Xjenza

JOURNAL OF THE MALTA CHAMBER OF SCIENTISTS

Volume 1 No 1

July 1996


FONTANA®
Sparkling
Partners in good taste!
FONTANA
Sparkling
PURE TABLE WATER
750ml
FONTANA
Sparkling
PURE TABLE WATER
330ml
LOW IN SODIUM • ENRICHED WITH FLUORIDE • PERFECT MIXER WITH FRUIT JUICES, WINES & SPIRITS • IDEAL COMPANION TO EVERY MEAL

Contents

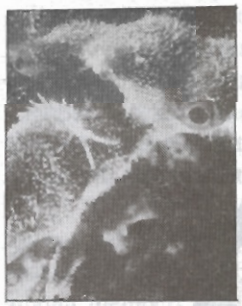
Xjenza
Official Journal of the Malta Chamber of Scientists

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: The sponge *Cacospongia sp.* with the opistobranch, *Cratena peregrina* grazing on its surface.
Picture by: Martin Vella

Malta Chamber *of* Scientists

The Malta Chamber Of Scientists,
P.O. Box 45,
Valletta B.P.O.,
Valletta,
Malta.

Printers: University Press, Msida

LONGINES®

More than 160 years of history and tradition. A dynamic and inventive tradition marked by a willingness to always be ahead of its time in watchmaking technology.

Registered models.

Longines Conquest VHP™ *(Very High Precision) for ladies and gents. Change of time zone with synchronization of the date. Quartz movements. Water-resistant to 100 metres. Sapphire glasses.*

THE PIONEERS' SWISS WATCH SINCE 1832

Available at: **HELVETIA** - 154 Melita Street, Valletta.
MALLIA BORG JEWELLERS - 140 St. Lucia Street, Valletta.
MICALLEF - 53 Zachary Street, Valletta.
A. AGIUS - 35 Tower Road, Sliema.
BOLIVAR LTD - 86 Tigne Street, Sliema.
MIDAS TOUCH - 37 Main Gate Street, Victoria, Gozo.

Editorial

***Xjenza*: The First Issue**

The need has long been felt among scientists in Malta for a local outlet for their work, particularly for work dealing with local issues that might not readily find acceptance by an international journal. It was from this need that the idea for the journal *Xjenza* was conceived about eighteen months ago, an idea put forward by a member of The Malta Chamber of Scientists. This proposal was later approved by the Council of the same Chamber. Since there was an encouraging response to a first call for submission of papers, it was decided to go ahead with the ambitious venture. What you see in front of you today is the fruit of six months hard work which led to the publication of this first issue of *Xjenza*.

All the research articles in this journal have been subjected to peer review. It is editorial policy to set high standards of peer review which should lead to equally high standards of scientific research. This does not mean that editorial policy will be solely about publishing the best work of established scientists; it will also be our policy to encourage young scientists with promising new lines of research to put pen to paper. In this way, *Xjenza* will provide a suitable training ground for young scientists seeking their first publication. Apart from original research articles, review articles on topical issues will also be featured.

Xjenza will be featuring a section devoted to current research profiles which will serve to increase awareness among members of the Chamber and the public, of the scientific research being done in Malta in the various disciplines of science. This section may include abstracts of papers already published in international journals. The Editorial Board welcomes submissions from scientists in Malta specifying their research interest and activities to ensure that the journal presents an opportunity to all researchers across the many scientific disciplines to feature on a regular basis. It is hoped that over time *Xjenza* would serve as a record of Maltese scientific development and achievement.

Xjenza will also serve as a forum for the initiation and development of debate among the Maltese scientific community on the central scientific issues of the day. It will be a matter for the Editorial Board to solicit contributions in this regard. The issues of debate will tend to be those identified within and reflecting the agenda of the Malta Chamber of Scientists, but not exclusively so, as the range of issues might well include a debate on the role of the Chamber itself. The journal will also carry a section on the activities of the Malta Chamber of Scientists. In subsequent issues, a correspondence column will be included in which we hope to publish letters from readers relating to articles featured in previous issues or on any topic of interest to our readers. We also welcome feed-back from readers on how, perhaps, we could improve the journal (assuming that such a thing is possible!). Any readers with ideas for a new section should let us know. So please write in with all your comments. We assure you that we are all grown-up women and men and can take criticism and ideas for change very well.

Another objective of *Xjenza* is to forge links between local scientists and Maltese scientists working abroad. A register of Maltese scientists working abroad will be compiled with a view to familiarising local colleagues with their work and encouraging cross-border Maltese collaboration. Attracting papers from such persons abroad would help in this familiarisation process but more importantly these scientists could be asked to act as referees for papers submitted to the journal. Such a register is being compiled. Readers who know of Maltese scientists working abroad and of their research interests, are asked to write in with these details. Who knows what projects and other benefits might be spawned in this way?

Since some of the articles published in this issue had been pending for some time because of a few teething problems, it was not possible this time round to include dates for submission and acceptance of these articles. It will become standard practice from the next issue of *Xjenza* which is planned for early 1997, by which time we also anticipate being on the Internet.

The first issue of *Xjenza* has come into being as a result of the support and dedication offered by my colleagues: Dr. Martin Ebejer, Dr. Richard Muscat, Dr. Emmanuel Sinagra and Dr. Christian A. Scerri. When spirits were low, they were always there with words of encouragement spurring me on. For this, and especially for their hard work, I do thank them.

I would also like to thank the many sponsors who have made this journal something akin to a viable proposition.

Finally I would like to call on all Maltese scientists. *Xjenza* is really your journal! Make it come to life!

We look forward to receiving the best work from you.

ANGELA XUEREB
EDITOR

Report

The Malta Chamber of Scientists - The Second Anniversary

Alex E. Felice and Richard Muscat
The Malta Chamber of Scientists, P.O. Box 45, Valletta B.P.O.

In the first issue of *Xjenza*, The Journal of the Malta Chamber of Scientists, it is our privilege as Foundation President and Foundation member of Council, to outline the activities of the first two years of the Chamber's life. This also gives us an opportunity to reflect on what we see as the future of the Chamber.

The Malta Chamber of Scientists has become the national representative organisation of practising scientists in Malta. There is now an organisation which is administratively sound, with a healthy budget, ready to face the future with confidence. The introduction of *Xjenza* roughly coincides with the second anniversary of the inaugural event with which the new Malta Chamber of Scientists was publicly launched. It completes a package of three publications that complement each other. The publications have been sustained successfully and it is hoped that they will be developed further. A quarterly newsletter, produced by Dr. Christian Scerri, serves to inform members of Chamber activities, and the monthly Malta Science Reports in "The Times", edited by Dr. Richard Muscat, provides space for the members of the Chamber to give visibility to their work in the eyes of the general public. Together they contribute substantially to increasing science awareness and science literacy especially among the younger generations. It is known that the Malta Science Reports are read with considerable interest by prominent members of the reading public including high officers in the civil service and diplomatic representatives.

The regular Business and Scientific Meetings have been prominent activities successfully sustained for the last two years. Many important and relevant topics have been covered ranging from basic or enabling sciences to science policy and industrial applications. One of the most notable events was the joint meeting that was held with the PUGWASH Conference on Science and World affairs. This was the first public appearance of

His Excellency Dr U. Mifsud Bonnici, President of the Republic of Malta addressing the Chamber at its inauguration on 28 July, 1994, at the University of Malta. Seen to his left are: Prof A.E. Felice, President of the Chamber, Rev Prof P. Serracino Inglott, Rector of the University, Mrs Mifsud Bonnici and Prof V. Ferrito, President-elect of the Chamber.

PUGWASH and of Nobel Laureate Joseph Rotblatt after they had been awarded the Nobel Peace Prize. Since then, the Malta Chamber of Scientists has represented the Pugwash Conference on Science and World affairs in Malta. It is hoped that the role of the Chamber in this prestigious group will be increased. Equally important meetings on science policy have been held in conjunction with the Malta Development Corporation and other business leaders. Following such a meeting, an agreement was reached with Messrs. Simonds Farsons Cisk Ltd., for financial support for the scientific meetings. Henceforth, these scientific meetings shall be known as the Farsons Series. The Chamber played an important part in the Malta Science Week by providing public speakers and many members contributed in various capacities. It is hoped that the Chamber will participate again next year.

The Chamber continues to grow and to establish itself on the Maltese professional scene. There are now 200 members drawn from all sectors of the national economy and from all scientific disciplines. A professional register of scientists has been compiled for the first time. This register will occupy an important position in the certification of science specialists in the context of professional recognition within the European Union. Further growth is possible by targeting new graduates for membership. There is scope for undergraduates and possibly sixth form science students to be affiliated with the Chamber of Scientists in a Junior Chamber. Also a Corporate Chamber deserves attention to sustain growth.

There have been areas in which less success has been achieved, such as in promoting accreditation and continuous professional development credits. This is important because shortly it will no longer be possible, in Europe, to use any professional scientific designation without an appropriate national accreditation process. Perhaps, recent, sad events in the industrial sector serve to emphasise the importance of this issue in a dramatic manner. The membership should be left in no doubt as to the importance of a properly conducted accreditation system which will serve all members in enhancing their professional standing in Malta and within the larger European context. The next council will undoubtedly have the onus of implementing a professional accreditation system linked to programmes for continuous professional development. The new council will also have to work harder at the political level to ensure stable and sustained funding for scientific research, and to improve the link between enabling sciences and social or economic development, perhaps, through a National Foundation for Scientific Research.

All the above mentioned activities would not have been possible without the effort put in by those members sitting on various committees of the Chamber. Also many companies have supported the Chamber and special mention should be given to the Malta Council for Science and Technology for a foundation grant of Lm 1,000 and Messrs. Lowenbrau Ltd. for cordially hosting council meetings. It is hoped that this support together with additional public and private sector financial support will be attracted in the future.

Membership

Full membership of the Malta Chamber of Scientists is open to all individuals possessing a first degree in a science related subject. Candidate membership is open to students reading for a science related degree. Membership fees are due on the 1 June of each year.

Yearly membership fees: Lm10 for full membership (Lm15 for a married couple both of whom are members).
Lm5 for candidate members.

Benefits include:
1. Subscription to ***Xjenza***
2. Right to attend all monthly scientific meetings
3. Quarterly newsletter

Please mail or fax your details to the:

The Malta Chamber of Scientists,
P.O. Box 45,
Valletta B.P.O.,
Valletta.
Tel:/Fax: 343535

Communication

Wind Energy in Malta

Pius J. Darmanin and Edward A. Mallia
Department of Physics, University of Malta, Msida

Wind energy is an attractive alternative source of energy. It is becoming increasingly popular in northern countries which have a good wind resource. It is estimated that there are about 17,000 machines providing about 2.7 GW in countries such as Denmark, Germany, Sweden, China, India, Hawaii, and the U.S.A. etc.

Wind energy is a renewable, freely available, non-polluting source of energy. Although the wind is notoriously variable, the mean energy available is generally reliable constant over long periods of time. The wind energy resource of any country depends on two main factors; the general meteorological conditions that effect the particular zone, and the land area available for farms.

Observations of wind speed and direction taken by the Meteorological Office at Luqa during the period 1972–1991 have been analyzed for a determination of wind energy in the Maltese Islands.

The Prevailing Wind Direction

The prevailing wind direction is North West (260°–340°). Wind in this sector blows for 41.4% of the time and contributes to 54% of the total energy content. East to South East wind (080°–160°) blows for 18.8% of the time contributing 19% of the total energy. These two reciprocal sectors contain 73% of the total wind energy. The best locations for wind energy converters (WECs) in Malta are those areas exposed to the North Westerlies.

Wind Energy to Electrical Energy

Wind turbines come in a range of sizes, the biggest have a rotor diameter of 100m, stand on a 100m high tower (Delimara P.S. stack 110m) and can produce 3MW at a wind speed of 11m/s. Smaller practical turbines have a 42m rotor, stand on a 50m tower and can produce 0.5MW at the same wind speed. This means that each large machine provides as much energy as 6 small ones. These machines have an electrical loading over 370W/m^2 which implies that they require an input wind energy of about 830W/m^2. Only part of the wind kinetic energy, can be converted to practical use due to aerodynamic and electrical constraints. It is necessary, therefore, to assume a typical WEC to evaluate the amount of electrical energy that can be produced.

Wind Characteristics and Energy

The mean wind speed, at 100m above ground level, is 6.2m/s and provides 142W/m^2, the median wind speed is 7.3m/s and provides 235W/m^2. Both values are small when compared to the required input loading of 830W/m^2. The estimated annual wind energy at Luqa is 2944 kW–hr/m^2.

The range of wind speeds observed, allow a typical WEC to provide electricity during only 62% of the time; it would be at a standstill for an accumulated period equivalent to 4.6 months, half of which would occur from July to September. Furthermore during the 7.4 months production period, the conversion efficiency varies from 8% to 46% as is shown in Table 1:

Time %	Conversion efficiency %	Remarks
26.1	11.8	Wind speed low (4.5 m/s - 11 m/s)
26.7	45.7	Wind speed ideal (ca. 11 m/s)
6.5	33.1	Wind speed high (>> 11 m/s); excess energy is spilled
2.1	15.4	Wind speed too high
0.2	7.9	Wind speeds too high

Table 1. Range of wind speed observed and conversion efficiencies

When the wind speed is higher than the rated capacity of the WEC, the excess energy is purposely spilled, keeping the machine producing at its maximum rated output. One typical WEC (irrespective of size) in the Maltese Islands can achieve a mean annual overall efficiency of 31%. This means that one 3MW WEC can produce 7154 MWhr annually.

Electricity Demand

During the period 1993–94 Enemalta produced a total of 1506 GWhr of electrical energy to meet demand. Thus, a typical output from a 3MW WEC represents only 0.48% of the national demand for electrical energy, i.e. 10 large WEC would supply ca. 5% of the demand.

In 1975, a German team who carried out a similar study, proposed a wind turbine farm of 10MW producing 22,000 MWhr annually, amounting to 7% of the energy generated in 1974/75. It works out that the demand for electricity then was 314 GWhr p.a. Due to the increased demand, the same farm would today produce only 1.4% of that supplied in 1994.

It is interesting to note that while the Maltese population has grown by 21% between 1975 and 1994, the electricity generated rose by 380% during the same period.

The feasibility of Using Wind Energy in Malta

We suggest that the corrected Luqa observations provide a sound basis for a decision on wind energy utilisation in Malta. Of course, the observations are site specific. The search for the best sites is still open, and it can be tackled with more refined methods of observation. It takes 12 large WECs to replace the gas turbines in current use, while 84 would be needed to replace completely one of Delimara's Steam turbines. Such a number of WECs would have a prohibitive impact on the aesthetics of the Maltese islands as the best sites are likely to be of high scenic, landscape and ecological value. Alternative sites may be found in industrial zones such as Hal Far which has a high exposure to the south sector. Such a site might take 5–10 large (3MW) WECs but may still suffer from proximity to the line of the main runway at Luqa airport. For workers in the area the WECs may produce an intolerable noise level. These considerations suggest that wind energy should be given a lower priority of exploitation than more direct solar alternatives like domestic solar water heating and distributed and centralised power generation via photo-voltaics. Moreover, serious consideration should be given to WECs dedicated to production of hydrogen by electrolysis rather than to augmenting power in the grid.

Communication

A Case for Biological Zeros

Adrian Mallia [1] **and Patrick J. Schembri**
Department of Biology, University of Malta, Msida, Malta

Most ecological work on rocky shores entails the quantification of species distributions and abundances relative to some convenient and accurate datum point. The most commonly used are tide levels and chart data. The problem arises when tides are virtually absent, as in most of the Mediterranean Sea or when accurate chart data are not available.

In the Maltese Islands there is only one datum point, located in the Grand Harbour, Valletta (14° 30.64' E, 35° 53.60' N). A number of bench marks and trigonometric stations of various orders are found scattered all over the Islands, but these are not always located very close to the shore so that levelling work from these stations to a particular study site may become quite a laborious task which may exceed the capabilities of most ecologists. Furthermore, some of these marks are old and location data for them are untraceable, while others have turned out to be inaccurate (Williams, E, personal communication, 1993). In fact, a project is currently underway by the Mapping Unit of the Planning Authority to recalibrate these stations and to establish new bench marks. A further complicating factor is that different charts make use of different datum points. Thus, while Admiralty Charts use a zero point (Chart datum) which is the level of lowest astronomical tide and the level to which all bathymetric soundings are referred, all heights shown on the official Government of Malta survey sheets use a datum point which is 0.5859m above the Admiralty Chart datum and which is taken to be mean sea level (MSL) for the Maltese Islands. Additionally, there is also a Public Works Department (PWD) datum which is 0.4100m above the Admiralty Chart datum. It is not always clearly stated which datum points charts and maps are based on.

This state of affairs has resulted in field workers having to resort to some other (possibly less accurate) datum point with which to relate all their data.

By far the most commonly used reference point is the so-called "biological zero", that is, the upper limit of phacophyceans of the genus *Cystoseira* in quantity (cfr. Boudouresque and Cinelli, 1976). These shrubby brown algae form a wide, mainly infralittoral, belt on most Mediterranean shores and in most places stop forming a consistent belt at about mean sea level. This has led some workers to utilise species of this genus as indicators of mean sea level. Different species or combinations of species occur in different parts of the Mediterranean. In the Maltese Islands the most commonly occurring species at sea level are *C. stricta*, *C. compressa*, *C. balearica* and *C. barbata* (Attard and Giglio, 1990; Borg, 1992; Calleja, 1991; Camilleri and Fleri-Soler, 1991; Vella, 1990).

Obviously, the position of this datum is somewhat subjective and cannot be determined with the same precision as for surveyed levels, which may be accurate to the nearest millimetre. Nonetheless there seems to be some relationship between this datum and mean sea level as any visit to the seashore on a calm day would suggest. It should also be kept in mind that the zonation of these algae on the shore is expected not to depend solely on physical factors, but also on biotic ones. The fact that different species, with different tolerances, occur in different parts of the Mediterranean and possibly also on different shores in the same geographical locality, also means that "biological zero" may not be the same everywhere.

As part of a wider project aimed at studying the zonation patterns of rocky shore communities in the Maltese Islands (Mallia, 1993), an attempt was made to quantify the relationship, if any, between this "biological zero" and mean sea level for the Maltese Islands.

This was done by fixing a 4cm long brass stud in a pre-drilled hole by means of Araldite adhesive at two stations, Qawra and Dahlet ix-Xmajjar, on the north-eastern coast of Malta. The height of this stud above biological zero was determined using a slightly modified version of the can and tube method described by Eifion Jones (1980). Next, the height of the studs above the MSL datum was determined using the level and staff method and starting from the nearest trigonometric station or bench mark. The difference between the two measurements was then calculated (see Table I).

These results show that although the biological zero does not coincide with the MSL datum, the difference between the two is fairly constant. The difference

[1]Present Address: Environmental Management Unit,
Planning Authority, St Francis' Ravelin, Floriana

LOCATION	Height of stud above "biological zero" (bottle and tube)	Height of stud above MSL (level and staff)	Difference in heights
Qawra	0.87m	1.10m	0.23m
Dahlet ix-Xmajjar	0.88m	1.12m	0.24m

Table I. Comparison of height data for the two localities.

between the two localties (0.01m) is very small and within the error range of the can and tube method. Therefore, these results indicate that in the absence of an accurate, levelled datum, the biological zero is a reliable substitute datum point for ecological work on rocky shores.

Recent work in the Maltese Islands has also shown that other organisms might also be useful as indicators of sea level. For example, Azzopardi (1992) found that the mean shell aperture of the reef-forming, vermetid gastropod *Dendropoma petraeum*, which on Maltese shores extends its range of distribution well into the lower mediolittoral zone, is lowest towards mean sea level and increases on either side of it. Such organisms may be used as indicators on shores where a definite *Cystoseira* belt is missing.

References

Attard L and Giglio MP (1990) *Zonation patterns on a Maltese rocky shore: Qalet Marku*. Unpublished B.Sc. dissertation, Faculty of Science, University of Malta; 167pp.

Azzopardi L (1992) *Aspects of the ecology of vermetid gastropods on Maltese rocky shores*. Unpublished B.Sc. dissertation, Faculty of Science, University of Malta; vi + 163pp.

Borg R (1992) *Zonation patterns around the coast of Qawra peninsula, Malta*. Unpublished B.Sc. dissertation, Faculty of Science, University of Malta; ix + 164pp.

Boudouresque CF and Cinelli F (1976) Le peuplement algal des biotopes sciaphiles superficiels de mode battu en Méditerranée occidentale. *Pubbl. Staz. Zool. Napoli*, **40**, 433-459.

Calleja M (1991) *Algal zonation and epiphytes along a Maltese rocky coast*. Unpublished B.Sc. dissertation, Faculty of Science, University of Malta; vii + 136pp.

Camilleri MV and Fleri Soler L (1991) *A comparative study of the macrophytic algae of three sites situated along the north-east coast of Malta*. Unpublished B.Sc. dissertation, Faculty of Science, University of Malta; iii + 126pp + appendices.

Eifion Jones W (1980) Field teaching methods in shore ecology. In: *The shore environment* Vol I: *Methods* (Eds WF Farnham, DEG Irvine and JH Price), pp. 19-44. Academic Press, London.

Mallia A (1993) *Effect of exposure on rocky shore zonation patterns in the Maltese Islands*. Unpublished M.Sc. dissertation, Faculty of Science, University of Malta; vii + 242pp.

Vella M (1990) *A study of the variation between the algal communities of an exposed and sheltered site along the Maltese coast*. Unpublished B.Sc. dissertation, Faculty of Science, University of Malta; viii + 124pp

Williams E (1993) Department of Land Surveying, University of East London, Essex.

Review Article

The Current Status of Predictive Genetic Testing for Cancer in Humans: Scientific, Clinical and Ethical Issues surrounding the p53 gene.

Mario Grixti
Cancer Research Laboratory, Department of Anatomy, University of Malta, Msida, Malta.

Our current understanding of the molecular basis of human cancers has raised a very critical issue which is that of predictive genetic testing for cancer in humans. Over the past few years so much data has been forthcoming that it is timely to review the situation.

For many years it has been hypothesized that cancer must have a genetic component. As long ago as 1914, Boveri suggested that an aberration in the genome might be responsible for malignant transformation. Subsequent work has supported this theory and we now define cancer in humans as being a genetic disease at cellular level. Evidence supporting the observation that cancer or the risk of cancer could be inherited comes from the work of Mullvihil et al (1997) and Li et al (1988) on familial cancers.

Almost every form of cancer in humans has been reported to cluster in families. This can be explained, either by the inheritance of a mutated susceptibility gene, or by chance association and shared exposures to environmental carcinogens (Knudson, 1989).

Since the early 1980s, extensive research world-wide has been undertaken in order to identify the genes responsible for malignant transformation of morphologically normal cells through their subsequent mutations. Some of these genes have been identified. These include the hereditary retinoblastoma (Rb) gene, the Wilms' tumour (WT1) gene, the neurofibromatosis type 1 gene, the familial polyposis APC gene, the Li-Fraumeni Syndrome (TP53) gene, the male breast cancer (AR) gene, the deleted colon cancer (DCC) gene and the recently discovered familial breast/ovarian cancer (BRCA-1) gene, and the familial breast cancer (BRCA2) gene (Knudson, 1989; Wooster et al, 1995; Miki, 1994; Stratton, 1995). Two recent studies have shown that mutations in the BRCA-1 gene which predisposes women to breast and ovarian cancer may also be associated with an increased risk of prostate cancer in men (Hall et al, 1990; Miki et al, 1994; Easton et al, 1995).

Mapping and identification of these cancer predisposing genes on different chromosomes have been facilitated in recent years by the application of new molecular biology techniques. The polymerase chain reaction has in fact revolutionized the approach to genetic research through its ability to cyclically amplify the genomic regions of interest. As a result of these technical advances we have a situation where many genes have been identified and others mapped so rapidly, that our understanding of their biological significance has not kept pace.

There is evidence to indicate that for many types of cancer, including the most common forms such as breast, lung, colon and bladder, there exists not only an environmental influence but also a hereditary predisposition to the development of cancer. This can now be clearly illustrated by the classical example of lung cancer as it is related to cigarette smoking. Whilst appreciating the evidence that cigarette smoking is clearly linked to the development of lung cancer in most instances, not all smokers necessarily develop lung cancer. Also, the mortality rate from lung cancer among non-smoking relatives of lung cancer patients (smokers) has been shown to be higher than that of non-smoking relatives of non-smokers used as controls (Garfinkel et al, 1985). Similar results were obtained when comparing the mortality rates from lung cancer of smoking relatives of lung cancer patients to that of smokers with no family history of lung cancer. So it appears that genetic influences must contribute to the development of cancer, even when there are clearly defined environmental factors.

A genetic predisposition to breast cancer has also been shown (Adami et al, 1980; Bain et al, 1980). The exact role that heredity plays in the predisposition to particular breast cancers cannot be quantified. Lynch (1971) states that approximately 10-15% of breast cancers have a hereditary background. Clearly, women who have no family history of breast cancer may also develop the disease, but those individuals from families with a history of breast cancer are at some increased risk, as shown by Anderson (1972, 1974, 1977). In general, the risk of the same neoplasm developing in close relatives of a cancer patient is approximately three times greater than in control populations (Knudsen, 1989).

A frequently asked question concerning both the cancer patients and their relatives is:-
"My mother or my father, or possibly both, died of cancer. Does this mean that I will certainly develop cancer? Under these circumstances, what are my percentage risks and what can be done to prevent the disease from developing?" This brings us to the very essence of the subject of predictive genetic testing for cancer in humans.

Genes predisposing to cancer

At molecular level, it is thought that cancer is the end result of an accumulation of genetic lesions occuring in key regulatory molecules. This widely held concept is gaining in importance as more information on growth arrest and cell death in the regulation of cell number becomes understood. The identification and functional role of these molecules are the subject of intense research. Positional cloning technology has begun to accelerate identification of genes that are responsible for familial cancers (Ruddon, 1994; Symonds et al, 1994). Over these last two years we have seen the cloning of two very important cancer predisposing genes, BRCA-1 (Miki, 1994) and BRCA-2 (Stratton, 1995; Tavtigian, 1995).

Genes that slow down cellular turnover, in other words growth inhibitors, are termed tumour suppressor genes (Knudson, 1989; Li, 1988; Weinberg, 1991). Seventeen years have passed since the original discovery of a nuclear phosphoprotein with a molecular mass of 53-kd that reacted with antiserum from animals with tumours induced by simian virus 40 (SV 40) (Linzer et al, 1979; Lane and Crawford, 1979).

The p53 gene is the most widely altered gene in human cancer (Hollstein et al, 1991; De Fromental and Soussi, 1992). It is a tumour suppressor gene which in its normal form codes for a 53-kd protein which binds to DNA and acts as a transcription factor to halt cells in the G1 to S transition of the cell cycle (Clarke et al, 1993). Mutant forms lack this DNA binding activity and therefore allows for abnormal proteins formed during malignant cell transformation to proceed in the cell cycle. The p53 gene spans a moderately sized segment of DNA (20 Kilobases long), located on the short arm of human chromosome 17, that is ultimately translated to a protein consisting of 393 amino acids contained in 11 exons, the first of which is non-coding. Five evolutionary conserved domains within the coding regions are regarded as essential to the functional activity of p53 (Malkin et al, 1990).

In the presence of DNA damage induced by gamma irradiation or chemotherapeutic drugs, intracellular levels of p53 rise and prompt the expression of a downstream gene WAF/CIP 1, whose protein product p21 binds to cyclin-depeudent kinases and inhibits their activity (Harper et al, 1993; El-Deiry et al, 1993). In this manner cell cycle is arrested prior to DNA synthesis and the cell is given the opportunity to repair the damaged DNA. If such repair does not occur, the presence of normal p53 induces the cell through a pathway of apoptosis or programmed cell death (Harris and Holstein, 1993). The apoptotic pathway is still poorly understood (Yonish, 1992; Stewart, 1994). The early chemical events that cause apoptosis have been so far hypothetical and these include: increases in ionisable calcium in the cytoplasm, drops in pH, generation of free radicals, and phosphorylation cascades. That p53 plays a cardinal role in the early events of apoptosis has been shown by the work of Stewart, (1994).

During the past seventeen years some 1300 mutations have been reported in more than 55% of all sporadically occurring tumours (Nigro et al, 1989). In 1992, the p53 gene was given the honour of being the second most significant scientific trend of the year in the Time Magazine (Time 1992). In 1993, p53 was named Molecule of the Year in Science (1993).

Understanding the role of p53 as a cancer predisposing gene comes from the clinical and scientific work on the Li-Fraumeni Familial Cancer Syndrome, first described as a clinical entity in 1969 by Li and Fraumeni, who noted the association between young onset sarcoma and other tumours in close relatives (Li, 1988). This syndrome is an autosomal dominant disorder that predisposes individuals to multiple forms of cancers occuring at a young age and in close relatives. It consists of a sarcoma developing in a first degree relative before the age of 45 and a second first degree relative who has developed any type of cancer under the age of 45 years or a sarcoma at any age Malkin, (1993). Other characteristic features of the syndrome include the occurance of multiple primary cancers in affected individuals, the early age of the patient at onset of most tumours and the autosomal pattern of inheritance of the disorder as determined by classical segregation analysis Malkin, (1992).

Component tumours of the syndrome include breast cancers, leukaemias, brain tumours and adrenocortical tumours. A recent study by Kyritsis et al (1994) reporting on germline mutations in the p53 gene on a set of glioma patients is important in that it sustains the recent observations that germline p53 mutations may occur outside the classically defined LFS families (Frebourg et al, 1992; Malkin et al, 1992; Toguchida et al, 1992). This observation complicates the scene of predictive genetic testing for the classical Li-Fraumeni Syndrome (Malkin et al, 1993).

Other cancer predisposing genes whose function is that of growth inhibition include: the retinoblastoma Rb gene, Wilms Tumour Gene WT1, WT2, the adeno polyposis coli gene APC and BRCA-1/ BRCA-2 genes.

Studies on the retinoblastoma gene demonstrate the correlation between hereditary mutation in tumour suppressor genes and genetic predisposition to develop cancer. Patients with the hereditary form (about 40%) have a high risk of multifocal retinoblastoma and other tumours in their family members and they may pass the disease to their progeny as an autosomal trait. The same features of hereditary cases of Rb can also occur in patients with no family history of the disease and are due to new mutations in the germ line. The non-hereditary cases are unifocal, with an older average age of onset (Vogel, 1979; Knudson et al, 1989). By analogy with retinoblastoma, some patients with sarcomas of bone and soft tissues and no family history of cancer may be carriers of new germline mutations at the p53 locus (Malkin et al, 1990).

Implications of Germline Mutations

Observational studies in the mutation spectrum of the p53 gene have been going on for quite some time (Bartek et al, 1992). We are now at a stage were the focus of current work is to correlate the significance of these mutations with clinical outcome (Harris et al, 1993). The frequency of cancers among carriers varies from 50% to 90% up to the age of 60. Above the age of 60 years the risk of developing cancer is said to be the same as that of the general population of the same age who do not carry a mutation in the p53 gene (Garber et al. 1991; Srivastava et al, 1990; Hollstein et al, 1991).

This may be explained by considering the fact that the presence of a particular mutation might just be a rare polymorphism and so there would be no biological significance of this mutation on cell growth. Therefore, any genetic tendency to develop cancer would manifest itself early in those individuals whose mutations are not just a rare variant of DNA, but would not do so in those individuals whose mutations are of no significant functional activity (Lynch and Kursh, 1971; Ory,1993). When discussing the relative risk for development of early onset breast cancer, the overall penetrance of gene carriers in the Li-Fraumeni Syndrome is 90% by the age of 50 years and the majority of cancers after childhood are breast cancers. Outside the Li-Fraumeni Syndrome families, germ-line p53 mutations have also been reported in patients who develop multiple primary cancers and in patients with a strong family history of cancer affecting multiple tissues (Frebourg and Friend, 1992; Malkin et al, 1992; Toguchida et al, 1992).

Biological and statistical issues also surronnd surveys for germ-line p53 mutations in population studies (Shapiro, 1989). The predictive power of a positive test for p53 is determined by three factors:

1. the prevalence of p53 mutations in the study population.
2. the sensitivity (the probability of detecting a true positive) of the test.
3. the specificity (probability of detecting a true negative) of the test.

Even when sensitivity and specificity are very high (99%), the predictive power of a positive test is only 50% when the prevalence of p53 mutations in the survey population is 1%; i.e., only one half of those with a positive p53 test actually are cancer-prone individuals. The power of the test is increased substantially by studying populations with a high prevalence, preferably greater than 10%. In predictive testing of siblings and offspring of cancer patients with a germ-line p53 mutation, the prevalence of mutation is as high as 50%. Available data suggest that the prevalence of this germline mutation might be 0.01% in the general population, 0.1-1% among various cancer patients, and 5-10 % among young patients with multiple primary cancers (Li et al, 1991).

Mutation-screening Techniques

The p53 protein can be detected immunohistochemically using monoclonal antibodies against this protein. During 13 years of work on this protein, it has been demonstrated that its overexpression can be detected in a wide variety of human malignancies including cancer of the breast, colon, lung, bladder, prostate and brain (Nigro, 1989).

The immunohistochemical technique has been shown to fail to stain both preneoplastic and neoplastic cells carrying a mutation of the p53 gene. Conversely, it has been shown to stain cells in a cancer family in which the p53 gene is normal (Barnes et al, 1992; Eeles et al, 1993). Normally, the p53 protein occurs at a very low concentration in cells because it is rapidly degraded by cellular proteases. However, using monoclonal antibodies against the mutant p53 protein, cancer cells often demonstrate high levels of the abnormal protein which accumulates in the cells. In transitional cell carcinoma of the urinary bladder, detection of p53 protein accumulation has been reported in up to 61% of invasive tumours (Sidransky et al, 1991; 1992). The immunohistochemical technique still remains useful for detecting p53 overexpression. It can be performed on biopsy material as well as on exfoliated cells such as in cervical smears, serous effusions and sputum. Morphologically normal cells, overexpressing the p53 protein, are presumed to indicate a preneoplastic stage of cellular differentiation.

There is no question that as far as testing for p53 gene mutation is concerned, the primary tools are those of molecular genetic techniques. Mutations within the gene are widely dispersed mainly between codons 130 and 290 and most of them involve the evolutionary conserved domains. In particular, at least three mutational hot-spots at codons 175, 248 and 273 have emerged. Mutations at these hot-spots are characteristically transitions at CpG dinucleotides. Cancers originating from various specific tissue sites differ with respect to the distribution and frequency of mutations at these hot-spots (Hollstein et al, 1991; Caron de Fromental and Soussi, 1992). Predictive testing for p53 gene mutation involves testing of the whole gene. A number of screening techniques for the detection of point mutations are available and provide the approximate location of the mutation.

Current methodologies use the polymerase chain reaction (PCR) to amplify a particular segment of the gene being investigated. The most commonly used mutation screening techniques are: single strand conformational polymorphism (SSCP) (Oritia et al, 1989), denaturing gel electrophoresis (DGGE or a variant CDGE) (Fisher et al, 1983; Borresen et al, 1991) and with chemical mismatch cleavage (CMC/HOT) (Montandon et al, 1989; Curiell,1990). The basic principles are as follows. In SSCP, single strands of DNA have a different secondary conformation depending on their base composition. In DGGE, double stranded DNA denatures at different

temperatures or concentrations of denaturant, dependent upon the base pair composition. The CMC/HOT technique mixes normal DNA with test mutant and allows single strands from each sample to reanneal. At the site of a base mutation, a mismatch occurs and can be identified by a chemical which binds to the mismatch and acts as a cleavage site for piperidine.

Each technique has its advantages and disadvantages. SSCP and CDGE are rapid, but each exon (or at the most, two exons together) of the p53 has to be analysed separately. Both have a sensitivity of nearly 90%. However, it is unlikely to be a long term solution for population screening in sporadic cancer because it is not so useful for analysing large PCR products where conformational differences become insignificant. CMC/HOT can analyse larger areas, but is laborious and uses hazardous chemicals. This latter method showed a higher sensitivity when compared to the other methods in a blind study of samples (Condie et al, 1993) but has been reported to miss G to T mutations.

Newer methods are now being sought, such as the analysis of Duplex DNA by triple helix formation and is applied to the detection of p53 microdeletions to facilitate DNA screening procedures (Olivas and Maher, 1994). This method exploits the ability of certain oligonucleotides to moniter DNA sequences iu the major groove without requiring denaturation of the double helical DNA target and might be directly applied to general screening of mutations affecting homopurine sequences.

All the above screening techniques indicate the approximate site of a mutation. The gold standard is direct sequencing and this will probably be the method of choice if clients wish to have a 100% reassurance that their p53 gene is normal.

It is recommended that once a mutation is found it should always be sequenced and also confirmed by at least one other technique, such as restriction enzyme or allele-specific hybridization.

Once a mutation is identified, tests can show with 100% certainty whether a relative is a carrier of the mutated gene or not (Eeles et al, 1993).

Early detection and prevention

The goal of predictive genetic testing in human cancer is to be able to predict the inheritance of a disease gene that is going to lead to a malignancy, and to initiate preventive measures before a person actually develops cancer. The best example so far of early detection and prevention is in the field of inherited cancer syndromes.

Since the localization of the multiple endocrine neoplasia type 2A (Mole et al,1993), it has been possible to develop a genetic test to screen for mutation in multiple endocrine neoplasia type 2A (MEN 2A) in the ret proto-oncogene on chromosone 10, (Donis-Keller et al, 1993). Multiple endocrine neoplasia (MEN 2A) is an autosomal dominantly inherited cancer syndrome comprising medullary thyroid cancer (MTC), adrenal gland phaeochromocytomas and hyperparathyroidism. Almost all patients with MEN 2A develop MTC during childhood or early adolescence. Genetic tests have been applied in the preclinical state to screen for MEN 2A, permitting early treatment (early curative thyroidectomy) in children predisposed to the disease (Calmettes et al, 1992; Marsh et al, 1994). This approach is now being performed at several centres, including Washington University and Cambridge University.

A different scenario is set up when discussing the problem of individuals carrying p53 mutations. There is lack of association between specific mutations and tumour histopathology. A situation is created whereby due to our current limitations in clinical diagnostic techniques, tumours cannot be effectively screened. In the case of the Li-Fraumeni Syndrome where families inherit a spectrum of cancers namely sarcomas, breast, brain, acute leukaemia, melanoma, germ-cell tumours, bladder cancers and adrenocortical carcinoma, current screening measures have shown to be ineffective in predicting disease in the preclinical state.

Proposed blood screening for leukaemias and magnetic resonance imaging for brain tumours have all proved to be unsuccessful for early detection. Mammography screening for breast cancer has been shown to decrease mortality in the over 50 years age group (Shapiro et al,1988; Shapiro, 1989), but its efficacy in women under 50 is uuknown. With the recent identification of the breast cancer gene BRCA1 (Miki et al, 1994), this approach to screening for predisposition to develop breast cancer can change completely.

Preventive measures such as chemoprevention may be of some value in certain cancers but, there is no evidence that it is of universal benefit. Chemoprevention studies should also include hormonal therapy such as the use of tamoxifen in the tamoxifen prevention trial for women at high risk of breast cancer (Cuzick and Baunm, 1985; Nayfield, 1991).

The inclusion of vitamins such as retinoids must also be given due consideration in chemoprevention studies. In women who are carriers of a mutated p53 gene, the risk of developing breast cancer before the age of 45 is 18 fold over the general population (Birch, 1992; Easton et al, 1993; Sidransky et al, 1992; Eeles et al,1993). Prophylactic subcutaneous mastectomy may not be an unreasonable preventive measure for breast cancer in those patients carrying p53 mutations.

Therefore, it may be concluded that so far, detecting individuals carrying germline p53 mutations is not technically impossible. However, monitoring these

individuals, for early detection of the different tumours which may develop, is not yet possible (Garber et al, 1991).

Gene Therapy

Gene therapy is the stable insertion of a functional gene into the genome of a host cell to alter the functional capabilities of the cell or to correct a specific genetic defect. This technique gives researchers the possibility to understand more about the regulation of gene function and at the same time find its applicability as a therapeutic approach in the treatment of cancer (Foa and Guarini, 1993; Miller, 1992; Gottesman, 1994). Optimization of both efficiency and safety of the ways in which a gene is transferred, is the crucial feature of all strategies seeking to exploit this technology.

The p53 tumour suppressor gene is a prime canditate for gene therapy (Takahashi et al, 1989; D'Amico et al, 1992; Chiba et al,1990). Genetic lesions in the p53 gene are the most commonly occuring changes found in all human cancers (Vogelstein, 1990). Several groups of scientists were able to show that the stable transfection (Takahashi et al, 1992), or retroviral transduction (Fujiwara et al, 1993) of wild type p53 gene into cancer cells with a mutant p53 dramatically inhibits cell growth in cultured cells despite the possible presence of other genetic lesions. The significance of this observation is that any other genetic lesion need not be corrected before an anti-tumour effect could be seen (Fujiwara et al, 1994).

In-vitro gene therapy experiments started in the late 1980s and it is only now that we are seeing the possible application of in-vivo gene therapy using wild type p53 gene (Carbone and Minna, 1994). News of the first proposed gene therapy for lung cancer to enter human trials has been reported in the (Journal of the National Cancer Institute, Vol. 86 No5 March 2, 1994). This trial is still pending approval by the U.S. Food and Drug Administration on the safety of the retroviral vectors being used (Anderson, 1992). In oncology, research into gene therapy is mainly concentrated on lung cancer. Mutations of the p53 tumour suppressor gene are the genetic abnormalities most frequently identified in non-small cell lung cancer (Takahashi et al, 1989).

Conventional methods of treatment have not resulted in a significant decrease in mortality from lung cancer and therefore this creates a political and economic power to assist researchers pursuing this novel therapy.

The immediate problem with application of gene transfer technology in patients is the delivery of the therapeutic gene to sufficient numbers of tumour cells to produce a clinically observable effect. There is also the consideration of safety of vectors. When a retrovirus infects a cell, its viral RNA is copied by the enzyme reverse transcriptase into DNA that enters the nucleus and integrates randomly into the genome of the host. These natural events are exploited for gene transfer by construction of retroviruses that do not contain the replication genes, and in which the viral structural genes are replaced by the new genes to be inserted into the cells. A very plausable biological model system is being currently promoted. This includes the direct administration of a retroviral wild-type p53 expression vector in orthotopic human lung cancer model in nu/nu mice (Fujiwara et al, 1994; Miller et al, 1989) resulting in growth inhibition of cancer cells.

As is true of interesting studies, these data raise a series of questions that should be considered in future experiments. Although the use of a retroviral vector favours integration in rapidly dividing cells, can all the growth supression be attributed to transfected cells only? Since it is likely that all cancer cells are tranfected, could the supression of growth also be due to a bystander effect? (Freeman et al, 1993). Does the growth suppression observed in transfected cells result from the induction of apoptosis (programmed cell death)? Do the bystander cells undergo the same growth suppression due to induction of apoptosis? Is there a bystander effect in metastatic cells? The bystander effect is an observation whereby transduced cells have been shown to inhibit the growth of nontransduced neighbouring cells in culture (Cai et al, 1993; Freeman et al, 1993). The molecular basis of this bystander effect is under investigation. Does this possible theraputic tool work in other sites of the body for other p53 mutations?

It is apparent that there are still some fundamental practical and clinical problems to be addressed. Lung cancers are rarely one cell layer thick and they are rarely confined to a closed space. Fujiwara and co-workers (1993) have shown that wild-type p53 is capable of multilayer penetration into the three-dimensional structure of multicellular tumour spheroids. Of clinical concern is the size of the tumour and the accessibility to metastatic sites. The potential toxic effects also need to be addressed. Retroviruses integrate stably into the genome of replicating cells. Therefore, it is important to consider the outcome of genetically altered normal epithelial cells.

Taking into consideration all the available results so far, it is being proposed that in the case of lung cancer, microscopically established tumours in the bronchial epithelium can be efficiently infected with a retroviral vector expressing wild-type p53 gene and that *in-situ* retrovirus-mediated gene transfer may be a useful strategy for manipulating genetic abnormalities of cancer cells in vivo (Roth et al, 1994). Using current molecular techniques it is possible to identify pre-neoplastic as well as microscopical neoplastic cells before these cells display the cytological and histological features of invasive cancers (Casson et al,1991; Sozzi et al, 1992; Kastan et al, 1992).

Much research still needs to be done. At present there are over 100 protocols accepted or under consideration by various advisory committees for gene targeted therapies

world wide, the majority being in the United States. Most are for cancer in which the ethics are perhaps simplest with the lowest risk benefit ratio.

Ethics of Predictive Genetic Testing

Issues regarding ethics of predictive genetic testing are emerging as themes of great concern. Over the past five years scientific publications have presented data ranging from screening for cancer predisposition (Markham et al, 1994) to screening for specific cancers (Kodish et al, 1994), genetic intervention studies (Roth, 1994) and recently pre-implantation diagnosis of inherited predisposition to cancer (Kogan et al, 1987; Handyside, 1993; Harper et al, 1994).

Testing young cancer patients and their unaffected relatives for p53 germline mutations presents a number of difficult clinical and ethical questions (Li et al, 1991; Prosser et al, 1991). As far as clinical management is concerned, the first set of problems arises because of the uncertainty about the risks conferred by germline p53 mutations. The spectrum of cancers so far reported in families with germline p53 mutations have been discussed previously (Li et al, 1988; Birch, 1992; Frebourg et al, 1992). The range of cancers include bone and soft tissue sarcomas, breast cancer, brain cancers, acute leukaemias, melanoma, germ-cell tumours, bladder cancer, adrenocortical carcinoma and prostate cancers. As more families are screened this list of associated cancers will probably increase even further.

Therefore, considering the limited potential for screening and early detection of cancers in carriers of p53 germline mutations, the question of whether it is ethical to test asymptomatic members of cancer families in whom such mutations have been found, must be addressed. What are the social, economic and psychological consequences resulting from such testing? What effective measures are available to carriers of the p53 mutations? At present the greatest benefit that can be derived from testing is reassurance and relief from anxiety in those familiy members found not to be carriers of a mutation. There are other benefits including ability to plan education, future careers and decisions on marriage and child bearing, taking into account the knowledge of cancer predisposition.

There are concerns that predictive testing in this particular setting will increase people's anxiety and may have a negative effect on psychological and economic issues, raising the question of selection of individuals for predictive testing (Kash et al, 1991). The effect on life insurance premiums is unclear, but individuals with a strong family history would have their premiums weighted and these would be reduced to normal if a relative of a person with a known mutation was shown not to carry the gene (Eeles et al, 1993).

Privacy and confidentiality of test results in identified carriers of germline p53 mutations have both social and economic impact. Employers for example, may be reluctant to appoint persons at high risk of developing cancer. The psychological effects of belonging to a family with high risk of cancer have not been studied and are not understood (Murray, 1991; Sujansky et al, 1990; U.S. President's commission for the study of ethics, 1990). One way to resolve this constallation of issues is to gain more insight into the biological significance of these mutations in correlation with particular patterns of cancer, i.e., specific mutations causing specific cancers. At present, testing for p53 germline mutations is performed only within the setting of research protocols but, it is expected that predictive testing will become more widely available. Predictive testing should be preceded by thorough counselling which should include psychological assessment and potential impact caused by a positive result. There should also be informed consent. A carefully planned long-term follow up is also needed in order to obtain data on the life experiences of carriers of mutations, as well as on cancer incidence.

Testing on children at risk is another contentious issue and it should be reserved only for cases in which a distinct and immediate benefit can be obtained. Such testing is usually postponed until adolesence, when the individuals can make their own informed decisions as to whether or not they wish to be tested, (Edwards and Hall, 1992; Wald and Law, 1992; Wald, 1993).

Advisory committees involved in the design of p53 testing programs, relate their recommendations based on their experience with Huntington's Disease (Went, 1990; Huggins et al, 1990; Lam et al, 1988; Bloch and Hyaden, 1990; Ford et al, 1994; Tyler et al, 1992). Predictive testing for Huntington's Disease involves an area where prevention is not possible and by using a set protocol, individuals having predictive testing for this disease helps to minimise the problems experienced and allows the individuals to have time to decide if they really want the test and for what reason. There may be many reasons why individuals may wish to have a predictive test. In the case of Huntington's Disease, 80% of individuals at risk said they wanted the test for planning their future and their family, and to relieve anxiety.

This experience has a significant relevance to the design of p53 testing programs. We cannot assume that the impact of testing for germline p53 mutations would have the same favourable outcome but there is definitely growing support in favour of such testing (Smigel, 1993; Berg, 1991; Li et al, 1992).

Conclusion

The concerted efforts from the work of the Human Genome Project will soon lead to the identification of many more genes responsible for hereditary diseases, including cancer (Watson, 1990). The genetic data that is presently available and that will be made available, in the near future, on populations and individuals is the subject of debate by all involved in this line of research. Issues such as confidentiality, the right to know or not to know, non-discrimination among carriers, insurance, disease specificity, availability of treatment, inheritance pattern

and gene penetrance and expressivity, feature prominantly in the ethics of any genetic screening program (Huggins et al. 1990).

The aim of this review was to update the present scene of cancer genetics and to promote the interest seen over the past few years on the concept of cancer genetics clinics. At present gene markers for the most commonly occuring cancers have been identified, i.e. bowel, breast and ovarian cancers (Fishel et al. 1993; Bronner et al. 1994; Miki. 1994; Wooster. 1995). This has generated a particular interest both from the general public and from clinicians dealing with cancer patients. More information is being demanded on the availability of genetic tests, cancer risks, screening measures, prevention and treatment.

Since 1989 the concept of cancer genetics clinics was already being proposed (Hoskins, 1989). To date there are more than 20 cancer genetics clinics set up in Europe. In the United Kingdom alone there are about 6 centres. One such centre is at the Royal Marsden Hospital where a genetic cancer clinic is held which also offers a cancer genetics screening service (personal communication, Eeles RA). Clients attending these clinics have access to information on their own genetic risk for cancer as well as to highly specialised screening tests available to date.

The clinical significance of being either a BRCA1 gene carrier or a BRCA2 gene carrier is at present still poorly understood. Large epidemiological studies have to be carried out to really assess the contribution of these genes to cancer development in familial and sporadic cases (Claus et al. 1994). At present, 10% of breast cancers have a strong family history and it is only possible to identify a definite dominant pattern of inheritance in approximately 1% of individuals. However, it is also possible that these genes could account for as little as 1-15% of breast cancers. Since breast cancer is the commonest tumour occurring in women world wide, one can appreciate the potential burden that individuals can place on cancer genetics clinics, given the current state of information.

There are as many questions as answers in the area of p53 cancer gene predisposition, in particular, the at risk groups need to be better defined and follow-up of known carriers is needed. The p53 gene is offering the most promising openings in the field of corrective gene therapy but it is unrealistic to think that their effectiveness would be immediate.

The advances in science and medicine have brought about yet another ethical issue to the scene. This is the ethics of preimplantation diagnosis of inherited predisposition to cancer. It is very likely that couples will seek prenatal diagnosis to prevent passing a mutant gene on to their children. Is the idea of terminating a pregnancy after a diagnosis by conventional means (chorion villus sampling) acceptable? Methods for diagnosing genetic defects in early embryos before implantation are being developed and heavily supported by investments (Harper et al, 1994). The challenge which we now face is how to relate all the information we have so far on cancer predisposing genes into effective screening and interventional programmes (Haward et al. 1991). Effective studies also have to be undertaken to define the cost effectiveness of population screening and the health gain to be anticipated from cancer predisposition screening.

Acknowledgements:
Sincere thanks go to Prof A Cuschieri, Prof A E Felice, Dr C A Scerri, and Dr F Portelli for giving me their academic and practical support to be able to initiate this line of Cancer Research at The University of Malta.

References:

Adami HL, Hansen J, Jung B et al. (1980) Familiality and breast cancer; a case control study in a Swedish population. *British Journal of Cancer*, 42, 71-77.

Anderson C (1992) Gene therapy researcher under fire over controversial cancer trials. *Nature*, 360, 399-400.

Anderson DE (1971) Some characteristics of familial breast cancer. *Cancer*, 28, 1500-1504.

Anderson DE (1972) A genetic study of human breast cancer. *Journal of the National Cancer Institute*, 48, 1029-1034.

Anderson DE (1974) Genetic study of breast cancer; Identification of the high risk group. *Cancer*, 34, 1090-1097.

Anderson DE (1977) Breast cancer in families. *Cancer*, 40, 1855-1860.

Anderson L A, Friedman L, Osborne-Laurence S et al. (1993) High density genetic map of the BRCA1 region of 17q 12 -q21. *Genomics*, 17, 618-623.

Berg RL (1991) Cancer prevention and screening in the light of health promotion of disability for the second fifty years. A report from the Institute of Medicine at the National Academy of Science. *Cancer*, 68, 2511-2513.

Bain C, Speizer FE, Rosner B et al. (1980) Family history of breast cancer as a risk indicator for the disease. *American Journal of Epidemiology*, 111, 301-308.

Barnes DM, Hanby AM, Gillet CE et al. (1992) Abnormal expression of wild type p53 protein in normal cells of a cancer family patient. *Lancet*, 340, 259-263.

Bartek J, Bartkova J, Vojtesek B, et al. (1992) Aberrant expression of the p53 oncoprotein is a common feature of a wide spectrum of human malignancies. *Oncogene*, 6, 1699-1703.

Birch M (1992) Germline mutations in p53 tumour suppressor gene: scientific, clinical and ethical challanges. *British Journal of Cancer*, 66, 424-426.

Block M, Hayden MR (1990) Opinion: Predictive testing for Huntington's Disease in childhood; challenges and implications. *American Journal of Human Genetics*, 46, 1-4.

Genetics, 46, 1-4.

Borresen AL, Hovig E, Smith-Sorensen B et al. (1991) Constant denaturing gel electrophoresis as a rapid screening technique for p53 mutations. *Proceedings from the National Academy of Science USA*, 88, 8405-8409.

Boveri A (1991) Oncogenes and Tumour suppressor genes. *BIOS Scientific Publishers*, Chapter 2, p19.

Bronner CE, Baker SM, Morrison PT, et al.(1994) Mutation in the DNA mismatch repair gene homologue *hMLH 1* is associated with hereditory non-polyposis colorectal cancer. *Nature*, 368, 258-261.

Cai DW, Mukhopadhyay T, Liu Y et al.(1993) Stable expression of wild-type p53 gene in human lung cancer cells after retrovirus mediated gene transfer. *Human Gene Therapy*, 4, 617-624.

Calmettes C, Ponder B, Fischer J, Raue F.(1992) Early diagnosis of the multiple endocrine neoplasia type 2A syndrome: consensus statement. European Community concerted action: medullary thyroid carcinoma. *European Journal of Clinical Investigations*, 22, 755-760.

Carbone DP and Minna JD (1994) In vivo gene therapy of human lung cancer using wild-type p53 delivered by retroviruses. *Journal of the National Cancer Institute*, 86(19), 1437-1438.

Caron De Fromental C, and Soussi T (1992) Tumour suppressor gene: a model for investigating human mutagenesis. *Genes Chromosomes and Cancer*, 4, 1-15.

Casson AG, Mukhopadhyay T, Cleary KR et al. (1991) p53 gene mutations in Barrett's Epithelium and esophageal cancer. *Cancer Research*, 51, 4495-4499.

Chiba I, Takahashi T, Nau M M et al. (1990) Mutations in the p53 gene are frequent in primary resected non-small-cell lung cancer. Lung Cancer Study Group. *Oncogene*, 5, 1603-1610.

Clark AR, Purdie CA, Harrison DJ et al. (1993) Thymocyte apoptosis induced by p53-dependent and independent pathways. *Nature*, 362, 849-852.

Claus EB, Risch NJ, Thompson WD (1990) Age at onset as an indicator of familial risk of breast cancer. *American Journal of Epidemiology*, 131, 961-972.

Claus EB, Risch N, Thompson D (1994) Autosomal dominant inheritance of early onset breast cancer. *Cancer*, 73, 643-651.

Condie A, Eeles RA, Borresen AL, Coles C, Cooper CS, Prosser J (1993) Detection of point mutations in the p53 gene: Comparison of SSCP, CDGE and Hot. *Human Mutation*, 2, 58-66.

Curiel DT, Buchhagen DL, Chiba I, D'Amico D, Takahashi T, Minna JD (1990) A chemical mismatch cleavage method useful for the detection of point mutations in the p53 gene in lung cancer. *American Journal of Respiratory Cell Mollecular Biology*, 3, 405-411.

Cuzick J, Baumin M (1985) Tamoxifen and contralateral breast cancer. *Lancet*, 1, 282.

D' Amico D, Carbone D, Mitsudomi T et al. (1992) High frequence of somatically acquired p53 mutations in small cell lung cancer cell lines. *Oncogene*, 7, 339-346.

De Frouental CC, Soussi T (1992) TP53 tumour suppressor gene: a model for investigating human mutagenesis. *Genes, Chromosomes and Cancer*, 4, 1-15.

Donis-Keller H, Dou S, Chi D et al. (1993) Mutations in the ret protooncogene are associated with MEN 2A and FMTC. *Human Molecular Genetics*, 2, 851-856.

Easton DF, Ford D, Bishop DY (1995) The breast cancer linkage consortium ; Breast and ovarian cancer incidence in BRCA1-mutation carriers. *Lancet*, 343, 692-695.

Easton DF, Bishop DT, Ford D, Crockford GP and the breast cancer linkage consortium (1993) Genetic linkage analysis in familial breast and ovarian cancer- results from 214 families. *American Journal of Human Genetics*, 52, 678-701.

Edery P, Lyonnet S, Mulligan L M et al. (1994) Mutations of the ret proto-oncogene in Hirshprung disease. *Nature*, 367, 378-380.

Edwards PJ, Hall DMB (1992) Screening,ethics and the law. *British Medical Journal*, 305, 267-268.

Eeles RA, Warren W, Knee G et al. (1993) Constitutional mutation in exon 8 of the p53 gene in a patient with multiple primary tumours; Molecular and Immunohistochemical findings. *Oncogene*, 8, 1269-1276.

Eeles RA. (1993) Predictive testing for germline mutations in the p53 gene; Are all the questions answered? *European Journal of Cancer*, 29A. 10, 1361-1365.

El Deiry WS, Tokino T Velculescu VE,et al. (1993) WAF1, a potential mediator of p53 tumour suppression. *Cell*, 75, 817-825.

Fischer SG, Lerman LS. (1983) DNA fragments differing by single base-pair substitution are separated in denaturing gradient gels : Correspondence with melting theory. *Proceedings from the National Academy of Science USA*, 80, 1579-1583.

Fishel F , Lescoe MK, Rao MRS, et al. (1993) The mutator gene homolog MSH2 and its association with hereditary non-polyposis colorectal cancer. *Cell*, 260, 1027-1038.

Foa R, Guarini A. (1993) Gene therapy in oncology: In vitro studies and potential clinical applications. *Forum*, 3, 399-410.

Ford D, Easton EF, Bishop DT, Narod SA, Goldar DE and Breast Cancer Linkage Consortium. (1994) Risks of cancer in BRCA-1 Mutation carriers. *Lancet*, 343, 692-695.

Frebourg T, Friend SH. (1992) Cancer risks from germline mutatious in p53 gene. *Journal of Clinical Investigation*, 90, 1637 -1641.

Frebourg T, Barbier N, Kassel J et al. (1992) A functional screen for germline p53 mutations based on transcriptional activation. *Cancer Research*, 52, 1-3.

Frebourg T, Kassel J, Lam KT et al. (1992) Germline

mutations of the p53 gene in patients with high risk for cancer inactivate the p53 protein. *Proceedings from the National Academy of Science USA*, 89, 6413-6417.

Freeman SM, Abbound CN, Whartenby KA, et al. (1993) The bystander effect; tumour regression when a fraction of the tumour mass is genetically modified. *Cancer Research*, 53, 5274-5283.

Fujiwara T, Cai DW, Georges RN, et al. (1994) Therapeutic effect of a retroviral wild type p53 expression vector in an orthotopic lung cancer model. *Journal of the National Cancer Institute*, 86, 1458-1462.

Fujiwara T, Grimm EA, Mukhopadhyay T, et al. (1993) A retroviral wild type p53 expression vector penetrates human lung cancer spheroids and inhibits growth by inducing apoptosis. *Cancer Research*, 53, 4129-4133.

Garber JE, Goldstein AM, Kantor AF, Dreyfus MG, Fraumeni JF jr. and Li FP. (1991) Follow up study of twenty-four families with Li-Fraumeni Syndrome. *Cancer Research*, 51, 6094-6097.

Garfinkel L, Auerbach O, Joubert L. (1985) Involuntary smoking and lung cancer. A case control study. *Journal of the National Cancer Institute*, 75, 4463-4469.

Gottesman MM, German UA, Aksentijevich I, et al. (1994) Gene transfer of drug resistance genes; implications for cancer therapy. *Annals of the New York Academy of Science*, 716, 126-139.

Hall JM, Lee MK, Newman B, Morrow JE, Anderson LA, Huey B, King M-C. (1990) Linkage of early onset familial breast cancer to chromosome 17q21. *Science*, 250, 1684-1689.

Handyside AH. (1993) Diagnosis of inherited disease before implantation. *Reproductive Medical Reviews*, 2, 51-61.

Harper JW, Adami GR, Wei N et al. (1993) The p21 Cdk-interacting protein Cip 1 is a potent inhibitor of G1 Cyclin-dependent kinases. *Cell*, 75, 805-816.

Harper JC, Coonen E, Ramackers FCS, et al. (1994) Identification of the sex of human preimplantation embryos in two hours using an improved spreading method and fluorescent in-situ hybridization (FISH) using directly labelled probes. *Human Reproduction*, 9, 721-724.

Harper JC, Handyside AH. (1994) The current status of preimplantation diagnosis. *Current Obstetrics Gynaecology*, 2, 331.

Harris CC. (1993) P53 at the crossroads of molecular carcinogenesis and risk assessment. *Science*, 262, 1952-1959.

Harris CC, Holstein M. (1993) Clinical implications of the p53 tumour suppressor gene. *New England Journal of Medicine*, 327(18), 1298-1305.

Haward RSA, Steinberg EP, Ford DE, Roizen MF, Roach KW.(1991) Preventive care guidelines 1991. *Annals of Internal Medicine*, 114, 758-583.

Helmrich SP, Shapiro S, Rosenberg L, et al. (1983) Risk factors for breast cancer. *American Journal of Epidemiology*, 117, 35-45.

Hollstein M, Sidransky D, Vogelstiin B, and Harris CC. (1991) P53 mutations in human cancers. *Science*, 52, 49-53.

Horio Y, Takahashi T, Kuroishi T et al. (1993) Prognostic significance of p53 mutations and 3p deletions in primary resected non-small cell lung cancer. *Cancer Research*, 53, 1-4.

Hoskins IA. (1989) Genetic councelling for cancer patients and their families. *Oncology*, 3, 84-92.

Huggins M, Bloch M, Kannani S et al. (1990) Ethical and legal dilemmas arising during predictive testing for adult onset disease; the experience of Huntington disease. *American Journal of Human Genetics*, 47, 4-12.

Kash KM, Holland JC, Halper MS, Miller DG. (1991) Psychological distress and surveilance behaviours of women with a family history of breast cancer. *Journal of the National Cancer Institute*, 1, 24-30.

Kastan MB, Zhan Q, el-Deiry WS et al. (1992) A mamnualian cell cycle check-point pathway utilizing p53 and GADD45 is defective in ataxia-telangiectasia. *Cell*, 71, 587-597.

Knudson AG jr.(1989) Hereditory cancers disclose a class of cancer genes. *Cancer*, 63, 1888-1891.

Kodish E, Murray TH, Shurin S (1994) Cancer risk research. *Clinical Research*, 42, 396-402.

Kogan SC, Doherty M, Gitschier J (1987) An improved method for prenatal diagnosis of genetic diseases by analysis of amplified DNA sequences; application to haemophilia A. *New England Journal of Medicine*, 317, 985-990.

Kyritsis AP, Bondy ML, Xiao M et al. (1994) Germline p53 mutations in a subset of glioma patients. *Journal of the National Cancer Institute*, 86, 344-349.

Lam RW, Bloch M, Jones BD et al. (1988) Psychiatric morbidity associated with early clinical diagnosis of Huntington disease in a predictive test program. *Journal of Clinical Psychiatry*, 49, 444-447.

Lane DP (1992) P53, the guardian of the genome. *Nature*, 358, 15-16.

Lane DP, Crawford LV (1979) Tantigen is bound to a host protein in SV 40- transformed cells. *Nature*, 278, 261-263.

Law JC, Strong LC, Chidambaram A, Ferrell RE (1991) A germline mutation in an extended cancer family. *Cancer Research*, 51, 6385-6387.

Levine AJ, Momand J, Finlay CA (1991) The p53 tumour suppressor gene. *Nature*, 351, 453-456.

Li FP (1988) Cancer families; Human models of susceptibility to neoplasia - the Richard and Hinda Rosenthal Foundation Award Lecture. *Cancer Research*, 48, 5381-5386.

Li FP, Correa P, Fraumeni JF jr (1991) Testing for germline p53 mutations in cancer families. *Cancer Epidemiology Biomarkers*. Prev 1, 91-94.

Li FP, and Franmeni JF jr (1969) Soft tissue sarcomas,

breast cancer, and other neoplasms. *Annals of Internal Medicine*, 71, 747-752.

Li FP, Fraumeni, Mulvihill JJ, Blattner WA, Dreyfus MG, Tucker MA, Miller RW (1988) A cancer family syndrome in twenty-four kindreds. *Cancer Research*, 48, 5358-5362.

Li FP, Garber JE, Friend SH et al. (1992) Recommendations on predictive testing for germline p53 mutations among cancer prone individuals. *Journal of the National Cancer Institute*, 84, 1156-1160.

Lin D, Shields MT, Ullrich SJ et al. (1992) Growth arrest induced by wild type p53 protein blocks cells prior to or near the restriction point in the late g1 phase. *Proceedings from the National Academy of Science USA*, 89, 9210-9214.

Linzer DIH, Levine AJ (1979) Characterization of a 52K dalton cellular protein present in SV 40 transformed cells and uninfected embryonal carcinomal cells. *Cell*, 17, 43-52.

Lynch HT, Kursh AG (1971) Genetic predictability and breast cancer risk. *Archives of Surgery*, 103, 84-88.

Malkin D, Li FP, Strong LC et al. (1990) Germline p53 mutations in a familial syndrome of breast cancer, sarcomas and other neoplasms. *Science*, 250, 1233-1238.

Malkin D, Jolly KW, Barbier N et al. (1992) Germline mutations of the p53 gene in children and young adults with second malignant neoplasms. *New England Journal of Medicine*, 326, 1309-1315.

Malkin D. The Li-Fraumeni Syndrome. In Principles and Practice of Oncology updates (De Vita VT Jr. Hellman S, Rosenberg SA, eds) Vol 7. Philadelphia: Lippincott, 1993, pp. 1-14.

Markham AF, Coletta PL, Robinson PA, Clissold P, Taylor GR (1994) Screening for cancer predisposition. *European Journal of Cancer*, 30A, 13, 2015-2029.

Marsh D, Robinson B, Andrew S et al. (1994) A rapid screening method for mutations in the Ret Proto-oncogene in multiple endocrine neoplasia type 2A and familial medullary thyroid carcinoma families. *Genomics*, 23, 477-479.

Miki Y (1994) A strong candidate for the the breast and ovarian cancer susceptibility gene BRCA1. *Science*, 266, 66-71.

Miller DA (1992) Human gene therapy comes of age. *Nature*, 357, 455-60.

Miller Da, Rossman GJ (1989) Improved retroviral vectors for gene transfer and expression. *Biotechniques*, 7, 980-6.

Montandon AJ, Green PM, Gianelli F, Bently DR (1989) Direct detection of point mutations by mismatch analysis; Application to haemophilia B. *Nucleic Acids Research*, 17, 3347-3357.

Molecule of the year -(1993) p53 sweeps through cancer research. *Science*, 262, 1985-1995.

Mole SE, Mulligan LM, Healey CS, Ponder BAJ, Tunnacliffe A (1993) Localization of the gene for multiple endocrine neoplasia type 2A to a 480 kb region in chromosome band 10q11.2. *Human Molecular Genetics*, 2, 247-252.

Mulvihill JJ, Miller RW, Fraumeni JF (1977) Genetics of human cancer. New York Raven Press, 1977.

Murray TH (1991) Ethical issues in human genome research. *FASEB J*, 5, 55 -60 .

Nayfield SG, Karp JE, Ford LG et al. (1991) Potential role of tamoxifen in prevention of breast cancer. *Journal of the National Cancer Institute*, 83, 1450-1459.

Neutra RR, Droletta ME (1978) Estimating exposure - Specific disease rates from case control studies using Bayes Theorem. *American Journal of Epidemiology*, 108, 214-222.

Nigro JM, Baker SJ, Preisinger AC et al (1994) Mutations in the p53 gene occur in diverse human types. *Nature*, 342, 705-708.

Olivas M, Maher LJ (1994) Analysis of Duplex DNA by Triple Helix Formation: Application of a p53 Microdeletion. *Biotechniques*, 16, 128-132.

Oritia M, Suzuki Y, Sekiya T, Hayashi K (1989) Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. *Genomics*, 5, 874-879.

Ory K, Legros Y, Auguin C et al. (1993) Codon arg 175: To be or not to be mutant? p53 in growth control and neoplasia. *Charles Rodolphe Brupbacher Foundation First Scientific Symposium, Zurich*, p32.

Ottman R, Pike MC, King MC, Henderson BE (1983) Practical guide for estimating risk for familial breast cancer. *Lancet*, ii, 556-558.

Prosser J, Elder PA, Condie A, MacFayden I, Steel CM, Evans HJ (1991) Mutations in the p53 do not account for heritable breast cancer: a study in five affected families. *British Journal of Cancer*, 63, 181-184.

Prosser J, Porter D, Coles et al. (1992) Constitutional p53 mutations in a non Li-Fraumeni cancer family. *British Journal of Cancer*, 65, 527-528.

Roth JA, Mukhopadhyay T, Zhang WW, Fujiwara T and Georges R (1994) Gene replacement stratages for the prevention and therapy of cancer. *European Journal of Cancer*, 30A, 13, 2032-2037.

Ruddon RW (1994) Cancer Biology. 2nd edition. *Oxford University Press*. 1994.

Ryan JJ, Danish R, Gottlieb CA et al. (1993) Cell cycle analysis of p53 induced cell death in murine erythroleukaemia cells. *Molecular Cell Biology*, 13, 711-719.

Santibanez-Koref MF, Birch JM, Hartley AL et al. (1991) p53 germline mutations in Li-Fraumeni Syndrome. *Lancet*, 338, 1490-1491.

Shapiro S (1989) Determining the efficacy of breast cancer screening. *Cancer*, 63, 1873-1880.

Shapiro S, Venett W, Strax P, Venet L (1988) Periodic screening for breast cancer. The health insurance plan and its sequela, 1963-86. Baltimore, John Hopkins University press 1988.

Sidransky D, Tokino D, Helzlsour K et al. (1992) Inherited p53 germline mutations in breast cancer. *Cancer Research*, 52, 2984-2986.

Sidransky D, Von Eschenbach A, Tsai YC et al.(1991) Identification of p53 gene mutations and product in bladder cancers and urine samples. *Science*, 252, 706-709.

Smigel K (1993) Major cancer screening trial in 10 US centers. *Journal of the National Cancer Institute*, 85, 1903.

Soussi T, Caron DE, Fromental C, May P (1990) Structural aspects of the p53 protein in relation to gene evolution. *Oncogene*, 5, 945-952.

Sozzi G, Miozzo M, Donghi R et al. (1992) Deletions of 17p and p53 in preneoplastic lesions of the lung. *Cancer Research*, 52, 6079-6082.

Srivastava S, Zou Z, Pirolla K, Blatter W, Chang EH (1990) Germline transmition of a mutated p53 gene in a cancer prone family with Li-Fraumeni Syndrome. *Nature*, 348, 747-749.

Stewart BW (1994) Mechanism of apoptosis; integration of genetic, biochemical and cellular indicators. *Journal of the National Cancer Institute*, 86, 1286-1296.

Stratton et al. (1995) Identification of six mutations in BRCA-2 in families with breast and ovarian cancer. *Nature*, 378, 781-789.

Sujansky E, Kreutzer SB, Johnson AM et al. (1990) Attitudes of at risk and affected individuals regarding presymptomatic testing for autosomal dominant polycystic kidney disease. *American Journal of Medical Genetics*, 35, 510.

Symonds H, Krail L, Remington L et al. (1990) p53 dependent apoptosis suppresses tumour growth and progression in vivo. *Cell*, 78, 703-712.

Takahashi T, Nau MM, Chibal et al. (1989) p53, a frequent target for genetic abnormalities in lung cancer. *Science*, 246, 491-494.

Takahashi T, Carbone D, Takahashi T et al. (1992) Wild-type but not mutant p53 suppresses the growth of human lung cancer cells bearing multiple genetic lesions. *Cancer Research*, 53, 2340-2343.

Tavtigian et al. (1995) Complete BRCA-2 gene sequence. *Nature Genetics*, 12, 333.

The Best Science of 1992. *Time*, 4 January : 62, 1091.

Toguchida J, Yamaguchi T, Dayton SH et al. (1992) Prevalence and spectrum of germline mutations of the p53 gene among patients with sarcoma. *New England Journal of Medicine*, 326, 1301-1308.

Tyler A, Ball D, Crawford D, on behalf of the United Kingdom Huntington's Disease Prediction consortium (1992) Presymtomatic testing for Huntington's Disease in the United Kingdom. *British Medical Journal*, 304, 1593-1596.

U.S. Presidents' Commission for the study of ethical problems in medicine and biomedical and behaviour research: Screening and counselling for genetic conditions. Washington, DC: 1983.

Vogel F (1979) Genetics of retinoblastoma. *Human Genetics*, 52, 1-54.

Vogelstein B (1990) *Nature*, 348, 681.

Vogelstein B, Kinzler KW (1992) p53 function and dysfunction. *Cell*, 70, 523-526.

Wald N, Law M (1992) Screening, ethics and the law. *British Medical Journal*, 305, 892.

Wald N (1993) Ethical issues in randomised prevention trials. *British Medical Journal*, 306, 563-565.

Watson JD (1990) The human genome project; past present and future. *Science*, 248, 44-49.

Weinberg RA (1991) Tumour suppressor genes. *Science*, 254, 1138-1146.

Went L (1990) Ethical issues policy statement on Huntington's Disease molecular genetics predictive test. *Journal of Medical Genetics*, 27, 34-38.

Wooster R, Bignell G, Lancaster J, et al. (1995) Identification of the breast cancer susceptiblity gene BRCA2. *Nature*, 378, 789-792.

Yonish-Rouach E, Resnitzky D, Lotem J et al. (1991) Wild type p53 induces apoptosis of myeloid leukaemic cells that is inhibited by interleukin-6. *Nature*, 352, 345-347

Commentary

Graphs and their Spectra

Irene Sciriha
Department of Mathematics, University of Malta, Msida, Malta.

From the contributions to conferences and journals, it is evident that most of the research in mathematics is directed towards the development of the theoretical aspect of its various areas. In this sense mathematics is an art fathomed and pondered on for the sake of its own beauty. It has determined the direction of most of the significant research in other fields. Being an incomparable human achievement, mathematical creativity has fathered our understanding about the nature of man and his universe. Invariably, apparently futile results find immediate applications in various fields.

Graph theory started as amusing brain teasers from the days of Euler, who showed, in the eighteenth century, that the seven Königsberg bridges cannot be crossed just once in a closed route, and of Guthrie and De Morgan who, in the nineteenth century, questioned the number of colours required to colour a map. Since then it has been taught under various guises including operational research in management studies, network theory in engineering and algorithms in computer science. It has become a valuable forecasting tool in several applications in industry and commerce.

Since 1939, chemists investigating molecular orbitals noticed a relationship between the energy levels and the stability of a molecule. The **Hückel molecular orbital theory** gives an approximation of the π-molecular orbitals of a molecule by expressing them as a linear combination of the atomic $p\pi$-orbitals (Cotton, 1963; Coulson and Rushbrook, 1940; Zivkovic, 1972). When **Schrödinger's equation**, that determines the molecular orbital energies, is simplified, the resulting equation is the characteristic equation $\mathrm{Det}(\lambda I - A) = 0$, where A is the adjacency matrix of the graph whose structure is the same as that of the molecule being considered (when hydrogen-suppressed, as in the case of hydrocarbons, which is the case most commonly quoted in chemistry literature) (Spialter, 1964). The spectrum of a graph is the solution of the characteristic equation of its adjacency matrix and the gen values in the spectrum describe the energy of the orbitals of the molecule whose structure is the same as that of the graph..

The investigation of **Graph Spectra** is one of the foremost problems of current research in the theory of graphs. Several important researchers have addressed the problem and the theory is sufficiently advanced so that state-of-the-art books synthesizing results have already appeared. In spite of this, there is ample scope for further research. One of the best books that offers a survey of the work done in this area up to a few years ago, is **Spectra of Graphs** by Cvetkovic and co-workers, 1980. When Cvetkovic was about to present his Ph.D. thesis on the spectra of graphs, an established physical chemist, I. Gutman showed a keen interest in his results. He realised that for a class of molecules the eigenvalues in the spectrum of a graph were precisely the energy of the orbitals of the molecule whose structure is the same as that of the graph.

The zero of the energy scale is that for no interaction between the separate atomic orbitals within the molecule (Karplus and Porter, 1970). Thus the zero eigenvalues, $\lambda=0$, determine the **non-bonding molecular orbitals** (NBMO) responsible for the instability of a molecule. The NBMO are described by the corresponding linearly independent kernel eigenvectors of A (Zivkovic, 1972). The solutions also shed light on the electron density distribution in a molecule (Cotton, 1963). So the results of graph spectra are of great interest in **chemistry**.

The mathematical analysis of an existing molecule in terms of its spectrum may be investigated if the bonds between the atoms are known. The data is represented as an adjacency list expressing the neighbouring atoms of each atom in the molecule or as a (0–1) adjacency matrix A in which an i–j entry is 1 if the corresponding atoms are bonded within the molecule and zero otherwise. The vanishing of the determinant of A is an indication of instability. Even the characteristic equation itself, $\mathrm{Det}(\lambda I - A)=0$, where A is the adjacency matrix of the graph whose structure is the same as that of the molecule being considered gives a number of results about the structure of the molecule.

A graph whose adjacency matrix has the number zero in its spectrum is said to be singular. Although some results regarding bipartite graphs have already appeared in the literature, identification, especially for arbitrary graphs, is complex as with increasing order the number of basic structures responsible for the singularities increases enormously.

As highly reactive molecules are difficult to prepare and to isolate, and very unstable, it would be useful to predict their possible structure. The characterisation of singular graphs is still an open question. In this respect the author has had some success in characterising minimal configurations (Sciriha, 1995a). A systematic search for the minimal

configurations in singular graphs that give rise to a singularity was carried out on the grounds that a linearly dependent set of t row vectors of A, the adjacency matrix of the graph, is present. The linear combination between the t row vectors is termed a **kernel relation** and is denoted R_t. It is noted that the coefficients of the kernel relation are the ordered entries of the corresponding **kernel eigenvector** in the nullspace of A. An algorithm was set up to identify the non-isomorphic singular graphs for successive values of t. This theory becomes more and more cumbersome when applied to large systems. One can practically **not** avoid the use of an appropriate software package, such as MATHEMATICA, used in programming mode, for values of t larger than or equal to 5

The first stage was to determine the **core**, χ_t, which is the subgraphs induced by the vertices corresponding to these t row vectors of A. From the core χ_t, a **minimal configuration** (Γ,χ_t,R_t) is "grown" by adding a **periphery**, a set of vertices adjacent to those of the core, until the number of zero eigenvalues in the spectrum of the resulting graph G, called the **nullity** of the graph G, is reduced to one. In the case when the core has one zero eigenvalue in its spectrum then the minimal configuration, Γ, is the core itself. Such a graph has been called a **nut graph** as the periphery is empty (Gutman and Sciriha, 1995). The core χ_t and kernel relation R_t of the minimal configurations Γ, thus obtained will be unique. There may be several peripheries for the same core and kernel relation so that there is a many–to–one correspondence between the structures of minimal configurations I and the pair $(\chi_t R_t)$. Such minimal configurations are the subgraphs found in all singular graphs. The 61 minimal configurations for t less or equal to 5 have been characterized. Several properties for larger t have also been established.

The core may shed light on the charge density distribution in a molecule. The vertices of the core have been shown to correspond to atoms that are charge rich in some ions and molecules. These are the atoms that are likely to be involved in chemical reactions.

As different minimal configurations may be "grown" from the same core, a **maximum configuration** may be set up by including all the peripheral vertices used to build up the minimal configurations from a core χ_t satisfying the relation R_t. In this way the largest graph with the number zero in its spectrum satisfying a particular kernel relation R_t, with all the vertices of the periphery joined to those of the core and themselves inducing a **null graph**, is obtained. This configuration may have more than one zero eigenvalue. Corresponding to each zero eigenvalue is a core and a particular kernel relation (Sciriha, 1995a).

The graph may be **enlarged** further by including edges between the vertices in the periphery and also by joining arbitrary graphs to the vertices in the periphery. In this way the order of the graph may be increased indefinitely without upsetting the core χ_t or the relation R_t. So will the **rank** of the graph which is equal to the order less the nullity.

In 1993 M. Ellingham published a paper showing that he has addressed the problem of singular graphs from a different point of view (Ellingham, 1993). He also built a singular graph G from "basic subgraphs". These he defined as non–singular graphs of order r where r is the rank of the adjacency matrix of G. It has also been interesting to investigate graphs of **small rank**. As the order of a graph is increased the rank is kept fixed or under control at small values by adding vertices which increase the nullity, that is the number of zero eigenvalues in the spectrum. Thus as new vertices are included more minimal configurations are discovered as subgraphs. For very small rank only complete k–partite graphs are admissible, allowing only **vertices of the same type**, that is vertices having the same set of neighbouring vertices. For rank greater than or equal to 4 more minimal configurations contribute to the nullity.

As the set of minimal configurations characterizing singular graphs is being collected and their properties studied, certain **common** properties are already apparent. In fact several **conjectures** have been made, the validity of which will be investigated. One such conjecture is on the common value L of the coefficient of λ of the **characteristic polynomial** $\mathrm{Det}(\lambda I - A)$ where A is the adjacency matrix of the graph, for minimal configurations grown from the same core for the same kernel relation. It is conjectured that L may be expressed in terms of the properties of the vertex–deleted . subgraphs of the corresponding minimal configuration (Sciriha, 1995b).

J.J. Seidel who is one of the pioneers of the development of Graph Spectra stressed his belief that the eigensolution holds the secrets to the understanding of graphs and the systems they model. The aim of this work is to unravel some solutions of significant importance.

References

Cotton FA *(1963)* Chemical Applications of Group Theory. *InterScience,* 117–182.

Coulson CA and Rushbrooke GS (1940) Note on the method of Molecular Orbitals. *Proceedings Cambridge Phil. Soc*, **36**, 193–200.

Cvetkovic D, Doob M and Sachs (1980) *Spectra of Graphs.* Academic Press (London).

Ellingham MN (1993) Basic subgraphs and graph spectra. *Australasian Journal of Combinatorics*, **8**, 247–265.

Gutman I and Sciriha I (1995) Graphs with Maximum Singularity. *Graph Theory Notes of New York.*

Sciriha I (1995a) On the Construction of Graphs of Nullity One. *Proceedings of the 15th British Combinatorial Conference,* International

Conference held at Stirling University.

Sciriha I (1995b) On the Coefficient of λ in the Characteristic Polynomial of Singular Graphs.

Spialter L (1964) The atom connectivity matrix and its characteristic ploynomial. *J. Chem. Doc.*, **4**, 261–274.

Zivkovic T (1972) Calculations of the non-bonding molecular orbitals in the Hückel Theory. *Croatica Chemica Acta*, **44**, 351-364.

Research Article

Preliminary Data On The Occurrence And Distribution Of Shallow Water Marine Sponges (Porifera) Around Maltese Coasts

Joseph A. Borg and Patrick J. Schembri
Department of Biology, University of Malta, Msida, Malta.

Summary. Data on the ecology of the Maltese Porifera is lacking altogether. Even documented basic information on the occurrence of commercial sponge species in Maltese coastal waters is unavailable. This study presents the results of a four year diving survey aimed at studying the occurrence and distribution of shallow water sponges around the Maltese Islands. In all, 33 species of Porifera have been identified, most of which are new records for the Maltese Islands. Information on the bathymetric distribution and abundance of these species is given. Although an extensive area has been covered in our survey, we have not recorded any commercial sponges. It is therefore likely that these species do not occur locally, at least in shallow inshore waters.

Keywords: Porifera, sponges, Maltese Islands, sponge disease, species lists, check-lists, sponge fisheries.

Sponges are a ubiquitous component of the marine benthos. Some species have considerable commercial importance as their fibrous skeleton is the familiar bathroom sponge and they have been exploited by man since antiquity. Until recently there has been a thriving fishery for sponges in the Mediterranean centered mainly on Greece, Tunisia and Turkey (FAO, 1994). Despite both their commercial importance and interesting biology, there is a lack of even the most basic biological and ecological information on the Maltese sponge fauna.

In July 1990, a FAO regional workshop was held in Malta to discuss the situation concerning the occurrence of a disease which had afflicted both commercial and non-commercial species of sponges since 1986 (FAO, 1994). This disease had a large economic impact on the Mediterranean commercial sponge fisheries, having practically eliminated this industry in some countries (Vacelet, 1991). Studies made during the past four years have suggested that the disease is due to a bacterium which normally plays a part in the digestion of the spongin skeleton of dead sponges by secreting a collagenase enzyme. In conditions stressful to sponges, this bacterium becomes virulent and also attacks the skeletal tissue of live sponges (Vacelet *et al.*, 1994). At the time of the Malta FAO workshop, the authors were asked to supply data on the local occurrence of commercial sponges and on the incidence of the sponge disease. The only information available concerning sponge fisheries in Malta were the recollections of some local fishermen (see below). A literature search for information on the local commercial and non-commercial sponge fauna revealed only a single publication, which recorded nine non-commercial species of Porifera (Micallef and Evans, 1968). These identifications are suspect, however, as the identification source used by the authors is a semi-popular guide to Mediterranean marine life which only features a handful of the species occurring in the Mediterranean, and in any case, identification of sponges is difficult without detailed histological examination. The lack of local information on commercial sponges and of a local sponge fishery contrasts with the important sponge fisheries in several nearby Mediterranean countries such as Italy, Tunisia and Greece. However, local fishermen from Wied iz-Zurrieq confirmed that foreign fishing boats had in the past collected commercial sponges off Filfla Island. From the fishermen's descriptions of the diving gear used by the sponge fishermen at that time, such an activity must have been carried out several decades ago. There is also a documented record in local newspapers of the 1890s concerning commercial sponge fishing activities in Malta (J. Inguanez, personal communication, 1991).

The lack of basic data on the local sponge fauna, such as an accurate species list, prompted the authors to initiate a survey having three primary aims:

1. To compile a checklist of the shallow water Porifera of the Maltese Islands and to provide basic ecological information on their bathymetric distribution and abundance.

2. To establish whether any commercial sponges exist locally.

3. To monitor the local occurrence of the sponge disease.

As a preliminary to this survey, J. Vacelet (Station Marine d'Endoume, Marseille) together with one of us (JAB), carried out five dives at different sites around mainland Malta to assess the incidence of the sponge disease amongst local sponge populations, to record the most commonly occurring non-commercial species, to gain experience in their field identification and to search for commercial species. Data on the incidence of the

sponge disease amongst non-commercial sponges obtained during this preliminary survey have been published in Vacelet *et al.* (1994) and FAO (1994).

Materials and methods

A total of 31 SCUBA dives were carried out at depths ranging from zero to 45m in 17 different localities around the Maltese Islands (Figure 1). In view of the indications of a past sponge fishery off Filfla, nine out of the 31 dives were made off this island. During most dives, divers working in pairs moved underwater along 6-metre wide belt transects at pre-determined bearings. The length of these transects varied depending on the depth of the water at the site concerned. All sponges encountered in the transects were identified and recorded *in situ* where possible, but specimens were also collected for later identification in the laboratory. An estimate was made of the abundance and a particular look-out was kept for diseased individuals and commercial species. A few specimens which are included in our species list were obtained from samples collected by trawling at 40-150m off mainland Malta (Tables I and II), whilst others were collected during other studies. Most of the identifications to species level have been checked by J. Vacelet of the Station Marine d'Endoume, Marseille, France. The collection has been deposited at the museum of the Department of Biology, University of Malta.

Results

In all, 33 species of sponges have been identified, most of which are new local records. Table I gives a classified list of the species recorded while Table II provides data on the localities surveyed.

The most abundant species in shallow (1-15m), exposed waters appeared to be *Sarcotragus spinosula* and *Ircinia variabilis*. The latter also occurred, although less abundantly, at depths of 20-25m. In more sheltered shallow waters (2-6m), especially along the rocky headlands of several inlets, *Chondrilla nucula* appeared to have the highest abundance. In sciaphilic environments throughout the 15-35m depth range, *Crambe crambe* and *Agelas oroides* had the highest abundance. *Chondrosia reniformis* and *Petrosia ficiformis* were common in the 5-25m depth range in some of the sites surveyed. At Filfla, *Cacospongia scalaris* was the most abundant species in the 20-35m depth.

No commercial sponge species were encountered during this survey. Only single individuals of non-commercial sponges apparently afflicted by the sponge disease were encountered during dives carried out in 1993 and 1994.

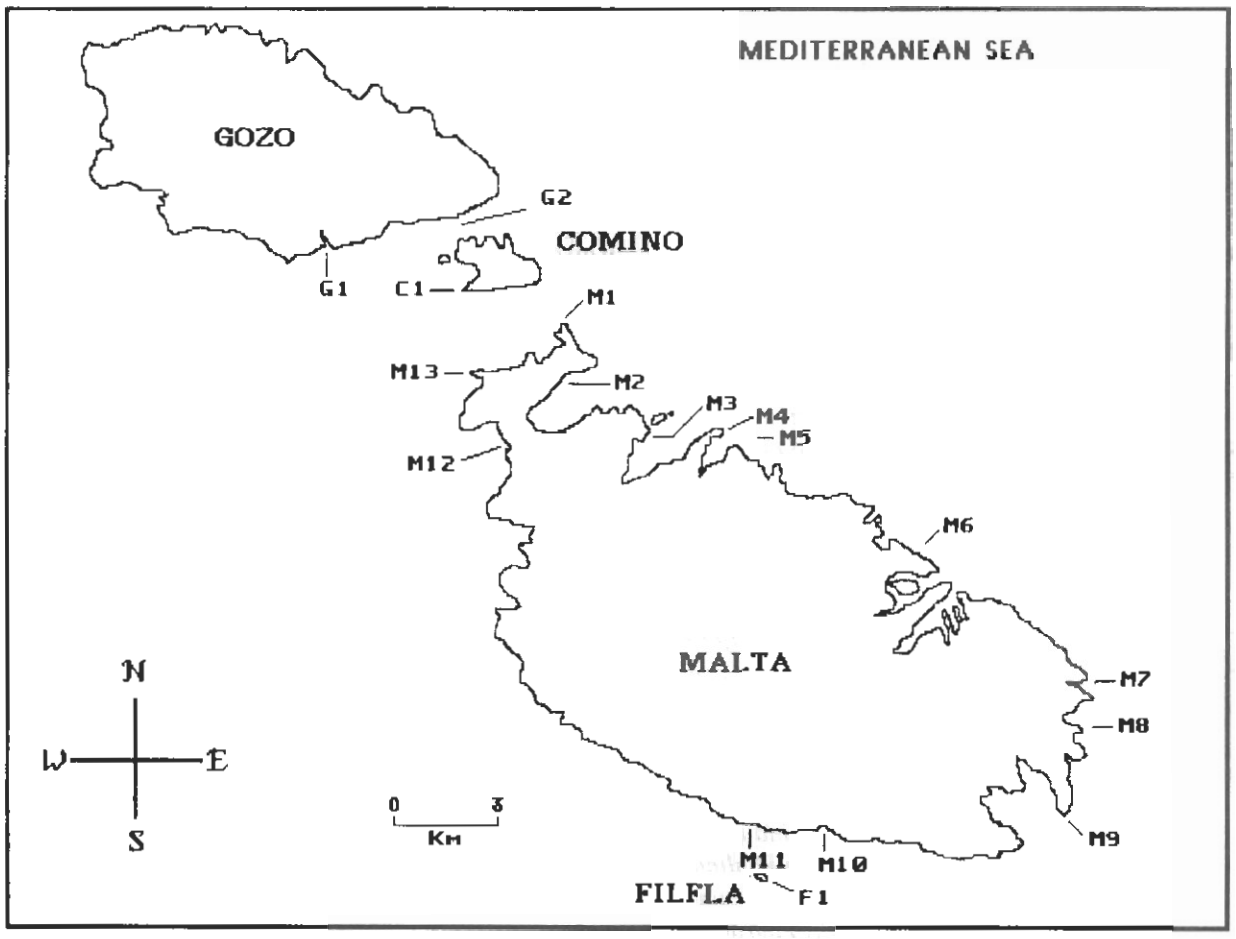


Figure 1: Map of the Maltese Archipelago showing the localities surveyed for sponges.

	Species	Site
Class CALCAREA		
Subclass CALCINEA		
1	*Clathrina clathrus* (SCHMIDT)	M6
Subclass CALCARONEA		
2	*Leuconia* sp.	Xghajra
3	*Petrobiona massiliana* (VACELET)	M13
4	*Sycon elegans* (BOWERBANK)	M12
5	*Ute glabra* (SCHMIDT)	Xghajra
Class DEMOSPONGIAE		
6	*Acanthella acuta* (SCHMIDT)	M6
7	*Agelas oroides* (SCHMIDT)	M1, M5, M9, M10, M12, M13, C1, F1
8	*Anchinoe* sp.	M1,
9	*Anchinoe paupertas* (BOWERBANK)	C1
10	*Aplysina aerophoba* (SCHMIDT)	M2, M4
11	*Axinella verrucosa* (ESPER)	C1
12	*Batzella inops* (TOPSENT)	M1, M10
13	*Cacospongia scalaris* (SCHMIDT)	M2, M3, M4, M6, F1
14	*Cacospongia mollior* (SCHMIDT)	M6, M13
15	*Chondrilla nucula* (SCHMIDT)	M2, M3, M4, M6, M7, M10, M10, M11, G2
16	*Chondrosia reniformis* (NARDO)	M1, M2, M4, M6, M7, M8, M9, M10, M11, M13, C1, C2
17	*Crambe crambe* (SCHMIDT)	M1, M2, M4, M5, M6, M9, M10, M11, M12, M13, C1, G1, G2, F1
18	*Dictyonella incisa* (SCHMIDT)	M3
19	*Dysidea* cf. *fragilis* (MONTAGU)	M1
20	*Dysidea* sp.	M1
21	*Fasciospongia* sp.	M1
22	*Haliclona* sp.	Off Ras il-Wahx
23	*Ircinia dendroides* (SCHMIDT)	C1
24	*Ircinia oros* (SCHMIDT)	M1, M8, M9, M13, C1
25	*Ircinia variabilis* (SCHMIDT)	M1, M2, M4, M5, M6, M8, M9, M10, M12, M13, C1, G1 M9, M10, F1
26	*Oscarella lobularis* (SCHMIDT)	M6
27	*Petrosia ficiformis* (POIRET)	M1, M5, M6, M8,
28	*Raspaciona aculeata* (JOHNSTON)	Off Qammieh
29	*Sarcotragus spinosula* (SCHMIDT)	M1, M2, M3, M4, M5, M6, M7, M8, M9, M10, M11, C1, G1, G2, F1
30	*Scopalina lophyropoda* (SCHMIDT)	Off Qammieh
31	*Siphonochalina* sp.	Off Qammieh
32	*Spiratrella cunctatrix* (SCHMIDT)	M1, M9, M10,M11
33	*Tethya aurantium* (PALLAS)	M12, G1

Table I. Classified list of species recorded. The sites where the species were recorded are indicated by a code corresponding to that in Fig. 1. Sites indicated by their actual name were not surveyed by SCUBA diving but the specimens were obtained from other workers.

Code	Name of site	Date of dive/s	Max. depth	Bottom type
M1	Ahrax Point	May '90	25m	Bedrock/Boulders/*Posidonia oceanica* meadows
		Aug '90	15m	Bedrock/Boulders/*Posidonia oceanica* meadows
M2	Mellieha Bay	Aug '91	15m	Bedrock/Boulders/*Posidonia oceanica* meadows
		Sept '91	20m	Bedrock/Boulders/*Posidonia oceanica* meadows
		Oct '91	15m	Bedrock/Boulders/*Posidonia oceanica* meadows
M3	St Paul's Bay	Oct '94	15m	Bedrock/*Posidonia oceanica* meadows
M4	Qawra Point	May '90	35m	Bedrock/*Posidonia oceanica* meadows
M5	Qawra reef	Sept '91	27m	Bedrock/*Posidonia oceanica* meadows
		Sept '91	27m	Bedrock/*Posidonia oceanica* meadows
M6	Sliema	May '90	30m	Bedrock/*Posidonia oceanica* meadows
M7	Zonqor Point	May '92	15m	Bedrock/*Posidonia oceanica* meadows
M8	Muuxar	Jau '93	35m	Bedrock/Boulders/*Posidonia oceanica* meadows
M9	Delimara	Aug '92	30m	Bedrock/*Posidonia oceanica* meadows
M10	Wied iz-Zurriecq	Aug '91	30m	Bedrock/*Posidonia oceanica* meadows
		Aug '91	35m	Bedrock/*Posidonia oceanica* meadows
M11	Ghar Lapsi	May '90	15m	Bedrock/*Posidonia oceanica* meadows
M12	Anchor Bay	Oct '90	15m	Bedrock/Boulders/*Posidonia oceanica* meadows
M13	Cirkewwa	May '90	30m	Bedrock/*Posidonia oceanica* meadows
		Aug '91	25m	Bedrock/*Posidonia oceanica* meadows
C1	Irqieqa Point	Oct '91	30m	Bedrock/Boulders/*Posidonia oceanica* meadows
G1	Imgarr ix-Xini	Oct '91	25m	Bedrock/*Posidonia oceanica* meadows
G2	Hondoq ir-Rummien	Aug '93	16m	Bedrock/Boulders/*Posidonia oceanica* meadows
F1	Filfla	Aug '92	30m	Boulders/Sand
		Aug '92	30m	Boulders/Sand
		Aug '92	25m	Boulders/Sand
		Aug '92	25m	Bedrock/Boulders/Sand
		Aug '92	25m	Bedrock/Boulders/Sand
		Sept '92	30m	Boulders/Sand
		Sept' 92	25m	Bedrock/Boulders/Sand
		Sept '94	30m	Bedrock/Boulders/Sand
		Sept '94	25m	Boulders/Sand
/	Off Qammieh	Aug '92	40m	Bedrock/Sand
/	Off Ras il-Wahx	Mar '93	150m	Sand/mud
/	Xghajra	Aug '90	0-0.5m	Mediolittoral/Upper infralittoral Bedrock

Table II. Details of the sites surveyed for sponges.

Conclusions

As expected, the majority of sponges recorded during this study belong to the class Demospongiae. Due to the search and sampling methods employed in this study, small sized epibenthic and epiphytic species such as those found in meadows of the seagrass *Posidonia oceanica* may have been overlooked. As a result, our species list is considerably shorter than for other parts of the Mediterranean (see for example Pansini and Pronzato, 1985; Carballo and Garcia-Gomez, 1994) and many more species no doubt occur; there are currently about 554 known species of marine Porifera in the Mediterranean (Pansini, 1990) Nevertheless, on the basis of our results we are able to state that:

1. The shallow water sponge fauna of the Maltese Islands appears to be similar to that of other parts of the Mediterranean.

2. There do not appear to be any large commercial sponge beds in Maltese shallow coastal waters that could be economically exploited, and indeed, no commercial sponge species were recorded.

We suggest that future work on the local marine sponge fauna should aim at quantifying species abundance, compiling a more complete species list and at identifying the more important species involved in interspecific relationships with other marine plants and animals.

Acknowledgements

This work has been partly financed by the Marine Resources Network of the Malta Council for Science and Technology (MCST), the Department of Agriculture and Fisheries of the Government of Malta, FAO programme TCP/RAB/8853 (C), and by research grants from the University of Malta. We are indebted to Prof J Vacelet of

the Station Marine d'Endoume, Marseille, France for identifying most of the species and for supporting us with his specialist knowledge. We are grateful to Mr Constantine Mifsud and Mr Adrian Mallia for donating several sponge specimens which they collected in the course of their work. We would also like to thank Mr Aldo Drago and Mr Anton Micallef for logistic assistance, and Mr Konrad Pirotta and all the other divers, especially members of the Calypso and ATLAM sub-aqua clubs, who have participated in the surveys.

References

FAO [Food and Agriculture Organisation of the United Nations]. (1994) The struggle against the epidemic which is decimating Mediterrauean sponges. *FAO Technical Report FI/TCP/RAB/8853*; Rome, FAO: v + 39pp.

Micallef H and Evans F (1968) *The marine fauna of Malta*. Msida: Malta University Press; vi + 26pp.

Pansini M (1990) Biogeographic and sytematic notes for an up-dating of the Mediterranean sponge fauna. In: D Bellan (ed.), *Speciation and biogeography in the Mediterranean Sea*. France: Perpignan.

Pansini M and Pronzato R (1985) Distribution and ecology of epiphytic Porifera in two *Posidonia oceanica* (L.) Delile meadows of the Ligurian and Tyrrhenian Sea. *P.S.Z.N.I. Marine Ecology*, 6(1): 1-11.

Vacelet J (1991) Statut des eponges commercialcs en Méditerranée. In: C F Boudouresque *et al.* (eds.), *Les espéces marines à protéger en Méditerranée*: pp. 35-42; France: G.I.S. Posidonie.

Vacelet J, Vacelet E, Gaino E and Gallissian M F (1994) Bacterial attack of spongin skeleton during the 1986-1990 Mediterranean sponge disease. In: R W M Van Soest *et al.* (eds.) *Sponges in time and space*. pp. 355-362. Rotterdaun: A A Balkema.

Research Article

The Cytotoxic Activity of Cucurbitacin E and Busulphan on Ovarian and Stomach Cancer Cells *In Vitro:* A Comparative Study

Everaldo Attard, Anthony Scicluna-Spiteri
Institute of Agriculture, University of Malta, Msida, Malta

Mario Grixti, Alfred Cuschieri
Department of Anatomy, University of Malta, Msida, Malta

Summary. *A comparative study of the cytotoxicity of cucurbitacin E, a natural product, and busulphan on human ovarian and stomach cell lines was carried out. The cells were exposed to different concentrations of these two compounds and cell viability was determined from day 0 to day 11. It was observed that cucurbitacin E had a marked effect on the ovarian cancer cell line while busulphan showed a similar effect when exposed to the stomach cancer cell line. These drug-cell combinations showed a pronounced cell kill exponential curve, leading to the conclusion that cucurbitacin E exerts its pinocytic activity on the ovarian cancer cells while busulphan exerts its alkylating effect on the stomach cancer cells.*

Keywords: *Ecballium elaterium*; *Cucurbitaceae*; cucurbitacin E; busulphan; ovarian cancer cell line; stomach cancer cell line

Cucurbitacin E and other cucurbitacins are highly oxygenated triterpenes which are found solely in plants grouped under the *Cucurbitaceae* family, including *Ecballium elaterium L.* (the squirting cucumber). *Ecballium elaterium L.* is a local medicinal plant which has been used in folk medicine as a cathartic (Cini, 1991) and as an emetic (Lanfranco, 1975). It has also been used in dropsy (Penza, 1969) and in the treatment of jaundice (Cini, 1991).

Experiments on the juice of the plant have shown that it is effective in the treatment of constipation, oedema, sinusitis, and the prevention of liver disease (Yesilada *et al.*, 1988). However, it has been found that the juice has a low therapeutic index (Farnsworth, 1992), but that it contains cucurbitacins, including cucurbitacins B and E, which have antitumour activity. Despite this, when individual cucurbitacins were tested on various normal cells, the cell viability was not affected (Gallily *et al.*, 1962).

Cytotoxicity (Gitter *et al.*, 1961) and metabolic studies (Shohat *et al.*, 1962) were performed on Sarcoma 180, Lettre Erhlich ascites carcinoma and Sarcoma Black using cucurbitacins D, E and I in mice. There was a higher cytotoxic effect shown by these compounds on Sarcoma 180 than on the other two cell lines. Metabolic studies showed that in Ehrlich ascites carcinoma cells, the oxygen uptake of cells was more sensitive to the action of cucurbitacins than the anaerobic glycolysis. It was observed that the inhibition of the oxidative metabolism of the cancer cells by the cucurbitacins was related to that observed by hydrocortisone. This may be due to the fact that the cucurbitacins have a steroid-like structure which may influence the permeability of the membranes of the cells and mitochondria. Combination therapy with cucurbitacins and X-rays on transplanted tumours in mice (Shohat *et al.*, 1965) was less effective on Ehrlich tumour than Sarcoma Black.

Cucurbitacins B and E showed an effect on cultured human nasopharyngeal carcinoma, and Sarcoma 37 implanted intramuscularly into right hind legs of CAF_1 mice when these compounds were injected intraperitoneally.

Cucurbitacin E (Figure 1) can exert its cytotoxic effect either on the cell membrane (Gallily *et al.*, 1962) or on the DNA in the nucleus of the cancer cells (Kupchan *et al.*, 1973). The cucurbitacin side chain is important for the observed cytotoxic activity (Kupchan *et al.*, 1970).

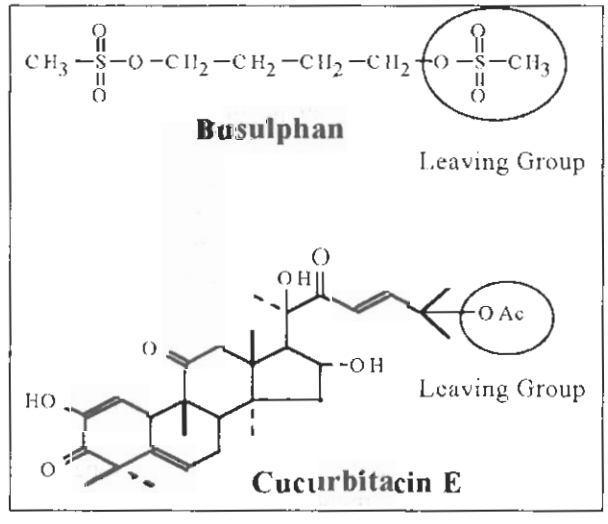


Figure 1: Structures of Busulphan and Cucurbitacin E

Busulphan (Figure 1) is an alkylating agent, which exerts its action by joining two guanine residues on two strands of the DNA, leading to cross-linking. This, in turn, prevents the uncoiling and replication of the DNA molecule, thus halting the multiplication of the tumour cells (Rogers et al., 1976).

Several studies have been carried out on the cell lines already mentioned using different cucurbitacins, but the effect of these compounds on ovarian and stomach cancer cell lines has not been studied. The present study was therefore undertaken to examine any possible effects of cucurbitacin E on ovarian and stomach cancer cell lines and also to compare the effect of cucurbitacin E with that of a widely used cytotoxic agent, busulphan.

Experimental Procedures

Cucurbitacin E was prepared by solvent extraction of the fruit of *Ecballium elaterium* (Lavie *et al.*, 1958), collected from Marsascala (Malta). 52% (w/w) of the pure compound was obtained. Its purity was confirmed using five analytical methods: UV and IR spectrophotometry, Melting Point Determination, TLC and HPLC against a known standard. A specimen is deposited at the Institute of Agriculture, University of Malta. From a stock solution of $1.8x10^{-4}$M, 1 in 10 dilutions were prepared.

Busulphan (Myleran® Wellcome, West Sussex, U.K.) 500mg tablets were ground in a mortar and then dissolved in RPMI 1640 medium to make a final stock solution of $4x10^{-4}$M. 1 in 10 dilutions were then prepared.

Single stomach (SNU-1) and ovarian (OVCAR-3) cell lines were obtained from the Department of Anatomy University of Malta. These cell lines were cultured and subcultured to propagate the cell lines. The cells were grown in RPMI 1640 medium in sterile Nunclon® culture flasks and incubated at 37°C in 6% CO_2. Subculturing was performed every seven days (Freshney, 1988).

A cell suspension was obtained by detaching the monolayer from the flask using trypsin and resuspending the cells in RPMI medium. Complete cell detachment was visualized under a x100 magnification microscope (Diavert Leitz-Wetzlar). 20ml of RPMI medium was added to each flask (x2) and the cell suspension was mixed. A small sample was withdrawn and cells were counted in an Improved Neubauer haemocytometer using the method described in the Sigma Cell Culture Catalogue (1994).

The drugs were added on day 0. Twenty-eight tubes were used in all. 1ml of ovarian cell suspension was added to each of fourteen tubes while 1ml of stomach cell suspension was added to another fourteen tubes. The concentrations of cucurbitacin E used were: $1.8x10^{-6}$M, $1.8x10^{-7}$M and $1.8x10^{-8}$M. The concentrations of busulphan used were: $4x10^{-6}$M, $4x10^{-8}$M and $4x10^{-10}$M. 2ml of the three solutions with different concentrations of cucurbitacin E were added to six tubes containing ovarian cancer cells, and to six tubes containing stomach cancer cells. The same procedure was repeated for busulphan. The rest (i.e. four tubes) acted as the control tubes in which 2 ml of RPMI medium were added to the cancer cell suspension.

From days 7 to 11, the number of viable and non-viable cells was counted using a haemocytometer and 0.4% trypan blue for the staining of non-viable cells (Freshney, 1988). The experiment was followed from day 7 to 11, as it was observed in our laboratory that there was no significant lethal effect on the cancer cells from day 0 to day 7. The five-day period, day 7 to 11, was sufficient to provide information on the cytotoxic activity of both cucurbitacin E and busulphan on the two cancer cell lines. A preliminary study had shown that the decrease in percentage cell viability was not significant after day 11.

The percentage cell viability was calculated using the number of viable and non-viable cells obtained. The four results were used to obtain an average percentage cell viability. The counting of non-viable cells was necessary to determine the LC_{50}, which is the concentration of cytotoxic compound required to kill 50% of the cells in suspension. The decrease in cell viability should depend on the cytotoxic activity of the compounds and not on the limited environmental factors, which include nutrient availability in the medium and the conditions inside the incubator. The cell counts for the tubes treated with the cytotoxic compounds were adjusted by taking the average cell count in the control tubes to be 100%.

Results

Tumour Cell Growth Inhibition. Figures 2 to 5 show the percentage log cell viability against number of days, for ovarian and stomach cancer cells, both treated with cucurbitacin E and busulphan.

As can be observed from Figure 2, at $1.8x10^{-6}$M, cucurbitacin E showed a lower terminal percentage log cell viability than at $1.8x10^{-7}$M, although cell viability, for the latter, was markedly reduced. Figure 3 shows that the inhibition of tumour growth is higher with increasing busulphan concentration. At the two lower concentrations ($4x10^{-8}$M and $4x10^{-10}$M) of busulphan used, a rapid fall in cell viability was evident after day 10 while at $4x10^{-6}$M, a rapid decrease was observed after day 9. Figure 4 shows that the effect of cucurbitacin E on stomach cancer cells varied with the three different concentrations used. At $1.8x10^{-8}$M cucurbitacin E, there was a rapid decrease in cell viability between day 8 and 9 but a slow steady fall thereafter. At $1.8x10^{-6}$M cucurbitacin E, there was a linear decrease in cell viability. At $1.8x10^{-7}$M cucurbitacin E, there was a slow decreases in cell viability after day 10. Stomach cancer cells treated with

$4x10^{-6}$M busulphan (Figure 5) showed a stepwise decrease in viability with time. A considerable decrease in cell viability after day 8 was observed at the three concentrations of busulphan used, but no further effect at $4x10^{-6}$M and $4x10^{-8}$M was observed after day 10. At $4x10^{-8}$M busulphan a higher cytotoxic effect was observed than at the lower concentration of $4x10^{-10}$M.

The four different cell-drug combinations were compared using the Probit analysis. The differences in the trends for these combinations were found to be statistically significant ($P<0.05$, $v=4$).

Cucurbitacin E showed a higher cytotoxic effect on the ovarian cancer cells than busulphan. It is worth noting

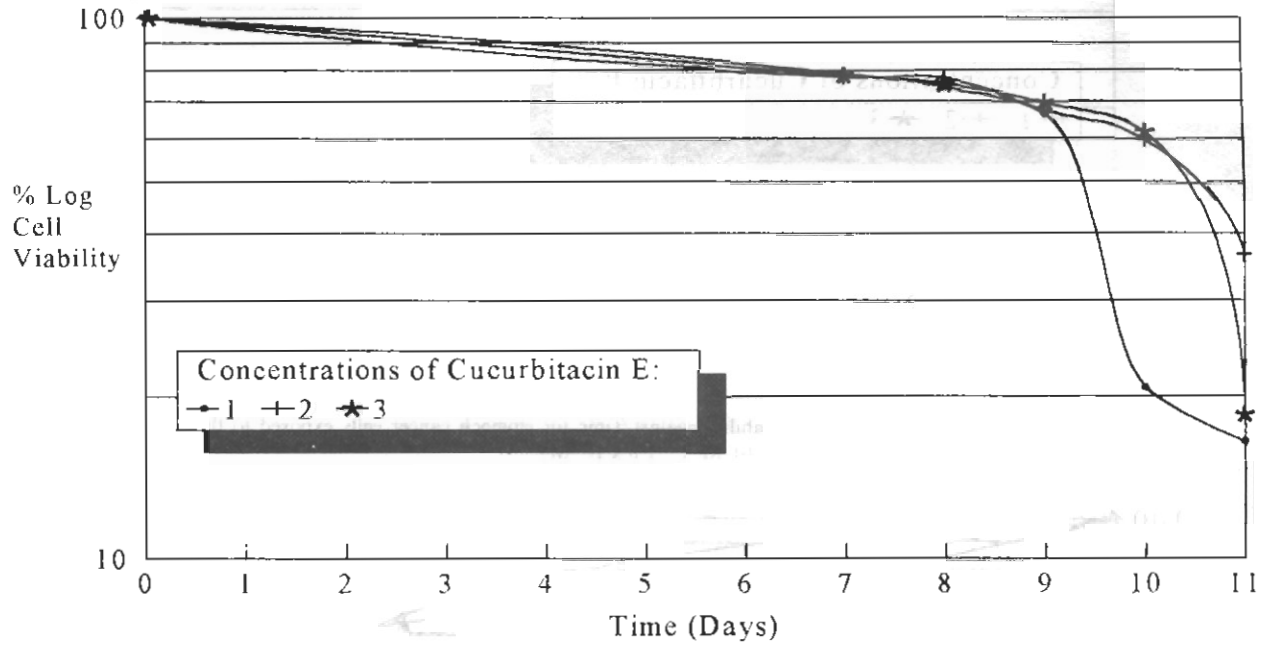


Figure 2: The Percentage Log of the Adjusted Cell Viability against Time for ovarian cancer cells exposed to three concentrations of Cucurbitacin E (Concentrations: 1 = $1.8x10^{-6}$ M, 2 = $1.8x10^{-7}$M, 3 = $1.8x10^{-8}$M).

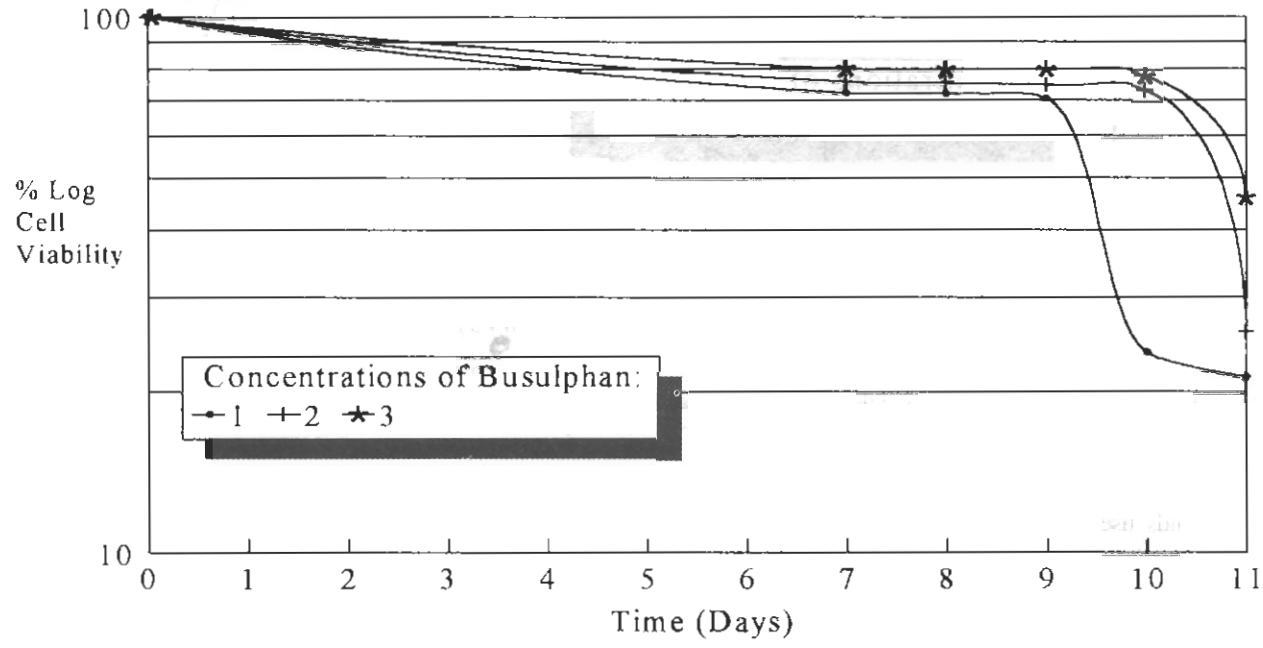


Figure 3: The Percentage Log of the Adjusted Cell Viability against Time for ovarian cancer cells exposed to three concentrations of Busulphan (Concentrations: 1 = 4×10^{-6} M, 2 = 4×10^{-8} M, 3 = 4×10^{-10}M).

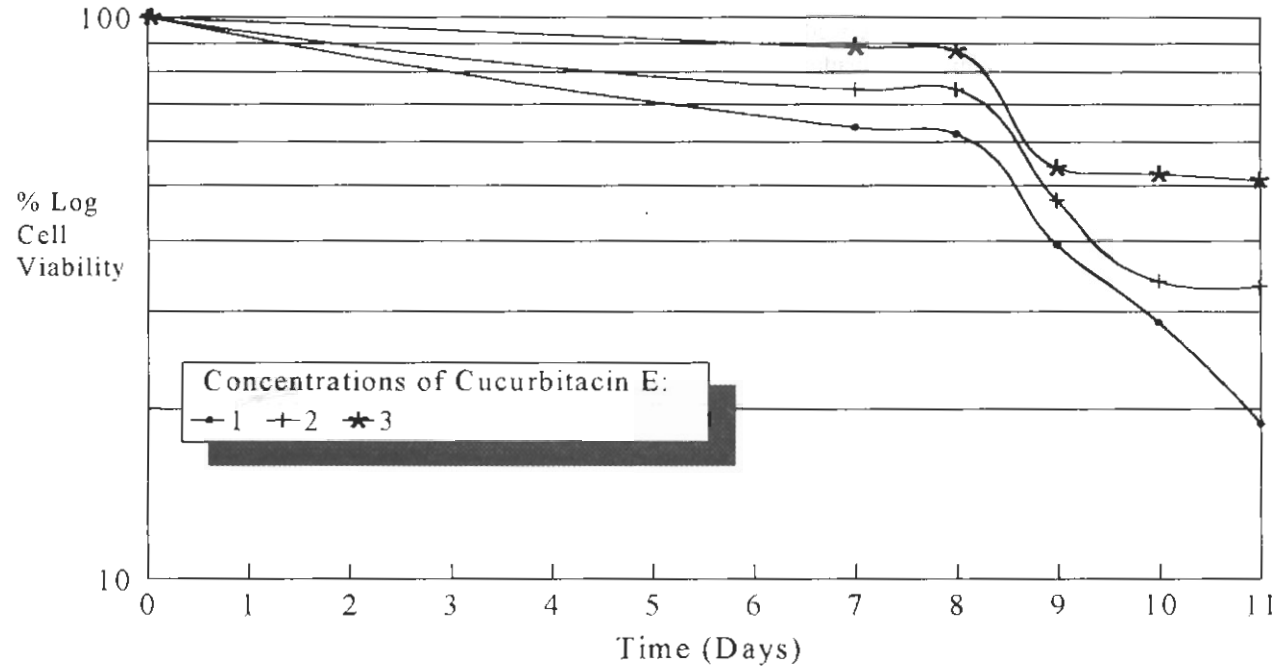


Figure 4: The Percentage Log of the Adjusted Cell Viability against Time for stomach cancer cells exposed to three concentrations of Cucurbitacin E (Concentrations: 1 = 1.8×10^{-6}M, 2 = 1.8×10^{-7}M, 3 = 1.8×10^{-8}M).

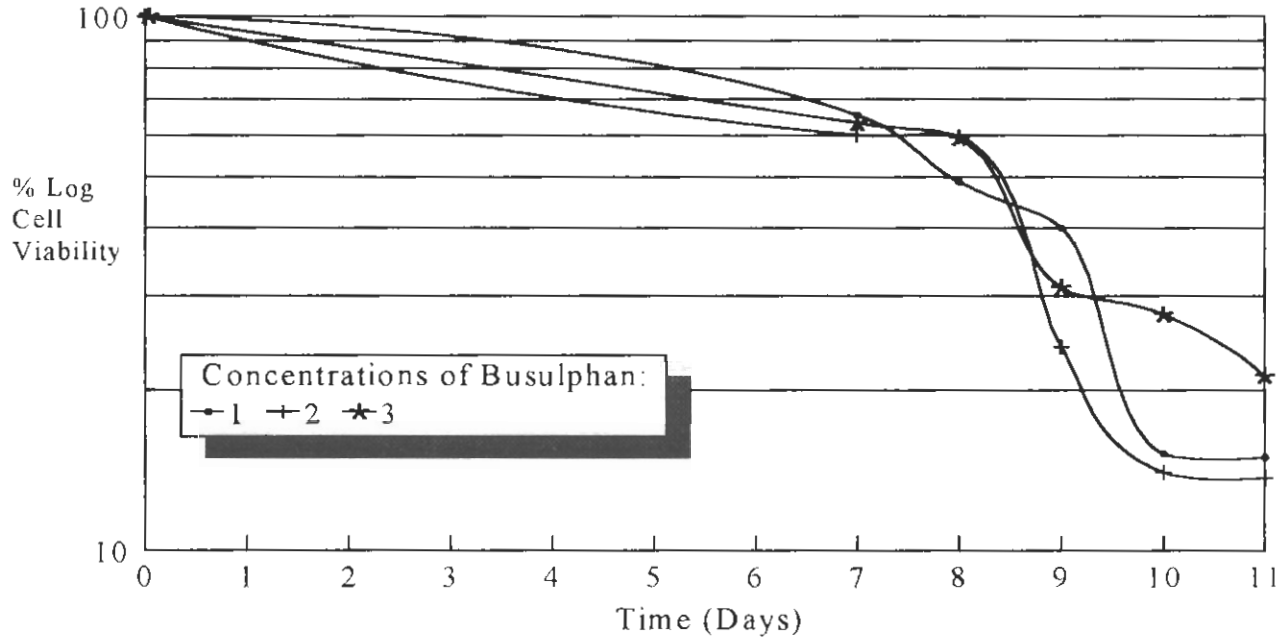


Figure 5: Graph of the Percentage Log of the Adjusted Cell Viability against Time for stomach cancer cells exposed to three concentrations of Busulphan (Concentrations: 1 = 4×10^{-6}M, 2 = 4×10^{-8}M, 3 = 4×10^{-10}M)

that at the highest concentration of both cytotoxic compounds used (1.8×10^{-6}M cucurbitacin E and 4×10^{-6}M busulphan), a similar pattern was observed, characterized by no further cytotoxicity at day 11. In the stomach cancer cells, a greater percentage cell death was obtained with busulphan than with cucurbitacin E.

Minimum Median Lethal Concentration. Table 1 shows the minimum median lethal concentration for the two different cell lines treated with the two cytotoxic drugs. Cucurbitacin E showed a minimum LC_{50} of 2.72×10^{-7}M for the ovarian cancer cells at day 10, and a minimum LC_{50} of 0.5269M for the stomach cancer cells at day 7. For busulphan, the minimum LC_{50} for ovarian cancer cells was 9.14×10^{-4}M at day 9, while that for the stomach cancer cells was 2.14×10^{-8}M on day 10.

From the values one may note that cucurbitacin E has a greater activity on ovarian cancer cells than busulphan (mLC_{50} E < mLC_{50} B). On the other hand, busulphanshowed a greater effect on the stomach cancer cells although this occurred on day 10 as opposed to the

	Ovarian Cancer Cells	Stomach Cancer Cells
	Cucurbitacin E	
mLC_{50}	2.72×10^{-7} M	0.5269 M
R value*	0.8795	0.7575
	Busulphan	
mLC_{50}	9.143×10^{-4} M	2.14×10^{-8} M
R value*	0.9006	0.9540

Table 1. Table showing the mLC_{50} for the two different compounds and cell lines. *All R values are taken at $p \leq 0.05$, $v = 4$.

mLC_{50} of cucurbitacin E which was found on day 7. The LC_{50} for cucurbitacin E-treated stomach cancer cells is too high (0.5269M) to be considered as an effective compound.

Discussion

Since cucurbitacin E has been shown to have an effect on DNA by alkylation (Kupchan *et al.*, 1973), and on the cell membrane by the process of pinocytosis (Gallily *et al.*, 1962), it was of interest in this study to compare its effects on cancer cells with those of busulphan and to draw some conclusions from the results obtained.

Tumour Cell Growth Inhibition. The results show that the *in vitro* cytotoxic effect of cucurbitacin E was best observed on ovarian cancer cell lines while busulphan showed a greater effect on stomach cancer cells.

If one considers that cucurbitacin E is taken up by the tumour cell by a rate limiting process, there might be sufficient uptake at low concentrations to have an alkylating effect on the DNA. This might explain the greater cytotoxic effect observed for cucurbitacin E on ovarian cancer cells at the lowest concentration (1.8×10^{-8}M) used. Whether this effect is due to the process of pinocytosis is still to be determined. However, this process is greatly influenced by high cucurbitacin E concentrations, where an increase in the uptake of fluid inside the cell leads to cell blistering and eventually cell death. This was observed by Gallily and co-workers (1962) on four cell lines, using elatericin A and B. At high concentrations, the effect on the cell membrane is more pronounced.

Busulphan, at a concentration of 4×10^{-10}M and 4×10^{-8}M, did not have an effect on the ovarian cancer cells. This is known as the tumerostatic effect. At these two concentrations, insignificant cytotoxicity was observed until day 10 after which a decrease in cell viability was observed. At the highest concentration (4×10^{-6}M) used, the same effect was observed until day 9 after which there was a better response.This might be explained by the fact that busulphan did not appear to affect the pinocytic activity of the tumour cells since cell morphological changes were not observed. The high rate of cell death observed for the high concentration might be due to the effects on the DNA by alkylation.

At the lowest concentration (1.8×10^{-8}M) of cucurbitacin E used, a small effect on the stomach cancer cell line was observed, probably due to the limited amount of drug in solution. However, at the highest concentration (1.8×10^{-6}M) a marked effect on cell viability was observed. It can be concluded that stomach cancer cells showed marked resistance towards cucurbitacin E, as was shown by the ovarian cancer cells toward busulphan. On the other hand, busulphan showed effective cytotoxicity in the stomach cancer cells. Although there was a great reduction in the viable count at the highest concentration (4×10^{-6}M) used, the 4×10^{-8}M concentration showed a lower end-point. However, at these two concentrations, after day 10, no further significant inhibition was observed.

It would appear that the activity of the cytotoxic compounds on stomach cancer cells does not depend on the pinocytic activity but on the alkylating effect on the DNA since busulphan had a greater activity than cucurbitacin E on these cancer cells.

It can be concluded from these results that cucurbitacin E lacks the pronounced alkylating effect of busulphan but the latter lacks the pinocytic activity of cucurbitacin E. It might also be postulated that cucurbitacin E increases the uptake of busulphan (and other alkylating agents) while the latter exerts its effects inside the cell. The effect of cucurbitacin E on the permeability of cell membranes (Shohat *et al.*, 1962) could be due to its steroid-like structure which is similiar to that of the cell membrane. It should also be stressed that cucurbitacin E has an effect on both cell lines, although a minimal one on the stomach cancer cells.

Minimum Median Lethal Concentration. The median lethal coucentrations (LC_{50}) for cucurbitacin E on the ovarian and stomach cancer cells show that for the ovarian cells the LC_{50} was quite satisfactory and hence merits further attention while for stomach cancer cells the high LC_{50} indicates a lack of sensitivity of these cells for the cytotoxic compound .

For busulphan the LC_{50} for the ovarian cancer cells is quite high and so it can be regarded as ineffective for the treatment of ovarian cancer. In fact the mLC_{50} for busulphan is 3361 times greater than that for cucurbitacin E in these cells. However, the low LC_{50} for stomach cancer cells suggests that it can be used. On the contrary, the mLC_{50} for busulphan is much smaller than that for cucurbitacin E, i.e. the mLC_{50} of the latter, being about 2.46×10^{7} times greater than the mLC_{50} of busulphan.

To substantiate the above finding, further iuvestigations would have to be carried out to determine the extent of the activity of the two cytotoxic agents *in vivo*, to determine morphological changes and to detect any DNA aberrations.

Acknowledgments

The authors are indebted to Prof D Lavie, Head of Department of Organic Chemistry in the Weizmann Institute of Science, Israel for the authentic sample of cucurbitacin E, and to Dr G Peplow of the Department of Chemistry, University of Malta, for the consultation in the use of analytical methods for the determination of the prepared cucurbitacin E.

References

Cini G (1991) Faqqus il-Hmir li faqqas it-Tmintax Irbighat. *L-Eghjun, Lehen il-Moviment Civiku Xaghra (MCV)*, **9**, 4.

Farnsworth NR (1992) Napralert (SM) Issue 2: Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois, Chicago, U.S.A.

Freshney RI (1988) Culture of Animal Cells: A Manual of Basic Technique, Alan R Liss, Inc., New York, 132-134.

Gallily R, Shohat B, Kalish J, Gitter S and Lavie D (1962) Further Studies on the Antitumor Effect of Cucurbitacins. *Cancer Research*, **22**, 1038.

Gitter S, Gallily R, Shohat B and Lavie D (1961) Studies on the antitumour effect of Cucurbitacins. *Cancer Research*, **21**, 516.

Kupchan SM and Tsou G (1973) The Structure and Partial Synthesis of Fabacein. *Journal of Organic Chemistry*, 38, 1455 - 6.

Kupchan SM, Smith RM, Aynehchi Y and Maruyama M (1970) Tumour Inhibitors. LVI. Cucurbitacins O, P and Q, the Cytotoxic Principles of *Brandgea bigelovii*. *Journal of Organic Chemistry*, **35**, 2891.

Lanfranco G (1975) Duwa u Semm il-Hxejjex Maltin. In: Edizzjoni Klabb Kotba Maltin Valletta, Malta, 33-40.

Lavie D, and Szinai S (1958) The constituents of *Ecballium elaterium* L. II. a-Elaterin. *Journal of the American Chemical Society*, **80**, 707.

Penza C (1969) Flora Maltija Medicinali, Progress Press, Malta, 29.

Roger H and Spectro R (1976) *An Introduction to Mechanisms in Pharmacology and Therapeutics. First Edition*, William Heinemann Medical Books Ltd., Great Britain, 383-385

Shohat B, Gitter S and Lavie D (1962) Antitumour Activity of Cucurbitacins: Metabolic Aspects. *Cancer Chemotherapy Reports*, **23**, 19.

Shohat B, Gitter S, Levy B and Lavie D (1965) The combined effect of cucurbitacins and X-ray treatment of transplanted tumours in mice. *Cancer Research*. **25**, 1828.

Sigma Cell Culture Catalogue (1994) Sigma Chemical Company, 205-206.

Yesilada E, Tanaka S, Sezik E and Tabata M (1988) Isolation of an anti-inflammatory principle from the juice of Ecballium elaterium. *Journal of Natural Products*. **51**, 504.

Research Article

Motor Vehicle Accidents: Analysis of Casualty Department Data, St. Luke's Hospital, Malta.

Maurice N. Cauchi
Department of Pathology, University of Malta Medical School , Guardamangia.

Summary: Motor vehicle accidents (MVAs) referred to St. Luke's Hospital Casualty Department were analysed. There were 616 MVAs referred to hospital during the year , an incidence of 170 per 100,000 of the population. There were 3 accident rate peaks, namely, one early in the morning, a second around mid-day, and a third around 6 pm. The majority (55%) involved young persons under the age of 30 years. The risk of hospitalisation was highest for males in the 20-29 age group (480/100,000). Nearly one third required hospitalisation for more serious injuries.This analysis emphasises the need for urgent measures to be taken to reduce the rate of MVAs and associated morbidity.

Keywords: motor vehicle accidents, Malta, St Luke's Hospital, emergency, admissions

The number of motor vehicle accidents is increasing at an alarming rate. This has resulted from a number of factors. In Malta, there is currently one car for every two residents (see Brockdorff 1995). Moreover, Maltese drivers have become more mobile, using their car more frequently, and younger in age as a result of the increasing purchasing power of the individual.

Studies relating to the incidence and pattern of car accidents in Malta are not readily available (see Camilleri 1969). In this study, an analysis of MVA casualties admitted to the Casualty Department of St. Luke's Hospital over a period of one year (1994) was carried out with a view to determine patterns which were significantly related to increased traffic accidents.

Materials and Methods

The registers of the Casualty Department, St. Luke's Hospital, were examined for the year 1994. The following information was obtained.

Age, sex, place of residence, date and time of accident, and outcome (whether discharged or transferred to a hospital ward). Data was stored on a data-base and analysed nsing a statistical analysis package (Manugistics Statgraphics Plus). Risk of admission to SLH for injury for MVAs was defined as:

$$\frac{\textit{Number of persons admitted during a defined time}}{\textit{Number of persons in that age/sex bracket in the Maltese population}}$$

Results

The number of injuries resulting from motor vehicle accidents admitted to St. Luke's Hospital (MVAs) per month varied from a low of 21 in May to a high of 97 in July (Table 1, Figure 1). As expected, there is a considerable male preponderance, with male:female ratios ranging from 1.4 to 3.2. There does not seem to be a seasonal distribution in this ratio.

	Total	Male	Female	Ratio M/F
Jan	37	26	11	2.36
Feb	45	29	16	1.81
Mar	38	22	16	1.37
Apr	34	24	10	2.40
May	21	16	5	3.20
Jun	70	48	21	2.28
Jul	97	62	32	1.94
Aug	43	26	15	1.65
Sep	44	26	16	1.69
Oct	34	20	13	1.70
Nov	42	28	14	2.00
Dec	61	41	19	2.16

Table 1: Motor vehicle injury admittance to SLH, by month and sex. 1994.

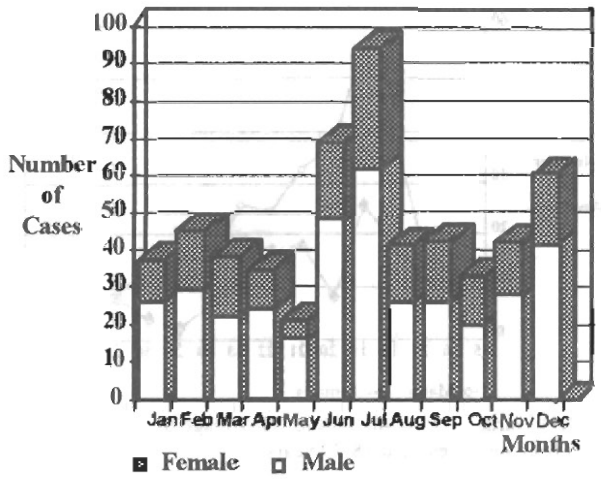


Figure 1: Monthly variation in the number of admissions to SLH casualty department.

There was a preponderance of accidents in the early hours of the morning. In males, the period from midnight till 4.00 am accounted for 28.5% of all accidents. There was a second peak during late

morning and another around 6 pm. In females, the periods between 4 and 6 pm accounted for the highest relative proportion of accidents (Figure 2, Table 2).

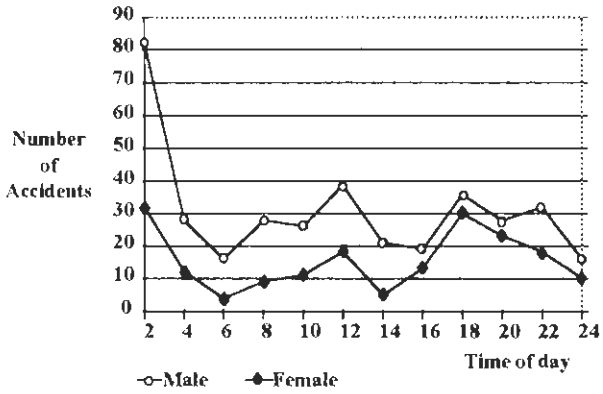


Figure 2. Variation in the number of admissions to SLH by time of day. Note the prominent early morning peak affecting mainly male individuals. Other peaks occur at around mid-day and around 6 p.m.

Time	Males		Females		Total		M/F
	No.	%	No.	%	No.	%	
<4.00hrs	113	28.5	54	25.3	167	27.6	2.09
4.00 -< 8.00hrs	46	11.6	14	6.6	63	9.8	3.29
8.00 -<12.00hrs	68	17.2	38	17.8	106	17.4	1.79
12.00 -<16.00hrs	45	11.4	21	9.8	66	10.8	2.14
16.00 -<20.00hrs	73	18.4	56	26.4	129	21.2	1.30
20.00 -<24.00hrs	51	12.9	30	14.1	81	13.3	1.7

Table 2: Number of persons hospitalised following MVAs, by time of occurrence.

The majority of accidents involved young persons under the age of 30 - in fact, 64% of injuries involved persons in this age group, with a sharp drop after the age of 30 years (Figure 3, Table 3).

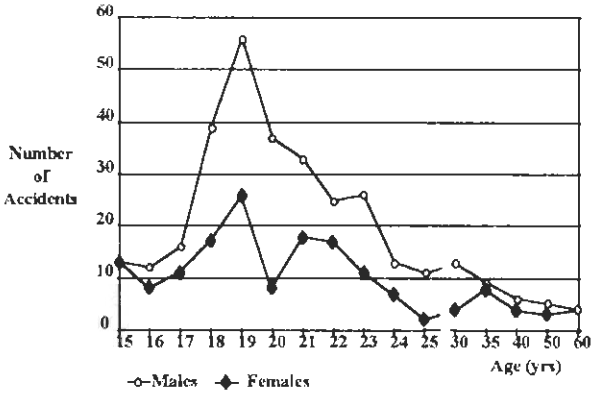


Figure 3: variation in admission to Slh by age and sex. Note the sharp peak for males aged 18-20 years.

There were 185 MVAs involving persons under the age of 20 years (33%), and 171 between the age of 20 and 30 years (23.4%). For the 20-29 age group, the risk of hospitalisation was 480/100,000 in males and 199/100,000 in females and was only slightly less for the 10-19 age group (373 and 181 respectively).

There were significant variations in the incidence of hospitalisation rates between the days of the week, with Sundays showing the highest incidence (21%) (Table 4)

	MVA's		Maltese Population		Risk per 100,000	
Age(Yrs)	M	F	M	F	M	F
0-9	13	16	27817	26502	47	60
10-19	107	49	28708	27086	373	181
20-29	123	48	25601	24112	480	199
30-39	59	32	28981	28102	203	114
40-49	37	23	27471	27736	134	83
50-59	17	11	16477	18669	103	59
>60	27	17	23507	30502	115	56
TOTAL	383	175	178560	182709	214	96

Table 3: MVAs: risk of Hospitalisation, by age and sex.

	Injuries	
	Number	Per cent
Monday	62	11.3
Tuesday	55	10.1
Wednesday	64	11.7
Thursday	90	16.5
Friday	80	14.2
Saturday	81	14.1
Sunday	113	20.9

Table 4: Variation in number of injuries, by day of week.

A measure of the degree of severity of the MVA may be obtained from an analysis of the discharge rates after attending the Casualty Department: obviously, those transferred to one of the hospital wards would account for the more severe cases. Table 5 shows that nearly 29% of all MVAs were referred. The proportion of males was slightly higher than that for females, but this was not statistically significant.

	Males	Females	Total
Discharged	251	147	404
Referred	104	49	153
Total	355	196	551
% Referred	29.3	25.0	27.8

Table 5: Proportion of MVA patients referred for further investigations and/or treatment (Note: Relative risk = 1.24, SE(RR) 1.22, 95% confidence interval for corrected Rr 0.38 ,- 1.84. c^2 for equal risk 0.96, N.S.).

Table 6 gives an analysis by area of residence, for localities where the total number of MVAs was 10 or more. When expressed as a proportion of the total

CITY	MALES	FEMALES	TOTAL	%	EXPECT	POPn
FLORIANA	10	4	14	4.86	5	2883
M'SCALA	10	5	10	3.95	7	3799
ST. JULIANS	16	8	24	3.42	12	7012
SANTA LUCIA	8	4	12	3.34	6	3594
MSIDA	12	10	22	3.21	12	6854
ST. PAULS BAY	11	5	18	3.00	10	6001
ZEBBUG	21	3	24	2.34	18	10248
B'KARA	31	18	50	2.33	37	21456
GZIRA	12	6	18	2.24	14	8032
SLIEMA	12	11	25	1.84	23	13567
ATTARD	11	3	14	1.79	13	7799
TARXIEN	8	5	13	1.75	13	7417
ZABBAR	16	7	23	1.69	23	13622
SAN GWANN	12	6	18	1.66	14	8032
HAMRUN	5	12	17	1.46	20	11653
ZURRIEQ	9	3	12	1.41	15	8518
MOSTA	11	9	21	1.36	26	15401
QORMI	17	7	24	1.34	31	17958
RABAT	9	7	16	1.27	22	12613
ZEJTUN	8	6	14	1.23	10	11411
PAOLA	9	2	11	1.15	16	9522

Table 6: Hospitalisation following MVAs: by place of residence

population residing in the particular locality, it is seen that certain areas of residence are associated with a greater than expected incidence of MVAs. For instance, Floriana, Marsascala, St. Juliaus, Santa Lucia, Msida and St. Pauls Bay (Bugibba) were associated with a MVA rate of more than 3 per 1000 population, whereas at the other end of the scale, Paola, Rabat, Qormi, Mosta, Zejtun and Hamrun had less than 1.5 per 1000 ($X2 = 64.5$; $P < .001$).

Discussion

This study represents an analysis of motor vehicle accidents presented at SLH during a period of one year. There were 616 MVAs treated during this year, representing 170 per 100,000 population. It is important to bear in mind that this represents only a proportion of MVAs and does not include the considerable number of minor accidents that do not require hospitalisation.

It is of interest to note that while June and July saw peak MVA rates, August was a relatively quiet month (with 43 MVA incidents, less than the number in February). Thus the distinction between the "busy" summer months and the rest of the year is not so clear-cut. The reasons for this low incidence in this relatively busy month are not clear, and could be due to random variation from year to year. Analysis over a number of years would be required before an analysable pattern can emerge.

The time distribution of MVAs calls for comment. There were three distinct peaks seen in the rate of accidents, namely (a) early hours of the morning, (b) around mid-day, and from 4-7 pm. While the daytime accidents might be correlated with the increase in motor traffic on the roads, the early morning peak is more likely to be associated with a younger age group. Possible factors accounting for this unnecessary loss of life include the following:

a. the marked tendency for Maltese youths to indulge in late night entertainment, particularly on a weekend, and, in the summer months, throughout the week.

b. the increased tendency to drink, which is now being offered in bulk (Disco clients are being offered the option of paying a once only fee of Lm10.00 and drink all they can).

c. increased availability of motor cars for younger age group drivers. Increased affluence has been a major factor in this phenomenon.

In fact, one of the most obvious findings in this study in comparison with the study carried out by Camilleri relating to traffic accidents in 1967 is the presence of the early morning peak of accidents which was non-existent at that time.

The day-to-day variation was significant, with lowest incidence during Monday-Wednesday and increasing for the rest of the week with a peak on Sunday (which includes the Saturday night / Sunday morning MVAs).

Whether there exists an accident-prone personality is difficult to prove. A recent study from Finland (Hilakivi et al, 1989) has shown that young male persons involved in traffic accidents are more likely to show impassivity, adventurousness, naivete, excessive trustfulness, and depression - factors relating to the control of emotions.

It is to be noted that wherever statistics over a long period of time have been analysed, the conclusion is confirmed that there is a tendency for accident death rates to increase over all ages, but the most striking increase is likely to affect youths aged 15-24 years of age (Millar & Last, 1988). These findings have been confirmed in Malta in a recent study (Galea, 1992). Any preventative aspects must take this into consideration, and special effort should be made to investigate the factors that could possibly be responsible for the escalation of this specific type of MVA.

The value of wearing seat belts in preventing serious injury has been emphasised again in a number of studies. For instance, Orsay et al (1988) conclude that "safety belt wearers had a 60.1% reduction in severity of injury, a 64.6% decrease in hospital admissions, and a 66.3% decline in hospital charges", emphasising the increase cost of medical care required for non seat-belt wearers.

The higher than expected incidence of MVAs associated with certain localities is of interest. Residents at Floriana, M'Scala St. Julians, Santa Lucia, Msida and St. Paul's Bay (Bugibba) are 2-3 times more likely to be involved in MVAs than residents of Paola, Zejtun, Rabat, Qormi, Zurrieq or Hamrun (Table 6). It is to be emphasised that these are residential addresses and not accident localities, and therefore do not

necessarily relate to local road or traffic conditions, although it is reasonable to argue that residents of high density traffic areas are more likely to be involved in car accidents than those residing in more rural areas. More study would be required to confirm these findings and to ferret out those factors associated with this increased accident rate.

While this study is restricted to only one year's MVAs, the pattern of accidents found is likely to be repeated. Efforts to reduce the number of MVAs must start with an analysis of the factors that lead to these accidents. While it was not the aim of this study to tease out these factors, serious efforts must be made by the relevant authorities to ensure that the multiple factors involved in car accidents as highlighted in the daily press (see eg. Cauchi M.N. 1995) are analysed and dealt with.

References

Brockdorff PV (1995) Facts, Figures and Myths on Malta, *The Sunday Times*, February 12, 6.

Cauchi MN (1995) Road Carnage: Need for Action *The Sunday Times*, March 5, 39.

Camilleri VT (1969) Traffic Injuries in Malta - some considerations and suggestions. *The St Luke's Hospital Gazette*. **iv**, 19-27.

Galea G (1992) A Young man's Death: An Analysis of Premature Mortality in Malta. University of Malta.

Hilakivi I, Veilahti J,Asplund P, Sinivuo J, Laitinen L, Koskenvduo K (1989) A sixteen factor test for predicting automobile driving accidents of young drivers *Accid Anal Prev*. **21**, 413-418.

Millar WJ and Last JM (1988) Motor vehicle traffic accident mortality in Canada, 1921-1984. *Am. J. Prev. Med*, 4(4) 220-230.

Orsay EM, Turnbull TL, Dunne M, Barrett JA, Langenberg P and Orsay CP (1988) Prospective study of the effect of safety belts on morbidity and health care costs in motor-vehicle accidents. *J.A.M.A.*, **260**, 3598-3603.

Current Research Profile

Dr. Robert Martin Borg Ph.D. (Dalhousie)

Department of Chemistry, University of Malta, Msida, Malta.

Robert M. Borg was born in Sliema in 1956. He attended secondary school at St. Aloysius College, B'Kara, followed by two years at the (old) Junior College, Valletta. In 1974, he joined the B.Sc. course at the University of Malta, and subsequently read for a Masters degree under the guidance of Professor Victor Ferrito. The title of his M.Sc. dissertation was "*The Analysis of the Oxazolone Derivatives of Amino Acids by Gas Chromatography-Mass Spectroscopy*". This work also led to his first academic paper.

His selection for a Canadian Commonwealth Scholarship in 1979 enabled him to read for a Ph.D. in chemistry at Dalhousie University, Halifax, Nova Scotia. The next four years, under the mentorship of Professor Donald R. Arnold, determined the direction his academic career would take, as a passion for organic photochemistry was kindled. Indeed, to this day, the title of his Ph.D. thesis: "*Radical Ions in Photochemistry*" best describes his current research interests. These involve the study of the light-induced generation of charged organic molecules, and their subsequent reactions.

Having graduated from Dalhousie in 1983, he spent the next five years as a post-doctoral fellow, the first two at the University of Maryland at College Park followed by three years at the University of Toronto both posts held with prominent photochemists. His research efforts in this period culminated in several publications, including the award of a U.S. Patent, which describes a novel process by which water-soluble polymers used extensively in the oil industry can be made fluorescent, a feature that makes their quantitative use easier, and thus might prevent pollution by avoiding waste.

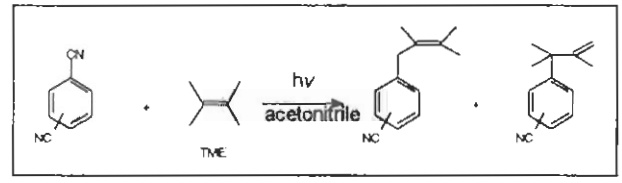
In 1988 Dr Borg returned from North America and joined the Department of Chemistry at the University of Malta as full-time lecturer, where he still is today (now as senior lecturer). He teaches organic chemistry, nuclear magnetic resonance (NMR) and mass spectroscopy, and photochemistry on the B.Sc. and M.Sc. courses.

In 1994, the Department of Chemistry was granted sufficient funds to purchase a 250MHz NMR spectrometer. This essential instrument generates some controversy due to the high running costs of its superconducting magnet (the only one in Malta), which requires cryogens (liquid helium and liquid nitrogen). However, by having access to this instrument (which is in use by research students practically 365 days a year), Dr Borg was able to publish the results of his research in a British journal within six months of its acquisition. Since then, another paper has been published, with one more manuscript on the way. These publications (described below) are also thanks to the hard work of the excellent graduate students currently reading for a Masters degree under Dr Borg's supervision.

Current Research Interests

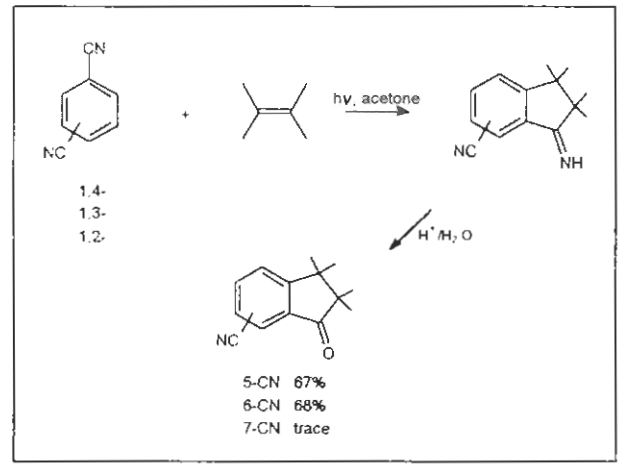
a) The photochemical reaction between cyano-aromatics and alkenes in acetone solution.

It has been shown previously by Arnold and co-workers (1984) that the irradiation of the dicyanobenzenes in the presence of 2,3-dimethyl-2-butene (tetramethylethene, TME) in polar acetonitrile solution yields substitution products as a result of photoinduced electron transfer, whilst in non-polar benzene solution, no reaction is observed to occur (Scheme 1).



Scheme 1

A new mode of photoreactivity of the dicyanobenzenes with TME, when acetone is used as a solvent, has recently been reported (Borg, 1994). Irradiation of 1,3- or 1,4-dicyanobenzene in the presence of TME, in acetone solution, affords cyano-substituted 2,3-dihydro-2,2,3,3-tetramethyl-1*H*-inden-1-ones in good yields. The *ortho* isomer of dicyanobenzene proved relatively unreactive.



Scheme 2

Evidence for the imines being intermediates, as well as

for the involvement of a charge-transfer interaction in the reaction mechanism was also presented.

These new photoreactions are significant, since they may provide a general synthetic methodology to indenone derivatives which are known to possess biological activity, or could serve as synthetic precursors to natural products such as steroids (Galatsis, 1994). Ongoing studies in the laboratory of Dr. Borg are therefore exploring the full scope of this reaction, so as to determine its synthetic utility. This work is currently being carried out by an M.Sc. student, Ms Mariella Berry, whose first research efforts at the undergraduate level were responsible for the implementation of this project.

References

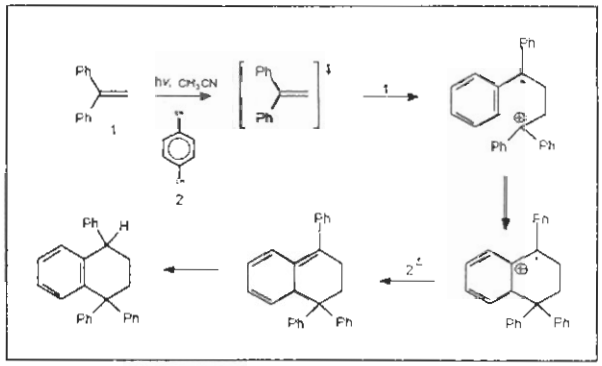
Borg RM, Berry M, and Mangion D (1994) The photochemical reaction between dicyanobenzenes and 2,3-dimethyl-2-butene in acetone solution: A potential route to substituted 2,3-dihydro-1H-inden-1-ones. *Tetrahedron Letters*, 35(45), 8485.

Borg RM, Arnold DR, and Cameron TS (1984) Radical ions in photochemistry. 15. The photosubstitution reaction between dicyanobenzenes and alkyl olefins. *Can. J. Chem.*, 62, 1785-1802.

Galatsis P, Manwell JJ, and Blackwell JM (1994) Indenone synthesis. Improved synthetic protocol and effect of substitution on the intramolecular Friedel-Crafts acylation. *Can. J. Chem.*, 72, 1656-1659.

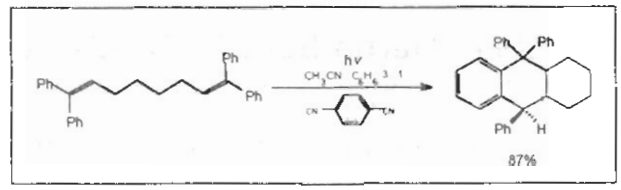
b) The photochemistry (electron transfer) of 1,1,ω,ω - tetraphenyl-1,(ω-1)-dienes.

The discovery, by Neunteufel and Arnold in 1973, that the irradiation of an acetonitrile solution of 1,1-diphenylethene and electron transfer photosensitizers such as 1,4-dicyanobenzene yields the tetrahydronaphthalene ring system via a formal [4e+2e] cycloaddition reaction (Scheme 3) led to a plethora of activity in this area.



Scheme 3

Despite the extensive nature of the above studies, the [4e+2e] cycloaddition reaction described above has never been applied to an intramolecular system and it was decided to investigate these reactions further. Indeed, in a preliminary report (Mangion, 1995) it has recently been demonstrated that the 1,4-dicyanobenzene-sensitized irradiation of 1,1,8,8-tetraphenyl-1,7-octadiene leads to the formation of the expected [4e+2e] cycloadduct in high chemical yield and stereoselectivity (Scheme 4).



Scheme 4

In Dr. Borg's laboratory studies have been extended to other tetraphenylated dienes of varying methylene chain length, with very interesting results. These will be presented at the American Chemical Society National meeting in Orlando, Florida this summer, with the full paper due to be submitted for publication shortly thereafter.

(These results are mainly the result of the hard work of an M.Sc. student, Dino Mangion, who is currently in the process of writing his dissertation. Dino will shortly be leaving for Canada, where he has been awarded a graduate fellowship to read for his Ph.D. - also at Dalhousie University under the guidance of Don Arnold!).

References

Arnold DR, and Neunteufel RA (1973) Radical ions in photochemistry. 1. The 1,1-diphenylethylene cation radical. *J. Am. Chem. Soc.*, 95, 4080.

Arnold DR Borg RM, and Albini A (1981) Photosensitized (electron transfer) [2e+4e] dimerization and cross-cycloaddition of phenylated olefins: trapping the intermediate. *J. Chem. Soc., Chem. Commun.*, 138.

Mangion D, Borg RM, and Errington W (1995) The photosensitized (electron transfer) [4e+2e] intramolecular cycloaddition of 1,1,8,8-tetraphenyl-1,7-octadiene; X-ray crystal structure of 4a,9a-trans-4a,10-trans-1,2,3,4,4a,9,9a,10-octahydro-9,9,10-triphenylanthracene *J. Chem. Soc., Chem. Commun.*, 1963.

c) Chemical Actinometry

Although this is not a research area as such, this article would not be complete without mentioning another M.Sc. project nearing completion. This involves the construction and subsequent use of an electronic actinometer. This is an indispensable instrument in photochemistry, and is used to quantify photochemical transformations (such as those described above) to the amount of light input into the system i.e. a measurement of the *quantum efficiency* of the photoreaction. This project is in the able hands of Nicky Gingell, who has almost completed the construction stage, in a highly professional manner. He will soon be embarking on some important measurements on the photochemical systems described in A) and B) , above.

Xjenza

Instructions for Authors

Xjenza will consider for publication manuscripts in the following categories:

- Original research articles
- Communications
- Reviews
- Commentaries

COMMUNICATIONS 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 :

Prof. Angela Xuereb,
Department of Pathology,
University of Malta Medical School,
Guardamangia..

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). 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 articles should give an appropriate introduction, a single methods section (with the methodology of all experiments reported), 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

Tables should bear Arabic 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, Greeb 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.

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

The Editorial Board would like to thank the Companies who have helped to make the publication of the journal possible:

Attard & Co. Ltd.,
67, South Street,
Valletta.
Tel: 238857, 220804, 237555

G.Borg Olivier Ltd.,
43, Old Bakery Street,
Valletta.
Tel: 237677

First International Ltd.,
Multitech Centre,
Regional Road,
Msida, MSD13.
Tel: 484458/9, 483819

H.H. Ltd.,
Wills Street,
Paola, PlA01.
Tel: 824920

Lowenbrau,
Triq l-Iljun,
Qormi, QRM11.
Tel: 485221

Oasis Ltd.,
Jasons Building,
Carmel Street,
Luqa, LQA04.
Tel: 803623/4

Simonds Farsons Cisk Ltd.,
The Brewery,
Mriehel.
Tel: 440331

Technoline Ltd.,
Edgar Bernard Street,
Gzira, GZR06.
Tel: 344345, 337975, 342366, 340537, 344749

Vivian Commercial Corporation Ltd.,
Sanitas Building,
Tower Road,
Msida, MSD01.
Tel: 344610

A wide range of advanced

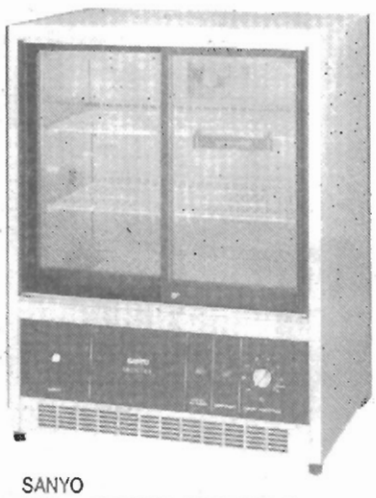
SANYO GALLENKAMP

technology in support of medicine and research.

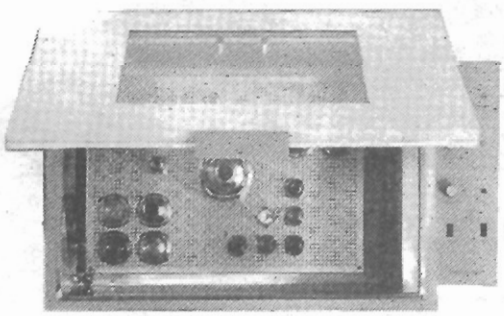
SANYO
-152°C FREEZER

SANYO

REFRIGERATORS & FREEZERS
Up to an unbelievable -152°C



SANYO
MPR 110 MEDICAL REFRIGERATOR

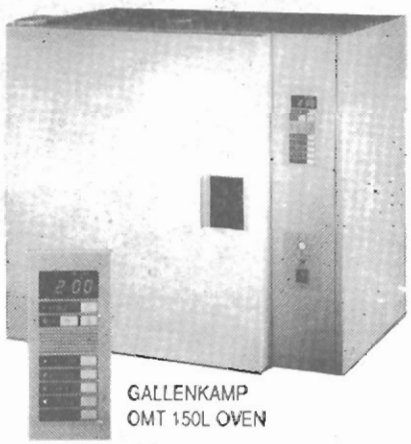


GALLENKAMP ORBITAL INCUBATOR

GALLENKAMP

INCUBATORS
From -10°C to +50°C

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



GALLENKAMP
OMT 150L OVEN

MSE MICROCENTAUR

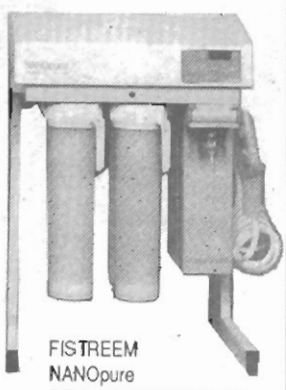
MSE

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

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.


Our heritage
· OUR LAGER ·

Mobile computing has never looked so good

Offering high performance multimedia computing at remarkably low cost, the Extensa range of notebooks feature Intel Pentium processors, massive, removable hard disk storage - from 810 MB up to 1.2 GB and an integrated CD-ROM. For the professional and business user, they are exceptional value.

A choice of brilliant, high contrast colour screens (dual scan and TFT), 16 bit sound, built-in speakers and microphone - plus support for full motion video - make these multi-talented notebooks ideal for product demonstrations and video presentations.

Yet all these features could'nt be more accessible. A dual boot facility, for example, enables you to take full advantage of Windows '95, while retaining the convenience of Windows 3.11 - both softwares are installed, ready for use.

An innovative touch mouse pad, serial infrared port for wireless communications, dual PCMCIA slots, fast (166550) serial port, Enhanced (ECC/ECP) parallel port, external keyboard and SVGA port are all standard features.

The Extensa series from Texas Instruments. Portable Pentium power has never been more affordable, or multimedia so accessible. Now there's nothing to stop you from doing extraordinary things.

For more information please contact *First International Limited* at *880 St. Joseph High Road, Hamrun. Tel. 235606* or *Multi-Tech Centre, Regional Road, Msida. Tel. 484458/9.*

TEXAS INSTRUMENTS

START DOING EXTRAORDINARY THINGS