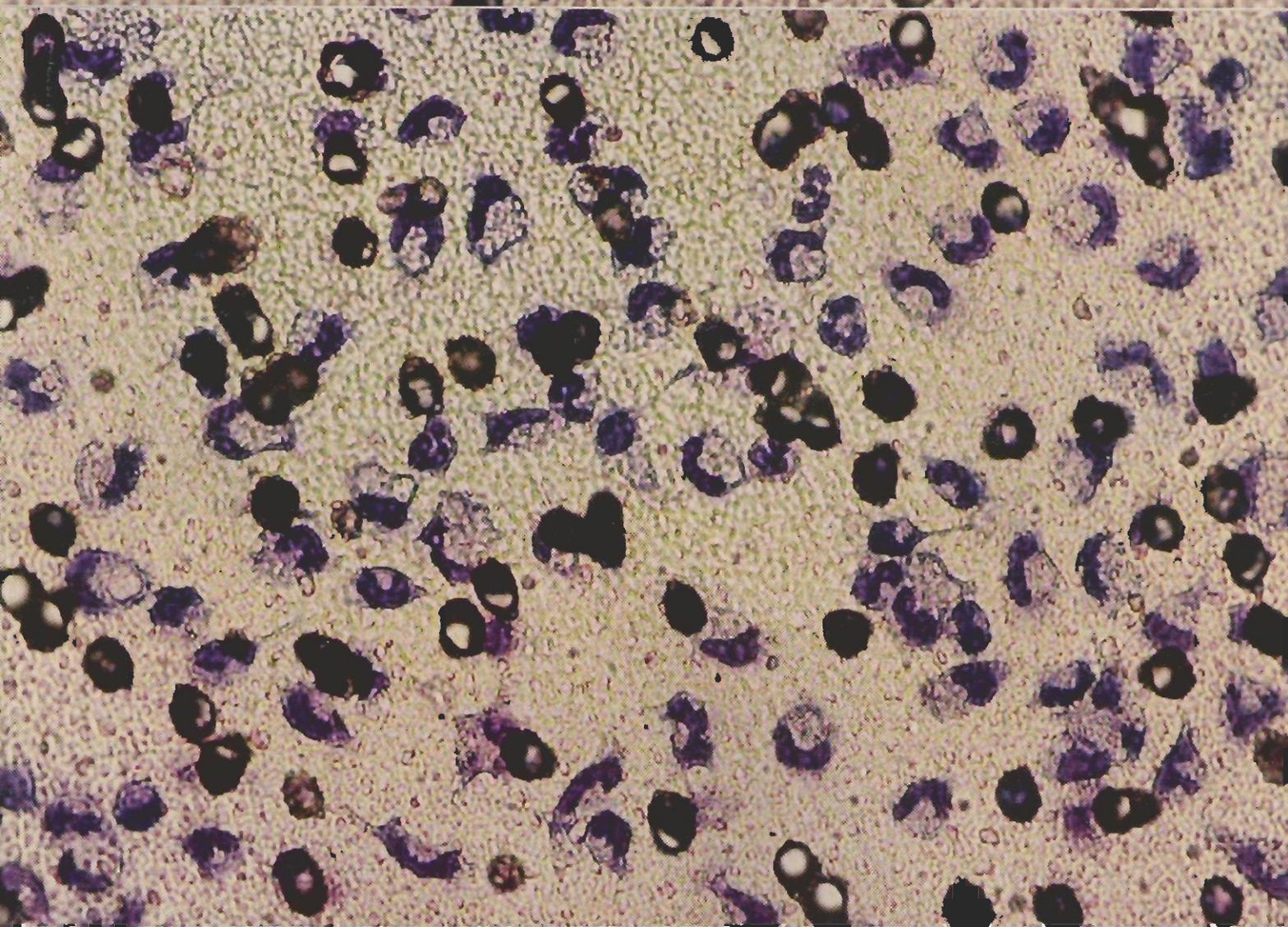


# Xjenza

JOURNAL OF THE MALTA CHAMBER OF SCIENTISTS

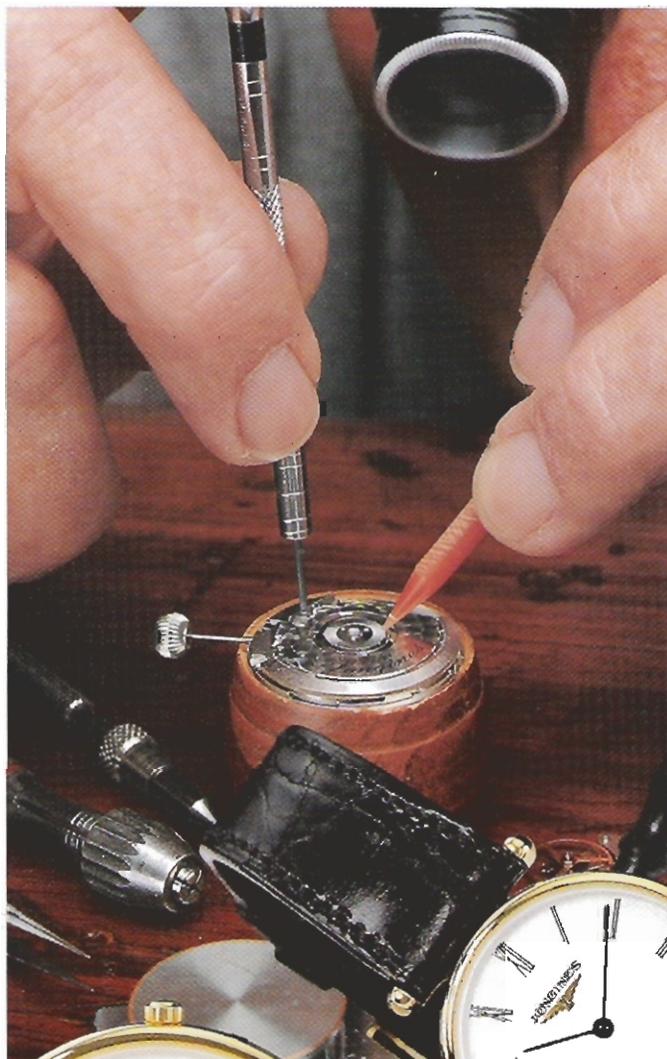
Volume 4 No 2

December 1999



# 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



*La Grande Classique by Longines™. Extra-slim models, water-resistant. Quartz movements. Sapphire glasses. Leather straps and metal bracelets.*

**'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.

---



---

# Contents

---



---

## Xjenza

Official Journal of the  
Malta Chamber of  
Scientists

Editor  
Angela Xuereb

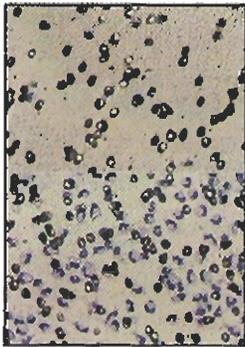
Associate Editors  
Martin Ebejer  
Richard Muscat

Editorial Address  
Xjenza,  
c/o University of Malta,  
Gwardamangia,  
Malta.

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

Typeset  
Anthony Sultana

Printers  
P.E.G. Ltd.,  
San Gwann.



### Cover Picture:

Immunostimulatory effect of bleomycin demonstrated by invasion of rat peritoneal macrophages through membrane sieves (8µm pore size). Top: Untreated (few macrophages). Bottom: Cells from treated rats (greater number of macrophages invading tumour tissue). Photograph supplied by Dr. M. Micallef

## editorial

The European Union's Fifth Framework for  
Research, Technological Development and  
Demonstration (FP5) (1998 - 2002) 2

## review articles

The Development of Effective Tumour Vaccines  
Mark J. Micallef 4

A Review of the Pleistocene Deposits in the  
Southwestern Coast of Malta  
Charles Savona-Ventura and Anton Mifsud 10

Neuroscience, the Bodymind and the Actor  
Albert Gatt and John Schranz 18

## article

History of the Practice of Chemistry  
- a Maltese perspective  
Charles Savona-Ventura and Michael Sammut 24

## abstracts

Biology Symposium 30

## instructions for authors

41



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

---



---

## Editorial

---



---

# The European Union's Fifth Framework for Research, Technological Development and Demonstration (FP5) (1998-2002).

### 1. What is the FP5 ?

The FP5 is the fifth in the series of 5-year E.U. Framework Programmes, launched in February 1999. It provides a coherent European framework for supporting research and technological development, as part of the E.U.'s research policy for the period 1998-2002. The FP5 confirms the E.U.'s increased commitment to research as the key to promoting an environment favourable to innovation, by encouraging technology transfer, ensuring the availability of venture capital, helping to protect intellectual property rights and developing human resources. The FP5 is aimed at promoting a number of the E.U.'s major policy thrusts into the year 2000, including strengthening the E.U.'s competitiveness in the global learning economy, creating new jobs, improving the quality of life, and meeting the challenge of enlargement.

The programme is concerned with societal and economic needs and how scientific research can contribute to these. The FP5 therefore reflects a problem-solving approach; instead of featuring a list of areas by scientific discipline, it has identified a limited number of problem-solving areas, targeting common priorities. The orientation of the FP5 is not on science *per se* but science at the service of the people, to improve their living conditions.

### 2. How does the FP5 work ?

The FP5 is made up of 4 thematic programmes and 3 horizontal programmes of research activity (Table 1). The European Commission published the first FP5 calls for proposals by programme in the Official Journal of the European Communities in March 1999. The calls for proposals were also published on the E.U.'s website for S&T information, CORDIS, which also provides all the background information and documents relating to the calls, including the Work Programme, Guide for Proposers and proposal forms for each of the seven programmes and the Evaluation Manual. An innovation of the FP5 is that the Commission is encouraging the submission of proposal forms electronically, using the Protocol which may be downloaded from the CORDIS website. Each of the work programmes provides an indicative road map of the calls to be issued over the next four years. It is important to note that a number of the calls are open calls and proposals may therefore be submitted at any time of the year. When a call does not appear on the stipulated date, an advance notice appears, indicating when the call in question will eventually appear.

### 3. Which organizations are eligible to participate in the FP5 ?

Legally established organisations and individuals in EU Member States and Associated States may participate with Community funding in all areas of the FP5.

### Thematic programmes :

1. Quality of Life and Management of Living Resources (16%)
  - 1.1 Food, nutrition and health
  - 1.2 Control of infectious diseases
  - 1.3 The "cell" factory
  - 1.4 Environment and health
  - 1.5 Sustainable agriculture, fisheries and forestry, and integrated development of rural areas
  - 1.6 The ageing population and disabilities
    - Generic RTD activities
    - Support for research infrastructures
2. User-friendly information society (24%)
  - 2.1 Systems and services for the citizen
  - 2.2 New methods of work and electronic commerce
  - 2.3 Multimedia content and tools
  - 2.4 Essential technologies and infrastructures
    - Generic RTD activities
    - Support for research infrastructures
3. Competitive and sustainable growth (18%)
  - 3.1 Innovative products, processes and organisation
  - 3.2 Sustainable mobility and intermodality
  - 3.3 Land transport and marine technologies
  - 3.4 New perspectives for aeronautics
    - Generic RTD activities
    - Support for research infrastructures
4. Energy, Environment and sustainable development (14%) and Euratom (7%)
  - 4.1 Sustainable management and quality of water
  - 4.2 Global change, climate and biodiversity
  - 4.3 Sustainable marine ecosystems
  - 4.4 The city of tomorrow and cultural heritage
  - 4.5 Clearer energy systems including renewables
  - 4.6 Economic and efficient energy for a competitive Europe
    - Generic RTD activities
    - Support for research infrastructures

### Horizontal programmes:

5. International role of Community research (INCO) (3%)
  - 5.1 States in the phase of pre-accession
  - 5.2 NIS and those CEECs not included in 5.1
  - 5.3 Mediterranean partner countries (INCO-MED)
  - 5.4 Developing countries (INCO-DC)
  - 5.5 Emerging economies and industrialised countries
  - 5.6 Training : INCO Bursaries
  - 5.7 Coordination
6. Promoting innovation and the participation of SMEs (2%)
  - 6.1 Promoting innovation.
  - 6.2 Encouraging SME participation
  - 6.3 Joint Innovation/SME activities
7. Improving human research potential & the socio-economic knowledge base(9%)
  - 7.1 Training and mobility of researchers
  - 7.2 Access to research infrastructures
  - 7.3 Promoting scientific and technological excellence
  - 7.4 Improving the socio-economic knowledge base
  - 7.5 Development of science and technology policies in Europe

Table 1. FP5's main programmes (Key Actions and budget breakdown).

---

Legally established organisations and individuals in other countries with or without cooperation agreements and international organisations may participate in all areas of the FP5 without Community funding on a self-funded project-by-project basis.

The second category of organisations and individuals may only benefit from Community funding:

- exceptionally, if it is essential for achieving the objectives of the project,
- under the relevant component of the INCO Programme (where applicable), and
- through subcontracting of work from one of the main project partners (Category a).

The following legally established entities may participate in the FP5:

- individuals
- industrial and commercial firms, with special incentives for small and medium-sized enterprises
- universities
- research organisations (private and government)
- international organisations

#### 4. How may these organisations participate ?

Participation in FP5 is on a partnership basis. Proposals submitted to the Commission should demonstrate a European dimension and as a general rule, should involve at least:

- two legal entities established in two different EU Member States, or
- a legal entity established in a EU Member State and an Associated State

#### 5. Malta's Status

Malta is currently negotiating the necessary Financing Agreement for its participation in FP5 as an Associated State. Individuals and legal entities established in Malta can participate with Associated Member status in calls for proposals with a closing deadline of September 2000 onwards.

Certain actions may vary from this rule - either by requiring more partners or by permitting a single one. It is therefore important to consult the relevant information package. The consortium jointly develops a research proposal and one of the EU partners in the consortium must agree to become the Project Coordinator. The latter, in consultation with the other partners, completes the necessary forms, including a detailed programme of work and the responsibilities and budgets for each of the partners. The Coordinator submits the duly completed forms directly to the Commission by the stipulated deadline. Projects are evaluated by the EU Commission in accordance with a set of specific selection criteria: eligibility, scientific excellence, economic development, Community added value and contribution to EU social objectives.

#### A. Shared-cost actions

- Research and technological development (R&D) projects - projects producing new knowledge intended to develop or improve products, processes or services and/or to meet the needs of Community policies. EU Funding: 50% of total eligible costs.
- Demonstration projects - projects designed to prove the viability of new technologies offering potential economic advantage but which cannot be commercialised directly. EU funding: 35% of total eligible costs.
- Combined R&D and demonstration projects - projects combining the above elements. EU funding: 35-50% of total eligible costs.
- Support for access to research infrastructures - (only implemented under the IHP Programme) actions enhancing access to research infrastructures for Community researchers. EU funding: 100% of the eligible costs.
- SME "Exploratory" awards - support for exploratory phase of a project of up to 12 months (e.g. feasibility studies, validation and partner search). EU funding: 75% of total eligible costs.
- SME "Cooperative" research projects - projects enabling at least 3 mutually independent SMEs from at least 2 Member States or one Member State and an Associated State to jointly commission research carried out by a third party. EU funding: 50% of total eligible costs.

#### B. Training Fellowships

- Marie Curie fellowships where individual researchers apply directly to the European Commission.
- Host fellowships where institutions apply to host a number of researchers.

In both cases, EU funding: up to 100% of additional eligible costs.

#### C. Research training networks and thematic networks

- Training networks for promoting training-through-research especially of researchers at pre-doctoral and at post-doctoral level (these are only implemented under the IHP Programme).
- Thematic networks for bringing together for example manufacturers, users, universities, research centres around a given S&T objective. These include coordination networks between Community funded projects.

For setting up and maintaining both types of networks, EU funding: up to 100% of eligible costs.

#### D. Concerted actions

- Actions coordinating RTD projects already in receipt of national funding, for example to exchange experiences, to reach critical mass, to disseminate results etc. EU funding: up to 100% of eligible costs.

#### E. Accompanying Measures

- Actions contributing to the implementation of a Specific Programme or the preparation of future activities of the programme. They may also prepare for or support other indirect RTD actions.

EU funding: up to 100% of total eligible costs.

#### Table 2. Types of proposals eligible for E.U. funding

More detailed information, including roadmaps of current and future calls can be obtained from

<http://www.cordis.lu>

A local web page is being set up at

<http://www.mcst.org.mt>

Dr. Joseph Micallef - FP5 Coordinator.

---

## Review Article

---

### The Development of Effective Tumour Vaccines

**Mark J. Micallef, Ph.D.**

*Department of Clinical Pharmacology & Therapeutics, Faculty of Medicine and Surgery on Campus, University of Malta, Msida MSD 06, Malta*

*Present address: Fujisaki Institute, Hayashibara Biochemical Labs., Fujisaki 675-1, Okayama 702-8006, Japan*

*Tel: 81 86 276-3141 FAX: 81 86 276-6885*

*E-mail: Fujisaki@hayashibara.com.jp*

**Summary:** *The search for effective tumour vaccines has been a very long one and has been fraught with many disappointments. The recent progress in our understanding of immune responses and tumour cell biology has produced new insights which many hope will lead to developing an effective cure for certain antigenic tumours. Tumour-associated antigens were first described many years ago. These antigens can be targeted by immunocompetent cells provided that an effective immune response, including antigen recognition, can be elicited. Therefore, tumour vaccines have been engineered such that there is better presentation of tumour-associated antigens and enhanced recognition. When coupled to effective adjuvants, anti-tumour responses were obtained in animal models and immune memory was elicited. These results and the ability to harvest professional antigen presenting cells from patients with different cancers have provided new hope for the development of anti-tumour strategies based on tumour vaccines. Clinical trials of tumour vaccines for prostate cancer and melanoma are reportedly in progress. The development of tumour vaccines from a hypothesis to near reality and the advantages of this form of treatment are discussed.*

#### Introduction

Effective tumour vaccines have been dubbed as the "Holy Grail" of immunologists and rightly so. This area of research has generated so much hope at times only to end up in failure and disappointment. Whereas vaccination for infectious agents is normally prophylactic, vaccination for tumours is normally therapeutic and is attempted only after diagnosis of the tumour. Thus, considering that tumours arising in different tissues have different biochemical and behavioural characteristics, tumour vaccines have to be tailor made for the different tumours in genetically distinct populations because different races may have genetically-linked discrete differences in immune functions. Tumour cells originate in normal tissues due to an accumulation of DNA damage which renders the cells independent of the growth control mechanisms which normally govern the life-span of healthy cells. DNA damage can be induced chemically, virally or through other agents such as exposure to UV radiation.

One of the many differences between normal and malignant cells is that tumour cells often express cell surface molecules or antigens which are usually expressed by developing tissues. The expression of these molecules is switched off in mature cells. This has led to researchers describing carcinogenesis as a process of de-differentiation. The surface molecules are expressed because the alterations in DNA function accompanying malignant cell development indiscriminately affect genes including those which code for cell surface antigens. It is also thought that de-differentiation reflects the lack of ability to undergo terminal maturation by malignant cells. The surface antigens predominantly expressed by tumour cells, have been labelled tumour-associated antigens but are not antigens specific to that tumour only. Normal tissues also express tumour-associated antigens (TAA) but do so in very small quantities or only transiently, for example during the embryonic stage. The

possibility that TAA may be targeted by the immune system leading to immunologically-mediated tumour regression is the ultimate goal of this area of research.

The idea of applying tumour vaccines as an anti-cancer therapy was originally met with scepticism because it was firmly believed that the immune system would never react against self antigens (including TAAs) and therefore, the application of tumour vaccines was contradictory to this belief. Cytotoxic T lymphocytes (CTL) which are antigen-specific killer cells and are potentially active against self antigens, were thought to be eliminated or down-regulated before these could harm normal tissues (Grooten et al, 1987). It was believed that CTL could not be generated against antigens which are shared between normal and malignant tissues (Dighiero and Rose, 1999). Animal models of the disease confirmed that CTL can be generated against self antigens provided there is a strong enough stimulus which overcomes tolerance to self antigens (Kurts et al, 1999). More recently, it was also shown that some cutaneous lesions in melanoma patients do contain CTL specific to melanoma antigens (Jager et al, 1999; Straten et al, 1999). Furthermore, some TAA may be viral products especially in tumours where a viral infection has been implicated as being the inducer of the malignancy or as being an accomplice in the process. These advances have added strength to the argument that tumour vaccines may have a deserved place in anti-cancer therapy, but as with other anti-cancer therapies, this will depend on the type of cancer and its characteristics.

#### Antigen recognition and the generation of anti-tumour immunity

Immune responses in humans can be generally classified into the humoral (or antibody) response and the cell-mediated cytotoxic response. In anti-cancer immune responses, the ultimate aim is to induce antigen-specific

CTL which will kill tumour cells but leave normal cells unharmed. This should also generate immune memory and theoretically prevent recurrence of the tumour. In viral infection, infected cells expressing viral antigen will die after producing viruses and the dead cell debris will be taken up by antigen-presenting cells (APC). Viral antigens will be processed into small 8-9mer amino acid sequences and presented to CD4+ T helper cells in association with major histocompatibility (MHC) class II molecules. The T helper cells will produce interleukin 2 (IL-2), the principal T cell growth factor, and expand the T cell population. Other cytokines such as interferon gamma (IFN- $\gamma$ ) are produced by the T helper cells in what is called a T helper type 1 (Th 1) response and are thought to be required for the functional development of the cytolytic T cells. APC will also interact with CD8+ T killer cells through MHC class I molecules and educate these cells to kill target cells expressing the viral antigen. Antigen-specific CTL generated in this fashion will recognise infected cells and eliminate them before they shed viruses. This mechanism is also true for cell-mediated anti-tumour effects. APCs also take up tumour cell debris arising from processes such as apoptosis of tumour cells, and present them to CD4+ T helper cells. These helper cells will develop into Th 1 cells and with the help of further antigen presentation, induce a tumour-specific cell-mediated immune response.

#### Early attempts at vaccination of animal models

Clinicians noted that some tumours regressed spontaneously and even observed that some subcutaneous melanoma lesions regress while others at different sites in the same patient would progress. This indicated that the body might be mounting a protective anti-cancer response to the tumour and inducing its regression. These and similar observations led researchers such as Hiroshi Kobayashi in Japan to try and chemically, virally or radiologically modify tumour cells and produce a crude tumour vaccine for tumour-bearing animal models, a process Prof. Kobayashi labelled as xenogenisation of tumour cells. Prof. Kobayashi's group infected rat fibrosarcoma (KMT-17) cells with Friend leukemia virus and injected the cells into syngeneic (same strain) rats bearing solid tumours of the parental cells. The majority of the established tumours regressed and a significant number of rats were cured (Kobayashi et al, 1969). Eventually, through immunisation procedures, a tumour-associated antigen, CE7, was identified. This antigen was a self TAA which was not recognised by the rat immune system when expressed alone but was recognised when co-expressed with the Friend virus envelope protein on infected tumour cells. What was significant was that when the rats that rejected the tumour were again subcutaneously injected with the same parental KMT-17 tumour cells, the tumour failed to "take". This showed that the rats had developed specific immune memory and that the immune system could be recruited against self antigens if the immune stimulation (in this case the viral product) is strong enough to act as an adjuvant. Eventually, it was discovered that CE7 is shed from the KMT-17 tumour cell surface and may act as a decoy to immune responses (Chiba et al. 1989). Bleomycin and its analogues of anti-tumour antibiotics were found to inhibit CE7 shedding from the cell surface making the cells more antigenic in

vivo (Micallef et al, 1992a). Besides this, Bleomycin is also immunostimulatory (Micallef et al, 1991; Micallef et al, 1992b) and tumour-bearing rats treated with Bleomycin reject tumour and develop immune memory (Micallef, 1993). These results indicated that chemical modulation of tumour cells in vivo by Bleomycin could also enhance tumour antigenicity and act as an adjuvant as well. Eventually, Shibata and co-workers showed that even irradiation from a  $^{60}\text{Co}$  source could inhibit CE7 antigen shedding and after fixation, this preparation proved to be an effective tumour vaccine in the KMT-17 rat fibrosarcoma model (Shibata et al, 1996).

The original observations of Prof. Kobayashi are very relevant to tumour immunology when one considers that there are strong implications for an association between certain viruses and tumour development. Certain papillomaviruses are associated with carcinoma of the cervix (Jarrett et al, 1990), human T-cell leukemia virus type 1 (HTLV-1) causes adult T-cell leukaemia (Poesz et al, 1980; Yoshida et al, 1982), while Epstein Bar virus is associated with Burkitt's lymphoma (Hoffbrand and Pettit, 1984). Thus, modifications of tumour cell vaccines based on viral products may have potent anti-tumour effects in some cancers with a strong implication of viral involvement in the multistep process of carcinogenesis (Jarrett et al, 1990).

#### The function of the adjuvant

Some tumours may produce factors which inhibit the proper functioning of the immune system and result in local or systemic immunosuppression. Transforming growth factor beta (TGF- $\beta$ ) (de Visser and Kast, 1999), IL-10 (Howard and O'Garra, 1992; Chen et al, 1994) and prostaglandin E2 (Botti et al, 1998) are well known immunosuppressing factors produced by tumours or bystander cells influenced by the tumour cells. Furthermore, anti-cancer chemotherapy is non-specific and affects all replicating cells, including cells of the bone marrow which give rise to immunocompetent cells. Therefore, cancer chemotherapy is frequently accompanied by immunosuppression. Under such conditions, the immune system is too weak to act against a TAA in the absence of an immunostimulant. Even if the immune system is intact, a stimulus is still required to overrule the inhibitory signal which normally prevents the immune system from acting against self antigens, which include the TAAs. Thus it becomes obvious that an adjuvant is required to help the weakened immune system react against the antigen. The adjuvant should be potent enough to both overcome anergy of the T cells induced by tolerance to the tumour antigen, and induce a Th 1 immune response. Some researchers have administered Th 1 cytokines such as interleukin 2 combined with tumour-infiltrating lymphocytes (TIL) directly in an attempt to enhance cell-mediated immune responses (Rosenberg et al, 1988). The problems with this approach are the dose-limiting side effects induced and that the cytotoxicity appears to be non-specific in vitro (Schomburg et al, 1992).

#### Early clinical applications

Early clinical trials employed modified autologous (self) tumour cells such as Newcastle Disease Virus (NDV)-infected colon carcinoma cells but these did not produce

antigen-specific effects (Patel et al, 1992). In contrast, when patients with renal cell carcinoma were vaccinated with autologous tumour cells, the majority of patients who developed delayed-type hypersensitivity (DTH) towards the antigen also had prolonged survivals when compared to the patients who did not develop DTH (McCune et al, 1990).

Dr. Donald Morton at the John Wayne Cancer Institute in California was convinced that tumour vaccines could be applied against melanoma, an aggressive form of skin cancer. After screening a large number of melanoma cell lines, Dr. Morton selected 3 cell lines which were the most antigenic. This meant that these three cell lines between them expressed a spectrum of melanoma TAAs and could be used for vaccination. *Bacillus Calmette Guerin* (BCG), an attenuated form of the tubercule bacillus, was chosen as an adjuvant and Dr. Morton proceeded to immunise patients subcutaneously with a cocktail of the three antigenic cell lines and BCG (Morton et al, 1992). It must be stated that it is now evident that BCG can stimulate the production of cytokines which normally induce Th 1 responses and cell-mediated immunity, such as IL-12 (Wang et al, 1999) and IL-18 (Okamura et al, 1995). In fact, both IL-12 (Brunda et al, 1993) and IL-18 (Micallef and Kurimoto, 1999) have been used successfully as anti-cancer therapy in animal models. Remarkable responses and regression of metastases were observed in a number of patients treated according to Dr. Morton's protocol, however, this was not always effective and its efficacy appeared to be related to the characteristics of the different tumours, the tumour burden and the degree of metastasis present. These inconsistent results have hindered the general application of this form of treatment for melanoma.

Conjugates of TAA with BCG were used to successfully immunise rats against a syngeneic hepatoma but both the BCG and the TAA alone failed to induce any tumour resistance (Crum et al, 1977). Thus, simultaneous stimulation with adjuvant and TAA induces direct activation of, and recognition of antigen by the rat immune system.

### **Tumour-derived antigenic peptides**

The vaccination of cancer patients with live tumour cells presents us with several problems of the ethical type. Therefore, other researchers prepared tumour cell extracts using different methods. Low molecular weight peptides were synthesized, and animals were stimulated with the peptides alone or combined with adjuvant (De Matos et al, 1998). Immune cells were also stimulated with the peptide extracts to determine whether the immune cells could be induced to kill tumour cells *in vitro* (Ikemoto et al, 1999). In both cases, results showed that even such tumour cell extracts could induce killer cell activity which was specific to the cells from which the extracts were derived. Nevertheless, there is some evidence to show that live modified tumour cells are a better tumour vaccine than dead cells or their extracts *in vivo*. To generate a specific anti-tumour response, professional APCs are required. Professional APCs express the MHC class II molecules required to present processed antigens to T cells. In addition, professional

APCs also express other accessory molecules such as CD80 and CD86 which are required as a co-stimulus to induce a response by the T cells. The latter molecules bind their ligands CD28 and CTLA-4 which potentiate and inhibit the immune stimulation, respectively. The most potent professional APC is the dendritic cell (DC) which can be generated from peripheral blood macrophages (M $\phi$ ) or bone marrow cells *in vitro*, based on the cytokine stimulus. Attempts have been made to generate DCs and stimulate them with crude tumour cell peptide extracts (Zitvogel et al, 1996) or TAA peptides (Mayordomo et al, 1995) before re-introduction into tumour-bearing animals. The stimulated DCs were found to induce anti-tumour responses. In other instances, allogeneic tumour cell lysates induced specific responses in melanoma patients (Mitchell et al, 1993).

Certain lesions in patients with melanoma regress without any apparent cause and some were found to harbour anti-tumour CTLs. Using these CTLs, researchers have identified the structure of the variable region in T cell receptors which recognise the melanoma-associated antigen presented on the HLA molecules. This led to the deciphering of the amino acid sequences of antigenic melanoma peptides presented to the CTL on HLA class II molecules. Thus, peptides such as the MAGEs (van der Bruggen et al, 1991), the BAGEs (Boel et al, 1995), Melanoma/MART-1 (Coulie et al, 1994; et al, 1994a), gp100 (Cox et al, 1994; Kawakami et al, 1994b) and others were identified. Different peptides are presented on different HLA class II sub-set molecules as targets for CTLs. What this implies is that there can be no single universal vaccine for a particular type of tumour but rather different vaccines for the same tumour depending upon the race involved (eg. oriental versus occidental). This is because different human races express different HLA molecules which would dictate the tumour peptide which can be best presented by the particular HLA molecules expressed by that race.

Heat shock proteins (HSPs) are a group of proteins whose physiological function is to chaperone antigenic peptides in cells. When HSP-peptide complexes were derived from murine tumours and used to vaccinate against the same tumours, a significant anti-tumour effect was noted in models of colon carcinoma and UV-induced spindle cell carcinoma (Tamura et al, 1997). HSPs on their own failed to induce an anti-tumour response in the animals.

### **Gene therapy**

The potential genetic modification of tumour cells has provided new opportunities to develop tumour vaccines. The successful enhancement of rat tumour cell antigenicity after viral infection described above led Prof. Kobayashi to attempt to duplicate the results he obtained using the intact virus by transfection of the target cells with the viral env gene (Sugiura et al, 1988). This result was among the first to show that direct genetic manipulation of tumour cells could modify their antigenicity and produce a tumour vaccine. Soon after Itaya and co-workers showed that mouse tumour cells could be made more antigenic by expressing allogeneic MHC class II molecules (Itaya et al, 1989). More

recently, Komata et al. transfected human glioma cells with the cDNA for the accessory molecule CD80 and these could stimulate allogeneic CD8+ T cells in the presence of IL-12, the principle cytokine responsible for the development of Th 1 responses and cell-mediated immunity (Komata et al, 1997). Studies such as these confirm the importance of accessory molecules and the induction of a Th 1 response in the generation of cell-mediated tumour immunity. Dendritic cells have also been genetically modified to express murine tumour-associated viral or self antigens and used successfully in treating tumour-bearing mice (Tuting et al, 1997). DCs were transfected with cDNA coding for human papilloma virus (HPV) 16 antigens which are associated with tumour-associated viral antigen E7 or with that coding for the tumour suppressor gene p53. These modified cells were used to treat mice bearing a HPV 16-transformed murine sarcoma or mutant p53-expressing sarcomas. Mice receiving the treatment were significantly protected from tumour growth and a significant number of the treated mice remained tumour-free throughout the experiments. In vitro experiments showed that treated mouse splenocytes that rejected the tumour could kill the respective parental cancer cells.

Transduction of human DC with a TAA-encoding gene would theoretically enhance antigen presentation by the cell. Reeves and co-workers showed that DC transduced with the gene for the melanoma-associated antigen MART-1, could activate MART-1 specific human T cells in vitro (Reeves et al, 1996).

### The role of antibodies

Although most of this discussion has centred around specific T lymphocyte responses to tumour cell antigens, which are thought to be the predominant responses against cancer cells, there is reason to believe that antibodies may also play a role in anti-tumour responses. Monoclonal antibodies against melanomas have been shown to have anti-tumour activity (Chapman et al, 1990) and there is strong correlation between specific antibody production and overall survival after vaccination with a melanoma cell vaccine (Jones et al, 1996). Melanoma patients immunised with a melanoma cell vaccine could also mount an antibody response against melanoma antigens (Hoon et al, 1995). Antibodies to melanoma antigens of the IgM class can also directly kill melanoma cells in the presence of complement.

### Conclusions

The search for effective tumour vaccines has been a long one, however, the results to date, coupled with our increasing understanding of immune responses have added strength to the quest for these potentially life saving therapeutic regimens. Immunostimulatory cytokines such as IL-12 and IL-18 may finally fill in the void that has been created by the lack of suitable adjuvants, required to stimulate the immune system. Clinical trials are now underway combining genetically modified tumour cells with potent adjuvants which, one hopes, will form the basis for the successful treatment of various types of antigenic cancers.

Because of the broad nature of this subject, new results

are being announced continuously and although every effort has been made to be as comprehensive as possible in this review, undoubtedly there are areas of interest which have not been discussed. Nevertheless, I sincerely hope that this article will give some information on the state of affairs in this field of research.

### References

- Boel P, Wildmann C, Sensi ML et al (1995) BAGE: a new gene encoding an antigen recognized on human melanomas by cytolytic T lymphocytes. *Immunity*, **2**, 167-175.
- Botti C, Seregini E, Ferrari L, Martinetti A and Bombardieri E (1998) Immunosuppressive factors: role in cancer development and progression. *International Journal of Biological Markers*, **13**, 51-69.
- Brunda MJ, Luistro L, Warriar RR, Wright RB, Hubbard BR, Murphy M, Wolf SF and Gately MK (1993) Antitumor and antimetastatic activity of interleukin-12 against murine tumors. *Journal of Experimental Medicine*, **178**, 1223-1230.
- Chapman PB, Lonberg M and Houghton AN (1990) Light chain variants of an IgG3 anti-GD3 monoclonal antibody and the relationship between avidity, effector functions, tumor targeting, and antitumor activity. *Cancer Research*, **50**, 1503-1509.
- Chen Q, Daniel V, Maher DW and Hersey P (1994) Production of IL-10 by melanoma cells: Examination of its role in immunosuppression mediated by melanoma. *International Journal of Cancer*, **56**, 755-760.
- Chiba I, Jin R, Hamada J, Hosokawa M, Takeichi N and Kobayashi H (1989) Growth-associated shedding of a tumor antigen (CE7) detected by a monoclonal antibody. *Cancer Research*, **49**, 395-397.
- Coulie PG, Brichard V, Van Pel A et al (1994) A new gene encoding for a differentiation antigen recognized by autologous cytolytic T lymphocytes on HLA-A2 melanomas. *Journal of Experimental Medicine*, **180**, 35-42.
- Cox AL, Skipper J, Chen Y et al (1994) Identification of a peptide recognized by five melanoma-specific human cytotoxic T cell lines. *Science*, **264**, 716-719.
- Crum ED and McGregor DD (1977) Induction of tumor resistance with BCG-associated tumor antigen. *International Journal of Cancer*, **20**, 805-812.
- De Matos P, Abdel-Wahab Z, Vervaert C, Hester D and Seigler H (1998) Pulsing of dendritic cells with cell lysates from either B16 melanoma or MCA-106 fibrosarcoma yields equally effective vaccines against B16 tumors in mice. *Journal of Surgical Oncology*, **68**, 79-91.
- De Visser KE and Kast WM (1999) Effects of TGF-beta on the immune system: implications for cancer immunotherapy. *Leukemia*, **13**, 1188-1199.
- Dighiero G and Rose NR (1999) Critical self-epitopes are key to the understanding of self-tolerance and autoimmunity. *Immunol Today*, **20**(9), 423-428.
- Hoffbrand AV and Pettit JE (1984) Malignant lymphomas. In: Essential Haematology (Eds AV Hoffbrand and JE Pettit) pp158-172. *Blackwell Scientific Publications*.

## United Kingdom.

- Hoon DSB, Yuzuki D, Hayashida M and Morton DL (1995) Melanoma patients immunized with melanoma cell vaccine induce antibody responses to recombinant MAGE-1 antigen. *Journal of Immunology*, **154**, 730-737.
- Howard M and O'Garra A (1992) Biological properties of interleukin 10. *Immunology Today*, **13**, 198-200.
- Ikemoto S, Sugimura K, Wada S, Yasumoto R, Yamamoto K and Kishimoto T (1999) Augmentation of antitumor activity of 5'-deoxy-5-fluorouridine by thymosin fraction 5 in mouse bladder cancer cells in vitro and in vivo. *Cancer Letters*, **145**, 121-126.
- Itaya T, Yamagiwa S, Okada F et al (1989) Xenogenization of a mouse lung carcinoma (3LL) by transfection with an allogeneic class I major histocompatibility complex gene (H-2Ld). *Cancer Research*, **47**, 3136-3140.
- Jager E, Jager D, Knuth A (1999) CTL-defined cancer vaccines: perspectives for active immunotherapeutic interventions in minimal residual disease. *Cancer Metastasis Rev*, **18**(1), 143-150.
- Jarrett WF, O'Neil BW, Gaukroger JM, Laird HM, Smith KT and Campo MS (1990) Studies on vaccination against papillomaviruses: a comparison of purified virus, tumour extract and transformed cells in prophylactic vaccination. *Veterinary Records*, **126**, 449 - 452.
- Jones RC, Kelley M, Gupta RK et al. (1996) Immune response to polyvalent melanoma cell vaccine in AJCC stage ??? melanoma: an immunologic survival model. *Annals of Surgical Oncology*, **3**, 437-445.
- Kawakami Y, Eliyahu S, Delgado CH et al. (1994a) Cloning of the gene coding for a shared melanoma antigen recognized by autologous T cells infiltrating into tumor. *Proceedings of the National Academy of Sciences USA*, **91**, 3515-3519.
- Kawakami Y, Eliyahu S, Delgado CH et al. (1994b) Identification of a human melanoma antigen recognized by tumor-infiltrating lymphocytes associated with in vivo tumor rejection. *Proceedings of the National Academy of Sciences USA*, **91**, 6458-6462.
- Kobayashi H, Sendo F, Shirai T (1969) Modification in growth of transplantable rat tumors exposed to Friend virus. *Journal of the National Cancer Institute*, **42**, 413-419.
- Komata T, Tanaka R, Yamamoto K, Oda T, Ono K, Yoshida S and Takahashi M (1997) B7-1 (CD80)-Transfected human glioma cells and interleukin-12 directly stimulate all ogenic CD8+ T cells. *Journal of Immunotherapy*, **20**, 256-264.
- Kurts C, Sutherland RM, Davey G, Li M, Lew AM, Blanas E, Carbone FR, Miller JF, Heath WR (1999) CD8 T cell ignorance or tolerance to islet antigens depends on antigen dose. *Proc Natl Acad Sci USA*, **96**(22), 12703-12707.
- Mayordomo JI, Zorina T, Storkus WJ, et al. (1995) Bone marrow-derived dendritic cells pulsed with synthetic tumor peptides elicit protective and therapeutic antitumor immunity. *Nature Medicine*, **1**, 1297-1302.
- McCune CC, O'Donnell RW, Marquis DM and Sahasrabudhe PM (1990) Renal-cell carcinoma treated by vaccines for active specific immunotherapy: a correlation of survival with skin testing by autologous tumor. *Cancer Immunology Immunotherapy*, **32**, 62-66.
- Micallef M (1993) Immunoregulatory cytokine production by tumor-bearing rat spleen cells and its modulation by bleomycin. *Anti-Cancer Drugs*, **4**, 213-222.
- Micallef M, Hosokawa M, Shibata T, Nakane A, Yang Z-B, Minagawa T and Kobayashi H (1991) Immunoregulatory cytokine release in rat spleen cell cultures after treatment with bleomycin and its analogues in vivo. *Cancer Immunology Immunotherapy*, **33**, 33-38.
- Micallef M, Hosokawa M, Togashi Y and Kobayashi H (1992) Rat macrophage activation after treatment with the bleomycin group of antitumor antibiotics in vivo. *Cancer Immunology Immunotherapy*, **35**, 106-112.
- Micallef MJ and Kurimoto M (1993) Interleukin 18: What role in anti-cancer strategies. Recent Developments in Cancer Research (Review, in press).
- Micallef M, Shibata T, Hosokawa M and Kobayashi H (1992) Bleomycin but not its derivatives inhibits the in vivo shedding of a rat tumor-associated antigen. *Anti-Cancer Drugs*, **3**, 659-664.
- Mitchell MS, Harel W, Kan-Mitchell J, et al (1993) Active specific immunotherapy of melanoma with allogeneic cell lysates. *Annals of the New York Academy of Sciences*, **690**, 153-166.
- Morton DL, Foshag LJ and Hoon DSB (1992) Prolongation of survival in metastatic melanoma after active specific immunotherapy with a new polyvalent melanoma vaccine. *Annals of Surgery*, **216**, 463-482.
- Okamura H, Tsutsui H, Komatsu T et al (1995) Cloning of a new cytokine that induces IFN- $\gamma$  production by T cells. *Nature*, **378**, 88-91.
- Patel BT, Lutz MB, Schlag P and Schirmacher V (1992) An analysis of autologous T-cell anti-tumor responses in colon carcinoma patients following active specific immunization (ASI). *International Journal of Cancer*, **51**, 878-885.
- Poiesz BJ, Ruscetti FW, Gazdar AF, Bunn PA, Minna JD and Gallo RC (1980) Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. *Proceedings of the National Academy of Sciences USA*, **77**, 7415-
- Reeves ME, Royal RE, Lam JS, Rosenberg SA and Hwu P (1996) Retroviral transduction of human dendritic cells with a tumor-associated antigen gene. *Cancer Research*, **56**, 5672-5677.
- Rosenberg SA, Packard B, Aebersold P, et al (1988) Use of tumor-infiltrating lymphocytes and interleukin-2 in the immunotherapy of patients with metastatic melanoma. *New England Journal of Medicine*, **319**, 1676-1680.
- Schomburg A, Menzel T, Korfer A, et al (1992) In vivo and ex vivo antitumor activity in patients receiving low-dose subcutaneous recombinant IL-2. *Natural Immunity*, **11**, 133-143.
- Shibata T, Hosokawa M, Micallef M, Mizukoshi T, Jin R,

- Chiba I, Takeichi N, Arisue M and Kobayashi H (1996) Modulation of the rat tumor-associated shedding antigen (CE7) and augmentation of immunogenicity by irradiation. *Anticancer Research*, **16**, 99-104.
- Thor Straten P, Becker JC, Guldborg P, Zeuthen J (1999) In situ T cells in melanoma. *Cancer Immunol Immunother*, **48**(7), 386-395.
- Sugiura C, Itaya T, Kondoh N, et al (1988) Xenogenization of tumor cells by transfection with plasmid containing env gene of Friend leukemia virus. *Japanese Journal of Cancer Research*, **79**, 1259-1263.
- Tamura Y, Peng P, Liu K, Daou M and Srivastava PK (1997) Immunotherapy of tumors with autologous tumor-derived heat shock protein preparations. *Science*, **278**, 117-120.
- Tuting T, DeLeo AB, Lotze MT and Storkus WJ (1997) Genetically modified bone marrow-derived dendritic cells expressing tumor-associated viral or "self" antigens induce antitumor immunity in vivo. *European Journal of Immunology*, **27**, 2702-2707.
- Wang J, Wakeham J, Harkness R and Xing Z (1999) Macrophages are a significant source of type 1 cytokines during mycobacterial infections. *Journal of Clinical Investigation*, **103**, 1023-1029.
- Yoshida M, Miyoshi I and Hinuma Y (1982) Isolation and characterization of retrovirus from cell lines of human adult T-cell leukemia and its implication in the disease. *Proceedings of the National Academy of Sciences USA*, **79**, 2031-
- van der Bruggen P, Traversari C, Chomez P et al (1991) A gene encoding an antigen recognized by cytolytic T lymphocytes on human melanoma. *Science*, **254**, 1643-
- Zitvogel L, Mayordomo JI, Tjandrawan T, DeLeo AB, Clarke MR, Lotze MT and Storkus WJ (1996) Therapy of murine tumors with tumor peptide-pulsed dendritic cells: dependence on T cells, B7 costimulation, and T helper cell. *Journal of Experimental Medicine*, **183**, 87-97.

---



---

## Review Article

---



---

### A review of the Pleistocene Deposits in the SouthWestern Coast of Malta

C. Savona-Ventura<sup>1</sup>, and A. Mifsud<sup>2</sup>

<sup>1</sup>*Institute of Health Care, University of Malta, Gwardamangia, Malta.*

<sup>2</sup>*"Mistpatch" De Paule Avenue, Balzan, Malta.*

**Summary:** *The various cave-fissure and coastal deposits described along the Southwestern coast of Malta have been shown to have had a fossil repertoire which corresponds with the 2<sup>nd</sup> faunal association described from Sicily dated to circa 455 +/- 90 ka and the 3<sup>rd</sup> Sicilian faunal association dated to circa 200 +/- 40 ka. The latter probably corresponds to the Gliridae/Hippopotamus Assemblage Biozone of Ghar Dalam Cave in Malta.*

**Keywords:** Pleistocene, paleofaunal assemblage

#### Introduction

The Pleistocene chronological sequence in Malta has generally been restricted to studies of the Ghar Dalam cave-floor deposits. These deposits have been described as consisting of four definite assemblage biozones which have been dated by absolute and relative dating to the Upper Pleistocene (Savona-Ventura & Mifsud, 1998). There has been very little attempt to assign a chronological sequence to deposits that may have different earlier faunal associations. This paper reviews the Pleistocene deposits of the Maghlaq and Benghisa regions to compare the sequence in this region with that at nearby Ghar Dalam Cave and with the Sicilian faunal assemblages.

#### Material and methods

A literature search was conducted in the various melitensia-holding libraries (the National Library, the University and Archaeology Museum Libraries) in Malta to identify the publications relating to the original descriptions of excavations carried out in the region. Several formal excavations and site descriptions were identified, these being published by several workers, notably T.A.B. Spratt and A.L. Adams. The Maghlaq deposits were reviewed by Galea Bonavia (1999). Still existent Quaternary sites in the locality were visited and studied with special attention being made as to the presence or absence of fossil remains.

#### Geology of the region

The Southwestern coast of Malta is characterized by a series of high cliffs caused by a major fault, named the Maghlaq Fault, which extends parallel to the coast in a NW-SE direction trending averagely N 120°. The stratigraphical evidence of the region suggests that the Maghlaq Fault is a relatively young tectonic feature (Reuther, 1984), possibly Middle Pleistocene. The effects of this fault can be noted by the preserved remnants of Upper Coralline Limestone deposits in the region of Halk it-Tafal close to Lapsi Cave and below Mnaidra. The fault line can also be clearly noted by the presence of escarpments in the region exhibiting very good examples of slickensides. During the Pleistocene, the region was characterized by some phases of increased water flow which helped cut out a number of valleys in the cliffs and which contributed to the

deposition of the alluvial and cave-fissure deposits of the region. The arid climate of the glacial periods resulted in the deposition of aeolian soils. After these deposits were laid down, undercutting of the fault remnant resulted in a landslide into the sea with a breaking up of the Pleistocene deposits and exposure of the fault line, particularly evident in the region below Mnaidra. The area has also been extensively modified by human intervention through quarrying for Upper Coralline Limestone.

#### Pleistocene deposits

The Maghlaq region was characterized by three cave-fissure deposits which were intimately associated. None of these deposits are extant at present (though Pleistocene conglomerate hard rock fragments containing bone and land shell fossils can still be encountered among the rocks of the region). The location and interrelationship of these cave-fissure deposits can only be extrapolated from the descriptions given by the original excavators. T.A.B. Spratt's (1867) description of Mnaidra Gap appears to be unreliable and his estimates of distances an exaggeration. His sea-view sketch of the area, depicting these deposits as being widely separated, is quite inaccurate. On the other hand the sketch prepared by Captain Goff and published by A.L. Adams (1865, 1870) is quite precise, and landmarks such as boulders, rubble walls and tafoni depicted by the artist can all still be readily identified today. In this sketch, all the three deposits are shown very close together indeed.

All the three cave-fissure deposits were associated with the valley sides of Wied Maghlaq. The Maghlaq cave with its associated terrace-deposit was located about 60 metres ("less than a stones throw") west of Mnaidra Gap. The Middle Cave was described to be some 3.5 metres below Mnaidra Gap and about 18 metres SE of the terrace deposit associated with the Maghlaq Cave (Adams, 1865; Adams 1870).

The Maghlaq region is also characterized by Pleistocene remains deposited at the mouth of the valley Wied Maghlaq. This deposit is still extant and available for study. Though best developed at the mouth of Wied Maghlaq, this deposit extends all the way from the cliff

face of Ghar Lapsi up to the Mnaidra region. Other valleys along the SW coast of Malta - Wied il-Mixta and Wied ix-Xaqqa - have also enabled the deposition of alluvial Pleistocene deposits notably that still extant at Benghisa (Adams, 1870; Cooke, 1896; Trechmann, 1938). A cave deposit at Wied il-Bieni at Kalafra in the region close to Benghisa also yielded fossil Pleistocene remains (Zammit, 1921).

#### Maghlaq Cave

The Maghlaq Cave (or Qrendi Cave of Spratt) was originally described by T.A.B. Spratt (1867) and re-described by A.L. Adams (1865, 1866, 1870) and J.H. Cooke (1892). The cave floor deposits were noted to consist of a series of three layers (Table 1). The uppermost layer consisted of a "calcareous grey sinter and small bands of a dark brown loam" containing remains of grilids (*Myoxus melitensis* = *Leithia melitensis*), avian bones (some of a large species), and land shells (Spratt, 1867; Adams, 1865; Adams, 1870). The middle layer consisted of a stalagmitic hardened conglomerate containing similar fossil remains as the previous layer. The lowermost layer consisted of a well-cemented, poorly sorted conglomerate containing remains of *Hippopotamus pentlandi*, *H. minutus* and remains of large birds (Spratt, 1867; Adams, 1870 p.307). This deposit extended outside the cave as a terrace deposit about twenty meters broad. Among the finds made by T.A.B. Spratt was a small canine or outer incisor of a carnivore the size of a fox, while A.L. Adams also notes the excavation of an incisor tooth described as being similar to that of a seal (Adams, 1870 p.204-205). Adams also described the presence of one solitary molar of Pigmy elephant *Palaeoloxodon* sp. (rolled and adherent to a large rounded pebble) discovered by C.A. Wright (Adams, 1865 p.259; Adams, 1870 p.1205). This specimen is suspect since Wright in an annotation in his copy of Adams' book commented that the specimen had not originated from this site but from amongst the debris of the Mellieha Cave (Zammit-Maempel, 1989 p.192 footnote 36). It was commented by Adams "that in no instance were the remains of the rodent (*Myoxus melitensis*) found in connection with the *Hippopotamus*, whereas they were always intimately associated with bones & teeth of the Proboscidian, and not only in this cave, but in several other localities in the island" (Adams, 1865) including the Mellieha Cave. Adams further noted the Maghlaq Cave deposits to be

similar to those from the Mellieha Cave. It is noteworthy that Adams, on the basis of the rounded pebbles and fragmented pachyderm bones, considered the lowermost level of the Maghlaq Cave floor to have been deposited by the influence of running waters. These were subsequently exposed to dry out and become sealed with a stalagmitic sheet. The upper deposits were considered to have been deposited *in situ*. The fossils of this drier period were considered by A.L. Adams to have possibly been deposited by predators (Adams, 1866, 1870).

#### Mnaidra Gap

The Mnaidra Gap deposit, situated within a few yards of the Maghlaq Cave and excavated by AL Adams (Adams, 1865; Adams, 1866; Adams, 1870). In contrast to the Maghlaq Cave in the vicinity, no *Hippopotamus* sp. remains were excavated from this cave. The Mnaidra Cave was characterized by a series of three fossiliferous layers overlying a series of sterile layers (Table 2). The sterile layers were considered by Adams to have been deposited through the gradual process of accumulation. In contrast the upper fossiliferous layers were deposited by the action of running water with the inclusion of rounded stones and pachyderm remains. The fossil content of these upper layers were similar throughout containing remains of gliridae - *Leithia melitensis*, *L. cartei*, and *Maltamys gollcheri*; elephant *Palaeoloxodon* sp. including *P. falconeri*, *P. mnaidrensis*, and *P. melitensis*; unidentified chiropteran remains; giant turtles *Geochelone robusta* and *G. spratti*; and several species of birds including *Grus grus*, *Grus melitensis*, *Cygnus falconeri* and large raptor species. Land shells were also abundant (Adams, 1865; Adams, 1865a; Adams, 1866; Adams, 1870; Adams, 1877; Borg, 1999; De Bruijn, 1966). The elephant remains in the lowermost fossiliferous layers was noted to be of large dimension (Adams, 1865; Adams, 1866; Adams, 1870; Adams, 1877). Amphibian remains attributed to Mnaidra Gap were assigned to the species *Discoglossus pictus* (Parker in Bate, 1935), though the specimens in question most likely referred to the amphibian remains excavated by Adams from the Middle Cave. Mnaidra Gap is the type locality for a number of giant dormice species including *Leithia melitensis* ADAMS 1868 (= *Myoxus melitensis*), *Leithia cartei* ADAMS 1868, and *Maltamys gollcheri* DE BRUIJN 1966. In addition the region is also the type locality of *Palaeoloxodon mnaidrensis* ADAMS 1870.

<b>MAGHLAQ CAVE STRATIGRAPHY</b>	
<b>Maghlaq-C1</b>	Thinly stratified stalagmitic layers with red earth/clay between them FOSSILS: <i>Leithia melitensis</i> ; large and small sized avian sp.; land shells
<b>Maghlaq-C2</b>	Stalagmitic layer. Fossil remains similar to above. No <i>Hippopotamus</i> sp. Fossils
<b>Maghlaq-C3</b>	Indurated brownish stalagmitic clay as hard as flint or jasper containing well-rounded pebbles of native rock FOSSILS: <i>Hippopotamus pentlandi</i> ; <i>Hippopotamus minutus</i> ; carnivore size of a fox/seal; large avian species; reported <i>Palaeoloxodon</i> remains suspect as to providence.

Table 1. Maghlaq Cave Stratigraphy

	<b>MNAJDRA GAP STRATIGRAPHY</b>		<b>MIDDLE CAVE STRATIGRAPHY</b>
Mnajdra-1	Superficial drift with masses of parent rock FOSSILS: <i>Leithia melitensis</i> <i>Leithia cartei</i> , <i>Maltamys gollcheri</i> ; a chiropteran species; <i>Palaeoloxodon falconeri</i> , <i>Palaeoloxodon mnaidrensis</i> , <i>Palaeoloxodon melitensis</i> ; <i>Geocelone robusta</i> ; <i>Grus grus</i> , <i>Grus melitensis</i> , <i>Cygnus falconeri</i> ; land shells	Middle-1 Middle-2	stalagmitic infill  Influx of Red earth
Mnajdra-2	Shelf of stalactite FOSSILS: land shells; <i>Leithia melitensis</i> ; <i>Palaeoloxodon melitensis</i> ; avian species	Middle-3	Shelf of stalactite FOSSILS: <i>Leithia melitensis</i> ; <i>Anseres sp.</i> (= ? <i>Cygnus sp.</i> ) and other smaller avian species, and an abundance of recent land shells
Mnajdra-3	Red clay and rounded stones FOSSILS: <i>Palaeoloxodon sp.</i> (larger dimension ? <i>P. mnaidrensis</i> ); avian species; <i>Leithia melitensis</i>		
Mnajdra-4	Shelf of stalactite and rounded stones at bottom; without organic remains		
Mnajdra-5	Red loam; without organic remains	Middle-4	Red earth; without organic remains
Mnajdra-6	Yellow band; without organic remains	Middle-5	seam of yellow earth interspersed with shelves and hardened masses of dripping, without organic remains
Mnajdra-7	Reddish black loam; without organic remains	Middle-6	reddish black loam hardened by stalagmitic infiltrations. FOSSILS: <i>Arvicola (?) pratensis</i> ; <i>Discoglossus pictus</i> ; avian sp.; pisces sp.
Mnajdra-8	White calcareous dripping with black seam on top; bare of organic remains	Middle-7	White calcareous dripping with a black seam on top with no organic remains

Table 2. Mnaidra Gap and Middle Cave Stratigraphy

### The Middle Cave

While in stratification, the Middle cave was similar to the Mnaidra Gap, this infill was, in contrast to the previous one comparatively rather poor in fossil remains (Table 2). The mode of deposition of the entire sequence was assessed by A.L. Adams to have been that of a gradual accumulation of soil, derived from infiltration into the cave via fissures and from weathering of the cave walls and roof. Dripstones and flowstones were also present (Adams, 1865; Adams, 1870). The upper layer consisted of red earth devoid of fossil remains. The middle layers contained remains of glirids (?*Leithia melitensis*), fragments of *Anseres sp.*, other smaller birds and an abundance of land shells. The lower layers contained only remains of *Arvicola pratensis* (?*Microtus Pitymys sp.*), frog bones (?*Discoglossus pictus*), fragments of bird bones and those of fish. No megamammals were recorded for the entire deposit. There were also two well abraded Miocene *Carcharocles megalodon* (AGASSIZ) teeth. It should be

noted that its physical location precluded its being reached by flowing waters, hence its complete lack of high-energy beds (Adams, 1865, 1870; Parker in Bate, 1935).

### The Maghlaq Coastal Deposit

The breccia talus-like deposit along the coast was described by A.L. Adams and subsequent geologists to consist of at least two or three layers (Adams, 1870; Cooke, 1892; Cooke, 1896; Trechmann, 1938). The lowermost strata was described (Adams, 1870) as composed of alluvial deposits and breccia formed of red earth containing angular and rounded native rock. This was overlain by an alluvial deposit. Adams (1870) describes finding *Palaeoloxodon* remains in this sequence, assigning these in 1870 to *P. falconeri* and in 1874 to *P. mnaidrensis* (Adams, 1870; Adams, 1874). The later deposit yielded remains of birds and two teeth of an unidentified ruminant similar to a goat/sheep (Adams, 1870). J.H. Cooke subdivides the deposits into

a tripartite division. Lying on the Upper Coralline Limestone was a deposit composed of yellowish clays containing small subangular fragments of rock without any evidence of organic remains. This is overlain by a talus of 6-10 feet in thickness consisting of angular and subangular boulders including *Paleoloxodon* sp. remains and a *Geochelone robusta* tibia. The uppermost layer of the deposit consists of angular rock fragments embedded in an extremely calcareous cement (Cooke, 1896; Cooke, 1890). C.T. Trechmann described this deposit as consisting of alternating horizontal beds of larger fragments and of red pebbly material. The uppermost less pebbly red matrix contained remains of land shells (Trechmann, 1938).

The present shore deposit consists of four definite strata (Table 3). The earliest stratum is found as patches infilling solution hollows in the karstic surface of the downthrown Upper Coralline Limestone. This deposit, previously described by J.H. Cooke (1896), is extremely indurated and appears to be devoid of vertebrate remains. These patches are obviously the remnants of a much thicker and more extensive deposit that was almost completely eroded away before the overlying beds were laid down. A long period may be inferred between deposition of this and the earliest overlying beds, an inference further supported by its induration which is much more advanced than that of any of the overlying beds.

This deposit is followed by a fluvial series some 3.9 metres thick, which infills the lower part of a shallow palaeochannel incised by Wied Maghlaq in the Upper Coralline bed. Each stratum of this series consists of very poorly sorted rounded (only *Globigerina*) and angular (the Corallines and *Globigerina*) pebbles, cobbles and boulders embedded in Terra Rossa. Several discrete lenses of Terra Rossa are also present. The presence of *Palaeoloxodon* sp. remains in this deposit has been confirmed.

Resting unconformably on these beds is another series that completes the burial of the palaeochannel and also extends laterally on both side. Vertebrate remains in this series are extremely scarce. Adams (1870) refers only to fragmentary bird bones and two teeth of a sheep/goat-sized unidentified ruminant (= ?*Cervus elaphus siciliae* Pohlig). It should be noted that both the two deposit sequences, besides exhibiting features indicative of water flow (e.g. channelling and imbrication), also show evidence of drier periods (e.g. caliche horizons and root-casts).

### Benghisa Gap Deposit

The Maghlaq coastal deposit is similar in structure to that found at Benghisa Gap deposited in association of another old waterway - Wied ix-Xaqqa - about five miles away from the Maghlaq deposits [bearing 584632]. The Benghisa Gap thirty-two foot deep deposits (Table 3) were described by Adams as being composed of six layers made up of distinct alterations of bands of large water-worn blocks and seams of pebbly loam, representing periods of turbulence and periods of comparative quiescence. The fossil remains characterized the middle layer composed of rounded

large stones bound by red soil. The vertebrate remains included *Paleoloxodon* sp. remains, gliridae, *Cygnus falconeri*, *Cygnus equitum*, *Otis tetrax*, *Branta bernida*, *Geochelone robusta*, and *Lacerta siculomelitensis*. Several species of land shells were also present (Adams, 1870; Adams, 1877; Borg, 1999). J.H. Cooke also described the deposits as containing "large, water-worn boulders with a black lustre occurring at different depths .... Intermixed with boulders and fragments of other colours .... Found as well-defined layers of several feet in thickness, alternating with beds of a rich red soil, containing elephant remains" (Cooke, 1892a). C.T. Trechmann described the deposit as consisting of large angular blocks at the base, overlain in the middle part by a series of alterations of brown clay and layers of pebbles. The uppermost layer was composed of a well calcareated red calcareous laminated soil with small stones. A *Palaeoloxodon* tooth fragment was excavated from the middle of the deposit (Trechmann, 1938). A review of the site confirmed the presence of several strata. The lowermost layers are made up of alluvial deposits containing rounded stones of variable sizes bound by a red loam. Fossil root casts were evident in this deposit. Bands of red earth layers containing pebbles and gravel suggest a subdivision of this alluvial deposit into two horizons. Overlying this alluvial deposit is a red stratified loam deposit containing small stones and pebbles. The uppermost layer consists of a whitish red soil containing variably sized stones. The uppermost two deposits extended inland under the road and into the industrial excavation at the Freeport.

### Il-Mara Deposit

Close to Benghisa Gap in association with another old waterway termed Wied il-Mixta and close to the stack known as Il-Mara [bearing 576628] is another superficial layer of breccia deposit made up of a conglomerate of rounded *Globigerina*, occasional Black Limestone and Lower Coralline rocks bound together with soil matter. The Il-Mara area is now inaccessible for study since it was used as a dumping site for coal ash, building materials and other waste items.

### Wied il-Bieni Cave Deposit

The cave detritus of a narrow cave in Wied il-Bieni near the shore of Kalafra consisted of a red soil with contained animal bones in a semi-fossilized state. The bones were identified by Dr. Smith Woodward of the British Museum of Natural History as belonging to sheep (? *Ovis aries*), goat (? *Capra hircus*), donkey (*Equus asinus* = ? *E. hydruntinus*), and deer (*Cervus barbarus* = ? *C. elaphus*). The presence of domesticated animals including sheep and goat remains suggests that these remains may have been deposited during Neolithic times (Zammit, 1922).

### Discussion

The Pleistocene deposits in the SouthWestern region of Malta can be interpreted as representing at least three definite assemblage biozones. The earliest faunal assemblage would be the *Palaeoloxodon falconeri assemblage biozone* represented by the beds of the Maghlaq Coastal Deposit, of Mnajdra Gap, and the Benghisa Gap deposit bearing *Palaeoloxodon falconeri* (Busk) and its associated fauna which shows a high

	MAGHLAQ COASTAL DEPOSITS		BENGHISA GAP DEPOSITS
Maghlaq-G1	Red ferruginous earth containing several variable sized stones, contained the remains of land shells and teeth of ruminant.	Benghisa-1	White calcareous drift soil with absence of organic fossil remains
		Benghisa-2	Pebbles and red earth layer
Maghlaq-G2	Compact red ferruginous earth		
Maghlaq-G3	Another similar layer containing small dimension rounded stones containing organic remains of <i>Palaeoloxodon</i> sp and also possibly <i>Geochelone robusta</i> .	Benghisa-3	Rounded large stones (some 15 ft circumference); rich in organic remains including <i>Palaeoloxodon</i> sp., gliridae (? <i>Leithia melitensis</i> ), <i>Geochelone robusta</i> , <i>Lacerta siculomelitensis</i> , <i>Cygnus falconeri</i> , <i>Cygnus equitum</i> , <i>Otis tetrax</i> , <i>Branta bernida</i> .
Maghlaq-G4	Alluvial breccia containing large rounded boulders bound together by a stalagmitic hardened red loam	Benghisa-4	Compact ferruginous red earth intermixed with a few pebbles and stones
		Benghisa-5	Gravel and rounded pebbles bounded by soil. Contains fossil root casts
		Benghisa-6	Large blocks of rock bounded by red soil and silt
Maghlaq-G5	Patches of an extremely indurated colluvium infilling solution hollows in the karstic surface of the Upper Coralline Limestone.		

Table 3. Maghlaq and Benghisa Gap costal deposits

degree of endemism including *Leithia melitensis*, *Leithia cartei*, *Maltamys gollcheri*, a unidentified chiropteran species; *Grus grus*, *Grus melitensis*, *Cygnus falconeri*; *Cygnus equitum*, *Otis tetrax*, *Branta bernida*, *Geochelone robusta*, and *Lacerta siculomelitensis*. On the basis of the very strong faunal similarities, this assemblage can with confidence be assigned to the Sicilian *Palaeoloxodon falconeri* - *Leithia melitensis* faunal assemblage of the early Middle Pleistocene which has been dated to about 500 k.y.a. by amino acid racemisation (Belluomini & Bada 1985, Bada et al. 1992).

It has traditionally been held or implied - e.g. Adams (1870:p.205,208), Zammit Maempel (1989a) - that the *Hippopotamus* sp. and the *Palaeoloxodon falconeri* fauna were sympatric and merely occupied separate niches. However it is inconceivable that such an ecological separation should have been so sharp that it only allowed *Hippopotamus* to accumulate in the Maghlaq Cave, while the *Palaeoloxodon falconeri* fauna

only accumulated in the Mnajdra Gap just a few metres away. The evidence presented by the Maghlaq Pleistocene deposits points towards a temporal rather than an ecological or depositional separation. A temporal separation suggests two distinct faunal assemblages in the Pleistocene mammal sequence (c.f. Hunt & Schembri, 1999).

The second assemblage biozone would thus be the *Hippopotamus assemblage biozone* represented by the basal conglomerate of the Maghlaq Cave and apparently characterised by the occurrence of *Hippopotamus* sp. as the dominant megamammal. This was apparently associated with a fox/seal-sized carnivore, and a large avian species. The *Hippopotamus* sp. was also apparently contemporary with *Palaeoloxodon* sp. even though the providence of the solitary elephant molar remains suspect. A providence from the Mellieha Cave rather than the Maghlaq Cave still confirms a contemporaneity of the two magamammals. The animals were also possibly contemporaneous at Ghar Dalam

SPECIES	MALTESE SOUTHWESTERN REGION					
	SW Coastal deposits; Mnaira & Middle Cave			Maghlaq (& Mellieha) Caves		Wied il-Bieni
	Upper I	Middle II	Lower III	Upper I	Lower II	
<b>Amphibia</b>						
<i>Discoglossus pictus</i>	-	-	+	-	-	-
<b>Reptilia</b>						
<i>Geochelone robusta</i>	-	+	-	-	-	-
<i>Lacerta siculimelitensis</i>	-	+	-	-	-	-
<b>Aves</b>						
undetermined sp.	-	+	+	+ (Small)	+ (Large)	-
<i>Cygnus equitum</i>	-	+	-	-	-	-
<i>Cygnus falconeri</i>	-	+	-	-	-	-
<i>Anseres sp.</i>	-	+	-	-	-	-
<i>Branta bernicla</i>	-	+	-	-	-	-
<i>Grus melitensis</i>	-	+	-	-	-	-
<i>Grus grus</i>	-	+	-	-	-	-
<i>Otis tetrax</i>	-	+	-	-	-	-
<b>Chiroptera</b>						
undetermined sp.	-	+	-	-	-	-
<b>Rodentia</b>						
<i>Eliomys (Maltamys) gollcheri</i>	-	+	-	-	-	-
<i>Leithia cartei</i>	-	+	-	-	-	-
<i>Leithia melitensis</i>	-	+	-	+	-	-
<i>Arvicola ?pratensis</i> (? <i>Microtus</i> sp.)	-	-	+	-	-	-
<b>Carnivora</b>						
undefined sp. ?fox/seal	-	-	-	-	+	-
<b>Proboscidae</b>						
<i>Palaeoloxodon sp.</i>	-	+	-	-	+	-
<i>Palaeoloxodon falconeri</i>	-	+	-	-	-	-
<i>Palaeoloxodon melitensis</i>	-	+	-	-	-	-
<i>Palaeoloxodon mnaidriensis</i>	-	+	-	-	-	-
<b>Perissodactyla Equidae</b>						
<i>Equus asinus</i>	-	-	-	-	-	+
<b>Artiodactyla</b>						
<i>Hippopotamus pentlandi</i>	-	-	-	-	+	-
<i>Hippopotamus melitensis</i>	-	-	-	-	+	-
undetermined ruminant sp.	+	-	-	-	-	-
<i>Capra hircus</i>	-	-	-	-	-	+
<i>Ovis aries</i>	-	-	-	-	-	+
<i>Cervus elaphus</i>	-	-	-	-	-	+

Table 4. Paleontological remains from the various sites

Cave, though it has been suggested that the Ghar Dalam fauna in the Bone Breccia deposit was probably a mixed one (Hunt & Schembri, 1999). This faunal assemblage may possibly represent the Gliridae faunal assemblage (= *Maltamys* sp. faunal assemblage of G. Storch) described from Ghar Dalam (Storch, 1974; Storch in Savona-Ventura & Mifsud, 1998). It may also represent the Carnivora faunal assemblage as described for Ghar Dalam (Savona-Ventura & Mifsud, 1998). Though the data is rather inadequate, it has been proposed that the *Hippopotamus* assemblage biozone (probably equivalent to the Ghar Dalam Gliridae faunal assemblage) may in fact be a hitherto unrecognised faunal assemblage biozone separate from the Sicilian *Hippopotamus-Palaeoloxodon* faunal assemblage (probably equivalent to the Ghar Dalam Carnivora faunal assemblage) (Galea

Bonavia, 1999).

The relationship between the *Palaeoloxodon falconeri* faunal assemblage and the *Hippopotamus* assemblage cannot be determined on the evidence of the Maghlaq Pleistocene deposits. The presence of glirid remains above the *Hippopotamus*-containing layer in the Maghlaq Cave is not a useful observation for chronological dating, since glirids have been associated with both the *Palaeoloxodon falconeri* faunal assemblage and also with the *Hippopotamus pentlandi* - *Palaeoloxodon mnaidriensis* faunal assemblage in Sicilian deposits (Belluomini & Bada 1985, Bada et al 1992).

In Sicily, *Hippopotamus pentlandi* and *Palaeoloxodon*

*mnaidrensis* form an faunal association characterizing a continental European affinity fauna, containing carnivores and as well as other herbivores. This association ranged from the late Middle Pleistocene to the early Late Pleistocene. Absolute dating of Sicilian *Hippopotamus pentlandi* and *Palaeoloxodon mnaidrensis* molars has given ages of 200 k.y.a. by amino-acid racemisation from various sites (Bada et al 1992) and an E.S.R. date of 88 k.y.a.- 146.8 k.y.a. for a series of molars from Contrada Fusco (Syracuse) (Rhodes 1996). A Maltese *Hippopotamus pentlandi* molar from Ghar Dalam - dated by E.S.R. and uranium series disequilibria by Bouchez et al (1988) has been variously reported in the literature as 110 k.y.a. - 130 k.y.a. (Reese 1996) and as 190 k.y.a. (Bonfiglio 1992). The exact stratigraphical providence of the specimen has not been defined and may have originated from the Ghar Dalam Lower Red Earth layer - Carnivora faunal assemblage or the Bone Breccia layer - Gliridae faunal layer (Savona-Ventura and Mifsud, 1998; Hunt and Schembri, 1999).

The significance of the glirid bed of the Maghlaq Cave, overlying the *Hippopotamus* bed, is difficult to interpret since the correlation of this deposit to the rest is by no means clear. The taphonomy of this bed is different from those containing the *Hippopotamus* faunas, which are clearly of a flowing water origin. Adams suggests that the Maghlaq Cave Glirid beds represent the food remains of predators, the glirids being taken selectively from a more diverse fauna and carried to the caves for consumption (Adams, 1866; Adams, 1870). A major problem in establishing a correlation for these beds is the fact that the glirid/s species involved were not specifically identified by the excavator. The absence of megamammals in these beds can be ascribed to the fact that megamammals were generally deposited by flowing water, and this had ceased to reach the Maghlaq Cave. Unfortunately the deposit is no longer extant. Certainly the stratigraphic location of the micromammal bed argues for a younger age for the Maghlaq Cave glirid layer than the *Hippopotamus* bed. The evidence suggests that the gliridae inhabited the Maltese Islands throughout a long span of the Pleistocene, certainly during both the *Palaeoloxodon falconeri* assemblage biozone assigned on the basis of the Sicilian faunal associations to circa

455 +/- 90 ka and the *Hippopotamus* assemblage biozone assigned to circa 200 +/- 40 ka (Zammit Maempel and de Bruijn, 1982)

The third faunal assemblage biozone - *Ruminant assemblage biozone* - represented by the deposits of this region includes the aeolian remains noted at the Maghlaq Costal and Benghisa Gap deposits. The former yielded two teeth of a sheep or goat-sized unidentified ruminant possibly belonging to the dwarf deer *Cervus elaphus siciliae* Pohlig. The evidence of these two non-identified teeth is far too sparse to allow any firm conclusions to be drawn, but this faunal assemblage may have been contemporaneous with the *Cervus* or *Pitymys* assemblage biozone described from Ghar Dalam (Savona-Ventura and Mifsud, 1998). The remains found at Wied il-Bieni Cave deposit may have belonged to this same faunal assemblage or to a later Neolithic deposit. Both the horse and the deer have been described from the Xemxija Neolithic tombs (Zammit, 1922; Pike, 1971).

The Maghlaq Pleistocene deposits highlight several problems relating to the mammalian faunal succession, particularly the clarification of stratigraphic relationships between the two *Hippopotamus* spp., with the phylogenetic, palaeogeographical and other implications. These problems need to be addressed before a reasonably accurate faunal assemblage sequence can be worked out. Furthermore a thorough revision of the Siculo-Maltese Gliridae is absolutely essential, since this would help to more precisely and with a finer resolution define the mammalian assemblage biozones involved (Kotsakis 1995).

#### References

- Adams AL (1865) Maltese caves. *Report on Mnaidra Cave. Report of the British Association for the Advancement of Science* 1865: 257-263.
- Adams AL (1866) Second report on Maltese fossiliferous caves, &c. *Report on Mnaidra Cave. Report of the British Association for the Advancement of Science* 1866: 458-462.
- Adams AL (1868) On a species of Dormouse (*Myoxus*)

SICILIAN FAUNAL ASSEMBLAGE BIOZONES	MALTA Ghar Dalam	MALTA Maghlaq-Benghisa
<i>Equus hydruntinus</i> assemblage	<i>Cervus</i> - <i>Pitymys</i> assemblage	Ruminant assemblage
<i>P.mnaidrensis</i> - <i>Hippopotamus</i> assemblage - dated circa 200 k.y.a.	Carnivora assemblage Dated circa 150 k.y.a. <sup>[1]</sup>	<i>Hippopotamus</i> assemblage
	Gliridae assemblage	
<i>Palaeoloxodon falconeri</i> assemblage dated circa 500 k.y.a.	Unrepresented	<i>Palaeoloxodon</i> assemblage
<i>Pellegrinia panormensis</i> assemblage		Unrepresented

[1] stratigraphic providence of *Hippopotamus* molar not defined. May have originated from the Carnivora or the Gliridae layers (quoted dates are approximate).

Table 5. Faunal assemblage correlations

- occurring in the fossil state in Malta. *Transactions of the Zoological Society*, 6:307-308.
- Adams AL (1870) Notes of a naturalist in the Nile Valley and Malta. Edmonston & Douglas, Edinburgh.
- Adams AL (1874) On the dentition and osteology of the Maltese fossil elephants, being a description of the remains discovered by the author. *Transactions of the Zoological Society of London*, 9, 1-124 + plates 1-22.
- Adams AL (1877) On Gigantic Land-Tortoises and a small freshwater species from the Ossiferous Caverns of Malta, together with a list of their fossil fauna; and a note on the chelonian remains of Gibraltar. *Quarterly Journal of the Geological Society of London*, 33, 177-191.
- Bada JL, Belluomini G, Bonfiglio L, Branca M, Burgio E and Delitala D (1991) Isoleucine epimerization ages of Quaternary mammals from Sicily. *Il Quaternario* 4(1a), 49-54.
- Belluomini G and Bada JL (1985) Isoleucine epimerization ages of the dwarf elephants of Sicily. *Geology* 13, 451-452.
- Bonfiglio L (1992) Middle and Upper Pleistocene mammal faunas in the islands of Sicily and Malta: analogies and palaeogeographic implications. *INQUA-MBSS Newsletter* 14, 52-56. [International Union for Quaternary Research - Subcommission on Mediterranean and Black Sea Shorelines].
- Borg JJ (1999) A Checklist to the Quaternary Avifauna of the Maltese Islands. A Preliminary report. In: *Facets of Maltese Prehistory* (Eds. A. Mifsud and C. Savona-Ventura), pp.77-89, Prehistoric Society, Malta.
- Bouchez R, Condomines M, Faure M, Guerin C, Jeunet A, Ma JL, Piboule M, Poupeau G, Rossi AM and Sarcia MNG (1988) Radiometric dating of Hippopotamus pentlandi from the Ghar Dalam cave, Malta. *Proceedings: International Conference 'Early man in island environments' September 25 - October 2, 1988, Oliena, Sardinia*, p.4.
- Bruijn H de (1966) On the Pleistocene Gliridae (Mammalia, Rodentia) from Malta and Mallorca. *Koninklijke Nederlandse Akademie van Wetenschappen, Proc.* B.69(4),481-496.
- Cooke JH (1892) Observations on the Geology of the Maltese Islands. *The Mediterranean Naturalist*, 1(9),120-133.
- Cooke JH (1892a) On the occurrence of Black Limestone in the strata of the Maltese Islands. *Geological Magazine*, 9,361-364.
- Cooke JH (1896) Notes on the Pleistocene Beds of the Maltese islands. *Geological Magazine*, 3,201-210.
- Galea Bonavia C (1999) The early stages of the Maltese Pleistocene mammalian sequence. Evidence from the Maghlaq Quaternary deposits. In: *Facets of Maltese Prehistory* (Eds. A. Mifsud and C. Savona-Ventura), pp.33-40, Prehistoric Society, Malta.
- Hunt C and Schembri PJ (1999) Quaternary environments and biogeography of the Maltese Islands. In: *Facets of Maltese Prehistory* (Eds. A. Mifsud and C. Savona-Ventura), pp.41-75, Prehistoric Society, Malta.
- Kotsakis T (1996) Anfibi e Rettili. In: *Siracusa - Le ossa dei giganti* (Eds B. Basile and S. Chilardi), pp.56-60, Arnaldo Lombardi, Italy.
- Parker HW and Bate DMA (1935) Two new Mammals from the Pleistocene of Malta, with notes on the associated fauna. *Proceedings of the Zoological Society*, 112, 261-262.
- Pike G (1971) The Animal bones from the Xemxija Tombs. In: *The Prehistoric Antiquities of the Maltese Islands: A Survey* (Ed JD Evans), pp.240-241. Athlone Press, London.
- Reese DS (1996) Cypriot Hippo Hunters No Myth. *Journal of Mediterranean Archaeology*, 9(1), 107-112.
- Reuther CD (1984) Tectonics of the Maltese Islands. *Centro*, B:1(1), 1-20.
- Rhodes EJ (1996) ESR dating of tooth enamel. In: *Siracusa - Le ossa dei giganti* (Eds B. Basile and S. Chilardi) pp.39-44, Arnaldo Lombardi, Italy.
- Savona-Ventura C and Mifsud A (1998) Ghar Dalam Cave: A review of the sediments on the cave floor stratigraphy. *Xjenza*, 3, 5-12.
- Spratt TAB (1867) On the bone caves near Crendi, Zebbug and Melliha on the island of Malta. *Quarterly Journal of the Geological Society, London*, 23, 283-297.
- Storch G (1974) Quartäre Fledermaus-faunen von der Insel Malta. *Senckenbergiana Lethaea*, 55, 407-434.
- Trechmann CT (1938) Quaternary deposits in Malta. *Geological Magazine*, 75, 1-26.
- Zammit T (1922) Wied il-Bieni near the shores of Calafra. *Reports on the working of government Departments during the financial year 1922-23*. Government Printing Office, Malta, E1.
- Zammit Maempel G (1989) *Pioneers of Maltese Geology*. Mid-Med Bank, Malta.
- Zammit Maempel G (1989a) *Ghar Dalam Cave and Deposits*. The author, Malta.
- Zammit Maempel G and De Bruijn H (1982) The Plio/Pleistocene Gliridae from the Mediterranean Islands reconsidered. *Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen* B85, 113-128.

---



---

## Review Article

---



---

### Neuroscience, the Bodymind and the Actor

- Reflections on consciousness, learning, memory and the actor in the post-Grotowski era.

Albert Gatt and John Schranz

*xHCA Programme, 55, Triq it Torri Gauci, San Pawl Tat-Targa.*

**NOTE:** Launched in February 1995, *xHCA* is an interdisciplinary research programme seeking interstices between contemporary theatre research and the Cognitive Neurosciences. It brings the instruments and the reflection of the Cognitive Neurosciences to bear on events taking place in the performer (a) as he works upon himself within the parameters of the contemporary theatre's rigorous training regimens and (b) as he engages in the performative act. *xHCA* was founded by the world-famous theatre pedagogue and director Ingemar Lindh (*xHCA* Director until his death in Malta in June 1997), Dr. Richard Muscat (Director, Laboratory of Behavioural Neuroscience), Herr Bernhard Plassmann (Administrator) and Dr. John Schranz (Vice-Director). In June 1998, its directorship was entrusted conjointly to the latter and Dr. Glyn Goddall, Cognitive Scientist at the University Victor Segalen, Bordeaux 2. Current research is directed to the fields of memory, attention and kinaesthesia.

#### Introduction

The following reflections have a number of characteristics which represent both their strength and their weakness: it is the paradox inherent in any exercise that attempts to be simultaneously factual and impressionistic. Factual, because neuroscientific data, insofar as they have been tested and have yielded positive results, constantly offer new insights on the nature of the human being. Impressionistic, because, in the past three years, the former has been involved in theatre praxis, which means that a lot of what will be said on the nature of the actor is a result of the *sketch* - preliminary at best - of oneself that one is able to form after this (admittedly short) period of time of involvement in what is, in itself, a research process.

At another level, we will try to relate neuroscientific findings to the implicit assumptions and observations that seem to underlie the writings of the late Jerzy Grotowski, and these observations are the fruit of a lifetime of research. Why should a theatre practitioner feature in any form of scientific discourse? Throughout his work, Grotowski insisted that he was not a scientist. Yet, any discipline whose focus is the human being as a conscious, behaving organism, with a capacity to learn and undergo change may be interested in the techniques and methods of an art in which the performer is engaged in a constant discovery which is also a learning process. Attempts to discuss the biological and cognitive foundations of performance are nothing new. Diderot proposed a biological theory of the actor, Evreinov linked theatre to human and animal behavioural science, while Meyerhold was fascinated by Pavlov's experiments (Pradier 1998). Meanwhile, the twentieth century has witnessed an entire reinterpretation of the heritage of masters such as Stanislavski and Meyerhold. Grotowski is one who set out to redefine theatre in terms of its barest essentials, that is, in terms of the organism of the actor and his/her relationship with the spectator (Grotowski 1968a). The research to date on the nature of the body's impulses and the basic resistance which must be overcome in the process of working upon oneself, that is, the *via negativa*, are essentially a learning process.

On the other hand, the sketch presented here is complicated by the fact that the numerous systems underlying human cognitive processes are multiplexed and often defy simplification (see Fentress 1991 for a discussion). For the present purposes, the discussion will take place on three levels: the molecular/biochemical, the organic, and the level of the organism as a conscious being. In short, in a discussion such as this one, molecular and genetic considerations, anatomical data and ethological observation will be of equal essence. Indeed, theatre praxis - as the work of the performer upon himself - is a research process which is reflected at each of these levels, and the very concept of a learning organism implies the possibility of change at each level. An operational definition of learning is therefore vital at this early stage:

*Learning is a process whereby an input - behavioural, sensory or somatosensory - results in a change in an organism's state and/or behaviour, requiring the mediation of encoding (memory) mechanisms that lead to the relative permanence of this change, within the architectural constraints imposed by the nature of the organism itself.*

#### Subjectivity and imagery

##### *The Problem of Models*

Various times, throughout his extant writings and speeches, Grotowski makes references to the personal associations of the performer. The research he proposes seems to be a thoughtless process; thinking is the arch-nemesis of creativity. The performer does not think, but what he does is given "meaning" in the personal, highly individual tangle of associations that make up the subjective state of the organism. What is subjectivity?

The question is inherently tied to the question of consciousness, of which more later. The point at issue is, at present, whether or not we can speak of subjectivity at all, without enmeshing ourselves in phenomenological and volitional fallacies (on which see Ryle 1949).

In 1949, Gilbert Ryle put paid to the conceptual fallacies that plague any discourse that treats such concepts as "will", "knowledge", etc. as anything more than conceptually useful items with little validity when grounded in the facts. Yet, the subject seems to be his own agent. What drives the performer? Why must he not think? ( we will never forget a seminar with Ingemar Lindh, back in 1997, in which, every day after the work was over, he would insist that "there was something to understand", but "without too much wanting, without too much thinking". The problem is certainly very real: we find we cannot "improvise" unless there is something resembling perfect silence and privacy. The director rarely interrupts this process, not, at any rate, until some action sequence is formalised and can finally be said to be "learned" and therefore open to dissection (improvisation is an exercise in what is not there yet and thus is hardly open to dissection, analysis, conceptualisation).

In actual fact, the more suitable question seems to be, "Does the subject/performer *need* to think at all?". There seems to be little doubt that there is such a thing as subjectivity, but what constitutes the subjective?

#### ***A contribution from Minsky***

Marvin Minsky (1968) posed the problem of subjective agency quite succinctly when he pointed out that, while attributing volition to any organism or automaton is merely a dead give-away for the lack of knowledge about that organism, "all such questions are pointed at explaining the complicated interactions between parts of the self-model". A man's or machine's strength of conviction about such things tells us nothing about the man or about the machine except what it tells us about his model of himself". The problem is further posed by Dennett (1991): Is there a picture in the mind? If so, does the mind have a picture of the picture? And a picture of the picture of the picture?

Talking about agency is more than vaguely reminiscent of Moliere's comic notion of *virtus dormitiva*: opium puts people to sleep because it has the virtue of putting people to sleep. (People have will - and are often wilful - because.....well.) But there is also the risk of infinite regression. Minsky's (1968) talk of models is grounded in the assumption that a human being, or machine, has a model of the world and a model of himself, and models of these models. Where does it all stop? Presumably, it doesn't, and the real answer does not lie in any arbitrary splitting up of subjectivity into models; the notion of "subjectivity" may well become nothing more than that - a notion or a reified construct - unless one can talk of the complex systems underlying the phenomenon as completely as possible (cf. Fentress 1991 on the problem of reification of constructs in ethology and neuroscience).

#### ***The impressionist club:***

##### ***Dennett's phenomenological garden***

Dennett (1991) pays a "visit to the phenomenological garden" and asks some pertinent questions. The implicit argument is that we cannot talk of pictures in the brain, nor can we extrapolate the argument to any form of mental imagery, including that pertaining to other

modalities such as audition. Similarly, emotions are not explainable by the self-defeating arguments such as " something is painful and I can imagine it so, because that particular something is painful".

What are impressions? What is mental imagery? They are not models - at least, not static models - and they are more than static memories. Imagery and subjectivity is a process. Otherwise, associations in the performer are simply vivid pictures, subject to intellectualistic analysis, and this is *not* the case (see above). Grotowski (1968) gives the example of the performer who's playing Macbeth. You don't have to be a killer, but it helps, he seems to be saying. Yet, killing, or the subjective impression of being a killer would certainly not be possible if this impression were merely an association with the actual act of killing a person, that is, if one were to succumb to the phenomenological argument. Yet, any performer who has had at least a week's experience of normal living is highly likely to have killed a fly. This is not murder, but it too helps.

#### **Imagology: the process and the Process**

##### ***Imagery***

It appears that mental imagery of motor activity is subject to the same constraints as the activity itself. Crammond (1997) argued that the act of imaging a simple motor action takes the same time, and is subject to the same constraints of speed and accuracy. Fitt's Law, a fundamental tenet of biomechanics and motor activity studies, states that there is a speed-accuracy trade-off in any action. A related finding is reported in Fentress (1991), to the effect that, a movement - such as grooming in rodents - is carried out oblivious of interruption and/or environmental stimuli the faster it is executed.

It seems that motor imagery utilises the same networks of connectivity as actual motor movements, with the exception of the efferent stimulation of the muscular effectors. The reason seems to be related to the efference copy, or corollary discharge, that is communicated to brain regions other than the effectors, resulting in a copy of the movement being synthesised and evaluated. The site for this evaluation is probably the parietal cortex. The discovery has a number of implications, among which, as Fuster (1992) observed, the brain never processes anything exclusively in serial mode, but reconciles serial and parallel. A similar hypothesis was put forward by Rumelhart, McClelland and Sejnowski (1987), who claimed that sequential thought processes are probably the result of the sequencing of multiple parallel processes. Their observation may however, be too simplistic, as it is based on the assumption that the processes which are placed sequentially are in themselves exclusively processed in parallel.

Images are therefore extant in the brain, and not only *motor* images at that. It seems possible therefore, to live in virtual reality, with the obvious trade-off that, if one were only to image walking, nothing would get done, which might be why one sometimes "resigns from not doing".

Motor activity and the evaluation thereof is also dependent on reafferent activity and kinaesthetic feedback. Gandevia and Burke (1992) reviewed the research on both active and passive movement and posture, particularly on the hand. The argument presented was that the motor system depends in part on kinaesthetic feedback to execute commands: knowledge of the "self" is vital. In this context, a small excerpt from Grotowski's Skara Speech probably speaks for itself:

*What is an association in our profession? It is something that springs not only from the mind but also from the body. It is a return towards a precise memory. [...] Memories are always physical reactions. It is our skin which has not forgotten, our eyes which have not forgotten.*

(Grotowski 1968: 225-226)

This then, is what is meant by "associations" and "contact". If the theatre is to be a process of uncovering "the truth", at least insofar as it avoids to be a contrived series of gymnastic activities, then the intention of the performer is one of constant self-assessment, and this is not an intellectualistic activity.

#### **Images and plasticity**

Perception and image-formation may well lead to the creation of large-scale topographical representations that are strengthened/weakened according to the amount of practise/learning. This has major implications for plasticity: strengthening of neuronal groups that represent images in any sensory modality, aided both by the highly ordered gating of inputs to early sensory cortices via topographically arranged thalamic nuclei and by practice may result in changes in representation (Merzenich and De Charms 1996).

Plasticity is a major factor in determining individuality, adding further weight to Grotowski's constant insistence that the first and major task of the performer is to determine the association (image) that the action holds for him/her. The task of delivering is relegated to a place of secondary importance. As regards individuality, Johnson (1997) concludes that, while the laminar and topological arrangement of the cortex is a given predetermined factor, epigenesis is probabilistic in the sense that, to a large extent, what determines the make-up and the structure of topological representations is experience, coupled with innate (genetic) mechanisms. In other words, the individual is a result of a dynamic interplay and interplay which Merzenich and de Charms (1996) view in terms of the strengthening of connections that are related to specific images via the constant reappraisal of the said images, through such processes as training. It is important to note that the "image" is hardly a picture as this would otherwise leave us open to Dennett's criticism (see above). Rather, the term image is to be understood as any form of representation. This also now invokes the "use it or lose it" principle inherent in such theory's as Edelman's *Neural Darwinism* in which connections and representations that are not strengthened tend to be weakened and/or lost. Furthermore, neurogenesis may well be an iterative process, influenced by a number of variables that determine the outcome in much the same way as "attractors" that

determine an oscillation's chaotic activity. In this connection, it is worth noting that were one to view the topographical structure of the cortex from a fractal geometric viewpoint, its topography is practically infinite.

These changes in representation may occur as a result of environmental stimulation, which creates an image that, juxtaposed against pre-existing images, may cause a change or perturbation in the pre-existent image of the self. Images seem to be stored topographically or otherwise in the early sensory cortices (including V1 and S1), but are somehow juxtaposed or otherwise interpolated in higher sensory cortices (Damasio and Damasio 1996). The hypothesis put forward by Damasio (1994) and Damasio and Damasio (1996) is that there is such a thing as dispositional representations that are activated and cause a distributed reactivation of images in sensory cortices. The fact that these images are distributed implies that there must be some kind of convergence zone and moreover, that some process of attentional orientation is required. Damasio (1994) hypothesises that the convergence zones may lie in the temporal and frontal cortices. In the temporal lobe, the hippocampus has long since been identified as a substrate for memory. Yet, the actual role of the hippocampus may well be more complex than simply that of a storage site for engrams. Eichenbaum et al. (1994), in an extensive literature review, propose *pattern-formation* as one of the functional components of the hippocampal memory system: while engrams may be encoded in the perirhinal and parahippocampal tissue, the hippocampus per se is probably a convergence site. Some more insight is provided by studies carried out by two groups of researchers on the nictitating membrane reflex in the rabbit. Thompson and colleagues identified the site of the engram for the (procedural) learned reflex in the cerebellum, but a parallel finding by the Disterhoff group identified a functional role of the hippocampus using a different conditioning paradigm - trace conditioning - in which the time window is such that the conditioned and unconditioned stimuli do not co-occur in time. If this is indeed a functional role of the hippocampus, it highlights the importance of convergence *in time* (see also Merzenich and de Charms 1996 for a discussion of temporal contiguity in the formation of images).

As regards the frontal region, dorsolateral prefrontal cortex is one site that seems to serve as a neural substrate for working memory, while the frontal cortex itself is involved, by and large, in decision-making and preparatory set for motor activity (see Fuster 1996). This planning and execution function is also coupled with the high connectivity of the frontal lobe, whose resources for aiding decision making are, among other things, emotional, since connections exist to the basal ganglia among other areas. Latash (1998) claims that, while a motor program that is learned after repetition and automated is probably relegated to subcortical brain structures for the purpose of reducing redundancy, it is nevertheless liable to subtle changes due to the active nature of what is known as the *orienting response* (OR), which has been found to have an emotional value, in the performance situation or just prior to initiation of action.

### **Creativity**

An important proviso should be added at this point. While it is possible to image a movement - or anything prelearned for that matter - the emphasis must be placed on *learned*. The movement studies reported by Crammond (1997) indicate that the movement as imaged is subjected to the same constraints *as the movement when it has been learned and carried out*. Put another way, without actually doing anything there would be no corollary discharge and thus no reafferent input to create images from. If researchers such as Fuster (1992) are correct in their judgements, there is very little that we actually do which is ever completely new: walking is not learned by the child out of nothing. Thus, we are not talking about an imaginative process, nor are we creating something out of nothing. The process of making images is therefore the result of learning: subjectivity is the result of ontogenetic development, plasticity and that infinitesimally subtle process called learning. And learning is a progression.

What, then, is creativity? In theatre research, the performer is engaged in various physical activities - exercises - which serve the research (Grotowski 1968). Creativity may well be a process of combination and interpolation of what is essentially part of oneself. Theatre research is a corroboration of what one knows, an exploration of its associations and derivations, and an elimination of all that is superfluous, the *via negativa*.

The implication for the performer is that thinking about doing is different from doing. Indeed, the possibility of actually thinking about doing is merely the result of doing, or having done. Once doing is in progress, the formation of images and the action itself are quasi-indistinguishable phenomena, a corollary of the observation by Fentress (1991) that it is often difficult to subdivide a process into its component parts without ignoring the fact that the components may be doing more than one thing at a time: the same neural substrates are used, by and large, for the formation of motor images and the execution of movements (Crammond 1997).

The performer is therefore left with the option of only executing the task and making the association in the Process. At first glance, the process called improvisation seems to be an unwieldy exercise in anarchic self-stimulation. The research that Grotowski makes reference to at every stage, from start to finish, in the description of the exercises carried out by the Theatre Laboratory constitutes a substantial denial of such a claim. What the actor is doing is forming associative images in the Process of doing, and since doing is never a complete novelty, training is a learning process, and improvisation is the culmination of such learning.

### **Concluding remarks: orienting, learning and being-in-the-act**

The picture sketched above is one of a self-conscious individual with the capacity to react in a specific time window to input that causes psychosomatic and behavioural changes. The Orienting Response - itself bearing emotional value - coupled with the decision-making capacity of the performer, as well as the integrative process of bringing together distributed

representations in convergence zones, via the mediation of dispositional representations, are all factors that bear on this point. In accordance with the operational definition of learning given in Section 1, it is possible to surmise as to the nature of the performance itself: given that the performer is not a solipsistic automaton, but has the capacity to orient and react *actively*, then the execution of a program of action - even if it is stored in subcortical regions (such as the reticular formation and the basal ganglia) for the purposes of reducing redundancy (Latash 1998) - may very well be in a state of constant flux. Damasio's (1994) somatic marker hypothesis, highlighting the interplay of environment, memory and current organismic state, gives us the picture of a conscious subject whose self-image is under constant perturbation. Moreover, if the act of creation in action (and improvisation) is viewed as a syntactic process of re-ordering of what is there already and what is new in terms of input, then, for the performer:

*Each night's "run" in(-)forms the performer at the same time that the performer forms it. Each night's run is considered to be (and it is indeed seen to be) a "revisiting", a step in the process of the performance's growth, a step in the learning of the possibilities of the performance, a step of the continuous change and flux of the process, night after night.*

(Schranz 1997: 2)

The problem of creativity is thus posed in terms of the capacity of the human being to create anew from the old, almost as though a pre-existing grammar were being utilised to string together new sentences. The temporal and co-ordinating function of some possible convergence zones may well be of this sort, further highlighting the conceptual appeal of the term so frequently used in neuroscientific literature: the syntax of action. It is comparable to the musician whose ultimate goal is to create a new semantics from an old syntax. This is why, in the theatre, "the exercise serves the research" and why, while everyone can walk and raise his hands, the performer can do it beautifully.

Another point that has been made implicitly throughout this section regards the nature of consciousness: the conscious human subject is a self-observer, and the self-observer has the capacity to assess perturbations in his own body proper (his most intimate space) and changes in the environment, the external space. The conscious subject must act upon these possibilities - that is what they are - and collapse the enormous number of possibilities into a single one by a process of conscious decision-making.

### **Micro phenomena and Macroscopic entanglement**

#### ***Quantum phenomena and the self-observer***

Some decades ago, Schroedinger made history by placing a cat in a box. The cat was accompanied by a radioactive molecule that could, if a certain oscillatory pathway within the molecule were taken and a radioactive energy release occur, break open a cyanide cylinder which would then kill the cat.

The intramolecular dynamics of the molecule are such that each subatomic particle exists in a number of

superposed states - the essence of quantum theory. But these superposed states, which when summed up form the *wave function*, resulted in a corresponding state of indeterminacy in the cat, a phenomenon known as macroscopic entanglement. The cat, once the lid was closed would also exist (to the external observer) in at least two superposed states: dead or alive. The fundamental point is that, once the box is opened - were Schroedinger to peer into the box as it were - the wave function would collapse: the cat would take up one of its two superposed states *via the mediation of the observer*. Are there such microphenomena in the brain and is the conscious (self-)observer constantly collapsing his/her own wave function?

### **Signaling cascades and knockouts**

Most of the ABook explored the possibilities of learning largely in terms of structural changes and constraints imposed by the brain's cytoarchitecture. At a more micro level, the changes occurring at synapses offer a different, if highly related, account of what happens when a process is learnt well enough for the neuronal circuits supporting the process to be facilitated in the long-term. This facilitation, and its counterpart, the actual weakening of the synapse, is the hallmark of long term potentiation (LTP) and long-term depression (LTD) (H.K. Lee et al., 2000). LTP is one way of increasing the brain's efficacy for storage.

A plausible site for LTP is, of course, the hippocampus. There are signaling cascades occurring in this region, specifically, the binding of glutamate to NMDA (N-methyl D-aspartate) receptors that causes the concomitant opening of calcium channels in the cell membrane. Calcium activates a number of enzymes, among them CAMKII, which influence the transcription of immediate early genes, and whose influence at the micro level has repercussions throughout the learning process. Indeed, genetic knockouts have highlighted the importance of this process: take a laboratory rat, knock out the relevant CAMKII gene, and the rat becomes incapable of learning the position of a submerged platform within the Morris water maze (see the report by Seife 1997).

In actual fact, there are other sites for calcium activity and the quantum phenomena sketched out in ABook that may actually be occurring in the brain. Miller (1997) made tentative proposals to the effect that signaling cascades in the frontal lobe, among other regions, may lead to the formation of calcium "hot spots", large pools of calcium ions within the cell. Prefrontal cortex, one of the phylogenetically youngest regions of the cortex, is, as has been pointed out above, highly implicated in the processes of decision making - the collapse of possibilities that was hinted at in ABook on Phineas Gage remains the paradigmatic example of what happens when the prefrontal cortex is damaged beyond repair: the capacity to react emotionally, to orient and to make effective decisions is lost (see Damasio 1994; Miller 1997). Phineas Gage is a rather bleak case of a human being who has lost the capacity to act on possibility, mainly because of an apparent deficit in his capacity to act upon perturbations in the self-image.

### **The Calcium Hot Spot and the small Big Bang**

The superposition of possible states of a calcium ion - its wave function - has been estimated to be quite large (Miller 1997); this could imply that the release of neurotransmitter which is calcium-mediated, or intracellular mechanisms that depend on calcium activity are quantum phenomena and may somehow form a possible substrate for the neural, continuous phenomenon called consciousness.

Miller (1997) suggested the possible relevance of calcium "hot spots" in this respect: calcium hot spots occur when as much as a single calcium ion enters the cell, encounters a ryanodine receptor gated pool of calcium and causes the release (by as much as four degrees of magnitude) of a huge cascade of intracellular calcium. The result may have consequences upon the memory processes outlined above as a function of gene transcription in the nucleus, and may also cause the phosphorylation of the cell's calcium channel, altering the consequent flow of calcium into the cell. In short, a small ion may cause a "big bang" in the neuron. Miller further suggested that, if one of these hot spots is viewed as a type of wave function collapse, about 10 trillion hot spot collapses are occurring in the brain every 25 msec. This collapse must be somehow synchronised, given that consciousness is a continuous, uninterrupted phenomenon. Somehow, the shortest period oscillator must be responsible for the eventual synchronisation of these oscillatory mechanisms. If 25 msec is the shortest period, then the oscillation will eventually be synchronised at about 40 Hz which has also been suggested by Crick in his book *The Astonishing Hypothesis*. This is also the global oscillation that has been recorded in wakeful and rapid eye movement conditions but not in slow-wave (dreamless) sleep. Moreover, these hot spots could behave like a Bose-Einstein condensate during which a population of atoms are forced into the same quantum mechanical state. The process of synchronisation discussed here could be such a mechanism.

In the right cortical areas, such as the prefrontal lobe, these oscillatory mechanisms could be a possible explanation of such theoretical constructs (or Cartesian fallacies) as volition; they moreover fit well into the description of the self-conscious observer. Is the conscious subject a person capable of collapsing his own wave function? (but see Hahnloser et al., 2000). The observer's decision to act is in a sense the culmination of the wave function collapse, the taking of one path out of many. This is subject to all of the constraints discussed. We have often encountered instances - both in our own work and in that reported to us by some colleagues - wherein decisions are taken without the slightest bit of hesitation, *even during performance*. This fact makes it hard to believe that a performance can ever be called a finished work. It is also possible that the decisions taken are instantaneous collapses of a series of possibilities that occur to a performer during the act, resulting in, say, the choice of one action as opposed to another (it is often the case that, at a fixed point in an action sequence, a number of different courses of action have been worked out and are therefore equally possible). This could also be a part of the decision-making process called

improvisation. All of the above is subject to how much one has learned, one's basic grammar and how the action can be strung together in time to produce a syntax of rhythm and melody.

### Concluding remarks

The foregoing is mainly the result of personal reactions, both to Grotowski's pedagogy and to our own preliminary explorations in theatre. There is much that remains unsaid, and, it should remain so. Grotowski has not been considered as a research scientist, but rather as one of a number of significant theatre masters who have proposed a methodology, or ways and means of arriving at one such. Some things remains a mystery: how, for instance, can the actor achieve "a state of grace"? We believe these questions, which are often considered with something of a cynical eye - except by a critic who, having witnessed Ryszard Cieslak's performance of the Constant Prince, found it necessary to make a public confession that he had been "converted from his cynicism" (see Grotowsky 1968: 97) - are best left unanswered, or put down to experience.

There are a number of possible answers to questions which arise when, having witnessed *results* in the theatre, one asks what are the possible explanation for such. However, most theatrical experience remains beyond rationalisation, and this fact alone is a good enough reason for further exploration.

### References

- Crammond DJ (1997) Motor imagery: never in your wildest dream. *Trends in Neurosciences*, **20**, 54-57.
- Damasio A (1994) *Descartes's Error: Emotion, Reason and the Human Brain*. London: Papermac.
- Damasio A and Damasio H (1996) Making Images and Creating Subjectivity. In: *The Mind-Brain Continuum* (Eds P Churchland and R Llinas R), MIT Press, Cambridge, Massachusetts.
- Dennett D (1991) *Consciousness Explained*. Penguin, UK.
- Eichenbaum E, Cohen NJ and Otto T (1994) Two Functional Components of the Hippocampal Memory System. *Behavioural and Brain Sciences*, **17**, 448-472.
- Fuster J (1992) Brain systems have a way of reconciling "opposite" views of neural processing; the motor system is no exception. *Behavioural and Brain Sciences*, **15**, 741-743.
- Fuster J (1996) Frontal Lobe and the Cognitive Foundation of Behavioural Action. In: *Neurobiology of Decision-Making* (Eds A Damasio, H Damasio and Y Christen). Springer-Verlag, Berlin, Heidelberg and NY.
- Fentress J (1991) Analytical Ethology and Synthetic Neuroscience. In: *The Development and Integration of Behaviour* (Ed P Bateson), CUP, Cambridge, UK.
- Gandevia SC and Burke D (1992) Does the nervous system depend on kinesthetic information to control limb movements? *Behavioural and Brain Sciences*, **15**, 614-632.
- Grotowski J (1968) *Towards a Poor Theatre*. Simon and Schuster, New York.
- Hahnloser RHR, Sarpeshkar R, Mohowald MA, Douglas RJ, and Seng HS (2000) Digital selection and analogue amplification in a cortex-inspired silicon circuit. *Nature*, **405**, 947-951.
- Johnson M (1997) *Developmental Cognitive Neuroscience*. Blackwell, Oxford, UK.
- Latash LP (1998) Automation of Movements: Challenges to the Notions of the Orienting Reaction and Memory. In: *Progress in Motor Control* (Ed M Latash), MIT Press Cambridge, Massachusetts.
- Lee HK, Barbarosie M, Kameyama K, Bear MF and Huganir RL (2000) Regulation of distinct AMPA receptor phosphorylation sites during bidirectional synaptic plasticity. *Nature*, **405**, 955-959.
- Merzenich M and de Charms RC (1996) Neural Representations, Experience and Change. In: *The Mind-Brain Continuum* (Eds P Churchland and R Llinas), MIT Press, Cambridge, Massachusetts.
- Minsky M (1968) Matter, Mind, Models. In: *Semantic Information Processing* (Ed. M Minsky), MIT Press, Cambridge, Massachusetts.
- Miller JD (1997) The Prospects for a quantum neurobiology. E-Print Archives, Neuroscience-Net/10011.
- Pradier J-M (1997) Ehtnoscenology. E-Print Archives, Limen.
- Rumelhart DE, Smolensky P, McClelland JL and Hinton GE.(1987) Schemata and Sequential Thought Processes. In: *Parallel Distributed Processing Vol II: Applications* (Eds JL McClelland, and DE Rumelhart), MIT Press, Cambridge, Massachusetts.
- Ryle G (1949). *The Concept of Mind*, Penguin, UK.
- Schranz JJ (1997) "Can there be 'real-time', instantaneous learning in the brain"? A reflection from within the praxis of the work of the performer upon himself. E-Print Archives, Cognitive Psychology Online List.
- Seife C (1997) New knockout mice point to molecular basis of memory. *Science*, **275**, 32-33.

---

---

## Article

---

---

### History of the Practice of Chemistry - a Maltese Perspective

C. Savona-Ventura<sup>1</sup> and M. Sammut<sup>2</sup>

<sup>1</sup>*Institute of Health Care, University of Malta, Gwardamangia, Malta*

<sup>2</sup>*Department of Pathology, University of Malta, Gwardamangia, Malta*

**Summary:** *The practice of chemistry has a long history on the Maltese Islands being closely related to the preparation of pharmaceutical substances. In line with events on the Continent, the initial investigations in non-pharmaceutical chemistry was closely linked to the practice of alchemy. Laboratory chemical investigations received as impetus during the nineteenth century with the introduction of medical chemical investigations. Academic curricula in non-medical chemistry were only established in the latter half of the twentieth century.*

**Keywords:** chemistry, alchemy, pharmacology

#### Introduction

Philosophical concepts in the make-up of matter started being entertained by the ancient Greeks during the fourth century B.C. Aristotle (384-322 BC) concluded that everything was made up five elements - earth, air, fire and water on Earth, and ether in the heavens. To Aristotle, the four traditional earthly elements were only aspects of a single matter affected by four fundamental qualities - hot, cold, fluid and dry. These qualities combined in pairs: cold and dry gave the element earth; cold and fluid gave water; hot and fluid gave air; hot and dry gave fire. While Aristotle's contemporary Democritus suggested that all matter was made of invisible particles which he called atoms, Aristotle's view of the composition of matter gained momentum and was extended to medical concepts by Galen (130-200 AD) with the identification of the four humours and four temperaments. The Medieval Italian monk Thomas Aquinas (1266-1273) in his attempt to compile the complete theory of the Christian doctrine adopted Aristotle's views thus giving a dogmatic seal to scientific concepts. These views on the make-up of matter led to practical science of the pre-17<sup>th</sup> century being practised by superstitious alchemists, while chemical substances were only utilised in the preparation of pharmaceutical substances. In spite of their limited views, the alchemists contributed towards several discoveries in chemistry. After the 16<sup>th</sup> century, the alchemists of Europe became divided into two groups. One group was composed of those who earnestly devoted themselves to the scientific discovery of new compounds and reactions. These scientists were the legitimate ancestors of modern chemistry. The other group took up the visionary, metaphysical side of the older alchemy and developed it into a practice based on imposture, necromancy, and fraud, from which the prevailing notion of alchemy is derived. It was only in the 17<sup>th</sup> century that Robert Boyle overturned many of the superstitious beliefs of alchemy. Boyle in *The Sceptical Chemist* (1661) outlined his ideas about the make-up of matter. He concluded that there were more than the four traditional elements. Furthermore matter was made up of primary particles which could combine together to form 'corpuscles'. After the set-back in progress caused by the invention of the phlogiston theory (concept of a 'volatile principle' contained in bodies freed by combustion) by

Stahl in 1697, chemistry took on a scientific direction with the work of Lavoisier in 1770. Lavoisier showed the determining role of oxygen in combustion and oxidation, and exploding the notion of phlogiston and inaugurating a quantitative chemistry. The nineteenth century became "the century of chemistry" with the establishment of the basic principles of chemistry and their practical application (Lonchamp, 1993).

#### Pre-Hospitaller Period

The investigations in chemistry in Malta followed similar trends as on the continent. No information is available as to the philosophical attitudes towards the earth sciences during the Classical period in Malta. However, a tomb slab from a catacomb at Rabat (Malta) dated to the Palaeo-Christian period suggests that the medical physicians practising on the Island during that period were familiar with the humoral theory of disease described by Galen. The tomb slab depicts a series of medical instruments including bleeding cups used to correct the humoral imbalances believed to be caused by disease (Cassar, 1974; Savona-Ventura, 1999). It is likely that these physicians also subscribed to the theory that matter was made up of the four elements. The Medieval Period period in Malta was dominated initially by Muslim rule and later (after 1127) by Latin influences. It is likely that the scientific community of the late Medieval period, including physicians and pharmacists, subscribed to Aristotelian-Galenic humoral views.

#### Hospitaller Period

By the late Medieval period, Maltese physicians were apparently well versed in and subscribed to the Galenic views on disease, besides the views of other ancient and medieval authors including Rhazes (860-932), Avicenna (980-1037), and Avenzoar (1072-1162). Evidenced for this comes from a medico-legal report dated 1542 presented by two Maltese doctors to the Ecclesiastical Court (Cassar, 1974a). The practise of chemistry in Malta became closely related to pharmacology. Pharmaceutical lists of the mid-sixteenth century from Malta confirm that while the overwhelming preponderance of pharmaceutical substances was of organic sources, a few mineral substances were regularly used (Table 1). Worthy of note is the use of nitric acid,

MINERAL MATERIA MEDICA	1546	1590	18 <sup>th</sup> cent.
<i>Alumen (Coctum (?))</i> : a mineral salt obtained by burning ( <i>alumen ustum</i> ) a type of rock; used for cicatrization of wounds.	❖		
<i>Aqua fortis</i> : Nitric acid or saltpetre acid prepared from potassium nitrate ( <i>saltpetre</i> ); a resolvent.	❖		
<i>Diaiteon (Ceratum)</i> : composed of alum, calcium and verdigris; use unknown.	❖		
<i>Diaquilon (Diachylon) Nigrum, Emplastrum</i> : Made from the mucilaginous roots of <i>Althaea officinalis</i> , litharge (lead monoxide) and oils; an emollient and resolvent of tumours.	❖	❖	❖
<i>Filonia persica (Philonium persicum)</i> : made up of white pepper, hyosciamus, sealed earth of Lemnos, dried preputial follicles from beaver, 'prepared pearls' and zedoaria; lozenge to arrest spitting of blood, vomiting and treat piles.		❖	
<i>Terra sigelata (Terra sigillata)</i> : Sealed earth from a cave in Island of Lemnos ( <i>terra lemnia</i> ), occasionally mixed with other substances ( <i>Confectio hyacinti</i> and <i>Theriaca</i> ); as lozenge for bleeding and diarrhoea.		❖	❖
<i>Coralli (Corallum)</i> : made up of ground calcareous corals mixed sometimes with opium, myrrh, cascarilla, and cinnamon; syrup to fortify liver and stomach.		❖	❖
<i>Bolo armenu (Bolo di Armenia)</i> : clay high in iron content from Armenia; used orally as an astringent in dysentery and in bleeding.		❖	
<i>Tucia</i> : made up of zinc oxide, lead, and white wax; ointment applied to ulcers		❖	
<i>Lapid. D. Pauli</i> and <i>Linguae D. pauli pulv.</i> : made up of the powdered Limestone obtained from St. Paul's Cave at Rabat (Malta) and from powdered fossil shark's teeth; antidote against poisons		❖	❖
<i>Silver nitrate</i> : as caustic against granulation tissue			❖

Table 1. Mineral Materia Medica

copper oxide, zinc oxide, alum, and salts of calcium, iron and lead. Many of the substances were probably imported from Sicily, since none were to be obtained from local sources (Cassar, 1976; Fiorini, 1988/89). The use of minerals in the local pharmacopoeia, including lead, mercury, silver nitrate, alum and calcium, continued well into the eighteenth century (Cassar, 1969; Cassar, 1964).

The formal study of Pharmacy in Malta was probably introduced with the establishment of the School of Anatomy and Surgery in 1676. The director of that School, Dr. Giuseppe Zammit, was also the teacher of botany and in 1690 set up at his own expense a botanical garden in the vicinity of the Sacra Infermeria with the aim of providing living plant specimens for the practical teaching of pharmaceutical botany. Dr. Zammit similarly introduced the teaching of chemistry in the school and gave practical demonstrations in the subject. Among the various chemical and pharmaceutical compounds prepared by Dr. Zammit was sodium sulphate. Until 1886 it was known in Malta as Zammit's salt (Cassar, 1964).

The Hospitaller Order of the Knights of St. John was made up of the elite of European nobility, and it is not surprising to find that many of its members showed an active interest in the various aspects of science. There is evidence to show that GrandMaster Pinto de Fonseca

practised alchemy. Fra Pinto, a native of Portugal and Bailiff of Acre, was elected GrandMaster to the Order on the 18th January 1741. His reign is usually described by historians as long, prosperous and glorious. It was certainly long-lasting 32 years, but its prosperity and glory appear to have been over-rated. Pinto died on the 23rd January 1773 at the veritable age of ninety-two. While his old age may reflect the successful discovery of a secret *elixir vitae*, the financial situation that Pinto left behind him definitely confirm that he was unable to discover the secret of transmutation of metal.

While the statutes of the Order of St. John strictly forbid the practice of alchemy, Pinto is known to have been very interested in alchemy and experimented in his personal laboratory in the Valletta Palace. The Order's statutes clearly defined that "*The same punishment (rowing on the galleys for five years) will be meted out to those goldsmiths and silversmiths who dare receive or work any kind of metal for alchemy, without earning any exemption of the penalty on the plea of ignorance*". Pinto's interest in alchemy appeared to be limited to the old school searching for the philosopher's stone of eternal life and for turning base metal into silver or gold. European alchemists are known to have been well received in Malta, and freely used Pinto's laboratory. A manuscript diary belonging to Ignatius Saverio Mifsud dated 1754 states that "For some months there has been lodging in the Palace of His Most Serene Highness a

man who claims to be a chemist. This man was received by the afore-mentioned Highness and admitted to his full confidence, being lodged in his Palace and maintained at his own expense, occupying rooms surrounding the loggia of the fountain made by His Highness in the Garden Court. And the reason of all this is that he has announced his intention to concoct a certain elixir of life designed to keep man sound in health and strength and mind" (Freller, 1997). During the period 1762-66, the alchemist Giuseppe Balsamo, known as Count of Cagliostro, may have also been lodged in Pinto's Palace. The notorious Balsamo, accompanied by his alchemist friend Althotas, were driven into Malta by stress of weather during their return to Europe after visiting Turkey. In Malta, they worked in Pinto's laboratory for some months and tried hard to change a pewter platter into a silver one. Balsamo, having less faith than his companions, was soon wearied and obtaining from his host many letters of introduction to Rome and Naples, left Pinto and Althotas to find the philosopher's stone and transmute the pewter platter without him. Althotas, who according to Balsamo was a member of the Order of the Knights of St. John, may have died in Malta (Mackay, 1841).

Experimentation in Malta was not only restricted to alchemy. Several members of the Order published theses that dealt with various aspects of science - particularly medical and natural sciences. Dr. Josephus Demarco (1718-1793) prepared a manuscript entitled "*De philosophiae Experimentalis Natura, constitutione, objecto etc. aliisque rebus quae philosophiae tyronibus cognitu maxime sunt necessariae*", which discussed experimental philosophy and its importance in the teaching of apprentices. In addition, his inquiring mind is reflected in other non-medical thesis dealing with logic, elementary arithmetic, plane trigonometry, experimental physics and hydrostatics (Vella, 1999).

The ousting of the Order of St. John from Malta in 1798 resulted in a short interlude whereby the Maltese Islands came under French dominion. On the 18th June 1798, the University was abolished and a central school established. This school envisaged the appointment of eight teachers or Professors including Professors of Chemistry and of Mechanics and Physics (Hardman, 1909). The civil strife that ensued in the two years at the end of the eighteenth century stopped all plans towards developing an education programme on the Islands.

#### **Nineteenth and early twentieth century**

The close links between the study of chemistry and pharmacology continued well into the subsequent period. Mineral substances continued to be used in the nineteenth and early twentieth century. The list included, among others, magnesium sulphate, bismuth subnitrate, bicarbonate of soda, hydrocyanic acid, ammonium carbonate, citrate of iron, calcium salts, lead plaster, potassium iodide and bromide (Cassar, 1969). The teaching of chemistry continued to be linked to medical education. The re-organisation of the university in 1800 after having been disbanded during the French interlude required the setting up of chairs in medicine, anatomy and surgery, pathology, and physiology. Forensic medicine, hygiene and pharmacology were introduced as

separate subjects during 1829-34. In 1836, a Royal Commission was appointed to investigate the general administration of the Islands, including the university. The Commissioners proposed five professorships - anatomy and surgery, medicine, obstetrics, chemistry, and botany - to bring the Malta University in line with other Medical Schools in England. These proposals were embodied in the 1838 Statute of the University. The first Professor of Chemistry to teach at the Malta University was Prof. G.G. Aquilina (1834-1859) who set up a regular chemical laboratory at the University. By 1856 Organic Chemistry was being taught for 41/2 hours each week. In the revised 1887 University Statute, the number of professorships was augmented to six, which included a Professor of Organic Chemistry, Practical Chemistry and Materia Medica. In 1900, a Professorship in Pathological Anatomy was further set up which ensured the training of medical students in the chemical, bacteriological and microscopical examination of pathological specimens (Cassar, 1964: p.448-461).

The teaching of Chemistry to medical students became more and more important with the increasing use of laboratory investigations in diagnosis and postmortem studies. A post-operative exudate was submitted by the surgeon Dr. P. Fabrizi who commented that "*siffatta sostanza essendo stata analizzata dal rispettabile mio amico Dr. Aquilina, Professore di Chimica in questa Università, fu da lui riconosciuta per un carbonato di rame*" (Fabrizi, 1841). Prof. G.G. Aquilina also performed chemical analysis on excretions, on urinary calculi, and cerebrospinal fluid (Cassar, 1964: p.540). Another contemporary proponent of laboratory investigations was Prof. C. Schinas (Professor of Medicine). In his medical journal *L'Ape Melitense* (published Sept-Dec 1838), Schinas encouraged the use of laboratory investigations, but warned against considering these as an easy solution to therapeutic problems (Schinas, 1838). The problems of laboratory diagnosis in the detection of blood in stains - using microscopy and chemical tests - were discussed by three contemporary doctors including Prof. G.G. Aquilina, Dr. F.L. Cravagna and Dr. T. Chetcuti in 1841 (Filologo Maltese, 1841).

The increasing interest shown by local practitioners towards laboratory diagnosis was probably in part stimulated by contacts with British doctors and researchers who visited and worked in Malta. One important visitor was Dr. John Davy, brother of Sir Humphrey Davy who invented the safety lamp for miners and recorded the effects of nitrous oxide. John Davy, a military medical officer, was posted to Malta in 1828-35, and again visited the Island in 1838, 1839 and 1840. In the interim, he probably kept up correspondence with the local practitioners, since in September 1840 he was elected honorary member of the Societa Medica d'Incoraggiamento di Malta. Besides his military duties, Davy, during his posting to Malta, also had a busy private practice and acted as President of the Medical Committee that supervised civilian medical services. Davy was definitely acquainted with Prof. G.G. Aquilina and in 1840 had attended one of his lectures (Cassar, 1986).

Even before he came to Malta, Davy had already conducted several scientific investigations, including the preparation of phosgene gas ( $\text{COCl}_2$ ). While in Malta, he carried out several physiological investigations on the human body and the effects of various substances/conditions on human tissue. He further carried out chemical studies publishing several papers including "Some Experiments and Observations on the Combination of Carbonic Acid and ammonia" (1833); "Some Observations on Phosphorus"; "Some Observations on a Note of M.A. Van Beek purporting to point out an error in the Bakerian Lecture of the late Sir Humphrey Davy on the Relations of Electrical and Chemical Changes" (1834); and "Some observations on Euchlorine relative to the question of its decomposition" (1834). His laboratory was sited in the Military Hospital. His instruments comprised a microscope, a galvanometer, Coulomb's electroscope, Harris's electrometer, a Leyden jar, a Voltaic cell and an electricity generating machine. Other laboratory items included glass retorts and tubing, a spirit lamp, an air pump with receiver, steam and mercurial baths, a fine balance, glass-stoppered bottles, wires of gold, steel, platinum and copper, and litmus paper. His list of chemicals included: alcohol, ammonia, manganese oxide, camphor, marble, hydrochloric acid, chlorate of potash, distilled vinegar, iron filings, hydrocyanic acid, mercury, nitrous oxide gas, oil of turpentine, silver nitrate, sulphuric acid, sulphuric ether, and aqueous solutions of chlorine, iodine and bromine (Cassar, 1986).

The use of laboratory investigations to augment the clinical diagnosis increased in the subsequent decades. Chemical and microscopical analysis of urine was repeatedly advocated in the local medical journal *Il Barth* edited by Dr. Giovanni Gulia (Gulia, 1871-72). The last decades of the nineteenth century (1875) saw the restructuring of the Sanitary Office, which was entrusted with the examination of articles of food and drink and with the investigation into the causes of infectious disease. This required the re-organisation of a Public Health Laboratory. The Professor of Chemistry at the University was ex officio appointed the analytical chemist of this laboratory. In 1895, the Public Health Department was revived. The first annual reports of the laboratory of the Public Health Department (1896-1899) prepared by the analytical chemist Prof. T. Zammit show that a large variety of investigations had been performed on a variety of foods and beverages. During these years, the Department was able to identify adulteration of coffee beans with lead chromate (Zammit, 1897-1900; Savona-Ventura & Sammut, 1998).

### Contemporary period

The close links between medical studies and practice and chemistry persisted until the post-Second World War period. The University had established the Faculty of Literature and Science, which served as a preparation for the course of Medicine. During the triennial course for 1912-15 of the Faculty of Literature and Science, the total number of students pursuing studies in science amounted to 33 (Magro, 1914). The situation persisted in spite of the new 1915 University Statute. The Faculty of Science was responsible for academic courses leading to a degree of Bachelor of Science, a course of Pharmacy

and preparatory courses for admission to the Faculties of Engineering and Architecture, and of Medicine and Surgery. The academic year 1930-31 had only 40 students enrolled with the Faculty of Science (Agius, 1932).

In 1957, a Commission was set up under the chairmanship of Sir. H. Hetherington to study the University System, its place and function in Maltese life, its relations with Government and the obligations of Government to the institution. The Commission reported that "*judged by the standards of most other Universities, science has hardly made more than a beginning. Both in equipment and in the depth of its scientific courses, the University seems to offer little more than is offered by an ordinary secondary school in the United Kingdom. .... the main business of the Faculty of Science is to provide a certain amount of basic science preparatory to other degrees. .... Hence the strengthening of the Faculty of Science appears to us to be one of the main and the first concerns of the University of Malta. .... In this faculty, the basic sciences of mathematics, physics, chemistry, biology and geology should be presented along fundamental (as opposed to applied) lines. .... It is therefore clear that the development of a sound Faculty of Science is a prime necessity in the University. The new laboratories are there. These need equipment, some of which, we understand, can be made available. They need also some suitably trained technicians. But above all, they need well-qualified professors of physics, chemistry and biology*" (Hetherington et al, 1957). Both the Government and the University accepted the recommendations, and by the passing of the 1958 Royal University of Malta (Commission) Ordinance, the B.Sc. course came into its own (G.o.V., 1959).

The setting up of a formal B.Sc. course of studies by the University initiated a move towards the training of young graduates in various fields of science, aiming to serve education and technology. The first Professor of Chemistry in the newly set-up Faculty of Science was Prof. Philip Farrugia who occupied the post until 1960. A medical graduate, Farrugia was the first Maltese associate of the Royal Institute of Chemistry (1949) and the first fellow of the same institute (1957). He was succeeded by Professor W.G.H. Edwards in 1960.

Professor Edwards had a purely chemistry background. His appointment to the post of Professor of Chemistry in the Faculty of Science ensured that the traditional links between chemistry and medicine were truly severed. His experience in academic and industrial chemistry ensured that the chemistry module offered by the Faculty of Science could improve its standards to European levels with the inclusion of all the formal branches of chemistry, i.e. physical, organic and inorganic chemistry. The Faculty of Science received a further impetus with the transfer of the Department of Chemistry from Evans Buildings in Valletta to the new laboratories at the Tal-Qroqq Campus in 1968. The Department of Chemistry also promoted post-graduate studies with research activities in various fields, including the Orobanch Research Project funded by the U.K. Overseas Development Fund. A number of the early chemistry graduates from the Faculty of Science proceeded

Year	Honorary Graduate
1947	❖ Prof. R.V. Galea
1954	❖ Prof. E. Lapira
1960	❖ E. Medi
1965	❖ Sir R. Bradlaw
1984	❖ Prof. H.M. Gilles
1987	❖ Dr. J.H. Mercieca
	❖ Dr. A. Pardo
	❖ Prof. A. Prophet
1988	❖ H.E. Mr. J. Perez de Cuellar
1989	❖ Prof. F. Vella
1993	❖ Dr. G. Zammit Maempel
	❖ Prof. A. Zichichi
1996	❖ Mr. P. Pistorio

Table 2. Honorary Graduates awarded "Doctor of Science" by University of Malta

overseas to read for post-graduate doctorate degrees in chemistry. The return of these doctorate-qualified Maltese in the late 1960s and early 1970s saw the gradual transition from expatriate academic staff to Maltese academic staff. During Professor Edwards's tenure, Chemistry became more popular with students attending the Faculty of Science at the Royal University of Malta. The science student population in 1973 amounted 123, of whom 88 (75.6%) were studying chemistry as one of their main subjects (Anon., 1973). Prof. Edwards was succeeded by Prof. V. Ferrito in 1976. Prof. Ferrito joined the Faculty of Science in its early years in 1959 from where he graduated B.Sc. and M.Sc. He pursued his doctoral studies at the University of Swansea, Wales, graduating a Ph.D. in 1968 (Schjivone, 1997). Besides Prof. Ferrito, a further eleven Maltese who read chemistry at the University of Malta have now attained a Ph.D., the majority from Universities in the United Kingdom with sponsorships by Rhodes, Commonwealth or University Scholarships.

Attempts were also made to promote an interest in Chemistry by the setting up of academic societies and the publication of journals. In 1948, Prof. Farrugia founded the Royal University of Malta Chemical Society with the scope of promoting an interest in chemistry, publishing its journal *The Chemist*. In May 1967, the RUM Chemical Society issued its second magazine *Orbital*, which continued publication for a year with the publication of three issues. The society persisted with its activities until 1969, the last activity in April 1969 being a Science Exhibition organised jointly with the Biological Society of the Royal University of Malta. After a period of hibernation, the various then-dormant science societies amalgamated in 1972 to form the Royal University of Malta Science Society. This included as members all the science students, academic and technical staff of the Faculty of Science as well as the University's Junior College (the latter students had organised their own science society - the Junior College Science Society in 1971 and published their own magazine *The Neutrino*. The Junior College Science Society survived until the educational reforms of 1974 whereby the Junior College administration shifted from the University to the Government Department of Education). The RUM Science Society issued another newsletter *Hybrid* in

1972. This ran for only a few years (Savona-Ventura, 1997; Ferrito, 1972).

Science education in Malta received another set-back with the education reforms engendered in 1980 when the Faculty of Science was abolished and amalgamated in the Faculty of Education as the Department of Mathematics and Science. The Faculty of Science was again re-established with the 1988 Education Act, which enabled the University to award Bachelor of Science and Master of Science degrees, and also Doctor of Science honoris causa degree, although the earliest award of a D.Sc. was awarded in 1947. The University science students set up the University Science Students Association (Univ., 1999). Interest in scientific matters continued to increase and a need was felt for a forum where graduates from various fields in science could freely discuss multi-disciplinary topics. The Malta Chamber of Scientists open to any graduate with a primary science degree, including medicine, dentistry and pharmacy, was inaugurated on the 28th July 1994 and issued its journal *Xjenza* in 1996 (Felice & Muscat, 1996).

#### References

- Agius T (1932) University of Malta. In: *Reports on the Working of Government Departments during the financial year 1930-31*. section k pp. 1-9, Government Printing Office, Malta.
- Anonymous [Anon.] (1973) About Professor Edwards. *Hyphen* 4,7
- Cassar P (1964) *Medical History of Malta*. Wellcome Historical Medical Library, London.
- Cassar P (1969) Two centuries of Medical Prescribing in Malta 1683-1882. *St. Luke's Hospital Gazette*, 4(2),105-112.
- Cassar P (1974) Surgical Instruments on a tomb slab in Roman Malta. *Medical History*, 18,89-93.
- Cassar P (1974a) A Medico-legal report of the sixteenth century from Malta. *Medical History*, 18,354-359.
- Cassar P (1976) Inventory of a Sixteenth Century Pharmacy in Malta. *St. Luke's Hospital Gazette*, 11(1),26-34.
- Cassar P (1986) Physiological and Pathological Research at the General Military Hospital of Valletta, Malta, in the early Nineteenth century. *Medi-scope*, 9,18-33.
- Fabrizi P (1841) *Sopra alcuni punti relative alla rinoplastia*. Izzo & Co., Malta.
- Felice AE and Muscat R (1996) The Malta Chamber of Scientists - Second Anniversary. *Xjenza*, 1(1),4-5.
- Ferrito V (1972) Address by the president of the Science Society. *Hyphen*, 1,6-7.
- Fiorini, S (1988/89) A Prescription list of 1546. *Maltese Medical Journal*, 1(1),19-31.
- Freller T (1997) *Cagliostro and Malta*, pp.26, 48-52, Colour Image, Malta.
- Government of Malta [G.o.V.] (1959) *Development Plan of the*

- Maltese Islands for the years 1959-64*. Government Printing Press, Malta.
- Gulia G (1871-72) *Ostetricia: Sulle convulsioni puerperali. Esame chimico-clinico della urina dei vaiuolosi. Reativo dello zucchero nell'urina. Il Barth*, 1(3), 45-48; 9(1) 87.
- Hardman W (1909) *A History of Malta during the periods of the French and British occupations 1798-1815*, pp. 86, Longmans, London.
- Hertherington, H et al (1957) *Report of the Commission: July, 1957*. Royal University of Malta, Malta.
- Lonchamp J.P (1993) *Science and Belief*. Society of St.Paul, Guernsey.
- Mackay C (1841) *Extraordinary popular delusions and the madness of crowds*, pp. 232-240, R. Bentley, London, [1995 reprint Wordsworth, Hertfordshire].
- Magro E (1914) Office of Public Instruction. In: *Reports on the Working of Government Departments during the financial year 1913-14*. section M pp. 1-7, Government Printing Office, Malta.
- Savona-Ventura C (1997) Farrugia, Philip (24.8.1900-29.12.77) Professor of chemistry. In: *Maltese Biographies of the Twentieth Century* (Eds. MJ Schiavone and LJ Scerri), pp. 254, PIN, Malta.
- Savona-Ventura C (1999) *L-Istorja tal-Medicina fil-Gzejjer Maltin*. PIN, Malta.
- Savona-Ventura C and Sammut M (1998) Lead in the Maltese Environment and its significance to man. *Journal of EuroMed Pharmacy*, 1(4),8-13.
- Schinas C (1838) *L'Ape Melitense*. 1,25,132.
- Schiavone MJ (1997) Ferrito, Victor (21.9.41-) Scientist. In: *Maltese Biographies of the Twentieth Century* (Eds. MJ Schiavone and LJ Scerri), pp. 265, PIN, Malta.
- Vella HCR (1999) Introduction. In: *Joseph Demarco. On the passage of air from the lungs into the blood system*. University Press, Malta.
- Filologo Maltese* (1841), 17<sup>th</sup> June, pp. 45.
- Universtity [Univ.] (1999) *University Calendar*. University of Malta, Malta.
- Zammit T (1896-1900) In: *Public Health Department: Reports for 1896-1899* (Ed. SL Pisani), 4 vols, Public Health Department, Malta.

---

## Abstracts

---

### **INDUSTRY AND ENVIRONMENTAL MANAGEMENT SYSTEMS IN MALTA - A FIRST APPRAISAL**

Shawn Abela  
Supervisor: Victor Axiak

Together with the construction and tourism industry, the manufacturing industry has been one of the major causes of the rapid rise in pollution witnessed in the past three decades. Initial efforts towards dealing with industrial pollution concentrated on 'end-of-pipe' treatment methods. As environmental regulations became more stringent, greater demands were made on companies to enhance their overall environmental profile. The result was a general shift from pollution control to pollution prevention. This change in focus has resulted in the development of cleaner technology options and environmental management systems.

The manufacturing industry accounts for 27% of Gross Domestic Product (GDP) of the Maltese Islands. In spite of this, studies on industrial development within an environmental framework are very sporadic. The objective of the current study, which was carried out within the ambit of an extensive E.U. funded programme aimed at introducing the Environmental Management and Audit Scheme (EMAS) into Small and Medium sized enterprises (SMEs), was to investigate industrial activity in relation to environmental performance. A total of 51 SME's participated in the EMAS project, but in view of time and budget constraints only 5 of the participating SMEs were studied intensively.

The first part of the study aimed at examining the current attitude held by local industrialists in relation to environmental issues. This involved reviewing questionnaires disseminated to the 51 SMEs participating in the EMAS project. The most salient feature emerging from this part of the study was that locally, industrialists still view wastes as rejects of little economic value. Recycling and resource reclamation are concepts which have not yet fully penetrated the local industrial society. In fact 61.9% of the firms reported that only 0-25% of wastes generated are reclaimed.

The second part of the study aimed at identifying areas within individual companies associated with waste generation and quantification of these wastes. For the first time this study reports the results of laboratory analysis carried out on the liquid waste streams emanating from four different industries. The study also presents the application of an environmental performance indicator as a tool that could be used locally to compare the degree of pollution caused by different firms.

This study has shown that industrial activities have a

substantial impact on the environment, both in terms of the raw material and energy that these require, and in terms of the waste products and emissions that are generated as a result of their operations. Of particular interest on the process inputs side, were the high demands on water consumption made by the foods industry and the plastic industry. The bacon and meat industry studied consumes 15,000m<sup>3</sup> per year, the dairy products industry consumes 17,364m<sup>3</sup>, while the plastic industry consumes 100,000m<sup>3</sup>.

Also of interest were the high BOD5 levels associated with the liquid waste stream from all industries. The highest levels were those recorded in the slaughtering industry, where BOD5 reached 10,555mg/l.

Another feature that emerged from this study is that two of the five industries studied still make use of ozone-depleting chlorofluorocarbons such as the degreaser Freon R113 and the refrigerant gas R11.

From an occupational standpoint, one of the major concerns was related to the high levels of noise associated with certain machinery in the shop floor, where noise sometimes exceeded the upper BS 4142 (1990) limit of 85dBA, the level beyond which auditory damage happens at an accelerated rate. The noise problem was encountered in all five companies investigated.

This study is particularly pertinent because it has indicated areas within specific firms where improvement is required and identified those areas that can be optimised. On a wider scope, this study has highlighted the need for preventive approaches by local industry and has introduced the concept of cleaner production, which is internationally viewed as the central element for industry to achieve environmental improvements while remaining competitive and profitable. Being the first one of its kind, this study is presented as a tool that can serve as a baseline for future studies where compliance environmental monitoring might be required as a platform to the implementation of an environmental management system.

### **THE POTENTIAL USE OF OPUNTIA EXTRACT IN THE REGULATION OF SUGAR METABOLISM**

Claire A Baluci

Supervisor: Carmelo Agius

It is of great importance that blood glucose in humans is maintained at a rather precise concentration. Many factors affect the circulating levels of glucose such as food intake, rate of digestion, excretion, exercise, psychological state and reproductive state. Diabetes

Mellitus represents a syndrome with disordered glucose metabolism and inappropriate hyperglycaemia due to either an absolute deficiency of insulin secretion or a reduction in its biological activity, or both.

Several are the pharmatherapeutical treatments used to try and normalise metabolic activities, namely glucose levels. The use of drugs is however limited due to adverse side effects that may cause incompatibility with other medicaments, and they also become less effective with prolonged use. Due to such limitations, there has been, and still remains, great interest in the use of alternative treatments. In traditional practices, various medicinal plants are used to control diabetes, where the hypoglycaemic effect of such plants has been confirmed. The prickly pear cactus, *Opuntia* spp., appears to be one of the most promising sources of plant-derived sugar metabolism regulators.

A wide variety of substances increase insulin secretion, especially sugars and amino acids. In some animals fatty acids or ketone bodies may be relatively important as physiological secretagogues. The aim of the experimental work carried out was to determine whether any of the major sugars or amino acids known to be potential insulin secretagogues are present in *Opuntia* extracts. High performance chromatography was the selected technique for amino acid analysis. A reverse-phase HPLC method using pre-column derivatization with phenylisothiocyanate (PITC) was found to be suitable for accurate determination because of its high sensitivity, simplicity, speed and reliability. For carbohydrate analysis, the analytes concerned lack convenient chromophores, and therefore the ease of using post-chromatographic chemical reactions with *p*-anisidine phthalate and aniline phthalate for their determination was a primary reason for choosing thin layer chromatography.

Results from HPLC and TLC analysis showed that none of the important insulin secretagogue carbohydrates and amino acids were present in significant amounts, and therefore, the hypoglycaemic effects of *Opuntia* extracts must be due to some other complex mechanism. Various studies regarding the significance of heat shock proteins in diabetes, the effects of *Opuntia* extract on blood glucose levels, and the effects of *Opuntia* on blood heat shock protein levels are also reviewed.

There is significant evidence that *Opuntia* extract can control sugar metabolism, and the observed anti-hyperglycaemic effects may possibly be due to the strong protective effect *Opuntia* has against physical and physiological stresses. The ability of *Opuntia* extract to increase levels of heat shock proteins, where usually an inability to mount such a protective stress response occurs in diabetic subjects, may avoid the promotion of islet cell death and thus initiation or propagation of the disease process.

## THE SESSILE EPIFAUNA OF A MAERL GROUND OFF THE NORTH-EASTERN COAST OF MALTA

Katielena Camilleri  
Supervisor: Patrick J. Schembri

Maerl beds are accumulations of unattached calcareous rhodophytes forming nodules known as rhodoliths, together with their debris and other sediment, and are of international conservation significance. In the Maltese islands, the largest such bed covers some 20km<sup>2</sup> of bottom between Ras il-Qala on the south-eastern tip of Gozo and St. George's Bay on the north-eastern coast of Malta. Here, maerl forms a transitional sedimentary bottom between the lower infralittoral and upper circalittoral zones.

Maerl beds support a diverse biota and may be significant nursery grounds for commercial species of fish and shellfish. However, ecological studies on the biota of maerl beds are scant, especially for European seas. The present study is concerned with one component of this biota - the sessile epifauna. Epifauna are animals living on the surface of a substratum. The aim of the present study is to characterise the sessile epifaunal assemblages that colonise rhodoliths and pebbles occurring in the maerl bed off the northeastern coast of Malta

Attention was focused on two areas within the maerl bed that are subject to different levels of anthropogenic disturbance. Periodic trawling was believed to occur in the site designated as 'Impacted' while the 'Control' site is not trawled. In a recent study Borg et al. (1998) have shown that trawling has no significant negative effects on the maerl ecosystem at these two sites. However, many pieces of evidence (differences in the amount and size of rhodoliths, in sediment composition, and in the abundance of certain biota) suggested that the 'Impacted' area is subjected to a higher disturbance regime than the 'Control' area. The source of the disturbance is difficult to identify but it might be due to one or more of these factors: (1) currents or episodic storm-induced disturbance, (2) bioturbation by large fish and (3) low-intensity trawling.

Sessile epifauna was studied on dry specimens of rhodoliths and pebbles collected using a van Veen grab as part of a previous study. For each season, four grabs were examined, two replicate grabs from the 'Control' site and another two from the 'Impacted' site. In the case of pebbles, which occurred in lower numbers than rhodoliths, three replicate grabs per season were sometimes examined.

A total of 4267 epifaunal organisms belonging to 45 species were recorded on 1698 rhodoliths examined, while 1352 epifauna organisms belonging to 35 species were recorded on 668 pebbles examined. The higher species richness on rhodoliths was attributed to their complex three-dimensional architecture, which provides a variety of different microhabitats and refuges for settling propagules. The two substrata (rhodoliths and pebbles) were found to support similar epifaunal groups,

which included bryozoans, serpulid polychaetes, sponges, foraminiferans, molluscs, hydroids and an unidentified 'brown tube'. Bryozoans and polychaetes were the most abundant groups on rhodoliths with a population density of about 11 individuals/100cm<sup>2</sup> of rhodolith surface.

On pebbles, the polychaetes were the dominant colonisers with an average density of 21 individuals/100cm<sup>2</sup> of pebble surface. Multivariate statistical analysis (cluster analysis and non-metric multi-dimensional scaling) showed that different rhodolith morphotypes supported different suites and densities of sessile epifaunal species. This is related to the different structural complexity of the different morphotypes. The close-branched morphotype F had the highest species richness (40 species) while the laminar morphotype C had the highest population density (170 individuals/100cm<sup>2</sup> of rhodolith surface).

Cluster and MDS plots revealed a difference between the 'Impacted' and 'Control' sites in terms of presence and abundance of epifauna on rhodoliths and pebbles. On rhodoliths, 44 different species with an average population density of 17 individuals/100cm<sup>2</sup> were recorded from the 'Impacted' area. For the 'Control' site, the equivalent figures were 37 species and 43 individuals/100cm<sup>2</sup>. These differences were attributed to the different microenvironments and different disturbance regimes present at the two sites. A chi-square ((2) test showed that there was no significant difference in the distribution of the epifauna between two arbitrarily chosen surfaces of the rhodoliths from the 'Impacted' area. This implied that periodic overturning of rhodoliths occurs in this area. However, for the 'Control' site the epifaunal species were preferentially distributed on one surface of the rhodoliths, implying that here the rhodoliths are somewhat more stationary than at the 'Impacted' site. A difference in the distribution of epifauna also occurs between the thallus and crevice microenvironments of rhodoliths. SIMPER analysis identified that such dissimilarity was mainly due to the serpulid *Spirorbis* sp., which was found predominantly on the exposed thalli.

Although pebbles offer similar microenvironments in the 'Impacted' and 'Control' sites, a difference in the presence and density of epifauna on this substratum resulted between the two sites: 31 species with an average population density of 33 individuals/100cm<sup>2</sup> of pebble surface were recorded from the 'Impacted' area, while 20 epifaunal species with an average density of 25 individuals/100cm<sup>2</sup> of pebble surface were observed from the 'Control' area. However, the higher epifaunal population density at the 'Impacted' site was not found to be statistically significant.

No seasonal patterns in the abundance of epifauna were recorded for either rhodoliths or pebbles at the two sites.

This is the first detailed study carried out on the sessile epifauna of any Mediterranean maerl bed. Most of the epifaunal species recorded are first time records for Maltese waters.

## A STUDY OF THE NON-CORALLINACEOUS MACROPHYTES, AND THEIR EPIBIOTA, FROM A MAERL GROUND OFF THE NORTHEASTERN COAST OF MALTA

Ritianne Cilia

Supervisor: Edwin Lanfranco

The term 'maerl' refers to biogenic sediments composed mainly of living and dead unattached, non-geniculate, coralline algae and their debris. These coralline algae form rhodoliths, which are nodules or unattached branched growths. The morphology of rhodoliths varies from open branched thalli to densely branched spheroidal bodies. Their complex structure provides a range of microenvironments for attached biota. In order to achieve their typical concentric growth, rhodoliths need to be regularly overturned so that all their surfaces are periodically exposed to light and would thus be able to photosynthesize. These structurally complex, perennial habitats form isolated habitats of high benthic biodiversity and biomass and some support rare, unusual or otherwise interesting species of benthic organisms and as such they are of particular international conservation interest. Maerl beds are a complex habitat composed of a number of strata or layers. The rhodolith thalli together with the non-living sediment make up a lower layer which provides a substratum for many animals as well as anchorage for the non-corallinaceous macroalgae which form an overlying stratum. The objective of this study was to characterise this stratocoenosis, that is, the non-corallinaceous macrophytes and their epibiota. To date there are no published studies on this assemblage in Malta.

Three grab samples were collected from two grounds - termed 'impacted' and 'control' which seem to have different disturbance regimes. Sampling was carried out once every three months (seasonally) on both grounds. The non-corallinaceous macrophytes were sorted from the seasonal grab samples and identified. They were also classified according to the type of substratum on which they were growing. The abundance of the macrophytes was estimated as wet weight, however, for the densely branched, filamentous, delicate, creeping rhodophyte *Polysiphonia setacea* which was often found entangling rhodoliths, the abundance was determined as percentage coverage. The distribution of the epibiota growing on the three most abundant erect macrophytes, *Flabellia petiolata*, *Vidalia volubilis* and *Codium bursa*, was studied by estimating the percentage cover of each species encountered and recording which part of the algae it grew upon.

Macrophytes belonging to 43 species were found growing on the maerl bed with the following taxonomic composition: Rhodophyta (56%), Phaeophyta (30%), Chlorophyta (12%) and Cyanophyta (2%). On both grounds, the most abundant species in terms of biomass were *Flabellia petiolata* (8.3421g mean wet wt/0.1m<sup>2</sup>), *Vidalia volubilis* (0.6427g mean wet wt/0.1m<sup>2</sup>) and *Cystoseira corniculata* (1.1543g mean wet wt/0.1m<sup>2</sup>). There was no significant difference between the control and impacted grounds in the biomass of these

macrophytes. However, the slow-growing chlorophyte *Codium bursa* was not found on the impacted ground. Overall, no seasonal patterns in abundance of the non-corallinaceous algae were observed.

The occurrence of non-corallinaceous macrophytes at depths between 43m and 56m implies the presence of primary production at very low light intensities. This primary production is in addition to that of the maerl-forming coralline algae which are known to be able to photosynthesise at very low light intensities. Many of the non-corallinaceous macrophytes species encountered in this study, such as *Flabellia petiolata* and *Dictyota dichotoma*, are known to produce secondary metabolites that deter grazing by herbivores. Thus, this primary production is probably not consumed in situ and enters the food web via the detrital pathway. In this aspect, the maerl ecosystem resembles the highly productive and species-rich Mediterranean *Posidonia oceanica* meadows.

Of the non-corallinaceous macrophytes, 43% by mass were found growing on rhodoliths while 57% were found growing on the non-living sediment. The latter was often stabilized by the rhizoids of *Flabellia petiolata* and the creeping, filamentous thalli of *Polysiphonia setacea*, forming a pseudo-hard 'crust', thus changing the substratum into a more stable type allowing the settlement of other algae. However binding by these algae also immobilized the rhodoliths. This may lead to a reduction in the growth and development of the rhodolith-forming algae since they are prevented from turning - a requirement for their survival if they are to photosynthesise and not become buried by sediment.

In addition, algae that grow epiphytically on the rhodoliths may lead to their death by decreasing the amount of photosynthetically active radiation reaching the algal tissues through shading, and perhaps through competition for nutrients. Moreover, the creeping filamentous algae such as *Polysiphonia setacea* can trap sediment, creating an anoxic boundary-layer around the thallus, smothering it and limiting rhodolith growth.

The epibiota growing on the three basiphytes *Flabellia petiolata*, *Vidalia volubilis* and *Codium bursa* were analysed. A total of 83 species (23 epifloral species and 60 epifaunal species) were found growing on the 1487 individual basiphytes analysed. The epibiotic assemblages included bryozoans, foraminiferans, serpulids, hydroids and rhodophytes. These are similar in type to the well-known and diverse epibiotic assemblages of the sea-grass *Posidonia oceanica*: Bryozoa had the highest faunal species richness (26 species) while with respect to the flora, Rhodophyta had the largest species richness (11 species).

The abundance of epibiota on *Flabellia petiolata* was low (3.4% coverage) possibly due to the short turn-over time of the individual host, which is known to shed its thallus periodically providing a dynamic substratum for the epibiota. Grazers therefore have a relatively poor food resource and therefore the number of grazers in these systems is expected to be low. No significant difference in the percentage coverage of epibiota was found

between the impacted and control grounds, which is not unexpected, since the basiphyte is offering the same type of substratum on both grounds. The same results were obtained for the basiphyte *Vidalia volubilis*. A significant difference was found in the distribution of epibiota on the fan-shaped blade of *Flabellia petiolata* as compared to its cylindrical stipe; the former offers more surface area for attachment and growth.

During this study three species of rhodophytes, *Aglaothamnion tenuissimum*, *Myriactula rivulariae* and *Myrionema magnusi*, that were previously unknown from Malta, were recorded for the first time.

## THE ASSESSMENT OF HEAVY METAL POLLUTION IN MALTA

Henrietta Debono  
Supervisor: Victor Axiak

Heavy metals are elements that are found naturally in seawater. They may, however, be present as a result of contamination caused by a variety of sources, mainly anthropogenic. Metals may exert toxic effects if present in sufficiently high concentrations.

The concentrations of heavy metals in the marine environment may be monitored through chemical analysis. In the last decade a new approach called biomonitoring has been used. This approach involves the measurement of a biological response that an organism exhibits as a result of stress factors. One such biomarker is metallothionein. Metallothionein is a low molecular weight, cysteine rich protein that is induced by heavy metals. A number of methods have been exploited to measure the concentrations of this protein. One of the most modern and simple methods is the spectrophotometric assay developed by Viarengo et al., in 1997. This assay was used successfully for the measurement of total metallothionein concentrations in the limpet *Patella rustica* by Pace in 1998.

In the present investigation, the spectrophotometric assay was used once again to measure the total MT levels in *P. rustica* collected from a total of ten sites along the coast of the Maltese Islands. The locations range from sites of waste and sewage disposal to areas used for fuel storage.

The results from biomonitoring by means of the spectrophotometric assay confirmed further the trends and 'hotspots' revealed by Pace (1998) namely, the gradient of pollution along the Wied Ghammieq-Xghajra-Marsascala coastal stretch, Bahar ic-Caghaq and Manoel Island. It also aided in the identification of new 'hotspots' of metal pollution. These are Ras il-Hobz (not to the same extent as Wied Ghammieq), and two sites along the coast of Birzebbuga, the Freeport Area and the area in the vicinity of the Fuel Storage facilities.

Although statistically significant seasonal fluctuations were identified only in the Fuel Storage area, the trends in seasonality were clearly evident. MT levels vary

seasonally according to the levels of some essential metals in the tissues of organisms, reflecting the physiological role of that some metals may possess. MT levels also vary among locations, indicating that the degree of impact by heavy metals is different in each location.

Confirmation of the results produced by the previous investigation provides good ground for use of the spectrophotometric assay in future biomonitoring programmes. Such programmes should be performed at least once yearly in order that the concentrations of heavy metals and other compounds are kept under constant surveillance.

## **A STUDY OF THE MACROBENTHIC ASSEMBLAGES IN THE VICINITY OF A MARINE FISH-FARM**

Mark Dimech

Supervisor: Patrick J. Schembri

Aquaculture in the Maltese Islands has developed mainly in sheltered embayments. Here, the bottom tends to be sandy some distance away from the shore, and the dominant benthic communities are sea-grass meadows. In shallow water these meadows are formed either by *Cymodocea nodosa* or are mixed meadows of *Cymodocea* and *Posidonia oceanica*. As the water becomes deeper, the latter species forms extensive monospecific stands. Most fish-farm cages therefore are located above these meadows. The importance of sea-grass meadows has long been recognised and is due to their high productivity, high diversity of the associated biota, stabilisation of the sediment, oxygenation of the water column, and their function as buffers for wave action and nursery grounds for a variety of species, some of which are commercially exploited. The aim of this study was to determine what effect, if any, fish-farm cages are having on local benthic assemblages, especially *Posidonia oceanica* meadows.

The benthic assemblages associated with *Posidonia oceanica* meadows were studied in the vicinity of a marine fish-farm located at Mistra Bay, north-east Malta. During the month of August 1998, six benthic stations arranged along a line transect at exponentially increasing distance from the fish-farm cages were sampled. Another station on the eastern side of the bay, far from the farm, was also sampled. Samples were collected using a specially constructed corer. This consisted of a 35cm diameter metal pipe fitted at one end with a 0.5mm mesh net, and having a serrated edge at the other. Divers pushed the corer into the bottom with a sawing action and it was able to cut through even *Posidonia* mat. The cores were then pushed through the corer and into the mesh bag. The core samples were sorted for biota, and this was preserved, identified and counted. Environmental parameters, including several related to the phenology of *Posidonia oceanica* and to the sediment, were measured.

A total of 1196 individuals belonging to 107 species of

macrofauna were identified. The application of multivariate analytical techniques showed that the benthic macrofauna was distributed into three distinct zones. Zone I comprised stations in the first 20m linear distance from the markers delimiting the boundaries of the cages, Zone II included those stations between 20m and 160m from the boundary markers, and Zone III stations at a distance greater than 320m from the boundary markers. The distribution of the benthic macrofauna in these zones was found to be related to the composition of the sediment, which in turn was related to the sedimentation of particulate organic matter from the fish-farm cages.

Zone II was slightly enriched with organic matter originating from the fish-farm. This slight enrichment seems to have a beneficial effect on the benthic assemblages: comparison of Zone II with Zone III (the clean zone) showed that a relatively higher species richness and abundance was present in the former.

From an analysis of the feeding type of the most abundant species that contributed to the observed zonation, it resulted that most of the ones in Zones I and II were feeders on particulate organic matter. The species within Zone III had more diversified food sources.

For *Posidonia oceanica*, the total number of leaves per shoot did not vary significantly with distance from the farm boundary, however, the number of adult leaves per shoot was significantly lower in Zone I. Also in Zone I, the total leaf length and the adult leaf length were nearly half the values in Zone III. Shoot density increased significantly with increasing distance from the farm boundary. Epiphyte load on the *Posidonia* leaves decreased with distance from the farm boundary.

In general, the fish-farming activity in the bay studied altered considerably the state of the *Posidonia oceanica* meadows, as well as the species richness and abundance, and the structure of the macrofaunal assemblages up to a distance of 160m from the cage boundary. The impact of a point source such as a fish-farm may be quite severe close to the farm.

## **BIOLOGICAL ANALYSES FOR THE CONSERVATION OF PIPISTRELLES (PIPISTRELLUS SPP.) IN MALTA**

Krista Falzon

Supervisor: Adriana Vella

In order to tackle conservation aspects of any organism, some knowledge is required about the biology of the species. Little work has been carried out on bats in Malta and therefore data was gathered in as many areas as possible regarding the biology of pipistrelles.

Roosts were located and monitored. Various environmental parameters were noted when surveys were undertaken, including light intensity. The local mean time at which pipistrelles left the roost was also

noted. Seasonal changes in behaviour were tested with respect to environmental parameters that affected the time at which pipistrelles left the roost. For two of the roosts studied, temperature had a significant effect on emergence of pipistrelles. One of the roosts showed a relationship with wind. Light intensity was also shown to be related to when the pipistrelles left the roost. This was concluded as there was no significant change observed in light intensity when pipistrelles left the roost between seasons. Time of emergence was, however, seasonal so this was not a dependent factor influencing when pipistrelles left the roost. The light intensity of emergence of pipistrelles was then compared between three roosts which did show a significant difference.

Foraging habitats were also studied according to habitat type and also seasonal differences. Upon carrying out statistical analyses, both the habitat type and season were seen to have an affect on pipistrelle numbers. Simar, a nature reserve, was found to be the preferred foraging habitat. Urban areas were the least preferred habitats. Urban areas might be expected to be ideal foraging areas due to high insect abundance. Thus, the fact that pipistrelles seemed not to choose to forage in these habitats may indicate a degree of disturbance met by pipistrelles in urban habitats.

Another aspect of pipistrelle biology studied was their diet. Faecal analyses was carried out in order to be able to obtain an indication of the insect orders which pipistrelles preyed upon and whether or not the former had any impact on the health of pipistrelles since most are found to be agricultural/human pests controlled using pesticides. A disadvantage of using this method to analyse pipistrelle diet involves bias in prey representation. Thus, prey with body parts that are not digested may be over represented in the faeces. Also, certain structures, for example, lepidopteran scales may remain in the digestive tract for some time before being represented in the faeces. However, the aim in this study was to obtain an idea of insect species that pipistrelles prey on rather than a complete picture. The method is reliable due to the fact that the hard structures found in the faeces are often (though not always) intact. Killing of pipistrelles to examine stomach content was avoided since this work aimed at their conservation and it was desirable to disrupt populations found.

Pipistrelles were captured and ringed twice from the same roost. The capture was carried out in two different seasons. Various body measurements were carried out before release of the animals. A difference in average forearm length of females with respect to males was noted. A significant difference in body weight was observed between the two seasons. Capture was kept to a minimum in order to minimise disturbance to the animals.

After analysing these various aspects in pipistrelle biology conservation strategies were suggested. Legislation and education are important factors and the place to start. Sites for conservation should be assigned and strategic planning is needed. Setting up bat boxes helps raise public awareness as well as providing alternative roosts for pipistrelles. The use of pesticides

should be reviewed and timber treatments where pipistrelles are known to roost should be avoided due to their toxicity.

Finally, areas for further research were outlined and the importance of monitoring populations stressed.

## **FOOD AND FEEDING HABITS OF JUVENILE AMBERJACKS, *SERIOLA DUMERILI* (RISSO, 1810) IN THE CENTRAL MEDITERRANEAN SEA**

Mark Gatt  
Supervisor: Carmelo Agius

Although both immature and adult specimens of the amberjack (*Seriola dumerili* Risso 1810) are of great commercial value, and have been acknowledged as a species with high potential to aquaculture, very little is known on the ecology and life history of this fish. The main approach of this study about *Seriola dumerili* involved stomach content analysis, since much of the current understanding of the ecological role of fish populations is derived from studies of the diet based on the analysis of stomach contents.

In this study, offshore samples (from 50 - 100 miles off the coast of Gozo) were provided by means of purse seine nets and the coastal samples were provided by means of trolling lines. Juvenile amberjacks appear in Maltese offshore waters, around FADs (Fish Aggregating Devices or kannizzati), from late August to early December. As the aim was to examine fish of different sizes, sampling started on the 31st of August, and stopped on the 25th November. Sampling of coastal fish also stopped around this time, as fish of similar sizes to those caught from offshore waters were needed for comparison purposes. A total of 264 fish were sampled from the two different habitats. These fish were divided into 4 size classes.

Results of the stomach content analysis showed that the diet of the offshore size classes 1 and 3 (16-26cm standard length) consisted mainly of Crustacea, particularly those of the zooplankton community. Fish increased in importance as the offshore fish grew larger, and these started to replace planktonic Crustacea in accordance to the weight eaten, as indicated by size class 4. Results of the coastal size classes showed that diet shift is strongly related to habitat in addition to size. Coastal fish showed different diet preferences when compared with offshore size classes of the same size. Offshore group 3, (22-26cm standard length) which was planktivorous, contrasted with coastal group 3, which was completely piscivorous, preying almost exclusively on fish of the Family Clupeidae. Offshore size class 4, (26-31cm standard length) which was showing a slight shift towards a piscivorous diet, contrasted more lightly with coastal size class 4, which had solely piscivorous habits as coastal size class 3.

The planktivorous phase in offshore fish, sampled from deep waters was much longer than that found by other

authors. The latter, sampling fish from the shallow gulf of Castellammare in Sicily found that diet shift towards fish occurs at a size range of about 12-20cm in standard length. In this study, this diet shift started much later (at 26-31cm in standard length), and was not so marked as at this size plankton were still abundant in number. It is possible that the fish studied could have acted in a more opportunistic way rather than in a specific way due to the need to consume what is available in environments where food is in shorter supply than in shallower waters. This reasoning can also be supported, in a way, by the fact that coastal fish were complete piscivores, although having the same size range of offshore size classes 3 and 4. This could mean that due to energetic reasons, amberjacks prefer to shift their diets towards fish at a size range of about 12-20cm in standard length, as shown by other authors and the results of the coastal fish. This is because juvenile *Seriola dumerili*, try to find an optimal balance between the quantity of energy contained in the prey consumed and the energy necessary for their capture. Every time the balance between these two components becomes unfavourable, a change in diet is detected. In the case of *S. dumerili* this change correlates with the size of the individuals. However, the results of this study showed that this diet shift is also strongly related to the type of habitat, in addition to energetic factors involved, as in the offshore fish feeding behaviour had to change considerably, and a diet shift did not occur at the particular size range which was the most energetically favourable.

Offshore amberjacks also preyed on smaller fish than did the coastal ones. Fish prey size usually increases in relation to predator size, as seen in the coastal size classes, but in the offshore size classes. This can also mean that the latter were acting more opportunistically due to the nature of their environment.

A circadian feeding rhythm was also observed for this fish. Feeding started at dawn and reached a peak before noon. This was followed by a period of resting and then another peak was observed before dusk. Observations also showed that this fish does not feed at night.

### **ASPECTS OF THE BEHAVIOUR OF THE MALTESE WALL LIZARD *PODARCIS FILFOLENSIS* IN COASTAL AREAS**

Ronald Grech  
Supervisor: Patrick J. Schembri

The Maltese Wall Lizard *Podarcis filfolensis* is a species endemic to the Maltese Archipelago, Linosa Island and the islet of Lampione. Of the named geographical races of this lizard, *P. f. laurentiimuelleri* (Fejervary, 1924), is endemic to the islands of Linosa and Lampione. All other subspecies are found exclusively in the Maltese Archipelago. *P. f. maltensis* (Mertens, 1921), is found on the three main islands as well as on the islet of Cominotto and on Manoel Island which has been linked to the mainland by a bridge since circa 1750; *P. f. filfolensis* (Bedriaga, 1876), inhabits only the islet of Filfla; *P. f. generalensis* (Gulia, 1914), resides on

General's Rock, while *P. f. kieselbachi* (Fejervary, 1924) is found only on the larger of the two Selmunett Islands (or St. Paul's Islands).

In small or medium sized diurnal lizards, precise control of body temperature is primarily achieved by shuttling between microenvironments that offer different heating or cooling rates through varying degrees of exposure to solar radiation. Adjustment of body temperature within a characteristic 'activity temperature range' is thought to involve two classes of hypothalamic temperature sensitive receptors which determine the upper and lower 'set points' that define the body temperature at which heat seeking ( $T_{bask}$ ) or cooling ( $T_{move}$ ) behaviour are initiated.

A study of the behaviour of *P. f. maltensis* was conducted by taking scan samples and collecting faecal pellets from three populations in coastal areas on the island of Malta (Ta' Xbiex, Manoel Island and Bahar ic-Caghaq). Field observations on behaviour were made from June 1998 to March 1999 and human activity in the study sites was also monitored. The data collected were then analysed statistically in order to determine the behavioural patterns of the lizards and how these are affected by factors such as intraspecific interactions, temperature variations, substratum type and human presence.

*P. filfolensis* was found to be active throughout the nine months of the study period. Temperature and photoperiod influenced daily and seasonal activity patterns. Duration of the activity period varied from 2-3 hours in late winter to 12-14 hours in summer. The daily activity pattern in summer was bimodal with peaks during the early or mid-morning and late evening. In autumn, bimodal activity was less pronounced and a unimodal pattern with peak activity in the mid-morning was more common. Peak activity in winter was confined to the warmest time of the day.

In populations of *P. filfolensis*, a size-determined hierarchy governed dominance in intraspecific interactions. Time of morning emergence and nocturnal resting was found to depend on temperature and on the dominance of the individual lizard. Furthermore, a smaller weight to length ratio in juveniles and hatchlings indicated a faster heating rate, so these individuals were the first to emerge and the last to retreat for the night. Dominance was also shown to influence the daily and seasonal patterns of basking, foraging and hiding behaviours normally controlled by temperature. Subdominant individuals showed a delay (time-lag) in this thermoregulatory pattern with respect to dominant individuals. Different substrata are preferred during different times of the day and during different seasons. This is most probably related to thermal input from the substratum.

*P. filfolensis* is the largest and commonest carnivore on rocky shores in the Maltese Islands but rarely ventures into the bare zone. For this reason, predation on the supralittoral isopod *Ligia italica* was only occasionally observed, in spite of the extreme abundance of these crustaceans. Human presence had minimal effect on

lizard activity. Walls provide suitable habitats and coastal roads reduce immigration/emigration rates and predation pressure. Adult lizards are territorial and have a core area in which they spend most time. Up to 99% of the home range of two lizards was found to overlap spatially but temporal separation exists between dominant and sub-dominant lizards. Dominant lizards have larger or better quality home ranges. Male-female partnerships form in the mating season and a common territory is defended. Agonistic behaviour and physical aggression are especially prominent in the mating season.

*P. filfolensis* has a generalised diet and in times when arthropod food is scarce it can resort to scavenging and herbivory. Consumption of 'low cost' food such as ants, picnic left-overs and vegetative material was especially prominent in summer. Statistical analysis of the weight of faecal pellets reflected differences between habitats with different arthropod food resources. Seasonal changes in food type availability were reflected in this analysis and confirmed the visual observations made.

### **DISTRIBUTION AND ABUNDANCE OF BAT SPECIES IN MALTA: IMPLICATIONS FOR THEIR CONSERVATION**

Clarissa Jones  
Supervisor: Adriana Vella

The abundance and distribution of bat species found in Malta have been investigated using various surveying techniques between summer 1998 and spring 1999. The surveying techniques included line transects, repeated transects, roadside counts and point surveys. In addition to these surveys, cave visits and mist netting were carried out.

Distribution of all European bats has been investigated to create a list of potential bat species inhabiting Malta. Eighteen species of the thirty European bat species have their distribution crossing over the Maltese Islands. This study confirmed the presence of three species, and thus highlights the requirement for the conservation of this mammal.

The three species of bats, namely *Rhinolophus hipposideros* (The Lesser Horseshoe Bat), *Myotis blythi punicus* (The Lesser Mouse Eared Bat) and *Pipistrellus pipistrellus* (The Common Pipistrelle) were identified, using a bat detector and observational techniques. The latter species was found to be the most abundance and distributed species. In fact only pipistrelle species were detected during transect surveys. The other two species were always found in caves. The roosts of these cave-dwelling species were visited at least once per season during the study period.

This study allowed the overview of the status of these three bat species, and the results of this project recognised their status as being vulnerable in Malta. These bats are not only vulnerable in Malta but also throughout other European countries. Previous authors

have listed several other species said to inhabit this island. Unfortunately, the lack of detection of any of these species is not a good sign. All this points towards the requirement for effective conservation management designed specifically for the bat fauna of Malta, and this can be achieved by implementing all the conservation strategies mentioned in the previous section, but most importantly through further scientific research. Such conservation strategies require the workers or scientists to have at least a basic knowledge of the animal's biology.

### **EVALUATION OF CLEANING AGENTS USED IN ELIMINATION OF FOOD-BORNE BIOFILM**

Shirley Mifsud  
Supervisor: Carmelo Agius

Microbes adhered to surfaces have a tendency to form protective extracellular matrices called biofilms. International and local legislation of food hygiene, together with an increasing public awareness of product quality make it essential for food processing industries to have the most efficient cleaning methods and cleaning agents.

In this project a comparison of detergents (caustic soda) and different sanitisers (quatarnary ammonium compound, peracetic acid and chlorine solution) were carried out to study their antibacterial effects on the non-pathogenic *Streptococcus bovis* and the pathogenic *Escherichia coli* bacteria growing in biofilms. Two types of surfaces were used; stainless steel and rubber surfaces since these were identified as the main surface type employed in food processing equipment. The method used in studying biofilm build-up was a conventional cultivation using the swabbing technique followed by standard plate counts.

The results of this work showed that none of the cleaning agents or sanitisers tested in the recommended concentrations was efficient enough to eradicate all the bacteria growing in biofilms on the surface. It however showed that the mean log<sub>10</sub> reduction in biofilm was always higher for the *Escherichia coli* bacteria and for the biofilm growing on stainless steel surface. Comparisons of the 3 disinfectant when applied to a specific bacteria type on either surfaces showed a non significant result at the 0.05 level of significance meaning that, any of the disinfectants can be used against the biofilms tested with the same degree of efficiency.

A possible explanation for the higher mean log<sub>10</sub> reduction for *Escherichia coli* biofilm may be related to the structure of the two bacteria where the Gram-positive *Streptococcus bovis* have a thick slime capsule which lacks in Gram-negative *Escherichia coli* and therefore makes the latter more susceptible to the cleaning agents. Another possible explanation can be related to the presence of appendages (pili) on *Escherichia coli* that

might enhance attachment to the surface and therefore be more difficult to remove by swabbing giving the impression of higher bacterial reductions. This shows the limitations of the swabbing method. The results also showed the importance of surface topography in the food industry where a smooth surface such as stainless steel is much easier to clean, giving higher mean  $\log_{10}$  reductions than non smooth surface such as rubber.

As an appreciation of in vivo formation of biofilms, a food processing line was analysed for regions most likely to be involved in bioburden. The identified regions were listed as those requiring highest monitoring in terms of hygiene.

## ENVIRONMENTAL IMPACT ASSESSMENT OF THE MAGHTAB LANDFILL ON THE MARINE ENVIRONMENT

Mario Saliba

Supervisors: Victor Axiak & George Peplow

In the early 1977, the Maghtab landfill started receiving waste on a regular basis. The landfill covers an approximate area of 800,000m<sup>2</sup>. The original site incorporated a valley and the geological map of the area shows that Coralline limestone is present, which is of a higher porosity and higher permeability. The water table in that area has an underground hydrology with a piezo gradient predominately seawards. About 2,200 metric tons of waste enters the landfill daily. 75% of this amount is debris material. Combustible gases, produced by decomposing organic matter, and other combustible material give rise to localised burning as a result of spontaneous combustion. Smoke and pungent gases are liberated into the surrounding atmosphere, resulting in a nuisance pollutant to the surrounding environment.

The objective of the present work was to investigate the impact of the Maghtab landfill on the adjacent marine environment from heavy metal and petroleum hydrocarbon leaching. The metals analysed were lead, nickel copper, chromium, manganese, cadmium and arsenic. The assessment was undertaken through the analysis of marine sediments from the coastal area adjacent to the landfill, surface runoff water from the landfill, and borehole water samples from inflow and outflow regions to the Maghtab landfill. The assessment also included leachate simulation tests on waste collected from the landfill.

A review was undertaken of available literature on the Maghtab landfill and on the waste generated by governmental and private sectors.

Petroleum hydrocarbons (PHC) were analysed by UV spectrofluorometry and levels quantified in terms of chrysene equivalents.

Levels of lead, nickel and copper in marine sediments were higher than those at a control site, especially in

Qala San Marku, which always showed the highest concentration. Qala San Marku had maximum lead, nickel and copper concentrations of 127.24, 10.10 and 15.51 mg/kg Dry Weight (DW) respectively, whereas the respective control values were 4.62, 6.71 and 3.6 mg/kg DW. The levels for the other metals were below that of the control.

Petroleum hydrocarbon concentration decreased from summer to winter in all sampling sites. Qala San Marku had the highest of 48.44(g/g DW chrysene equivalents while the control value was 3.26(g/g DW chrysene equivalents. PHC pollution was found to be mainly due to anthropogenic activity related to the marine environment. Runoff water had very low values for heavy metals and petroleum hydrocarbon. Heavy metal values were less than 1mg/L runoff while petroleum hydrocarbon levels were less than 3.06µg/ml chrysene equivalents runoff water.

Borehole water showed a considerable increase from the inflow to the outflow samples for lead, nickel and copper. In an inflow region, lead, nickel and copper concentrations were 5.72 mg/kg DW, 7.62 mg/kg DW and below detection limit, respectively, while the outflow region the levels 9.56, 165.00, 19.02 mg/kg DW respectively.

The leachate results indicate that lead, nickel and copper are probably leaching in quantities higher than those for other metals.

The overall indication is that the landfill could be leaching lead, nickel and copper into the marine environment.

Other sources of heavy metals and petroleum hydrocarbon contributing to the pollution of the marine environment, apart from the landfill, such as lead from cars powered by leaded fuel adsorbing to road dust, were also discussed. Potential sources of these metals into the landfill are reviewed in the introductory chapter and later discussed in relationship to the indicative results. It may be pinpointed out that solid waste containing high percentage of nickel and copper does reach the landfill.

Malta currently requires the implementation of a waste management strategy with a priority in environmental protection. Monitoring of the existing landfill for assessing the environment impact will be an essential part of such a strategy. The results obtained during the assessment will therefore provide baseline values for further work related to the site studied and to environmental impact of the landfill. Constant monitoring over a number of years is required to strengthen the indications put forward in this study and to determine the contribution of the landfill with respect to these metal anomalies. The strategy has to focus on the principle of waste minimisation at source, reuse, recycling and composting. Characterisation of waste entering the landfill has to be performed in greater detail. The data collected by characterisation must also include the specific source and the actual amount it is providing. The process of characterisation must lead to the separation of hazardous waste from inert waste with the

consequence of different disposal procedures.

## LOCAL OIL POLLUTION: ESTABLISHING LEVELS, TRENDS AND FINANCIAL COSTS

Mark Scicluna Bartoli  
Supervisor: Victor Axiak

The study sets out to achieve two tasks. The first one is to carry out a comparative analysis of the levels of petroleum hydrocarbon pollution in sediments around the Maltese Islands, through the sampling of previously studied sites. Baseline levels of these contaminants for particular touristic bays, that have not been studied previously, were also identified.

The second task is to establish financial costs for specific oil spill pollution using a number of valuation methodologies. The four valuation methods chosen are the Production Valuation, Contingency Valuation, Travel Cost Valuation and Hedonic Valuation methods respectively. Realistic values of costs and incomes from various economic activities are used in order to provide some benchmarks of the aggregate costs of oil spill pollution.

The results of the levels and trends of petroleum hydrocarbon pollution indicate that oil pollution around the Maltese shoreline is on the increase. However, unlike past studies the present and immediate future threats are not originating mainly from industrial activity around the harbour area but from marine recreational boating activity around Malta's popular bays.

With regards to financial costing it has been found that the costs established by the four methods vary somewhat from each other. However, they strongly show that an approximate Lm2 million figure is a reasonable value, to associate with the overall cost of an oil spill accident in one of our more popular bays.

## THE STRUCTURE OF THE MOLLUSCAN ASSEMBLAGES OF SEA-GRASS BEDS IN THE MALTESE ISLANDS

Hassan M Howege  
Supervisor: Patrick J Schembri

Macrobenthic molluscs associated with meadows of the sea-grasses *Posidonia oceanica* and *Cymodocea nodosa* were sampled between November 1993 and November 1995 at about bimonthly intervals. Two sets of samples were collected from the *Posidonia* meadow at four depths (6m, 11m, 16m and 21m), one set using a suction sampler and the other, a hand-towed net. Samples were collected from the *Cymodocea* meadow at two depths (4m and 8m) using a suction sampler. Phenology of both sea-grasses was studied, and sediment granulometry and other sediment parameters were measured. Species composition, abundance and biomass of Mollusca were determined for each sample and community parameters

(species richness, diversity, evenness and dominance) were estimated. The molluscan assemblages were analysed using classification and ordination, analysis of similarities (ANOSIM), and similarity percentages (SIMPER).

The structure of the *P. oceanica* and *C. nodosa* meadows appeared to be influenced by water depth, resulting in distinct types of meadows at different depths. Shoot density of *Posidonia* decreased steadily with depth. The number of leaves per shoot varied more with season than with depth. Leaf length and width changed significantly with season but not with depth. The leaf area index (LAI) and leaf standing crop (LSC) showed significant seasonal changes; higher values were recorded in the warmer than in the cooler months. Only LAI showed significant variation with depth. For *Cymodocea*, shoot density varied significantly with depth, but not with season; the LAI and LSC were higher at the shallow (4m) station than at the deeper (8m) one. Seasonal changes in LSC were significant between but not within stations.

Sediment granulometry of the substratum of both *Posidonia* and *Cymodocea* meadows was very similar at all depths. The near absence of mud and of very fine sand indicates that the areas appear to be under the influence of considerable water movement, and to be little influenced by the presence of the sea-grasses. The organic carbon content of the sediment was not significantly different between stations for either meadow.

The Mollusca of the *Posidonia* meadow were differentiated into foliar and rhizome layer/matte assemblages. Altogether, five polyplacophorans, 99 gastropods and 28 bivalve species, represented by 7559 individuals, were collected. Of the total individuals collected, about 65% were inhabitants of the rhizome layer/matte. Only 16 species of molluscs were widespread and abundant, most of them gastropods of the leaf stratum. Molluscan species richness, abundance, and distribution along the depth gradient were influenced mainly by the structure of the *Posidonia* meadows and by water movement. The biomass of Mollusca (totalling 22878.3mg AFDW) was essentially contributed by gastropods (72-99.5%). Significant changes in biomass generally occurred with season and with depth. The use of multivariate analyses delimited the molluscan taxocene into (1) a shallow water (6m and 11m) assemblage; and (2) a deep water (16m and 21m) one.

A comparison between the two sampling methods used showed that the suction sampler collected twice the number of species and half the number of individuals of the hand-towed net. The suction sampler is probably a better method for qualitative and quantitative sampling of the molluscan assemblages associated with *Posidonia* meadows.

The Mollusca of the *Cymodocea* meadow were separable into two groups a very rich shallow water (4m) assemblage, and a very poor deep water (8m) one. A total of 56 species (43 gastropods and 13 bivalves, represented by 4081 individuals) was identified. Most

individuals occurred at the 4m station. Seven gastropod species made up ca.93% of the total number of individuals collected. The pattern of species richness and abundance was different from that found in studies made in other Mediterranean localities (where a stable molluscan assemblage occurred throughout the year). Community parameters of the meadow studied were in general significantly different between stations but not between seasons. The total biomass of Mollusca was 4449.3mg AFDW. It was much higher at the 4m than at the 8m station and was mostly contributed by gastropods (ca.82%). Eighteen species made up ca.93% of the total biomass, and these species were divided between (1) the large-sized (high biomass) but less common (low abundance) species; and (2) the small-sized (low biomass) but common (high abundance) species. Biomass varied more with depth than with season. Multivariate analyses differentiated the 4m samples from the 8m ones and the assemblages from the two depths were significantly different in both abundance and biomass.

Comparison of the molluscan assemblages of the two sea-grasses showed that the numbers of species and individuals per sample were much higher for *Posidonia* than for *Cymodocea*. About 26% of the total species

collected (150) were common to the two sea-grasses. These species mainly belonged to the Trochidae, Cerithiidae, Rissoidae, Muricidae and Turridae. Similarities between the two molluscan assemblages were low, which reflects the differences between the two habitats, although other factors such as the number of samples, sites and the sampling season may also contribute. The estimated total biomass of molluscs was much higher for *Posidonia* than for *Cymodocea*. Gastropods made the highest contribution in both sea-grasses. Biomass fluctuated with depth and season in both sea-grasses; high biomass was generally recorded from shallow depths and during the cooler months. A small number of species were the main contributors to total biomass in each sea-grass, however, only one of these (the gastropod *Hexaplex trunculus*) was common to both sea-grasses.

Trophic guild analysis of the molluscan assemblages showed six feeding-types to be present in beds of both sea-grass species. In terms of abundance, herbivores, carnivores and detritivores were dominant among gastropods and filter feeders among the bivalves. Biomass was dominated by carnivorous gastropods and by filter-feeding bivalves.

# Xjenza

## Instructions for Authors

Xjenza will consider for publication papers in the following categories:

- Original research reports
- Short reports
- Reviews
- Commentaries

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

### Submission of Manuscripts

Manuscripts should be sent to the Editor.

Prof. Angela Xuereb,  
Department of Pathology,  
University of Malta Medical School,  
G'Mangia..  
Tel: (+356) 2595 1882  
Fax: (+356) 235638

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

### Conflict of Interest

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

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

### Permissions

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

### Arrangement of Manuscripts

Preface each paper with a suitable title, the author(s) name(s), and the full address of the institution in which the work was carried out (please identify the author for proofs - giving telephone and fax numbers - for correspondence and reprint requests), an abstract of no more than 200 words, and up to

**8 keywords.** Research reports should give an **appropriate introduction**, a single **methods** section (with the methodology of all experiments reports), **results**, and **discussion**. Either British or American spellings are acceptable, but please be consistent. Authors should express **measurements** in SI units, although they may include older conventional units in parentheses if they wish. Except for units of measurement, **abbreviations** should be spelled out on first use, and should be standard. **Drug names** should be generic, although authors may add brand names in parentheses if they wish.

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

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

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

Scerri CA, Abela W, Galdies R, Pizzuto M, Grech JL and Felice AE (1993) The b+ IVS, I-nt No. 6(T->C) Thalassemia 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 Epidemiology of Haemoglobin, and the Molecular biology of Normal and Abnormal Globin gene expression. In: *Collected Papers* (Eds R Ellul-Micallef and S Fiorini), pp. 357-391. University Press, Malta.

David Rawn J (1989) *Biochemistry*. Neil

Patterson Publishers, North Carolina

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

### Proofs, Reprints and Copyright

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

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

### ETHICAL POLICY

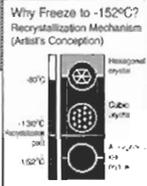
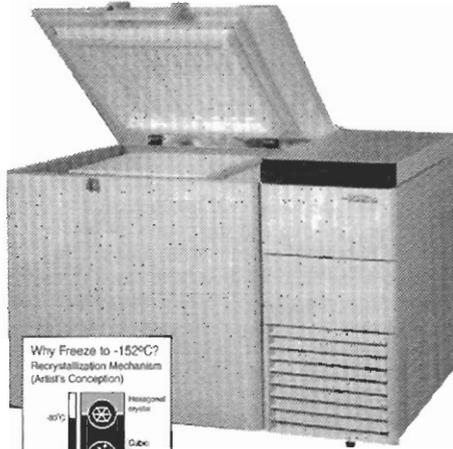
Studies using human subjects should include a statement within the manuscript that subjects provided informed consent for their participation and that the study was approved by a local ethics committee.

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

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

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

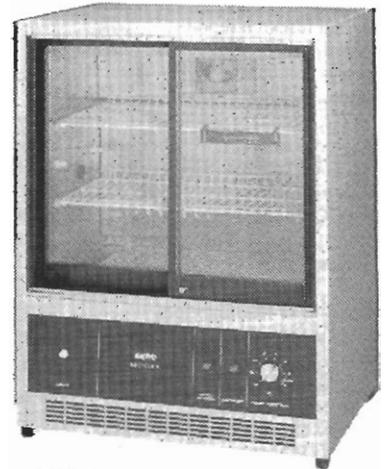
A wide range of advanced  
**SANYO** GALLENKAMP  
 technology in support of medicine and research



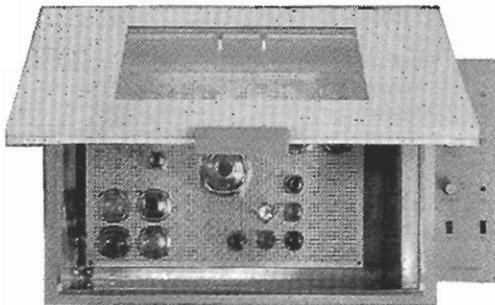
SANYO  
 -152°C FREEZER

**SANYO**

REFRIGERATORS  
 & FREEZERS  
 Up to an unbelievable -152°C



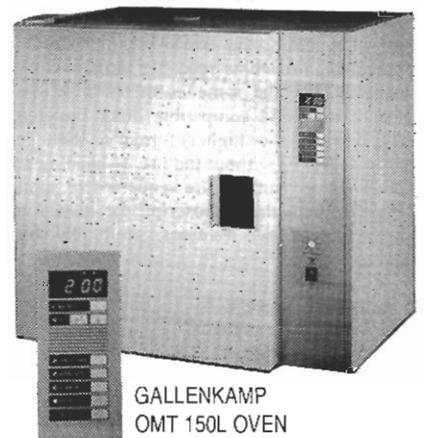
SANYO  
 MPR 110 MEDICAL REFRIGERATOR



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**

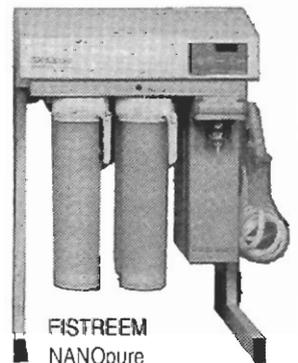
Wide range of  
 Micro to Ultra  
 Centrifuges



MSE MS80  
 ULTRACENTRIFUGE

**FISTREAM**

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



FISTREAM  
 NANOpure  
 DEIONIZATION SYSTEM

**SANYO**

SANYO Gallenkamp PLC

Sole  
 Agents:



67, South Street, Valletta.

ATTARD & CO. LTD. Tel: 237555 (6 lines) Fax: 220186

**GALLENKAMP**

**MSE**

**FISTREAM**

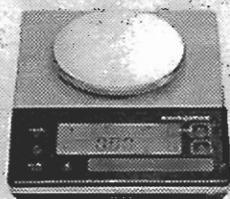
**Fytotron**

# LÖWENBRÄU



Your  
favourite  
Lager

# 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.

GENE DELIVERY

FUNCTIONAL  
GENEOMICS

CHROMATOGRAPHY

FLUORESCENCE

MICROSCOPY

IMAGE  
ANALYSIS

BLOTTING

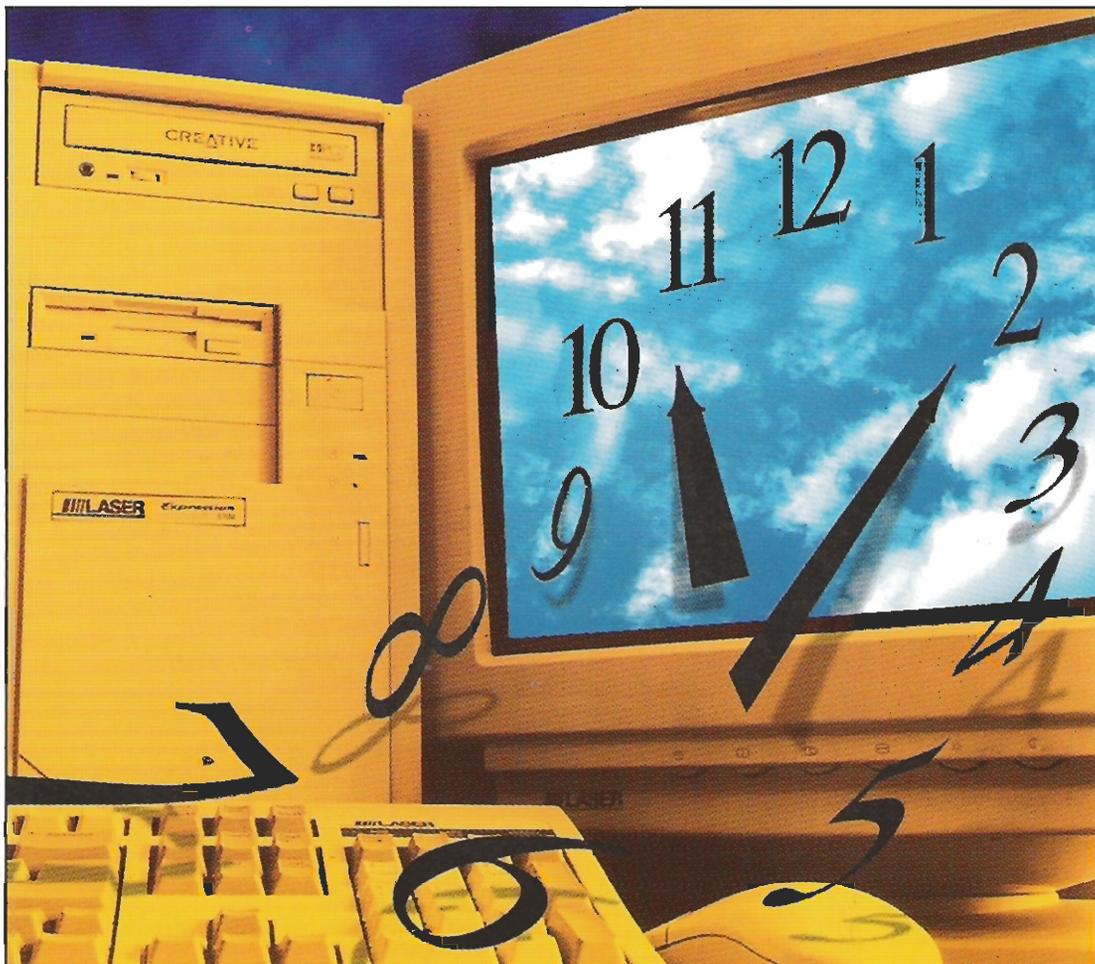
PROTEOMICS

ELECTROPHORESIS

Discover  
Bio-Rad

REPRESENTED IN  
MALTA BY:

E. J. BUSUTTIL LTD  
MRIEHEL STREET  
B'KARA  
TEL: 447184, 445885  
FAX: 445366



## We've added the speed and taken out the time!

Laser Computers achieve more, in less time at a better price. They are dependable, reliable partners that will deliver superior performance for your business or home at the right price.

Laser's manufacturing facility carries full ISO 9002 Certification and all our computers are bristling with the latest technology. Standard features like SDRAM, high capacity Fast ATA-3 hard disk, and a high performance

graphics accelerator ensure top performance. To deliver the ultimate multimedia experience, we only use the World's renowned Creative Labs CDROM's and Sound Blaster wavetable sound cards.

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

**/// LASER**  
computer

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