods

Passive etched-track detectors were used in this survey (Figure 1). Such detectors are composed of a polyallyldiglycol carbamate detecting element enclosed in a polypropylene holder (Hardcastle et al., 1996). Radon and its decay products are electrically charged when formed so that any electrostatic charges inside the holder will influence the radon concentration inside the detector. Thus the holders and element were treated with a dilute detergent solution during the assembly stage in order to impart an antistatic property to the detector. Radon enters the holder by diffusion. The half-life for entry is 25 minutes which is short compared with radon's decay half-life of 3.82 days. Thus the radon level inside the holder quickly approaches that outside.

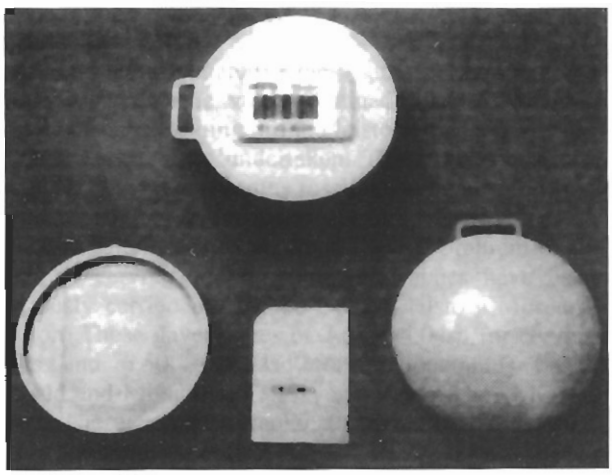

Figure 1. Passive etched-track detectors.

Inclusion Criteria

Participants in this survey were instructed to place the detector in rooms where they spend a considerable time, particularly bedrooms or living rooms, and to use only ground floor locations, where radon gas would be expected to accumulate with respect to higher elevations.
Exclusion Criteria:
Dwellings falling under the following categories were excluded from this survey:
(1) those expected not to be inhabited for an extended period (more than one month),
(2) those expected to undergo structural alteration, and
(3) rooms which were artificially ventilated by means of air conditioners.

Two batches of detectors were distributed to 25 residents in different localities (four localities in Gozo and 21 localities in Malta). Another three detectors were kept as controls during the survey period. The first batch of detectors was exposed between May and October 1997 and the second batch was exposed between November 1997 and April 1998. Three detectors, all from localities in Malta, were lost or damaged at the end of the exposure period. At the end of the survey 21 readings were available corresponding to a 12 month exposure period.

Results and Discussion

The computed geometric mean was 32 Bq m^{-3} with a corresponding geometric standard deviation of 2.0. Figure 2 compares the radon readings for the two batches of detectors. Radon values from the second batch of detectors, which corresponded to the exposure period November to April, were consistently higher than those of the first batch (geomean of 39 Bq m^{-3} compared to 23 Bq m^{-3}) the latter corresponding to the exposure period May to October. The warmer period of the year (May to October) is usually associated with increased room ventilation resulting in the lower radon concentration indoors. During the cooler months room ventilation is often reduced voluntarily in order to minimise heat loss from dwellings. This may contribute to the increase in radon concentration indoors.

The highest level recorded was that in the November/ April batch in Kercem, Gozo, with a detected radon level of 117 Bq m^{-3} (corresponding May/October reading 75

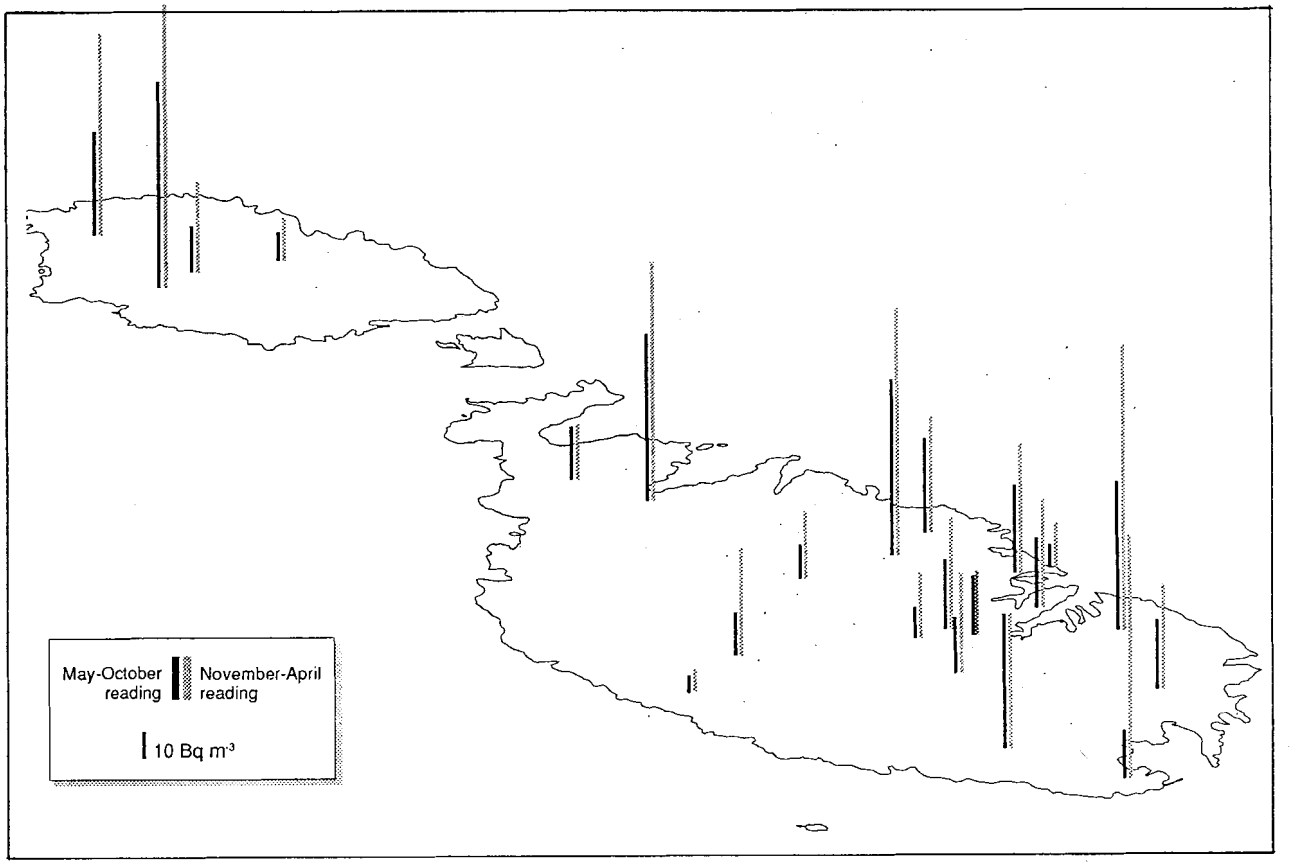

Figure 2. The computed geometric mean.

No. of dwellings sampled	Period and duration of exposure	Type of detector	Arithmetic mean in Bq m^{-3}	Geometric mean in Bq m^{-3}	Geometric Standard deviation
68	1994 - 95 24 hour	electronic radon monitor	55	40	2.3
21	1997 - 98 1 year	passive etch-track detector	40	32	2.0

Table 1

Bq m^{-3}). The lowest value recorded was that in Dingli with 6 Bq m^{-3}) during the May/October period (corresponding November/ April reading 8 Bq m^{-3}).

Conclusions

When compared with the results of the survey carried out in 1994/95 these were lower than that in the original survey (geomean of 32 Bq m^{-3} compared to 40 Bq m^{-3}). This difference may be due to the averaging out effect of long term monitoring which takes into consideration seasonal variations as against 24 hour snapshot readings utilised in the pilot phase of the study.

Acknowledgements

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Article

The Genetics of Mortality and Immortality

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Introduction

In Man's perpetual concern about life and death, the topic of mortality and immortality is undoubtedly an attractive and intriguing topic, especially if combined with the even more attractive and intriguing subject of genetics. From a strictly scientific and genetic point of view, the meaning of mortality and immortality is, perhaps, somewhat different from what immediately comes to mind when one is talking about these topics. The main concept underlying mortality and immortality is not concerned with death and dying but is centred on life, and this paper will consider the subject of mortality from the point of view of life.

This paper will not attempt to give a metaphysical discussion on life after death. From a strictly scientific viewpoint, this is a contradiction in terms. There are many different aspects of mortality and immortality. This paper will define the meanings of mortality and immortality in the context of the present discussion and will attempt to explain the genetic aspects of mortality and immortality and how these two contrasting concepts can be reconciled in scientific terms.

Death is a Part of Life

Paradoxically mortality is a feature of living organisms. We cannot speak of mortality in relation to inanimate objects. Death can only ensue where there is life and we generally consider death to be the termination of an individual's life. We may think of death as being caused by severe disease or injury which may occur at any age of one's life. We may also think of death as the ultimate stage that follows senescence in the life of an individual's physical existence. Although one may be fortunate enough to escape fatal disease or injury, no one can escape the ravages of time and ultimately everyone will meet with death. From the epidemiological viewpoint mortality is largely concerned with the ages at which people die. In the last century there have been social changes and medical advances, which have radically altered the pattern of mortality. Up to about 60 years ago infections were the main killers. Babies died of enteritis, children died of diphtheria and other infectious diseases and adults died in the prime of life of tuberculosis. People of all ages died of epidemics of plague and cholera. All these diseases have now been almost entirely relegated to the past. Now more and more people are living to a ripe old age because they are not dying younger, although they are increasingly subject to chronic diseases such as diabetes, hypertension and cancer. These diseases too are gradually finding more effective cures. Changes in the way of life, education, and social improvements, scientific discoveries and medical progress have all contributed to alter drastically the life expectancy of individuals over the last century.

Maximum Life Span

However, none of these advances have altered at all the maximum life span of people. There are plenty of recorded instances from time immemorial of people living to over 100 years, but nowhere can one find reliable evidence of people living to be more than about 125 years. The person who is considered to hold the record of longevity is the late Madame Jeanne Calment who died in France in 1997 at the age of 122 years and 164 days (Robine and Allard, 1998). The previous record holder was a Japanese person who also died at the age of 122 years, but was a few days younger than Madame Calment. This can be considered to be close to the maximum life span of humans, the limit of longevity. It is thought that the maximum life span of humans has not been affected at all by the changes that have promoted the dramatic increase in life expectancy over the last century. Today, centenarians are becoming commoner than they used to, but there is no evidence that people are exceeding the limit of the maximum life span. This maximum limit ensures that all people die.

It is believed that the maximum life span is fixed for every animal species and cannot be altered. Mice, for example, have a maximum life span of 4 years. No matter how well a mouse is cared for, it cannot live for 20 years. Every animal has an innate, genetically determined maximum life span.

Maximum Life Span is Part of the Programme of Life

The maximum life span may be considered to be part of the programme of life of animals. Taking human beings as an example, the programme of life begins at conception and passes though innumerable series of perfectly timed and co-ordinated developmental stages. The subject of human development is very vast and has many aspects. I will only mention a few notable landmarks just to set the scene of the programme of life. Embryonic life is the period of differentiation, which transforms one cell into a variety of tissues and organs. Foetal life is the period of growth and shaping of organs. Birth heralds the beginning of an independent existence; childhood is the great period of learning, which opens the way to a creative life. Puberty marks the beginning of reproductive life and the transition to the fully-grown and mature adult. Finally, the post-reproductive stage is characterised by a marked and progressive physiological decline, ultimately leading to inevitable death. All these stages are controlled by a genetic programme of life and accurately timed by a biological time clock. For example, the heart is formed and begins to pump at 4

weeks; birth occurs after 40 weeks of gestation; we begin to walk at the age of 12 months; puberty occurs at the age of about 12 years; the menopause occurs at 45 to 50 years. Life takes us through a series of developmental milestones at appropriate time intervals. If these times are not adhered to within rather narrow limits, there will almost certainly be a problem. As the clock ticks away we approach closer and closer to the ultimate notable landmark, the maximum permitted limit of life.

Genetics Regulates the Programme of Life

This biological programme is all encoded in the genome. Genetics is the basis of all life. In the genetic molecules of DNA are encoded the messages which regulate all biological processes. They regulate all embryological and post-natal events, which, in a precisely regulated manner, gradually unfold the development of the biochemistry, anatomy, and physiology and of the body. The genetic messages regulate with molecular precision the differentiation of cells, tissues and organs from the time of conception to full maturation of the individual. They also regulate the functioning of all body systems throughout life.

The saga of development includes the processes of cell proliferation and cell differentiation. As the cells multiply, the pluri-potent cells of the early embryo differentiate and undergo morphological and functional changes leading to the formation of specialised tissues of the brain, kidney, liver, heart, limbs and so on. Development also includes the co-ordination of these events so that the differentiated tissues organise themselves to form organs in precisely determined positions, and communicating with one another to function in perfect harmony as faithful members of one individual body. Development includes modelling of the body and its individual components to form a precise and remarkably constant anatomy.

The remarkable paradox is that cell death is also an integral part of this developmental process of sprouting life. Programmed cell death, which also goes by the euphemistic term "apoptosis", is a genetically determined and pre-programmed cell death, which is essential for normal development. Some developmental defects are precisely the direct results of failure of cell death to occur at the right place and the right time. Some cells, having outlived their pre-determined functions at a particular stage of development, need to make way for other specialised cells with different functions to take their place. Programmed cell death is an integral part of life.

The Conflict of Life and Death

It appears that the programme of life includes an in-built mechanism whereby it is ensured that all individuals die. But does it make sense that self-sustaining life in all its variety and beauty should programme itself to ensure that all individuals die? That life terminates at the time when people are finding their fulfilment of life? As Oscar Wilde remarked in: A Woman of No Importance, "The soul is born old, but grows young. That is the comedy of life. And the body is born young and grows old. This is life's tragedy."

However, there is more to life than the comedy and the tragedy, the emotions created by this conflict. There is also the beauty of life in which we might find some explanation for this paradox. The beauty of life lies in its variation. Not only is there the almost infinite variety of plants and animals and other living organisms to capture our admiration but even more impressively there is the individual variation which makes us all different from one another physically, psychologically and emotionally, which makes each one of us unique.

Genetics is the sum and substance of life, that which makes life self-perpetuating and self-regulating; genetics is the basis of variation; genetics is the driving force behind evolution. Evolution is survival. To appreciate the significance of evolution we must look at organisms in the wild. Evolution is the mechanism of natural adaptation to ensure survival in the face of new, adverse or hostile environments brought about by factors such as physical isolation, availability of food, the threat of predators and other life-threatening situations. Genetic mutations are nature's experiments to produce variation. Among these variants, a few would have a distinct survival advantage in the prevailing circumstances, and so continue to propagate themselves from one generation to the next by natural selection. This variation necessarily appears in new individuals and for these to be evolutionarily useful they must replace others. Death of the individual, therefore, is an integral part of the evolutionary process that ensures survival of the species.

This is the genetic justification of mortality! In the mind of Homo sapiens, however, death presents the harsh reality of an end to the beauty of life, an end to our physical sensation of life. Death is therefore interpreted by the conscious mind as void and darkness. But there is also a dim vision that death has a purpose, which, however, cannot be interpreted in terms of natural experience but is projected in terms of the supernatural.

In this light it, would be absurd to suggest that the justification for death is evolution and the preservation of the species. Man is situated at the apex of the evolutionary pyramid, at a point beyond which there appears to be nothing but an infinity of space and time. The evolutionary forces that have culminated in Man have produced a being that does not need to rely on chance events and natural selection to survive adverse and new environments. Instead, he has achieved an intellectual capacity of such a degree that he is capable of adjusting almost any environment to suit his needs. This enables man to live in extremes of climatic conditions or even in outer space and to survive where no other organism can survive. Man has intellectual capacity to understand and control life itself. He can now create genetic mutations almost instantly to suit his needs and whims. He can create new species and clones. Potentially he can do in a few days what evolution would do in thousands of years. Is it possible that man will come to understand life to such a degree that he might even learn how to exert a genetic control over the maximum life span? Before discussing this issue I would like to have a brief look at the biological aspect of immortality.

Immortality of Unicellular Organisms

The pattern of life as presented does not apply to all living organisms. A programmed time clock is not present among primitive organisms, particularly the unicellular ones such as amoeba or bacteria. Take bacteria as an example - they can easily be grown and studied in cultures in the laboratory. Each bacterium divides to produce two organisms, which then divide again and again and can continue to do so indefinitely producing an infinite number of generations. There is no limit to the number of proliferation times and the number of generations and so we can call such cells "immortal". The individual organisms do not die between generations. If any of the individual organisms die it is because of accidental circumstances such as toxic substances or lack of nutrients. Colonies of bacteria may die at a particular location where a hostile environment prevails but others will continue to proliferate.

In adverse environments, which are harmful but not quite lethal, the organisms may gradually undergo genetic mutations, which make them capable of surviving the adverse conditions. A familiar example is when bacteria become resistant to antibiotics, thus creating new strains. These mutations provide the mechanism to ensure survival of the species and continuity of life. Potentially the organisms can live indefinitely. Death of individual organisms is an incidental chance occurrence. The concept of mortality here is not that death is an inevitable and inescapable occurrence, but that living organisms require certain conditions beyond which they cannot survive. Within those limits, life is self-perpetuating and self-adjusting. Within those limits, life is immortal.

The Concept of the "Individual" in Higher Organisms

Does this concept of immortality apply only to primitive organisms, or can it be extended to all life, even human life? Here some clarification is required about the meaning of an "individual". Among higher organisms the concept of an individual is different from that among primitive organisms. Although in both cases the unit of life is the cell, the complex body that constitutes an individual in higher organisms is much more than its component cells. In higher organisms the multiplicity and variety of the component cells of the body contrast with the unity in the genetic composition of the body and its uniqueness. The genes contained in all the cells of the body are identical, no matter how diverse their functions may be. The genome belongs to the individual as a whole, and its component cells are all regulated by this singular genome. Each cell contains a copy of the individual's genome. Furthermore the genome of each individual is unique. The genome of each individual is different from that of other individuals of the same species. By contrast, the individuals in unicellular organisms are clones, all of which are genetically and structurally identical to one another and to the individuals from which they were derived.

The Mortal Soma and the Immortal Germ Line

In higher animals the propagation of life from one individual to another is restricted to only one particular cell line, the germ cell line, which produces spermatozoa and ova. The germ cell line is responsible for the continuity of the species in perpetuity. There is no limit to the number of generations that can be produced, and in this sense we can speak of an "immortal" germ cell line. This perpetuity, however, does not affect the rest of the body, the soma, which is in fact genetically programmed to die within the specified maximal life span. In higher organisms, including man, we can speak of the mortal soma and the immortal germ line!

Mortality in Individual Cells

The inherent, in-built programmed mortality mentioned earlier, refered to the mortality of the body as a whole. However, death of the body is not the same as death of the component cells, although the two may be interdependent. An in-built, genetically programmed mortality is also to be found in the component somatic cells. If somatic cells, such as fibroblasts, are isolated from the body and nurtured in cell culture, providing all the nutrients and environmental conditions necessary to support growth, they will proliferate repeatedly. In this respect they are rather like unicellular organisms and bacteria. However, they differ from these organisms in one crucial factor - they will not proliferate indefinitely. They have a limited maximal life span, characteristic for the organism from which they were taken. The life span of human cells is different from that of similar cells derived from mice, sheep or other animals. And when they approach their maximum limit, they become unhealthy and aged, lose their ability to divide further and die.

Interestingly, the maximal life span of cultured cells is not measured in chronological time but in the number of cell divisions, or the number of times the cell population doubles itself. In fact, it is quite independent of time. Under optimal conditions the limited number of divisions may be exhausted within several weeks. The process may be slowed down under certain conditions but the number of permitted doubling times remains the same. The process may even be interrupted by putting the cells in a deep freeze for a prolonged period of time. When the cells are again placed in culture, even after several years, they resume proliferation, retaining a memory for the number of their previous divisions, and continue to proliferate until they reach their limit. The component cells of the body, therefore, and not only the body as a whole, have in-built life-limiting biological clocks.

Telomeres and Telomerase

While the somatic cells are mortal and have a limited time span, the germ cells are not similarly programmed. What is it that creates this dichotomy? What genetic mechanism ensures that somatic cells die while germ cells continue to propagate life and proliferate indefinitely through the generations? Cell biology has provided a likely answer to this question. The thin strands of DNA, which carry along their lengths the genetic messages encoding the programme of life, are coiled in a complex manner to form the chromosomes. The ends of each of these strands are called telomeres. They contain repeated coded signals. With each cell division the telomeres shorten slightly so that the number of repeat signals decreases slightly until, eventually, they are completely exhausted. This, it is thought, is the

genetic time clock that determines cell longevity. Germ cells are, however protected from this shortening and life-limiting mechanism by the enzyme telomerase. This enzyme actually promotes the telomeres to be re-built. Telomerase prevents cells from the life-limiting mechanism of mortality (Bodnar 1998). It enables cells to become immortal. Like all enzymes and all other proteins in cells, the gene for telomerase is encoded within the genome of the organism. The genome, therefore, has simultaneously incorporated into it both the genetic mechanisms, which ensure mortality of somatic cells and immortality of the germ line for propagation of the species.

Can Somatic Cells be Immortalised?

The logical reasoning is that, if the same mechanism were to be applied to somatic cells, they too would become immortal. An interesting and very illustrative story emerging from experiments with cultured cells illustrates this point. Somatic cells were growing in culture for some time so that they had exhausted most but not all of their telomeres. These cells were infected purposely with a certain type of virus, such as the Rous Sarcoma virus. As expected, the virus invaded the cells, monopolised the genetic mechanism of the infected cells and used it to propagate itself, producing millions of viruses, which occupied the cells. The viruses caused havoc and the cells died. In this scenario of devastation, destruction and death there were a few lonely cells which survived this terrible ordeal. They began to recover and once again began to proliferate. They continued to proliferate over and over again and continued to do so. They had become transformed into immortal cells. They had been genetically altered by the virus, which, among other things, had activated their telomerase and so were liberated from the life-limiting telomere shortening.

A similar mechanism also operates in most cancer cells. These too are genetically altered cells, which have been liberated from the normal mechanisms regulating cell proliferation. They proliferate without restraint. Since then they have been grown in laboratories world-wide and are acknowledged as immortal cells which will continue to proliferate as long people continue to culture them.

Genes for longevity

The mechanisms controlling cell mortality and immortality that have been referred to are not the same as those imposing mortality on the body as a whole. In fact, the two are quite distinct, although they are related. The aged body does not die because its component cells have reached their maximum limit of longevity. So why does the aged body die?

Over the years there has been a shift of thought in this regard. The original idea that people died of old age has long been discarded. In 1819 Sir Anthony Carlisle commented: "It seems little more than a vulgar error, to consider the termination of advanced life as the inevitable consequence of time, when the immediate cause of death in old persons is generally known to be some well-marked disease". People do not die of old age but, people die in old age because of a cardiac infarct, a stroke, gangrene, cancer or some other condition. So can we dismiss the notion that there is a maximum limit of longevity?

The actual life span of an individual is determined by a multitude of factors including lifestyle, diet, socio-economic status, environmental conditions and genetic inheritance. Most of these factors are alterable, and influence the mean life span of individuals. They have been instrumental in altering the life expectancy in communities.

Many inherited genes are known to limit longevity in individuals, such as the genes for hypercholesterolaemia, and for diabetes and familial genes predisposing to cancer. These genes affect mortality indirectly, and death results from the disease condition rather than the direct effect of the gene itself. The disease-causing genes responsible for such conditions are mutated genes and their normal counterparts, found in normal individuals, do not influence longevity. We now know that even in the absence of these specific inherited disease-causing genes, there are other genes which undergo mutation in occasional cells (somatic mutations); these are the underling causes of non-inherited cancer, auto-immune diseases and other age-related diseases. While it is now generally accepted that people, even in extreme old age, always die of illness or accident and not of the passage of time per se, are we to believe that all these illnesses occur purely as chance occurrences. One of the theories of ageing postulates that the chance of getting a serious and eventually fatal disease or injury increases with time so that fewer and fewer people will be fortunate enough to survive to extreme old age. If we were to rely on chance alone, however, we would expect that a small, perhaps very small number of individuals would escape fatal disease and survive to two, three or even four hundred years. This would not be different from the increasing chance of destruction of artefacts with the passage of time. We still find artefacts dating back to thousands of years, which have escaped chance destruction, but we never find people who live to exceed the specified age limit.

The programmed time clock that mentioned earlier does not refer to a sort of alarm clock that, at the pre-set time, suddenly activates a genetic switch. It is a gradual process, evidenced in the gradual physiological decline that is invariably noticed in ageing individuals. The genetic time clock is a sort of in-built obsolescence, which causes organs to age and to decline in function in spite of the very effective and remarkable repair mechanisms with which our body is endowed.

So now scientists are asking the question: "Are there longevity genes that directly determine how long an individual is permitted to live? Which genes determine the maximal life span?" We can expect that if such genes exist, they can be mutated so that their life-limiting effect would be modified, extending the maximum limit of longevity.

Over the past decade in several laboratories, the life span of at least two multicellular animal species has been significantly altered by genetic manipulation - one in

Drosophila and the other in Cyaenorhabditis elegans. In Drosophila (Jaswinski, 1996), mutations induced in the SOD1 gene, which controls free-radical metabolism, increased the animals' life span to about twice the normal span. SOD (superoxide dismutase), which is the product of this gene is essential for removing harmful free radicals, which are thought to be an important factor in ageing. In Cyaenorhabditis elegans (Ewbank, 1997), the situation is much more impressive. Here, two gene mutations were induced resulting in a sixfold increase in the maximal life span of this species. This is no mean achievement.

In more complex organisms, the problem is not as simple as this because we would expect that there would be several genes interacting with one another in complex fashions. This makes investigation of possible longevity genes very difficult. At present, there is still no concrete evidence that it is possible to induce life-lengthening mutations in higher animals and humans. However, there has certainly been a shift in our belief that the maximal life span is immutable. The possibility of extending the maximum life span in humans has now gone from legend to laboratory. It is being taken very seriously.

Is Man immortal?

In a discussion about mortality and immortality, it is inevitable that the question, " Is Man immortal?" should crop up. My first reaction was to keep well away from this question and not even to mention it. It is not possible to discuss the topic of immortality, which is entirely spiritual, in terms of genetics, which is entirely materialistic. The concept of Man's immortality, which dates back to the earliest cultures, is based on belief rather than on visible and tangible facts. Therefore, I will not try to answer the question "Is man immortal?" Instead I will take as my starting point my personal belief in Man's spiritual immortality. The question that I asked myself became "What is immortal in man if the body dies?" I tried to answer this question for my own personal satisfaction.

We speak in a rather "matter of fact" way, that man and all living things are made up of living matter and of the molecules of life. Is there such a thing as living matter? The genetic material and all that constitutes living cell and organisms are made up of atoms, just like all other matter. These are the same atoms that participate in all of nature's re-cycling of carbon, hydrogen, oxygen, nitrogen and so on. No particular molecule or substance in the cells is living. The body as a whole and its constituent, intact cells are living. If we think of the moment just after death, a person is made up of exactly the same matter as that immediately before death. So what has happened to the matter when an individual dies?

When we speak of "living matter" we are really referring to matter that has been animated by life. Life is the moving force that enables the matter to function in a perfectly harmonised way. Life becomes an integral part of the matter it animates. However, life does not originate of its own accord. Life is transmitted from one generation to the next through two germ cells, which fuse to form a small mass of matter, the zygote.

Here life assumes a new identity, a new individuality and a new character. And as the zygote develops into the human body, this life becomes one with the body with its personality and uniqueness. The individual, component cells share of the same life, the singular genome. When the body dies we are left with inanimate, lifeless matter. There comes a stage when for one reason or another the body is so deranged as a consequence of damage or disease that it can no longer support the life that animated it and dies. That life which had assumed the identity, the individuality, the personality and the character of a person lives on and is truly immortal.

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Errata

The Application of Multivariate Analytical Techniques to the Study of Marine Benthic Assemblages: A Review with Special Reference to the Maltese Islands.

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In the Contents page, the first author's name was incorrect. It should have read *Rene' M. Micallef.*
Also, in Figure 1 (page 10) *Manhatten* was misspelt. It should have read *Manhattan.*

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