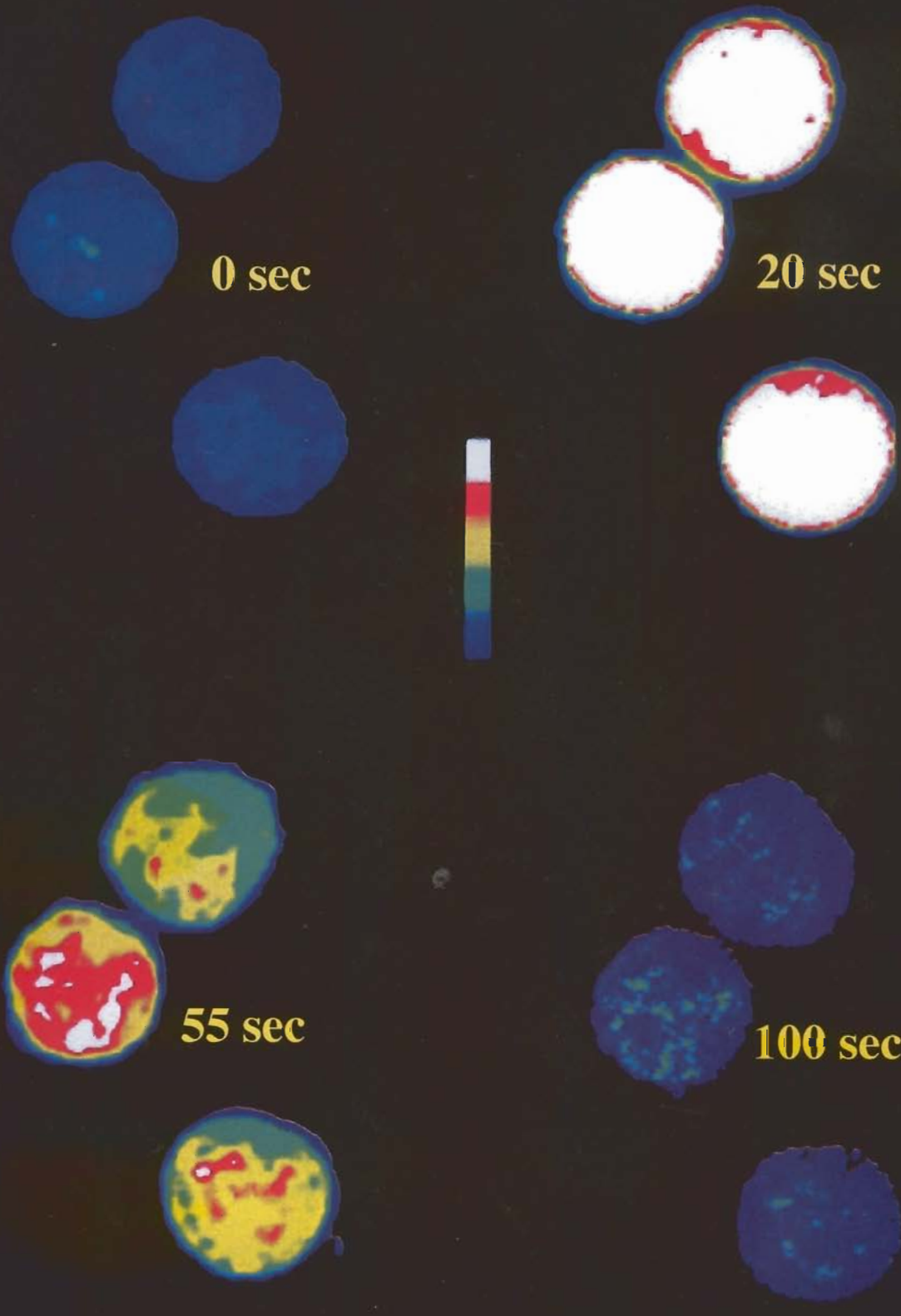
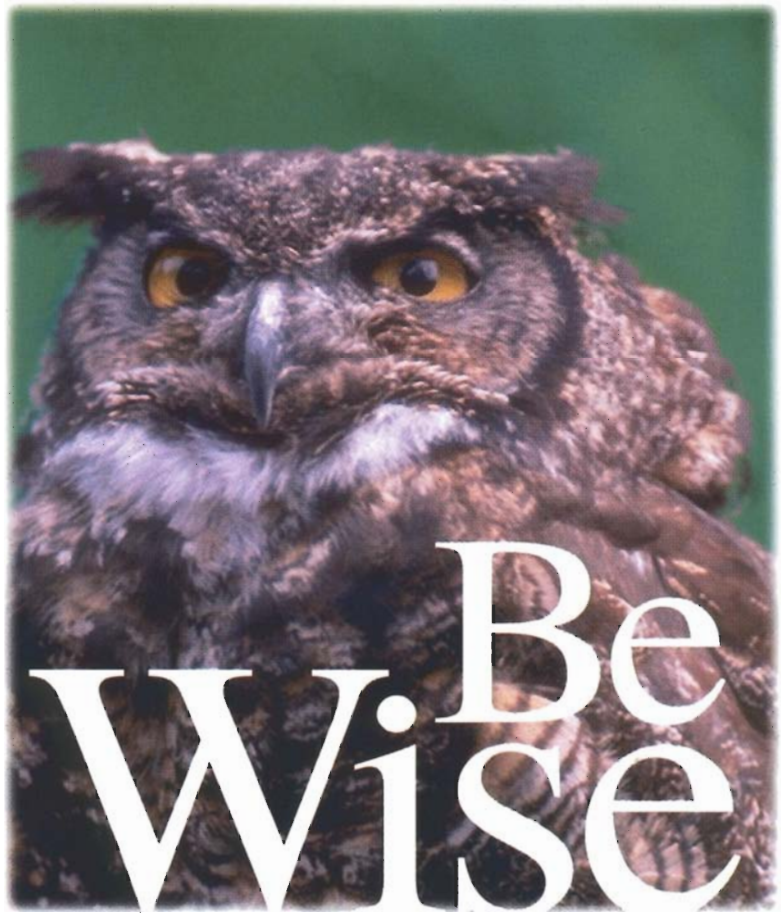
Xjenza

JOURNAL OF THE MALTA CHAMBER OF SCIENTISTS

Volume 2 No 2 December 1997





In a world of stiff competition, being spoilt for choice is inevitable. However making a wise choice is what counts. Mita will spoil you for choice even further. Because Mita offers you a vast range of high speed photocopiers to suit any type of business - from the AO size down to the friendly home-user - backed by a prompt and efficient after-sales service. And above all, it's a Mita.

mita

.... more than just copiers

Strand Electronics Ltd., Multi-Tech Centre, Regional Road (off Valley Road), Msida • Tel 484458/9, 483819 • Fax 483820

A wide range of advanced

SANYO GALLENKAMP

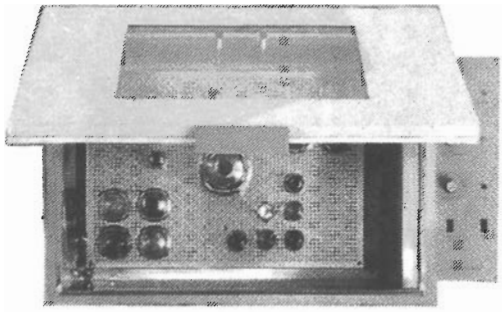
technology in support of medicine and research.

SANYO
-152°C FREEZER

SANYO

REFRIGERATORS & FREEZERS
Up to an unbelievable -152°C

SANYO
MPR 110 MEDICAL REFRIGERATOR



GALLENKAMP ORBITAL INCUBATOR

GALLENKAMP

INCUBATORS
From -10°C to +50°C

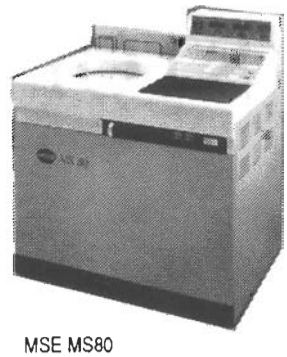
OVENS & FURNACES
From ambient +50°C to 1100°C

GALLENKAMP
OMT 150L OVEN

MSE MICROCENTAUR

MSE

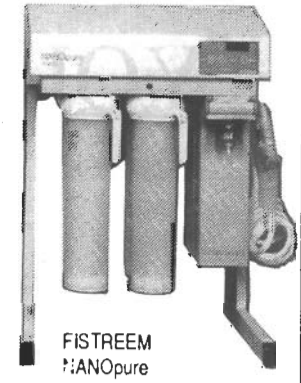
Wide range of Micro to Ultra Centrifuges.



MSE MS80
ULTRACENTRIFUGE

FISTREEM

For ultra pure water with conductivity as low as 0.06 µs cm.



FISTREEM
NANOpure
DEIONIZATION SYSTEM

SANYO

SANYO Gallenkamp PLC

Sole Agents:
ATTARD & CO. LTD.
67, South Street, Valletta.
Tel: 238857, 220804, 237555.

GALLENKAMP MSE FISTREEM Fitotron

BOOKSTOP

YOUR ONE STOP SHOP ON CAMPUS

Tal-Qroqq Tel: 3290 2996 Fax: 311082
Junior College Tel: 225090 Fax: 224169
Gozo Center Tel: 564559
Postal Address PO Box 52, Msida MSD 01, Malta

Contents

Xjenza
Official Journal of the Malta Chamber of Scientists

Editor
Angela Xuereb

Associate Editors
Martin Ebejer
Richard Muscat
Christian A. Scerri
Emmanuel Sinagra

Editorial Address
Xjenza,
c/o University of Malta
Medical School,
Guardamangia.,
Malta.

Tel: (+356) 25951882
Fax: (+356) 235638

Cover Picture: Hepoxilin evoked changes in intracellular calcium in human neutrophils loaded with fluo-3 AM. Colour bar range: blue-white (approx. 150->400 nM calcium). Details on page 38.

Malta Chamber *of* Scientists

**P.O. Box 45,
Valletta B.P.O.,
Valletta, Malta.**

Printers: Printcare, Valletta.

Editorial

Drug and Alcohol Misuse in Malta

The use of alcohol and other drugs in society today has taken a significant twist in that hitherto the problems, related to the misuse and abuse of these substances, were entirely the domain of the professionals involved in the front line. With the advent of the, so called, "permissive society" that, in turn, is fuelled by the increase in material wealth and the need to have it now rather than later, these problems have alas become part and parcel of such a culture and thus of every day living. The media and the political parties have also made important in-roads as far as bringing the problem "out into the open" without necessarily having the answers or the means to address the situation. It is imperative before hastily concocting any half-baked solution to have the necessary information in hand along with the context under which the data was gathered, if one is to tackle a particular facet of the problem in a comprehensive manner.

In the field of jurisdiction, Malta has now acceded to the 1988 United Nations Convention against Illicit Trafficking in Narcotic Drugs and Psychotropic Substances. At present a bill is on its way through Parliament to include psychotropics as well as the conventional narcotics. In 1994, The Prevention of Money Laundering Act was passed and in the same year the Dangerous Drugs Ordinance 1939 was also amended to include coerced treatment and a more complete definition of a trafficking offence. These positive changes in the law following those of 1986, in which stiffer penalties for drug traffickers were introduced along with the freezing and forfeiture of assets, are a serious attempt to limit illicit drug trafficking.

It is now apparent that there is a need to introduce laws in relation to the consumption of alcohol under specific circumstances. One may argue that a "Breathalyser Test" shortly to be introduced is the result of the media hype over the visible increase in fatal traffic accidents. However, there has been no research done to date in this area which would uphold this view. In contrast, conclusive data has been drawn from the European School Study on Alcohol and Other Drugs in 26 European countries, which demonstrated that Maltese sixteen-year olds topped the European order of merit when it came to imbibing wine or spirits three times or more during the last thirty days. Malta also figured high up on the list when it came to age (13-year old or younger) at first use of alcohol. In a further study, attempting to clarify at what age our youngsters first drank alcohol, which was conducted on 9- to 11- year old primary school children, it was found that a significant number of the 400 or so cohort had already imbibed alcohol, mainly wine or beer, procured from home. The recent National Census Study, conducted in 1995, also supports the findings of these two specific studies and provides further evidence that within the population as a whole a number of us Maltese drink more than the recommended WHO directive of 14 units (female) and 21 units (male) of alcohol per week.

There are other situations where it might be prudent to limit the consumption of alcohol, namely, at the place of work. Mark Gauci and Noel Vella, in their article in this issue of Xjenza (see page 24), make a first attempt to establish the attitudes of managers of various industries throughout Malta, towards the use of alcohol and other drugs both on and off the premises. The results of their study have significant implications in the way we approach such problems. Both Caritas and Sedqa, have different programmes to tackle separate aspects of this issue. Caritas mainly involves itself in the preventative and counselling side of things whereas Sedqa, following the results of the Gauci and Vella study, has developed the programme "**SAFE**" which is based on a similar successful American programme. It is envisaged that further talks with the GWU will result in most companies adopting the programme for the safety of both the staff and management. It would be fruitful, however, if the study could be repeated amongst the staff, this time to assess their attitudes towards the use of these substances at work. It is commendable that the authors, Gauci and Vella, have managed to succeed with this first study and I would like to take this opportunity to recommend that they follow this through with the study suggested above.

Finally, it would be of great advantage to all policy makers and strategists in this field if more similar studies were undertaken. Such studies have the potential of providing specific and detailed information without which any policy document or strategy might not be worth the paper it is written on!

Richard Muscat
Associate Editor

Review Article

The Lipoxygenases: a mini review

Cecil R. Pace-Asciak[1] and Santosh Nigam[2]

[1]Research Institute, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario M5G 1X8 and Department of Pharmacology, Faculty of Medicine, University of Toronto, Ontario M5S 1A8, Canada; [2]Eicosanoid Research Division, Department of Gynaecology, University Klinikum Benjamin Franklin, Free University of Berlin, D-12200, Berlin, Germany.

An International Conference on Lipoxygenases, organized by the authors, was held in Malta on May 21-24, 1997. This article reviews the field in general, involving to some extent the topics discussed during that conference. The proceedings of that conference will comprise a series of review articles covering specific aspects of the field. Publication is expected early in 1998 by Plenum Press.

Lipoxygenases (abbreviated LOX or LO) are a group of enzymes derived from a multi-gene family, identified mostly in mammalian systems, but also occur in plants. They incorporate molecular oxygen into an all *cis*-methylene-interrupted polyunsaturated fatty acid to produce a hydroperoxide with a conjugated Z,E-diene structure (Hamberg, 1971). The location of entry of oxygen into the fatty acid depends on the type of lipoxygenase; for example, 5-LO incorporates oxygen at carbon atom 5 in the fatty acid, 12-LO at the 12 position and 15-LO at the 15 position (Yamamoto, 1992). In fact, the various LO proteins have been named according to the point of entry of the molecule of oxygen into the fatty acid. The immediate product from the LO reaction is a hydroperoxide (HPETE) of distinct optical activity (*S*-configuration) (Hamberg, 1971; Nugteren, 1975; Yamamoto, 1991). It is further transformed by other enzymes into a variety of biologically active families of products, including the leukotrienes (or LTs formed from 5*S*-HPETE) (Samuelsson et al, 1987), lipoxins (or LXs formed from 5*S*- and 15*S*-HPETE) (Serhan and Romano, 1995) and hepoxilins (Hx formed from 12*S*-HPETE) (Pace-Asciak, 1994). The various LO and their products have been implicated in a variety of diseases including hypersensitivity reactions and asthma (5-LO) to atherosclerosis (15-LO), diabetes (12-LO), obesity (8-LO), and cancer (12-LO)(vide infra).

Three mammalian LO have been isolated, highly purified and their cDNAs cloned (Yamamoto, 1992; Funk, 1996). They are derived from reticulocytes (15-LO) (Kuhn et al, 1990; Sloane et al, 1990), platelets (12-LO) (Hada et al, 1991) and neutrophils and basophilic leukaemic cells (5-LO) (Furukawa et al, 1984). There are some distinguishing features in these enzymes. All contain a catalytic non-heme iron and a non-catalytic sulphur atom. Functionally, they can be distinguished by their substrate specificities; human platelet 12-LO and basophilic leukaemia cell 5-LO are highly specific for arachidonic acid (AA or ω6-C20:4) and eicosapentaenoic acid (EPA or ω3-C20:5) as substrates, while the porcine 12-LO has a broad spectrum of substrates ranging from ω6 and ω3-C18:2 and :3 to the C20 and C22 series of polyunsaturates. The rabbit reticulocyte 15-LO works best with the C18:2, :3 and C20:3 series (9, Hada et al, 1991; Yokoyama et al, 1986; Takahashi et al, 1988). Recent structural studies have shown great homology between the 15- and 12-LO. In fact site directed mutagenesis of the native 15-LO to replace the residues isoleucine (417) and methionine (418) with the smaller residue, valine, transformed 15-LO activity to form 12*S*-HPETE by the mutant, a product of the 12-LO (Sloane et al, 1991. Additional recent evidence indicated that the amino acid 353 appears to be the primary determinant of product specificity (Borngraber and Kuhn, 1997). Thus, if aa353 is occupied by a small amino acid such as valine, 12-oxygenation is preferred and aa417 and aa418 do not play a role; while if aa353 is occupied by a bulky amino acid such as phenylalanine, then aa417 and aa418 become important for determining 12-or 15-oxygenation. This reflects the extent of penetration of the substrate into the active pocket of the enzyme. Some evidence of this diversity may, in fact, also be evident in the native enzyme as the porcine 12-LO forms a small amount of 15*S*-HPETE in addition to the expected 12*S*-HPETE. Platelet 12-LO is devoid of this activity and forms exclusively 12*S*-HPETE. 5- and 15-LO appear to be multifunctional as their hydroperoxide product is further transformed to an epoxide i.e. 5,6- from 5-HPETE and 14,15- from 15-HPETE (Yamamoto,1991).

Important information on the role of proteins in mammalian physiology and patho-physiology can theoretically be obtained from animals incapable of expressing that protein through gene disruption techniques. Funk et al have bred mice made defective of a specific single LO gene (see review by Funk in the Malta LOX proceedings). They have generated animals deficient in either of the three LO (Chen et al, 1994; Chen et al, 1995; Copeland et al, 1993). Each LO-deficient mouse lacked the ability to form the HPETE specific to that LO. Results have indicated that although LO-gene

disruption did not produce gross abnormalities and hamper the animals' fertility, some interesting insights into the importance of LO and LO products have been obtained.

It is well known that LTs, historically known as SRS-A (slow reacting substance of anaphylaxis) are mediators of hypersensitivity reactions causing potent bronchoconstriction of the human airways (Dahlen et al, 1981; Samuelsson et al, 1980; Samuelsson, 1983). In fact the cysteinyl-LTs contract the isolated airways preparation about 1000-fold greater than that caused by histamine (Dahlen et al, 1980). Considerable interest has therefore been placed by the pharmaceutical industry to produce selective inhibitors of the biosynthesis or action of these compounds. LTB_4, a dihydroxy metabolite formed through the 5-LO pathway, is a potent mediator of inflammation with chemotactic and chemokinetic properties, able to recruit inflammatory cells to the site of injury (Samuelsson et al, 1980; Samuelsson, 1983; Borgeat et al, 1979; Sameulsson et al, 1979). The involvement of the cysteinyl-LTs in asthma is well established, as these compounds are potent smooth muscle constrictors, cause mucus hypersecretion, decrease mucus transport and contribute to the onset of inflammation by causing plasma extravasation and recruiting inflammatory cells (Hay et al, 1995; Dahlen et al, 1983).

5-LO disruption has led to the finding that the knock-out mice were unresponsive to aerosol antigen challenge whereas the wildtype mice developed markedly enhanced cholinergic responsiveness of similar extent to that seen in normal asthmatics (See Funk, proceedings of the Malta LOX conference). Pronounced eosinophilia (70-80% of the total bronchoalveolar lavage) was observed in wildtype mice, whereas eosinophils accounted for a small proportion of the bronchoalveolar cells in the knockout mice. This is the first evidence of the result of a functional deficiency in LT production (through the 5-LO pathway). Of significant interest is the effect of 5-LO deficiency in protection against PAF-induced shock. PAF causes a dose-dependent increase in mortality in wildtype mice, while 5-LO deficient mice show great resilience in this shock model (Chen et al, 1994). This is an important demonstration of the involvement of 5-LO products in shock and the potential rescue by inhibiting the generation of these products. Although this was achieved through LO-deletion, it paves the way for the development of selective 5-LO inhibitors for the pharmacological manipulation of 5-LO. PAF causes the lowering of arterial blood pressure leading to shock and mortality in the wildtype mouse. This is overcome in the 5-LO knockout mice after an intial drop in blood pressure resulting in survival of these mice. This may be due to the known effects of LTs in causing plasma microvascular leakage and bronchoconstriction, vide supra. These studies reaffirm rather conclusively the pathophysiological role of the LTs in asthma and related inflammatory states.

Within the 12-LO pathway, two major pathways have been identified. The extent of direction of the initial 12-LOX product, 12-HPETE, is fine tuned by the action of glutathione peroxidase, an abundant enzyme in the cell cytosol. One pathway involves the reduction of 12*S*-HPETE to 12*S*-HETE by glutathione peroxidase (Bryant and Bailey, 1980), while the second pathway involves the isomerization of 12*S*-HPETE by hepoxilin synthase into the hepoxilins (Pace-Asciak et al, 1983; Pace-Asciak and Martin, 1984; Pace-Asciak, 1984; Reynaud et al, 1994). 12*S*-HETE has been shown to potently promote the attachment of tumour cells to vascular endothelium, implicating a role for this compound in tumour metastasis (Raz et al, 1993; Liu et al, 1994; Tang et al, 1993; Tang et al, 1995). In this process, 12*S*-HETE inhibits cell apoptosis, and promotes tumour cell survival, a condition that favours tumour cell attachment and metastasis. In fact inhibitors of 12-LO appear to favour tumour cell apoptosis and therefore may find application in cancer therapy (see review by Honn in the Malta LOX proceedings). Hepoxilins, the other half of the equation, have been identified as intrinsic factors which regulate platelet cell volume (Margalit et al, 1993) and which play a role in ion (Ca^{2+} and K^{+}) fluxes in the cell (see review in Pace-Asciak, 1994). They have also been implicated in insulin secretion and brain function. Use of leukocyte-type 12-LO knockout mice have not been as successful in identifying a phenotype as has been stated above with the disruption of the 5-LO gene (Sun and Funk, 1996). One should remember that with 12-LO, two types of enzyme have been described, i.e. the platelet-type and the leukocyte-type. Leukocyte-type 12-LO knockouts appear normal, and their macrophages appear to still be able to form about 20% of 15-LO products (normally these are side products of this type of 12-LO) and an enhanced production of 5-LO products (probably through a redirection of fatty acid substrate). The leukocyte-type 12-LO knockout mice appear to have normal behaviour in tumour metastatic potential (Sun and Funk, 1996), while other parameters (involved in hepoxilin action) have not yet been investigated. It is possible that the other phenotypes may become apparent when the platelet-type 12-LO gene is deleted. Currently little information is available on these knockouts, but platelet-type 12-LO knockout mice (see review by Funk in the Malta LOX proceedings) also appear to show similar platelet and megakaryocyte numbers as wildtype mice, and to show similar adherence to extracellular matrix proteins. However, platelets from the deficient mice show enhanced responsiveness to ADP (3 - 4 times) in both in vitro and in vivo thromboembolism studies induced by ADP administration. It is not known which particular 12-LO product may be responsible for this effect, either 12-HETE or any of the hepoxilins. It is attractive to link the known inhibitory effects of 12-HPETE or 12-HETE on platelet thromboxane production and aggregation to the hyperresponsiveness of platelets from 12-LO knockout mice. These experiments would support the involvement of 12-LO in platelet aggregation and tumour metastasis, which requires tumour cell docking at sites on the vascular endothelium rich in platelet clumps. Recent

studies with 12*S*-HPETE have suggested that at micromolar concentrations, 12*S*-HPETE (but not 12*S*-HETE) causes a potentiation of aggregation induced by subaggregating concentrations of arachidonic acid (see review by Lagarde in the Malta LOX proceedings). Hence platelets appear to be 'primed' to aggregate by physiological concentrations of 12*S*-HPETE.

15-LO has been implicated in lipoprotein modification through oxidation of HDL. Together with 5-LO, 15-LO is involved in the synthesis of lipoxins (LX), trihydroxytetraene-containing derivatives of arachidonic acid (Samuelsson et al, 1987; Serhan and Drazen, 1997; Serhan, 1989) LX are formed within the vascular lumen through the interaction of platelets and leukocytes and at mucosal surfaces by leukocyte-epithelial interactions. LX are vasoactive hormones displaying selective actions on human leukocytes including inhibition of neutrophil chemotaxis, neutrophil transmigration through epithelial and endothelial cells. They are consequently involved in multicellular responses such as inflammation, atherosclerosis and thrombosis (see review by Serhan in the Malta LOX proceedings). But to date, no information is available on 15-LO knockouts, or implications of specific phenotypes relating to inhibition of LX formation. But this is certainly an area that we will be hearing about in the near future.

Other LOs are known to exist on the basis of the isolation of the corresponding HETEs although little is known regarding the purification or properties of these LOs. Of the products formed by these LOs, 8-HETE has been shown to have interesting biological actions relating to specific binding to the nuclear receptor, PPARα (Evans, 1997). Peroxisome proliferator activating receptors (PPARs) are members of the steroid/thyroid/retinoid nuclear receptor superfamily of transcriptional factors implicated in the control of lipid metabolism (Lemberger et al, 1996; Devchand et al, 1996). Three receptor subtypes have been identified, α, β and γ (see review in Lemberger et al, 1996). PPARs appear to play a role in the regulation of lipid metabolism (fatty acid oxidation) but recent studies have indicated that these receptors may also be involved in cell growth, differentiation and death and therefore involved in tumour progression. The subtype appears to be upregulated in intestinal epithelial cells in some human polymorphs (Samid et al, 1997; Brockman et al, 1997), but the biological significance of this finding is not yet understood. Prostaglandins of the J_2 type, i.e. 15-deoxy-Δ-12,14-prostaglandin J_2 appear to have the highest activity of all ligands tested to bind to and activate PPARγ promoting differentiation of pre-adipocytes into mature triglyceride forming fat cells (Forman et al, 1995), while 8S-HETE appears to be selective for PPARα (Evans, 1997). The latter appears to regulate fatty acid oxidation. Hence the LOs and their products, may be involved in the regulation of obesity and diabetes as well as in cancer therapy through their actions on PPARs. 8-HETE, among many eicosanoids tested, has also been implicated in oocyte maturation (Meijer et al, 1986).

LO enzymes appear to be derived from separate genes, each consisting of 14 exons located on chromosome 11 (Funk, 1996; Funk, 1993), although the 5-LO appears distinct being located on chromosome 11 in man and central chromosome 6 in the mouse (Chen et al, 1994; Funk et al, 1992). Some LO have a greater degree of homology than others as shown in the following phylogenetic scheme which depicts the various mammalian LO types and the relationship to each other. The x-ray crystal structure of 15-LO from soybean has been reported (Boyington et al, 1994).

LOs have also been proposed in plants and seeds. An 8R-LO has been isolated from the marine coral *P. homomalla* and cloned (Brash et al, 1996), and a complex series of LO products (collectively called oxylipins) have been isolated from the marine red algae and proposed to have osmoregulatory functions (see review by Gerwick in the Malta LOX proceedings). In seeds, the activation of an LO is proposed to be involved in the germination process (Feussner and Kuhn, 1995). 15-LO products analogous to the hepoxilins derived from 12-LO, have been isolated from roots of the common edible garlic (Pace-Asciak et al, unpublished) but its significance is not well understood at this time.

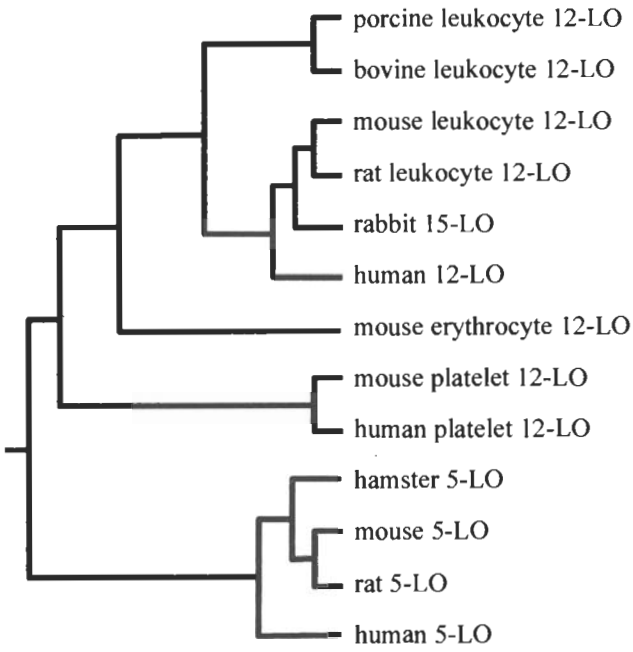


Figure 1. Phylogenetic tree of the LO gene family (courtesy of Dr. Colin Funk, University of Pennsylvania)

Future directions in this field will certainly involve the knockout mouse models, possible involving multiple knockouts, to better understand the physiological and pathophysiological role(s) of specific LOs and their immediate products. Studies with 5-LO knockouts have already provided good evidence of the implication of LTs in asthma. Cancer, obesity, diabetes and cardiovascular disease may be obvious phenotypes to investigate future gene disruption studies.

References

Borgeat P and Samuelsson B (1979) Transformation of

arachidonic acid by rabbit polymorphonuclear leukocytes. Formation of a novel dihydroxy eicosatetraenoic acid. *Journal of Biological Chemistry,* **254**, 2643-2646.

Borngraber S and Kuhn H (1997) Sequence determinants for the positional specificity of mammalian lipoxygenases. *In: Eicosanoids & other bioactive lipids in cancer, inflammation and related diseases, 5th International Conference, La Jolla, California, September 17-20, 1997; Abstract #188.*

Boyington JC, Gaffney BJ, Amzel LM, Doctor KS, Mavrophilipo DV, Mavrophilipos ZV, Colom A and Yuan SM (1994) The x-ray structure and biophysical studies of a 15-lipoxygenase. *Annals of the New York Academy of Sciences,* **744**, 310-313

Brash AR, Boeglin WE, Chang MS and Shieh B-H (1996) Purification and molecular cloning of an 8R-lipoxygenase from the coral Plexaura homomalla reveal the related primary structures of R- and S-lipoxygenases. *Journal of Biological Chemistry,* **271**, 20949-20957.

Brockman JA, Gupta R and DuBois RN (1997) The identification of PPAR activity in intestinal epithelial cells. *In: Eicosanoids & other bioactive lipids in cancer, inflammation and related dieases. 5th International Conference, La Jolla, California, September 17-20, 1997, Abstract #67.*

Bryant RW and Bailey JM (1980) Altered lipoxygenase metabolism and decreased glutathione peroxidase activity in platelets from selenium deficient rats. *Biochemistry and Biophysics Research Communications*, **92**, 268-276.

Chen X-S, Kurre U, Jenkins NA, Copeland NG and Funk CD (1994) cDNA cloning, expression, mutagenesis of C-terminal isoleucine, genomic structure, and chromosomal localizations of murine 12-lipoxygenases. *Journal of Biological Chemistry*, **269**, 13979-13987.

Chen X-S, Naumann TA, Kurre U, Jenkins NA, Copeland NG and Funk CD (1995) cDNA cloning, expression, mutagenesis, intracellular localization, and gene chromosomal assignment of mouse 5-lipoxygenase. *Journal of Biological Chemistry,* **270**, 17993-17999

Chen X-S, Sheller JR, Johnson EN and Funk CD (1994) Role of leukotrienes revealed by targeted disruption of the 5-lipoxygenase gene. *Nature (London),* **372**, 179-182.

Copeland NG, Gilbert DJ, Jenkins NA, Nadeau JH, Eppig JT, Maltais LJ, Miller JC, Dietrich W F, Steen RG, Lincoln SE et al. (1993) Genome maps IV 1993. Wall chart. *Science,* **262**, 67-82.

Dahlen SE, Bjork J, Hedovist P, Arfors KE, Hammarstrom S, Lindgren JA and Samuelsson B (1981) Leukotrienes promote plasma leakage and leukocyte adhesion in postcapillary venules: In vivo effects with relevance to the acute inflammatory response. *Proceedings of the National Academy of Science (USA),* **78**, 3887-3891.

Dahlen S-E, Hansson G, Hedqvist P, Bjorck T, Granstrom E and Dahlen B (1983) Allergen challenge of lung tissue from asthmatics elicits bronchial contraction that correlates with the release of leukotrienes C4, D4 and E4. *Proceedings of the National Academy of Science (USA),* **80**, 1712-1716.

Dahlen S-E, Hedqvist P, Hammarstrom S and Samuelsson B (1980) Leukotrienes are potent constrictors of human bronchi. *Nature (London),* **288**, 484-486.

Devchand PR, Keller H, Peters JM, Vazquez M, Gonzalez FJ and Wahli W (1996) The PPARalpha-leukotriene B4 pathway to inflammation control. *Nature (London),* **384**, 39-43.

Evans RM (1997) Nuclear receptors as targets for hypolipidemic drugs, fatty acids, and eicosanoids. *In: Eicosanoids & other bioactive lipids in cancer, inflammation and related dieases. 5th International Conference, La Jolla, California, September 17-20, 1997, Abstract #60.*

Feussner I and Kühn H (1995) The lipid body lipoxygenase from cucumber seedlings exhibits unusual reaction specificity. *FEBS Letters,* **367**, 12-14.

Forman BM, Tontonoz P, Chen J, Brun RP, Spiegelman, BM and Evans RM (1995) 15-Deoxy-Delta12,14-prostaglandin J2 is a ligand for the adipocyte determination factor PPARgamma. *Cell,* **83**, 803-812.

Funk CD (1993) Molecular biology in the eicosanoid field. [Review, 176 rfs]. *Progress in Nucleic Acid Research. and Molecular Biology,* **45**, 67-98.

Funk CD (1996) The molecular biology of mammalian lipoxygenases and the quest for eicosanoid functions using lipoxygenase-deficient mice. [Review, 139 refs]. *Biochimica et Biophysica Acta,* **1304**, 65-84.

Funk CD, Funk LB, FitzGerald GA and Samuelsson B (1992) Characterization of human 12-lipoxygenase genes. *Proceedings of the National Academy of Science (USA),* **89**, 3962-3966.

Furukawa M, Yoshimoto T, Ochi K and Yamamoto S (1984) Studies on arachidonate 5-lipoxygenase of rat basophilic leukemia cells. *Biochimica et Biophysica Acta,* **795**, 458-465.

Hada T, Ueda N, Takahashi Y and Yamamoto S (1991) Catalytic properties of human platelet 12-lipoxygenase as compared with the enzymes of other origins. *Biochimica et Biophysica Acta.* **1083**, 89-93.

Hamberg M (1971) Steric analysis of hydroperoxides formed by lipoxygenase oxygenation of linoleic acid. *Analytical Biochemistry,* **43**, 515-526.

Hay D, Torphy TJ and Undem BJ (1995) Cysteinyl leukotrienes in asthma: old mediators up to new tricks. *Trends in Pharmacological Science,* **16**, 304-309.

Kühn H, Sprecher H and Brash AR (1990) Occurrence of lipoxygenase products in membranes of rabbit reticulocytes. Evidence for a role of the reticulocyte lipoxygenase in the maturation of red cells. *Journal of Biological Chemistry,* **265**, 1454-1458.

Lemberger T, Desvergne B and Wahli W (1996) Peroxisome proliferator-activated receptors: a nuclear receptor signaling pathway in lipid physiology. *Annual Review of Cell and Developmental Biology,* **12**, 335-363.

Liu B, Maher RJ, Hannun YA, Porter AT and Honn KV (1994) 12(S)-HETE enhancement of prostate tumour cell invasion: Selective role of PKCalpha. *Journal of*

the National Cancer Institute, **86**, 1145-1151.

Margalit A, Sofer Y, Grossman S, Reynaud D, Pace-Asciak CR and Livne A (1993) Hepoxilin A3 is the endogenous lipid mediator opposing hypotonic swelling of intact human platelets. *Proceedings of the National Academy of Science (USA),* **90**, 2589-2592.

Meijer L, Brash AR, Bryant RW, Ng K, Maclouf J and Sprecher H (1986) Stereospecific induction of starfish oocyte maturation by (8R)-hydroxyeicosatetraenoic acid. *Journal of Biological Chemistry,* **261**, 17040-17047.

Nugteren D-H (1975) Arachidonate lipoxygenase in blood platelets. *Biochimica et Biophysica Acta,* **380**, 299-307.

Pace-Asciak CR (1984) Arachidonic acid epoxides. Demonstration through oxygen-18 labeled oxygen gas studies of an intramolecular transfer of the terminal hydroxyl group of 12S-hydroperoxy-eicosa-5,8,10,14-tetraenoic acid to form hydroxy epoxides. *Journal of Biological Chemistry,* **259**, 8332-8337.

Pace-Asciak CR (1994) Hepoxilins: a review of their cellular actions. *Biochimica Biophysica Acta,* **1215**, 1-8.

Pace-Asciak CR and Martin JM (1984) Hepoxilin, a new family of insulin secretagogues formed by intact rat pancreatic islets. *Prostaglandin Leukotriene and Medicine,* **16**, 173-180.

Pace-Asciak CR, Granström E and Samuelsson B (1983) Arachidonic acid epoxides: Isolation and structure of 2 hydroxy epoxide intermediates in the formation of 8,11,12-trihydroxy eicosatrienoic acid and 10,11,12-trihydroxy eicosatrienoic acid. *Journal of Biological Chemistry,* **258**, 6835-6840.

Raz A, Silletti S, Timar J and Honn KV (1993) Effect of 12-HETE on the expression of autocrine motility factor-receptor and motility in melanoma cells. *In: Eicosanoids and other bioactive lipids in cancer, inflammation and radiation injury, Ed. S. Nigam, L. J. Marnett, K. V. Honn, T. L. Walden Jr., Kluwer Academic Publishers (London), pp 645-649.*

Reynaud D, Demin P and Pace-Asciak CR (1994) Hepoxilin A_3 formation in the rat pineal gland selectively utilises 12(S)-HPETE but not 12(R)-HPETE. *Journal of Biological Chemistry,* **269**, 23976-23980.

Samid D, Wells M, Kulkarni M, Liu L and Thibault A (1997) The nuclear receptors PPARs as novel targets in cancer therapy: experience with phenylacetate and analogous aromatic fatty acids. *In: Eicosanoids & other bioactive lipids in cancer, inflammation and related dieases. 5th International Conference, La Jolla, California, September 17-20, 1997, Abstract #66.*

Samuelsson B (1983) Leukotrienes: Mediators of immediate hypersensitivity reactions and inflammation. *Science,* **220**, 568-575.

Samuelsson B Borgeat P Hammarstrom S and Murphy RC (1980) Leukotrienes: a new group of biologically active compounds. *Advances in Prostaglandin and Thrombosis Research,* **6**, 1-6.

Samuelsson B, Borgeat P, Hammarstrom S and Murphy RC (1979) Introduction of a nomenclature: Leukotrienes. *Prostaglandins,* **17**, 785-787.

Samuelsson B, Dahlen SE, Lindgren AA, Rouzer CA and Serhan CN (1987) Leukotrienes and Lipoxins: Structures, Biosynthesis and Biological Effects. *Science,* **237**, 1171-1176.

Serhan CN (1989) On the relationship between leukotriene and lipoxin production by human neutrophils: Evidence for differential metabolism of 15-HETE and 5-HETE. *Biochemica et Biophysica Acta,* **1004**, 158-168.

Serhan CN and Drazen JM (1997) Antiinflammatory potential of lipoxygenase-derived eicosanoids: A molecular switch at 5 and 15 positions? *Journal of Clinical Investigation,* **99**, 1147-1148.

Serhan CN and Romano M (1995) Lipoxin biosynthesis and actions: Role of the human platelet LX-synthase. *Journal of Lipid Mediation in Cell Signalling,* **12**, 293-306.

Sloane DL, Browner MF, Dauter Z, Wilson K, Fletterick RJ and Sigal E (1990) Purification and crystallization of 15-lipoxygenase from rabbit reticulocytes. *Biochemical and Biophysical Research Communications,* **173**, 507-513.

Sloane DL, Leung R, Craik CS and Sigal E (1991) A primary determinant for lipoxygenase positional specificity. *Nature (London),* **354**, 149-152

Sun D and Funk CD (1996) Disruption of 12/15-lipoxygenase expression in peritoneal macrophages. Enhanced utilizationof the 5-lipoxygenas pathway and diminshed oxidation of low density lipoprotein. *Journal of Biological Chemistry,* **271**, 24055-24062.

Takahashi Y, Ueda N and Yamamoto S (1988) Two immunologically and catalytically distinct arachidonate 12-lipoxygenases of bovine platelets and leukocytes. *Archives of Biochemistry and Biophysics,* **266**, 613-621.

Tang DG, Grossi IM, Chen YQ, Diglio CA and Honn KV (1993) 12(S)-HETE promotes tumour-cell adhesion by increasing surface expression of alphaVbeta3 integrins on endothelial cells. *International Journal of Cancer,* **54**, 102-111.

Tang DG, Renaud C, Stojakovic S, Diglio CA, Porter A and Honn KV (1995) 12(S)-HETE is a mitogenic factor for microvascular endothelial cells: Its potential role in angiogenesis. *Biochemical and Biophysical Research Communications,* **211**, 462-468.

Yamamoto S (1991) "Enzymatic" lipid peroxidation: Reactions of mammalian lipoxygenases. *Free Radical Biology in Medicine,* **10**, 149-159.

Yamamoto S (1992) Mammalian Lipoxygenases: Molecular Structures and Functions. *Biochimica Biophysica Acta* **1128**, 117-131.

Yokoyama C, Shinjo F, Yoshimoto T, Yamamoto, S, Oates JA and Brash AR (1986) Arachidonate 12-lipoxygenase purified from porcine leukocytes by immunoaffinity chromatography and its reactivity with hydroperoxyeicosatetraenoic acids. *Journal of Biological Chemistry,* **261**, 16714-16721.

Research Article

Effective Assessment of Professional Development in Primary Science: A Case Study from the Open University, UK – Primary Teachers Learning Science

Susan Tresman *with* Valda Stevens and Sue Spurr
Centre for Science Education, Open University, Science Faculty, Walton Hall, Milton Keynes U.K.

Keywords: primary science, professional development, primary teachers.

The introduction of the national curriculum in science in England and Wales has resulted in a significant change in the emphasis and content of courses which promote professional development for teachers of primary aged children.

Work at the Centre for Science Education (CSE) at the Open University (OU) in England over the past six years has focused on developing courses in partnership with LEA advisory teams, mentored by academic staff at the CSE. These courses are based on distance learning materials which provide additional subject knowledge in science at the level of the teacher and explore ways in which links can be made between subject content and pedagogy.

Teachers for whom these courses are designed are generally experienced primary practitioners but they are likely to have low levels of subject knowledge and confidence in science.

In this paper, the impact on teaching and learning in primary schools of a new course providing professional development in primary science, 'Primary Teachers Learning Science', is considered via its assessment strategy. The assessment aims to reinforce the learning objectives of the course and help participants to acquire and apply appropriate concepts and skills.

What is to be assessed? An outline of the course.
Designing a course which is both accessible, challenging and appropriate to experienced primary teachers requires explicit links to be made between selected content and practice, so that the teachers' investment in time and effort in the course will be professionally relevant.

This was one of Harlen's main thrusts in her research into the development of science in the Primary School (Harlen 1992).

Six Workbooks are provided that focus on different areas of science and its teaching in school and these are linked with BBC broadcasts for teachers and children. The Workbooks are entitled Life: Diversity and Evolution; Materials: Chemical and Physical Changes; Forces and Energy; Electricity: Making Connections; The Planet Earth and Ecosystems. The Course Team advise Local Education Authority (LEA) groups on planning an appropriate in-service training (INSET) programme around the course materials. This programme will vary according to local needs and finances. All students have a tutor who provides support through face to face and distance contacts. Tutorial support is available to assist teachers' preparation and planning for assessment.

The 'Primary Teachers Learning Science' Workbooks take the reader through a learning process of exploring — planning — implementing — reviewing in a reflective learning cycle, based on the Mackinnon cycle (1987). Such a model for learning facilitates a move forward into new learning through the ability to process information gleaned during the course and insights gained with experience to move forward into new learning (Baird et al, 1991). The assessment woven into the course is a key element in establishing whether new learning has occurred and whether teachers have been able to use this new knowledge to make changes to established practice. Two strands of assessment are likely to be particularly revealing in establishing whether this has occurred: personal learning and the associated learning strategies and their impact on practice.

Effective guidance is required for the process of learning and its application to practice and to assist teachers in the production of assessed pieces of work. This is outlined in the opening section of each of the six workbooks and supported by tutors during the course.

Teachers are introduced to the concept of a learning journey which includes developing a model of reflective learning. In this way, teachers are encouraged to become actively involved in the learning process and to document and take responsibility for discovering changes which occur in themselves as they implement their learning.

There are explicit statements on general and subject specific outcomes expected after studying each workbook; performance criteria relevant to each specific outcome are given as is guidance in how to use them to map out an appropriate route through the workbook.

The Learning File

Teachers are asked to compile a learning file (in a ring binder which they receive as part of the course materials) to enable them to keep a record of their learning journey through notes, responses to activities, inclusion of pieces of children's work, results of reflection etc. Since most learning takes place when we are actively processing information (Wood, 1988) the activities and questions in the workbook are designed to assist teachers in achieving their chosen personal learning outcomes for a given area of science and absorbing these into their science teaching and 'practical' theory as described by Handel and Lauvas (1987).

Learning files can be considered as a record or evidence of their work in science. They might be used to contribute to course assignments for the OU's Certificate in Science for Primary Teachers, as the basis for a claim for a vocational qualification, in an application for promotion or a new job, or in staff development within their school.

Assessment strategy

Assessment aims to provide teachers with opportunities to demonstrate the processes involved in the development of science knowledge and skills in the 'real' teaching context. Teachers are required to evaluate their learning in science and apply their learning in their professional capacities.

As already implied, self assessment plays a leading role in evaluating learning. Within the workbooks are embedded activities designed to develop teachers' skills in interaction with concepts and ideas in science and science teaching introduced in the text. 'Core skills activities' develop skills that underpin all learning and are intended to assist evaluation of learning and professional performance using cyclical, reflective learning processes (Tresman and Edwards, 1993). Responses to 'Core skills activities' may contribute in the process of planning and structuring of tutor marked assignments.

Other activities are concerned with exploring specific concepts linked to the subject areas covered in the particular workbook. These are structured to develop knowledge and ideas, reinforce understanding and provide opportunities to practice and apply newly acquired concepts.

The Summative Assessment for the course comprises two assignments and one project. All are included in a Course and Assessment Guide provided to teachers and tutors at the outset of the course. All count towards the final assessment score. They have a dual focus: on personal learning; how the processes of learning science as an adult occur and on professional learning, i.e. how the subject knowledge is used within the context of primary teaching in science.

Tutors are briefed on the course and assessment strategy and are provided with detailed guidance notes for grading assignments. Feedback, through correspondence tuition, is provided to all students.

Assignment marking is monitored by a member of the Course Team or experienced Course Tutor as part of the quality assurance process to check that grading and type and amount of teaching comments are appropriate. Feedback and suggestions are given to tutors through the monitoring process to assist them in their support of students to critically reflect on their learning and its application to their classroom teaching.

The First Strand: personal learning in science

The first strand of the personal learning journey requires teachers to analyse the methods they use to learn science during the early stages of the course.

As part of the first assignment, they examine their progress in acquiring scientific knowledge in one area of science of their choice. They do this by constructing an audit of knowledge and skills after working on the particular section of the course in question. Estimations of progress in science have to be illustrated by evidence (derived from the course) in support of the statements contained in the audit, e.g. responses to related activities in the workbooks. A self-assessment of their competence in teaching this area of science and confidence in supporting colleagues in their learning and teaching of the same is an integral part of the audit.

In an analysis of responses to this first assignment, teachers clearly explained areas of uncertainty in their knowledge and skills at the start of the course. Many had difficulty realising precisely what they did not know until they began study, after confronting areas that were unfamiliar. Many demonstrated improvements in the first 3 months of the course, and there was a readiness to take knowledge beyond that needed in the primary classroom as personal development by some teachers.

Many teachers

- felt confident about knowledge in many science areas up to Key Stage 1 but not beyond
- felt that they lacked confidence in teaching/supporting colleagues and this was due to limited knowledge and understanding in science
- hoped that an increase in knowledge understanding would heighten their own awareness and stimulate interest in science which would
- enable them to feel more confident in supporting colleagues and identify more easily with children learning science.

At this stage, many students had difficulty in providing evidence in support of an evaluation of their current levels of knowledge. In some cases, tutors needed to give guidance on disentangling knowledge and skills when students considered their current levels and priorities for development. Priorities for future development were based on the teachers' science needs but also on the needs

of their schools and there were tensions when these two needs were not the same and where the latter needs were imposed by senior management teams/emphasis of school development plans. Support of the headteacher and colleagues was significant in fostering a positive approach to the course.

The personal learning strand is developed progressively in the second assignment. Students are required to give evidence (e.g. responses to Workbook activities) to show progression in understanding in a given area of science. This may build on priorities given in their previous audits. Teachers are guided to discuss strategies used to develop their ideas in a particular area of science and how they confronted difficult concepts, analysing why these ideas were problematic, and discuss strategies used to overcome them.

In the second assignment, the teachers described a number of ways in which they had worked to develop their knowledge in science and to confront difficult concepts. These included:

- reading and re-reading text,
- discussion with colleagues (found to be particularly useful by many teachers), family, friends and other students on the course,
- performing tasks in the course activities,
- making notes,
- drawing diagrams,
- participating in activities at tutorials,
- constructing concept maps,
- thinking of everyday/familiar i.e. real life examples helps to clarify ideas,
- making own glossary of terms — writing own definitions from memory — more effective than memorising or copying from text book
- 'reading round' — where time permits including visits to museums, lectures, TV and radio programmes as well as other books
- constructing learning timetable helped to provide focus as opposed to 'meandering through the workbooks'
- breaking text up into small chunks i.e. proceed in small steps (perhaps in conjunction with above)

Sometimes it was necessary to leave the task and return to it at a later time because of an initial lack of success. Allowing time to think and reflect on complex ideas was recognised as very important — time needs to be allowed to assimilate information. It was noted that this has interesting implications for the notion of primary aged children remaining 'on task' in order for learning to be effective.

A number of teachers commented that thinking about how they learned helped them to recognise that 'because I tell the children something does not mean that they will be able to accept it for themselves'. Some also commented that they recognised that their own role learning in the past had not led to sound understanding. The teachers became more aware of their own learning strategies, which in turn helped them to recognise the different strategies their pupils adopt. They could more readily identify with the situation of pupils confronted with new concepts. Some teachers also had a heightened awareness of the need to help children to understand how to select what is relevant in their ideas and observations and what to reject. They also highlighted the need to promote interaction between ideas and observations if learning in science is to be supported.

Teachers' evaluations of the learning and development achieved on the course, along with their expectations of the durability of the impact this will have on their practice are further developed in the project. The course takes each teacher on a personal learning journey where each finds their own level and makes progress on an individual basis.

The second strand: teaching science

Here the focus switches to an analysis of approaches to teaching science, requiring a critical evaluation of practice from a variety of perspectives. The balance of tasks and their timing is crucial here, so that they build sequentially, coherently and progressively across a period of a number of months.

The SPACE project constructivist approach (Russell and Watt, 1990) has been a major influence in bringing together ideas about the processes of learning and teaching of science concepts building on what children already know. The main focus of the teaching strand in the first assignment is to enable teachers to find out, and then use, the ideas which children hold about a given concept in science to plan learning objectives and select appropriate activities to help children realise them. In the second assignment these activities are taught and the learning experiences of the children and the contribution of the selected activities to that learning are analysed.

The children's learning is evaluated and appropriate evidence provided to support the analysis. The teaching is evaluated in terms of its appropriateness in terms of realising the original learning objectives identified in the first assignment.

Through 'bridging' questions, explicit links between acquisition of an enhanced personal knowledge in science and impact on practice are explored. A review of professional practice is sought, with supporting evidence, which demonstrates how practice has changed or modified as a result of studying the course.

In the first assignment some teachers experienced difficulty in focusing on a defined concept — the area of knowledge they selected and presented was too broad. This then led to difficulties in defining specific learning objectives for children and providing appropriate activities which were not too ambitious. Providing practical activities does not necessarily mean that learning will take place, and being able to spot when children have

difficulties or misconceptions has implications for the personal knowledge of teachers. Increased knowledge and understanding by the teachers appeared to enable them to intervene more effectively and support pupil learning. Tutor feedback on the assignment encouraged greater precision in stating concepts — breaking down complex ideas into a number of points.

Teachers used a variety of methods for finding out pupils' existing ideas in a science area.

Methods used to find out about children's ideas included:

- concept maps (useful tool for establishing misconceptions but does not give insight into extent or level of understanding)
- open questions (allows children to answer individually, to share ideas but depends on language/listening skills/confidence of children; particularly useful as it does not depend on reading/writing skills
- sorting (useful 'hands-on' method which does not depend on reading/writing skills) on-going diary (time consuming but valuable; children (or teaching on their behalf) recording their own ideas.

Many teachers used a combination of methods eg concept mapping coupled with questions to children individually or in small groups. Methods of recording ideas included:

- taped recording of open questions/dialogues occurring between children as they shared ideas. These proved invaluable for accurately establishing and subsequently analysing children's ideas in order to plan learning objectives. However, it was recognised that these strategies were more difficult to implement in whole class situations — and had significant resource implications.

Many teachers had difficulty selecting methods that were entirely effective in leading to detailed learning objectives for each child in their selected group. Many commented that they had tried concept mapping for the first time, found it worked very well, and would use this method again.

In their second assignment, teachers commented on their increasing confidence in planning and teaching areas of science where they previously had little confidence — or which they had avoided. Teachers acknowledged that they had avoided areas, particularly Forces, because of their lack of knowledge and understanding and correspondingly low levels of confidence in their ability to teach effectively. A wider knowledge of science concepts enabled them to provide a wider spectrum of appropriate activities in the classroom.

Many teachers recognised the beginning of changes in their practice. Notable was finding out children's existing ideas at the start of a topic and to plan the teaching to begin from 'where the pupils are at as opposed to where the teacher thinks they are at'.

The learning processes that the teachers were experiencing and their reflections on learning strategies were beginning to impact on the ways in which they approached science work in the classroom. The emphasis was moving away from 'telling' information and providing very structured activities towards more questioning and probing of children's ideas and this helped to make science more enjoyable!

Teachers noted that children also need time to reflect on what they have done and learned. Often they tried to cover too much in a given time. Increased knowledge in an area of science appears to give the teacher increased confidence to encourage children to articulate where they are still confused, so that the teacher can plan appropriate further work.

The second assignment demonstrated that working on the course had helped teachers broaden their knowledge of science and begin to reconsider their ideas on how to teach it more effectively in the classroom.

The project

The classroom based research project represents a shift in the level of demand to build on previous work and provides a challenge in the context of whole school science. In each case, teachers are required to demonstrate, through evidence, how they have translated their own science knowledge and understanding to inform their practice in the chosen area. Teachers are encouraged to include or make reference where appropriate to work from previous assignments.

A choice of three contexts is provided:

- to write a report of a project to develop a portfolio of assessed and moderated children's work in one area of science for use in school;
- to write a report of a project to plan science in the curriculum over a range of time scales;
- to write a report on the planning, running and evaluation of a science school-based in-service training session.

The evidence to show the range of contributions the project has made to assessing personal and professional development in primary science and the potential for primary teachers to work as researchers in their classrooms, will be considered in a later paper.

Conclusions

Evidence produced in an examination of responses by teachers to the assessment events punctuating the course indicates that this assessment strategy can contribute to a reinforcement of learning objectives by requesting teachers to analyse the extent and nature of their learning journey in science.

A measure of their acquisition of science concepts and skills is provided in the nature of students' responses to questions about personal learning. Some of the ways in which this learning has informed and impacted on practice have been revealed in answers to classroom-focused questions in both tutor marked assignments. There has been a move towards identifying child starting points and planning learning outcomes.

The personal assessment of professional development has revealed increasing confidence in understanding science and transferring the acquired knowledge and skills to the classroom situation through a constructivist approach in teachers' own learning and teaching.

References:

Baird J, Fensham P, Gunstone R and White R (1991) The importance of reflection in improving science teaching and learning. *Journal of Research in Science Teaching*, **28**, 163–182.

Harlen W (1992) Research and the development of science in the Primary School. *International Journal of Science Education*, **14**, 491–503.

Handel G and Lauvas P (1987) The Practical Theory of Teachers. *Promoting Reflective Teaching: Supervision in Action*, SRME and Open University Press.

Mackinnon A (1987) Detecting reflection-in-action among preservice elementary science teachers. *Teaching and Teacher Education*, **3**, 135–145.

Russell T and Watt D (1990) Science Processes and Concept Exploration (SPACE): Growth, Liverpool University Press, Liverpool.

Tresman S and Edwards D (1993) Reflecting on practice: some illustrations. *Challenges and Opportunities for Science Education*, Paul Chapman, pp.27–43.

Wood D (1988) How Children Think and Learn, Blackwell, Oxford.

Research Article

Mu, Delta and Kappa Opioid Receptor Involvement in the Hypothermic Response to Caffeine and Theophylline

Ivan Debono and Charles Scerri
Department of Biomedical Sciences, University of Malta, Msida MSD06, Malta.

Summary. *This study describes the effect of the methylxanthines, caffeine and theophylline, on core body temperature (T_b) of unrestrained mice at normal ambient temperature. Acute administration (IP) of caffeine and theophylline produced a dose-dependent hypothermia; caffeine induced hypothermia was of a greater magnitude and longer time-course than theophylline. The hypothermic response was attenuated (50%) by naloxone HCl, a peripheral and central non-selective opioid antagonist, showing that methylxanthine-induced hypothermia is partly mediated by opioid receptors. In part the hypothermic response appeared to be naloxone-insensitive (50%) indicating that other mechanisms may mediate this effect. Only theophylline-treated mice exhibited an attenuation by 25% of this response when pretreated with naloxone methiodide which only acts peripherally, indicating that part of the opioid receptor mediation of theophylline-induced hypothermia is dependent on a peripheral mechanism. No attenuation occurred when theophylline- and caffeine-treated mice were pretreated with low-dose naloxone HCl, a Mu- selective antagonist while naltrindole HCl, a Delta-selective antagonist produced a mild attenuation. The greater attenuation produced by nor-binaltorphimine, a Kappa-selective antagonist, would suggest that the Kappa receptors are mainly responsible for the opioid receptor mediation of both caffeine- and theophylline-induced hypothermia. The acute effects of caffeine and theophylline on thermoregulation are not unlike those of morphine and endogenous opioids. The pharmacological, clinical and biological significance of the thermoregulatory effects of caffeine and theophylline are discussed.*

Keywords: *caffeine, theophylline, naloxone, naltrindole, nor-binaltorphimine, body temperature, mouse*

Drugs exerting their effects through the opioid system have profound effects on body temperature. The particular effect seen is dependent on species, ambient temperature, degree of restraint imposed on the subject and route of drug administration (Adler et al, 1988).

Moderate to high doses of caffeine (>30mg/kg) have been reported to induce dose-related reductions in core body temperature in rats and mice (Durcan et al, 1991) kept at ambient temperatures in the standard range. Doses of caffeine in the range from 37.5mg - 300mg/70kg in stimulant drug abusers have also been reported to cause up to 4°C drop in skin temperature (Rush et al, 1995).

There are a number of reports of methylxanthines exerting their effects through interactions with opioid receptors, although the majority of studies have used caffeine. High doses of caffeine and other methylxanthines have been reported to potentiate the antinocioceptive actions of opioid receptor agonists such as morphine (Misra et al, 1985; Sweeny et al, 1991; Nicholson et al, 1991). Narcotic administration particularly of morphine sulphate and fentanyl can be safely carried out in the preterm infant when using intravenous caffeine simultaneously to offset the risk of apnea (Mainous, 1995).

It has been reported that naloxone hydrochloride (a non-selective opioid antagonist) attenuates the hypothermic action of caffeine (Durcan et al, 1992). The attenuation of the hypothermic effect should apply to other methylxanthines provided that the dose of naloxone used does not have an intrinsic hypothermic effect. Further studies are thus needed to fully define the role, precise mechanisms and the extent of interaction.

At present there are no studies on the involvement of the main opioid receptor types (i.e., *mu, delta* or *kappa*) in the hypothermic effect caused by methylxanthines. Discrimination between the individual involvement of each opioid receptor type would enable the determination of the exact molecular target of methylxanthines as well as to determine the involvement of central and/or peripheral effects.

In this series of experiments reported here we examined whether the hypothermic effects of caffeine and theophylline are opioid-dependent, receptor type specific and peripheral or central in origin with respect to the opioid system.

Materials and methods

Subjects

Twenty male albino mice (inbred), 8 weeks old, weighing 24g - 34g at the start of the experiment were used. They were singly housed on a 12:12 hr light:dark cycle with food and water available *ad libitum* except during testing. The mice were handled daily to reduce stress trauma to a minimum.

Temperature studies were carried out in the animal house

(temperature 20 ± 1oC and 60% ± 5% relative humidity). Experiments were performed between 10.00 hrs and 17.00 hrs and were carried out every 4 days to ensure wash out and complete metabolism of the drug.

Before the experiments were initiated, the mice were acclimatised to the surrounding environment for a period of 15 days. On each experimental day, food was removed from the cage (but not water). Food consisted of sunflower seeds, wheat, green rice, peas, sorghum seeds and green pellets mainly made up of dehydrated alfalfa meal (Kik Rico, Encia, Italy).

Temperature Recording

Core body temperature was measured using a rectal thermistor probe for mice and a digital thermometer (Physitemp Inc. formerly Sensortek Inc. New Jersey, USA). The probe was inserted 2.5 cm into the colon of each mouse.

During temperature measurements, the mice were unrestrained and were simply held gently at the base of the tail. A baseline temperature was taken before the animals were treated.

Procedure

In PART I of the study 2 groups of mice (N = 10), either received caffeine or theophylline. Animals were injected intraperitoneally (i.p.). 5 doses of caffeine and theophylline were selected: vehicle, 30mg/kg, 60mg/kg, 90mg/kg and 120mg/kg. These doses were administered in a couterbalanced order. Caffeine and theophylline (aminophylline) were dissolved in distilled water and then warmed for complete dissolution.

Colonic temperature of each mouse was taken every 2 minutes in the injecting sequence such that 20 minutes would have elapsed between injection and the first temperature recording. Colonic temperatures were recorded every 20 minutes for each mouse for a period of 140 minutes post drug administration.

In PART II of the study 2 groups of mice (N = 10), received a fixed dose of naloxone followed by either a fixed dose of caffeine or theophylline. From Part I of the study it was determined that 90 mg/kg caffeine and 120 mg/kg theophylline would be used as the fixed dose. 3 mg/kg naloxone was selected on the basis of previous reports in the literature (Durcan et al, 1992). 1 mg/kg naloxone methiodide, injected subcutaneously, was also selected on the basis of previous reports in the literature (Bhandari et al, 1992).

Each mouse received the following combinations in a counterbalanced order: Vehicle followed by vehicle, Vehicle followed by caffeine/theophylline, Naloxone HCl followed by vehicle, Naloxone HCl followed by caffeine/theophylline, Naloxone MeI followed by vehicle or Naloxone MeI followed by caffeine/theophylline.

Each mouse received 2 injections. Treatment sequence adopted was such that each treatment group (caffeine or theophylline) received their 2 administrations separated by a lag time of 15 minutes. 20 minutes following the second injection temperature recording (T_{20}) was started according to the injecting sequence. Prior to each injection a baseline colonic temperature was recorded.

In PART III of the study 2 groups of mice (N = 10), received a fixed dose of a selective opioid receptor antagonist followed by either a fixed dose of caffeine or theophylline. 90 mg/kg caffeine and 120 mg/kg theophylline were selected as the fixed dose. 0.3 mg/kg Naloxone hydrochloride (low dose), a selective *mu* opioid receptor antagonist was administered intraperitoneally (i.p.). The dose and route of administration was selected on the basis of previous reports in the literature (Kamei et al, 1992). 10.0 mg/kg Naltrindole hydrochloride, a selective *delta* opioid receptor antagonist was administered intraperitoneally (i.p.). The dose and route of administration was selected on the basis of previous reports in the literature (Toyoshi et al, 1992). 5.0 mg/kg Nor-Binaltorphimine, a selective *kappa* opioid receptor antagonist was administered subcutaneously (s.c.) in the nape of the neck. The dose and route of administration was selected on the basis of previous reports in the literature (Suzuki et al, 1992; Endoh et al, 1992).

Each mouse received the following combinations in a counterbalanced order: Vehicle followed by vehicle, vehicle followed by caffeine/theophylline, Naloxone HCl followed by vehicle, Naloxone HCl followed by caffeine/theophylline, Naltrindole HCl followed by vehicle, Naltrindole HCl followed by caffeine/ theophylline, Nor-binaltorphimine followed by vehicle, Nor-binaltorphimine followed by caffeine/ theophylline.

Each mouse received 2 injections. The lag time between the first and second treatment (I2) was 15 minutes for naloxone hydrochloride and 15 minutes for Naltrindole hydrochloride (based on previous reports by Toyoshi et al, 1992), 2 hours for Nor-Binaltorphimine (based on previous reports by Suzuki et al, 1992; Endoh et al, 1992). 20 minutes following the second injection temperature recording (T_{20}) was started according to the injecting sequence. Prior to each injection a baseline colonic temperature was recorded.

Drugs

Anhydrous caffeine (Sigma Chem. Co., Poole, UK), Theophylline as aminophylline (Sigma Chem. Co., Poole, UK), Naloxone hydrochloride (Sigma Chem. Co., Poole, UK), Naloxone methiodide (Research Biochemicals Inc., Natick, USA), Nor-Binaltorphimine (Research Biochemicals Inc., Natick, USA), Naltrindole hydrochloride (Research Biochemicals Inc., Natick, USA).

Statistics and Analysis

Data were analysed using analysis of variance (ANOVA) supplemented where appropriate by tests of simple main effects. A two way analysis with drug/drug dose and time as within subject variables were used for the three studies. All data are shown as means of change in temperature from baseline at 20 minute intervals for 140 minutes.

Results

Acute effects of caffeine on body temperature

Caffeine, produced a dose-dependent decrease in body temperature which followed different time-courses, main effects of dose [F(4, 36) = 11.93, p<0.001], and time, [F(7, 63) = 37.20, P<0.001], and the dose x time interaction, [F(28, 252) = 6.76, P<0.001].

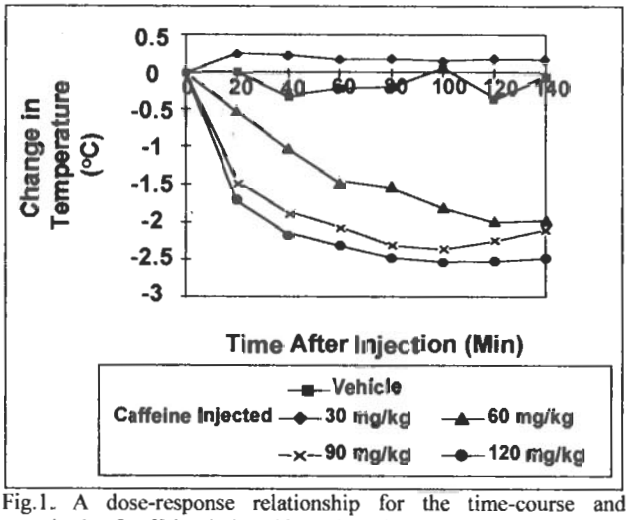


Fig.1. A dose-response relationship for the time-course and magnitude of caffeine-induced hypothermia in mice,

The onset of the caffeine-induced hypothermic effect was dose dependent. The 60 mg/kg induced an initial (20 minutes) drop in body temperature of around 0.52°C, [F(1, 315) = 4.46, p<0.050, from baseline, but was not statistically significant [F(1,288) = 1.30, p>0.100]. However, the drop in body temperature continued to develop over the test period reaching a peak change in temperature of 2.00°C below baseline at 120 minutes post caffeine administration, [F(1, 315) = 65.91, p<0.001].

The hypothermic response to the highest doses of caffeine, 90 and 120 mg/kg, were observed soon after drug administration, with a significant mean drop of 1.44°C, [F(1, 315) = 34.17, p<0.001] and 1.69°C, F(1, 315) = 47.06, p<0.001] respectively, 20 minutes post caffeine administration.

Acute effects of theophylline on body temperature

Theophylline, also produced a dose-dependent decrease in body temperature which however followed different time-courses, main effects of dose [F(4, 36) = 9.32, p<0.001], and time, [F(7, 63) = 13.35, P<0.001], and the dose x time interaction, [F(28, 252) = 5.76, P<0.001]. Doses of 60 mg/kg and above resulted in a biphasic response, that is, a period of temperature drop followed by a rise in temperature back towards baseline levels.

60 mg/kg induced an initial drop in body temperature by 0.88°C, [F(1, 315) = 12.91, p<0.001], below baseline, 20 minutes post theophylline administration. This initial drop in core body temperature was of short duration and coincided with the peak drop in body temperature.

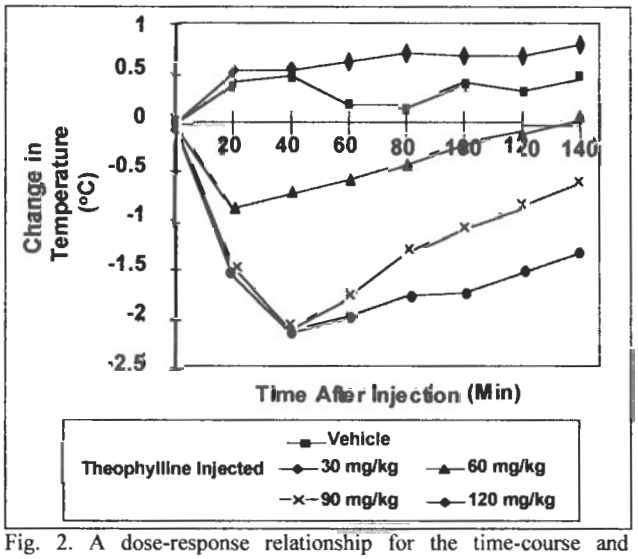


Fig. 2. A dose-response relationship for the time-course and magnitude of theophylline-induced hypothermia in mice

Following the initial temperature drop, core body temperature gradually but consistently approached baseline levels. At 100 minutes post theophylline administration no statisticaly significant effect was found [F(1, 315) = 0.81, p>0.100].

The hypothermic response following the highest doses of theophylline, 90 and 120 mg/kg, were observed soon after drug administration. At 20 minutes, 90 mg/kg theophylline produced a mean drop of 1.44°C below baseline, [F(1, 315) = 34.57, p<0.001].

Naloxone effects on caffeine-induced hypothermia.

Pretreatment with the centrally- and peripherally-acting non-selective opioid antagonist, naloxone hydrochloride, at a dose of 3 mg/kg attenuated the hypothermic action of 90 mg/kg caffeine as reflected by an upward shift in the temperature-time chart.

The attenuation was by approximately 1.23°C as reflected by the magnitude of the hypothermic effect at 100 minutes (time of peak hypothermia) post caffeine administration, [F(1, 378) = 42.23, p<0.001]. This temperature elevation was however significantly lower than that of naloxone HCl-pretreated, vehicle-treated mice, [F(1, 360) = 23.58, p<0.001].

The significance of this attenuation can be seen when contrasted with the temperature drop (at 100 minutes) of the vehicle-pretreated, caffeine-treated mice (2.29°C) and that of naloxone HCl- pretreated, caffeine-treated mice (1.23°C), [F(1, 360) = 11.94, p<0.001]. The attenuation at peak hypothermia was not the most pronounced. Attenuation was greatest at 20 minutes post caffeine administration, reaching, 0.7°C above that of the caffeine-treated mice.

Pretreatment with the peripherally-acting non-selective opioid antagonist naloxone methiodide, at a dose of 1 mg/kg did not attenuate the hypothermic action of 90 mg/kg caffeine. Naloxone methiodide-pretreated, caffeine-treated mice showed a larger drop below baseline

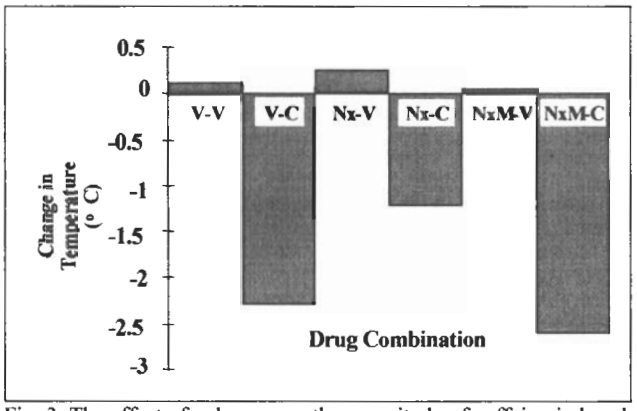


Fig. 3. The effect of naloxone on the magnitude of caffeine-induced hypothermia with time. Vehicle-vehicle (V), vehicle-caffeine (VC), naloxone-vehicle (NxV), naloxone-caffeine (NxC), naloxone methiodide-vehicle (NxMV), naloxone methiodide-caffeine (NxMC), *** P<0.001.

but the difference between the two combinations was not significant as demonstrated at 100 minutes post caffeine administration, [F(1, 360) = 1.02, p>0.100].

At the doses used neither Naloxone methiodide (1 mg/kg) nor Naloxone HCl (3 mg/kg) produced an intrinsic effect on core body temperature.

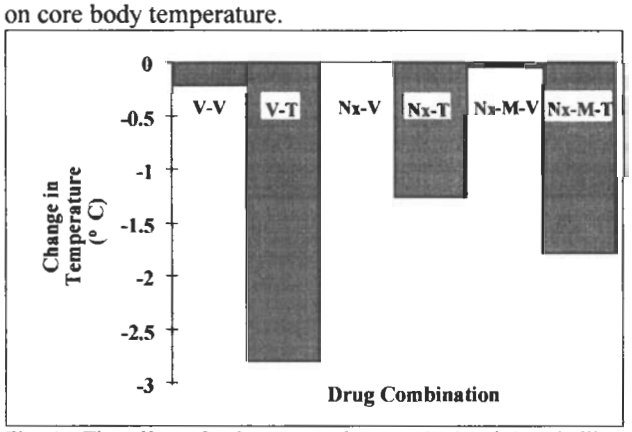


Fig. 4. The effect of naloxone on the magnitude of theophylline-induced hypothermia with time. Vehicle-vehicle (VV), vehicle-theophylline (VT), naloxone-vehicle (NxV), naloxone-theophylline (NxT), naloxone methiodide-vehicle (NxMV), naloxone methiodide-theophylline (NxMT), *** P<0.001.

Naloxone effects on theophylline-induced hypothermia

Pretreatment with the centrally- and peripherally-acting non-selective opioid antagonist, naloxone hydrochloride, at a dose of 3 mg/kg attenuated the hypothermic action of 120 mg/kg theophylline as reflected by an upward shift in the temperature-time chart.

The 120 mg/kg theophylline-induced hypothermia in mice pretreated with naloxone hydrochloride was significantly attenuated by approximately 50% as reflected by the magnitude of the hypothermic effect at 60 minutes post theophylline administration [F(1, 378) = 26.97, p<0.001].

Pretreatment with the peripherally-acting non-selective opioid antagonist, naloxone methiodide, at a dose of 1 mg/kg attenuated the hypothermic action of 120 mg/kg theophylline.

The 120 mg/kg theophylline-induced hypothermia in mice pretreated with naloxone methiodide was significantly attenuated by about 35% as reflected by the magnitude of the hypothermic effect at 60 minutes post theophylline administration (time of peak hypothermia). Temperature attenuation was of the order of 1.80°C.

Neither, naloxone hydrochloride nor naloxone methiodide pretreatment disrupted the biphasic temperature-time profile of 120 mg/kg theophylline.

Opioid selective antagonist effects on caffeine-induced hypothermia.

Pretreatment with naloxone hydrochloride (0.3 mg/kg), acting as the *mu*-selective opioid antagonist did not result in any attenuation of the hypothermic action of 90 mg/kg caffeine during the 140 minutes test period [F,max(1, 504) = 2.14, p>0.100].

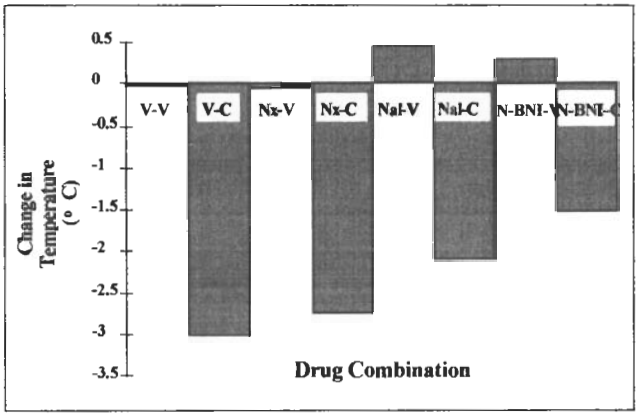


Fig. 5. The effect of selective opioid antagonists on the magnitude of caffeine-induced hypothermia with time. Vehicle-vehicle (VV), vehicle-caffeine (VC), naloxone-vehicle (NxV), naloxone-caffeine (NxC), naltrindole-vehicle (NalV), naltrindole-caffeine (NalC), nor-binaltrophimine-vehicle (NBNIV), nor-binaltrophimine-caffeine (NBNIC), *** P<0.001.

Pretreatment with 10 mg/kg naltrindole hydrochloride (the *delta*-selective opioid antagonist) resulted in an attenuation of the hypothermic effect of 90 mg/kg caffeine. This was reflected by an upward shift in the temperature-time chart.

The 90 mg/kg caffeine-induced hypothermia in mice pretreated with naltrindole hydrochloride (60 - 140 minutes post caffeine administration) was attenuated by about 30% as reflected by the magnitude of the hypothermic effect at 100 minutes (maximum attenuation) post caffeine administration [F(1, 504) = 7.41, p>0.010].

Pretreatment with 5 mg/kg Nor-binaltorphimine dihydrochloride (Nor-BNI), *kappa*-selective opioid antagonist, resulted in an attenuation of the hypothermic effect of 90 mg/kg caffeine as reflected by an upward shift in the temperature-time chart.

The difference between the two combinations was significant throughout the 140 minutes test period, [Fmax(1, 504) = 21.95, p<0.005].

The 90 mg/kg caffeine-induced hypothermia in mice pretreated with nor-BNI was (40 - 140 minutes post

caffeine administration) below baseline and was significantly attenuated.

The attenuated temperature at peak hypothermia that is, at 100 minutes post caffeine administration was 1.52°C below baseline, [F(1, 504) = 50.13, p<0.001]. This temperature was also significant when compared to that of nor-binaltorphimine-pretreated, vehicle- treated mice, [F(1, 504) = 28.40, p<0.001].

Opioid selective antagonist effects on theophylline-induced hypothermia.

Pretreatment with low dose naloxone hydrochloride (0.3 mg/kg), as the *mu*-selective opioid antagonist, resulted in no attenuation of the hypothermic action of 120 mg/kg theophylline.

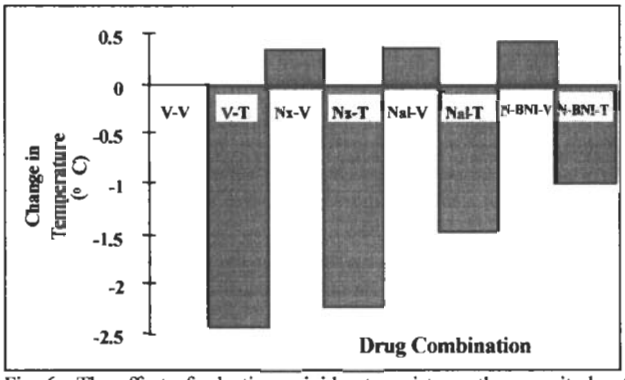


Fig. 6. The effect of selective opioid antagonists on the magnitude of theophylline-induced hypothermia with time. Vehicle-vehicle (VV), vehicle-theophylline (VT), naloxone-vehicle (NxV), naloxone-theophylline (NxT), naltrindole-vehicle (NalV), naltrindole-theophylline (NalT), nor-binaltrophimine-vehicle (NBINV), nor-binaltrophimine-theophylline (NBINT), *** P<0.001.

Although, the temperature-time profile of low dose naloxone hydrochloride/theophylline combination was above that of the theophylline-treated mice, the magnitude of the hypothermia of the two combinations was not significantly different, [Fmax(1, 504) = 1.91, p>0.100] throughout the 140 minutes test period.

Pretreatment with 10 mg/kg naltrindole hydrochloride, *delta*-selective opioid antagonist, resulted in an attenuation of the hypothermic effect of 120 mg/kg theophylline as reflected by an upward shift in the temperature-time chart.

Attenuation at peak hypothermia (60 minutes post theophylline administration) was by 39%, the attenuated temperature at 60 minutes being 1.46°C below baseline, [F(1, 504) = 44.05, p<0.001]. The significance of the attenuation was evidenced by the contrast of T_b at 60 minutes, of naltrindole/theophylline combination (1.46°C) and that of theophylline-only treated mice (2.41°C), [F(1, 504) = 7.34, p<0.010].

Pretreatment with 5 mg/kg Nor-binaltorphimine dihydrochloride (Nor-BNI) *kappa*- selective opioid selective antagonist, resulted in an attenuation of the hypothermic effect of 120 mg/kg theophylline as reflected by an upward shift in the temperature-time curve.

The 120 mg/kg theophylline-induced hypothermia in mice pretreated with nor-BNI was below baseline throughout the 140 minutes test period and was significantly attenuated by a maximum of 40% occurring at 60 minutes [F(1, 504) = 16.98, p<0.001] (maximum attenuation) post theophylline administration and a minumum of 26% occurring at 20 minutes post theophylline administration [F(1, 504) = 10.72, p>0.005].

Discussion

The results of the present study demonstrate that caffeine and theophylline, induce a dose-dependent hypothermia similar to that reported previously for caffeine and other alkylxanthines (Schlosberg, 1983; Spindel et al, 1983; Pohorechy et al, 1989; Kalhorn et al, 1990; Carney et al, 1985; Durcan et al, 1991). This hypothermic effect, in both high-dose caffeine and theophylline was seen to persist for greater than 2 hours post drug administration.

In the present study the animals (mice) were active throughout the testing period due to the repeated body temperature measurements.

From the results it would appear that doses of 60 mg/kg and above cause a dose-related hypothermia.

As opposed to caffeine, theophylline showed a biphasic response with respect to core body temperature. This is similar to what is observed with an acute dose of morphine. However, the drug did not change the set-point around which body temperature is maintained because the animals would have maintained the temperature at which T_b originally dropped if that were the case. The effect of caffeine should not be inferred to a change in set-point but rather a more persistent hypothermic effect than that produced by theophylline.

The biphasic response demonstrated by theophylline may be attributed to the shorter half-life of theophylline (Goodman-Gilman et al, 1991).

The effects of naloxone on caffeine- and theophylline-induced hypothermia.

The results of the present study confirm previous reports (Durcan et al, 1992) that caffeine/theophylline-induced hypothermia is partly mediated by opioid receptors as reflected by the partial attenuation of the hypothermia when the subjects were pretreated with naloxone HCl. Part of the caffeine-induced hypothermia seems to be naloxone- insensitive indicating that mechanisms other than opioid receptors are responsible. Theoretically, higher doses of naloxone could have resulted in a greater attenuation of the response but in practice this was not possible as doses of naloxone greater than 3mg/kg cause a hypothermic effect (Durcan et al, 1992).

Pretreatment with naloxone methiodide, a derivative of naloxone, which does not cross the blood-brain-barrier, resulted in a slight downward shift in the temperature-time curve of caffeine. However, the magnitude of the change in T_b was not significant when compared to the vehicle-pretreated, caffeine-treated mice.

The caffeine-induced hypothermia is thus partly mediated by central opioid receptors and it seems that there is little or no involvement of peripheral opioid receptors.

Thus, contrary to what was observed with caffeine, theophylline-induced hypothermia is partly mediated by central opioid receptors and partly by peripheral opioid receptors.

Since both caffeine and theophylline cross the blood-brain-barrier efficiently, the fact that part of the theophylline-induced hypothermia is peripherally mediated, shows that theophylline interacts with peripheral opioid receptors with greater affinity than caffeine. The shorter time-course of the hypothermia seen with theophylline may be due to this peripheral site of action, which is less efficacious than those found centrally.

Moreover, the difference with regards to peripheral versus central action of these drugs, is indicative of the finding that the conformation of opioid receptors in the CNS and the peripheral nervous system, are different. This is in accordance with various reports in the literature (Siegel et al, 1989) which differentiate between various opioid receptor subtypes, such as *mu*$_1$, *mu*$_2$ *and kappa*$_1$, *kappa*$_2$.

Although the attenuation by naloxone suggests that caffeine- and theophylline-induced hypothermia is opioid receptor mediated, a factor which must be considered is the interaction of caffeine and theophylline with the various opioid receptor types. Morover, caffeine is reported to alter with the basal tone of the opioid system suggesting that the induced hypothermia may be an indirect effect through the release of endogenous opioids such as *Beta*-endorphin (Arnold et al, 1983). Hence the use of naloxone at doses at which it is non-selective, make interpretation of results rather speculative as naloxone is reported to decrease *Beta*-endorphin release and also block caffeine-induced (Arnold et al, 1983) and possibly theophylline-induced stimulation of this release. Additionally, naloxone blocks *mu* opioid receptors to which *Beta*- endorphin is the natural ligand. *Beta*-endorphin and other opioid agonists, including dynorphin and U-50,488H have been shown to effect body temperature in rodents (Bhargava et al, 1989; Cavicchini et al, 1989; Olson et al, 1989; Spencer et al, 1989). However, it is not clear if caffeine and possibly other methylxanthines stimulate the release of endogenous opioids other than *Beta*-endorphin.

The effect of selective opioid antagonists on caffeine- and theophylline-induced hypothermia.

Regulation of body temperature by the opioid system is not clear cut, but generally it appears to be receptor type specific (Olson et al, 1992).

As in the case of morphine, selective mu, delta and kappa opioid agonists have also been reported to produce a differentiable profile of body temperature changes. DAMGO, a selective mu receptor agonist, caused a primary decrease in body temperature of restrained rats and an increase in body temperature of unrestrained rats (Spencer et al, 1988).

Low doses of both DPDPE, a selective delta receptor agonist, and U-50,488H a selective kappa receptor agonist, caused a decrease in body temperature of both restrained and unrestrained rats (Spencer et al, 1988, 1990).

In the present experiment, the contribution of opioid receptor types (i.e., the *mu*, *delta*, and *kappa* opioid receptors) was studied using selective opioid receptor antagonists. Pretreatment with low-dose naloxone HCl (0.3 mg/kg), acting selectively on *mu* opioid receptors, resulted in a non-significant upward shift in the temperature-time curve.

Thus, it appears that *mu* receptors of the CNS are not responsible for caffeine-induced hypothermia. This is in accordance with reports in the literature (Adler et al, 1988) that the *mu* receptors are associated with mechanisms of heat gain.

Although there is no evidence to date for the effect of low dose naloxone on *Beta*-endorphin release, one may exclude until proved otherwise, that low dose naloxone was able to decrease *Beta*-endorphin and hence produce the caffeine-induced and possibly theophylline-induced stimulation of this release meaning that caffeine and theophylline-induced hypothermia is unrelated to the Mu opioid receptors.

Although, the results suggest that *delta* opioid receptors might be involved in mechanisms of heat loss, these should not be interpreted as a possibility that *delta* opioid receptors are tonically active in thermoregulation, since the selective antagonist did not have a significant intrinsic effect on body temperature.

Therefore, the attenuation by naltrindole was not due to a counter thermoregulatory mechanism that masked the caffeine-induced hypothermia, but blockade of *delta* receptors and thus prevention of the access to caffeine (or endogenous opioid released as a result of caffeine stimulation) to the receptor site.

The administration of nor-binaltorphimine, resulted in a significant attenuation of caffeine-induced hypothermia. Consequently, it seems that the *kappa* opioid receptors are also involved in the hypothermic action of caffeine. This is in accordance with previous reports in the literature which associate dynorphins and *kappa* receptors with mechanisms of heat loss (Adler et al, 1988).

However, the attenuation obtained with nor-binaltorphimine was more significant than that obtained with naltrindole. Thus, the results show that although both *delta* and *kappa* receptors are involved in caffeine/theophylline-induced hypothermia, the *kappa* receptors are the major contributors to the mediation of the hypothermic effect.

The *Kappa* opioid receptor seems to be the major contributor towards the opioid receptor mediation of theophylline-induced hypothermia, with a minor involvement of *delta* opioid receptors. However, the

percentage attenuation of theophylline-induced hypothermia by nor-binaltorphimine was greater than that demonstrated with caffeine.

Chronic administration of U-50,488H (a selective *kappa* agonist) resulted in the development of tolerance to its analgesic and hypothermic effects (Reddy et al, 1992, Tejwani et al, 1992). These findings are in accordance with observations that methylxanthine-induced hypothermia resembles that of morphine with respect to chronic administration and the results of the present study which demonstrate that methylxanthine-induced hypothermia is partially mediated by kappa receptors.

In view of the fact that part of the opioid receptor mediation of theophylline-induced hypothermia is of peripheral origin, the greater attenuation in the case of theophylline is in accordance with reports (Adler et al, 1988) that a significant proportion of *kappa* opioid receptors are found primarily outside the brain. This, however, also suggests that binding to central *kappa* opioid receptors results in a more profound hypothermia as reflected by the dose-response relationships of caffeine.

The fact that naltrindole and nor-binaltorphimine caused an attenuation of the hypothermia, suggests that the attenuation caused by naloxone in Part II was not due to interference with the phosphodiesterase-inhibiting properties of caffeine and theophylline but due to blockade of the *delta* and *kappa* opioid receptors. Rather, it was the part of the hypothermia insensitive to the antagonists which could be the result of phosphodiesterase inhibition by caffeine and theophylline.

References

Adler MW, Geller EB, Rosow CE and Cochin J (1988) The opioid system and temperature regulation. *Annual Review of Pharmacology and Toxicology,* 28, 429-449.

Arnold MA, Carr DB, Togasaki DM, Pian MC, Martin JB (1983) Caffeine stimulates *Beta*-endorphin release in blood but not in cerebrospinal fluid. *Life Science,* **31**, 1017.

Bhandari P, Bingham S and Andrews PL (1992) The neuropharmacology of loperamide-induced emesis in the ferret: the role of the area postrema, vagus, opiate and 5-HT3 receptors. *Neuropharmacology,* **31,** 735-742.

Bhargava HN, Gulati A and Ramarao P (1989) Effect of chronic administration of U50,488H on tolerance to its pharmacological actions and on multiple opioid receptors in rat brain regions and the spinal cord. *Journal of Pharmacology and Experimental Therapeutics,* **251**, 21.

Carney JM, Seale TW, Logan L and McMaster SB (1985) Sensitivity of inbred mice to methylxanthines is not determined by plasma xanthine concentration. *Neuroscience Letters,* **56**, 27.

Cavicchini E, Candeletti S, Spampinato S and Ferri S, (1989) Hypothermia elicited by some prodynorphin-derived peptides: Opioid and non-opioid actions. *Neuropeptides,* **14**, 45-50.

Durcan MJ and Morgan PF(1991) Hypothermic effects of alkylxanthines: evidence for a calcium-independent phosphodiesterase action. *European Journal of Pharmacology,* **204**, 15.

Durcan MJ and Morgan PF(1992) Opioid receptor mediation of the hypothermic response to caffeine. *European Journal of Pharmacology,* **224**, 151-156.

Endoh T, Matsuura H, Tanaka C and Nagase H (1992) Nor-binaltorphimine: a potent and selective *kappa*-opioid receptor antagonist with long-lasting activity in *vivo*. *Archives of Internal Pharmacodynamics and Therapeutics,* **316**, 30-42.

Goodman-Gilman A, Rall TW, Nies AS and Taylor P (1991) *The Pharmacological Basis of Therapeutics*. Pergamon Press, New York.

Kalhorn TF, Lee CA, Slattery JT and Nelson SD (1990) Effect of methylxanthines on acetaminophen hepatotoxicity in various induction states. *Journal of Pharmacology and Experimental Therapeutics,* **252**, 112.

Kamei J, Katsuma K and Kasuya Y (1992) Involvement of *mu*-opioid receptors in the antitussive effects of pentazocine. *Naunyn Schmiedebergs Archives of Pharmacology,* **345**, 203-208.

Mainous RO (1995) Research utilization, pharmacologic management of neonatal pain. *Neonatal-Network,* **14**, 71-74.

Misra AL, Pontani RB and Valdlamani NL (1985) Potentiation of morphine analgesia by caffeine. *British Journal of Pharmacology,* **84**, 789.

Nicholson D, Reid A and Sawynok J (1991) Effects of forskolin and phosphodiesterase inhibitors on spinal antinociception by morphine. *Pharmacology, Biochemistry and Behavior,* **38,** 753.

Olson GA, Olson RD and Kastin AJ (1990) Endogenous opiates: 1989. *Peptides,* **11**, 1277-1304.

Pohorecky LA, Roberts P, Cotler S and Carbone JJ (1989) Alteration of the effects of caffeine by prenatal stress. *Pharmacology, Biochemistry and Behavior,* **33**, 55.

Reddy VPL and Bhargava HN (1992) Abstinence from U50,488H, a *kappa*-opiate receptor agonist, decreases the binding of [H]DPAT to 5-HT_{1A} receptors in the hypothalamus of the rat. *Neuropharmacology,* **31**, 1243-1249.

Rush CR, Sullivan JT and Griffiths RR (1995) Intravenous caffeine in stimulant drug abusers, subjective reports and physiological effects. *Journal of Pharmacology and Experimental Therapeutics,* **273**, 351-358.

Schlosberg AJ (1983) Temperature responses in rats after acute and chronic administration of caffeine. *Pharmacology, Biochemistry and Behavior,* **18**, 935.

Siegel GJ, Agranoff BW, Albers RW and Molinoff PB (1989) *Basic Neurochemistry*, Raven Press, USA.

Spencer RL, Hruby VJ and Burks TF (1988) Body temperature response profiles for selective *mu*, *delta* and *kappa* opioid agonists in restrained and unrestrained rats. *Journal of Pharmacology and Experimental Therapeutics,* **246**, 92-101.

Spindel E, Griffith L and Wurtman RJ (1983) Neuroendocrine effects of caffeine. II. Effects on thyrotropin and corticosterone secretion. *Journal of Pharmacology and Experimental Therapeutics,* **225**, 346.

Suzuki T, Narita M, Takahashi Y, Misawa M and Nagase H (1992) Effects of nor-binaltorphimine on the development of analgesic tolerance to and physical dependence on morphine. *European Journal of Pharmacology,* **213**, 91-97.

Sweeny MI, White TD and Sawynok J (1991) Intracerebroventricular morphine releases adenosine and adenosine 3',5'-cyclic monophosphate from the spinal cord via a serotonergic mechanism. *Journal of Pharmacology and Experimental Therapeutics,* **259**, 1013.

Tejwani GA, Rattan AK, Koo KL, Tijoe SA and Bhargava HN (1992) Effect of U50,488H induced tolerance and abstinence on met-enkephalin levels in brain, spinal cord pituitary and peripheral tissues of the rat. *Society of Neuroscience Abstracts,* **18**, 369.

Toyoshi T, Ukai M and Kameyama T (1992) Combination of a *delta* opioid receptor agonist but not a *mu* opioid receptor agonist with the D1- selective dopamine receptor agonist SKF 38393 markedly potentiates different behaviours in mice. *European Journal of Pharmacology,* **213**, 25-30.

Research Article

Drug and Alcohol Abuse in the Workplace; A Survey of Managers' Awareness of the Problem

Noel Vella and Mark Gauci
Occupational Health and Safety Unit, Department of Labour, Valletta.

Summary. *A questionnaire was sent to managers of several companies. The aim was to collect data on the use of alcohol and illicit drugs at work. Occasional or experimental drug use was considered unacceptable and harmful by the great majority (88% and 86%). A very small proportion of respondents took drugs or drank alcohol heavily at work (1.1% and 1.5%). A significant number of managers (21.4%) were aware of workers who drank alcohol at work, and to a lesser extent, of workers taking drugs at work (4%). A considerable proportion of respondents reported that alcohol consumption was allowed at work (18.4%) and this was related to the type of employing industry. Measures must be taken to educate employers and workers about the possible harmful consequences of alcohol and drug use at work.*

Keywords: drugs, alcohol, Malta, work, workplace, managers, employees

The negative effects which alcohol and drug abuse have on workers' health and on their efficiency in the workplace are well documented. In Malta, whilst anecdotal evidence exists that persons under the influence of drugs or alcohol have caused a number of accidents and near-misses in workplaces, data regarding their prevalence in industry is conspicuous by its absence.
This paper presents the results obtained from part of a survey by questionnaire which dealt with attitudes toward occasional drug use, prevalence of current and past drug use amongst responders, knowledge of any workers in the company taking drugs at and off work, the level of alcohol consumed by managers, knowledge of any workers drinking alcohol at work or drinking alcohol heavily off work and sale of alcohol at work.

Method

A survey by questionnaire was conducted in April and May 1996 to investigate managerial attitudes towards drug and alcohol use in the Maltese workplace. 250 companies were randomly chosen from the 666 companies included in the METCO publication "Made in Malta - 1996 - Manufacturers and Exporters". The activities of these companies were various and included the manufacture of textiles, furniture, metal, machinery, as well as printing, paper, rubber, chemical, transport, construction, food and beverage industries and business services. The persons occupying managerial grades in the companies whose general manager showed interest in taking part were sent the questionnaire. In view of the fact that some of the information being requested was personal and involved an admission of indulging in an illegal activity, the questionnaire was designed so as to guarantee the respondents' anonymity. An attempt was made to minimise the number of non-responders by sending a second letter addressed to all participants urging them to return any unsent questionnaires.

Heavy alcohol drinking was defined as the consumption of more than an average of 3 units of alcohol per day, whilst drug use was limited to the use of substances of abuse. Table 1 shows the format of 2 of the questions in the survey.

	Yes	No	Uncertain
Do you know of any workers in your company who take drugs at work?	☐	☐	☐
who take drugs when off work?	☐	☐	☐

What is your average daily unit* intake of alcohol?	0	☐	1	☐	2 ☐
* 1 unit of alcohol is equivalent to 1 bottle of beer, or 1 glass of wine or 1 tot of spirits.	3	☐	>3	☐	

Table 1 Format of questionniare

Results were analysed using BMDP statistical software. Significance was accepted at $p < 0.05$.

Results

Of the 250 general managers invited to participate in this survey, 190 (76%) agreed to take part and sent details of their managerial staff to be included in this study. A total of 764 managers were sent a copy of the questionnaire, and responses were obtained from 457 of them giving a final response rate of 59.8%.

The majority of participants consisted of male managers

(89.5%). The age of the respondents was normally distributed, with about 15% and 17% in the younger and older age groups, respectively, and about 34% in each of the two middle age groups. The distribution of participants by company size appears in Fig. 1.

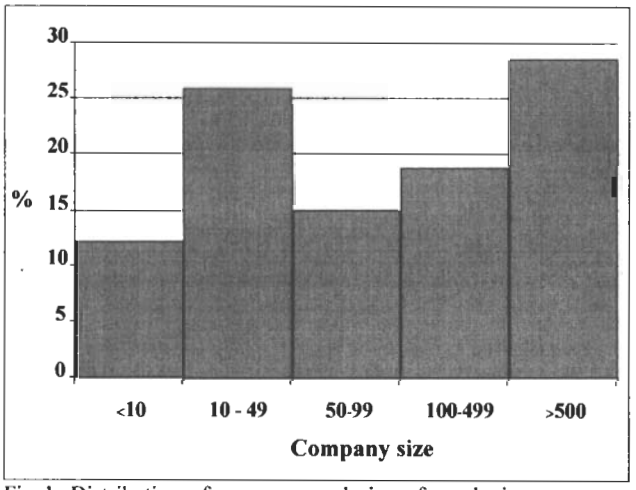


Fig 1. Distribution of managers and size of employing company (n=457)

The majority of managers surveyed considered occasional/experimental drug use to be unacceptable (88%) and harmful (86%).

There was a positive association between the perception that occasional drug use is unacceptable and that it has lasting effects ($p<0.01$) and also with it being harmful ($p<0.001$). Acceptability of drug use was related to the age of responders, the largest proportion of those considering it acceptable being in the youngest age group with the proportion decreasing progressively with age ($p<0.001$) (Fig. 2).

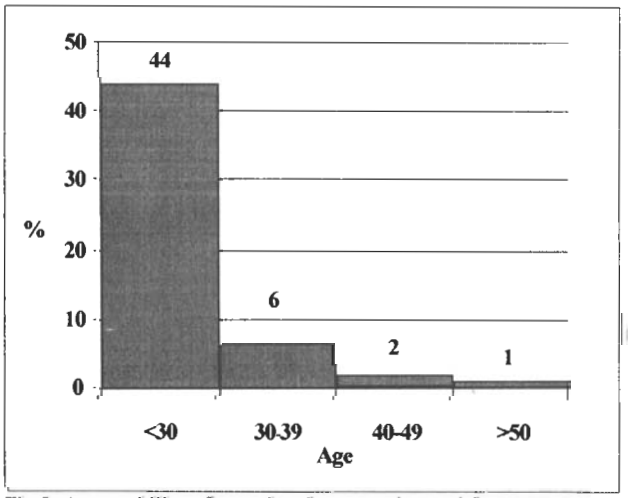


Fig 2. Acceptability of occasional or experimental drug use

Whilst there was a positive association between managers having a past history of drug use and the perception that occasional or experimental drug use is acceptable ($p<0.001$), a past history was negatively correlated with the perception of it being harmful ($p< 0.01$).

8.5% of managers returning the questionnaire claimed that they had taken drugs in the past, whilst 1% stated that they were currently taking drugs.

There was a significant difference in the age groups of respondents claiming past drug use ($p<0.001$) (Fig. 3), with the largest proportion being under 40 years of age. A past history of drug use was significantly associated with an increased knowledge of other persons taking drugs at work ($p<0.02$) and also with an increased knowledge of persons using drugs off work ($p<0.001$).

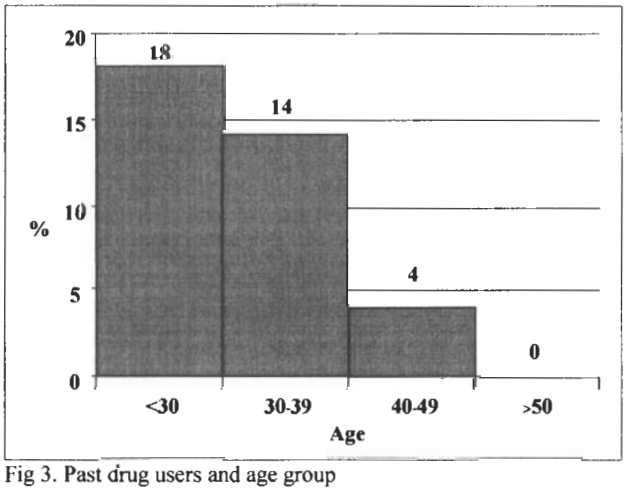


Fig 3. Past drug users and age group

About 5% of managers claimed that they were aware of workers within their company who took drugs at work, whilst this figure rose to 13.6% for workers who took drugs away from work (Fig 4).

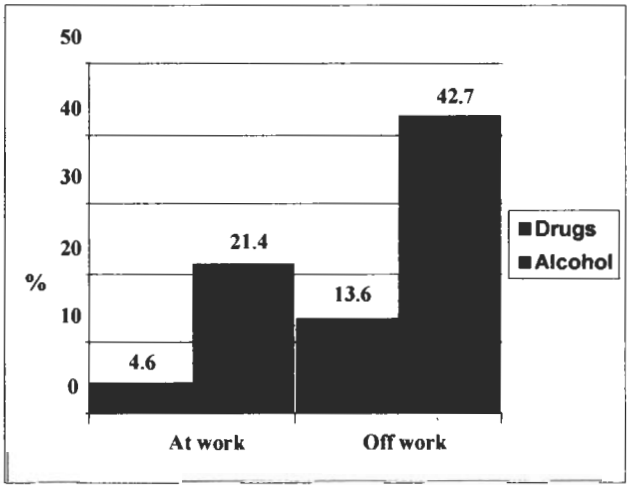


Fig 4. Knowledge of workers taking drugs and alcohol (n=457)

It could not be ascertained whether workers known to use drugs or drink alcohol at work also figured in the proportion of those known to use drugs or drink alcohol heavily off work. Managers aware of workers taking alcohol at work were more likely to be aware of workers drinking alcohol heavily off work, ($p<0.001$), as well as workers who took drugs at work ($p<0.01$).

The proportion of managers aware of workers taking drugs at work was significantly associated with an increased awareness of drug-taking off work

($p<0.001$), and with company size ($p<0.001$), with managers of companies employing over 100 workers reporting an increased awareness of such workers (Fig 5). The amount of alcohol consumed by the respondents is shown in Fig 6.

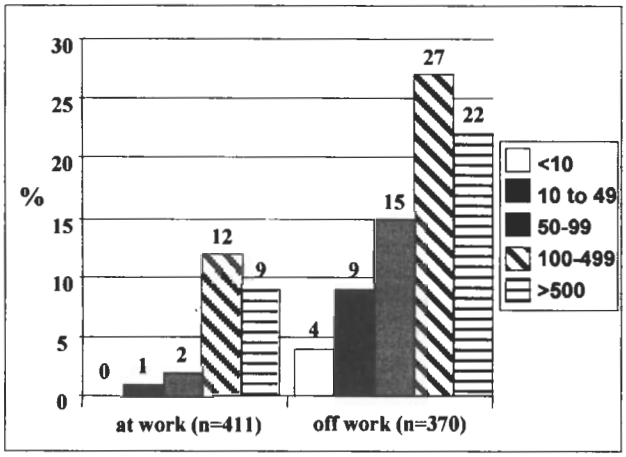


Fig. 5. Managers' awareness of workers taking alcohol. (Categories represent size of establishment in menpower)

Managers' awareness of workers taking alcohol both at and off work was significantly related to the company size ($p<0.001$ in both cases) (Fig 7), and with type of industry ($p<0.001$ and $p<0.01$ respectively) (Table 2).

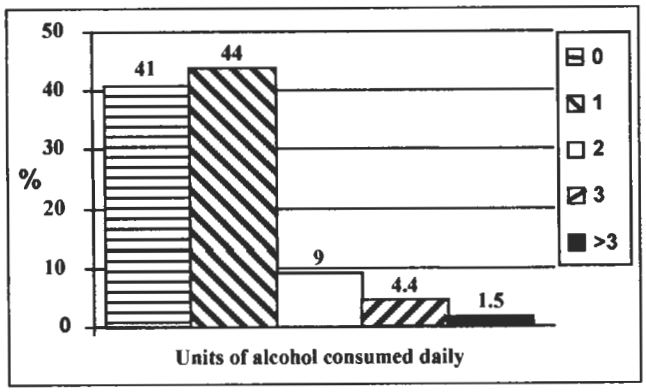


Fig 6. Alcohol consumption by respondents (n=457)

9.2% (42) of the managers who said that they had a canteen facility at their workplace admitted that alcoholic beverages were sold from these facilities. However, a total of 64 respondents (18.4%) said that alcohol consumption was permitted at their workplace.

Sale of alcohol at work was associated with an increased likelihood of allowing alcohol to be consumed at work ($p<0.001$), and with company size ($p<0.05$). Company size was also significantly correlated with the practice of allowing alcohol consumption at work ($p<0.05$), medium sized and large companies being twice more likely to allow this to occur (Fig 8). The type of respondents' employing industry was significantly related to the presence of a canteen facility, the sale of alcoholic beverages, and the practice of allowing alcohol consumption at the place of work ($p<0.001$ in all cases) (Table 3).

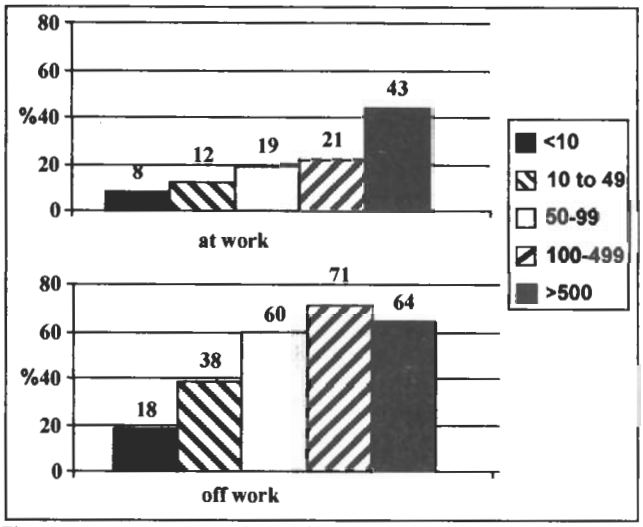


Fig 7. Knowledge of workers taking alcohol at and off work in different sized companies

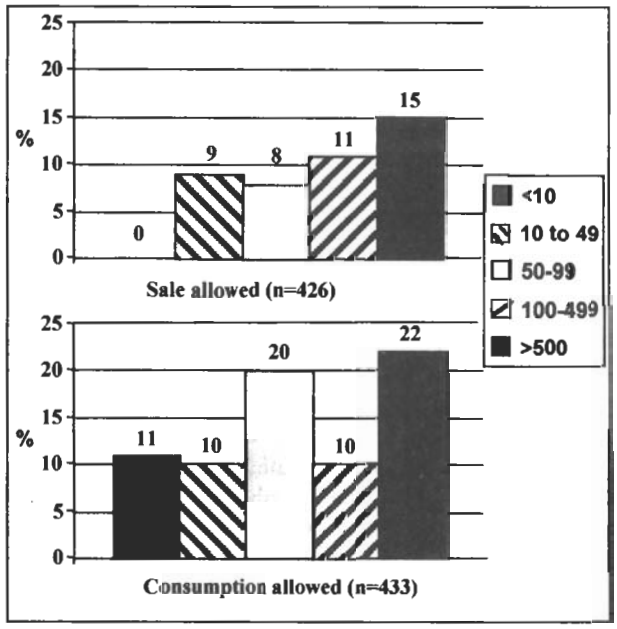


Fig 8. Sale of alcohol and alcohol consumption at work in different sized companies

Type of employing industry	Knowledge of workers: taking alcohol at work	Knowledge of workers: drinking alcohol heavily off work
Rubber & Plastics	53	56
Transport equipment	55	65
Beverage	66	62
Metal products	30	48
Community & Business services	14	5

Table 2. Managers' awareness of workers taking alcohol at and off work in different industries (% of managers in each industry).

Discussion

Malta, like any other country, may have a problem of increasing magnitude caused by drugs and alcohol use. Whilst media attention focuses on the problems caused by illicit drug use, particularly since there are indications that

Type of employing industry	Canteen facility	Sale of alcoholic beverages	Alcohol consumption allowed
Rubber & Plastics	90	0	0
Transport equipment	56	19	5
Beverage	59	34	60
Metal products	39	12	3
Community & Business services	11	0	37

Table 3. Proportion of managers (% in type of industry) reporting the presence of a canteen facility, sale of alcoholic beverages, and permitted alcohol consumption at the place of work.

this is on the increase in Malta, (as evidenced by the number of drug related arraignments in Court, persons referred to detoxification and rehabilitation centres and drug related fatalities), problems related to alcohol are not given as much prominence.

The effects of both alcohol and drug use on the work and the worker have been extensively researched. These include poor job performance, absenteeism, increased absence due to sickness (Atwell, 1995; Bertera, 1991), increased probability of having an occupational accident and injury (Gutierrez-Fisac, 1992), reduced productivity, wasted training, problems arising from disciplinary issues, late arrival at and early departures from work, long breaks, aggression (Bassett P, 1988), irregular patterns of work and difficulty in concentration and deteriorating appearance (Terrell, 1988), unexplained disappearances, personality changes, disinterest in work, and loss of time for medical treatment, damage to equipment and materials, higher insurance rates, sharp increase in crime especially theft on the premises (Baldwin, 1975). In fact, alcohol consumption on its own is also considered to be a safety hazard (Moll-Van-Charante, 1990; Smith, 1988). Harig, (1989) reported that alcoholics often encounter difficulty in facing their problems due to denial, a situation which is often compounded by co-workers or supervisors who ignore or excuse certain behavioural patterns, thus unwittingly enabling such workers to continue these activities which may constitute a risk both to themselves and to others.

This study showed that whilst the observed prevalence of current drug use amongst respondents was low (1.1%), the number of managers admitting to a past history of drug use (8.5%) was rather high. As was to be expected, the prevalence of managers who stated that they consumed alcohol was much higher than that for drug use but the proportion of heavy alcohol users was low and very similar to those admitting to drug use (1.1% vs. 1.5%). Managers knew of more workers taking alcohol rather than drugs at work (21.4% vs. 4.6%). This could be due either to the fact that alcohol use is carried out in a more overt manner, particularly since its use is not illegal and is culturally acceptable, or because alcohol use is more common than drug use at work, or for both reasons. The fact that a significant number of managers are aware of workers who use either drugs or alcohol at work is a cause for concern since no initiatives are being taken to curb these highly dangerous practices at the workplace.

The problem of alcohol consumption at work will obviously be much more difficult to solve in those workplaces where the sale of alcohol is allowed, and 14% of participants claimed that alcohol consumption at work was allowed. This is indeed worrying in view of the potential dangers associated with alcohol consumption.
Another finding which is a cause for concern is that a high proportion of managers are aware of workers drinking heavily or taking drugs away from work (43.6% vs. 21.4%). The substance taken may still be present in sufficient amounts by the time a worker returns to work, to cause undesired effects (Wolkenberg, 1975). Various studies have shown that between 9% and 16% of fatalities at work have detectable blood alcohol levels (Alleyne, 1991; Sniezek, 1989). Whilst acute alcohol intoxication is responsible for only a small proportion of fatalities and serious injuries at the workplace, it does account for a large proportion of minor and damage-only accidents (Ide, 1995).

It is of interest to note that managers from small companies do not perceive drug use to be as significant a problem as their colleagues from larger companies. The latter were also more aware of drug and alcohol use at and off work. Although there may be a certain degree of overconfidence in these perceptions, particularly as regards the identification of the occasional user, it is highly unlikely that a person showing overt manifestations of dependence will not be noted by a manager in a very small enterprise.

This study had some limitations in that information about the type, amount, and duration of previous drug use, was not surveyed in order to keep the questionnaire as concise as possible. Drugs prescribed as medication may also have undesirable effects which also influence performance at work, irrespective of whether they are taken as prescribed or misused. This aspect was not looked into. Another limitation is that larger companies may have a larger complement of managers, which could have resulted in overrepresentation of a particular sector.

It is to be noted that the majority of past drug users amongst respondents was in the lower age groups (<40 years), which can be partly attributed to the increasing availability of drugs in Malta over the last 20 years. Younger managers were also more tolerant towards occasional drug use although, in general, respondents showed a low tolerance towards occasional or experimental drug use.

Not surprisingly, past exposure to drugs affected the individual manager's outlook on drug use. Individuals in this subgroup were found to be more tolerant to occasional drug use and less likely to consider drug use as having deleterious effects. Whilst various factors may affect an individual's decision to stop taking drugs, the data obtained suggests that there is a strong need to actively counter any misconceptions that these individuals have about drug use. These findings might also indicate a certain readiness to resume drug use under the right

conditions.

A number of differences were noted in the responses obtained from managers coming from different types of industry. However, in view of the fact that certain types of industry remained underrepresented in this survey, and due to problems with regards to statistical analysis, it was decided to limit analysis to those companies represented by more than 20 managers. A tolerant outlook towards alcohol use at work was particularly evident in the attitudes shown by managers employed in the beverages industry and they were also the most likely to be aware of workers actually taking alcohol at work. It is also interesting to note that managers from the transport equipment industry were most likely to know of workers who consumed alcohol at and off work, and also of workers taking drugs off work. This correlates with the fact that this industry had the second largest proportion of managers reporting the sale of alcohol at work. The results obtained show that in several instances there is an association between the type of industry and managerial attitudes/perceptions with regard to drug and alcohol use. Whilst these attitudes may be the result of an actual difference in prevalence of these problems in workplaces, or of different conditions of work in different types of industry, (such as high temperatures, monotonous or repetitive work), they may also reflect reduced awareness of the effects of drug and alcohol use.

This study indicates that a substantial number of those surveyed drink alcohol at work or know of workers who do so. Alcohol consumption at work is a relatively common phenomenon in the Maltese workplace and is also more widespread than drug use. Certain types of employment were shown to be associated with increased tolerance to alcohol use at work, and this will obviously indirectly encourage such a practice. Whilst legislation banning the sale and use of alcohol in the workplace is needed, this will not have the desired effect unless efforts are made to educate more extensively, from an early age, our society as a whole, and persons at work in particular, about the personal and sociological effects of drug and alcohol consumption in general. Industry and workers' representatives, possibly helped by government, need to increase efforts to adopt and commit themselves to policies aimed at introducing and implementing measures to designate workplaces as alcohol- and drug-free areas. Adequate resources need to be channelled in ongoing education campaigns and preventive programmes for managers and workers about the effects of substance abuse, particularly in companies which have a higher prevalence of this problem and in designing and introducing appropriate initiatives to attempt to control this practice. Such initiatives should attempt to offer not only rehabilitation services but also other forms of support to the affected individual and his family, such as assurances regarding job security whilst undergoing rehabilitation. These measures will serve to heighten managers' and workers' awareness of the problem thereby facilitating prevention. They will also help to identify workers already suffering from these problems and offer them counselling or rehabilitation, thus facilitating self-referral of the substance abuser to supportive services.

References

Alleyne BC (1991) Alcohol and other drug use in occupational fatalities. *Journal of Occupational Medicine*, **33**, 496-500.

Atwell C (1995) Drug and alcohol screening in the workplace. *Occupational Health*, **47**, 51-54.

Baldwin DM (1975) The trouble with drugs. *Job Safety and Health*, **3**, 4-10.

Bassett P and Leadbeater C (1988) Warning on alcohol abuse. *Financial Times* No, 30680, 7.

Bertera RL (1991) The effects of behavioural risks on absenteeism and health-care costs in the Workplace. *Journal of Occupational Medicine*, **33**, 1119-1124.

Gutierrez-Fisac JL(1992) Occupational accidents and alcohol consumption in Spain. *International Journal of Epidemiology*, **21**, 1114-1120.

Harig PT (1989) Alcoholism in the workplace. *Occupational Medicine*, **4**, 213-221.

Ide CW (1995) Time gentlemen please. Time to re-examine the relationship between drinking and work. *The Safety and Health Practitioner*, **13**, 23-26.

Lewis RJ and Cooper PS (1989) Alcohol, other drugs and fatal work-related injuries. *Journal of Occupational Medicine*, **31**, 23-28.

Moll-Van-Charante AW and Mudler PGH (1990) Perceptual acuity and the risk of industrial accidents. *American Journal of Epidemiology*, **131**, 652-663.

Smith GS and Kraus JF (1988) Alcohol and Residential, Recreational and Occupational injuries, A review of the Epidemiological evidence. *Annual Review of Public Health*, **9**, 99-121.

Sniezek JE and Horiagon TM (1989) Medical-examiner-reported fatal occupational injuries, North Carolina, 1978-1984. *American Journal of Industrial Medicine*, **15**, 669-678.

Terrell A (1988) Screening for Drug Abuse. *Journal of Occupational Health and Safety Australia and New Zealand*, **4**, 441-445.

Webb GR, Redman S, Hennrikus DJ, Kelman GR, Gibberd RW and Sanson-Fisher RW (1994) The relationships between high-risk and problem drinking and the occurrence of work injuries and related absences. *Journal of Studies on Alcohol*, **55**, 434-446

Wolkenberg RC, Gold C and Tichauer ER (1975) Delayed effects of acute alcoholic intoxication on performance with reference to work safety. *Journal of Safety Research*, 7, 104-118.

Article

First Checklist of the Myxomycetes of Malta

Michael Briffa
'Durham House', 20, Creche Street, Sliema SLM09, Malta.

Summary: *71 species of Myxomycetes occurring in Malta are reported for the first time in the form of an annotated Systematic checklist.*

Among the most curious groups of living organisms are the *Myxomycetes,* meaning 'slime fungi', or as they are popularly known the 'slime moulds'. They are minute creatures, mostly measuring no more than a few millimeters. Their life-cycle is divided into two main stages: the assimilative, vegetative stage, in which they take the form of a moving mass of protoplasm, and the reproductive stage, in which they assume the form of fungus-like structures reproducing by spores. When the spores 'germinate', they release haploid protoplasts, which later unite in pairs to form diploid zygotes. Successive mitotic nuclear division in the zygotes takes place without cell division, thus giving rise to an acellular, multinucleate, slime-looking, naked mass of protoplasm, the 'plasmodium' , to which the 'slime moulds' owe their name. When it reaches the reproductive stage, the plasmodium gradually transforms itself into fungus like fruiting bodies. In fact many of the species were mistaken for 'puff-balls', and included in the order *Gasterales,* by the early mycologists. It was only in 1833 that Wallroth introduced the name *Myxomycetes*, separating the slime moulds from the rest of the fungi. In 1864, following intensive investigations, De Barry established that slime moulds are closer to protozoa than to fungi, and he called them *Mycetozoa* meaning 'fungous animals'. He was followed by distinguished authorities, including Rostafinski, the Listers, Hagelstein, and more recently by Olive. However, this designation has never been fully accepted, and students of slime moulds kept using both names: mycologists calling them *Myxomycetes* and zoologists calling them *Mycetozoa*. At present, although everybody is convinced that slime moulds are not fungi, the name *Myxomycetes* seems to have prevailed. In fact, practically in all the published scientific papers, both in Europe and the United States , not to mention the far east, slime moulds are being referred to as *Myxomycetes*.

Although Myxomycetes have been studied for over two centuries, there seems to be no trace of any records from the Maltese Islands. The only mention of Malta that could be found in the available literature was in Carlos Lado's 'Checklist of Myxomycetes of the Mediterranean Countries' (1994), in which he includes Malta together with San Marino, Sardinia, Sicily, and the Vatican with Italy. However, Dr. Lado (pers. comm.) confirms that the inclusion of Malta with Italy was not based on any specific records. Of the 71 species occurring in Malta, 12 have not been reported from Italy (Lado, 1994). These include: *Comatricha anomala, Craterium rubronodum, Didymium quitense, D. trachysporum, Echinostelium colliculosum, Enteridium splendens, Physarum echinosporum, P. lividum, P. perfectum, Stemonitis virginiensis, Trichia contorta* and *Tubifera microsperma.* Of these, *Craterium rubronodum, Physarum echinosporum* and *Tubifera microsperma* are new records for the Mediterranean Region. Much remains to be done. There are other equally suitable localities to be explored. No field work has yet been done in our sister island of Gozo and as yet, no moist chamber culture has been attempted.

Material and Method

Six localities in Malta were chosen for their suitable topography and ecology. Over a three year period (1995/97), 43 excursions were made to search for specimens of Myxomycetes. Numerous specimens belonging to 66 species were collected. These together with a few others collected previously, and information received on *Echinostelium colliculosum,* make up the present checklist. All the specimens supporting this checklist are deposited at the author's herbarium.

The localities explored were the following:

a) **Ghajn il-Kbira** a deep, sheltered, damp, cultivated fault-valley planted with old fruit trees including *Prunus spp.* , *Citrus spp.* , and *Eriobotrya japonica.* At the bottom of the valley there are also small surviving native stands of *Populus alba* and *Ulmus canescens.*

b) **Buskett** dominated by *Pinus halepensis.* Hitherto, however, no Myxomycetes have been found on pine needles. On the contrary many have been collected from a neglected part of the valley among the debris of old planted trees of *Carya olivaeformis.*

c) **Maqluba**, at the bottom of a circular fault about 60 meters broad and 30 meters deep. It was used as an orchard in the 16th century. In fact *Punica granatum* still survives, together with *Laurus nobilis* and *Ceratonia siliqua,* which are possibly of indigenous origin.

d) **Wied Babu**, a deep damp river-valley of the last

glacial (stadial) period, dominated chiefly by dispersed old trees of *Ceratonia siliqua,* under which many specimens were found.

e) **Ballut tal-Wardija,** a residual population of *Quercus ilex*, believed to be a surviving relic of the primitive Maltese evergreen forests. Some trees are estimated to be more than 800 years old. Among these also occur a few trees of *Ceratonia siliqua* and *Olea europea.*

f) **Ballut tal-Imgiebah,** another native stand of *Quercus ilex* and *Ceratonia siliqua,* sheltered from the prevailing north westerly winds by a high cliff.

The identification of the collected material was mainly based on the keys and descriptions in the monographs of Lister (1925) and Martin & Alexopoulos (1969). Taxonomic difficulties and doubtful identifications, were resolved with the expert help of many colleagues abroad. These persons are listed in the acknowledgement section at the end of this article.

Explanatory note on the Checklist

The classification follows the one adopted by Martin & Alexopoulos (1969), except for *Reticulariaceae* which has been replaced by *Enteridiaceae* and *Dictydiaethaliaceae* and for the insertion of the subclass *Stemonitomycetidae,* separating the order *Stemonitales* from the subclass *Myxogasteromycetidae.*

Each species listed is followed by the herbarium reference-number of the selected examined specimen/s supporting it, together with the relative data. For the sake of brevity, the names of the collectors and the authorities who determined or confirmed the identification of the specimens, are replaced by their initials. They are those of the author and of those whose names are mentioned in the acknowledgements. In some cases remarks concerning certain specimens have been added.

The frequency of occurrence of each species, based on the collected material, is indicated by the following: "rare", collected from only one locality; "frequent", collected from two localities; "common", collected from three or more localities.

THE CHECKLIST

Division: MYCOTA

Subdivision: MYXOMYCOTINA

Class: **MYXOMYCETES**

Subclass: **CERATIOMYXOMYCETIDAE**
Order: CERATIOMYXALES

Family: Ceratiomyxaceae

Ceratiomyxa fruticulosa (O.F. Muell.) T. Macbr.
MB1036 Maqluba 8 Oct. 1996 on fallen branch of *Ceratonia siliqua.* Leg. AB & MB, det. MB, test. WN. Frequent.

Subclass: **MYXOGASTEROMYCETIDAE**

Order: LICEALES

Family: Liceaceae

Licea variabilis Schrad.
MB1003 Qormi 6 Nov.1983 on dead leaves. Leg. C. Briffa & MB, det. PC. Rare.

Family: Cribrariaceae

Cribraria argillacea (Pers.) Pers.
MB1196 Buskett 28 Oct.1997 on dead stump of *Ceratonia siliqua.* Leg. AB & MB, det. MB, test. CL. form with distinctly warted spores. Rare.

Cribraria cancellata (Batsch) Nann. -Bremek.
MB1028 Ghajn il-Kbira 30 Oct.1995 on dead wood of *Populus alba.* Leg. AB & MB, det. MB. Frequent.

Cribraria violacea Rex
MB1209 Ghajn il-Kbira 27 Nov.1997 on unidentified stem among debris of *Ulmus canescens.* Leg. AB & MB, det. MB, test. WN. Rare.

Family Enteridiaceae

Enteridium splendens (Morgan) T. Macbr.
MB1035 Maqluba 8 Oct.1996 on dead branch of *Ceratonia siliqua.* Leg. AB & MB, det. WN. This specimen is referable to *v. juranum* (Meyl.) Haerk. (WN). Frequent.

Lycogala epidendrum (L.) Fr.
MB1018 Ballut tal-Wardija 29 Sept.1995 on fallen branches of *Quercus ilex.* Leg. MB, det. MB. Common.

Lycogala flavofuscum (Ehrenb.) Rostaf.
MB1006 Wied il-Luq 1 Oct.1985 high up living trunk of *Fraxinus angustifolia.* Leg. A. Valletta & MB, det. MB, test. WN. Rare.

Tubifera microsperma (Berk. & M.A. Curtis) G.W. Martin.
MB1194 Buskett 28 Oct.1997 on decaying stump of *Ceratonia siliqua.* Leg. AB & MB, det. MB. test. CL
First record for the Mediterranean. Rare.

Family Dictydiaethaliaceae

Dictydiaethalium plumbeum (Schumach.) Rostaf.
MB1137 Ghajn il-Kbira 3 Feb.1997 on unidentified dead branch. Leg. AB & MB, det. MB. Common.

Order: ECHINOSTELIALES
Family: Echinosteliaceae

Echinostelium colliculosum K.D. Whitney & H.W. Keller

Moist chamber culture 1 May 1995 on bark of living unidentified tree from Malta. Leg. & det. H. Mueller. (WN pers. com.)

Order: TRICHIALES

Family: Arcyriaceae

Arcyria cinerea (Bull.) Pers.
MB1063 Buskett 5 Jan.1996 on the hymenial surface of *Ganoderma lucidum.* Leg. AB & MB, det. MB. Common.

Arcyria denudata (L.) Wettst.
MB1053 Buskett 16 Nov.1995 on dead wood. Leg AB & MB , det. MB. MB1045 Ghajn il-Kbira 10 Nov. 1995 on remains of *Prunus sp.* Leg. AB & MB, det. MM, (faded form). Common.

Arcyria incarnata (Pers.) Pers.
MB1112 Ballut tal-Imgiebah 24 Oct.1996 on dead branch of *Quercus ilex.* Leg. AB & MB, det. WN. Common.

Arcyria insignis Kalchbr. & Cooke
MB1033 Maqluba 8 Oct.1996 on dead bark of *Laurus nobilis.* Leg. AB & MB, det. WN. Frequent.

Arcyria minuta Buchet
MB1107 Ballut tal-Wardija 23 Sept.1996 on dead branch of *Quercus ilex.* Leg. AB & MB, det. WN, spores 10-11µm. Macroscopically 1107 looks like *A. insignis,* but large spores indicate *A. minuta.* (WN). MB1111 Ballut tal-Imgiebah 24 Oct.1996 on dead wood of *Quercus ilex.* Leg. AB & MB, det. WN, spores 8µm (Type 2 = *A. gulielmae* Nann. -Bremek.). Common.

Arcyria obvelata (Oeder) Onsberg
MB1037 Maqluba 8 Oct 1996 on fallen branch of *Ceratonia siliqua.* Leg. AB & MB, det. MB. Common.

Arcyria oerstedtii Rostaf.
MB1044 Ghajn il-Kbira 10 Nov.1995 on decaying wood of *Prunus sp.* Leg. AB & MB, det. MB. test. WN. Rare.

Arcyria pomiformis (Leers) Rostaf.
MB1059 Ballut tal-Wardija 18 Dec.1995 on dead wood. Leg AB. & MB, det MB. MB1152 Wied Babu 13 Feb.1997 on dead wood of *Ceratonia siliqua.* Leg. AB & MB, det. YY, (a variant; it had been mistaken for a form of *A. nigella* showing affinity with *A..glauca*). Common.

Metatrichia vesparium (Batsch) Nann. -Bremek.
MB1017 Ghajn il-Kbira 23 Jan.1995 on dead wood of *Populus alba.* Leg. AB & MB, det. MB. Rare.
Perichaena chrysosperma (Curr.) A. Lister
MB1073 Girgenti 26 Feb.1996 on dead wood. Leg. AB & MB, det. MB, test. WN. Frequent.

Perichaena corticalis (Batsch) Rostaf.
MB1022 Buskett 16 Oct 1995 on fallen trunk. Leg. AB & MB, det. MB. Rare.

Perichaena depressa Lib.
MB1090 Ghajn il-Kbira 23 Mar.1996 on dead wood. Leg. AB & MB, det. MB, test. WN. Rare.

Family Trichiaceae

Trichia contorta (Ditmar) Rostaf.
MB1014 Ghajn il-Kbira 23 Jan.1995 on dead wood of *Populus alba.* Leg. AB & MB, det. PC. MB1015 Ghajn il Kbira 23 Jan.1995 along with typical form (1014). Leg. AB & MB, det. PC. 1015 is referable to var. *inconspicua* (Rostaf.) A. Lister, with unbranched elaters (PC). Rare.

Trichia decipiens (Pers.) T. Macbr.
MB1087 Ghajn il-Kbira 23 Mar.1996 on dead wood of *Populus alba.* Leg. AB & MB, det. WN. Rare.

Trichia lutescens (A. Lister) A. Lister
MB1066 Ballut tal-Wardija 16 Jan.1996 on dead branch of *Quercus ilex.* Leg. AB & MB, det. MB test. WN. Rare.

Trichia persimilis P. Karst.
MB1105 Ballut tal-Wardija 23 Sept.1996 on dead branch of *Olea europea.* Leg. AB & MB, det. WN. Frequent.

Trichia varia (Pers.) Pers.
MB1086 Ghajn il-Kbira 23 Mar.1996 on dead wood. Leg. AB & MB, det. MB. test. WN. Rare.

Order: PHYSARALES

Family: Physaraceae

Badhamia foliicola A. Lister
MB1060 Ballut tal-Wardija 18 Dec.1995 on dead leaf of *Ceratonia siliqua.* Leg. AB & MB, det. MB. MB1181 Wied Babu 17 Mar.1997 on dead wood of *Ceratonia siliqua.* Leg. AB & MB, det. WN, test. GM & CI, a critical form with limeless sporangia! Total absence of lime confirmed by author: sporangia show no reaction at all to lactic acid in lacto-phenol medium. Common.

Badhamia utricularis (Bull.) Berk.
MB1012 Ghajn il-Kbira 15 Nov.1994 on dead branch of *Prunus sp.* Leg. AB & MB, det. WN. Rare.

Craterium aureum (Schumach.) Rostaf.
MB1052 Ballut tal-Wardija 13 Nov.1995 on dead leaf of *Ceratonia siliqua.* Leg. AB & MB, det. MB. Frequent.

Craterium leucocephalum (Pers.) Ditmar
MB1119 Ballut tal-Imgiebah 16 Jan.1997 on dead leaves of *Quercus ilex.* Leg AB & MB, det. MB. test. WN, specimen referable to var. *scyphoides* (Cooke & Balf.) G. Lister, (WN). Rare.

Craterium rubronodum G. Lister
MB1206 Ballut tal-Imgiebah 21 Nov.1997 on leaf litter of *Quercus ilex.* Leg. AB & MB, det. MB, test. CL. First

record for the Mediterranean Region. Rare.

Leocarpus fragilis (Dicks.) Rostaf.
MB1013 Ballut tal-Imgiebah 23 Dec.1994 on debris of *Quercus ilex.* Leg. MB, det. PC. Frequent.

Physarum bitectum G. Lister
MB1140 Wied Babu 13 Feb.1997 on dead wood of *Ceratonia siliqua.* Leg. AB & MB, det. WN, (spores 10-11μm). MB1159 Wied Babu 20 Feb.1997 on dead leaf. Leg. AB & MB, det. WN, (spores 13-14μm). Rare.

Physarum bogoriense Racib.
MB1075 Maqluba 1 Mar.1996 on moss growing on dead wood. Leg. AB & MB, det. MB. Rare.

Physarum cinereum (Batsch) Pers.
MB1008 Ballut tal-Imgiebah 10 Dec.1993 on dead leaf. Leg. MB, det. PC. MB1160 Wied Babu 20 Feb.1997 on decaying legume of *Ceratonia siliqua.* Leg. AB & MB, det. GM & Cl. Unlike all specimens of this species seen so far, MB1160 is a critical form consisting of groups of heaped sporangia with no sign of any plasmodiocarps. Common.

Physarum compressum Alb. & Schwein.
MB1148 Wied Babu 13 Feb.1997 on dead herbacious stem. Leg. AB & MB, det. MB, test. WN. Frequent.

Physarum echinosporum A. Lister
MB1208 Imgiebah 21 Nov.1997, on dead wood of *Quercus ilex*, leg. AB & MB, det.WN. First record for the Mediterranean Region. Rare.

Physarum gyrosum Rostaf.
MB1139 Wied Babu 13 Feb.1997 on dead twig of *Rubus ulmifolius.* Leg. AB & MB, det. MB, test. WN. An atypical form, however, spores indicative of species (WN). Rare.

Physarum leucophaeum Fr.
MB1009 Ghajn il-Kbira 28 Dec.1993 on stump of *Prunus sp.* Leg. AB & MB, det. PC. Frequent.

Physarum leucopus Link
MB1024 Ballut tal-Wardija 25 Oct.1995 on dead leaf. Leg. AB & MB, det. MB typical form, (several other variants were determined by WN and MM). Common.

Physarum lividum Rostaf.
MB1176 Wied Babu 17 Mar.1997 on decaying legume of *Ceratonia siliqua.* Leg. AB & MB, det. WN. Specimen with defective maturation (WN). Rare.

Physarum melleum (Berk. & Broome) Massee
MB1050 Ballut tal-Wardija 13 Nov.1995 on dead leaves of *Quercus ilex.* Leg. AB & MB, det. WN. Specimen not fully mature, but identifiable (WN). Frequent.

Physarum nutans Pers.
MB1040 Buskett 30 Oct.1995 on dead wood. Leg. AB & MB, det. MB. Occurs on dead wood of various tree species in all localities visited. Common.

Physarum perfectum Peck
MB1147 Wied Babu 13 Feb.1997 on dead leaf of *Ceratonia siliqua.* Leg. AB & MB, det. WN. Frequent.

Physarum pusillum (Berk. & M.A. Curtis) G. Lister
MB1177 Wied Babu 17 Mar.1997 on dead wood of *Ceratonia siliqua.* Leg. AB & MB, det. MB, test. WN. Frequent.

Physarum viride (Bull.) Pers.
MB1088 Buskett 23 Mar.1996 on dead wood of *Carya olivaeformis.* Leg. AB & MB, det. MB. Several specimens were met with on dead wood of various tree species in all localities visited. Common.

Family: Didymiaceae

Diderma hemisphaericum (Bull.) Hornem.
MB1005 Maqluba 10 Feb.1985 on leaf of *Ceratonia siliqua.* Leg. E. Lanfranco & MB, det. MM. Common.

Diderma spumarioides (Fr.) Fr.
MB1132 Ballut tal-Wardija 17 Jan.1997 embracing living herbacious stem. Leg. AB & MB, det. MB. Rare.

Didymium clavus (Alb. & Schwein.) Rabenh.
MB1122 Ballut tal-Wardija 17 Jan.1997 on dead leaves. Leg. AB & MB, det. MB. Rare.

Didymium difforme (Pers.) Gray
MB1070 Ghajn il-Kbira 12 Feb.1996 on dead leaves of *Populus alba.* Leg. AB & MB, det. MM. Rare.

Didymium iridis (Ditmar) Fr.
MB1164 Wied Babu 20 Feb.1997 on decaying legumes of *Ceratonia siliqua.* Leg. AB & MB, det. MB. Rare.

Didymium melanospermum (Pers.) T. Macbr.
MB1165 Wied Babu 20 Feb.1997 on dead leaves of *Ceratonia siliqua.* Leg. AB & MB, det. WN. Rare.

Didymium minus (A. Lister) Morgan
MB1098 Ghajn il-Kbira 8 Apr.1996 on dead leaves of *Carya sp.* Leg. AB & MB, det. WN. MB1095 Ghajn il-Kbira 23 Mar.1996 on dead leaves of *Eriobotrya japonica.* Leg AB & MB, det. MM (a sessile form). Common.

Didymium nigripes (Link) Fr.
MB1021 Ghajn il-Kbira 2 Oct.1995 on dead leaves. Leg. AB & MB, det. MB. Common.

Didymium quitense (Pat.) Torrend
MB1174 Maqluba 17 Mar.1997 on dead wood of *Punica granatum.* Leg. AB & MB, det. MB, test. WN. A form with very large spores showing affinity with *D. rugulosporum* (WN). Rare.

Didymium squamulosum (Alb. & Schwein.) Fr.
MB1048 Ballut tal-Wardija 13 Nov.1995 on dead leaf of *Ceratonia siliqua.* Leg. AB & MB, det. MB. MB1134 Buskett 3 Feb.1997 on dead leaf of *Carya olivaeformis.* Leg. AB & MB, det. MM, a sessile form. Common.

Didymium trachysporum G. Lister
MB1173 Maqluba 17 Mar.1997 on dead wood of *Laurus nobilis.* Leg. AB & MB. det. BI. Rare.

Subclass **STEMONITOMYCETIDAE**

Order: STEMONITALES

Family: Stemonitaceae

Comatricha anomala Rammeloo
MB1183 Ballut tal-Wardija 27 Aug.1997 on dead wood of *Quercus ilex.* Leg. AB & MB, det. GM & CI. From all the countries bordering the Mediterranean, this species had only been reported from Spain (LADO 1994). Frequent.

Comatricha nigra (Pers.) J. Schroet.
MB1102 Ghajn il-Kbira 6 Feb.1992 on stump of *Prunus sp.* Leg. AB & MB. det. PC, test. WN, (with ovate or shortly cylindrical sporangia on relatively short stipes). MB1079 Maqluba 1 Mar.1996 on dead wood. Leg. AB & MB, det. MB, test. WN, (with globose sporangia on tall stipes). These represent two extreme forms of *C. nigra.* Nannenga-Bremekamp includes several varieties of this species in an unpublished key to a planned Monograph (WN). Common.

Comatricha tenerrima G. Lister
MB1200 Ghajn il-Kbira 6 Nov.1997 on stem of *Acanthus mollis.* Leg. AB & MB, det. MB. Rare.

Diachea leucopodia (Bull.) Rostaf.
MB1004 Ballut tal-Imgiebah 10 Jan 1985 on leaf of *Ceratonia siliqua.* Leg. MB, det. MB. MB1166 Wied Babu 20 Feb.1997 on leaf of *Ceratonia siliqua.* Leg. AB & MB, det. MB. test. WN, a form with fused stipes. Occurs practically everywhere, common.

Enerthenema papillatum (Pers.) Rostaf.
MB1031 Maqluba 8 Oct.1996 on branch of *Ceratonia siliqua.* Leg AB & MB, det. MB. Frequent.

Lamproderma scintillans Morgan
MB1199 Ghajn il-Kbira 6 Nov.1997 on twig and leaf of *Hedera helix.* Leg. AB & MB, det. MB. Rare.

Stemonitis flavogenita E. Jahn
MB1019 Ghajn il-Kbira 2 Oct.1995 on dead wood of *Populus alba.* Leg. AB & MB, det WN, a short form, total height 2.7-3.6mm. MB1034 Maqluba 8 Oct.1996 on dead wood of *Ceratonia siliqua.* Leg. AB & MB, det. YY, a critical form with undeveloped surface net showing affinity with *S. splendens.* (YY); at first it was mistaken for a *Stemonaria sp.* (See iconography). Frequent.

Stemonitis fusca Roth
MB1141 Wied Babu 13 Feb.1997 on dead branch of *Ceratonia siliqua.* Leg. AB & MB, det. GM & CI, a critical atypical form with total height up to 2.6mm and stipe more than one half of total height. GM & CI studied the type material of *S. fusca, S. nigrescens* and *S. virginiensis* and concluded that *S. fusca* is a variable species, *S. nigrescens* is synonymous to *S. fusca,* and *S. virginiensis* is characterised by small size and small spores with little reticules. (GM & CI pers. com.). Rare.

Stemonitis smithii T. Macbr.
MB1032 Maqluba 8 Oct.1996 on dead wood of *Ceratonia siliqua.* Leg. AB & MB, det. WN. Identification based on small size of spores (4µm). Rare.

Stemonitis splendens Rostaf.
MB1010 Buskett 10 Nov.1994 on fallen trunk of *Carya olivaeformis.* Leg. MB, det. WN. Rare.

Stemonitis cf. *virginiensis* Rex
MB1092 Ghajn il-Kbira 23 Mar.1996 on dead herbacious stem. Leg. AB & MB, det. WN. MB1096 Maqluba 1 Mar.1996 on dead wood. Leg. AB & MB, det. WN. Surface net of 1096 is strikingly finer than the one of 1092 (WN). The identification of these specimens may have to be reviewed following research on type material by GM & CI. Frequent.

Stemonitopsis typhina (F.H. Wigg.) Nann. -Bremek.
MB1016 Ghajn il-Kbira 23 Jan.1995 on dead wood of *Populus alba.* Leg. AB & MB. det. PC. Rare.

Acknowledgements
Thanks are due: to Wolfgang Nowotny of Riedau, Austria for determining 21species and confirming 15 others, for his information on *Echinostelium colliculosum,* for his continuous help and valuable advice, and for critically reviewing this paper; to the late Pierre Chauvin of Paris, France for determining 5 species, and for his help in my initial attempts at identification; to Marianne Meyer of Maison Forestiere Rognaix, France for determining 5 species; to Dr. Bruce Ing of University College, Chester, UK for determining a species; to Yukinori Yamamoto of Ohtsu-ko, Japan for examining two critical specimens and his permission to reproduce his iconography of one of them;to Dr. Gabriel Moreno and Dr. Carlos Illana of Universidad de Alcala de Henares, Spain for identifying two critical specimens and confirming two others; and to Dr. Carlos Lado of Consejo Superior de Investigaciones Cientificas, Madrid, Spain for examining a critical specimen and confirming 3 species. Finally, special thanks go to Mr Anthony Bonnici who accompanied me on most of the field excursions and collected many of the specimens himself.

References

Castillo A et al (1993) A new species of Comatricha with incompletely reticulated spores from Spain. *Mycotaxon* XLVI pp. 315-319.

Castillo A et al (1996) Badhamia melanospora Speg. , a species wrongly interpreted. *Mycotaxon* LVII pp. 163-170.

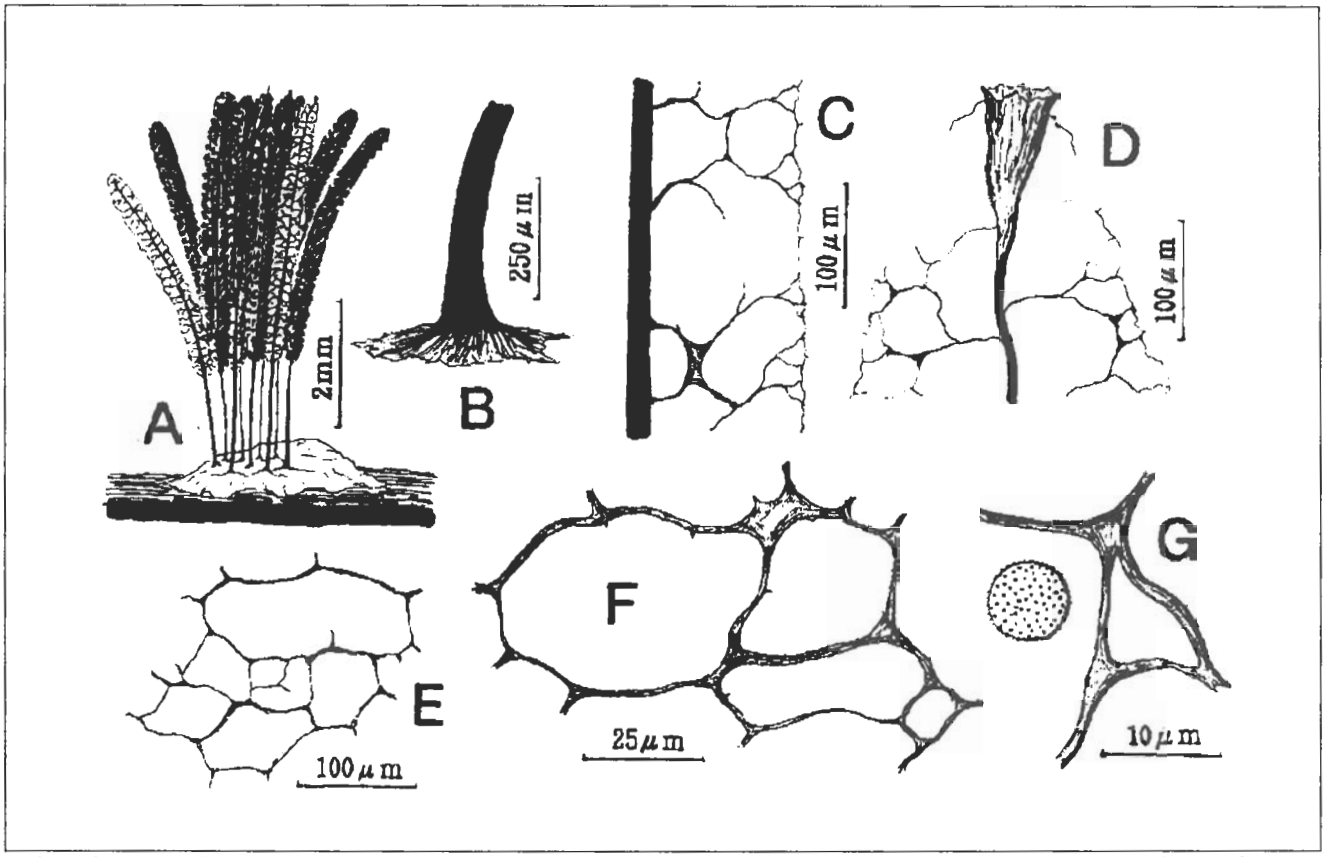


Figure 1 MB1034 *Stemonitis cf flavogenita* showing very fine traces of the undeveloped surface net, which at first seemed to be absent. (Iconography by Yukinori Yamamoto).

A. Habit.
B. Base of stipe.
C. Branches of capillitium uniting with traces of surface net.
D. Apex of columella with cupulate expansion characteristic of *S. flavogenita.*
E. & F. Different magnifications of sections of surface net with large irregular meshes indicative of *S. splendens.*
G. Spore

Chassain M (1979) *Myxomycetes* I Ed. Lechevalier S.A.R.L. Paris.

Chassain M (1979) Obtention de Myxomycetes par le procede dit 'De la Chambre Humide'. *Documents. mycologiques*, Tome X, Fasc. 37-38.

Chassain M (1980) Essai sur la place ecologique des Myxomycetes. . *Documents mycologiques*, Tome XI, Fasc. 42.

Chassain M (1981) Myxomycetes de France. *Documents mycologiques*, Tome XI, Fasc. 44.

Chassain M (1982) Essai sur l'ecologie des Myxomycetes. Note No. 2. *Documents mycologiques*, Tome XII, Fasc. 46

Chassain M (1987) Fisches technique de Myxomycetes. *Documents mycologiques*, Tome XVII, Fasc. 67.

Chassain M (1987) Fisches technique de Myxomycetes. *Documents mycologiques*, Tome XVIII, Fasc. 69 pp. 1-20.

Farr M L (1981) *How to know the true slime molds.* The pictured key nature series. Wm. C. Brown Co. Publishers Dubuque, Iowa.

Gracia E et al (1996) Enteridium rubiginosum sp. nov. A new Myxomycete from Spain. *Cryptogamie Mycol.* 17 (I) pp. 33-38.

Illana C et al (1992) Spanish Myxomycetes. V. A new species of Badhamia, and a new variety of Physarum. *Mycotaxon* XLV pp. 241-247

Lado C (1994) A Checklist of Myxomycetes of the Mediterranean Countries. *Mycotaxon* LII/1 pp. 117-185.

Lister A (1925) *A Monograph of the Mycetozoa. ed. 3,* revised by G. Lister. British Museum (Natural History). London.

Lizarraga M et al (1997) The Myxomycetes from Baja California (Mexico) I. *Mycotaxon* LXIII pp. 287-300.

Lizarraga M et al (1997) Didymium clavodecus (Myxomycetes) Una Especie Americana Nueva para Europa. *Cryptogamie Mycol.* 18 (I) pp. 87-90.

Moreno G et al (1992) Spanish Myxomycetes. VI. Four interesting species belonging to Stemonitales. *Cryptogamie Mycol.* 13(4) pp. 295-303

Moreno G et al (1993) Spanish Myxomycetes. VII. (Province of Castellon) *Mycotaxon* XLVI pp. 407-424.

Martin GW and Alexopoulos C J (1969) *The*

Myxomycetes. University Iowa Press. Iowa City.

Meyer M et al (1994) Une espece nouvelle du genre Lamproderma Rost. (Myxomycetes). *Bull. Fed. Myc. Dauphine-Savoie.* 132, pp. 34-38.

Nowotny W (1990) Beitraege zur Kenntnis der Myxomyceten Oberoesterreichs. V. *Linzer biol. Beitr.* 22/1 pp. 97-142.

Nowotny W (1991) Beitraege zur Kenntnis der Myxomyceten Oberoesterreichs. VI. *Linzer biol. Beitr.* 23/1 pp. 79-128.

Nowotny W (1992) Beitraege zur Kenntnis der Myxomyceten Oberoesterreichs. VII. *Linzer biol. Beitr.* 24/1 pp. 151-206.

Nowotny W (1992) Beitraege zur Kenntnis der Myxomyceten Oberoesterreichs. VIII. *Linzer biol. Beitr.* 24/2 pp. 863-892.

Nowotny W (1993) Beitraege zur Kenntnis der Myxomyceten Oberoesterreichs. IX. *Linzer biol. Beitr.* 25/1 pp. 321-337.

Nowotny W (1995) Schleimpilze oder Myxomyceten - unbekannte Organismen - faszinierend im Lebenszyklus und im Erscheinungsbild. *Oeko.* L 1995 pp. 32-38.

Orsino F et al (1983) Mixomiceti della Liguria. *Mic. Ital.* 1983 n. 3, pp. 23-28.

Orsino F et al (1986) I Mixomiceti. *Monti e Boschi* 1986 n. 3, I-VIII.

Orsino F et al (1987) Nuovi Mixomiceti della Liguria. *Mic. Ital.* 1987 n. 2, pp. 3-7.

Orsino F et al (1987) I Mixomiceti II. *Monti e Boschi* 1987 n. 3, I-VIII.

Orsino F et al (1988) I Mixomiceti III. *Monti e Boschi* 1988 n. 5, I-VIII.

Orsino F et al (1991) I Mixomiceti IV. *Monti e Boschi* 1991 n. 3, I-VIII.

Yamamoto Y et al (1995) *Additions to the Myxomycetes of Hokkaido.* Reports of the Taisetsuzan Institute of Science. No. 30.

Abstracts

PERIOTRON 8000: Calibration characteristics and reliability

M Ciantar and DJ Caruana
Journal of Periodontal Research (submitted, 1997)

The Periotron is an instrument designed to quantitate submicrolitre volumes of fluid sampled on a filter paper strip. To date, three models have been manufactured: the Periotron 600 (1976), the Periotron 6000 (1983) and more recently, the Periotron 8000 (1995). This paper investigated for the first time the calibration characteristics and reliability of the Periotron 8000. The fluids under investigation were: deionised water, human serum, foetal bovine serum and an ultrafiltrate of foetal bovine serum. Quantitative analysis was studied by recording a series of Periotron readings over a volume range of 0-1.0ml for each fluid. The average of five Periotron values for each particular fluid was then plotted versus the respective fluid volume. Qualitative changes in fluid composition versus Periotron Scores were analysed. Volume conversion for Periotron scores using both Periotron MLCONVRT software and a best fit equation selected from TableCurve 2D software compared well. The results of this study revealed that: 1) differences in calibration fluid composition (e.g. protein content) are reflected in the Periotron scores; 2) positioning of filter paper strip between the jaws of the Periotron should be standardised, 3) calibration of the Periotron 8000 seems to be consistent over a one week interval.

Modification of Glucose Oxidase by the Covalent Attachment of a Tetrathiafulvalene Derivative

PN Bartlett, S Booth, DJ Caruana, JD Kilburn and C Santamaria
*Analytical Chemistry 1997, **79**, 734-742*

4-[(3'-Carbohydroxypropyl)thio]-5 (methylthio)tetrathia-fulvalene was synthe- sized and used as a mediator for the oxidation of glucose oxidase both free in solution and after covalent attachment to the enzyme through carbodiimide coupling to amine residues in the protein. The modified enzyme was characterised by isoelectric focusing gel electrophoresis and found to have a higher pI than the native enzyme. Electrochemical studies show that the singly oxidised tetrathiafulvalene derivative attached to the enzyme can act as a mediator for the direct reoxidation of the enzyme at electrode surfaces, whereas the doubly oxidised dication is not a mediator. Similar results are found for mediation by the tetrathiafulvalene derivative in solution. The application of the modified enzyme in membrane enzyme electrodes was investigated, and the response was analysed to give kinetic information about the modified enzyme kinetics. Our studies show that the modified enzyme has good stability on storage in the absence of glucose but is less stable during continuous operation in a glucose sensor. This appears to arise from reactions between the tetrathiafulvalene groups attached to the modified enzyme and traces of hydrogen peroxide generated by the enzymatic reaction of glucose with oxygen present in the solution.

Electrochemical Detection of Halothane

Daren J Caruana
American Chemical Society Books Series (in print) due in July 1997
Book entitled The Chemistry and Technology of Chemical Sensors, Edited by N Akmal and A Usmani

The novel electrochemistry of halothane in aqueous acidic solution is described. The irreversible reduction at -0.15 V vs. SCE results in the deposition of an intermediate which may be oxidised at +0.65 V vs. SCE. The strength of adsorption is dependent on pH, and the oxidation peak only occurs in the presence of chloride. Previous studies on halothane electrochemistry have been concerned with the reduction process in alkali conditions. Under these conditions a high current density is obtained, however, concurrent reduction of oxygen occurs in the same potential region which makes electrochemical detection of halothane problematic. In acidic conditions anodic stripping voltammetry may be used to measure halothane concentrations, free from interference of oxygen. A thin film sensor is described which may be used to measure halothane at clinically relevant concentrations (0 to 5%) in the presence of oxygen. The sensor is inexpensive to produce, has a fast response time (700 ms), and is unaffected by vapour flow rate.

Surface properties of fractured and polished platinum microelectrodes

DJ Caruana, JV Bannister
*Journal of Electroanalytical Chemistry, 1997, **242**, 119-131.*

An investigation of the surface properties related to the method of microelectrode fabrication is presented and discussed. The voltammetric response in acidic media (sulphuric and formic acids) of fractured 25 μm diameter wire which had been sealed in soft glass was used to investigate the surface microstructure. SEM studies showed that the crystallite size of the fractured wire, which had previously been sealed in glass, was large, and that a single crystallite may be exposed to the solution surface. The effect of sealing the wire in glass on the bulk properties of the wire is related to the voltammeter response in acid media. Thus, the surface of a polished platinum microelectrode sealed in glass cannot be considered as truly polycrystalline. The responses of fractured platinum wire sealed in soft glass in sulphuric acid are predominantly Pt(111), when compared by non-linear regression and congruent analysis with the three low index faces of platinum in the same acidic conditions

under the same conditions. This is explained by the process of slip deformation, which involves the slipping of Pt(111) planes (close packed planes or planes of highest atomic density) forming steps with the exposed surface predominantly Pt(111).

Electrochemical detection of halothane by anodic stripping voltammetry

D J Caruana and J Giglio (1996)
Journal of the Chemistry Society, Faraday Transeactions, 92, 3669-3670

The anaesthetic vapour halothane is detected by anodic stripping voltammetry at an unmodified gold electrode. The reduction of halothane in acidic aqueous conditions results in adsorbed products which may be oxidised at +0.62 V. The technique is shown to be suitable for measuring halothane at clinically relevant concentrations, free from oxygen interference.

Neutron reflection studies of poly(phenol) films

SJ Roser, DJ Caruana, M Gerstenberg (1996)
Journal of Electroanalytical Chemistry, 411, 153-160

The detailed structure of an electrochemically produced poly(phenol) film is investigated in situ by neutron reflection. The surface density of the polymer is measured, and its thickness is determined accurately from fits to reflectivity data. From contrast variation measurements, it is seen that there is penetration of solvent into the growing film, but only in the topmost part. Kinetic measurements are made on the deposition process for the polymer film. The potential of the technique for studies of protein adsorption is discussed.

Report

Pugwash Conferences On Science And World Affairs

Pugwash is an international movement of scientists concerned with the social impact of science and seeking ways to prevent its misuse. Particular attention is given to banning weapons of mass destruction (nuclear, chemical and biological), to the solution of conflict without resort to force, to the creation of a sustainable environment, and to bettering the conditions of life of all people. The Movement has its origin in the Russell-Einstein Manifesto of 1955, which called on scientists to meet and find ways to avert the threat to civilization created by the advent of thermonuclear weapons. It took its name from the venue of the first meeting in 1957 – the Canadian fishing village of Pugwash on the Atlantic coast.

One of the roles of Pugwash is to organise meetings (6-8 a year) for scientists and scholars from all over the world with the aim of influencing government policies and public thinking on topical problems of global security. Meeting in private, as individuals representing only themselves, they are able to reach conclusions which often pave the way to governmental agreements and international treaties. The topics on the agenda of the Workshops and at the Annual Conferences are various and include amongst others nuclear forces, foreign debts, social tensions and ethnic conflict. Reports on activities are published in the *Pugwash Newsletter* and *Proceedings of Conferences*. Pugwash also runs projects (e.g. A Nuclear-Weapon-Free World; Conversion of Military R&D; Education for World Citizenship) which are published as books.

Amongst its achievements, Pugwash provided a channel of communication between influential scientists and scholars from East and West during the Cold War period, facilitating better understanding between the adversaries. This in turn helped to make possible agreements on important issues, such as the Partial Test Ban Treaty, the Nuclear Non-Proliferation Treaty, the Anti-Ballistic Missile Treaty and the Biological Weapons Convention. More recently it has contributed to making possible a large reduction of nuclear arsenals, the Chemical Weapons Convention and the negotiations on a Comprehensive Test Ban Treaty. However, much still needs to be done to secure peace in the world.

Throughout the years Pugwash has gained significant international recognition. In fact Pugwash Conferences have received many international awards: in 1987, they were awarded the *Olympia Prize* by the Onassis Foundation (US$ 100,000, shared with the Archaeological Society of Greece), and the *Feltrinelli Prize* by the Accademia Nazionale dei Lincei (Lit. 100.000.000 – awarded every four years for work having a high moral and humanitarian value). This money was placed in the International Pugwash Foundation (located in Geneva, and on whose Administrative board both Robert McNamara, ex-US Secretary of Defense and later President of the World Bank, and Prince Sadruddin Aga Khan take part), which was set up with the goal (still far off) of raising US$ 5,000,000 as a financial base. In 1989, UNESCO awarded to the Pugwash Conferences the *Einstein Gold Medal*. In 1992, The *Albert Einstein Peace Prize* (US$ 50,000) was awarded to Hans Bethe and Joseph Rotblat, who donated his half to the Pugwash foundation.

The 1995 *Nobel Peace Prize* was awarded in two equal parts, to Joseph Rotblat, President of Pugwash, and to the Pugwash Conferences on Science and World Affairs, with the following statement (released on Friday, October 13, 1995):

> *The Norwegian Nobel Committee has decided to award the Nobel peace Prize for 1995, in two equal parts, to Joseph Rotblat, President of Pugwash, and to the Pugwash Conferences on Science and World Affairs, for their efforts to diminish the part played by nuclear arms in international politics and in the longer run to eliminate such arms.*
>
> *It is fifty years this year since the two atomic bombs were dropped on Hiroshima and Nagasaki, and forty years since the issuing of the Russel-Einstein Manifesto. The Manifesto laid the foundations for the Pugwash Conferences, which have maintained a high level of activity to this day. Joseph Rotblat was one of the eleven scientists behind the Manifesto, and has since been the most important figure in the Pugwash work.*
>
> *The Conferences are based on the recognition of the responsibility of scientists for their inventions. They have underlined the catatstrophic consequences of the use of the new weapons. They have brought together scientists and decision-makers to collaborate across political divides on constructive proposals for reducing the nuclear threat.*

The Pugwash Conferences are founded in the desire to see all nuclear arms destroyed and, ultimately, in a vision of other solutions to international disputes than war. The Pugwash Conference in Hiroshima in July this year declared that we have the opportunity today of approaching those goals. It is the Committee's hope that the award of the Nobel Peace Prize for 1995 to Rotblat

and to Pugwash will encourage world leaders to intensify their efforts to rid the world of nuclear weapons.

The Malta Pugwash Group, represented by the Malta Chamber of Scientists, forms part of the network of over 30 National Groups supporting the International Pugwash Movement. Membership is open to anyone living in Malta and qualified by profession or experience to contribute to the work of Pugwash. The Malta Pugwash Group holds seminars and public meetings on relevant international topics as well as topics specifically relevant to Malta; it prepares papers for the international Pugwash meetings; and nominates participants from Malta to the Annual Conferences. It also runs research projects aiming at informing public opinion and influencing government policy, especially on international security issues. Interested persons are invited to join and help to continue this important work! Applications may be sent to The Malta Chamber of Scientists, P.O. Box 45, Valletta B.P.O.

London Office:
Flat A Museum Mansions,
63A Great Russell Street,
London WC1B 3BJ, UK,
Tel: (+44) 171-4056661, Fax: (+44) 171-8315651,
E-mail: pugwash@qmw.ac.uk

Geneva Office:
63 rue de Lausanne,
1202 Geneva, Switzerland,
Tel: (+41) 22-7383294, Fax: (+41) 22-7383292

Rome Office:
Palazzine dell'Uditorio,
Accademia Nazionale dei Lincei,
Via della Lungara 10,
I-00165 Rome, Italy,
Tel: (+39) 6-6872606, Fax: (+39) 6-6878376,
E-mail: calogero@sci.uniromal.it

Hepoxilin-evoked Changes In Intracellular Calcium

Hepoxilin-evoked changes in intracellular calcium in human neutrophils loaded with fluo-3AM. Note the rapid rise in intracellular calcium after addition of hepoxilin A_3-methyl ester at 1μg/μl in DMSO in 1ml calcium-free medium containing 1mM EGTA. The same three cells are shown at four different time points, i.e. before addition and after addition of the hepoxilin. Colour bar range: blue – white (approx. 150 - >400nM calcium).

Acknowledgements: Supported by an NSERC grant to L.R. Mills, Department of Physiology, Faculty of Medicine, University of Toronto and The Toronto Hospital, and an MRC grant to C.R. Pace-Axiak, Research Institute, Hospital for Sick Children, Toronto and Department of Pharmacology, Faculty of Medicine, University of Toronto, Canada.

Xjenza

Instructions for Authors

Xjenza will consider for publication papers in the following categories:

- Original research reports
- Short reports
- Reviews
- Commentaries

SHORT REPORTS should be of no more than 1,500 words and 2 tables or figures. Please contact one of the editors to discuss the suitability of topics for REVIEW up to 7,500 words, COMMENTARY - up to 2,500 words, or other material falling outside the usual categories.

Submission of Manuscripts
Manuscripts should be sent to the Editor.

Prof. Angela Xuereb,
Department of Pathology,
University of Malta Medical School,
G'Mangia..

Tel: (+356) 2595 1882
Fax:(+356) 235638

Please submit 4 copies, typewritten, double spaced with wide margins, keep a copy yourself. After an article has been accepted for publication, the final version should be provided in machine readable form with hard copy exactly the same as the data on disk. Please use IBM compatible disks preferably Word (ver. 2.0 or higher) format or Rich Text Format (RTF) without the use of heading and body styles. Authors are requested to submit together with the manuscript, the names and addresses of two referees, preferably from overseas.

Conflict of Interest
Authors are expected to disclose any commercial or other associations that might pose a conflict of interest in connection with the submitted article. All funding sources supporting the work, and institutional or corporate affiliations of the authors, should be acknowledged on the title page.

Articles are considered for publication on the understanding that neither the article nor its essential substance has been or will be published elsewhere before appearing *in Xjenza.*

Permissions
Materials copied from other sources must be accompanied by a written statement from both author and publisher giving permission to *Xjenza* for reproduction. Obtain permission in writing from at least one author of papers still in press, of unpublished data, and of personal communications. It is the author's responsibility to ensure that permissions are obtained.

Arrangement of Manuscripts
Preface each paper with a suitable **title,** the author(s) name(s), and the full address of the institution in which the work was carried out (please identify the author for proofs - giving telephone and fax numbers - for correspondence and reprint requests), an **abstract** of no more than 200 words, and up to 8 **keywords.** Research reports should give an appropriate **introduction,** a single **methods** section (with the methodology of all experiments reports), **results,** and **discussion.** Either British or American spellings are acceptable, but please be consistent. Authors should express **measurements** in SI units, although they may include older conventional units in parentheses if they wish. Except for units of measurement, **abbreviations** should be spelled out on first use, and should be standard. **Drug names** should be generic, although authors may add brand names in parentheses if they wish.

Illustrations should be lightly numbered on the back in pencil, with orientation and your name. We prefer glossy photographs or professionally prepared line drawings; any lettering and symbols *must* be large enough to stand reduction in size - publication could be delayed if lettering or symbols are too small. For halftones send unmounted glossy photographs with explanatory legends on a separate sheet of paper. Please send one set of photographs and attach a set of photocopies to each copy of the manuscript. Please contact the publishers for an estimate of the cost for colour illustrations. Illustrations on Electronic Media should be in a common format (Bitmap, JPG or TIFF) and of publishing quality.

Tables should bear Roman numerals and be typed on separate sheets of paper. Each table requires a title, but no legend; identify footnotes by superscripts [1,2,3], etc.

References follow the Harvard system. In the text give the author(s) name and, in parentheses, the date of the paper/book being cited. Differentiate between papers by the same author in the same year by a, b, c etc., immediately after the date. Where there are three or more authors use et al. in the text and a, b, c to resolve ambiguities. All works cited must be listed at the end of the paper, ordered alphabetically by first author's name. For each first author, list single author works, next joint authored works in **alphabetical** order, last multi-authored works in **chronological** order. Each reference should give the names and initials of each author, the year of publication, the title of the paper, the name of the journal or book in full, the volume or edition, the first and last page, and, for books, the publisher and town of publication. For example:

Scerri CA, Abela W, Galdies R, Pizzuto M, Grech JL and Felice AE (1993) The β^+ IVS, I-nt No. 6(T->C) Thalassaemia in heterozygotes with an associated Hb Valletta or Hb S Heterozygosity and homozygotes from Malta. *British Journal of Haematology,* **83,** 669-671.

Felice AE (1992) Molecular Epidemiolgy of Haemoglobin, and the Molecular biology of Normal and Abnormal Globin gene expression. In: *Collected Papers* (Eds R Ellul-Micallef and S Fiorini), pp. 357-391. University Press, Malta.

David Rawn J (1989) *Biochemistry.* Neil Patterson Publishers, North Carolina

Muscat R, Papp M and Willner P (1992a) Antidepressant-like effects of dopamine agonists in an animal model of depression. *Biological Psychiatry,* **31,** 937-946.

Proofs, Reprints and Copyright
Authors will normally be sent page proofs by fax where available. Such corrections as are necessary should be listed in the form of a table, with the location of the correction in the left hand column and the correction required in the right hand column. This table should be faxed to the editors within three days of proof receipt. Alterations, other than essential corrections to the text of the paper, should not be made at this stage; the cost of such alterations may be passed on to the author.

A form for ordering reprints will be supplied with the proofs and should be returned direct to the Publisher. Manuscripts are accepted for publication on the understanding that exclusive copyright is assigned to the Publishers. However, this does not limit the freedom of the author(s) to use material in the papers in any other published works.

ETHICAL POLICY

Studies using human subjects should include a statement within the manuscript that subjects provided informed consent for their participation and that the study was approved by a local ethics committee.

Studies using animal subjects must conform to internationally accepted standards of animal welfare, and authors should include an appropriate statement to that effect, either within the manuscript or in their covering letter. Every effort should be made to minimize the number of animals used.

The editors will welcome the submission of work in any area of science. However, studies that subject animals to pain or stress require special consideration and three principles will be used to judge the ethics of such work. First, experiments of this kind will be required to reach a higher threshold of scientific quality in order to be publishable. Second, the utility of the study will be assessed, in terms of the balance between the distress inflicted and the likelihood of benefit. Third, it must be demonstrated that the objectives of the experiment could not have been achieved by the use of less stressful procedures; the fact that a particular procedure has been used in previously published work will not in itself suffice. The editors believe that scientists apply strict ethical criteria to their work, and will have no difficulty in meeting these requirements.

Published by
Malta Chamber of Scientists
P.O. Box 45, Valetta B.P.O.,
Valletta, Malta
Tel: (+356) 3290 2774
Fax:(+356) 343535

Sartorius Portable Series.

Portable fun for everyone!

Technoline

Technoline Ltd. Edgar Bernard Street, Gzira.
Tel: 337975, 342366, 344149, 344345 Fax: 343952

Technoline's service department is the only local service entity able to offer precision scale and analytical balance calibrations up to the international ISO 9000 standard.

TO LOWER YOUR
CHOLESTEROL
HAVE CEREALS FOR BREAKFAST

We hear so much about Cholesterol nowadays, what is it ? Cholesterol is a type of fat which is essential for your body. Most of it is made by your liver. However, this amount increases when we eat lots of foods rich in fat. If there is too much cholesterol in our blood, problems can arise.

Heart problems are partly caused by too much cholesterol in the blood. The worrisome fact is that according to the Dept. of Health (1993) Food & Health in Malta, three out of every four adults in Malta have elevated Cholesterol levels. That could very likely mean you.

The Good News ! - Yes there is good news! By eating less fat and cholesterol and more carbohydrate foods you can help reduce blood cholesterol levels. This is where high fibre cereals fit in - they are very very low in fat - there is even scientific evidence[1] that shows that people who have cereals for breakfast tend to have lower fat intakes, they also tend to have lower cholesterol levels.

So when you wake up tomorrow morning - remember that high fibre cereals are low in fat, and cholesterol free the perfect complement to a low fat diet !

1. *Morgan et al (1986) The role of breakfast in diet adequacy on the US adult population. Journal of the American College of Nutrition.*

Stanton & Keast (1989) Serum Cholesterol, fat intake and breakfast consumption in the United States adult population, journal of the American College of Nutrition.

Kellogg's

Nutritious Breakfast For a Healthier You

Caution!

Fasten your seatbelt before takeoff

Test drive a Laser Computer today and feel the difference. We have harnessed the power of Intel's newest Pentium MMX processors to deliver awesome performance at an incredible price. Whether you are surfing the Internet, researching your latest project or playing the latest game, your Laser Computer will fly through the task.

Laser's manufacturing facility carries full ISO 9002 Certification and all our computers are bristling with the latest technology. Standard features like SDRAM, high capacity Fast ATA-3 hard disk, and a high performance graphics accelerator ensure top performance. To deliver the ultimate multimedia experience, we only use the World's renowned Creative Labs CDROM's and Sound Blaster wavetable sound cards.

Quick delivery and our prompt efficient service are our hallmarks. Visit our showroom today and test drive a Laser Computer. You will be pleasantly surprised!

LASER computer

OFFICE: First International Ltd., Multi-Tech Centre, Regional Road (off Valley Road), Msida Telephone: 483819
SHOWROOM: 880 St. Joseph High Road, Hamrun Telephone: 235606