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Invertebrates in the medical service of man:
Part 1 – The Biotherapeutic Worms

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The invertebrates have long received particular attention from the medical profession. Their medical importance lies primarily in their role as parasites or harbingers of disease conditions, and in the potential toxic stings or bites of some species. While the faunal diversity of the Maltese Islands is limited because of the restricted ecological diversity, a number of invertebrate species present in the Maltese Islands and in the surrounding sea have medical significance resulting from their parasitic or toxic capabilities. These species have been previously reviewed.[1,2]

While the medical import of the invertebrates remains generally an adverse one, there are a number of invertebrate species which have in the past and in present times been utilised to serve the medical needs of mankind giving rise to biotherapeutic options in the management of disease. Invertebrates which have contributed to biotherapy include members of the worms and insects.

Phylum ANNELIDA; Class: HIRUDINEA
The Medicinal leech – Hirudo medicinalis

The humoral aetiology of disease introduced by Galen during the Classical Age considered that disease was due to alterations in the composition of these humors. Thus medical therapy aimed to restore the balance of these humors; thus venesection was a primary therapeutic option in a large majority of disease states. Venesection generally took the form of using a knife or lancet to open a vein, but a gentler and more desirable form of bleeding was to put a leech on the affected part and to let the animal engorge itself with the bad blood thought to dwell below the point of application. Leeches have been used medically for centuries; in Europe the use of leeches to drain off blood reached its height of popularity in the 19th century and persisted into the 20th century. Its use decreased pari passu with the introduction of effective pharmaceutical tools. The practice of bloodletting in the Maltese Island

Figure 1: Hirudo medicinalis

dates at least to about the 2nd century AD as evidence by depictions on a tomb-slab from the late Roman/Palaeo-Christian period which depicts a set of surgical instruments that include two Roman cupping vessels. The gentler form of bloodletting through the use of leeches was also practiced in Malta. The first traced reference to the use of leeches in relation to the Maltese Islands dates to the late 16th century during the Plague epidemic that ravaged the Maltese Islands during 1592-93. centuryThe use of leeches became increasingly fashionable in Europe during the 19th century. The situation was similar in Malta, where medical practice shifted from the French school to the British school. By 1840 the Civil Hospitals in the Maltese Islands were using 2600 leeches per month. The smaller hospital – Santo Spirito Hospital – with an average population of twenty-five patients, was in 1851 using 300 leeches per month. Instructions about the application and care of leeches were given to the nursing and midwifery personnel during the late 19th and early 20th century. The use of medicinal leeches in Malta continued throughout the first half of the 20th century. The last reported use was in the late 1960s to manage severe congestive heart failure in an elderly patient.[3]
a therapeutic option is now rarely considered, and situations where it is considered, preference is given to the formal collection of blood to use in cases of need. Leeches no longer play a role in venesection, however in recent years the useful medicinal properties of the blood-sucking leech are again being investigated, particularly in the field of plastic surgery. The salivary glands of leeches produce a cornucopia of pharmacologically active substances, including the anticoagulant hirudin (identified in 1884), an antihistamine, proteases, and possibly an anaesthetic and an antibiotic. In the 1980s leeches again "crawled out of their therapeutic closet" when plastic surgeons found its local anticoagulant and bloodletting properties useful for relieving venous congestion in grafts and transplants.[4] Failure of adequate venous return from a graft reduces blood supply, causing tissue necrosis. Placing a leech on the congested skin flap, finger, or other compromised area removes the congested blood and enables the graft to be salvaged. Leeches are currently used during postoperative care of reimplanted fingers, skin grafts, and breast reconstructions. Fresh leeches are applied as required for several days or weeks until the venous congestion is relieved and normal venous drainage of the graft has had time to develop. Other described uses for the medicinal leech include haematomas, purpura fulminans, paronychia, and even vascular congestion of the penis.

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Figure 2: *Bonellia viridis*

Phylum ECHIUROIDA; Class: BONELLIIDAE
The Spoonworm – Bonellia viridis

This species is a marine worm which is found in crevices and holes on the seabed, from where its proboscis projects, feeling around for food. Its beneficial role in medicine has yet to be determined but studies carried out by the Chemistry Department of the University of Malta have shown that the animal contains a substance named bonellin which causes haemolysis of erythrocytes, besides exhibiting other in vitro bioactivity such as depressing oxygen uptake of spermatozoa.[5]

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Invertebrates in the medical service of man:
Part II – The Insect Surgeons

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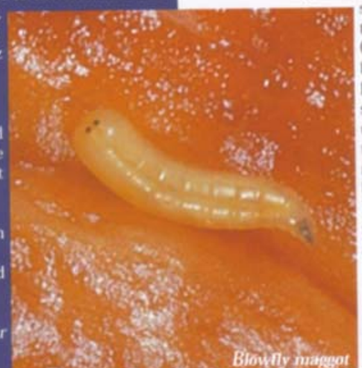
The invertebrates have generally been regarded as direct or indirect harbingers of disease conditions. Some species throughout history have however been used in lieu of surgical procedures.

The example par excellence is the leech which was introduced in medical practice to perform venesection aimed at restoring the body humors envisaged by the Galenic theory of disease. These invertebrate surgeons were considered as a more gentle method for the procedure. Their use for venesection has slowly decreased in the latter part of the 20th century, but the use of these animals has been resurrected in surgical practice for the biotherapeutic properties of their secretions.[1] Beside the Medicinal leeches, other insect species have been used as surgical assistants.

Phylum Arthropoda; Class: Insecta; Order: Diptera; Family: Calliphoridae
Bluebottle Fly – Calliphora vicina [Maltese – Zarzura]
Greenbottle Fly – Lucilia sericata [Maltese – Dehbija tal-Hmieg]

The primitive, carrion-breeding habit of the blowflies has been known and recorded for centuries. A very early reference can be found in the *Hortus Sanitatus* published at Mainz in 1491. The use of maggots to clean suppurating wounds was graphically demonstrated during the film 'Gladiator' when the protagonist played by Russell Crowe was healed of his battle wounds through the use of maggots. It was however at the turn of the 19th century that the beneficial role of maggots was noted. Napoleon's Surgeon in Chief, Baron Dominic Larrey reported that when maggots developed in battle injuries, they prevented the development of infection and accelerated healing. "*These insects, so far from being injurious to their wounds, promoted rather their cicatrization by cutting short the process of nature and causing the separation of cellular eschars which they devoured. These larvae are indeed greedy only after putrefying substances and never touched the parts endowed with life.*" There is no evidence, however, that Larrey deliberately introduced maggots into his patients' wounds. Similarly, during the American Civil War, a Confederate medical officer Joseph Jones noted the beneficial effects of wound myiasis commenting that "*I have frequently seen neglected wounds filled with maggots, as far as my experience extends, these worms only destroy dead tissues, and do not injure specifically the well parts. I have heard surgeons affirm that a gangrenous wound which has been thoroughly cleansed by maggots heals more rapidly than if it had been left to itself.*" During that same conflict, the Confederate surgeon J. Zacharias may have been the first western physician to intentionally introduce maggots into wounds for the purpose of debriding the wound, writing that "*During my service in the hospital in Danville, Virginia, I first used maggots to remove the decayed tissue in hospital gangrene and with eminent satisfaction. In a single day they would clean a wound much better than any agents we had at our command.... I am sure I saved many lives by their use, escaped septicaemia, and had rapid recoveries.*"



Blowfly maggot

The thought of intentionally introducing wriggling worms in gangrenous wounds rather than opt for a clean surgical debridement brings visions of medieval horror to doctors trained in a modern sterile hospital environment. Few of the doctors practicing in Malta today have experienced the surgical capabilities of fly maggots. I do however recollect one case of a maggot-performed amputation during the late 1970s. This occurred in a rather neglected geriatric female patient who had been admitted to the surgical ward with gangrene of the terminal part of one foot. When her home dressings were removed, the nurses were shocked to see a mass of wriggling maggot within the dead tissue of the foot. The Consultant Surgeon shocked everyone by deciding to utilize the maggots as his assistants rather than undertake surgical debridement in a medically-unstable individual. Within a few days the dead tissue had been completely removed by the insects and the dead bone remnants fell off, leaving a clean "surgical" healed plane. The patient recovered fully enabling discharge into a residential home.

The real founder of modern maggot therapy or wound myiasis was William Baer (1872-1931), Clinical Professor of Orthopaedic Surgery at the Johns Hopkins School of Medicine in Maryland. He first experienced the therapeutic potentials of maggot surgeons during the First World War. In 1928, he set out to experiment with the use of maggots in the treatment of intractable osteomyelitis in children. Repeated successes encouraged him to use the technique more widely, until several of his patients developed tetanus. In response to this complication, Baer set out to develop a suitable sterilizing process of the larvae and the eggs. Other workers enthusiastically took up this work and in the absence of any equally effective alternative for the treatment of osteomyelitis or infected soft tissue injuries, the use of maggots spread quickly during the 1930's. These developments however coincided with the development of the antibiotic era which gave an effective medical therapeutic option. By the mid-1940s, the enthusiasm for maggot therapy virtually ceased and maggot therapy was only used sporadically.

A resurgence of interest in maggot therapy recurred in the late 1980's when Robert Sherman, an entomologist and physician in Los Angeles, noticed healthy infection-free tissue in a leg wound crawling with 'worms'. He established an insectary in California to breed maggots for clinical use, and carried out the first controlled study showing that maggot therapy significantly increases the rate of healing of chronic pressure sores – at much lower cost than the usual regimen of repeated surgery and antibiotic treatments. The results of these preliminary investigations indicated that maggot therapy offered several advantages over other wound treatments currently employed. Maggot therapy has since grown in popularity and has met with success in Europe. It has recently been used in Germany, Hungary, Sweden, Belgium and the Ukraine. In 1996, the International Biotherapy Society was founded to investigate and develop the use of living organisms, or their products, in tissue repair, and the first in a series of international conferences on maggot therapy and other similar topics also took place in that year.[2,3]

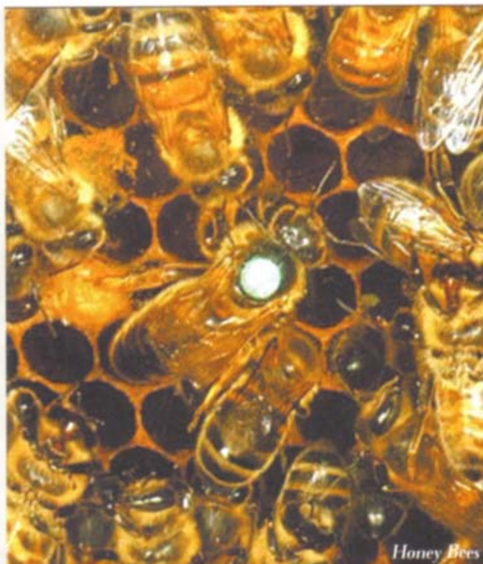
Order: Hymenoptera; Family: Formicidae
Carpenter ant – Camponotus barbaricus [Maltese: Zokkrin]

Other members of the insect family have also been utilized in surgical practice since ancient times. Hindu writings dating to 1000 B.C. describe the use of ant mandibles for closing incisions and wounds. The insect's head is positioned so that the two parts of the mandible are situated on either side of the cut. They are then allowed to bite thus holding the wound shut like staple-stitches. The ants' bodies are then pulled off, leaving only the heads and jaws locked in place until the wound heals. Ant stitching is reportedly still used by primitive societies in Brazil today. In Algeria and Turkey, beetle mandibles were the stitches of choice.

Carpenter ant

Order: Hymenoptera; Family: Apidae
Honey Bee – Apis mellifera [Maltese: Naħla ta' l-Għasel]

Honey has long been recognized as a sweetening food product and an 'over-the-counter' medicine being utilized not only to make certain medicines palatable but also to sooth chest infections. It has also found use as a wound dressing, this medicinal function being described by the ancient Egyptians.



Honey Bees

Its role in controlling bacterial invasion and growth is based on its low pH value of 3.9, its hyperosmolarity and the presence of inhibine or glucose oxidase which liberates peroxide acting as a strong antibacterial agent. Apitherapy, i.e. the medical use of honeybee products, is also gaining momentum. Anecdotal claims from the beekeeping community have suggested that bee-venom has a therapeutic value for joint inflammatory conditions. Bee-venom can be injected artificially via a syringe or simply by allowing live bees do the stinging. Bee-venom contains dozens of active chemical substances; some of which are known to have analgesic and anti-inflammatory properties. Bee-venom has been said to be useful for the management of migraine headaches and chronic pain. Bee-stings have also been reported to ease the symptoms of arthritis, bursitis and rheumatism. The sting regimen for arthritis includes several stings per treatment performed two to three times per week for a period of up to three months.

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Invertebrates in the medical service of man:
Part III – The Research Assistants

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The invertebrates have generally been medically classed as disease vectors, though a number of species have been utilized in the management of surgical conditions or for the biotherapeutic properties of their secretions.[1,2] A number of species have been essential tools in frontline research studies which have in some instances received international acclaim.

Phylum Mollusca; Class: Cephalopoda;
Several species of Squids, Cuttlefish and Octopi
Sepioteuthis lessoniana; Sepioteuthis sepioidea; Lolliguncula brevis; Loligo opalescens; Loligo pealei; Illex illecebrosus; Sepia officinalis; Octopus vulgaris

Loligo sp.

In 1909, L.W. Williams noted that the squid possessed a giant nerve cell or more specifically a large projection from the cell which is called an axon. These observations slipped into obscurity until 1936, when the Professor of Anatomy J.Z. Young rediscovered this curiosity. Using a pair of electrodes, Young stimulated surrounding nerve fibers and found that he could only produce large muscle contractions in the mantle when the large vessel-like structure remained intact, confirming that the structure was indeed a single, huge nerve axon. Scientists quickly appreciated the significance of Young's finding in presenting a working neurological model. This enabled scientists to initiate a number of research projects aimed at understanding neural function.

Based on squid models, the British scientists, Alan Hodgkin and Andrew Huxley, in a series of papers published during the 1950s described the behavior of nerve impulses. This sterling work was in 1963 awarded the Nobel Prize. Further researchers working in the field of neural physiology using the squid model were also in subsequent years made Nobel Laureates. These included the American George Wald, honored in 1967 for his research on chemical and physiological processes in the eye, and the British Bernard Katz, honored in 1970 for his discoveries concerning the role played by chemicals in nerve impulses.

Squid research was hampered by the difficulty in breeding these animals in captivity. Several species of squid were experimented with but problems were encountered either because the species was too small or because it generally lived in cold waters. Finally, in 1987 experimentation with the warm-water species *Sepioteuthis lessoniana* yielded promising results and presented researchers with a laboratory-bred animal. The cephalopods have been useful in elucidating physiological information on membrane biophysics, the ion channel, neuron development and injury, cell biology, muscle biomechanics, brain physiology, chemical neural reception, vision and oculomotor functions, equilibrium receptor systems, behavior and neural pharmacology, transmitter functions, melanin synthesis and developmental biology.

Phylum: Arthropoda; Class: Merostomata; Order: Xiphosura
Horseshoe Crab – Limulus poluphemus

Another invertebrate contributor to the understanding of neural processes was the Horseshoe Crab. Much of what we know about the function of our eyes is the result of studies that began over 50 years ago on the large, compound eyes of the horseshoe crab. Its eyes have a relatively simple construction, and the optic nerve is readily accessible. In addition, it is easy to keep *Limulus* alive in the laboratory, making it an ideal animal for eye research. The cornea of *Limulus* eyes was first examined in 1782, while the 19th century was to result in the investigation into the structure of the median eyes (each with a single lens) and the lateral eyes (composed of small hexagonal eyes called *ommatidia*). Physiological studies on the electrical impulses in the horseshoe crab optic nerve were carried out in 1928 by Dr. H. Keffer Hartline, whereas in 1932, electrical responses were recorded for the first time from a single visual receptor of the *Limulus* eye. In 1967, Dr. Hartline was awarded the Nobel Prize for his continuing research on horseshoe crab vision. His researches had elucidated how sensory cells in the retina help the brain process visual cues, enabling horseshoe crabs to see lines, shapes, and borders. This mechanism, called *lateral inhibition*, allows horseshoe crabs to distinguish mates in murky water. Research of this type was helpful for understanding human eye diseases like retinitis pigmentosa, which causes tunnel vision and can lead to total blindness.[3] Other researchers have utilized the horseshoe crab's eyes to identify the visual pigment as rhodopsin (1960), and also related light sensitivity to a circadian clock mechanism in the horseshoe crab's brain that enhances night vision. Building on the Hartline's lateral inhibition and circadian clock mechanism research, the American ophthalmologist Dr. Robert Barlow and his colleagues are investigating the role of vision in potential mate selection. By using dedicated computer models, Dr. Barlow has analyzed how the brain of a horseshoe crab processes signals transmitted from the eyes and optic nerve. It is hoped that in the future, decoding this pathway may provide valuable information for correcting human vision disorders. An extract of the horseshoe crab's blue copper-based blood – lysate – is used to test the purity of a number of medicinal properties. Furthermore certain properties of the shell are used to help speed blood clotting mechanisms and to produce absorbable sutures.

Phylum: Arthropoda; Class: Insecta; Order: Diptera; Family: Drosphilidae
Fruit Fly - Drosophila melanogaster [Maltese – Ferminalle]

Drosophila melanogaster

The fruit fly *Drosophila* has long been a major contributor to our fundamental understanding of genetic processes serving as the primary organism in the development of the chromosome theory of heredity. As with most of the long-established model

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organisms, the initial choice for using this species was for practical reasons. The fruit fly is small and has a simple diet and hence large numbers of flies can be maintained inexpensively in the laboratory. The life cycle is also very short, taking about two weeks, so large-scale crosses can be set up and followed through several generations in a matter of months. Fruit flies also have large polytene chromosomes, whose barcode patterns of light and dark bands allow genes to be mapped accurately.

In the early 1900s, American-based geneticists began to make important contributions to genetic research. Working at Columbia University in 1910, Thomas Hunt Morgan conducted experiments in fruit flies, mainly *Drosophila melanogaster*, and established that some genetically-determined traits were sex-linked. Morgan's work was followed up by his students – Calvin Bridges in 1913 established that genes were located on chromosomes; while Alfred Sturtevant in 1913 determined that the genes were arranged on the chromosomes in a linear fashion and further demonstrated that the gene for any specific trait was in a fixed location or locus. Yet another of Morgan's students, Herman J. Muller, in 1926 discovered methods for artificially producing mutants in fruit flies by ionizing radiation and other mutagens, thus discovering the origin of new genes by mutations. These studies opened a new vista for understanding the principles of genetics, mutations and chromosome behavior. A second major contribution of *Drosophila* research has been in our understanding of development. The development of a single egg cell into a many-celled organism is a complex process. The system of patterning genes and signaling molecules in the fruit fly leads to the direct development of different organs in their proper locations. In 1980, Christiane Nusselein-Volhard and Eric Wieschaus performed the first genome-wide mutational screen in an attempt to identify all genes involved in development. In acknowledgement for the work on Drosophila developmental genetics, Ed Lewis, Christiane Nusslein-Volhard and Eric Wieschaus were awarded the Nobel Prize for medicine and physiology in 1995.[4]

The fly genome was sequenced in the year 2001 by J. Craig Venter and the Celera corporation, although much of the work had already been laid by the Berkelely, European, and Canadian *Drosophila* genome projects. The genome is made up of 165 million base pairs in length (spread over four chromosomes) and contains approximately 14,000 genes. The full sequence of the *Drosophila* genome adds to the usefulness of the fly as a model organism. Now that the *Drosophila* genome has been sequenced, the genetic code will greatly facilitate positional cloning methods. The new sequence has also revealed previously unknown counterparts to human diseases, most notably cancer and neurological diseases. With this new knowledge, it is possible to model human diseases and disease pathways in flies. Additionally, it is now possible to systematically study entire networks of genes at once, rather than individual pathways. Consequently, studies in fruit flies will reveal important insights into human physiology and medicine. Finally, the fruit fly could be the key to answering the great question of how a genome can give rise to an organism without any instructions for doing so, possibly serving as the key for artificial organ production from stem cells.

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