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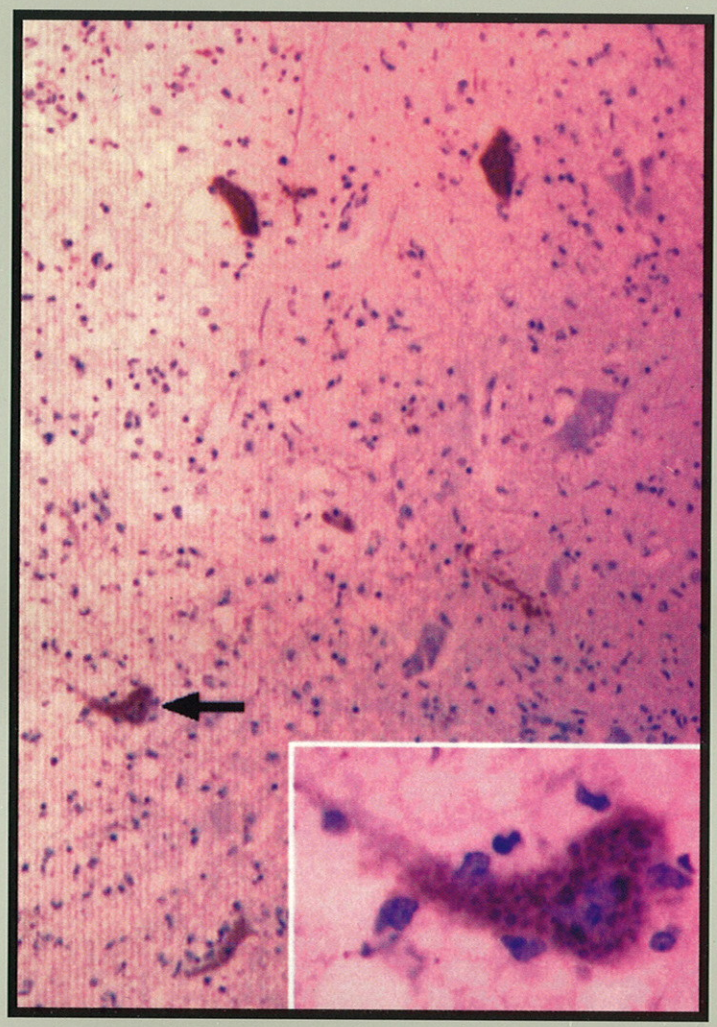

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Immunohistochemistry of human spinal cord showing presence of Enterovirus 71 in neurones.
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Manuscripts: All manuscripts should be submitted in duplicate, typed on one side of A4 size paper and double-spaced with at least 2.5 cm margin. A computer diskette (3.5 in) containing the manuscript in MS Word 6.0 or Word Perfect and a covering letter, stating that the work has not been published or under consideration for publication elsewhere, should be submitted to the Editor. Presentations at meetings do not class as prior publication. The text of the manuscript should be in the following form:

Title page: The title page should contain a concise title of the article. It should identify all the authors, the name(s) of the institution(s) and their full addresses where the work was carried out. The initial and address of the corresponding author should also be indicated.

Abstract and Keywords: The second page should contain an abstract of about 150-200 words. It should state the purpose of the study, a brief description of the procedures employed, main findings and principal conclusions. Three to ten key words should also be listed below the Abstract.

Text: Wherever possible, the text should consist of an introduction, materials and method, results, discussion and references.

References: Number references consecutively in the order in which they are first mentioned in the text. References in the text should be indicated by a figure within parenthesis. The titles of journals in the list should be abbreviated according to the Index Medicus. Authors are responsible for the accuracy of all references. Examples of correct forms of references are given below:

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ii) ***Personal author(s) of book:***
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iii) ***Chapter in book:***
Weinstein L., Swartz MM. Pathogenic properties of invading microorganisms. In: Sodeman WA Jr, Sodeman WA, Eds. Pathologic physiology: mechanisms of disease. Philadelphia: WB Saunders; 1974; 457-72.

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STROKE – TIME FOR GREATER EFFORTS AND ENTHUSIASM

Stroke is a very common clinical problem. It has been estimated that in the United Kingdom, the lifetime cumulative incidence of stroke is one in three in the population, and the mortality from stroke is one in seven. There are evidences to support that stroke incidence is higher in the developing as compared with the developed world. The WHO's MONICA Project provides a standardised comparative data of stroke incidence in 17 populations mainly from Europe during the period 1985 to 1990. Novosibirsk from Russia ranked number one for both men and women in this multinational "league table" for stroke occurrence (1). Beijing was the only oriental population represented in this study. The stroke attack rate ranked sixth among men and second among women. Stroke is not just a problem in the elderly. In the University Hospital, Kuala Lumpur's Stroke Registry, out of the 413 stroke patients seen in year 1994, half of the patients were ≤62 years and 19% were ≤ 50 years (2). Thus, in Malaysia, more than half of the stroke patients were of middle age or younger. Not only does stroke carry a significant mortality, it also often has a devastating effect on the patient's physical and mental function, resulting in dramatic changes in the daily life of the patients and their families.

On the other hand, there are grounds for optimism in the prevention and treatment of stroke. There has been a decrease in stroke mortality between 3% to 5% per annum in most Western countries over the period from 1970 to 1985 (3). Similar trend was also seen in the neighbouring Singapore, the age and sex-standardised mortality rates declined from 99/100,000 in 1976 to 59/100,000 in 1994 (4). Unfortunately, no reliable stroke mortality trend is available in Malaysia. However, the Ministry of Health has reported stroke as accounting for about 10% of death in the Government Hospitals. The decline in the mortality is probably due to decreasing severity as well as incidence of stroke, although the data supporting the later is conflicting.

In mainland China, a door-to-door survey in six cities in 1983 showed a marked geographical variation in the incidence of stroke. The northeast city of Harbin had the highest figure of 441 per 100,000 population, while the southwest city of Chengdu had the lowest of 136 per 100,000 population (5). Another survey between 1986 to 1990 yielded similar results, with the Harbin at 486 per 100,000 population, and Shanghai had 81, per 100,000, a six folds difference (6). A study from Taiwan showed that the annual incidence of stroke in the rural areas was twice that in the urban (7).

The declining stroke mortality and the wide variations in stroke incidence among populations with similar racial origins demonstrate the importance of modifiable risk factors in stroke. Stroke is clearly a largely preventable condition. The known modifiable risk factors related to life-style are: smoking, alcohol, obesity, physical activity, oral contraceptives, diet particularly salt and lipid. The risk factors which can be modified by medications or surgical procedures are: hypertension, atrial fibrillation, transient ischaemic attack, carotid stenosis, diabetes mellitus and lipid status (8).

There are also significant recent advances in the treatment of stroke. In particular, thrombolysis with recombinant tissue plasminogen activator (rt-PA) for ischaemic stroke within the first three hours of onset is able to improve the neurological outcome (9). Patients treated by a stroke team in a stroke unit is also able to reduce the mortality, morbidity, disability, institutionalisation, length of stay and health costs of the patients (10).

It is not just the Malaysian medical professionals who often have a nihilistic attitude to stroke and place stroke in low priority. According to information from the National Institute of Health in US, the money spent on research per death for 1996 was US$ 43,207 for AIDS, US$ 4,723 for cancer, US$ 1,270 for heart diseases, but only US$ 750 for stroke. It is time we change our priority and devote more efforts and enthusiasm for stroke.

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MAGICAL MOMENTS IN MEDICINE
Part 5: Medieval Medicine

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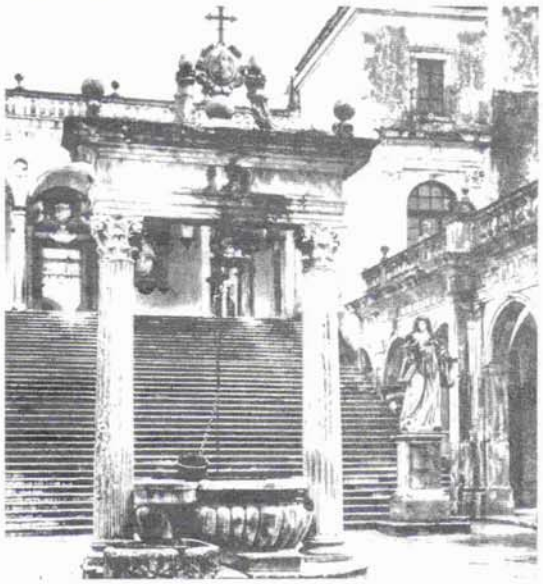
Prologue

As the curtains lift for the next act of this drama on Medical History, we now find the stage dominated by a multitude of characters consisting of clergymen, scholars and doctors, as well as some pestilent epidemics, who steal the show as veritable villains. The Middle Ages, which span a period of a thousand years from 500 to 1500 AD, were so inert, there were no noteworthy advances in medicine during this period. Some historians even consider this era unscientific and unworthy of serious attention. However, this is the time where we see radical changes in perspective, especially about the universe, the human being and the physical and spiritual interaction between the two. In a partial eclipse, religion blurs scientific insight. This naturally causes a lot of emphasis to be shifted on the Creator or the Supreme Being. Historical records lead us to believe that Jesus influenced the course of medicine during his lifetime. Brian Inglis, in his book states that like Prophet Mohammed, Jesus had no interest in disease as such, but was concerned deeply about the sick. The scientific basis of his healing, however, is poorly understood and has been a topic argued right till this day. While some believe that they were healing miracles, others believe that they were either legends or case histories with a natural explanation. However, caring for the sick soon became a noble and charitable notion in the community, emphasised and encouraged by the church. As a result, spiritual intermediaries, namely the Church and the clergy attain importance and exert powerful influence over the public, at least during the early middle ages. Ancient medicine, magical in Egypt, mythical in Greece and methodical in Rome, becomes monastic during medieval times.

When Rome fell to German tribes in the 5th century AD, Constantinople (now Istanbul) the capital of the Byzantine Empire, became the centre of Western learning. Being predominantly Christian, the Church gained power, leading to a complete change in the attitude of the people. People were led to believe that sickness was divine retribution for their sins. Therefore, prayers became prescriptions and meditations became medications. The forceful influence of the Church pushed practitioners to the background and scientific inquiry almost became non-existent. Monasteries became healing centres, which admitted and cared for the sick and the suffering. They later transformed into institutions of hospitality which have, to a very great extent, remained unchanged in some of their conceptions up to the present day - the hospitals.

Hospitals

Hospitals are largely a medieval legacy and possibly the only significant contribution of this era that was otherwise scientifically sterile. However, the concept of hospitals has been known to exist several centuries earlier. Records tell us about the existence of hospitals in Ceylon (now Srilanka) in 5 BC and in India as early as 260 BC. The hospital-like buildings for treatment of troops and the valetudinaria for the care and sick civilians established in ancient Rome were discussed in our previous episode. The first regular hospital or *nosocomium* was established by a nobly born Roman woman called Fabiola in 398 AD. Situated in Constantinople, it could accommodate 7000 patients. Fabiola herself was moved to work among the patients in repentance for her sins. Early medieval hospitals rarely treated the sick. They just received them, supplied their bodily wants and ministered to their spiritual needs until they were well enough to return to work. But soon they became separate entities, with their own identity. Benedictine of Nursia (480-543 AD), the founder of the Benedictine Order, introduced a new concept in health care when he founded the first monastic medical centre in 529 AD in Monte Cassino, (Picture A) among the



Picture A. The first monastic hospital at Monte Cassino.

ruins of a temple of Apollo. He urged the monks in the monastery to read and copy ancient manuscripts of medicine and also encouraged them to practice medicine. Monasteries doubled as hospitals and had pharmacies manned by monks. The monks later started practising "theurgic" therapy, which was religious psychotherapy and physiotherapy based on saints' miracles and magical herbs. Monks practised medicine only within the premises of the monastery. Presumably, religious commitment and sentiment became *sine qua non* for practitioners, since there came a time when a medieval physician first had to be ordained a priest before practising medicine.

The oldest hospital in France was the Hotel-Dieu founded in 652 AD by the then Bishop of Paris. In a new wave that started in 1180, the foundation of the Order of the Hospitalers of the Holy Ghost opened hospitals all over Europe. In Germany, municipalities established and operated hospitals. In 937 AD, during the Saxon times, England instituted her first hospital in York, which was quickly followed by numerous others. St. Barthelomew's of London is one of the earliest hospitals which, since its inception in 1123, has functioned with distinguished elegance.

In the Islamic world, numerous hospitals were erected in major cities. Caliph El Welid is reported to have founded a hospital in Damascus as early as 707 AD and there were more to follow in Baghdad, Egypt, and Cairo. By 1160, Baghdad alone is reported to have had 60 operating hospitals. Al-Mansur, in Cairo, was the greatest of them all, with a reported income equal to about 400,000 ringgit a year. This money came from landed property assigned to the hospital by the royalty as well as philanthropists. It had every facility conceivable, including lecture rooms, a library, isolation wards, diet kitchens, pharmacy and an orphan asylum. Admission was unrestricted and duration of stay unlimited. On top of it all, each convalescent - on discharge - was given a suitable amount of money so that he/she need not have to return to work before having fully recovered.

During the late middle ages we find a radical reorientation of medicine, wherein medieval superstition is gradually abandoned in favour of logic and simple scientific notions. The patient once again becomes the centre and focus of attention. The medical school at Salerno, a Greek colony in Italy, is seen as the cultural melting pot of this era.

Medieval medical schools

The School of Salerno, first heard of in 848 AD, deserves special mention not because it represented medieval traditions, but because it transcended it. Legend has it that it was founded by a Jew, an Arab, a Greek and a Roman. By the 10th century AD, it had become a well-reputed medical school. The director of the school was Nicolaus Praepositus, author of Antidotarium, the first medieval pharmacoepeia. Described as the first secular institution of higher learning in the West, it is said to have had an extensive curriculum and emphasised on practical skills rather than theoretical knowledge. The curriculum included three years of logic, five years of medicine and one year of resident training. The Anatomy department used pigs for dissection in the theatre. The institution seems to have been an equal opportunity employer with a lay faculty, which included several women professors. Dame Trotula, who held the Chair of gynaecology, is dubiously credited with the authorship of two great texts on female diseases. Passionibus Mulierum Curandorum or Trotula Major was written to educate male medics about the female body, because such knowledge was generally lacking. The book comprised of sixty-three chapters and gives information about menses, conception, pregnancy, childbirth, as well as general diseases and their treatments. The majority of remedies are herbs, spices, oils and of animal origin. She recommended long periods of convalescence and a positive mental attitude for good recovery. She asserted that both men and women could have physiological defects that affected conception. This was asking for trouble, since even the thought that a man could be responsible for infertility was a cheeky notion at that time. She also described the use of opiates to dull the pain of childbirth and incurred the wrath of the church, which maintained that women, as sisters of Eve, should suffer the ordeal of childbirth without any relief. Trotula's other book was De Aegritudinum Curatione or Trotula Minor. Dame Trot, seems to be an ethereal character whose sex has been questioned and very existence disputed by some scholars. However, she is generally accepted and acclaimed as a great teacher and midwife.

The Salerno medical school was co-educational. Pretty female students must have inspired boys and tapped their poetic potential as evidenced by the mnemonic, *"Agnes attracts the boys like iron to lodestone."* Most modern day mnemonics are also female-centred (*"She looks too pretty; try to catch her,* " for carpal bones and "*Sister Lucy's powdered face often attracts medical students*" for branches of the external carotid artery) and therefore, I would think that students have not changed much from medieval to modern times. New arrivals were ragged and often compelled to wear costumes depicting animals. However, students in medieval universities led tough lives. Fees were low with scholarships for the poor, but books were expensive. Classes began at six o'clock in the morning. Students wore long cloaks and black, monkish gowns. Long hair, lace and ornaments were prohibited. Teacher and students were seated on straw mats on the floor during academic discussions. Exams were not easy to pass

in Salerno. All examinations were oral and perhaps very aggravating for the student, since some universities had rules against stabbing examiners. It was a common practice by the students to get the masters drunk on the eve of the examination. After about eight years of specialised training, the students had to appear for a rigorous final exam before the faculty and the Royal Commissioner. Successful candidates were rewarded with a ring and a laurel wreath and licensed to practise.

When some of the professors of Salerno sat down to produce a simplified textbook of medicine and hygiene, little did they know that they were about to produce a bestseller. The Regimen Sanitatis Salernitanum, a neat and compact handbook with over 300 rules for healthy living, became famous all over Europe and had 24 manuscript editions, all of which exist till this day. It had eight hundred and forty two lines of verse, to help doctors remember the rules. Here are some catchy jingles from the Regimen:

If thou to health and vigour wouldst attain,
Shun weighty cares-all anger deem profane,
From heavy suppers and much wine abstain.

If in your teeth you hap to be tormented,
Burn Frankincense (a gum not evil scented)
And in a tunnel to the tooth that's hollow,
Convey the smoke thereof and ease shall follow.

Shun idle slumber nor delay
The urgent calls of nature to obey.

But as all practice shows, no doctor can
Make life anew, though he may stretch its span.

Nor trivial count it after pompous fare
To rise from the table and to take the air.

Use three physicians still-first Dr. Diet
Next Dr. Merryman, third Dr. Quiet.

In 1170 AD, Roger Frugardi of Salerno brought out the first ever western book on surgery. Guy de Chauliac revised the same and published the book Great Surgery in 1363 AD. Although principles of surgery were taught in Salerno, practice of surgery was still done by barbers and barber surgeons. Surgical procedures were often crude, but nevertheless effective. Finely chopped hair of the hare (called 'mummy powder') was used as a styptic to coagulate bleeding wounds. Anaesthesia was administered with sponges impregnated with narcotics (opium or mandragora) and placed in the nose or mouth. Torn intestines were reportedly sewn together over an animal's trachea. Plaster of Paris was yet to be invented, but Salernitians used bandage and a flour-and-egg mixture just as effectively to splint fractured parts. The adjustable surgical operating tables, designed to tilt the patients to optimal positions, are just Trendelenburg's reintroduction of the equipment and procedure used by Salernitians. (Picture B)

Picture B. The tilting operating table. Invented by Salerno doctors and reintroduced by Frendelenburg in 1881.

The doctors of Salerno were real trailblazers in the field of medical practice and education. The medicine man, until now known as '*medicus*' was renamed '*physicus*' or physician, which emphasises the doctors' scientific skills over and above their medical skills. For the first time, a curriculum based on medical textbooks was established. The title 'doctor' was first legally used here to denote a physician in 1180 AD.

Formal medical education spread from Salerno to various parts of Europe, England, France and most of Northern Europe with the establishment of several new universities in Paris, Bolgna, Padua, Montpellier and other major cities. But medieval men could not, however, digest the idea of women practising medicine. Women were rejected from Universities and refused medical practising licenses. Jacqueline Felicie de Almania was one of five women who defied the law in Paris in 1322. In spite of eight patients testifying that she was able to cure them when male doctors had failed, she was found guilty by a chauvinistic Court of Justice and punished.

The University of Bologna was a 'lay' university administered by students themselves. Among its best outgoing students were Dominican friar Theoderic of Lucca, who first used a sponge dipped in opium and mandrake oil for anaesthesia and Copernicus, the great astronomer. In 1281 AD, students of Bologna witnessed perhaps, the first autopsy in history. Anatomy advanced in Bologna under Mondino de Luzzi, author of *Anathomia*, who was also the first man to dissect a cadaver in public in 1315 AD. Bologna had rigid rules for its teaching staff and stipulated that teachers could not be absent for lectures even for a day. If a teacher had to leave town for whatever reason, he had to deposit a certain sum to insure his return.

The school in Paris, by contrast, was managed by masters and more rigid and bureaucratic in its principles. In order to retain their titles as doctors, students were forced to be bachelors. The University rejected Lanfranc, an Italian surgeon, when he tied the knot. He is famous for coining the words "healing by primary intention" found in the opening chapters of most textbooks of pathology. He must have been worldly-wise and a practical man, since he is quoted to have said, "Do all you can for the poor, but get all you can from the rich". His contemporary Henri de Mondeville, a gifted teacher, however was cynical about fee collecting. With a passion to 'preach' and not 'practice', he used anatomical wall charts to enliven his lectures and his efforts represent the first known audio-visual teaching attempt in the history of medical education. Albert Magnus, the most famous medieval physician and Roger Bacon, the first modern scientist who described the magnetic needle and reading glasses (and also reportedly predicted radiology and the discovery of America) were from this school. Petrus Hispanus, who later became Pope John XXI was also from this school. Being the only physician to attain this office, he authored Thesaurus Pauperum and Liber de Oculo. In the former, he advises blowing pepper and salt up the patient's nose in hysterical fainting to make the patient come around. In the latter, he prescribes infant urine as eyewash for ocular infections.

Uroscopy was given great importance during medieval times as evidenced by medieval art, where every medical scene invariably has a physician, in his unfathomable wisdom, poring pensively over the urine glass. Telemedicine began when people carried urine in handsome flasks in wicker baskets and travelled great distances for expert opinion and accurate diagnosis. Physicians had remarkable skills in analysing urine. Duke Henry of Bavaria sent a lady's urine to Notker, a monastic physician, labelling it as his own. Notker inspected the urine and declared that God is about to work a hitherto unheard of miracle, whereby a man would be giving birth to a child. The Duke, failed in his attempt of crafty deception, blushed and accepted defeat.

The four-humour theory of Hippocrates evolved into a humoural theory of complexion or temperament, which described the social, psychological and physical characters of a person. A person was thought to be made sanguine by excess of blood, phlegmatic with excessive phlegm and choleric due to yellow bile, while black bile caused melancholy. (Picture C). As a result, people bled themselves at least twice a year (during spring and fall) to keep in tune with the climatic conditions. In fact, bloodletting or phlebotomy was almost a fashion during the Middle ages. It was a voluntary and often pleasant ritual, which according to the Regimen, brought joy to the sad, kept minds of monks from mundane thoughts and even helped flirtatious women to forget lovers and remember husbands. Therefore barbers and barber surgeons had good business. However, in their zealous enthusiasm, some tactlessly went overboard by displaying buckets of blood and hanging bloody rags on poles to advertise their services. The people must have taken this ethical violation quite seriously, since the University of Paris subsequently started obtaining sworn declarations from all aspirants to the medical school that they would never perform surgery.

Herbs

Herbs were used extensively during the Middle ages. Europe's oldest surviving Herbal that was written in the vernacular is The Leech Book of Bald, and was written in the first half of the tenth century. In this book some of the most used herbs of the Saxon times were Wood Betony, Vervain, Mugwort, Plantain, and Yarrow. The herbalists believed that the outward appearance of the plant lets you know what illness it could cure. Unbelievably, this was often accurate. For example, Lady's Mantle was said to look like a woman's cervix, and thus was deemed useful in childbirth. Anything that was yellow was thought to help liver conditions, or choleric conditions, and one of these plants was the Dandelion. Till this day we find Dandelion featuring in natural health and beauty care catalogues, claiming that it helps to enhance the flow of bile from the liver and assists in healthy digestion. Galen created a system for food, classifying it into categories of hot, cold, dry, and damp and food intake was thought to have a great effect on the four humours, described by Hippocrates. The Upanishads of India (500 B.C) stressed the importance of food in the saying, "From food are born all creatures, which live upon food and after death return to food.

Picture C. The Four Temperaments

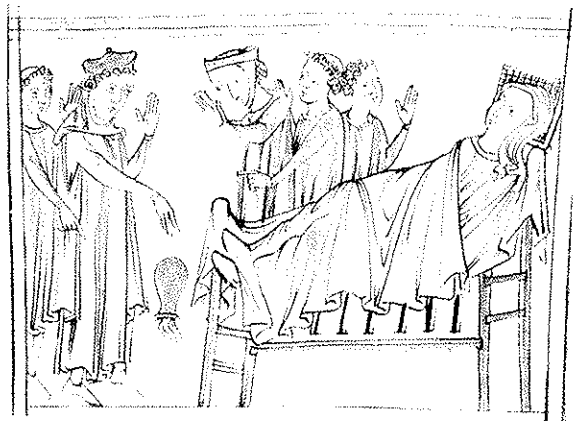
Food is the Chief of all things. It is therefore said to be the medicine of all diseases of the body." The medieval housewife realised this and knew how to make a Galenical balanced meal by adjusting her ingredients. When cooking fish, she would add "hot and dry" fennel; she would add pepper to foods that were "cold and moist", like beans. Hot foods would include onions and almonds. Asparagus and coriander were considered dry foods. Damp foods were grapes and barley. Lettuce would be a cold food.

Yarrow (Achillea mullefolium) is said to have been used in the Trojan War to treat the wounds of the soldiers. Its folk name, "nosebleed", proves that it was used as a styptic to stop bleeding. In medieval times it was a remedy for toothache. Even today, Yarrow is considered a "heal-all" reputed to cleanse your system and reduce inflammation. St. John's Wort (Hypericum perforatum) contains tiny oil sacs that have red oil in them. When the sun shines on it, the plant looks like it is covered with red holes. This herb was used for stopping bleeding and as an antiseptic. It was proven helpful for burns, sprains, cramps and also believed to dispel evil spirits. In the thirteenth century, physicians of Mydafai, Wales, prescribed it for mental illnesses. Amazingly, the early herbal healers were right on target. The aerial tops of the plant can be taken internally and science proves that it lightens mood, lifts the spirits, acting as a nerve tonic for anxiety, irritability, and nervous exhaustion. Of course, they had no means of knowing that St. John's Wort contains the active ingredient hypericin, which contains flavonoids and xanthones, which are monoamine oxidase (MAO) inhibitors. We now know that MAO inhibitors increase the level of the nerve impulse transmitters in the brain that maintain normal mood and emotional stability. A weed called Plantain (Plantago species) was called "waybread" and was an important healing herb. It is still widely used for medicinal purposes, especially for bee-stings. The seeds of a related species, P. pysllium, are sold over-the-counter as a natural laxative. Look for these and other simple, inexpensive ancient herbs, packaged in all those expensive health products being sold straight to you!

Health and life style

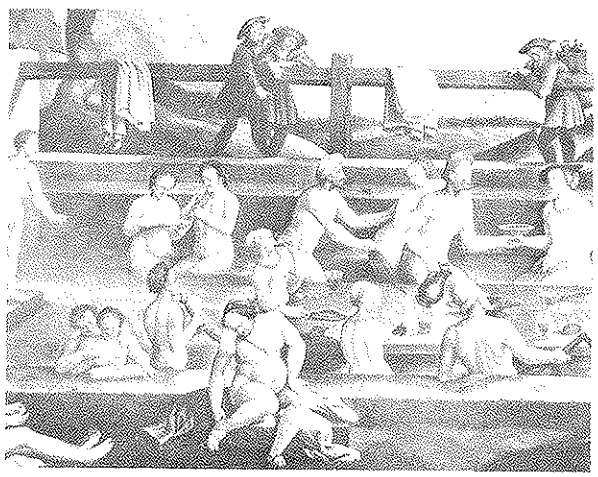
Medical treatment was available mainly to the wealthy, and those living in villages rarely had the help of doctors, who practised mostly in the cities and courts. Though most remedies were herbal in nature, the medications also included ground earthworms, urine, and animal excrement. Medieval doctors stressed prevention, exercise, a good diet, and a good environment. Apart from uroscopy, one of the best diagnostic tools of that time, other diagnostic aids existed, including taking the pulse and collecting blood samples. Treatment ranged from administering laxatives and diuretics to fumigation, cauterisation, and the taking of hot baths and herbs.

Professionals often have dramatic ways of declaring their judgement; judges who pronounced a death sentence broke the nibs of the pen used; medieval doctors dropped urine glasses to signal bad prognosis. (Picture D)



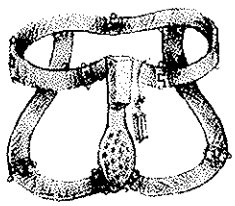
Picture D. Doctor dropping urine glass as a sign of bad prognosis.

To begin with, the lavish baths of Roman society did not find a counterpart during early medieval times. But when Charlemagne built a *therma* in his palace grounds, the interest was revived and the custom of hot baths gradually spread. A weary knight, resting in an aristocratic home, could enjoy a sauna bath being scrubbed by the ladies of the manor. By the end of the twelfth century, when public bathhouses were instituted by the Town Hall, they became an enthusiastic diversion for noblemen and common men alike. Men and women bathed together with spectators watching from a balcony (Picture E). With time, modesty became non-existent and bathing houses became base brothels. Fun and frolic with song, drink and dance escalated as people "scratched each others' back" among other things. Defeating the good intentions with which they were built, they became centres of public contagion. By the 14th century, civic authorities began to restrict entrance to the public baths. Syphilis dealt the death



Picture E. The bathing mania. Men and women frolic in a communal bath as spectators watch from a balcony. From A History of Medicine.

blow to public bathing, when the rapid incidence of the disease instilled fear in the community, leading to the eventual closure of all public bathhouses. Decreasing moral values led to an increase in venereal diseases. Paradoxically, a concurrent increase in female promiscuity led to a decrease in male trust leading to the development of the chastity belt, a typical middle age invention. (Picture F)



Picture F. Chasity belt Illustration from medieval manuscript.

The Black Death

Even as we live in a civilised and technologically advanced world, we are grappling with HIV, flesh-eating bacteria and the Ebola virus in recent years, not knowing when, where and how we would be able to conquer them. During the Middle Ages, our ancestors had the same predicament with two diseases, which by relative standards were no less scary or miserable. Plague and leprosy were the greatest villains of their time and many uncertainties still linger regarding the origin, impact and future threat of these catastrophic perils.

In 1346 rumours reached Europe of strange and terrible things happening in the East. People heard stories of a horrible scourge supposedly arising in China and spreading to India, Persia, Mesopotamia, Syria, Egypt and all of Asia Minor, told of a devastating death toll. India was reported to be depopulated, whole territories covered by dead bodies. There were no actual eyewitnesses, but a Flemish priest, basing his remarks on a letter from a friend at the papal court, mentioned that in a certain eastern province, unheard of tempests overwhelmed for three days. A rain of venomous beasts and reptiles on the first day, massive hail stones on the second and fire on the third supposedly slew most of the population. This was the first exaggerated news that the Europeans heard of impending disaster. But this phantom enemy, which had no name yet, caused little concern in Europe, since stories of natural disasters from the East were common and without the concept of contagion no serious alarms were felt. They were for a period, nonchalant about the rumoured pestilence, which, with unprecedented magnitude would soon be embracing them, shattering lives, families, institutions, and the very fabric of medieval society.

The Black Plague, or bubonic plague, appears to have begun in the eastern provinces of China, perhaps a result of the forced movement of peoples under the Mongol Empire. However, the plague bacillus was alive and active as early as the 6th century, when Europe suffered an epidemic (albeit in minor proportions) known as the Justinian plague. But the disease had lain relatively dormant in the succeeding centuries. Scientists say that the climate of Earth began to cool in the 14th century, and some think that perhaps this so-called little Ice Age had something to do with the rejuvenation of the bacillus.

It is not clearly known how or when exactly the plague first reached Europe. G.G. Coulter, a 20th century medieval historian wrote that it reached Constantinople in 1347, and from there followed the well-established Silk Road, making its way across the Eurasian continent until it reached the Middle East and the Eastern Mediterranean. From here, much of the Eastern spice and silk were carried in galleys to European merchants. A Flemish chronicler records that in January, 1348, three galleys from the East, laden with a variety of spices and other valuable goods put in at Genoa. The sailors were horribly infected and irremediably infected other people. Other accounts say that infected trading ships had reached Messina in October of 1347 with dead and dying men at the oars. At any rate, by January 1348 the Black Death had established itself in Sicily and on the Italian mainland, penetrated France via Marseilles, and North Africa via Tunis. From Marseilles it spread westward to Spain and northward to Avignon. It soon hit Rome, Florence and Paris. Crossing the channel, it reached southern England and crossing the Alps it conquered Switzerland before moving eastward into Hungary.

Michael Platiensis, in his account of the plague says that the disease appeared so virulent that anyone who only spoke to the patient was seized by a mortal illness and in no manner could evade death. The infection not only spread to everyone who had intercourse with the diseased, but also to those who had touched or used any of their things. Soon men hated and shunned each other- mother abandoned child; father abandoned son. Many drew their last will and testament and confessed their sins to the priests. Corpses lay forsaken in the houses. Since no man dared to enter, hired servants were paid high wages to bury the dead. The hired help incidentally helped themselves to all the valuables, gold and jewels of the house. However, the plague raged with such vehemence that soon there was a shortage of servants and finally none at all. People who migrated to flee from the epidemic, only transmitted the disease.

What the world didn't know at this time was that this was bubonic plague, a contagious, fatal epidemic disease caused by the bacterium *Pasturella (Yersinia) pestis*. The bacteria were transmitted by fleas, which travelled by virtue of *Rattus rattus*, the small medieval black rat that lived on ships, as well as the heavier brown or sewer rat. This infection was transmitted from person to person by normal contact or by the bite of fleas from an infected host. The flea infected the human by regurgitating the blood containing the bacteria from the rat. The rat dies. The human dies. The flea usually lives happily, unaffected by the bacteria, but may be choked to death by them

when they multiply in millions within the flea's alimentary tract. Nature sure has a morbid sense of humour.

The disease progression of plague was typical. The blood stream of the bitten or infected person then carried the bacteria throughout the body. The body temperature rose to about 104 degrees Fahrenheit and the victim became very ill, disoriented, vomited and experienced severe muscular pain. Swellings about the size of an egg or an apple appeared in the armpits and groin due to inflamed lymph nodes (buboes). Suppurated swellings oozed blood and pus and were followed by spreading boils and black blotches on the skin from internal bleeding. This gave the disease the name of "Black Death." The sick suffered severe pain and died quickly within five days of the first symptoms.

A second form, known as the Pneumonic Plague, was the most contagious and virulent form of the disease. It ravaged concurrently with its sibling. Respiratory contact and droplet infection spread the infection. The bacteria invaded the victim's lungs, which filled up with frothy, bloody liquid. The victims of this type, who vomited blood died in about twelve hours, or even less. As the disease progressed, the symptoms were continuous fever and hemoptysis, rather than bubonic swellings. These victims coughed and sweated heavily and died within three days or less, sometimes in 24 hours. In both types everything that issued from the body — breath, sweat, blood from the swellings and the lungs, bloody urine, and blood-blackened excrement — smelled foul. Depression and despair followed the physical symptoms, and before long "death is seen seated on the face." The malignancy of the pestilence appeared particularly terrible because it's victims had no idea of what was killing them, knew no prevention, and had no remedy.

Ignorance of this medical knowledge only augmented the horror of the time. The 14th century had no suspicion that rats and fleas were the carriers, perhaps because they were so common, familiar and perhaps, even innocuous. Fleas, a common household pest, were never mentioned in contemporary plague writings. Although folklore did associate rats with pestilence, they were not even suspected guilty in the plague crime. This is evident from the legend of the Pied Piper of Hamlin, which originated after a minor outbreak in 1284. The actual plague bacteria were to remain undiscovered for another five hundred years.

Leaving a strange pocket of immunity in Bohemia and Russia unattacked until 1351, the plague had passed on to most of Europe by mid-1350. The effects were devastating. In the words of Jean Froissart, the most famous historian at that time - "A third of the world died." By modern calculations, we now know that the population decreased from 125 million to 90 million- his was quite an accurate estimate.

Medical thinking, trapped in astrology and leechcraft, stressed air as the communicator of the disease, ignoring sanitation or visible carriers. Absurdity reached dizzy heights when people widely accepted rumours that the plague was caused by a corrupted cloud of mist or smoke carried by "foul blasts of wind" into Europe. Some declared that the sun had drawn up this cloud from the stagnant depths of the sea. Others blamed zodiacal influences and planetary alignments. In France, King Philip VI asked the medical facility of the University of Paris for a report on the affliction, which was threatening all human life. With careful thesis, antithesis, and proofs, the doctors ascribed it to a triple conjunction of Saturn, Jupiter, and Mars in the 40th degree of Aquarius said to have occurred on March 20th, 1345. They acknowledged, however, effects "whose cause is hidden from even the most trained of intellects." The verdict of the masters of Paris became the official version. Borrowed, copied from Latin into various vernaculars, and carried abroad, it was accepted everywhere, even by Arab physicians, as the scientific answer. But to the people at large there was only one real explanation - whether from bad air or planets, the plague was the wrath of God and divine punishment upon mankind for its sins.

When the plague eventually extinguished itself by the end of 1350 AD, the survivors were totally disillusioned. They believed that they were saved by the grace of God, chosen to inhabit the "better-conditioned, humble and virtuous" world. But society was never to be the same again. Feudalism started to decline. The church, unable to stop the pestilence or even come up with a satisfactory explanation for what was happening, lost much of its credibility and importance, when the common man realised the helplessness of the Church during the epidemic. The seeds of the Renaissance were planted.

The last outbreak in England was the Great Plague of London in 1665. During this epidemic, doctors began wearing a robe of toile-cirée, which was linen coated with a wax paste (Picture G). The idea was that the plague came from "venomous atoms" which infected salubrious air making it "miasmatic" or disease causing. These atoms were "sticky", clinging to things the way smoke or perfume clings to things. The waxed robe presumably provided no surface to cling to. The breathing tube beak was filled with materials imbued with perfume. A priest in Italy complained that the robe was useless against plague, saying, "it is good only to protect one from the fleas which cannot nest in it". This friar (who came close to guessing the cause of the plague without knowing it) complained of being "devoured by fleas, armies of which nest in my gown."

The sensible thing to do when the plague struck was to get out of town. Aristocrats could do this because they had estates in the countryside. The poor, who had nowhere to go, had no option but to remain and die. One of those in 1665 who had a country estate was a young Cambridge professor, Isaac Newton. He had been working on some theories and mathematical problems regarding the physics of motion, but his teaching duties allowed him little time to work on them. The plague of 1665 forced him into isolation and idleness. It was while at his country estate in the summer of 1665 that Newton solved the mathematical problems associated with his theory of gravitation. So, the plague was not without any virtue, after all.

Picture G. The plague robe of a medieval doctor.

The bubonic plague has not gone away. It still exists, everywhere in the world. In 1994, outbreaks of bubonic and pneumonic plague were reported in northern India. Because most of the reports were unconfirmed, the extent of the outbreaks is unclear. Following reports of a rat die-off, a few cases of pneumonic plague were reported, followed by several pneumonic plague cases and numerous deaths. It is quite common among rodent populations—rats, of course, but squirrels, rabbits and skunks as well. The Rocky Mountains in the US is one of the places where it is still endemic. Every few years, a hunter contracts the disease. Although we now have a cure for it, the disease progresses very quickly and there have been many instances where the patient doesn't make it.

The plague phenomenon has so far been very much a paranormal activity. But the plague is still very much with us. And the bacillus is out there.

Leprosy

Leprosy, which is supposed to have come from the East through Sicily, was the other disease, which became a pandemic in the twelfth century. Lepers were treated shabbily and with utter disgust. The all-powerful Church considered them unclean and unfit to be part of society. In a gruesome ritual, the victim was forced to wear burial shrouds and lie in a coffin before the altar, when earth was thrown on the afflicted and the priest declared him officially dead. From then, he had to wear gloves and fur shoes, clack a rattle warning others of his approach and beg for a living (Picture H). However, during the 12th century, the Church itself started building rehabilitation homes for them. This saw the emergence of more than 19,000 leprosoria all over Europe during the 13th century. By ostracising the patients, medieval society had unwittingly contained the contagion, which eventually led to the complete removal of this dreadful health threat by the 16th century. The plague, by natural decree, also did its part by wiping out a majority of leprous victims.

Picture H. Leper with his rattle.

The Dancing mania

Amidst our people here is come
The madness of the dance.
In every town there now are some
Who fall upon a trance.
It drives them ever night and day,
They scarcely stop for breath,
Till some have dropped along the way
And some are met by death.

Thus goes a grim ditty from the Straussburgh Chronicle of Kleinkawel, Germany in 1625, describing another outbreak of 'dancing mania', the last mass contagion of the Middle Ages. Manic dancing was first mentioned in the 14th century in Germany and sporadic outbreaks are described right until the 17th century. The first major outbreak of dancing mania was in Aix-la-Chapelle in July 1374, where a group of people was seen to dance uncontrollably in the streets, foaming at the mouth and screaming of wild visions. They kept on dancing until they collapsed from exhaustion, but even then they flailed about in agony. They had to be forcefully restrained in swaddling clothes, the primitive version of the straightjacket. The mania caught on and spread rapidly throughout France and some parts of Europe and had its peak around 1418.

Dancers filled the streets around the clock, accompanied by musicians. In the High Middle Ages, music was considered a cure for the ills of the mind as well as the society. (Picture I) The wild gyrations of the dancing mania were treated by the playing of music, similar to the way seizures were treated at that time. In Straussburg, Germany, where the disease was at its worst, thousands had either been afflicted with the

dancing mania, or caught up in the dancing, or trying to help, or gawking from the sidelines, that the normal activities of the town were brought to a complete standstill on several occasions. The frenzied people danced to the tunes of instruments through the streets until they dropped down exhausted. They were eventually exorcised in the chapel of St. Vitus, who soon became the patron saint of the dancers. A clinical condition called as Sydenham's chorea was also known 'St. Vitus' Dance' because of the similarity of symptoms in both. However, it is unrelated to manic dancing. According to the Cologne chronicle, many dancers became victims of much "fraud and knavery" and more than a thousand women and virgins in Cologne became pregnant during the dancing epidemic.

leads to another hypothesis that the manic dancers (at least some of them) were victims of ergot poisoning. Ergotism, known in the Middle Ages as "St. Anthony's Fire", is a toxic condition in humans and animals who inadvertently eat rye and other grasses parasitized by *Claviceps purpurea* (ergot). This small brown fungus produces an amazing array of dangerous chemicals, including lysergic acid diethylamide (LSD). Symptoms of ergotism may include psychotic delusions, nervous spasms, spontaneous abortion, convulsions, and gangrene. LSD, in particular, causes intensely coloured hallucinations, perhaps explaining the visions of some of the dancers, like those who claimed to have seen the heavens open up to reveal the Gods. Ergotism is also frequently fatal and could have been the cause of the death of the dancers.

Picture I. The Dancing Mania. Drawing by Brueghel (1564) showing hysterically dancing women restrained by their companions, as musicians continue to provide music - from A History of Medicine.

In the late 15th century, one particular outbreak in the town of Taranto in Southern Italy gave rise to an actual dance form. Here, it was believed that the manic dancing was caused by the bite of a local spider. Again, music was employed to try to cure the dancers. The name of the local dancing mania became known as '*tarantism*', after the town of Taranto, and the indigenous tarantula spider. This species is not poisonous and could not have been responsible for the mania.

The dancing mania eventually died out or assumed other forms, leaving others to wonder as to how and why it happened. The manic dancers are first described in the late fourteenth century, a time of beautiful art, music, and poetry. Several hypotheses exist to explain the dancing manias and the true cause will perhaps never be known. One hypothesis suggests that the dancing mania arose as a form of mass hysteria. The spectre of the Black Death could have struck massive terror and despair, engendering mass hysteria and manic dancing could have been an expression of this hysteria. The dancing mania may also have had a physical cause. The descriptions of the symptoms of some of the sufferers

Conclusion

In a curious coincidence, the Middle Ages began and ended with the catastrophic plague; between them the plagues of the sixth century and the fourteenth century wiped out a big percentage of the population. The Middle Ages saw leprosy at its worst. Victims of leprosy, with their peaked hood, grey sackcloth and sinister rattle became the dreaded phantoms of this age. Latin replaced Greek as the new universal language. The Church wielded great authority during the early half of this period, but lost its supremacy when the public lost confidence, observing the helplessness of the Church during the plague epidemic. In fact, the decline of monastic medicine started even in the twelfth century, when the Church authorities realised that monks were far too preoccupied with healing activities and neglecting their religious vows and commitments. So, for a period, monasteries employed lay physicians on a contractual basis, paying them meagre salaries. However, famous ones like John de Bosco, were given beer and a horse, in addition to the remuneration.

Monastic practice was finally banned in the early part of the thirteenth century, but by that time the ancient knowledge had been transmitted to the lay schools and universities.

Medieval medicine has been largely described as an uneventful night. However, Greek medical thought transmitted through the Islamic world was preserved in the vaults of the monasteries and transmitted to European universities. The changes that occurred during this period are numerous; they include the introduction of gunpowder, increased importance of cities, economic and demographic crises, political dislocation and realignment, and powerful new currents in culture and religion. In a dark, hostile and violent environment, tormented with epidemics and punctuated with wars (especially the Crusades), learned men tried to light candles and strove hard to bring light. The greatest boon derived from this age was the establishment of hospitals and the development of formal medical teaching in universities. The late medieval times also saw some enforcement of public health measures. The concept of quarantine arose during this period when Italy first enforced *quarantenaria* – a forty-day isolation period for suspected disease carriers. In short, the pillars of modern medicine were erected and the stage is now set for the Renaissance and the beginning of science.

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THE USE OF BISPHOSPHONATES IN OSTEOPOROSIS: A REVIEW

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ABSTRACT: **Bisphosphonates are synthetic analogues of pyrophosphate. Their main pharmacological effect is to inhibit bone resorption by a variety of mechanisms, not all of which are clearly understood. The activity of the bisphosphonates varies depending on the compound. In clinical trials, they have been shown to stop postmenopausal bone loss and increase bone density, with a concomitant reduction in fracture rate with some agents. This article reviews the currently known mechanisms of action of the bisphosphonates and the evidence that they are useful in the treatment of osteoporosis.** *(JUMMEC 1998 1&2: 13-17)*

Introduction

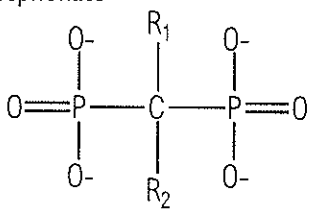
The bisphosphonate class of drugs have been developed over the last 20 years following the discovery in blood and urine of pyrophosphate, a naturally occurring inhibitor of calcification (1). Pyrophosphate consists of a P-0-P back bone which is easily hydrolysed and therefore does not allow for therapeutic use. Analogues of pyrophosphate, with the oxygen atom in the P-0-P backbone being replaced by a carbon atom to form a P-C-P backbone, were developed which are resistant to hydrolysis (2) and are called bisphosphonates (previously diphosphonates) (Figure 1).

Modification of the side chains of the carbon atom has led to the development of a variety of compounds with different properties. The aim of these modifications has been to increase anti-resorptive activity without a similar effect on inhibition of mineralization, since the latter has little clinical potential (2). The addition of amino groups (pamidronate, alendronate and risedronate), increasing the length of the side chains on the carbon atom (alendronate) and more recently adding methyl and pentyl groups (ibandronate) have led to high anti-resorptive properties without the concern of inducing defective mineralization (Table 1).

Mechanism of Action

All bisphosphonates bind strongly to hydroxyapatite, inhibit calcium phosphate formation and ectopic mineralization through a physicochemical inhibition of crystal growth. Bisphosphonates have similar effects on calcium carbonate, which is why they were originally developed as washing powders. Apart from this, they also inhibit bone resorption by inhibiting osteoclastic activity. The bisphosphonates are deposited onto bone because of their strong affinity for the mineral, and the ostcoclasts are then inhibited when they start to

Basic bisphosphonate



Pyrophosphate

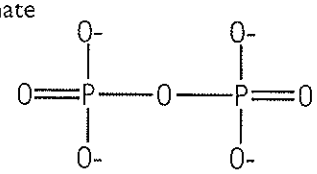


Figure 1. Chemical structure of a bisphosphonate and pyrophosphate

Table 1. Relative anti-resorptive properties of bisphosphonate compounds (4)

Compound	Anti-resorptive Potency *(in vivo)*
Etidronate	1
Clodronate	10
Tiludronate	10
Pamidronate	100
Alendronate	700
Ibandronate	4000
Risedronate	5000
Zolendronate	10,000

engulf the bisphosphonate containing bone. They seem to be preferentially deposited under the osteoclasts (3). Experiments with bisphosphonates on osteoclast cells show a reduction in acid production, reduction in lysosomal enzyme activity, reduction in prostagiandin secretion and increased membrane permeability (4). In addition, bisphosphonates also reduce the formation of osteoclasts from their mononuclear haematopoietic precursors in marrow cultures (5) and there is evidence that they can cause osteoclast apoptosis *in vitro* and *in vivo* (6). However, bisphosphonates will inhibit *in vitro* resorption of mineralized substrate even if they are added directly to the cells only, which suggests that they will also act in ways apart from binding onto bone mineral. This was found by Sahni and colleagues (7) who showed *in vitro* that inhibition of bone resorption is dependent on the presence of osteoblasts. Recently, it has been shown that in the presence of bisphosphonate, osteoblasts are stimulated to produce an inhibitor of osteoclast resorption which is heat and proteinase labile and has a molecular mass between 1 - 10 kDa (8).

The current evidence suggests that the mechanism by which bisphosphonates inhibit bone resorption is complex and involves a combination of a direct effect on osteoclasts and precursors and an indirect effect on osteoclasts via osteoblasts. The relative importance of these two mechanisms remains unclear.

Most of the pharmacokinetic data for the bisphosphonates come from animal studies with limited data on humans. Gastrointestinal absorption is poor with only 1 - 10 % of the ingested dose absorbed (2). Absorption occurs in the small intestine (in rats) and is markedly decreased by the presence of food, especially those which contain calcium. Therefore when these compounds are administered orally they need to be taken on an empty stomach (2). Metabolism does not occur, at least of the P-C-P bond, and approximately 20-50% of a given dose (depending on the compound) is taken up by bone, the remainder undergoing renal excretion (9). The half-life in the blood is therefore short - between 30 minutes and 2 hours. However, the half-life in bone is very long, as the compounds are buried within the skeleton. Although skeletal blood flow partly determines uptake of bisphosphonate into bone, the other important factor is that there is preferential uptake to bones that have high turnover rates, especially at sites undergoing resorption, probably due to the larger exposure of hydroxyapatite in these areas, onto which bisphosphonates are adsorbed. Once in bone, they are liberated again only when the bone in which it was deposited is resorbed and thus the bisphosphonate half-life in bone is related to the rate of local bone turnover (2).

Clinical Uses

Etidronate

Etidronate was the first bisphosphonate compound used clinically to treat Paget's disease of bone. At higher doses, it had the disadvantage of impairing mineralization of newly formed bone matrix as well as inhibiting resorption (10). However, when used at low doses (400 mg daily) in an intermittent cyclical regimen, it has been shown to improve bone mineral density (BMD) and reduce fracture rates in women with spinal osteoporosis compared to placebo-treated controls who lost BMD and had an increased risk of fracture. It was the first bisphosphonate used for the treatment of postmenopausal osteoporosis (11- 13).

Treatment with etidronate for 3 years led to an increase in lumbar spine BMD of between 5-8% (11,13) and, to an increase in proximal hip BMD of 1.44 % (13). Further treatment for another year maintained the gain in BMD obtained over the first 3 years (13). In 17 patients that took etidronate for 5 years, there was an additional 1.4% increase during the fourth and fifth years on top of the 5.5% gain in vertebral BMD after the first 3 years (14).

Looking at the more important clinical end-point of fractures, in the 2 year study with etidronate, there were 29.5 in the treated group compared to 62.9 new vertebral fractures per 1000 patient years in the placebo group (12). In contrast, Storm and colleagues (11) found no overall significant difference in the rate of new vertebral fractures in their 3 year study with etidronate (18 in the treated compared to 43 in the placebo group per 100 patient years) but the difference was significant between weeks 60 to 150 (6 in the treatment group compared to 54 new fractures in the placebo group per 100 patient years). After 5 years of etidronate therapy, there was no further reduction in the rate of new vertebral fractures (14). Harris and colleagues (13) found no overall significant differences between the fracture rate in their bisphosphonate-treated group compared to placebo. However, there was a significant reduction in the new vertebral fracture rate in etidronate-treated patients at high risk for fracture (that is, those with low spinal bone density and 3 or more vertebral fractures at study entry), compared with non-etidronate-treated patients (228 compared to 412 fractures per 1000 patient years respectively). There is no data with etidronate on non-vertebral fracture rates.

Alendronate

Alendronate, an amino-bisphosphonate, has recently become available in Malaysia for the treatment of osteoporosis. It has been shown to be useful in the treatment of postmenopausal women with osteoporosis (15-18). After 2 years of alendronate therapy, there

was an increase in lumbar spine BMD of 7.2 %, an increase in femoral neck BMD of 5.0 % and an increase in total body BMD of 2.5% (16). After 3 years of therapy, there was an increase in lumbar spine BMD of 6.2-8.8%, an increase in femoral neck BMD of 4.1-5.9% and an increase in total body BMD of 1.8-2.5%, compared to placebo (17, 19).

The rate of new vertebral fractures was reduced by approximately 50%; the incidence of new vertebral fractures in the alendronate-treated group was reduced to 3.2% compared to 6.2% in the placebo group overall (17). In addition, alendronate has been shown to reduce the incidence of new hip and wrist fractures, as well as new vertebral fractures, by approximately 50% after 3 years treatment (19). However, the numbers that needed to be treated to prevent a fracture were large; the 1022 women in the study that took alendronate for 3 years had 11 hip fractures (1. 1%) compared to the 1005 women on placebo who suffered 22 hip fractures (2.2%). For wrist fractures, 2.2% of women taking alendronate suffered a fracture compared to 4. 1 % of those on placebo (19).

Alendronate has also been shown to prevent postmenopausal bone loss (20). In a study of 1174 healthy postmenopausal women, 5 mg of alendronate increased lumbar spine BMD by 3.5% and increased femoral neck BMD by 1.3% after 2 years treatment, compared to the placebo groups which lost 1.8% and 1.6% at the lumbar spine and femoral neck respectively. The gains in BMD with alendronate were only slightly less than those obtained with a oestrogen-progestin regime given to an additional 110 women in the same study (20). This suggests that alendronate can be used to prevent postmenopausal bone loss with a similar efficacy to hormonal regimes, which makes it a useful alternative to women unable or unwilling to take hormone replacement therapy.

Other Bisphosphonates

Other bisphosphonates that have been shown to improve BMD in postmenopausal women are pamidronate, both intravenously (21) and orally (22,23), clodronate, both intravenously (24) and orally (25), tiludronate (26), risedronate (27), and ibandronate, both intravenously (28) and orally (29). However, only the first 3 compounds are currently licensed overseas, and, apart from pamidronate (22) and clodronate (24), there is no fracture data with these other bisphosphonates.

Intravenous pamidronate infusion of 30 mg every month over 2 years have been shown to increase lumbar spine BMD by 10. 1% and femoral neck BMD by 4.8% (21). Oral pamidronate at a dose of 300 mg daily will improve lumbar spine BMD by 3. 1% and femoral neck BMD by 3.2% after 1 year of treatment (23). At a lower dose of 150 mg oral pamidronate daily over 2 years, there was an increase in lumbar spine BMD of 7% and femoral neck BMD was maintained, compared to a fall in the placebo group (22). In this study, there was also a non-significant reduction in vertebral fracture rates in the pamidronate group of 13/ 100 patient years compared to 24/ 100 patient years in the placebo group (22).

Cyclical oral clodronate 400 mg daily for 1 month, followed by 2 months without any treatment, will increase lumbar spine BMD by 3.88% compared to a loss of BMD of 2.34% in the placebo group after 1 year (25). Giving oral clodronate continuously daily does not result in a better gain in BMD (30). In a 6-year trial, 200 mg intravenous clodronate given every 3 weeks lead to a 5.69% increase in lumbar spine BMD compared to controls and reduced the incidence of new vertebral fractures after the third year of treatment (24).

Tiludronate has been shown to maintain lumbar spine BMD in postmenopausal women compared to a 2. 1% loss in the placebo group after a 6 month course of oral tiludronate 100 mg daily (26).

Oral risedronate can be taken either cyclically, 5 mg daily for 2 weeks followed by no treatment for 2 weeks, or 5 mg daily. When taken cyclically for 2 years, it will prevent postmenopausal bone loss at the lumbar spine compared to a 4.3% reduction in the placebo group. Daily treatment with risedronate will increase lumbar spine BMD by 1.4% after 2 years, an increase of 5.7% compared to placebo. At the femoral neck, BMD is maintained with either cyclical or daily risedronate compared to a decrease of 2.4% in the placebo group after 2 years (27).

Ibandronate is another of the bisphosphonates that can be given orally or intravenously. Oral therapy at a dose of 2.5 mg daily will lead to a 4.6% increase in lumbar spine BMD after 1 year (29). Cyclical bolus injection of 2 mg of ibandronate every 3 months increased lumbar spine BMD by 5.2% after 1 year and maintained femoral neck BMD (28).

Side-Effects

The most common side-effects of the oral bisphosphonates are gastro-intestinal, most frequently nausea, but also abdominal pain and dyspepsia. However, in most of the clinical trials, the frequency of adverse events have been similar in the placebo and treatment groups. Alendronate has been associated with severe oesophagitis and oesophageal ulceration (31) although the actual incidence is very low. It is reversible on stopping therapy with alendronate and its occurrence prevented by careful instructions on taking the drug, i.e. the tablet should be taken with at least 180 ml of water and the patient should maintain an upright position for 30 minutes after swallowing the tablet. Concomitant use of acid-suppression drugs together

with, alendronate do not seem to heal the oesophageal lesions(31).

Response to Therapy

The majority of patients seem to respond to bisphosphonate therapy with a gain in bone mass. Of the limited data that is available, over 96% of women treated with alendronate 10 mg daily showed increases in lumbar spine BMD (17) and between 60-80% of patients will respond to oral pamidronate 300 mg daily (23). However, although the bisphosphonates have a long half-life in bone, it is disappointing that once treatment is stopped, the bone loss resumes, similar to the patients on placebo (27,32). But, reassuringly, there is no evidence that bone loss is accelerated once these agents are stopped.

Conclusion

Bisphosphonates have potent inhibitory effects on bone resorption due a variety of mechanisms. They have been shown to stop postmenopausal bone loss and increase BMD. In addition, some agents have been shown to reduce the incidence of fracture, the end result of low bone mass. The currently available bisphosphonates are taken orally, which can create problems because of poor/variable absorption and the potential for gastro-intestinal side-effects. A more promising approach would be the use of intermittent intravenous infusions/injections of potent bisphosphonates which would avoid the above problems with similar improvements in BMD.

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DNA VACCINES

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General overview

Vaccines have proven to be the most successful medical intervention of human morbidity and mortality. Bacterial vaccines have resulted in the marked decrease in the incidence of human infections such as tetanus, diphteria and whooping cough. Viral vaccines have not only reduced the incidence of paedeatric diseases such as measles and poliomyletis but have resulted in the complete eradication of small pox.

Current vaccines that are available can be broadly categorised into two groups: live and dead. Live vaccines encompass attenuated microbes which are viral or bacterial that were selected for their reduced pathogenecity but with maintained immunogenicity, and recombinant vaccines which are foreign antigens expressed in a bacterial or viral vector.

Dead vaccines, on the other hand, include killed, whole pathogens, as well as soluble pathogen proteins and protein subunits.

The nature of a vaccine determines the type of immune response induced. Dead vaccines cannot efficiently enter the MHC I pathway, and may be less effective in inducing the cell-mediated immune response that is critical in protection against many diseases caused by intracellularly replicating organisms. Live vaccines on the other hand may be dangerous to immunocompromised hosts as they can revert to pathogenecity within the vaccinated person. They could also be contaminated by potentially harmful chemicals during production.

The ideal vaccine should be safe, cheap, heat stable, containing protective immunogenic sequences from multiple pathogens, and administered preferable as a single dose. To date, no such vaccine for human use meets all these requirements.

There is, however, a novel approach to the control of infectious agents in the form of DNA vaccines which could prove to be the answer to the ideal vaccine.

DNA vaccines, which are currently under development, utilize genes encoding the proteins of pathogens or tumour, rather than the pathogen or their subunits as in the more conventional approaches.

The principles of DNA vaccines is outlined in Figure 1 (1). Briefly, recombinant DNA technology is used to clone in the genes which encode one or more microbial antigens of interest (potential immunogen) into an eukaryotic expression vector. The constructed plasmid is transformed into a bacterial host (*E. coli*), grown up in large quantities and purified from bacterial contaminants. The purified plasmid construct is then directly inoculated into the host via intramuscular or intradermic injection. The DNA enters some cells where RNA transcription and protein translation of the genes encoding the bacterial antigens occurs. The expressed antigen is taken up by specialized cells of the immune system and transported to draining lymph nodes where an immune response to the disease is then elicited.

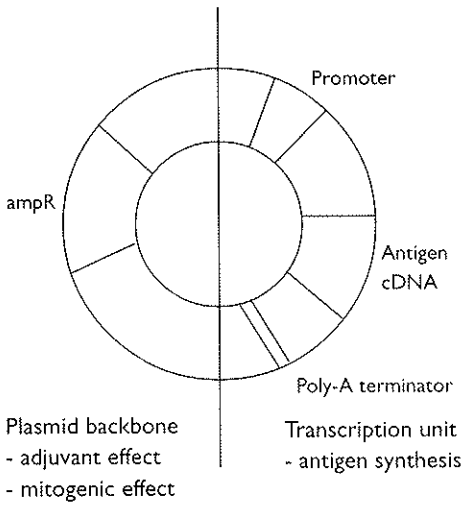

Figure 1. Principle of DNA Vaccines (adapted from Lambert and Siegrist (1997)(1)

Advantages of DNA vaccines

The advantages of DNA vaccines over conventional vaccines are numerous.

Firstly, they are easy to manufacture and much cheaper. They provide prolonged antigen expression that continuously stimulates the immune system (2). DNA vaccines also elicit qualitatively different immune responses which include induction of MHC-class I-restricted CTL and Th 1-biased immune responses (3). The technology with which they are constructed enables manipulation of the antigenicity of the protein at DNA level without the need for protein production and purification. Genes inserted into a plasmid can be modified readily, allowing the removal or insertion of carbohydrate side chains or other residues that could

affect the processing of the protein. The sequence could also be modified by site-directed mutagenesis resulting in single-amino acid changes that could enhance the antigenecity of the protein. Parts of the gene sequence could be deleted that encode for epitopes that trigger unwanted immune responses. When co-delivered with plasmid DNA-encoded cytokines or co-stimulatory molecules, a DNA vaccine offers the possibility for enhancement or modulation of the subsequent response to the DNA-encoded antigen (4). Resistance to heat would enable the use of DNA vaccines in countries where "cold chains" are difficult to maintain. DNA vaccines lack a replicating agent therefore are safer to be administered to pregnant women or immunocompromised patients. They have the capacity to induce, in murine models, adult-like antibody, Th1 and CTL responses in early life when the immune system is still immature (5).

DNA vectors

Expression of the protein of interest encoded in the vector of the DNA vaccine is influenced by several factors, one of the most important being the plasmid used. Basically, the plasmid used for DNA vaccination (see figure 2) comprises of 2 major units:

1. plasmid backbone that delivers adjuvant and mitogenic activity via immunostimulatory sequences, and
2. transcription unit comprising a promoter, antigen cDNA and polyadenylation (A) addition sequence, which together direct protein synthesis.

Most of the commercially available mammalian expression vectors carry a promoter from the human cytomegalovirus (HCMV) which has been shown to induce high level expression in many cell types (6), although alternative promoters are being studied namely plasmids containing control sequences from human papilloma viruses which result in longer term gene expression (7) Furthermore, the addition of introns and efficient transcription termination/processing units have been shown to increase gene expression in the mamalian host (8,9).

Route of administration

Protective immune responses can be generated by skin, muscle and intravenous innoculations of DNA (10). DNA immunization can been carried out by a number of methods which include direct injection of the naked DNA in saline (11), of DNA complexed with lipids (12) , and by impelling DNA either by an aerosol or using a gene-gun to propel DNA-coated gold beads into cells (13).

The most widely used methods for immunization has been the direct injection of the "naked" plasmid DNA

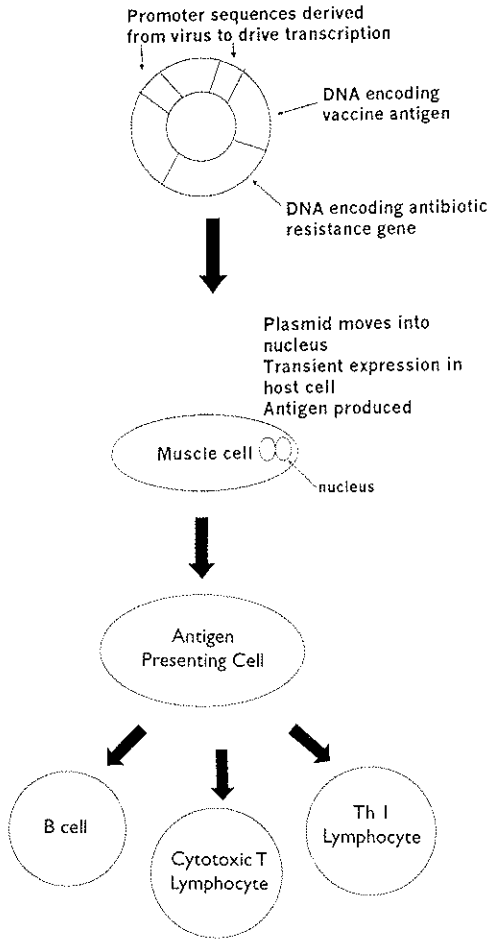

Figure 2. Schematic representation of plasmid DNA used in gene vaccination (adapted from Tighe *et al.*, 1998 (3)

into skeletal muscle and the gene-gun administration to the skin. When compared with intra-muscular immunization (i.m.), the gene-gun method requires almost 100-fold less DNA and the injections are more reproducible (14). However i.m. is easier to carry out and is more cost-effective.

Site of gene expression differs with mode of administration. Following muscle innoculations, most of the antigen expression occurs in the skeletal muscle (15) whereas following skin inoculations, expression is mostly in keratinocytes (2,16). The success of these DNA immunizations gave rise to the suggestion that the skeletal muscle and keratinocytes might be presenting the antigen to the immnune system as opposed to the conventional immunizations where the immune response is initiated by bone marrow-derived antigen presenting cells (17).

A recent study by Grillot-Courvalin *et al.* (18) has shown that a better, safer method of delivery is by using bacteria that are non-pathogenic that have been genetically modified to enter cells and release their plasmid DNA, for example E. coli bearing a deficiency in cell wall

biosynthesis and transformed with the gene encoding the protein invasin from Yersinia pseudotuberculosis, that die after entry into mamalian cells thereby releasing their contents which include plasmid DNA.

Immune response

DNA immunization results in the uptake of the DNA into cells close to the injection site. The DNA that remains episomally is subsequently transcribed and translated causing expression of the vector-encoded protein (15,19). The protein is processed like a virus-encoded antigen, resulting in presentation of antigenic fragments in association with MHC Class I molecules which result in the activation of cytolytic T cells (11). The immune response, as studied in mice, is weaker than that of conventional vaccines but has proven to be exceptionally long lasting and requires only a single dose (13,17,20). Presentation of the antigen is thought to be by the muscle cells but transfection of the plasmids into antigen-presenting cells (such as dendritic cells) residing in the muscle tissue presumably occurs (1). Activated dendritic cells up-regulate MHC and co-stimulatory molecules, secrete cytokines, and migrate to the lymph nodes where they initiate an immune response (21). One activation signal of dendritic cells is the cytokine granulocyte-macrophage colony-stimulating factor (GM-CSF) (22). In addition, stimulation of T helper cells and B cells occur (23,24) resulting in protection to the challenge.

Safety

Questions that must be addressed regarding the safety of DNA vaccines include: Does integration of the plasmid lead to insertional mutagenesis of the host genome? Are anti-DNA antibodies induced? Does immunological tolerance against the antigen occur if produced over a lengthy period? (25). Studies have shown that the integration of plasmid after intra-muscular injection is very low (26) and that seems to be no induction to tolerance whatsoever (27). Furthermore, there seems to be no significant increase in the levels of pathogenic anti-DNA antibodies (28). However, more studies are being carried out to determine the safety of these viruses vaccines.

Conclusion

DNA vaccines seem to have all the potential qualities of an ideal vaccine. However the safety, feasibility and immunogenecity of these vaccines in humans are currently under investigation. To date, many experimental trials have been successfully conducted in a variety of disease models including HIV (29), malaria (23), Hepatitis B(30) rabies (24) and cancer, specifically B-cell lymphoma (31). If DNA vaccines can be established in regard to their safety and efficacy, this may eventually lead to the replacement of existing conventional vaccines and allow the prevention of diseases that were previously unable to benefit from vaccine intervention.

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AGE-RELATED NEURONE-LOSS AND THE OCCURRENCE OF DARK AND LIGHT NEURONES IN THE GANGLIA OF CRANIAL NERVES AND AUTONOMIC NERVOUS SYSTEM: A COMPARATIVE EVALUATION OF THEIR DEVELOPMENTAL AND GROWTH CHANGES

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ABSTRACT: Senescent-decline in the nervous-system functions is very frequently attributed to age-related neurone-loss. Processes and mechanisms involved in neuro-degeneration form part of the structural frame-work for interpreting the functional consequences. The literature concerning this matter are confusing and contradicting. The behaviour of cranial nerve ganglia was studied using neuro-histological techniques. On the evidence available in the present study, the dark cells are considered as active ones; the light cells are considered as those which have failed to establish functional projections, inactive, dying, dead or degenerating ones. Probably it is during the medium-sized stage of cell growth, the peripheral and central processes (of axons) begin to grow from the cell body and attempt to get established in their projection fields. The light cells have appeared among the very-small cells just on the day of hatching. This probably signifies the possible attempt to eliminate the growing cells since they are no longer needed to replace larger categories of cells which have already well-developed neuronal connections at this stage. It is assumed that the time of appearance of light cells might be indirectly related to the onset of establishment of active functional connections of neurones and to the functional importance of the organs which it supplies. *(JUMMEC 1998 1&2: 22-46)*

KEYWORDS: Neurone-Loss and Ageing, Dark and Light Neurones, Sensory and Autonomic Ganglia

Introduction

Senescent-decline in the nervous-system functions is very frequently attributed to age-related neurone loss. Processes and mechanisms involved in neuro-degeneration form part of the structural framework for interpreting the functional consequences of age-related changes in other parameters. The literature concerning age-related neurone loss give confusing and sometimes contradicting data and therefore, remain with controversy (1, 2, 3, 4, 5, 6). However, age-related neurone-loss represents a structural basis of senescent-decline in nervous system functions.

Dark and Light types of neurones, based on staining properties have been documented in many vertebrates (7, 8, 9, 10) including primates (11, 12). A few investigators (9, 13, 14) have found differences in chemical constituents in these two types of neurones in sensory ganglia of rodents. Similar observations have been reported in mammals (9, 15, 16) and reptiles (17).

The significance of Dark and Light neurones in different ganglia in different animal species has been controversial in available literature. Dual embryonic origin (of epidermal placode and of neural crest origin) (18), as fixation artefacts (19), difference in central and peripheral projections (20, 21), different sensory functions (22, 23), different histogenetic characteristics (10), difference in distribution of cytoplasmic organelles and relative density of cytoplasm (8, 12, 24), fluid shift between cells and the surrounding extra-cellular spaces (25) have been offered as different hypotheses. From available literature, there is no report of a study in the whole life cycle of any one animal species so as to infer a conclusive significance of this dual cytology of neurones. All these works have been done in adult animals or in certain stages of development or growth.

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Therefore, it is thought useful to study this aspect in different ganglia related to different functions, in the whole life cycle of any one animal species during embryonic development through adult, so as to infer the conclusive significance and hypothesis regarding the occurrence of this dual cytology and to see whether their occurrence is related to age-changes. This has been really rewarding to achieve this conclusive information from the results obtained as described below. However, the discussion is restricted to some useful points (rather than description) in order to simplify a great deal of repetition. (However, the detailed descriptions in relation to individual ganglia can be found elsewhere.) The results in the present study can be of great value to correlate and evaluate the functional status of similar ganglia during development and in relevant clinical conditions and ageing in the human.

Material and methods

The chicks *Gallus gallus domesticus*, White Leghorn breed were used in this study. Fertilised eggs were collected in groups of 25 - 30, and incubated at a temperature of 37.5 degree Centigrade. The date and time while beginning the incubation were recorded every time when a new set of eggs was kept for incubation. After every 24 hours from this time, it was considered as Embryonic Day 1 (E1), Embryonic Day 2 (E2) etc till hatching (H). Embryos from E3 till hatching were removed carefully without causing damage and fixed in 10 % formaldehyde solution at least for two weeks. Larger (older) embryos were cut transversely into suitable smaller pieces and labelled serially for future orientation. The tissues of older embryos (i.e., E15 and onwards till adult) were usually decalcified after fixation. In the adult, and in those belonging to later stages of development, the head at the level of C6 was separated and the brain was exposed by a longitudinal cut on the skull by means of a thin bone-saw, to facilitate proper fixation of the brain tissue. After making paraffin blocks, serial sections of 8 - 10 microns were stained by Cresyl Fast Violet for Nissl granules.

Only a few selected stages which showed some remarkable changes are described in this work. These include E6, E8, E10, E13, E15, E18, chick on the day of hatching and adult. In all, three animals in each group, having a total of twenty four animals were used. Ganglia of both sides in each animal were used for observation. Average of the results of all six ganglia is described in the results. Every section of the ganglion was observed, drawn and the cells were plotted in diagram with the help of light microscope having a camera lucida attachment. Different categories of neurones were classified into Dark and Light neurones according to the difference in the intensity of cytoplasmic stain. Each of these types is again subdivided into various subclasses (according to size) represented in the diagram by a symbol. Only those cells having a clear nucleus and a nucleolus were counted. Dimensions of cells were measured with the help of an eye piece graticule. The following categories of cells (based on average dimensions) were classified. Tiny (< 5 microns), very small (6 - 10 microns), small (11 - 15 microns), medium sized (16 - 20 microns), big (21 - 25 microns), very big (26 - 30 microns), large (31 - 35 microns), very large (36 - 40 microns), giant (41 - 45 microns), gigantic (46 - 50 microns), gigantic giant (> 51 microns).

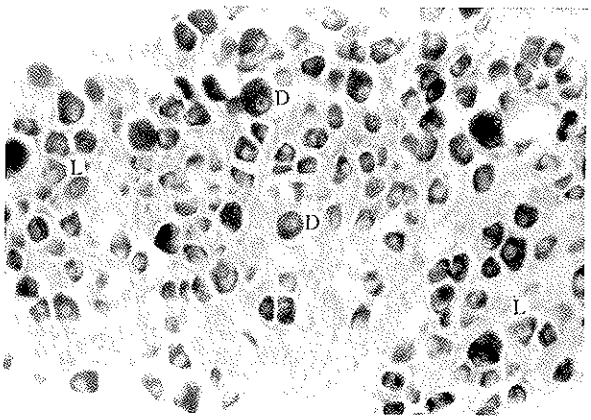

Figure 1. Shows Dark (D) and Light (L) cells observed in the ganglion

The following sensory and autonomic ganglia have been studied.

Trigeminal (Sensory)
Genicular (Sensory)
Vestibular (Sensory)
Acoustic (Sensory)
Prox. G. Comp.of N. IX and X (Sensory)
Petrous (Sensory)
Nodose (Sensory)
Ciliary (Parasympathetic)
Superior Cervical (Sympathetic)

Results

The ganglion showed great difference in different age groups of animals and in different areas of the same ganglion. These changes during successive embryonic days, on the day of hatching and in the adult situation were studied in greater detail. The most striking changes are as follows. When the dark neurones alone are present in the ganglion, they are represented just by their numbers; when they are mixed with light neurones, D= dark neurones, and L= light neurones.

1. Trigeminal Ganglion

The trigeminal ganglion could be recognised clearly on E6 while it had a rostro-caudal length of 0.376 mm and a volume of 0.0485 mm^3. The ganglion had 73862 cells, all which were dark type. In all, there were 4923 (6.67

%) tiny cells, 24453 (33.11 %) very small ones, 41267 (55.87 %) small ones, and 3219 (4.36 %) medium sized ones. On E8, the ganglion had a length of 0.600 mm, a volume of 0.1414 mm^3 and had 259405 cells. Among these cells, 259327 (99.97 %) were dark type and 78 (0.03 %) were light ones. In all, there were 63347 (24.42 %) tiny cells, 168400 (64.92 %) very small ones, 23973 (D= 23970 + L= 3) (9.24 %) small ones and 3685 (D= 3610 + L= 75) (1.42 %) medium sized ones. On E10, the ganglion had a length of 0.594 mm and a volume of 0.1909 mm^3. There were 101199 cells. Among them, 100603 (99.41 %) cells were dark type, and 596 (0.59 %) were light ones. In all, there were 581 (0.57 %) tiny cells, 17067 (16.86 %) very small ones, 59443 (D= 59095 + L= 348) (58.74 %) small ones, 24043 (D= 23804 + L= 239) (23.76 %) medium sized ones, 52 (D= 45 + L= 7) (0.05 %) big ones and 13 (D= 11 + L= 2) (0.01 %) very big ones. On E13, the ganglion had a length of 0.780 mm, a volume of 0.4641 mm^3 and had 84493 cells. Among these cells, 52199 (61.78 %) were dark type and 32294 (38.22 %) were light ones. In all, there were 510 (0.6 %) tiny cells, 27203 (32.2 %) very small ones, 25841 (D= 10824 + L= 15017) (30.58 %) small ones, 22442 (D= 9856 + L= 12586) (26.56 %) medium sized ones, 7062 (D= 3131 + L= 3931) (8.36 %) big ones, 1140 (D= 503 + L= 637) (1.35 %) very big ones, 283 (D= 163 + L= 120) (0.33 %) large ones, 4 (D= 3 + L= 1) very large ones, and 8 (D= 6 + L= 2) gigantic type. On E15, the ganglion had a length of 1.300 mm, a volume of 0.2004 mm^3 and had 62441 cells. Among these cells, 34032 (54.5 %) were dark type and 28409 (45.5 %) cells were light ones. In all, there were 1004 (1.61 %) tiny ones, 19330 (30.96 %) very small ones, 17374 (D= 5046 + L= 12328) (27.82 %) small ones, 16694 (D= 5742 + L= 10952) (26.74 %) medium sized ones, 5554 (D= 1833 + L= 3721) (8.89 %) big ones, 2479 (D= 1071 + L= 1408) (3.98 %) very big ones.

On E18, the ganglion had a length of 1.300 mm, a volume of 0.7688 mm^3 and had 306498 cells. Among these cells, 288252 (94.05 %) were dark type and 18246 (5.95 %) were light ones. In all, there were 113491 (37.03 %) tiny cells, 106003 (34.59 %) very small ones, 49614 (D= 43706 + L= 5908) (16.18 %) small ones, 32053 (D= 22305 + L= 9748) (10.46 %) medium sized ones, 4346 (D= 2154 + L= 2192) (1.42 %) big ones, 964 (D= 578 + L= 386) (0.31 %) very big ones and 27 (D= 15 + L= 12) large ones. On the day of hatching, the ganglion had a length of 1.950 mm, a volume of 0.7873 mm^3 and contained 58779 cells. Among these cells, 36116 (61.44 %) were dark type and 22663 (38.56 %) were light ones. In all, there were 781 (1.33 %) tiny cells, 17223 (D= 12288 + L= 4935) (29.3 %) very small ones, 19557 (D= 11275 + L= 8282) (33.27 %) small ones, 14890 (D= 8138 + L= 6752) (25.33 %) medium sized ones, 3841 (D= 1856 + L= 1985) (6.53 %) big ones, 1733 (D= 1116 + L= 617) (2.95 %) very big ones, 679 (D= 605 + L= 74) (1.16 %) large ones, and 75 (D= 57 + L= 18) (0.13 %) very large ones. In the adult situation, the ganglion had a length of 3.750 mm, a volume of 2.7904 mm^3 and had 36826 cells. Among these cells, 29475 (80.04 %) were dark type and 7351 (19.96 %) were light ones. In all, there were 548 (1.49 %) tiny ones, 5512 (D= 3886 + L= 1626) (14.97 %) very small ones, 3576 (D= 2539 + L= 1037) (9.71 %) small ones, 6167 (D= 4567 + L= 1600) (16.75 %) medium sized ones, 522 (D= 435 + L= 87) (1.42 %) big ones, 4381 (D= 3568 + L= 813) (11.9 %) very big ones, 3996 (D= 3277 + L= 719) (10.85 %) large ones, 10191 (D= 8841 + L= 1350) (27.67 %) very large type, 10 (D= 7 + L= 3) (negligible) giant cells, 1917 (D= 1803 + L= 114) (5.21 %) gigantic type of cells and 6 (D= 4 + L= 2) cells of gigantic giant type in the ganglion.

2. *Geniculate Ganglion*

The geniculate ganglion could be clearly recognized on E6 while it had a rostro-caudal length of 0.176 mm and a volume of 0.0055 mm^3. The ganglion had 3428 cells and all of them were dark type. In all, there were 95 (2.77 %) tiny cells, 1232 (35.94 %) very small ones, 1815 (52.95 %) small ones, and 286 (8.34 %) medium sized ones. On E8, the ganglion had a length of 0.208 mm and a volume of 0.0049 mm^3. The ganglion had 6705 cells, all of which were dark type. In all, there were 1952 (29.11 %) tiny cells, 3111 (46.4 %) very small ones, 1542 (23 %) small ones and 100 (1.49 %) medium sized ones. In a few sections in the rostral part of the ganglion, the cells were more crowded towards their periphery. On E10, the ganglion had a length of 0.243 mm and a volume of 0. 0054 mm^3. The ganglion had 1361 cells and all of them were dark type.). In all, there were 81 (5.95 %) tiny cells, 160 (11.76 %) very small type, 677 (49.74 %) small ones), 343 (25.2 %) medium sized ones and 100 (7.35 %) big ones. On E13, the ganglion had a length of 0.270 mm and a volume of 0.0180 mm^3. The ganglion had 4764 cells of which 4252 (89.25 %) were dark type and 512 (10.75 %) were light ones. In all, there were 78 (1.64 %) tiny cells, 1841 (38.64 %) very small type, 774 (D= 693 + L= 81) (16.25 %) small ones, 964 (D= 807 + L= 157) (20.24 %) medium sized ones, 1017 (D= 765 + L= 252) (21.35 %) big ones, 61 (D= 45 + L= 16) (1.28 %) very big ones and 29 (D= 23 + L= 6) (0.61 %) large ones. On E15, the ganglion had a length of 0.250 mm and a volume of 0.0071 mm^3. It had 2869 cells of which 1442 (50.26 %) were dark type and 1427 (49.74 %) were light ones. In all, there were 98 (3.42 %) tiny cells, 935 (32.59 %) very small type, 515 (D= 150 + L= 365) (17.95 %) small ones, 908 (D= 147 + L= 761) (31.65 %) medium sized ones, 317 (D= 74 + L= 243) (11.05 %) big ones, and 96 (D= 38 + L= 58) (3.35 %) very big ones. On E18, the ganglion had a length of 0.300 mm, a volume of 0.0418 mm^3 and contained 17592 cells. Among these cells, 16543 (94.04 %) were dark type, and 1049 (5.96 %) were light ones. In all, there were 8092 (46 %) tiny cells, 5533 (31.45 %)

very small type, 2288 (D= 1971 + L= 317) (13.01 %) small ones, 1559 (D= 920 + L= 639) (8.86 %) medium sized ones, 106 (D= 20 + L= 86) (0.6 %) big ones and 14 (D= 7 + L= 7) (0.08 %) very big ones. On the day of hatching, the ganglion had a length of 0.350 mm, a volume of 0.0264 mm^3 and contained 2093 cells. Among these cells, 1112 (53.13 %) were dark type and 981 (46.87 %) were light ones. In all, there were 20 (0.96 %) tiny cells, 183 (D= 59 + L= 124) (8.74 %) very small type, 328 (D= 69 + L= 259) (15.67 %) small ones, 603 (D= 289 + L= 314) (28.81 %) medium sized ones, 480 (D= 301 + L= 179) (22.93 %) big ones, 473 (D= 368 + L= 105) (22.6 %) very big ones, 2 (0.1 %) large ones, 1 very large ones and 3 (0.15 %) giant dark type of cells. In the adult situation, the ganglion had a length of 0.550 mm, a volume of 0.0457 mm^3, and contained 1021 cells. Among these cells, 904 (88.54 %) were dark type and 117 (11.46 %) were light ones. In all, there were 15 (1.47 %) tiny cells, 7 (0.69 %) very small type, 36 (D= 22 + L= 14) (3.53 %) small ones, 96 (D= 84 + L= 12) (9.4 %) medium sized ones, 22 (D= 13 + L= 9) (2.15 %) big ones, 212 (D= 194 + L= 18) (20.76 %) very big ones, 198 (D= 175 + L= 23) (19.39 %) large ones, 349 (D= 315 + L= 34) (34.18 %) very large ones, 2 (D= 1 + L= 1) (negligible) giant ones, and 84 (D= 78 + L= 6) (8.23 %) gigantic type of cells.

3. Acoustic Ganglion

The acoustic ganglion could be clearly recognised on E6 while it had a rostro-caudal length of 0.312 mm and a volume of 0.0085 mm^3. The ganglion had 10060 cells and all of them were dark type. In all, there were 334 (3.32 %) tiny cells, 4710 (46.82 %) very small ones, 4841 (48.12 %) small ones and 175 (1.74 %) medium sized ones. On E8, the ganglion had a length of 0.416 mm, a volume of 0.0165 mm^3 and contained 20415 cells and all of them were dark type. In all, there were 5863 (28.72 %) tiny cells, 9660 (47.32 %) very small ones, 4728 (23.16 %) small ones and 164 (0.8 %) medium sized ones. In most of the sections, the larger sized cells were more numerous medially mixed with a few tiny cells, whereas compactly arranged tiny cells were found laterally. On E10, the ganglion had a length of 0.558 mm, a volume of 0.0287 mm^3 and contained 42668 cells, and all of these cells were dark type. In all, there were 6495 (15.22 %) tiny cells, 33229 (77.88 %) very small type), and 2944 (6.9 %) small ones. On E13, the ganglion had a length of 0.600 mm and a volume of 0.0537 mm^3 and contained 39097 cells. Among these cells, 36809 (94.15 %) were dark type, and 2288 (5.85 %) were light ones. In all, there were 325 (0.83 %) tiny cells, 16320 (41.74 %) very small type, 20640 (D= 18817 + L= 1823) (52.79 %) small ones 8) and 1812 (D= 1347 + L= 465) (4.63 %) medium sized ones. On E15, the ganglion had a length of 0.700 mm, a volume of 0.0769 mm^3 and 49313 cells. Among these cells, 47807 (96.95 %) were dark type and 1506 (3.05 %) were light ones. In all, there were 6482 (13.14 %) tiny cells, 28864 (58.53 %) very small, 8808 (D= 8290 + L= 518) (17.86 %) small ones, 4640 (D= 3969 + L= 671) (9.41 %) medium sized ones, 474 (D= 193 + L= 281) (0.96 %) big ones and 45 (D= 9 + L= 36) (0.09 %) very big ones. On E18, the ganglion had a length of 0.720 mm, a volume of 0.2386 mm^3 and 220338 cells. Among these cells, 220314 (99.99 %) were dark type and 24 (0.01 %) were light ones. In all, there were 57962 (26.31 %) tiny cells, 93394 (42.39 %) very small, 63328 (D= 63318 + L= 10) (28.74 %) small ones, 5617 (D= 5603 + L= 14) (2.55 %) medium sized ones and 37 (0.02 %) big (dark) cells. In some of the sections, smaller cells were found to be more numerous in the medial part of the ganglion whereas the larger cells were found to be more in their lateral part. On the day of hatching, the ganglion had a length of 0.910 mm, a volume of 0.1568 mm^3 and 26701 cells. Among these cells, 13037 (48.83 %) were dark type and 13664 (51.17 %) were light ones. In all, there were 219 (0.82 %) tiny cells, 3977 (D= 840 + L= 3137) (14.89 %) very small, 16771 (D= 8018 + L= 8753) (62.81 %) small ones, 5714 (D= 3943 +L= 1771) (21.4 %) medium sized ones and 20 (D= 17 + L= 3) (0.07 %) big ones. In the adult situation, the ganglion had a length of 1.000 mm, a volume of 0.1420 mm^3 and 14633 cells. Among these cells, 14524 (99.26 %) were dark type and 109 (0.74 %) were light ones. In all, there were 3329 (22.76 %) tiny cells, 6617 (D= 6557 + L+ 60) (45.22 %) very small ones, 4488 (D= 4450 + L= 38) (30.67 %) small ones and 199 (D= 188 + L= 11) (1.36 %) medium sized ones.

4. Vestibular Ganglion

The vestibular ganglion could be recognised on E6 while it had a rostro-caudal length of 0.304 mm and a volume of 0.0094 mm^3. The ganglion had 32924 cells. Among these cells, 32916 (99.98 %) were dark type and 8 (0.02 %) were light ones. In all, there were 2220 (6.74 %) tiny cells, 14803 (44.96 %) very small ones, 15197 (46.16 %) small ones and 704 (2.14 %) medium sized ones. On E8, the ganglion had a length of 0.480 mm and a volume of 0.0240 mm^3, and contained 42695 cells, and all of these cells were dark type. In all, there were 874 (2.05 %) tiny cells, 30870 (72.3 %) very small ones, 10750 (25.18 %) small ones and 201 (0.47 %) medium sized ones. On E10, the ganglion had a length of 0.963 mm, a volume of 0.0442 mm^3 and 61936 cells and all of these cells were dark type. In all, there were 10268 (16.58 %) tiny cells, 40467 (65.34 %) very small ones, 9418 (15.21 %) small ones and 1783 (2.88 %) medium sized ones. In a few sections of the ganglion the larger sized cells were more at their periphery while the smaller cells were more in their centre. On E13, the ganglion had a length of 1.020 mm, a volume of 0.1232 mm^3 and 43894 cells. Among these cells, 40267 (91.74 %) were dark type and 3627 (8.26 %) were light ones. In all, there were 2674 (6.09 %) tiny cells, 27480 (62.61 %) very small ones, 9251 (D= 6818 + L= 2433) (21.08 %) small ones, 3655 (D= 2665 + L= 990) (8.33 %) medium sized ones, 763 (D= 583 + L= 180) (1.74 %) big ones and 71 (D= 47 + L= 24) (0.16 %)

very big ones. Many smaller groups of cells were observed in the ventral part of some sections of the ganglion. On E15, the ganglion had a length of 0.940 mm, a volume of 0.0770 mm^3 and 53439 cells. Among these cells, 47465 (88.82 %) were dark type and 5974 (11.18 %) were light ones. In all, there were 3449 (6.45 %) tiny cells, 31886 (59.67 %) very small ones, 10090 (D= 7501 + L= 2589) (18.88 %) small ones, 5654 (D= 3195 + L= 2459) (10.58 %) medium sized ones, 1609 (D= 953 + L= 656) (3.01 %) big ones, and 751 (D= 481 + L= 270) (1.41 %) very big ones. On E18, the ganglion had a length of 1.230 mm, a volume of 0.3874 mm^3 and 237191 cells. Among these cells, 234877 (99.02 %) were dark type and 2314 (0.98 %) were light ones. In all, there were 91241 (38.47 %) tiny cells, 100958 (42.56 %) very small type, 32555 (D= 31988 + L= 567) (13.73 %) small ones, 10248 (D= 8981 + L= 1267) (4.32 %) medium sized ones, 1827 (D= 1491 + L= 336) (0.77 %) big ones and 362 (D= 218 + L= 144) (0.15 %) very big ones. On the day of hatching, the ganglion had a length of 1.200 mm, a volume of 0.2079 mm^3 and 18067 cells. Among these cells, 4871 (26.96 %) were dark type and 13196 (73.04 %) were light ones. In all, there were 105 (0.58 %) tiny cells, 4179 (D= 428 + L= 3751) (23.13 %) very small type, 4987 (D= 924 + L= 4063) (27.6 %) small ones, 6640 (D= 2178 + L= 4462) (36.75 %) medium sized, 1531 (D = 693 + L= 838) (8.47 %) big, 498 (D= 425 + L= 73) (2.76 %) very big ones and 127 (D= 118 +L= 9) (0.7 %) large ones. In the adult situation, the ganglion had a length of 1.290 mm, a volume of 0.2325 mm^3 and 12483 cells. Among these cells, 12189 (97.64 %) were dark type and 294 (2.36 %) were light ones. In all, there were 1183 (9.48 %) tiny cells, 4935 (39.53 %) very small ones, 3800 (D= 3645 + L= 155) (30.44 %) small ones, 1961 (D= 1860 + L= 101) (15.71 %) medium sized ones, 182 (D= 168 + L= 14) (1.46 %) big ones, 295 (D= 278 + L= 17) (2.36 %) very big ones and 127 (D= 120 + L= 7) (1.02 %) large ones.

5. *Proximal Ganglionic Complex of Cranial Nerves IX and X*

The proximal ganglionic complex of cranial nerves IX and X could be recognised on E6 while it had a rostro-caudal length of 0.328 mm and a volume of 0.0078 mm^3. The ganglion had 17905 cells all of which were dark type. In all, there were 785 (4.38 %) tiny cells, 8222 (45.92 %) very small type, 8444 (47.16 %) small cells and 454 (2.54 %) medium sized ones. On E8, the ganglion had a length of 0.360 mm, a volume of 0.0236 mm^3 and contained 31016 cells all of which were dark type. In all, there were 492 (1.59 %) tiny cells, 22987 (74.11 %) very small type, 7342 (23.67 %) small ones and 195 (0.63 %) medium sized ones. On E10, the ganglion had a length of 0.612 mm, a volume of 0.0445 mm^3 and 28813 cells all of which were dark type. In all, there were 2810 (9.75 %) tiny cells, 18610 (64.59 %) very small type, 7019 (24.36 %) small ones and 374 (1.3 %) medium sized ones. On E13, the ganglion had a length of 0.660 mm, a volume of 0.0676 mm^3 and 26208 cells (P. D= 388843). Among these cells, 19277 (73.55 %) were dark type and 6931 (26.45 %) were light ones. In all, there were 302 (1.15 %) tiny cells, 13166 (50.24 %) very small type, 6342 (D= 2956 + L= 3386) (24.2 %) small ones, 5142 (D= 2274 + L= 2868) (19.62 %) medium sized ones, 1161 (D= 537 + L= 624) (4.43 %) big ones and 95 (D= 42 + L= 53) (0.36 %) very big ones. On E15, the ganglion had a length of 0.660 mm, a volume of 0.0505 mm^3 and 24677 cells. Among these cells, 16770 (67.96 %) were dark type and 7907 (32.04 %) were light ones. In all, there were 281 (1.14 %) tiny cells, 7451 (30.19 %) very small type, 5435 (D= 4221 + L= 1214) (22.02 %) small cells, 8131 (D= 3905 + L= 4226) (32.95 %) medium sized ones, 3282 (D= 867 + L= 2415) (13.3 %) big ones, 82 (D= 38 + L= 44) (0.33 %) very big ones, 9 (D= 5 + L= 4) (0.04 %) large ones and 6 (0.02 %) very large ones. On E18, the ganglion had a length of 0.900 mm, a volume of 0.2469 mm^3 and 106131 cells. Among these cells, 103947 (97.94 %) were dark type and 2184 (2.06 %) were light ones. In all, there were 37646 (35.47 %) tiny cells, 36189 (34.1 %) very small ones, 17228 (D= 16518 + L= 710) (16.23 %) small ones 12344 (D= 11356 + L= 988) medium sized ones, 2018 (D= 1657 + L= 361) (1.9 %) big ones and 706 (D= 581 + L= 125) (0.67 %) very big ones. On the day of hatching, the ganglion had a length of 0.980 mm, a volume of 0.2480 mm^3 and 17536 cells. Among these cells, 10354 (59.04 %) were dark type and 7182 (40.96 %) were light ones. In all, there were 182 (1.04 %) tiny cells, 2136 (D= 945 + L= 1191) (12.18 %) very small type, 4620 (D= 2251 + L= 2369) (26.35 %) small ones, 6380 (D= 3942 + L= 2438) (36.38 %) medium sized ones, 3142 (D= 2185 + L= 957) (17.92 %) big ones, 1013 (D= 799 L= 214) (5.78 %) very big ones and 63 (D= 50 + L= 13) (0.36 %) large ones. In the adult situation, the ganglion had a length of 1.300 mm, a volume of 0.6408 mm^3 and 13105 cells. Among these cells, 10757 (82.08 %) were dark type and 2348 (17.92 %) were light ones. In all, there were 509 (3.88 %) tiny cells, 3192 (D= 2517 + L= 675) (24.36 %) very small type, 2658 (D= 2047 + L= 611) (20.28 %) small ones, 2641 (D= 2052 + L= 589) (20.15 %) medium sized ones, 1052 (D= 1004 + L= 48) (8.03 %) big ones, 1664 (D= 1494 + L= 170) (12.7 %) very big ones, 1196 (D= 987 + L= 209) (9.13 %) large ones and 193 (D= 147 + L= 46) (1.47 %) very large ones.

6. *Petrous Ganglion*

The Petrous ganglion could be recognised on E6 while it had a rostro-caudal length of 0.336 mm and a volume of 0.0077 mm^3. The ganglion had 7778 cells all of which were dark type. In all, there were 205 (2.64 %) tiny cells, 3681 (47.33 %) very small ones, 3484 (44.79 %) small ones and 408 (5.25 %) medium sized ones. On E8, the ganglion had a length of 0.360 mm, a volume of 0.0118 mm^3 and had 8379 cells, all of which were dark type. In all, there were 106 (1.27 %) tiny cells, 3446 (41.13 %) very small ones, 4579 (54.65 %) small ones, 235 (2.8 %) medium sized ones and 13 (0.16 %) big ones. On E10, the ganglion had a length of 0.360 mm, a volume of 0.0157 mm^3 and had

6866 cells. Among these cells, 6858 (99.88 %) were dark type and 8 (0.12 %) were light ones. In all, there were 839 (12.22 %) tiny cells, 3244 (47.25 %) very small ones, 2218 (D = 2211 + L = 7) (32.3 %) small ones, 476 (D = 475 + L = 1) (6.93 %) medium sized ones and 89 (1.3 %) big ones. On E13, the ganglion had a length of 0.370 mm, a volume of 0.0243 mm^3 and had 10022 cells. Among these cells, 7960 (79.43 %) were dark type and 2062 (20.57 %) were light ones. In all, there were 128 (1.28 %) tiny cells, 6191 (61.78 %) very small ones, 1843 (D = 853 + L= 990) (18.39 %) small ones, 1015 (D= 440 + L = 575) (10.13 %) medium sized ones, 789 (D= 326 + L= 463) (7.87 %) big ones and 56 (D = 22 + L = 34) (0.56 %) very big ones. On E15, the ganglion had a length of 0.450 mm, a volume of 0.0425 mm^3 and had 8126 cells. Among these cells, 6673 (82.12 %) were dark type and 1453 (17.88 %) were light ones. In all, there were 399 (4.91 %) tiny cells, 3097 (38.11 %) very small ones, 2030 (D = 1556 + L = 474) (24.98 %) small ones, 2366 (D = 1521 + L = 845) (29.12 %) medium sized ones, 221 (D = 93 + L = 128) (2.72 %) big ones and 13 (D = 7 + L= 6) (0.16 %) very big ones. On E18, the ganglion had a length of 0.470 mm, a volume of 0.0561 mm^3 and had 32203 cells. Among these cells, 31462 (97.7 %) were dark type and 741 (2.3 %) were light ones. In all, there were 10948 (34 %) tiny cells, 9937 (30.86 %) very small ones, 6333 (D = 6136 + L = 197) (19.67 %) small ones, 3917 (D = 3577 + L = 340) (12.16 %) medium sized ones, 756 (D = 635 + L= 121) (2.35 %) big ones and 312 (D = 229 + L = 83) (0.97 %) very big ones. On the day of hatching, the ganglion had a length of 0.540 mm, a volume of 0.0548 mm^3 and had 3859 cells. Among these cells, 1711 (44.34 %) were dark type and 2148 (55.66 %) were light ones. In all, there were 42 (1.09 %) tiny cells, 459 (D = 50 + L = 409) (11.89 %) very small ones, 1087 (D = 288 + L= 799) (28.17 %) small ones, 1872 (D = 992 + L = 880) (48.51 %) medium sized ones, 313 (D = 258 + L = 55) (8.11 %) big ones and 86 (D = 81 + L = 5) (2.23 %) very big ones. In the adult situation, the ganglion had a length of 0.940 mm, a volume of 0.2390 mm^3 and had 2992 cells. Among these cells, 1870 (62.5 %) were dark type (D= 7824) and 1122 (37.5 %) were light ones. In all, there were 52 (1.74 %) tiny cells, 235 (D = 85 + L = 150) (7.85 %) very small ones, 749 (D = 304 + L = 445) (25.03 %) small ones, 1516 (D = 1053 + L = 463) (50.67 %) medium sized ones, 349 (D = 291 + L = 58) (11.66 %) big ones and 91 (D = 85 + L = 6) (3.04 %) very big ones.

7. *Nodose Ganglion*

The nodose ganglion could be recognised on E6 while it had a rostro-caudal length of 0.496 mm, a volume of 0.0127 mm^3 and 10740 cells. Among these cells, 9225 (85.89 %) were dark type and 1515 (14.11 %) were light ones. In all, there were 161 (1.5 %) tiny cells, 2488 (23. 17 %) very small ones, 4545 (D= 3731 + L= 814) (42.32 %) small ones and 3546 (D= 2845 + L= 701) (33.02 %) medium sized ones. On E8, the ganglion had a length of 0.536 mm, a volume of 0.0251 mm^3 and had 17167 cells. Among these cells 16958 (98.78 %) were dark type and 209 (1.22 %) were light ones. In all, there were 116 (0.68 %) tiny cells, 6425 (37.43 %) very small ones, 6729 (D= 6645 + L= 84) (39.2 %) small ones, 3735 (D= 3627 + L= 108) (21.76 %) medium sized ones and 162 (D= 145 + L= 17) (0.92 %) big ones. On E10, the ganglion had a length of 0.603 mm, a volume of 0.0484 mm^3 and had 16181 cells. Among these cells, 16014 (98.97 %) were dark type and 167 (1.03 %) were light ones. In all, there were 135 (0.83 %) tiny cells, 4774 (29.5 %) very small ones, 7626 (D= 7584 + L= 42) (47.13 %) small ones, 2370 (D= 2300 + L= 70) (14.65 %) medium sized ones, 1226 (D= 1178 + L= 48) (7.58 %) big ones and 50 (D= 43 + L= 7) (0.31 %) very big ones. On E13, the ganglion had a length of 0.630 mm, a volume of 0.0474 mm^3 and contained 8972 cells. Among these cells, 6613 (73.71 %) were dark type and 2359 (26.29 %) were light ones. In all, there were 94 (1.05 %) tiny cells, 3812 (42.49 %) very small ones, 2504 (D= 1645 + L= 859) (27.91 %) small ones, 1656 (D= 714 + L= 942) (18.46 %) medium sized ones, 587 (D= 229 + L= 358) (6.54 %) big ones, 271 (D= 99 + L= 172) (3.02 %) very big ones and 48 (D= 20 + L= 28) (0.53 %) large ones. On E15, the ganglion had a length of 0.740 mm, a volume of 0.0870 mm^3 and had 46803 cells. Among these cells, 44857 (95.84 %) were dark type and 1946 (4.16 %) were light ones. In all, there were 979 (2.09 %) tiny cells, 35572 (76 %) very small ones, 4964 (D= 4388 + L= 576) (10.39 %) small ones, 5027 (D= 3769 + L= 1258) (10.74 %) medium sized ones, 204 (D= 128 + L= 76) (0.44 %) big ones, 43 (D= 14 + L= 29) (0.09 %) very big ones and 14 (D= 7 + L= 7) (0.03 %) large ones. On E18, the ganglion had a length of 0.790 mm, a volume of 0.1071 mm^3 and had 80720 cells. Among these cells, 79253 (98.18 %) were dark type and 1467 (1.82 %) were light ones. In all, there were 30278 (37.51 %) tiny cells, 25074 (31.06 %) very small ones, 16309 (D= 15800 + L= 509) (20.2 %) small ones 6921 (D= 6154 + L= 767) (8.57 %) medium sized ones, 1778 (D= 1594 + L= 184) (2.2 %) big ones, 322 (D= 315 + L= 7) (0.4 %) very big ones and 38 (0.05 %) large ones. On the day of hatching, the ganglion had a length of 1.350 mm, a volume of 0.1368 mm^3 and had 11464 cells. Among these cells 7808 (68.11 %) were dark type and 3656 (31.89 %) were light ones. In all, there were 66 (0.58 %) tiny cells, 351 (D= 259 + L= 92) (3.06 %) very small type, 1855 (D= 908 + L= 947) (16.18 %) small ones, 4253 (D= 2709 + L= 1544) (37.1 %) medium sized ones, 2574 (D= 1654 + L= 920) (22.45 %) big ones, 2053 (D= 1903 + L= 150) (17.91 %) very big ones and 312 (D= 309 + L= 3) (2.8 %) large ones. In the adult situation, the ganglion had a length of 2.100 mm, a volume of 1.4497 mm^3 and had 9130 cells. Among these cells, 8784 (96.21 %) were dark type and 346 (3.79 %) were light ones. In all, there were 275 (3.01 %) tiny cells, 1526 (16.71 %) very small ones, 1551 (D= 1527 + L= 24) (16.99 %) small ones, 2342 (D= 2245 + L= 97) (25.65 %) medium sized ones, 973 (D= 902 + L= 71)

(10.66 %) big ones, 1500 (D= 1418 + L= 82) (16.43 %) very big ones, 505 (D= 467 + L= 38) (5.53 %) large ones, 413 (D= 388 + L= 25) (4.52 %) very large ones, and 45 (D= 36 + L= 9) (0.49 %) giant ones.

8. Ciliary Ganglion (Parasympathetic)

The ciliary ganglion could be recognised on E6 while the ganglion had a rostrocaudal length of 0.240 mm and a volume of 0.0174 mm^3. The ganglion had 16629 cells all of which were dark type. In all, there were 2053 (12.35 %) tiny cells, 8820 (53.04 %) very small ones, 5707 (34.32 %) small ones, and 49 (0.29 %) medium sized ones. On E8, the ganglion had a length of 0.296 mm, a volume of 0.0274 mm^3 and had 17646 cells all of which were dark type. In all, there were 114 (0.65 %) tiny cells, 9452 (53.56 %) very small ones, 5684 (32.21 %) small ones and 2396 (13.58 %) medium sized ones. On E10, the ganglion had a length of 0.387 mm, a volume of 0.0261 mm^3 and had 23618 cells all of which were dark type. In all, there were 2918 (12.35 %) tiny cells, 14152 (59.92 %) very small ones, 4337 (18.36 %) small ones, and 2211 (9.36 %) medium sized ones. On E13, the ganglion had a length of 0.460 mm, a volume of 0.0758 mm^3 and had 21867 cells. Among these cells, 21093 (96.46 %) were dark type, and 774 (3.54 %) were light ones. In all, there were 156 (0.71 %) tiny cells, 14142 (64.67 %) very small ones, 5124 (D= 4559 + L= 565) (23.43 %) small ones, 2256 (D= 2057 + L= 199) (10.32 %) medium sized ones, and 189 (D= 179 + L= 10) (0.86 %) big ones. On E15, the ganglion had a length of 0.510 mm, a volume of 0.0674 mm^3 and had 9365 cells. Among these cells, 6909 (73.77 %) were dark type, and 2456 (26.23 %) were light ones. In all, there were 413 (4.41 %) tiny cells, 2519 (26.9 %) very small ones, 3355 (D= 1887 + L= 1468) (35.82 %) small ones, 3062 (D= 2078 + L= 984) (32.7 %) medium sized ones, 9 (D= 6 + L= 3) (0.1 %) big ones, and 7 (D= 6 + L= 1) (0.07 %) very big ones. On E18, the ganglion had a length of 0.540 mm, a volume of 0.2390 mm^3 and had 186557 cells. Among these cells, 186379 (99.9 %) were dark type and 178 (0.1 %) were light ones. In all, there were 155843 (83.54 %) tiny cells, 16125 (8.64 %) very small ones, 9924 (D= 9923 + L= 1) (5.32 %) small ones, 3315 (D= 3237 + L= 78) (1.78 %) medium sized ones, 935 (D= 876 + L= 59) (0.5 %) big ones, and 415 (D= 375 + L= 40) (0.22 %) very big ones. On the day of hatching, the ganglion had a length of 0.600 mm, a volume of 0.0903 mm^3 and had 10521 cells. Among these cells, 5610 (53.32 %) were dark type and 4911 (46.68 %0 were light ones. In all, there were 319 (3.03 %0 tiny cells, 2867 (D= 1486 + L= 1381) (27.25 %) very small ones, 3361 (D= 1639 + L= 1722) (31.95 %) small ones, 2976 (D= 1594 + L= 1382) (28.29 %) medium sized ones, 856 (D= 472 + L= 384) (8.14 %) big ones and 142 (D= 100 + L= 42) (1.35 %) very big ones. In the adult situation, the ganglion had a length of 0.660 mm, a volume of 0.2977 mm^3 and contained 2535 cells. Among these cells, 2079 (82.01 %) were dark type and 456 (17.99 %) were light ones. In all, there were 259 (10.22 %) tiny cells, 336 (13.25 %) very small ones, 313 (D= 249 + L= 64) (12.35 %) small ones, 605 (D= 449 + L= 156) (23.87 %) medium sized ones, 373 (D= 274 + L= 99) (14.71 %) big ones, 195 (D= 150 + L= 45) (7.69 %) very big ones, 234 (D= 178 + L= 56) (9.23 %) large ones and 220 (D= 184 + L= 36) (8.68 %) very large ones.

9. Superior Cervical Ganglion (Sympathetic)

The Superior Cervical Ganglion could be recognised on E6 while it had a rostro-caudal length of 0.560 mm and a volume of 0.0083 mm^3 and, had 14489 cells all of which were dark type. In all, there were 687 (4.74 %) tiny cells, 5986 (41.31 %) very small type, 7250 (50.04 %) small ones, and 566 (3.9 %) medium sized ones. On E8, the ganglion had a length of 0.600 mm, a volume of 0.0325 mm^3 and had 30832 cells all of which were dark type. In all, there were 5230 (16.96 %) tiny cells, 12194 (39.55 %) very small type, 13190 (42.78 %) small ones and 218 (0.71 %) medium sized ones. On E10, the ganglion had a length of 0.657 mm, a volume of 0.0387 mm^3 and had 47681 cells all of which were dark type. In all, there were 10832 (22.72 %) tiny cells, 31144 (65.32 %) very small ones, 3929 (8.24 %) small ones and 1776 (3.72 %) medium sized ones. On E13, the ganglion had a length of 0.580 mm, a volume of 0.0582 mm^3 and had 39322 cells all of which were dark type. In all, there were 347 (0.88 %) tiny cells, 22999 (58.49 %) very small ones, 12004 (30.53 %) small ones and 3972 (10.1 %) medium sized ones. On E15, the ganglion had a length of 0.690 mm, a volume of 0.1075 mm^3 and had 74974 cells all of which were dark type. In all, there were 1292 (1.72 %) tiny cells, 59282 (79.01 %) very small ones, 11798 (15.72 %) small ones and 2602 (3.47 %) medium sized ones. On E18, the ganglion had a length of 0.720 mm, a volume of 0.1963 mm^3 and had 127722 cells. Among these cells, 127231 (99.62 %) were dark type and 491 (0.38 %) were light ones. In all, there were 38420 (30.08 %) tiny cells, 42647 (33.39 %) very small ones, 44575 (D= 44252 + L= 323) (34.9 %) small ones and 2080 (D= 1912 + L= 168) (1.63 %) medium sized ones. On the day of hatching, the ganglion had a length of 0.810 mm, a volume of 0.1166 mm^3 and had 55244 cells. Among these cells, 18172 (32.89 %) were dark type and 37072 (67.11 %) were light ones. In all, there were 675 (1.22 %) tiny cells, 38083 (D= 9404 + L= 28679) (68.94 %) very small ones, 15240 (D= 7086 + L= 8154) (27.59 %) small ones, and 1246 (D= 1007 + L= 239) (2.26 %) medium sized ones. In the adult situation, the ganglion had a length of 1.430 mm, a volume of 0.4928 mm^3 and had 34374 cells. Among these cells, 18271 (53.15 %) were dark type and 16103 (46.85 %) were light ones. In all, there were 475 (1.38 %) tiny cells, 20026 (D= 8228 + L= 11798) (58.26 %) very small ones, 12481 (D= 8365 + L= 4116) (36.31 %) small ones, 1212 (D= 1057 + L= 155) (3.53 %) medium sized ones and 180 (D= 146 + L= 34) (0.52 %) big ones.

Table 1. Illustrates the total number of dark and light cells in the trigeminal ganglion in different age groups of animals in the ontogeny of the chick

Size / Age	Tiny <5u	Very small 6-10u	Small 11-15u	Medium 16-20u	Big 21-25u	Very big 26-30u	Large 31-35u	Very large 36-40u	Giant >40u	Total Number	Grand Total
E6-D	4923	24453	41267	3219	0	0	0	0	0	73862	
E6-L	0	0	0	0	0	0	0	0	0	0	73862
E8-D	63347	168400	23970	3610	0	0	0	0	0	259327	
E8-L	0	0	3	75	0	0	0	0	0	78	259405
E10-D	581	17067	59095	23804	45	11	0	0	0	100603	
E10-L	0	0	348	239	7	2	0	0	0	596	101199
E13-D	510	27203	10824	9856	3131	503	163	3	6	52199	
E13-L	0	0	15017	12586	3931	637	120	1	2	32294	84493
E15-D	1004	19330	5046	5742	1833	1071	0	0	0	34026	
E15-L	0	0	12328	10952	3721	1408	0	0	0	28409	62435
E18-D	113491	106003	43706	22305	2154	578	15	0	0	288252	
E18-L	0	0	5908	9748	2192	386	12	0	0	18246	306498
H-D	781	12288	11275	8138	1856	1116	605	57	0	36116	
H-L	0	4935	8282	6752	1985	617	74	18	0	22663	58779
A-D	548	3886	2539	4567	435	3568	3277	8841	1814	29475	
A-L	0	1626	1037	1600	87	813	719	1350	119	7351	36826

(D = Dark cells E=Embroynic age H=Day of hatching A=Adult)

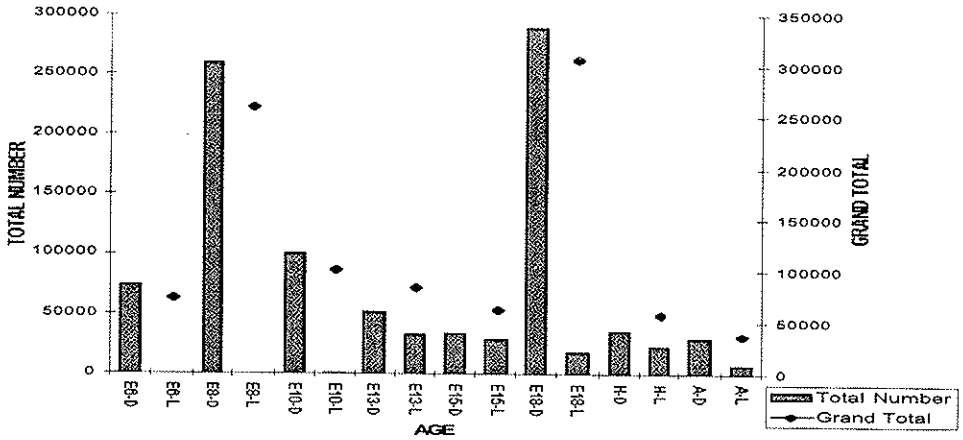

Figure 1 A. Total number of dark & light cells in the trigeminal ganglion in different age groups of animals in the ontogeny of the chick

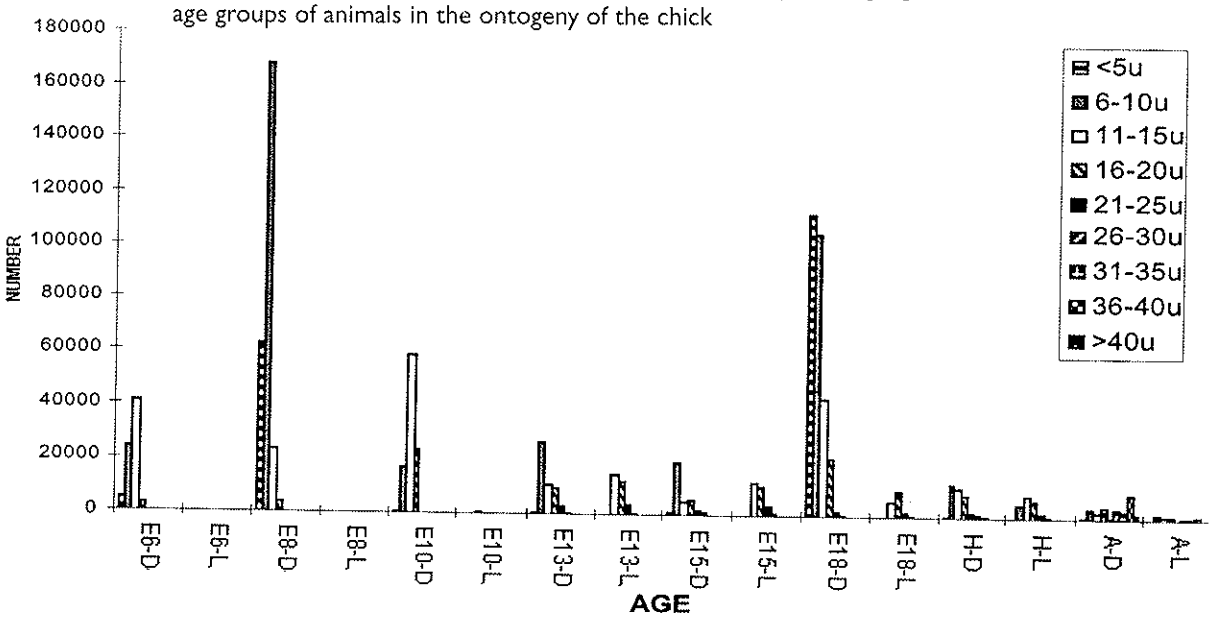

Figure 1B. Total number of dark & light cells in the trigeminal ganglion in different age groups of animals in the ontogeny of the chick

Table 2. Illustrates the total number of dark and light cells in the genicular ganglion in different age groups of animals in the ontogeny of the chick

Size / Age	Tiny <5u	Very small 6-10u	Small 11-15u	Medium 16-20u	Big 21-25u	Very big 26-30u	Large 31-35u	Very large 36-40u	Giant >40u	Total Number	Grand Total
E6-D	95	1232	1815	286	0	0	0	0	0	3428	
E6-L	0	0	0	0	0	0	0	0	0	0	3428
E8-D	1952	3111	1542	100	0	0	0	0	0	6705	
E8-L	0	0	0	0	0	0	0	0	0	0	6705
E10-D	81	160	677	343	100	0	0	0	0	1361	
E10-L	0	0	0	0	0	0	0	0	0	0	1361
E13-D	78	1841	693	807	765	45	23	0	0	4252	
E13-L	0	0	81	157	252	16	6	0	0	512	4764
E15-D	98	935	150	147	74	38	0	0	0	1442	
E15-L	0	0	365	761	243	58	0	0	0	1427	2869
E18-D	8092	5533	1971	920	20	7	0	0	0	16543	
E18-L	0	0	317	639	86	7	0	0	0	1049	17592
H-D	20	59	69	289	301	368	2	1	3	1112	
H-L	0	124	259	314	179	105	0	0	0	981	2093
A-D	15	7	22	84	13	194	175	315	79	904	
A-L	0	0	14	12	9	18	23	34	7	117	1021

(D = Dark cells E=Embroynic age H=Day of hatching A=Adult)

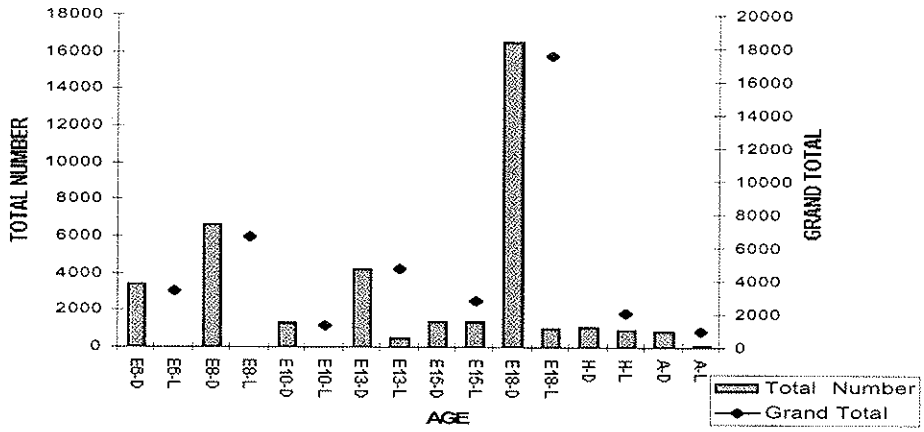

Figure 2 A. Total number of dark & light cells in the genicular ganglion in different age groups of animals in the ontogeny of the chick

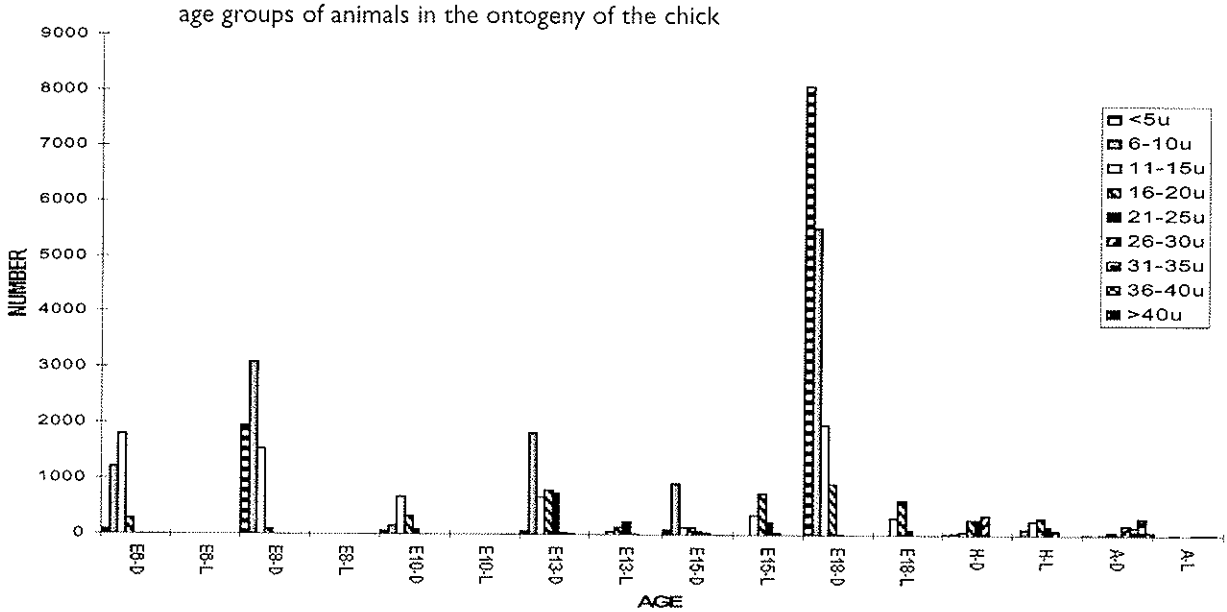

Figure 2B. Total number of dark & light cells in the genicular ganglion in different age groups of animals in the ontogeny of the chick

Table 3 Illustrates the total number of dark and light cells in the acoustic ganglion in different age groups of animals in the ontogeny of the chick

Size / Age	Tiny <5u	Very small 6-10u	Small 11-15u	Medium 16-20u	Big 21-25u	Very big 26-30u	Large 31-35u	Very large 36-40u	Giant >40u	Total Number	Grand Total
E6-D	334	4710	4841	175	0	0	0	0	0	10060	
E6-L	0	0	0	0	0	0	0	0	0	0	10060
E8-D	5863	9660	4728	164	0	0	0	0	0	20415	
E8-L	0	0	0	0	0	0	0	0	0	0	20415
E10-D	6495	33229	2944	0	0	0	0	0	0	42668	
E10-L	0	0	0	0	0	0	0	0	0	0	42668
E13-D	325	16320	18817	1347	0	0	0	0	0	36809	
E13-L	0	0	1823	465	0	0	0	0	0	2288	39097
E15-D	6482	28864	8290	3969	193	9	0	0	0	47807	
E15-L	0	0	518	671	281	36	0	0	0	1506	49313
E18-D	57962	93394	63318	5603	37	0	0	0	0	220314	
E18-L	0	0	10	14	0	0	0	0	0	24	220338
H-D	219	840	8018	3943	17	0	0	0	0	13037	
H-L	0	3137	8753	1771	3	0	0	0	0	13664	26701
A-D	3329	6557	4450	188	0	0	0	0	0	14524	
A-L	0	60	38	11	0	0	0	0	0	109	14633

(D = Dark cells E=Embroynic age H=Day of hatching A=Adult)

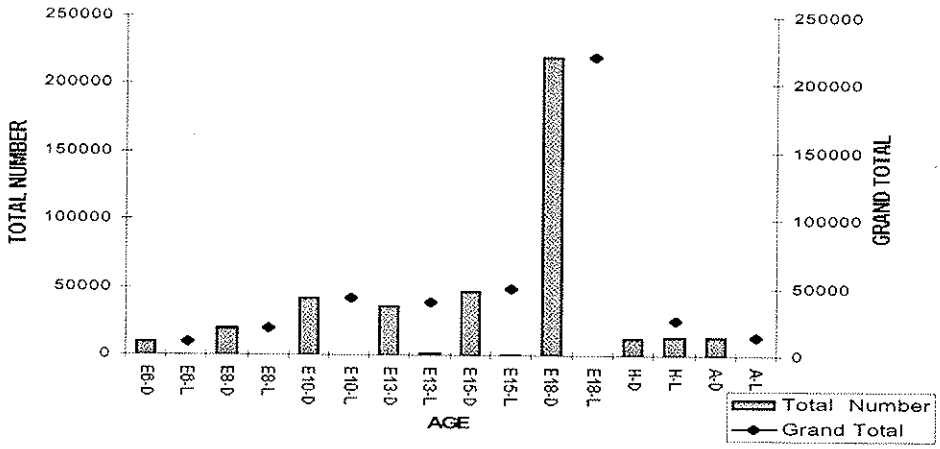

Figure 3 A. Total number of dark & light cells in the acoustic ganglion in different age groups of animals in the ontogeny of the chick

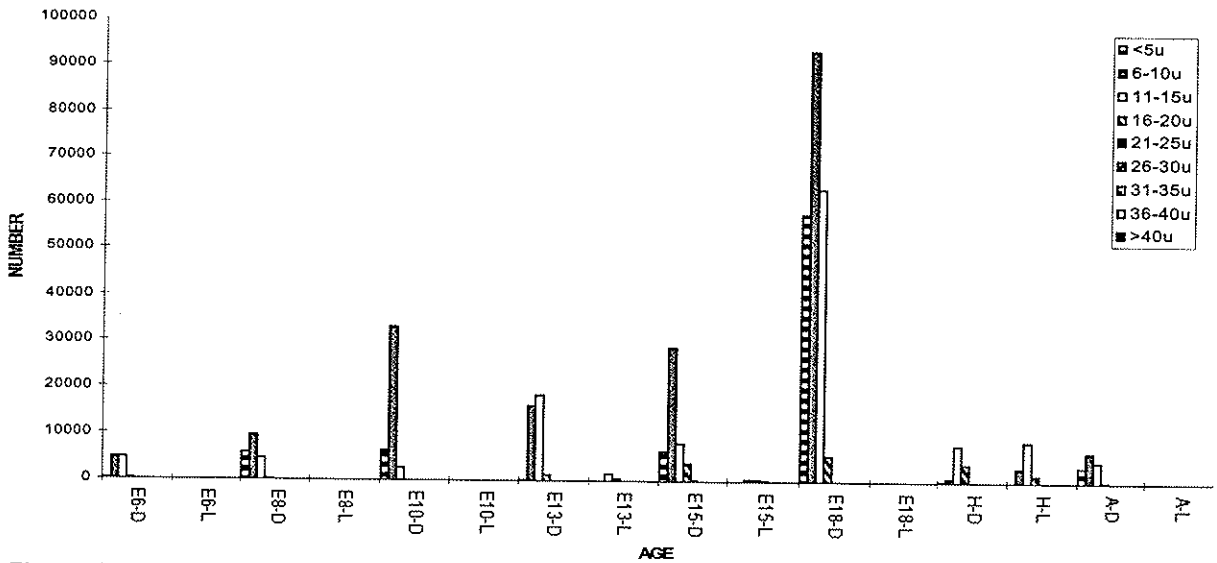

Figure 3 B. Total number of dark & light cells in the acoustic ganglion in different age groups of animals in the ontogeny of the chick

Table 4 Illustrates the total number of dark and light cells in the vestibular ganglion in different age groups of animals in the ontogeny of the chick

Size / Age	Tiny <5u	Very small 6-10u	Small 11-15u	Medium 16-20u	Big 21-25u	Very big 26-30u	Large 31-35u	Very large 36-40u	Giant >40u	Total Number	Grand Total
E6-D	2220	14803	15197	696	0	0	0	0	0	32916	
E6-L	0	0	0	8	0	0	0	0	0	8	32924
E8-D	874	30870	10750	201	0	0	0	0	0	42695	
E8-L	0	0	0	0	0	0	0	0	0	0	42695
E10-D	10268	40467	9418	1783	0	0	0	0	0	61936	
E10-L	0	0	0	0	0	0	0	0	0	0	61936
E13-D	2674	27480	6818	2665	583	47	0	0	0	40267	
E13-L	0	0	2433	990	180	24	0	0	0	3627	43894
E15-D	3449	31886	7501	3195	953	481	0	0	0	47465	
E15-L	0	0	2589	2459	656	270	0	0	0	5974	53439
E18-D	91241	100958	31988	8981	1491	218	0	0	0	234877	
E18-L	0	0	567	1267	336	144	0	0	0	2314	237191
H-D	105	428	924	2178	693	425	118	0	0	4871	
H-L	0	3751	4063	4462	838	73	9	0	0	13196	18067
A-D	1183	4935	3645	1860	168	278	120	0	0	12189	
A-L	0	0	155	101	14	17	7	0	0	294	12483

(D = Dark cells E=Embroynic age H=Day of hatching A=Adult)

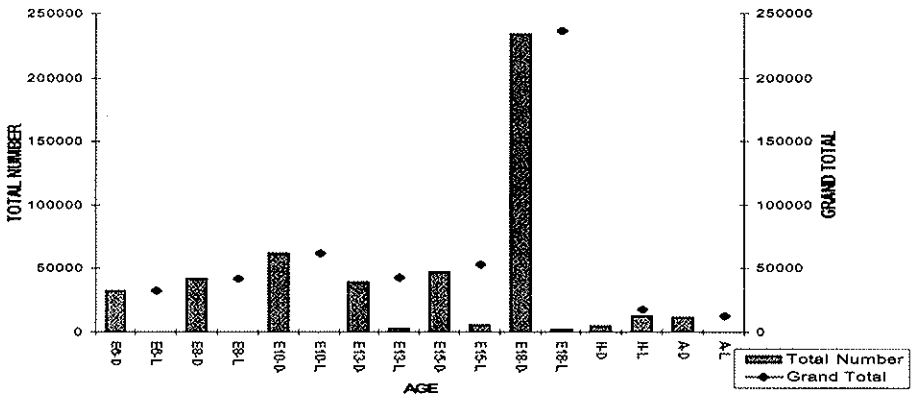

Figure 4A. Total number of dark & light cells in the vestibular ganglion in different age groups of animals in the ontogeny of the chick

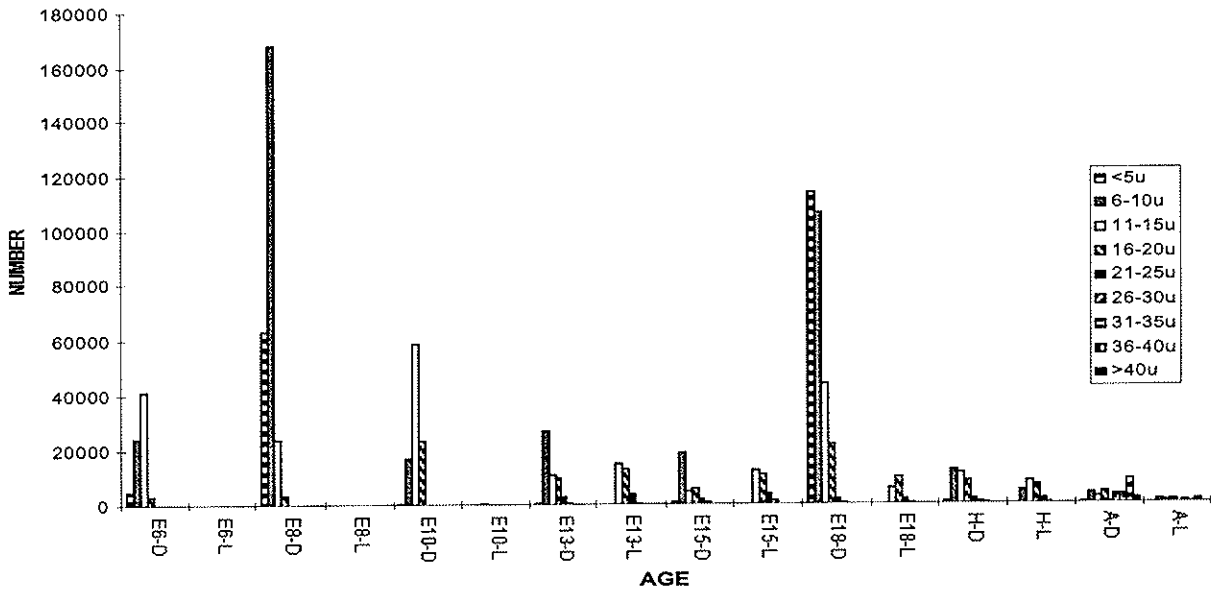

Figure 4 B. Total number of dark & light cells in the vestibular ganglion in different age groups of animals in the ontogeny of the chick

Table 5. Illustrates the total number of dark and light cells in the proximal ganglionic complex of cranial nerves IX & X in different age groups of animals in the ontogeny of the chick

Size / Age	Tiny <5u	Very small 6-10u	Small 11-15u	Medium 16-20u	Big 21-25u	Very big 26-30u	Large 31-35u	Very large 36-40u	Giant >40u	Total Number	Grand Total
E6-D	785	8222	8444	454	0	0	0	0	0	17905	
E6-L	0	0	0	0	0	0	0	0	0	0	17905
E8-D	492	22987	7342	195	0	0	0	0	0	31016	
E8-L	0	0	0	0	0	0	0	0	0	0	31016
E10-D	2810	18610	7019	374	0	0	0	0	0	28813	
E10-L	0	0	0	0	0	0	0	0	0	0	28813
E13-D	302	13166	2956	2274	537	42	0	0	0	19277	
E13-L	0	0	3386	2868	624	53	0	0	0	6931	26208
E15-D	281	7451	4221	3905	867	38	5	2	0	16770	
E15-L	0	0	1214	4226	2415	44	4	4	0	7907	24677
E18-D	37646	36189	16518	11356	1657	581	0	0	0	103947	
E18-L	0	0	710	988	361	125	0	0	0	2184	106131
H-D	182	945	2251	3942	2185	799	50	0	0	10354	
H-L	0	1191	2369	2438	957	214	13	0	0	7182	17536
A-D	509	2517	2047	2052	1004	1494	987	147	0	10757	
A-L	0	675	611	589	48	170	209	46	0	2348	13105

(D = Dark cells E=Embroynic age H=Day of hatching A=Adult)

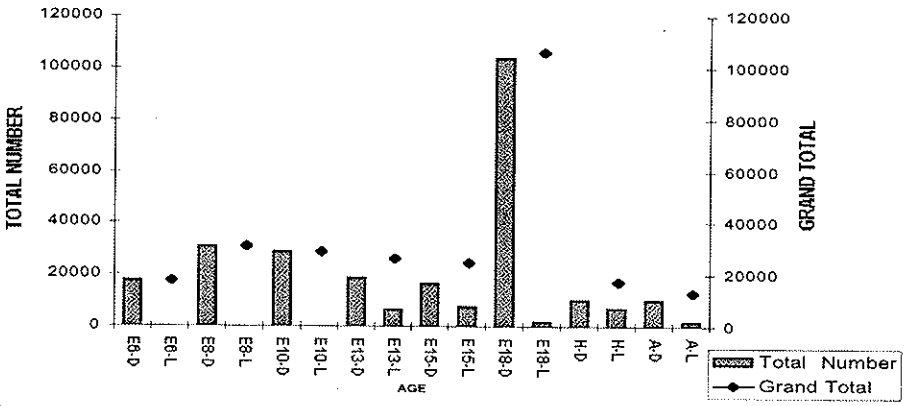

Figure 5A. Total number of dark & light cells in the proximal ganglionic complex of cranial nerves IX & X in different age groups of animals in the ontogeny of the chick

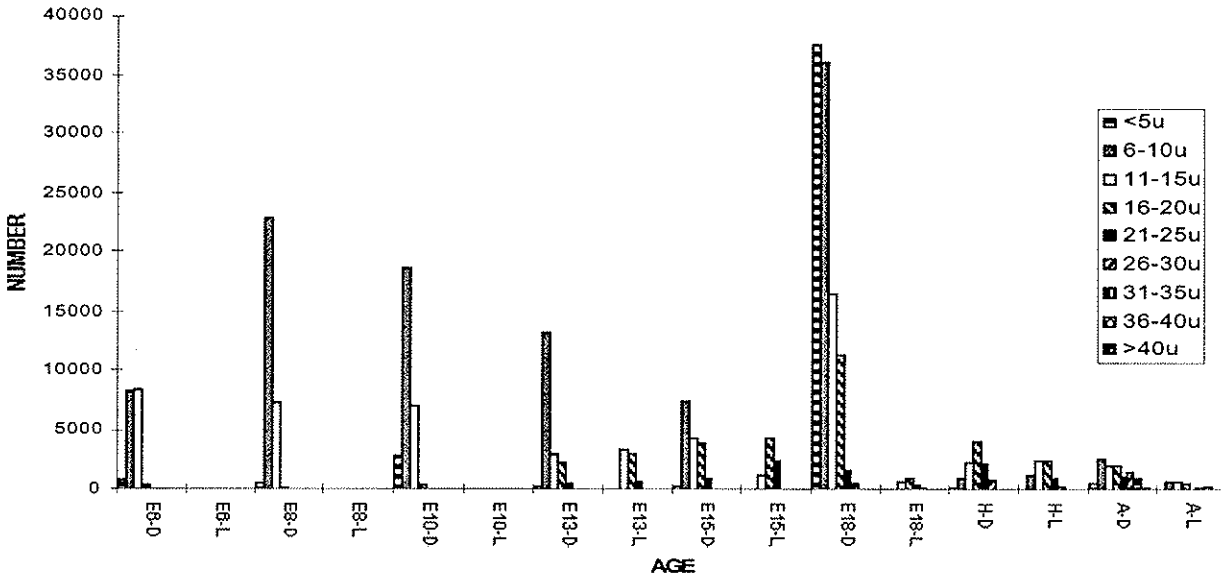

Figure 5B. Total number of dark & light cells in the proximal ganglionic complex of cranial nerves IX & X in different age groups of animals in the ontogeny of the chick

Table 6. Illustrates the total number of dark and light cells in the petrous ganglion in different age groups of animals in the ontogeny of the chick

Size / Age	Tiny <5u	Very small 6-10u	Small 11-15u	Medium 16-20u	Big 21-25u	Very big 26-30u	Large 31-35u	Very large 36-40u	Giant >40u	Total Number	Grand Total
E6-D	205	3681	3484	408	0	0	0	0	0	7778	
E6-L	0	0	0	0	0	0	0	0	0	0	7778
E8-D	106	3446	4579	235	13	0	0	0	0	8379	
E8-L	0	0	0	0	0	0	0	0	0	0	8379
E10-D	839	3244	2211	475	89	0	0	0	0	6858	
E10-L	0	0	7	1	0	0	0	0	0	8	6866
E13-D	128	6191	853	440	326	22	0	0	0	7960	
E13-L	0	0	990	575	463	34	0	0	0	2062	10022
E15-D	399	3097	1556	1521	93	7	0	0	0	6673	
E15-L	0	0	474	845	128	6	0	0	0	1453	8126
E18-D	10948	9937	6136	3577	635	229	0	0	0	31462	
E18-L	0	0	197	340	121	83	0	0	0	741	32203
H-D	42	50	288	992	258	81	0	0	0	1711	
H-L	0	409	799	880	55	5	0	0	0	2148	3859
A-D	52	85	304	1053	291	85	0	0	0	1870	
A-L	0	150	445	463	58	6	0	0	0	1122	2992

(D = Dark cells E=Embroynic age H=Day of hatching A=Adult)

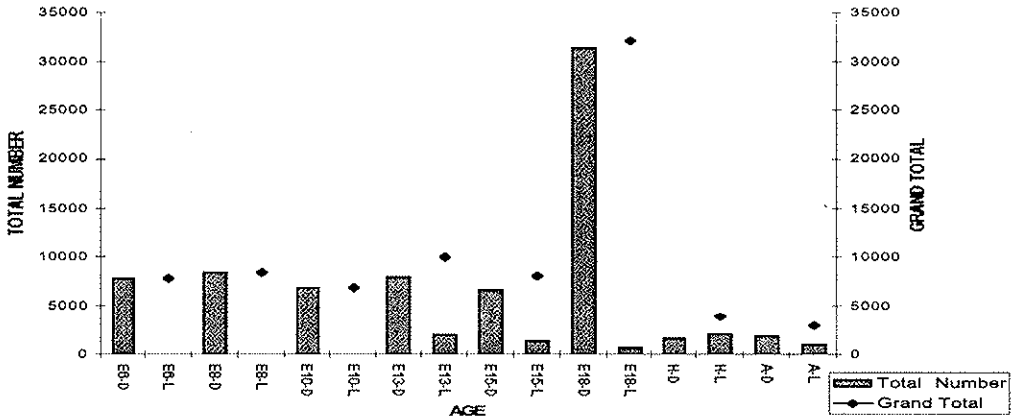

Figure 6A. Total number of dark & light cells in the petrous ganglion in different age groups of animals in the ontogeny of the chick

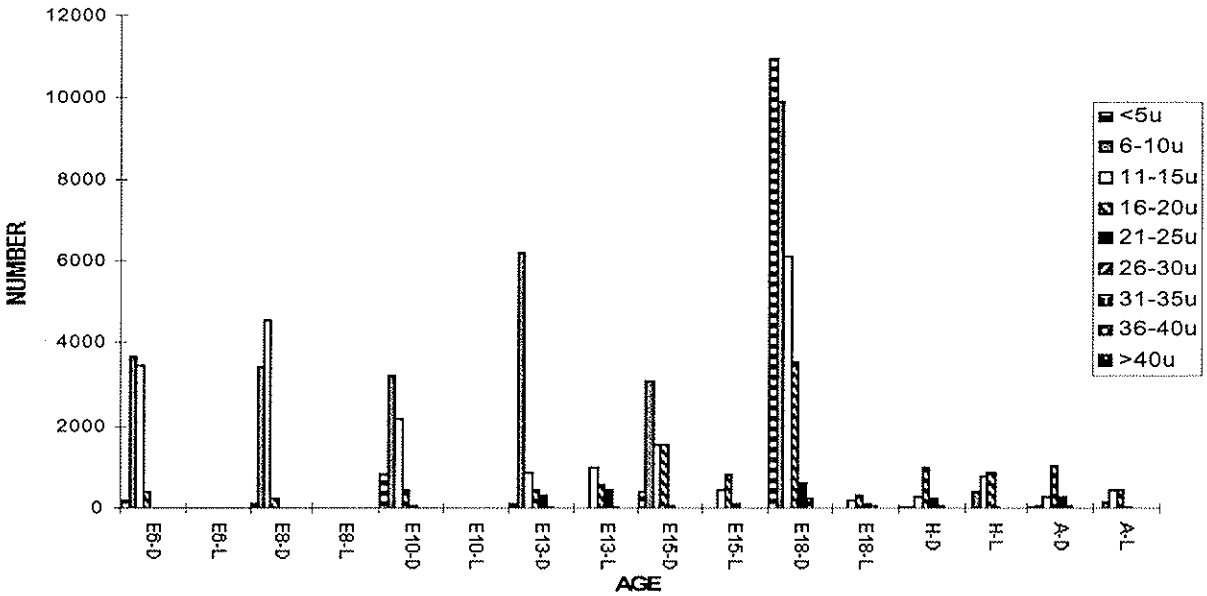

Figure 6B. Total number of dark & light cells in the petrous ganglion in different age groups of animals in the ontogeny of the chick

Table 7. Illustrates the total number of dark and light cells in the nodose ganglion in different age groups of animals in the ontogeny of the chick

Size / Age	Tiny <5u	Very small 6-10u	Small 11-15u	Medium 16-20u	Big 21-25u	Very big 26-30u	Large 31-35u	Very large 36-40u	Giant >40u	Total Number	Grand Total
E6-D	161	2488	3731	2845	0	0	0	0	0	9225	
E6-L	0	0	814	701	0	0	0	0	0	1515	10740
E8-D	116	6425	6645	3627	145	0	0	0	0	16958	
E8-L	0	0	84	108	17	0	0	0	0	209	17167
E10-D	135	4774	7584	2300	1178	43	0	0	0	16014	
E10-L	0	0	42	70	48	7	0	0	0	167	16181
E13-D	94	3812	1645	714	229	99	20	0	0	6613	
E13-L	0	0	859	942	358	172	28	0	0	2359	8972
E15-D	979	35572	4388	3769	128	14	7	0	0	44857	
E15-L	0	0	576	1258	76	29	7	0	0	1946	46803
E18-D	30278	25074	15800	6154	1594	315	38	0	0	79253	
E18-L	0	0	509	767	184	7	0	0	0	1467	80720
H-D	66	259	908	2709	1654	1903	309	0	0	7808	
H-L	0	92	947	1544	920	150	3	0	0	3656	11464
A-D	275	1526	1527	2245	902	1418	467	388	36	8784	
A-L	0	0	24	97	71	82	38	25	9	346	9130

(D = Dark cells E=Embroynic age H=Day of hatching A=Adult)

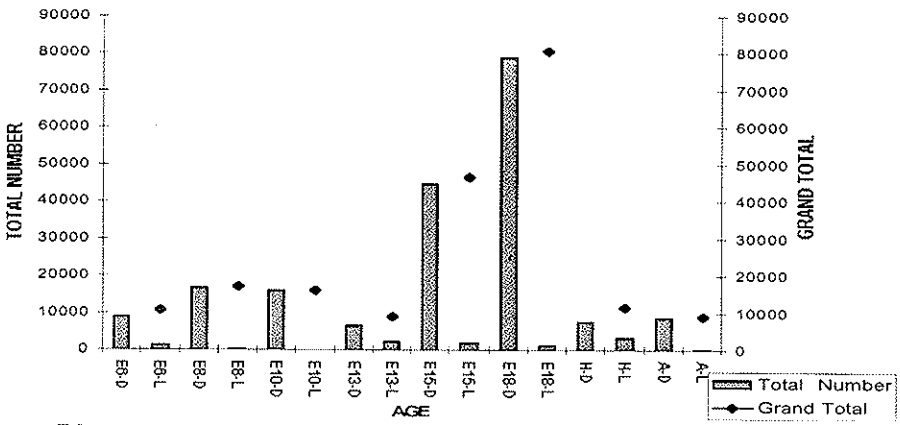

Figure 7A. Total number of dark & light cells in nodose ganglion in different age groups of animals in the ontogeny of the chick

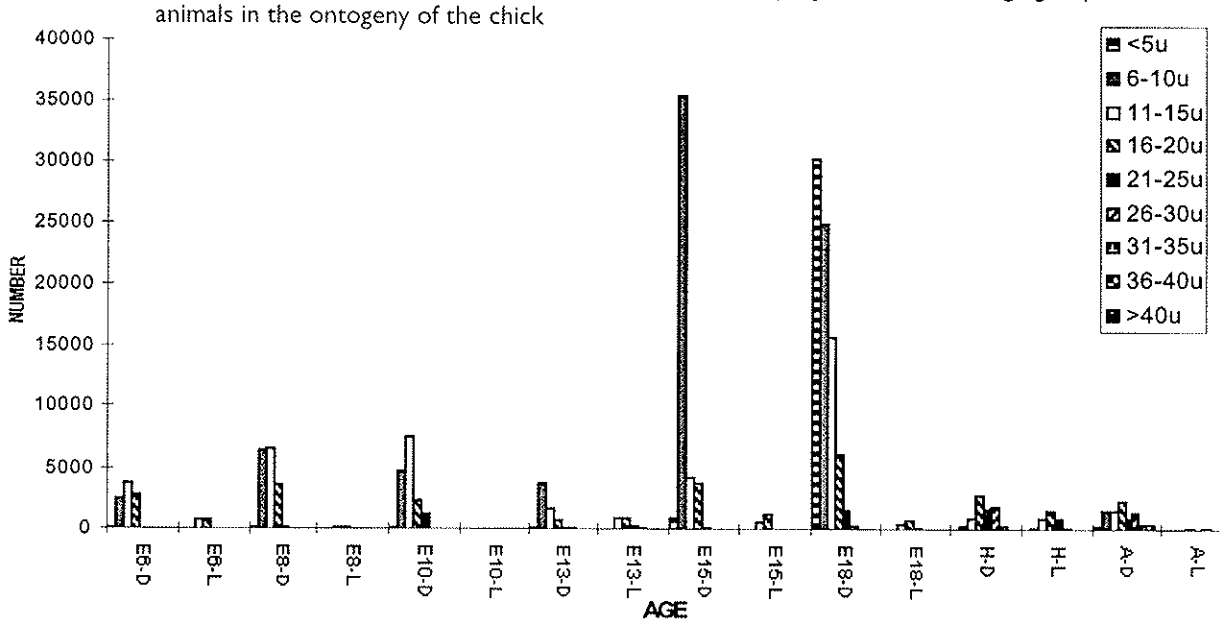

Figure 7B. Total number of dark & light cells in nodose ganglion in different age groups of animals in the ontogeny of the chick

Table 8. Illustrates the total number of dark and light cells in the ciliary ganglion in different age groups of animals in the ontogeny of the chick

Size / Age	Tiny <5u	Very small 6-10u	Small 11-15u	Medium 16-20u	Big 21-25u	Very big 26-30u	Large 31-35u	Very large 36-40u	Giant >40u	Total Number	Grand Total
E6-D	2053	8820	5707	49	0	0	0	0	0	16629	
E6-L	0	0	0	0	0	0	0	0	0	0	16629
E8-D	114	9452	5684	2396	0	0	0	0	0	17646	
E8-L	0	0	0	0	0	0	0	0	0	0	17646
E10-D	2918	14152	4337	2211	0	0	0	0	0	23618	
E10-L	0	0	0	0	0	0	0	0	0	0	23618
E13-D	156	14142	4559	2057	179	0	0	0	0	21093	
E13-L	0	0	565	199	10	0	0	0	0	774	21867
E15-D	413	2519	1887	2078	6	6	0	0	0	6909	
E15-L	0	0	1468	984	3	1	0	0	0	2456	9365
E18-D	155843	16125	9923	3237	876	375	0	0	0	186379	
E18-L	0	0	1	78	59	40	0	0	0	178	186557
H-D	319	1486	1639	1594	472	100	0	0	0	5610	
H-L	0	1381	1722	1382	384	42	0	0	0	4911	10521
A-D	259	336	249	449	274	150	178	184	0	2079	
A-L	0	0	64	156	99	45	56	36	0	456	2535

(D = Dark cells E=Embroynic age H=Day of hatching A=Adult)

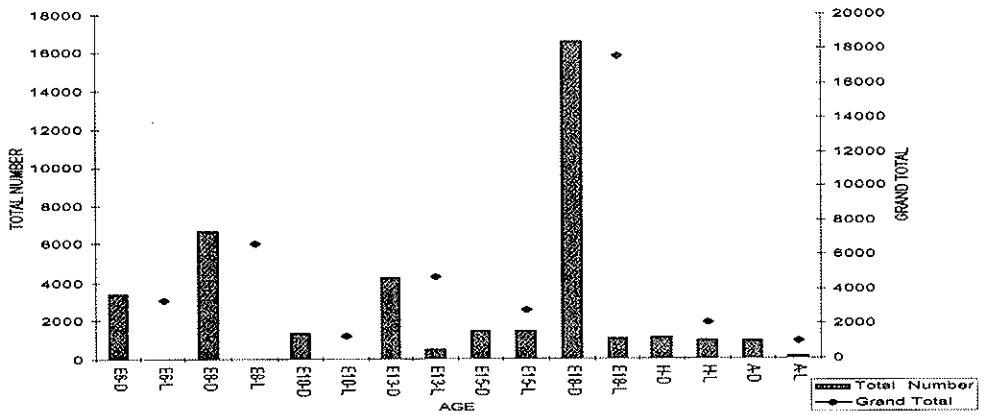

Figure 8A. Total number of dark & light cells in ciliary ganglion in different age groups of animals in the ontogeny of the chick

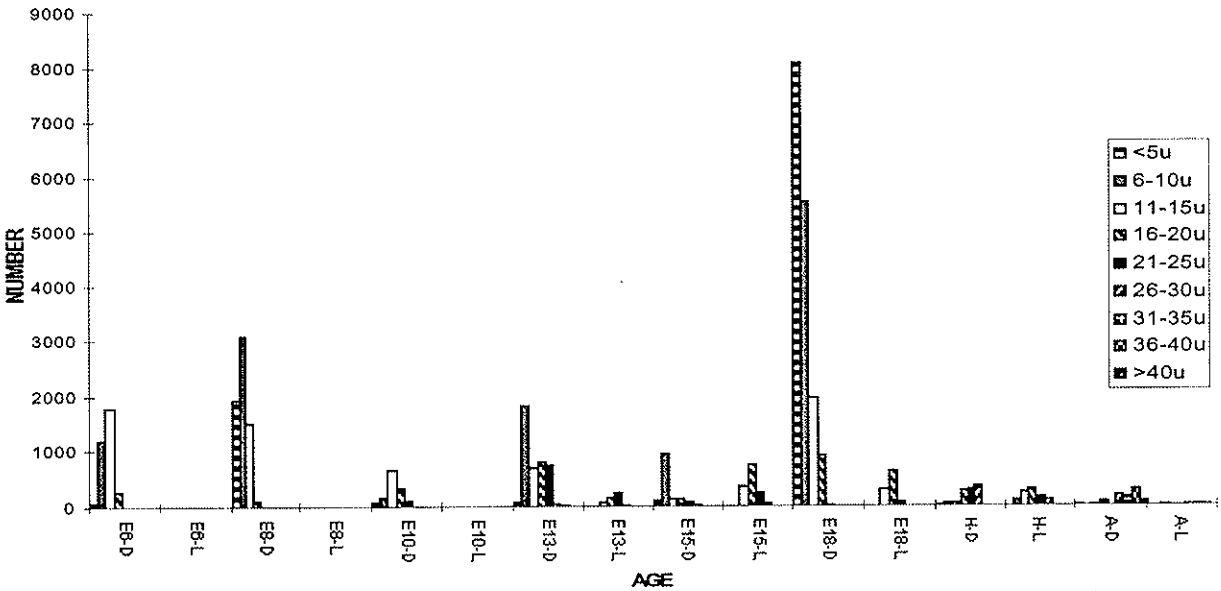

Figure 8B. Total number of dark & light cells in ciliary ganglion in different age groups of animals in the ontogeny of the chick

Table 9. Illustrates the total number of dark and light cells in the superior cervical ganglion in different age groups of animals in the ontogeny of the chick

Size / Age	Tiny <5u	Very small 6-10u	Small 11-15u	Medium 16-20u	Big 21-25u	Very big 26-30u	Large 31-35u	Very large 36-40u	Giant >40u	Total Number	Grand Total
E6-D	687	5986	7250	566	0	0	0	0	0	14489	
E6-L	0	0	0	0	0	0	0	0	0	0	14489
E8-D	5230	12194	13190	218	0	0	0	0	0	30832	
E8-L	0	0	0	0	0	0	0	0	0	0	30832
E10-D	10832	31144	3929	1776	0	0	0	0	0	47681	
E10-L	0	0	0	0	0	0	0	0	0	0	47681
E13-D	347	22999	12004	3972	0	0	0	0	0	39322	
E13-L	0	0	0	0	0	0	0	0	0	0	39322
E15-D	1292	59282	11798	2602	0	0	0	0	0	74974	
E15-L	0	0	0	0	0	0	0	0	0	0	74974
E18-D	38420	42647	44252	1912	0	0	0	0	0	127231	
E18-L	0	0	323	168	0	0	0	0	0	491	127722
H-D	675	9404	7086	1007	0	0	0	0	0	18172	
H-L	0	28679	8154	239	0	0	0	0	0	37072	55244
A-D	475	8228	8365	1057	146	0	0	0	0	18271	
A-L	0	11798	4116	155	34	0	0	0	0	16103	34374

(D = Dark cells E=Embroynic age H=Day of hatching A=Adult)

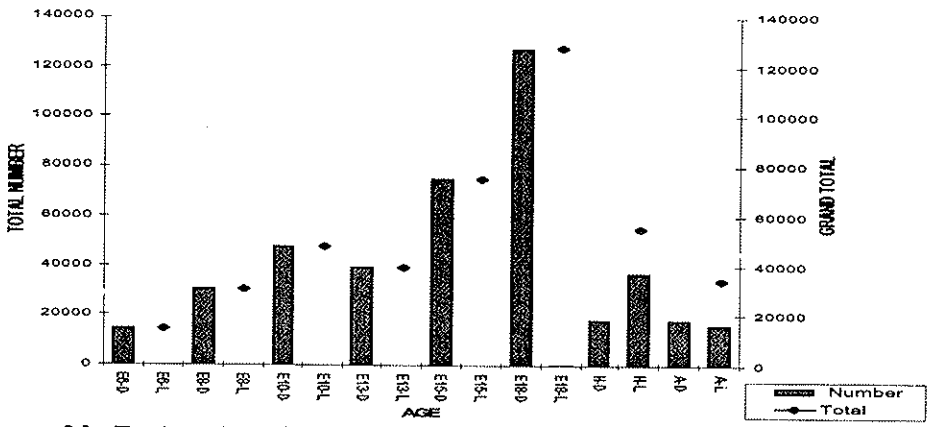

Figure 9A. Total number of dark & light cells in superior cervical ganglion in different age groups of animals in the ontogeny of the chick

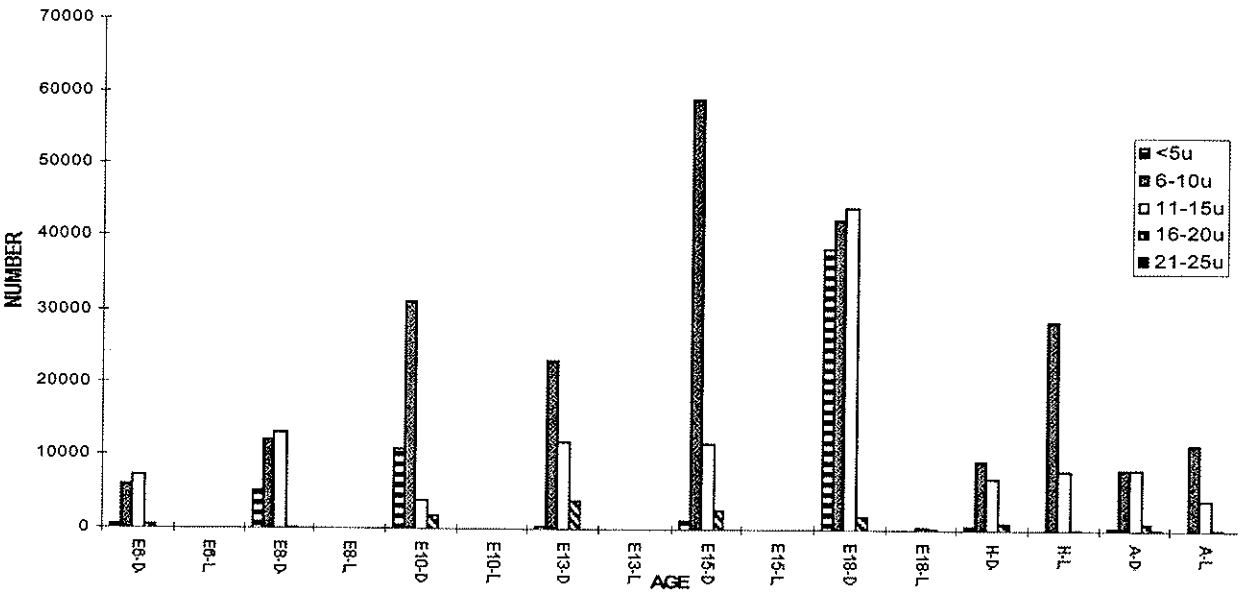

Figure 9B. Total number of dark & light cells in superior cervical ganglion in different age groups of animals in the ontogeny of the chick

Discussion

A. Dark and Light Cells

In general, by a critical analysis and evaluation of the results in all the ganglia studied in the present series of investigation, it is assumed that

a. the dark cells represent a group of functionally active cells which might proliferate, grow, mature, establish proper connections and continue to perform their functions. However, these cells may lose their activity and become inactive or die at any stage of their development, growth or activity and change to a light coloured cell on staining.

b. the light cells represent a group of inactive, dying, dead or degenerating cells. In many situations, the occurrence of light cells in the ganglion for the first time is associated with loss of cells. These cells might become inactive or die due to some inherent defects developed within themselves or to some adverse factors found in the micro-environment. These light cells are found to appear around the time when the cells begin to establish their projections and represent those which fail to establish functional connections. However, sometimes when the adverse factors are rectified, these cells which have at first started to lose their functions might be re-activated and become normal active cells again and, therefore, might turn to be a dark type.

i. by re-activation of their same original cell-processes which have first failed to establish a functional projection into their peripheral field of innervation, by rectifying the defects (found either within the cells themselves or in their micro-environment) by developing some favourable conditions, or

ii. by the development of new collateral branches from the main process, which might grow new and establish functional connections to their innervation fields.

A brief explanation is given below to illustrate the factor: b given above, i.e., to show that the light cells (inactive cells) might revert to dark cells (active ones). As an example, please refer to the results of the superior cervical ganglion on the day of hatching (H) and in the adulthood(A). Please note the number of total cells (55244 on H, 34374 in A), dark cells (18172 on H, 18271 in A) and light cells (37072 on H, 16103 in A), and the proliferative capacity which is represented by the number of tiny cells (675 on H, 475 in A) in these stages (H and reduced in A). This indicates that the number of dark cells have increased in the adulthood whereas the light cells have reduced, in comparison to that observed on the day of hatching. This is clearly suggestive of the assumption that some of the light cells have changed themselves to become an active type again (i.e., dark cells), probably by establishing a viable functional connection as a result of newly-formed collateral branches as a result of some favourable micro-environment, or by regaining the functional capacity of the original fibres by a process of re-activation, after the fibres have started to lose their functional capacity. Almost similar results can be noticed in the vestibular ganglion, proximal ganglionic complex of cranial nerves IX and X, petrous ganglion, nodose ganglion etc. However, in these ganglia, the increased number of tiny cells in the adulthood in comparison to that observed on the day of hatching might suggest that the increased number of dark cells in the adult situation might be related either to a re-activation of light cells (inactive cells) as explained above, or to the growth, maturation and establishment of active functional connections of the new-generation of tiny cells, or by both these processes.

The following evidences are presented in support of this assumption about the significance of the dark and light groups of cells. However, these statements are given in the form of brief points in order to avoid unnecessary long descriptions which might also need repetitions for every ganglion studied. Later, the facts are described in relation to some of the relevant available literature. Whenever necessary, for more details, the results of that particular ganglion may be verified and the facts be confirmed. The percentage or ratio of the dark and light cells in the ganglion in any age group may be found in the description of the results.

1. During the periods of active proliferation and growth especially in the early stages of development, while there is a continuous increase in the total number of cells, all the cells among all categories found in the ganglion are dark type; no light cells are found during these active periods of development. Therefore, the dark cells are considered as a group of active cells which might divide, proliferate, grow, mature and thereby help to increase (or add) the number of all classes of cells in the ganglion. It may be noticed that the light cells begin to appear in the respective ganglia just after the stages given below. The time of appearance of light cells in the ganglion is presumed to be related to the time of their failure to establish functional connections. The beginning of establishment of such connections should be earlier than the time of occurrence of these light cells in the ganglion. Of course, this period varies from one ganglion to the other. The following description shows the developmental stages where all the ganglion cells are dark type after which the light cells begin to appear.

Trigeminal ganglion	E6, probably the establishment of functional connection begins early.
Geniculate ganglion:	E6, E8, E10
Vestibular ganglion:	E6, E8, E10; Please refer to the explanation given for the presence of a few (8) light cells on E6

Acoustic ganglion:	E6, E8, E10
Prox. G. Comp. of IX and X:	E6, E8, E10
Petrous ganglion:	E6, E8
Nodose ganglion:	Light cells are found even on E6; Probably the establishment of functional projections begins very early, even before E6. Thus this is the first ganglion to developfunctional connections, possibly related to its supply tovital organs such as heart, lungs and alimentary canal and its importance.
Ciliary ganglion:	E6, E8, E10
Superior cervical ganglion:	E6, E8, E10, E13, E15; Very late appearance of light cells

Exceptionally, in certain stages of development, in some of these ganglia (given below), the number of cells are reduced instead of a continuous increase, indicating probably the presence of an active phagocytic process which would remove the inactive or dead cells (so-called light cells) during developmental period. However, the light-cell stage is not represented in them probably because the phagocytosis is so active and so-fast to leave this light-cell stage for clear observation. These developmental stages probably represent some critical periods (in that particular ganglion) in their attempt to establish functional projection to their innervation fields. It may be noticed that most of these periods of cellular loss are just before the occurrence of light cells in the ganglion.

Geniculate ganglion:	E10
Prox. G. Comp. of N. IX and X:	E10
Superior cervical ganglion:	E13

2. As soon as the light cells have appeared in the ganglion, the total cells have reduced in number representing a loss of cells which, in turn, suggests that these light cells play a role in the loss or reduction of cells in the ganglion, or in other words, these light cells might represent a group of resting, inactive, dying, dead or degenerating cells which will, in course of time, be removed from the ganglion by phagocytes. It may be noticed that the loss or reduction in the total number of cells in the ganglion is occurring around the time when the light cells make their first appearance.

Trigeminal ganglion:	E10, the very few (78 cells) negligible number of light cells ound on E8 probably have formed justthen, which is the beginning of cell death where the total cells, however, are greater in number.
Geniculate ganglion:	E15, the few (512 cells) light cells found on E13 probably is the beginning of an observable cell death. The cell loss found on E10 is explained in the Discussion; probably the removal is too fast so as not to observe the light-cell stage.
Vestibular ganglion:	E13
Acoustic ganglion:	E13
Prox. G. Comp. of N. IX and X:	E13
Petrous ganglion:	E10
Nodose ganglion:	Light cells are found even on E6; earlier stages are not observed in this ganglion.
Ciliary ganglion:	E13
Superior cervical ganglion:	A few (491 cells = 0.38 %) light cells have appeared only on E18, which is probably the beginning of the appearance of light cells, however, on the day of hatching a gross reduction in the total number of cells having an increased number of light cells is found. However, in the earlier stages of cell loss (E13) light cells are not found.

3. On E18, there is usually a greatly reduced number of light cells (compared to E15) in relation to the tremendously increased number of dark cells (predominantly smaller categories), most of which are probably phagocytes (please refer to Part C : Removal of Dead Cells in the end) (also assumed from the present results found on the day of hatching where there is a great loss in the total number of cells in the ganglion). It is possikåe that the phagocytic activity is so great and so-fast that the light cell stage is not always observable since most of these inactive cells are actively removed from the vicinity of the ganglion before they become observable. This is a constant feature in all the ganglia in order to free the tissue from the noxious effects of the remnants of dead cells before the delicate and young animal is exposed to an independent living on the day of hatching.

This is true in all the ganglia studied except a small difference observed in the superior cervical ganglion where the light cells have appeared for the first time only on E18.

4. a. But on the day of hatching, there is usually a greater proportion of light cells in the ganglion while there is a greatly reduced total number of cells because most of the unwanted cells have been removed by the greatly increased phagocytic activity found around E18. During post-hatching period, the light cells are greater in

number. Probably, most of these light cells are in a temporary resting or inactive stage; many of them might become active functional cells again. It is also assumed that a proportion of the smaller categories of dark cells might represent the continued presence of phagocytes, ready to remove the inactive or dead cells.

This is true in all the ganglia studied except Geniculate ganglion where some differences are found. That is, here in the geniculate ganglion greatest number of light cells are found on E15; later the light cells reduced in number throughout embryonic development, on the day of hatching as well as in the adult situation.

b. The tiny cells are found to be always dark. The very small type of cells are also found to be dark through the whole embryonic period till E18. Later, however, the light cells have appeared among the very small type also on the day of hatching, but they may or may not continue to be present in the adult situation. This might imply that even though the very small type of cells appear to keep themselves to be an active group till the day of hatching, and be ready to replace the dead cells occurring as a result of several adverse factors, cell death and degeneration begin among these cells also as from the day of hatching. It may be assumed that normally there cannot be any more necessity for the establishment of new functional projections after the day of hatching since all these connections might have been already established by this time while the animal is ready to lead an independent living. Therefore, there is no need for further growth and maturation of this smaller category of cells and the cell death begins even among this very small type of cells as from the day of hatching. Thus the appearance of light cells among this group just on the day of hatching is suggestive of evident cellular inactivity, death and degeneration process.

Trigeminal ganglion:	True : Continue to be present in the adult
Geniculate ganglion:	True : Disappear in the adult
Vestibular ganglion:	True : Disappear in the adult
Acoustic ganglion:	True : Continue to be present in the adult
Prox. G. Comp of IX and X:	True : Continue to be present in the adult
Petrous ganglion:	True : Continue to be present in the adult
Nodose ganglion:	True : Disappear in the adult
Ciliary ganglion:	True : Disappear in the adult
Superior cervical ganglion :	True : Continue to be present in the adult

5. Even the larger classes of cells (having a diameter greater than 30 microns) whenever they have appeared in the ganglion contain both dark as well as light cells. (However, the dark and light cells are also found among smaller classes as well). This contradicts the descriptions of many earlier workers (26, 27, 28) that the ganglion contains small dark cells and large light cells and also contradicts their attribution of different functions to these cells because, in the present study the dark and light cells are found distributed among all categories whose diameter is greater than 10 microns irrespective of their small or large size. Therefore, such classification and functional attribution are disputed.

Trigeminal ganglion:	E13, E18, on the day of hatching and adult; on E15 these larger classes of cells have totally disappeared
Geniculate ganglion:	E13 and adult; on E15 and E18 the larger classes of cells have totally disappeared; on the day of hatching only dark cells are found.
Vestibular ganglion:	On the day of hatching and adult when these larger classes of cells have appeared.
Acoustic ganglion:	Such large class of cells has never appeared in the ganglion
Prox. G. Comp. of IX and X:	E15, day of hatching and adult when the larger classes of cells have appeared. On E18 these larger classes have totally disappeared
Petrous ganglion:	Such large class of cells has never appeared
Nodose ganglion:	E13, E15, day of hatching and adult when these larger classes of cells have appeared. On E18 only dark cells are found among them.
Ciliary ganglion:	In the adult, only when these larger classes of cells have appeared
Superior cervical ganglion:	Such large class of cells has never appeared

6. The light cells continue to be present in the ganglion even in the adult situation while the total number of cells also continue to reduce. This is probably due to cellular inactivity and death as a result of ageing process while the functional reduction or functional loss is found in all organs including the organs of special sensibility and nervous control. This factor is uniformly noticed in all the ganglia studied.

7. The number of light cells lost in the ganglion is almost equal to the loss in the total number of cells, at a stage

while the proliferation has stopped or reduced as evidenced by the number of tiny cells. Even though such condition is observed in a few instances in the whole investigation, this cannot be neglected as invalid because such incidence or circumstance cannot be expected to occur frequently in a constantly changing life cycle in the ontogeny, when such a change has to coincide with the time of observation.

Petrous ganglion: and Superiorcervical ganglion:	Compare the results on the day of hatching and the and adult situation, at a time while the tiny cells are almost equal in number which indicates the stoppage of proliferative activity.

8. In the early stages of development, only dark cells are found in all the ganglia studied in the present series of investigation indicating that these are active cells. The light cells appear only after certain period of embryonic growth, probably at a time when the cells fail to establish proper functional projection onto their innervation fields. For example, in the present study, the structural evidence of the occurrence of light cells in the trigeminal ganglion on E8 seems to coincide with the appearance of physiological evidence occurring on the same embryonic day (E8) of exhibiting reflexogenic response to tactile stimulus of the beak of the chick embryo (29). Similarly there are suggestions (22,26) that the placode-derived neurones in the trigeminal ganglion have well-established peripheral projections by the end of the first week of incubation. In contrast, the neurones derived from the neural crest in the trigeminal ganglion (30, 31) do not cease dividing until the seventh day of incubation. It may be recalled that the process-formation of neurones begins after the terminal mitosis which is considered as neurone's birth date (31). Even though there is a slight variation in these suggestions given by different workers, it may be assumed that such degeneration is quite likely to happen around the seventh day of incubation. Thus the coincidence of appearance of light cells in the trigeminal ganglion in the present study indicating cell death on their failure to form proper connections suits very well with all these descriptions. However, similar physiological observations and reports for other ganglia are not available in the literature in order to make a comparison with the results in other ganglia studied in the present series of investigation.

9. It may also noticed that the light cells appear in the ganglion for the first time usually among small and medium sized cells (having a diameter of 11 - 20 microns) and later once they grow into larger classes, the light cells continue to be present among them. This is suggestive that the establishment of functional projection begins during this stage and that once they are unable to make functional connections, these cells become inactive or die and change into light coloured cells on staining. Such light cells may represent cellular inactivity or death. The light cells among larger classes may also represent cellular inactivity or death during successive growth periods, before establishing functional connections which may be due to any defect, either within the cell, in the micro-environment, inadequate supply of nerve growth factor (neurotrophic factor), or to ageing process in the case of adult.

10. However, it may be thought that the period of active cell loss (death) coincides with the period of active establishment of functional connections and in the present study, this period varies from ganglion to ganglion. This may be judged by the time of occurrence of light cells for the first time in the ganglion. However, this active establishment of functional connections may extend for longer periods as indicated by greater cellular loss in other stages. The following stages show the time of first appearance of light cells in the ganglion.

Trigeminal ganglion:	E8
Geniculate ganglion:	E13
Vestibular ganglion:	E6, even though light cells are missing on E8 and E10
Acoustic ganglion:	E13
Prox. G. Comp. of N. IX and X:	E13
Petrous ganglion:	E10
Nodose ganglion:	E6, early and frank appearance of light cells
Ciliary ganglion:	E13
Superior cervical ganglion:	E18

The descriptions of the dark and light neurones by different investigators in different animals vary greatly in available literature. Some of these conflicting views from the literature which are considered more useful are described below. Some investigators (9, 26) have described in the trigeminal ganglion of the chick that the large neurones contain many isolated clumps of basophilic material in a neurofilament-rich cytoplasmic matrix having a diameter ranging from 19 - 40 microns and that the smaller neurones having a diameter ranging from 11 - 30 microns tend to be multipolar and have a greater concentration of ribosomes and granular endoplasmic reticulum which are dispersed through a dense matrix. Based on their staining properties, these larger ones are called light neurones and the smaller ones are called dark neurones. In the adult rats, trigeminal neurones have been classified into dark and light types on the basis of distribution of cytoplasmic organelles within the neurones and on the relative density of cytoplasm (7, 12, 24). In a comparative ultra-

structural study of the trigeminal ganglion (8) small dark cells were densely packed with rough endoplasmic reticulum and ribosomes, and large light cells with a dispersed and occasionally clumped ergastoplasm. It may be noticed that the classification (9, 26) of dark (small) and light (large) neurones overlap in size. However, all the above investigators have found that the dark (smaller) neurones have a greater concentration of ribosomes and granular endoplasmic reticulum, which is suggestive of a more active role than that of their lighter (larger) counterparts. This observation also supports the present results that the dark cells represent a group of active cells and the light cells represent a group of resting, inactive, dying, dead or degenerating cells.

However, in subsequent reports on human autopsied trigeminal ganglia, it was not easy for these workers (25) to readily identify dark and light neurones. The majority of cells were intermediate in appearance. On rare occasions when they did observe these two neuronal types, the cells differed only in their cytoplasmic density and not in the arrangement of any of their organelles. These observations led these investigators to conclude that the dark and light cells were not real entities, but resulted from fluid-shifts between the cells in question and the surrounding extra-cellular spaces. By the same token, other investigators (32, 33) working with cat and monkey trigeminal ganglia found that if proper tissue fixation techniques were used, there were no base for classifying the trigeminal neurones into dark and light cell types. It is possible that these conflicting reports given by different investigators may be attributed to the species difference of the experimental animals used.

There has been suggestions(26) that shortly before hatching (E18 onwards), there appears two classes of interposed neurones characteristic of the mature trigeminal ganglion : large light types and small dark type. Neither of these two populations corresponds uniquely to either of the two segregated populations (large peripherally and distally located cells, and small centrally and proximally located groups) found in the embryo. In the present study in the chick, the dual cytology of neurones (dark and light cells) is found in all the ganglia studied. They were observed not only in the mature ganglia (from 18th day of incubation to adult) as stated by earlier investigators (9, 26, 27, 30, 34, 36), but also in earlier developmental stages (please refer to the results). It is not clear whether the above workers failed to observe these classes of cells in earlier developmental stages because of unreliable staining techniques or whether they did not attempt to investigate their presence during early embryonic periods. It is also noticed in the present investigation that in the early stages of development, only dark cells are found in all the ganglia. The light cells begin to appear only after certain period of embryonic growth which varies from ganglion to ganglion : probably the time of their occurrence coincides with their failure to establish proper functional projection to their innervation fields. For example, in the present study in the trigeminal ganglion, the light cells have appeared for the first time on E8 which seems to coincide with the day (E8) of establishment of functional connections as observed on the basis of the presence of reflexogenic responses to tactile stimulus of the beak (29).

It is frequently suggested (18, 26, 27, 30, 31, 37, 38) that the large light and small dark neurones found in the trigeminal ganglion are correlated with the dual embryonic origin as derived from both the epidermal placode and neural crest. Through most of the second week of development the large cells are found in the distal and ventro-lateral parts of the ganglion and the small cells are found in the proximal (core) and medio-dorsal parts (39, 40). Even though the present study is not aimed at finding out the embryological origin of these two categories of cells, the segregation of large cells in the distal and ventro-lateral parts and the small cells in the proximal (core) and medio-dorsal parts of the ganglion is evident from the present results similar to these findings.

Also there is both circumstantial and direct evidence that cytological dichotomy in the adult ganglion is not based on separate embryological origin. First, other sensory ganglia that are exclusively of neural crest origin such as trunk dorsal root ganglia, or of placodal origin such as acoustic ganglion have both dark and light types of neurones in the present study. Similar view has been advocated by previous investigators (41, 42) also. In addition, the light and dark neurones are found interspersed at random throughout the ganglion during embryonic development and post-hatching periods in the present study, whereas the segregation of crest and placode-derived neurones (small dark and large light cells respectively) are found only in the embryo. Both transplantation experiments (27) and birth-date analysis (31) have proved that neurones from each of these anlagen retain their original separate locations during later stages of development and maturation. Thus the dual cytology of the mature trigeminal ganglion is not based on separate embryonic origins.

Since there is no clear evidence of trigeminal neuron projection to the solitary nuclear complex which normally receives only visceral input (43, 44, 45), there should be no visceral neurones in the trigeminal ganglion. The work on trigeminal ganglion (28) have demonstrated that the trigeminal ganglion had the smallest proportion of dark cells (46.8 %) whereas in the proximal ganglionic complex of the cranial nerves IX and X and the distal ganglion of cranial nerve IX the proportion of the dark cells was much greater (84.7 % and 70.3 % respectively).

Since these two ganglia have both somatic and visceral neurones (46) the large proportion of small dark cells suggests that at least some of the dark cells are visceral neurones. In a work on spinal ganglia (47), the visceral neurones had a tendency to be smaller than somatic neurones. If this is true, then the larger portion of dark cells in the proximal ganglionic complex of cranial nerves IX and X observed by the above workers suggests that this ganglion has more visceral neurones than the distal ganglion of cranial nerve IX. The specific functional attribution for the neurones, based only on size difference (small and large cells) or staining properties (dark and light cells) as suggested by the above-mentioned workers, however, could not be confirmed in the present study because of the following reasons : a) In the present investigation in the chick in the adult situation, even though the proportion of dark cells in the proximal ganglionic complex of cranial nerves IX and X (82.08 %) and that of the petrous ganglion of cranial nerve IX (62.5 %) are very close to the observations of the above mentioned workers in the same ganglia, the proportion of the dark cells found in the trigeminal ganglion (80.04 %) in the present study is quite different from that (46.8 %) observed by these investigators. This suggests that such proportion of dark and light cells in the ganglion might not be a constant factor so as to generalise their functional significance as has been suggested by them, or such changes could be due to species difference of the experimental animals as well. b) In the above-mentioned ganglia as well as in other ganglia studied in the present series of investigation, the proportion of dark and light cells shows a constant fluctuation through the whole ontogeny, i.e., during embryonic period, on the day of hatching as well as in the adult situation. Therefore, similar observation done at any one stage of the whole life cycle cannot prove valuable or give concrete evidence for such functional attribution for any cell. c) Again, this proportion of dark and light cells in the same ganglion is strikingly different in the adult situation even that observed on the day of hatching. The fluctuations in the number of these cells observed during development are considered as a necessary change for the establishment of a suitable functional organisation in the animal. However, similar suggestion cannot be attributed to an animal on the day of hatching, a stage while the animal is already prepared for an independent living (just as the adult animals themselves). d) In the adult situation, in all the ganglia studied in the present series of investigation, there is not only a change in the proportion of dark and light cells but also a great reduction in the total number of cells in the ganglion. Similar variation again confirms that such method of functional attribution of cells, that too found just on one stage of a long life-cycle, and just on the basis of their staining characteristics cannot be validated because this staining characteristics may change in different conditions such as change in pH and functional state of that particular cell. Thus, on analysing the results in the present series of investigation, it is assumed that the light cells represent a group of resting, inactive, dying, dead or degenerating cells and the dark cells represent a group of functionally active cells in the ganglion. It is also assumed that the time of appearance of light cells in the ganglion is directly related to the onset of establishment of functional connections whose importance is related to the organs which their fibres supply, for example, early appearance of light cells in the nodose ganglion is probably related to the functional importance of the vagus nerve which supplies the vital organs such as the heart, lungs and alimentary canal which should be properly innervated as early as possible.

It is also thought useful to quote the views of a few earlier investigators on Cell Death and Degeneration, and Removal of Dead Cells in order to complete the full evaluation of the dark and light cells. In this view, the following points are given for relevant reference.

B. Cell Death and Degeneration

One feature of development of many parts of the nervous system is the occurrence of two opposing processes : cellular proliferation which leads to the production of large numbers of neurones and massive cellular degeneration which results in the loss of many of these same neurones. These two processes ultimately control the final number of neurones of a neural centre. Even though no attempt was taken to investigate the purpose, reasons or ways of causing cell death which occur in different ganglia studied in the present series of investigation, the suggestions of some of the earlier investigators which are found suitable are given below in order to supplement the present assumption derived from a critical analysis of all these results. In the present study, cell death occurs mainly around E10 - E13 after which the ganglion prepares itself for a massive phagocytic activity which nearly comes to an end by the day of hatching. This resembles the descriptions (48) that there are corresponding and parallel changes in the brain stem auditory nuclei and their peripheral ganglionic projections.

The observations (48, 49, 50, 51) support the idea that it is the events in the target tissue (i.e., the periphery) which normally regulate cell survival. They demonstrated that the periphery both controls the proliferation and initial differentiation of undifferentiated cells and also provides the conditions necessary for continued growth and maintenance of neurones in stages following the first outgrowth of neurites. Several experiments (49, 52, 53, 54, 55, 56, 57, 58, 59) have led to a general acceptance of the idea that neuronal death regulates nerve cell numbers in response to peripheral demands by eliminating those nerve cells whose fibres

fail to establish proper peripheral connections. There are evidences (51, 53, 60) suggesting that events at the target tissue controlling normal cell-death, involve the notion of either a competition between neurones for a limited number of synaptic sites and / or for a limited amount of trophic substances supplied by the target. Failure of the neurones to either make or receive the appropriate synaptic connections has been attributed to most neuronal deaths during embryogenesis (51,61). It also seems more likely that the main function of cell death in this system is probably to remove redundant neurones which though being in the correct muscle, have failed to form a contact (62). According to this argument, the nervous system is programmed to over-produce cells in order to saturate the target and insure that all muscle fibers become innervated. The cell death presumably plays a role in the normal formation of orderly connections.

In some experiments (52, 60) in which it has been possible to experimentally enlarge the projection fields of the neuronal population, or to increase the number of afferents which it receives (a technique known to experimental neuro-embryologists as " peripheral over-loading or peripheral enlargement") the number of neurones found in the population at later stages of development has been significantly greater than normal. In contrast to these experiments, peripheral ablation experiments (48,57,58,60) have shown that the severity of the normal neuronal degeneration is much increased resulting in the additional cell death usually occurring over the same period as the naturally occurring neuronal loss. That is, natural cell death is known to be greatly enhanced by peripheral depletion.

From the available evidence, it is convincing to accept the idea that the peripheral influences, peripheral demands, trophic factors supplied by the target organs etc are important factors in controlling the neuronal death and the number of functional neurones available in the ganglia and that the neuronal death plays a role in the normal formation of orderly connections.

C. Removal of Dead Cells

Several investigators have suggested that macrophages are important in removing debris of dead cells (63). Different sources of these macrophages have been suggested. One possibility is the transformation of mononuclear leucocytes in the circulatory system into tissue macrophages (64, 65, 66, 67). It has also been demonstrated (68) many radioactively labelled mononuclear leukocytes can infiltrate into nervous system and aggregate around damaged nervous tissue. There is also electron-microscopic evidence that the leukocytes invade nervous system by crossing through the wall of the blood capillaries (69). It has also been demonstrated in an ultra-structural study (70) that the phagocytic cells contain neuronal debris that exhibit most of the characteristics of mononuclear leukocytes. The phagocytic activity of the satellite cells in the chick embryonic spinal ganglia are attributed to the removal of cellular debris of degenerated cell (71) during early development. There are also reports (72, 73) that phagocytosis is accomplished by glial cells. However, some investigators (74) believe that the degenerating cells produce hydrolytic enzymes for their own degeneration. Therefore, there is obvious reason to believe that the leukocytes from the blood stream can penetrate through the wall of the capillaries into the nervous system and function as tissue macrophages to remove the neuronal debris during cell death and that the glial and satellite cells also can act as phagocytes.

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CHANGES IN SERUM PROTEINS, ERYTHROCYTE SEDIMENTATION RATE AND MANTOUX TUBERCULIN SKIN TEST REACTIVITY IN ACTIVE TUBERCULOSIS

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ABSTRACT: The records of 141 consecutive patients with proven tuberculosis (TB) were reviewed to examine for changes in their serum proteins, erythrocyte sedimentation rate (ESR) and tuberculin skin test reactivity. Hypoalbuminaemia was present in 73% of patients and hyperglobulinaemia was seen in 92% of patients. ESR showed a negative correlation with the serum albumin level but a positive correlation with the serum globulin level. In the case of pulmonary TB, ESR was higher in patients with radiologically more extensive disease. Tuberculin reactivity was reduced in patients who were older, those with more severe hypoalbuminaemia and those with disseminated TB. *(JUMMEC 1998 1&2: 47-53)*

KEYWORDS: Albumin, erythrocyte sedimentation rate, globulin, tuberculin reactivity, tuberculosis

Introduction

Protein-energy undernutrition and hypoalbuminaemia are common observations in patients with active tuberculosis (TB). (1-8) The tuberculin skin test response in TB is related to the nutritional status of the patient which is reflected by the serum albumin level (9-11). Negative response to tuberculin may be seen in 8% to 30% of patients with active TB (1,12,13). Hyperglobulinaemia is a feature in patients with newly diagnosed TB and is mainly due to an increase in the gamma-globulin fraction (1,5). The erythrocyte sedimentation rate (ESR) is usually raised in active TB (1,13,14) but a normal value does not exclude active disease (15).

The objectives of this study are 1) to determine the frequency of abnormalities in the levels of serum albumin and globulin in a group of Malaysian patients with newly diagnosed TB, and 2) to examine the relationships between the different forms of TB and their severity on the one hand, and the serum albumin level, globulin level, ESR and tuberculin skin test reactivity, on the other.

Patients and methods

This is a retrospective analysis of patients with TB diagnosed at the University Hospital, Kuala Lumpur. The records of consecutive patients with newly diagnosed TB which was proven by bacteriology and/or histology in the hospital from September 1994 to December 1996 were reviewed. Confirmation of the diagnosis of TB was based on one or more of the following criteria in the relevant tissue or specimen: 1) positive smear for acid-fast bacilli (AFB) by the Ziehl-Neelson method, 2) positive culture for *Mycobacterium tuberculosis in* Lowenstein-Jensen medium, and 3) typical histology showing epithelioid granulomas with or without caseous necrosis and with or without positive staining for AFB. Disseminated TB was considered to be present when the chest radiograph showed miliary mottling in both lung fields and at least one of the above three criteria was met or when TB could be proven in at least two different organs.

Blood specimens were taken from patients with newly diagnosed TB for the measurement of ESR and liver function test which included serum total protein and albumin. The level of serum total globulin for each patient was obtained by subtracting the level of serum albumin from the total protein measured at the same time. ESR was considered elevated if it exceeded 35 mm/hr for elderly patients aged 65 years and above and for those below 65 years, if it exceeded 10mm/hr for male patients and 20 mm/hr for female patients. Testing for HIV antibody was not routinely done unless a patient belonged to a high risk category or other acquired immunodeficiency syndrome (AIDS) defining illnesses were present.

Plain postero-anterior chest radiographs taken at the time of presentation were reviewed and graded 1 to 6 to assess the extent of pulmonary disease (Table I) (16). The Mantoux test was performed by injecting 10 tuberculin units of Tween-80 stabilised purified protein derivative (PPD) (CSL Limited, Victoria, Australia) intradermally into the ventral surface of the upper part of the forearm and reactions were read at 72 hours. Reactions with indurations of 10 mm or more in diameter measured across the transverse axis of the forearm were regarded as positive and those with no induration or with indurations of less than 10 mm in diameter were considered negative (10,12,13,17,18).

Results were expressed as mean (± one standard deviation). Correlation coefficients (r) between variables were determined by simple linear regression analysis. Statistical comparisons of continuous variables were assessed by unpaired Student's *t*-test and analysis of variance using the Newman-Keuls multiple comparison technique. Categorical data were analysed using chi-square test (χ^2) or the Fisher's exact test. *P* values of less than 0.05 were accepted as statistically significant.

Results

Patient characteristics

During the period of review, tuberculosis was diagnosed and confirmed in 141 patients (87 male, 54 female) in the hospital. Table 2 summarises the distribution of the methods of confirming the diagnosis of TB. There were 77 (54.6%) cases of pulmonary TB of which 19 cases were associated with pleural effusions (Table 3). Thirty-one patients had pleural TB without chest radiographic evidence of lung parenchymal infiltrate, 15 had tuberculous lymphadenitis, 14 had disseminated TB and four had bone and/or joint TB. The age distribution of the patients is shown in Figure 1. The mean age of the patients was 41.2 (± 17.0) years (range, 17 - 84 years). Seventy-five (53.2%) patients were aged 40 years or below while 17 (12. 1 %) were 65 years or older. The mean age of male patients, 41.9 (± 16.9) years was not significantly different from the mean age of female patients which was 40.0 (± 17.3) years ($p = 0.516$). The mean age of patients with lymph node TB, 27.3 (± 9.5) years was significantly younger than those of patients with the other forms of TB; while patients with pulmonary TB alone was significantly older than the other patients (($p = 0.003$) (Table 3). Of the 50 patients who had pleural involvement by TB, those without chest radiograph evidence of lung infiltrate (n = 31) was younger [mean age 38.4 (± 17.1) years] than those with

Table 1. Grading of anatomical extent of lung involvement in pulmonary tuberculosis (16)

1. Trivial: that is, minimal lesions which the assessor regarded, purely on radiographic grounds, as inactive
2. Slight: that is, minimal or rather larger lesions which he regarded as radiographically active
3. Limited: that is, lesions of greater extent than in (2) but involving a total area of lung less than that occupied by the right upper lobe as visualized on a posteroanterior radiograph
4. Moderate: that is, lesions of greater extent than in (3) but whose total extent, even if bilateral, did not exceed an area equivalent to the whole of one lung
5. Extensive: that is, lesions which involved an area of more than the whole of one lung
6. Gross: that is, very extensive bilateral disease

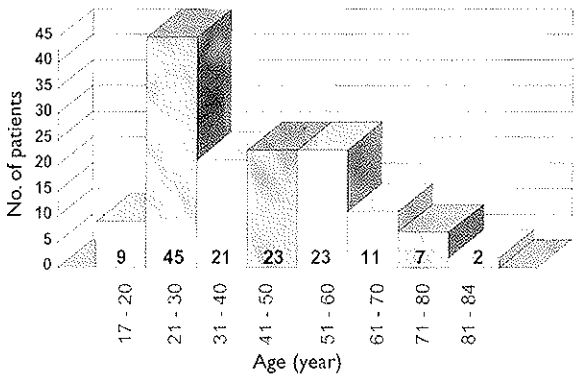

Figure 1. Age distribution of patients with tuberculosis

Table 2. Methods of confirming the diagnosis of tuberculosis

Bacteriological or histological confirmation*	No. of patients#
Sputum smear- and culture-positive	47
Bronchial washing smear- and culture-positive	8
Bronchial washing smear-negative but culture-positive	7
Bronchial biopsy positive histology	8
Lung biopsy positive histology	4
Pleural biopsy positive histology	49
Pleural effusion culture-positive	1
Lymph node biopsy positive histology	16
Vertebral biopsy positive histology	2
Psoas abscess culture-positive	2
Hip joint synovial biopsy positive histology	1
Liver biopsy positive histology	1
Pericardial biopsy positive histology	1
Peritoneal biopsy positive histology	1

*Smear-positive for acid-fast bacilli; or culture-positive for Mycobacterium tuberculosis; or biopsy showing epithelioid granulomas with or without caseating necrosis and with or without the presence of acid-fast bacilli

#Seven patients had positive results from 2 diagnostic procedures

of lung infiltrates (n = 19) [mean age 39.3 (± 15.1) years]. However, this difference did not reach statistical significance.

The distribution of the radiographic extent of lung involvement in the 77 patients who had pulmonary TB with or without pleural involvement is shown in Table 4. There was no relationship between the radiographic extent of lung involvement and the age of the patient.

The most common underlying disease was diabetes mellitus which was present in 35 patients. AIDS was present in three patients with pulmonary TB, one patient with pulmonary TB accompanied by pleural involvement and three patients with lymph node TB. One patient with CD4+ T-lymphocytopenia of unknown cause had pulmonary TB. Three patients had end-stage renal failure. Four patients were on prednisolone for underlying collagen vascular disease and one was receiving cytotoxic chemotherapy for seminoma.

Tuberculin skin test (Mantoux test)

Eighty-four (59.6%) patients showed positive reactions to the Mantoux test. All seven patients with AIDS and the patient with idiopathic CD4+ T-lymphocytopenia did not react to the Mantoux test. Excluding these eight patients (mean age, 31.5 years, range 24 - 46 years) who had obvious reasons for nonreactivity to the tuberculin skin test, patients who were Mantoux test negative (n=49), whose mean age was 46.2 (± 17.0) years, were significantly older than patients who were Mantoux test positive, mean age 39.1 (± 17.0) years (p = 0.021). Significantly more patients with disseminated TB were Mantoux test negative than Mantoux positive (p = 0.02) (Table 5). There was no relationship between Mantoux test reactivity and the radiographic extent of lung involvement in pulmonary TB with or without pleural involvement (Table 6). Although seven (58.3%) out of 12 patients with grade 5 and 6 chest radiograph changes were Mantoux test negative, this proportion was not significantly higher than that of 18 (31.3%) of 65 patients with lower grades of X-ray changes who were also Mantoux test negative (χ^2 =3.053, p = 0.081).

Table 3. Type of tuberculosis and age, serum albumin and globulin levels, and ESR

Type of tuberculosis	No. of patients (n = 141)	Mean age year	Mean serum albumin# g/L	Mean serum globulin* g/L	Mean ESR mm/hr
Pulmonary TB alone	58	46.8 (17.2)	27.8 (7.2)	46.1 (7.9)	69.0 (40.0)
Pulmonary TB with pleural involvement	19	39.3 (15.1)	28.3 (6.2)	48.5 (9.9)	58.2 (44.1)
Pleural TB alone	31	38.4 (17.1)	29.6 (5.5)	45.4 (6.5)	76.9 (30.6)
Lymph node TB	15	27.3 (9.5)	34.6 (7.5)	48.7 (9.3)	73.2 (46.3)
Bone and/or joint TB	4	39.8 (11.4)	31.8 (9.2)	47.0	72.6 (47.6)
Disseminated TB	14	42.0 (18.0)	28.1 (6.3)	49.1 (12.5)	66.1 (45.2)

136 patients: pulmonary TB alone (58 patients), pulmonary TB with pleural involvement (18), pleural TB alone (29), lymph node TB (13), bone and/or joint TB (4), disseminated TB (14)

* 97 patients: pulmonary TB alone (30 patients), pulmonary TB with pleural involvement (17), pleural TB alone (28), lymph node TB (11), bone and/or joint TB (1), disseminated TB (10)

Numbers in parentheses are the standard deviations

Table 4. Radiographic extent of pulmonary tuberculosis and age, serum albumin and globulin levels, and ESR

Grade of chest radiograph extent	No. of patients (n = 77)	Mean age years	Mean serum albumin# g/L	Mean serum globulin* g/L	Mean ESR mm/hr
2	4	54.5 (5.5)	34.3 (2.1)	40.3 (4.2)	23.8 (29.0)
3	33	46.0 (19.4)	29.1 (6.9)	46.6 (9.1)	67.6 (38.1)
4	28	43.8 (15.1)	27.5 (6.9)	46.7 (9.3)	59.6 (35.6)
5	9	46.8 (14.9)	24.0 (6.4)	50.8 (7.1)	101.6 (18.3)
6	3	25.0 (6.9)	22.7 (5.8)	51.7 (5.0)	78.0 (46.9)

76 patients: grade 2 (4 patients), grade 3 (32), grade 4 (28), grade 5 (9), grade 6 (3)

* 47 patients: grade 2 (3 patients), grade 3 (22), grade 4 (15), grade 5 (4), grade 6 (3)

Numbers in parentheses are the standard deviations

Serum albumin and total globulin levels

The liver function test including the serum albumin was not performed in 5 patients while an additional 39 patients did not have the serum total protein measured so the serum globulin levels for these patients could not be calculated. The mean serum albumin level of the patients at the time of diagnosis was 29.0 (± 6.9) g/l (range, 12 - 45 g/l) (n = 136). Hypoalbuminaemia (serum albumin level less than 34 g/l, the lower limit of normal in our laboratory) was present in 72.8% (99/136) of patients at diagnosis before commencement of anti-tuberculosis treatment. The serum albumin level showed a modest negative correlation with the patient's age ($r = -0.199, p = 0.02$). Patients with lymph node TB had the highest mean serum albumin level at 34.6 (±7.5) g/l and this was significantly higher than those of patients with the other forms of TB ($p = 0.038$) (Table 3). Patients who had pulmonary TB alone had a significantly lower mean serum albumin level at 27.8 (± 7.2) g/l compared to patients with the other forms of TB.

In patients with pulmonary TB with or without pleural involvement, those with chest radiographs showing more extensive lung involvement tended to have lower serum albumin levels although the differences in albumin levels of patients with the various grades of radiographic extent were not significant (Table 4). The mean serum albumin level of Mantoux test positive patients (n = 79) which was 31.0 (± 6.1) g/l, was significantly higher than the mean serum albumin level of 26.4 (± 7.1) g/l of patients who were Mantoux test negative (n = 57) ($p < 0.001$).

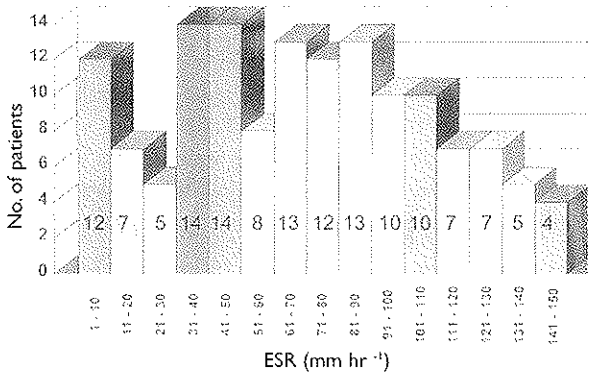

Figure 2. Distribution of ESR in patients with tuberculosis

The mean serum total globulin level of the patients before the commencement of anti-tuberculosis chemotherapy was 46.9 (± 8.5) g/l (range, 26 - 70 g/l) (n = 97). There was no significant correlation between the serum albumin and globulin levels ($r = -0.094, p = 0.362$). Hyperglobulinaemia (serum globulin above 35 g/l) was seen in 93.8% (91/97) of patients. The serum globulin level also showed a modest negative correlation with age ($r = -0.205, p = 0.044$). The serum globulin level was not affected by the form of TB (Table 3) or the extent of lung involvement in patients with pulmonary TB with or without pleural disease (Table 4). The mean serum globulin level of Mantoux test positive patients (n = 62), 47.1 (± 7.9) g/l was not

Table 5. Types of tuberculosis and Mantoux test reaction

Type of tuberculosis	Mantoux positive patients (%) (n = 84)	Mantoux negative patients (%) (n = 57)	P value
Pulmonary TB alone	37 (44%)	21 (37%)	0.497
Pulmonary TB with pleural involvement	12 (14%)	7 (12%)	0.928
Pleural TB alone	19 (23%)	12 (21%)	0.989
Lymph node TB	11 (13%)	4 (7%)	0.282
Bone and/or joint TB	1 (1%)	3 (5%)	0.303
Disseminated TB	4 (5%)	10 (18%)	0.02

Table 6. Extent of lung involvement on chest radiograph and Mantoux test reaction in patients with pulmonary tuberculosis with or without pleural involvement

Grading of extent of lung involvement on chest radiograph	Mantoux positive patients (%) (n = 52)	Mantoux negative patients (%) (n = 25)	P value
1	0	0	
2	2 (4%)	2 (8%)	0.592
3	24 (46%)	9 (36%)	0.551
4	21 (40%)	7 (28%)	0.421
5	3 (6%)	6 (24%)	0.051
6	2 (4%)	1 (4%)	0.698

significantly different from that of Mantoux test negative patients (n = 35) which was 46.6 (± 9.7) g/l (p = 0.759).

Erythrocyte sedimentation rate

The ESR distribution is shown in Figure 2. The mean ESR of the patients was 69.6 (± 38.9) mm/hr (range, 2 - 150 mm/hr). The mean ESR of male patients, 67.8 (± 39.3) mm/hr was lower than the mean ESR of female patients which was 72.4 (± 38.4) mm/hr. The difference, however, was not statistically significant (p= 0.502). The ESR was not elevated in 18 (12.8%) patients. These patients included two out of 17 who were aged 65 years and above. In patients younger than 65 years, 9 of 75 male patients and 7 of 49 female patients had normal ESR. There was no relationship between ESR and the patient's age. The ESR showed a weak negative correlation with the serum albumin level (r = -0.32, p <0.00 1) but a weak posit ive correlation with the serum globulin level (r = 0.213, p = 0.036). No differences in the ESR were observed with respect to the various forms of TB (Table 3). In the case of pulmonary TB with or without pleural involvement, the ESR of patients with grade 5 and grade 6 chest X-ray changes was higher than the ESR of those with radiologically less extensive lung involvement (Table 4) (p = 0.005). The mean ESR of Mantoux test positive patients, 67.0 (± 37.0) mm/hr, was not significantly different from that of Mantoux test negative patients, 73.4 (± 41.6) mm/hr (p = 0.339).

Discussion

This study confirms the observation of other investigators that hypoalbuminaemia (2-8) and hyperglobulinaemia (1,5) are common in patients with active TB. Hypoalbuminaemia was present in 73% of the patients and 92% of them had hyperglobulinaemia. The serum albumin level showed a negative correlation with the patient's age and in the case of pulmonary TB, a negative correlation with the radiographic extent of lung involvement. Chan *et al* (19) found that low serum albumin level is more common in patients with TB who are older than 65 years. Patients with lymph node TB in the present study had significantly higher serum albumin levels than patients with the other forms of TB. This is probably related to the fact that the patients with lymph node TB were significantly younger than the rest. In a study on pulmonary tuberculosis, (20) a negative correlation was similarly demonstrated between the serum albumin level and the radiographic extent of lung disease. Other workers (21) have also shown that patients with whole lung TB to have lower serum albumin than those with nonwhole lung TB. Deficit in the nutritional status as assessed by anthropometric measurements, hand-grip dynamometry and serum albumin in patients with pulmonary TB has been shown to increase with the radiographic extent of the disease. (3)

In patients with TB, there is a decrease in serum total protein and albumin with a corresponding increase in globulin, mainly due to an increase in gamma-globulin fraction.(5,7) Total plasma globulin consists of alpha- beta- and gamma-globulins or immunoglobulins. Serum levels of beta- and gamma-globulins, particularly IgG and IgM and acute phase proteins including alpha 1-antitrypsin and haptoglobulin are increased in active pulmonary TB.(8,22) In the present study, the serum globulin level showed a negative correlation with age. However, there was no relationship between the serum globulin level and the type of TB, the extent of lung involvement in patients with pulmonary TB, or Mantoux test reactivity. Onwubalili *et al* (1) found no correlation between cellular immunity and the levels of serum immunoglobulins in patients with active TB.

The Mantoux test response in tuberculosis has been shown to be related to the serum albumin level and the nutritional status of the patient. (9-11) Although no relationship between the serum globulin level and Mantoux test reactivity was found in the present study, hypoalbuminaemia was worse in patients who were Mantoux test negative. As the patient's nutritional status is reflected by his serum albumin level, (1,2,23,24) a higher serum albumin level would mean a better cellular immunity, delayed hypersensitivity reaction and cutaneous reactivity to tuberculin. (21) Malnutrition is a well documented cause of cutaneous anergy. (10) Non-reactors to tuberculin skin test among patients with untreated TB have been found to be more malnourished in one study.(11) The same study also showed an increase in dermal reactivity to tuberculin as abnormal nutrition-related indices improved during anti-tuberculous chemotherapy which supports the notion that tuberculin skin anergy in patients with TB may be a temporary phenomenon.(12)

In the present study, negative tuberculin skin test was seen in about 40% of patients, a proportion which is comparable to that of 30% in a study by Onwubalili and colleague (1) but much higher than that of 8% reported by Maher et *al* (12) and 16% of patients with pulmonary TB in a BCG vaccinated area reported by Hussain *et al.* (13) The acquisition of tuberculin sensitivity as a result of previous vaccination with Bacille Calmette-Guerin (BCG) makes the interpretation of tuberculin skin test more difficult. (17) As this is a retrospective study, the number of patients who had been vaccinated with BCG was not known. There is no reliable way to distinguish tuberculin reactions due to BCG vaccination from those due to natural mycobacterial infection. Tuberculin sensitivity induced by BCG vaccination often diminishes considerably over a period of years. The longer the period between vaccination and skin testing, the less likely it is that a large reaction is due to the BCG. Unless tuberculin sensitivity is maintained by repeated tuberculin testing, repeated vaccination, or repeated

mycobacterial infections, most persons vaccinated ten or more years ago are unlikely to manifest large reactions to tuberculin (17). The pathophysiology and immunology of the disease may be modulated due to vaccine intervention. However, little is known regarding the pathophysiology of tuberculosis following BCG vaccination.

As reported in previous studies, (12,25,26) TB patients who were Mantoux test negative were older than Mantoux test positive patients in the present study. Patients with disseminated TB were more likely to be non-reactive to tuberculin.

In the study by Hussain *et al,* (13) tuberculin test responsiveness as measured by the size of skin induration was found to have a negative correlation with the extent of lung involvement in pulmonary TB. Other researchers have also found tuberculin skin test anergy to be more common in TB patients with chest radiographs showing more advanced or bilateral disease or miliary changes (12, 21). On the other hand, one study has demonstrated that cellular immunity as assessed by in vitro cellular responses and cutaneous reactivity to tuberculin has no correlation with the radiological extent of pulmonary TB (1). In this study, although the proportion of pulmonary TB patients who were Mantoux test negative was higher in those with radiologically more extensive lung infiltrates compared to those with radiologically less extensive disease, the difference was not significant probably because of the small number of patients in the former category.

The ESR is usually elevated in active tuberculosis (1,13,14) but a normal value does not exclude active disease (l) as demonstrated by the present study in which about 13% of the patients had a normal ESR. Elevated ESR at diagnosis has been found by Maher *et al* (12) to be more common among tuberculin skin test anergic patients. However, the present study did not find any correlation between the ESR and Mantoux test reactivity which is similar to the finding by Onwubalili *et al.* (1) In the present study, even though the ESR tended to be higher in Mantoux test negative patients, it was not significantly different from the ESR in Mantoux positive patients. The ESR showed a negative correlation with the serum albumin level but a positive correlation with the serum globulin level. In the case of pulmonary TB, the ESR was significantly higher in patients with radiologically more extensive disease. A positive association between the radiological extent of pulmonary TB and ESR elevation was found in a study by Yanagisawa *et al .,* (27) while the study by Hussain *et al* (13) finds no such correlation. In the study by Yanagisawa *et al.,* (27) the ESR in TB patients tends to be higher in aged subjects but in this study there was no relationship between the ESR and the patient's age.

In conclusion, patients with newly diagnosed active TB frequently had hypoalbuminaemia and hyperglobulinaemia. A small proportion of patients with active TB had a normal ESR. The ESR in patients with pulmonary TB was higher in those with radiologically more extensive disease. Cutaneous response to tuberculin is more likely to be negative in patients who were older, those with more severe hypoalbuminaemia and those with disseminated TB.

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TRANSTHYRETIN GLU18, A NEW VARIANT ASSOCIATED WITH FAMILIAL AMYLOID POLYNEUROPATHY

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ABSTRACT: **Familial amyloid polyneuropathy is most commonly associated with variant plasma transthyretin (TTR) although it has been described in association with variant apolipoprotein AI and gelsolin. More than 40 TTR variants, all consisting of single amino acid substitutions distributed widely along the length of the 127 residue TTR subunit have now been described. We report here a novel TTR variant, Glu18, in a Colombian woman with TTR amyloidosis.** *(JUMMEC 1998 1&2: 54-55)*

Introduction

Familial amyloid polyneuropathy (FAP) is an adult onset autosomal dominant disease characterised by systemic amyloid deposition which most frequently involve the nerves, heart, vitreous and kidneys (1). The disease is most commonly associated with variant plasma transthyretin (TTR) and more than 40 different variants have now been identified (2). We report here a novel TTR variant in a woman with TTR amyloidosis.

Material and Methods

The proband, who was originally from Colombia, first presented age 51 years with floaters in her right eye which was unsuccessfully treated with laser therapy. The following year investigations for symptoms of heart failure revealed cardiac amyloidosis on biopsy. She then developed features of autonomic neuropathy characterised mainly by postural hypotension and diarrhoea. Although there were no symptoms referable to the peripheral nervous system, nerve conduction studies demonstrated a degree of small fibre neuropathy but no evidence for large fibre disease. Her floaters were successfully treated by excision of the vitreous opacities.

There is a strong family history of heart disease in the proband's family who remains in Colombia. Her mother died age 61 years of heart disease. Both her siblings and one maternal uncle also had heart disease. She is married but has no children.

Cardiac biopsy and the excised vitreous mass were examined by Congo red staining (3) and by immunohistochemical staining using a wide range of antibodies directed against known amyloid proteins (4). Amyloid fibrils extracted from the vitreous mass was characterised by immunoblotting with anti-TTR antibodies (5). DNA extracted and amplified from the proband's white blood cells was subjected to direct dsDNA sequencing (4). A ^{123}I-labelled SAP scan, an *in vivo* technique for the identification of systemic amyloid deposits was performed (6, 7).

Results

Amyloid deposits in the cardiac biopsy and vitreous mass were identified by Congo red staining. Immunohistochemical studies of vitreous amyloid demonstrated positive staining only with anti-TTR antibodies and the staining was abolished by absorption with pure human TTR. Immunoblot of fibril proteins extracted from the vitreous mass confirmed that the fibrils are derived from TTR. Direct dsDNA sequencing confirmed that the proband was heterozygous for a novel point mutation in exon two of the TTR gene resulting in a codon change from GAT to GAG, encoding a single amino acid (AA) substitution of aspartic acid by glutamic acid in position 18 of the mature protein (Figure). There was no evidence for significant extra-cardiac systemic amyloid deposits on scintigraphy.

Discussion

Genetic analysis revealed a new TTR variant, Glu18, in a patient with TTR amyloidosis. The genotype-phenotype relationship between TTR variant and TTR amyloidosis is well established (1, 2, 8) and this patient's clinical course is consistent with disease due to FAP. The hereditary nature of the disease is suggested by the strong family history of heart disease but unfortunately tissue was not available for analysis to demonstrate the Mendelian inheritance in this kindred.

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The TTR subunit is rich in β-sheet structure (9), a property commonly found in amyloid precursor proteins and indeed may be an important factor determining the amyloidogenicity of wild-type TTR associated with senile systemic amyloidosis. Point mutations encoding single AA substitutions appear not only to increase the amyloidogenic potential of the TTR molecule, probably by a destabilising effect on the secondary structure of the protein, but also results in disease of a different phenotype. However, not all TTR mutations are amyloidogenic (2) and amongst the 40 amyloidogenic variants, there is no obvious pattern to the AA substitutions which are widely distributed throughout the length of the protein, although there are several mutation hot spots. There is also considerable heterogeneity in the clinical spectrum of FAP, even amongst kindreds with the same mutation, suggesting that factors other than the mutations are important in determining pathogenesis in this apparently monogenic disease. The importance of these non-genetic factors is further illustrated by the finding that unlike most autosomal dominant diseases, gene dosage may not adversely influence the age of onset and severity of symptoms in FAP. Indeed there are individuals homozygous for TTR Met30 who have remained asymptomatic throughout life (10).

Our description of TTR Glu 18 adds to the increasingly heterogeneous mixture of TTR variants associated with FAP. This is also the first Colombian family reported to have the disease which most commonly affect populations in Portugal, Sweden, and Japan. However, with the increasing recognition of this disease and ready availability of molecular biological techniques, the wider population distribution of the disease will continue to be increasingly recognised and reported.

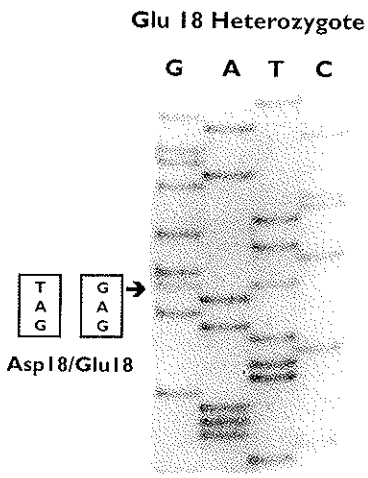

Figure 1. Nucleotide sequence of part of exon 2 of transthyretin gene of patient

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A RAPID AND SIMPLE TEMPLATE PREPARATION METHOD FOR DIRECT SEQUENCING OF RESOURCE LIMITED SPECIMENS

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Application of molecular techniques has greatly improved our ability to detect the presence of pathogenic microorganisms in patients specimens. The RT-PCR and direct sequencing of the amplification products for example has enabled enteroviruses detection from patients tissues. For direct sequencing of the PCR products, the template purity and amount are important factors which can affect the accuracy and throughput of the sequencing result. Consequently, more starting materials (DNA or RNA) are often needed for the RT-PCR because substantial amount of the amplified DNA products are lost during purification. Problem arises in dealing with clinical specimens where the resources are limited especially in cases of sudden death or specimens available are to be distributed between units and diagnostic centers. Sufficient DNA for sequencing can be obtained by cloning the initial amplified DNA products after purification into suitable cloning vectors, transforming bacteria, screening and isolating the recombinant plasmids. However, this method is laborious and time consuming. We report here application of a simple procedure to overcome this problem. Briefly, purified PCR products from the initial amplification were ligated into a suitable vector and then reamplified to obtain adequate DNA for direct sequencing. This method was successfully employed to detect the presence of Enterovirus 71(EV71) in tissues of children who died from brainstem encephalomyelitis (1).

The post-mortem tissues (~ 100 µg each of brain material from patient 1 and spinal cord from patient 2) of patients who succumbed to an acute childhood viral infection were homogenized and total RNAs were isolated using TRIzol™ Reagent (GIBCO BRL, Life Technologies Inc., USA). The reverse transcription polymerase chain reaction (RT-PCR) was performed using Access RT-PCR System (Promega, USA) and the RT-PCR parameters were 42°C for 90 minutes, 95°C for 2 minutes followed by 30 cycles of 95°C for 30 seconds, 55°C for 30 seconds, 72°C for 1 minute and final extension at 72°C for 5 minutes at the end of 30 cycles. The primers, 5' GGCGTTAGCACACTGGTATCAC 3' and 5' CTAGCTCAATAGACTCTTCGCA 3', annealed to positions 44 to 65 and 418 to 440 of the Coxsackievirus A9 genome respectively (2). The RT-PCR reagents were used following the manufacturer's protocols. Amplified

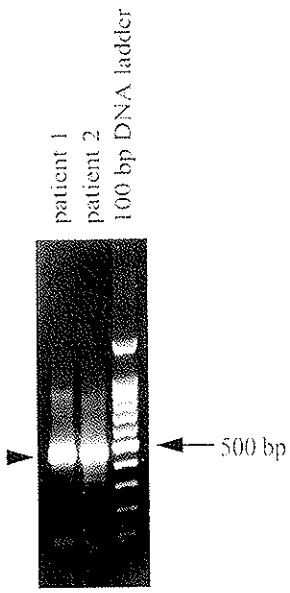

Figure 1. Amplification of enterovirus 5' UTR sequence from the ligation mix. The sequence was amplified from 1 µl of ligation mix using T7 and reverse primers. The amplified DNA fragment (8 µl) was electrophoresed in a 1.5% agarose gel. 100 bp DNA ladder was used for size indication. Arrow head indicates the amplified DNA fragments of about 460 bp.

DNA fragments (~400 bp) were separated on 1% agarose gel and purified using silica particles (3). The purified DNA fragments (4 µl) were ligated into pGEM-T PCR cloning vector (Promega, USA), which contained T7 and SP6 promoter sequences flanking the multiple cloning sites, for 16 hours at 15°C. Following day after, 1 µl of the ligation mix was used for a second step amplification using T7 universal primer and the Coxsackievirus reverse primer (Figure 1). The amplified DNA fragments (~460 bp, including 60 flanking nucleotides from the vector) were purified from agarose gel using silica particles, followed by column purification using Wizard™ PCR Preps DNA Purification System (Promega, USA) prior to sequencing. Using this method, the ligation mix could be fully utilized to prepare enough

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DNA fragments for direct sequencing (manual or automated) instead of the viral RNA which was limited. Furthermore, the use of T7 primer in the PCR enabled some of the vector sequences to be amplified together with the insert DNA and when the T7 primer was used in sequencing, full length nucleotide sequence of the gene of interest was obtained (4). In addition, the T7 primer used for sequencing was provided free by the automated sequencing service center (ACGT Inc., USA), therefore, preclude the need to synthesize expensive sequencing primers. In some cases, when sequencing were needed from the reverse end, the SP6 primer, another universal primer given free by the automated sequencing service centers was used. The method as outlined in Figure 2 is rapid and simple where only 2 days are needed in comparison to 3-5 days for cloning, transformation, and the messy screening steps. The saved viral RNA can also be used for other investigations.

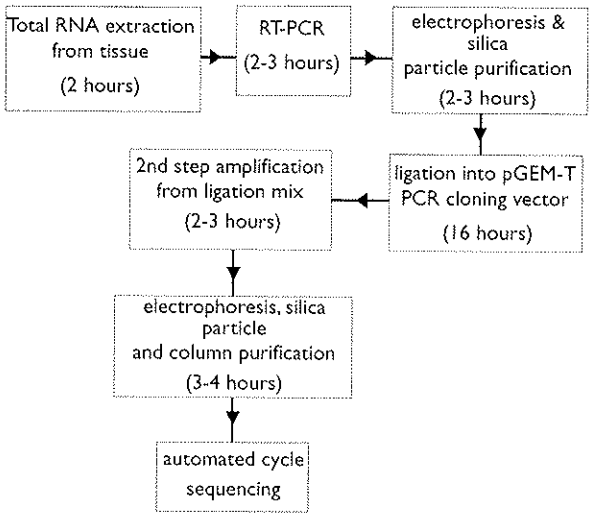

Figure 2. A rapid and simple method for preparation of template for automated cycle sequencing of resource limited specimens.

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THE YIELD OF LABORATORY INVESTIGATIONS FOR INFECTIVE AGENTS - A PILOT STUDY FINDINGS ON FOREIGN WORKERS

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SUMMARY: A total of 245 foreign workers was screened for various microbial and parasitic infections, as part of the pilot study on the health problems of foreign workers. The sample comprising of Indonesian and Bangladeshi workers, was selected on a non-probability basis from two sources, i.e. University Hospital and a private sector. This investigation revealed substantive number of workers with positive cases to some of the microbial and parasitic infections.

KEYWORDS: Pilot study, infective agents, foreign workers

The continuous influx of foreign workers into the country brings with it, its share of social economic and health problems. A pilot study was undertaken to assess the impact of foreign workers on our health system as well as to determine the prevalence rate of microbial and parasitic pathogens in these workers.

A pilot study which was clinic based and involved face to face interview was carried out in 1997. The information obtained using a structured questionnaire included data pertaining to social, demographic, environmental, medical, and recent illness. Physical examinations were also performed together with the collections of stool, venous blood, and urine specimens for microbiological, parasitological and clinical laboratory investigations.

Of the 245 subjects who participated in the study, 133 were Bangladeshi (all males) while the rest were Indonesian comprising of 84 males and 28 females. Most of the Indonesian workers (84%) were from Jawa Timur and Jambi, Sumatra, while majority of the Bangladeshis (67.7%) were from two neighbouring administrative districts of Dhaka and Chittagong. Majority of the Indonesians (50.0%) were working in service industry, while 53.5% Bangladeshis were in the manufacturing industry. One-fifth of the workers lived in squatter areas, and nearly half of them were working for the service industry.

About 70 % of the workers had at least one infection. The proportion was slightly higher among the Indonesians (72.3%) compared to the Bangladeshis (67.7%). Of the microbiological investigations, 18 (8.5%) was positive to HbSAg, 5 (2.2%) to RPR/ THPHA. and 5 (3.5%) stool positive for Salmonella. There was one positive case for HIV.

Examination for fecal pathogens revealed that 51 (31.9%), *Blastocystis hominis* 34 (21.4%) for hookworm, 23 (14.4%) for *Trichuris*, 8 (5.0%) for *Giardia*, 2 (1.3%) for *Ascaris*. Blood serology for parasitic infections showed that there were 61 (78.2%) positive for *Toxoplasma*, 60 (77.9%) for Filaria, 70 (29.8%) *Entamoeba histolytica* . 22 (9.4%) *Schistosoma*, and 13 *(5.5%) Echinococcos*.

It is of interest to point out that 40.0% had multiple infections. (Figure 1) It seemed that the proportion of multiple infections was higher (67.3%) in Bangladeshi workers compared to the Indonesian (50.9%). Thirteen subjects had 5 or more infections. A brief profile of two of these subjects are shown in the Box below.

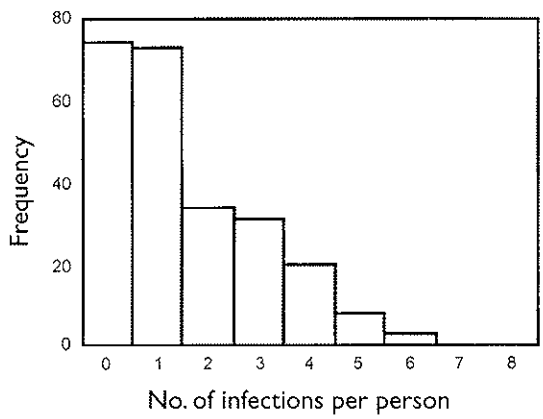

Figure 1. Distribution of number of infections per person

Case 1

GJ is a 47 year old Indonesian Muslim from Jambi province of Sumatra. He is married and stays home with both his wife and 3 children. He is employed as a janitor at the University Hospital Kuala Lumpur. He is staying in a rented house with a piped-water supply and pour latrine. He claimed that he used the same type of basic toilet and water supply facilities in Jambi.

Microbiology & Parasitology findings::

HbSAg for Hepatitis B
Ova for Hookworm, Trichuris
Blastocystis – diarrhoea causing pathogen

Serology positive for:

Amoebiasis, Filariasis, Schistosomiasis
Toxoplasmosis

Case 2

MNI is a 28 year old Bangladeshi Muslim from the administrative division of Dhaka. He is single. He works at a construction site. The "Kongsi" in which he stays has a piped-water supply and pour flush latrines. He used well water and a pour flush at the place where he comes from.

Microbiology & Parasitology findings:

HbSAg for Hepatitis B
Blastocystis – diarrhoea causing pathogen

Serology positive for:

Amoebiasis, Echinocosis, Filariasis,
Schistosomiasis, Toxoplasmosis

Conclusion

It is evident that from the pilot study, the high prevalence of microbial and parasitic pathogens these workers have, warrants attention. It is common knowledge that these workers are generally housed in cramped surroundings or over crowded dwelling and this would facilitate easy transmission of infectious organisms from one worker to another. The findings show that these workers may be prone to take leave from work as a result of symptoms that may be caused by these infections. The economic loss as a result of this and their probable dependence on our health system for recovery suggest that an extensive study be executed to assess the problems at large.

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ABSTRACTS

FOREIGN WORKERS'HEALTH: PILOT STUDY SOCIOLOGICAL ASPECTS

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The majority of migrant workers studied in this pilot survey were male, from Bangladesh and Muslim. The mean age was 30 years and the majority were aged between 21-30 years. Although almost half of them had 7-13 years of schooling, or an equivalent to secondary education, the majority were working in the Service industry, predominantly in cleaning services. It is noted that this employment trend varied from the national situation, whereby majority of legal migrant workers (Indonesian and Thai) are found in the agricultural sector. More than two thirds of the migrant workers were provided with various forms of housing by the employer. However, it is not known if such accommodation was adequate or not, as there were no questions about housing structures and extent of overcrowding. Majority of them stated that they had better amenities, such as piped drinking water and sanitary toilets, here in Malaysia compared to those in their home countries. Yet, the real extent of better sanitation is difficult to assess since verification of such amenities could not be done. From their self-reports, it appears that the majority did not engage in risk behaviours, such as smoking, alcohol and drug abuse. It is pertinent, however, to include other risk behaviours in the study, particularly the area of sexual behaviour. The pilot study yielded 28 female Indonesian migrant workers. More than two thirds of them were married. Although none of the married women reported that they were pregnant at the time of the survey, more than two thirds of them had between 1-3 children in Malaysia. Since the age range of these children would be an important indicator of the need for preventive health care, it is proposed that age range of the accompanying children and their immunisation status be included in the questionnaire. Just below half of them were currently practising family planning, and more than two thirds were using modern methods, such as, pill, Norplant and IUD. It is recommended that in addition to pregnancy and family planning information, the study could also collect data on gynaecological health and the health seeking behaviour for these problems.

CLINICAL FINDINGS IN MIGRANT WORKERS - A PILOT STUDY

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This section only examines the clinical findings and some blood chemistry in these workers. A total of 222 men and 28 women were studied. Their ages ranged from 12 to 57 years, the mean being 30.1(±7.4). Generally most of the physical examination was normal and no external features of infectious diseases were seen.

The mean systolic and diastolic blood pressure was 120(±13) and 76(±8.7) mm Hg respectively. About 8.4% of the population had elevated blood pressure of 140/90nim Hg or greater.

About 12.4% of these man and women were underweight (Body mass index (BMI) less than 19 kg/m^2) while 11.2% were either overweight or obese (BMI>25) with the mean being 21.8 (± 2.7). Only 3 had BMI greater than 30.

Three subjects had a mitral regurgitation murmur thought to be due to mitral valve prolapse. Four others had tinea cruris, six had insignificant axillary lymph-nodes, five had cervical lymph-nodes of which one was due to carcinoma of the tonsil 30 with shotty inguinal lymph-nodes which was thought to of no pathological significance. Four subjects had crepitations and five had rhonchi in their lungs.

A full blood count revealed that 16.65% of the man and 32.1% of the women had haemoglobin levels of less than 14gm/dl and 12gm/dl respectively. The most striking abnormality was the high prevalence of eosinophilia. 37% of the subjects had eosinophilia counts of greater than 450/dl.

About 19.4% of this study population had fasting blood glucose of greater than 6mmol/l but only 1.3% with fasting blood glucose of greater than 7.8mmol/l. About 22% of the urine examined revealed proteinuria but were otherwise unremarkable for the other parameters.

This group of foreign workers was made up of a presumably fairly healthy young population. Attempts to look for infectious disease on physical examination, not surprisingly did not reveal any remarkable findings. It could be that the majority of these subjects already had a examination prior to coming into

the country and another one soon after arrival. However an indirect measurement of infectious diseases via the eosinophilic count revealed a high prevalence of parasitic infestations. Attempts to examine the end results of social hardship, be it intrinsic before or appearing after arrival indirectly shoved some degree of suffering. There was a fairly high prevalence of anaemia, especially amongst the women. The body mass index also revealed this population to be generally less obese than other populations.

The value of medical check-ups has been debated, especially if it were done as a pre-employment procedure. This pilot study has shown that it is not cost-effective to do physical examination or blood chemistry and urine analysis in trying to identify infectious diseases in the migrant workers.

In the light of the paucity of clinical findings in this pilot study, it would be prudent to review the strategy for examining the health status of migrant workers. Perhaps the physical examination can be dispensed with, and blood and urine analysis be very focused and directed in order to maximise the cost-effectiveness of this programme. Certainly the high prevalence of eosinophilia needs further evaluation.

FOREIGN WORKERS STUDY MICROBIOLOGICAL INVESTIGATIONS

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During the study period, a total of 241 foreign workers were examined. The countries represented were Indonesia (103), Bangladesh (133), Myanmar (1), Pakistan (3) and others (1). The specimens collected were blood (238) and stool samples (173). The tests conducted on blood samples were for syphilis by RPR and TPHA, HIV, Hepatitis B, and from stool samples, enteric pathogens such as *Salmonella spp, Shigella spp.* and *Vibrio cholerae.*

Table I shows the type of tests performed on the various nationalities and Table 2 the results of testing. Of the 230 blood samples tested by RPR/TPHA, five were positive, one from Indonesia (1.09%) and four from Bangladesh (3.79%). There was only one sample of blood out of 238 tested which was HIV positive (0.42%) and this was in an Indonesian. Twenty three workers were found to be Hepatitis B antigen positive (9.66%), 10 out of 102 (9.80%) from Indonesia and 13 out of 131 from Bangladesh (9.92%).

As for the entric bacterial pathogens, only six out of 173 stool samples tested were positive, five for *Salmonella Spp.* and one for *Shigella sp.* Of the five positives for Salmonella, one was from Indonesia and four from Bangladesh. The single isolate of Shigella was from Pakistan.

From this preliminary study, it is obvious that hepatitis B is the most important problem among the workers from Indonesia and Bangladesh. The second of importance is venereal disease and enteric bacteria among Bangladesh workers. The other three national groups are too small to be analyzed.

It is interesting to note that although these workers are supposed to have been screened for venereal diseases, a number of them were still found to be positive. However, we are not certain that these might not have been acquired locally. There was only one case of HIV detected but if the foreign workers continue with their promiscuous lifestyle they are likely to pick up other sexually transmitted diseases including HIV and chlamydial infections. For those who were found to be stool positive for enteric pathogens, it is important to determine whether they are food-handlers as they will prove a significant risk for the spread of infections.

Originally, it was intended to test blood samples for hepatitis C and E markers since the incidence in foreign countries from which the workers come are higher. However, due to the shortage of the samples, this had to be deferred. In the light that hepatitis carriage rate is the highest for the microbes tested, it is important to include these two markers in future studies.

Table 1. Number of Tests Performed

Country	No.	RPR/TPHA	HIV	HB	Salmonella	Shigella	Vibrio
Indonesia	103	92	102	102	53	53	53
Bangladesh	133	133	131	131	115	115	115
Myanmar	1	1	1	1	1	1	1
Pakistan	3	3	3	3	3	3	3
Others	1	1	1	1	1	1	1
Total	241	230	238	238	173	173	173

Table 2. Number of Positive Samples (%)

Country	RPR/TPHA	HIV	HB	Salmonella	Shigella	Vibrio
Indonesia	1/92 (1.09)	1/102(0.98)	10/102 (9.80)	1/53 (1.69)	0/53 (0)	0/53 (0)
Bangladesh	4/133 (3.01)	0/131 (0)	13/131 (9.82)	4/115(3.48)	0/115 (0)	0/115(0)
Myanmar	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)
Pakistan	0/3 (0)	0/3 (0)	0/3 (0)	0/3 (0)	1/3(33.33)	0/3 (0)
Others	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)
Total	5/230(2.18)	1/238(0.42)	23/238(9.66)	5/173(2.89)	1/173(0.58)	0/173(0)

A CASE REPORT OF VISCERAL LEISHMANIASIS IN A FOREIGN WORKER

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We report a case of visceral leishmaniasis (kala azar) in a 28 year old Bangladeshi migrant. The patient had migrated to Malaysia 9 months prior to admission to our hospital. He was employed in a glove factory. His illness began one week prior to presentation with high swinging fever, chest pain and substantial weight loss.

On examination, he was found to be cachetic, with cervical and inguinal lymphadenopathy and massive hepatosplenomegaly.

Investigations revealed a pancytopaenia with a Hb of 9.9 g/L, WBC 3.10 x 10^9/L and a platelet count of 29 x 10^9/L. Liver function test revealed an elevated alkaline phosphatase 380 I.U./L and transaminases AST 169 I.U/L and ALT 95 I.U./L. The serum albumin was 19 g/L. Blood for malaria parasite was negative. A bone marrow examination was performed to look for LD bodies and to exclude haematological malignancies. The bone marrow examination revealed multiple LD bodies. Serology for leishmania was strongly positive.

The patient was treated with amphotericin B to a total dose of 0.6 g. There was resolution of his fever and reduction in the size of the liver and spleen at the end of therapy. There was also a steady gain in his weight.

The patient unfortunately failed to return for subsequent follow-ups.

FAECAL PATHOGENS IN FOREIGN WORKERS

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One hundred seventy three stool samples were obtained from workers from Indonesia, Bangladesh, Myanmar, Pakistan and others. The stool samples were examined for Ascaris, Trichuris, Hookworm, Schistosomes, trematodes and cestodes. The protozaon parasites included *Balantidium coli, Blastocystis* hominis, *Cyclospora cryptosporidium, Microsporidium, Entainoeba histolytica, Giardia lamblia, Iodamoeba butschilli.*

Of these 21.9%, 17% and I% of the population studied had hookworm, *Trichuris trichiura* and *Ascaris Lumbricoides* infections respectively. There was only one Indonesian reported to have Hymenolepis nana infections. The most common protozoan seen in the faecal sample is *Blastocystis hominis* (36%) followed by *Giardia lamblia* (4%). Most of the stools positive with these faecal pathogens were semi-solid especially the ones positive for the protozoan. We have also shown Blastocystis from the Indonesian workers show very small forms almost 3-5 μm in size compared to the normal size of 10~15 μm in the other nationalities. These forms show a distinct growth profile in cultures and appears to be more resistant to temperature changes than Blastocystis seen in the other two nationalities. The high incidence of Hookworm and Trichuris infections is suggestive that if these workers are left untreated their productivity will be hampered by other possible serious complications such as anaemia, weight loss, abdominal pain with diarrhoeal stools and nausea. There are increasing reports that *Blastocystis*

hominis is pathogenic. Flatulence, abdominal discomfort and the increase in the frequency of the passing watery stool has been noted in patients infected with the parasite.

Since most of the workers are generally housed in crowded rooms it is highly likely that this will facilitate transmission through the faecal-oral route of both Giardia and Blastocystis possibly increasing the incidences of these infections among workers.

PARASITIC INFECTION IN FOREIGN WORKERS: SEROLOGICAL FINDINGS

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We describe the results of serology for parasitic infection of 250 foreign workers who were seen at the University of Malaya Medical Centre, UMMC during 7-months period. The 250 foreign workers participated included 114 from Indonesia, 142 from Bangladesh, two from Myanmar and two from Pakistan. Blood samples were taken from these workers and eight tests (amoebiasis, echinococcosis, filariasis, leishmaniasis, malaria, schistosomiasis, toxoplasmosis, and trypanosomiasis) were performed on serum. Among the 250 sera tested, 92 (36.8%) were found to be positive for at least one parasitic infection. There was one case where the serum was found positive for 5 tests. The most common antibody detected in those positive sera was antibody for toxoplasma (80.%), followed by filaria (32.8%) and amoeba (30%). Other tests showed low percentage of infection with schistosomiasias, 10%; echinococcosis, 6% and malaria, 3.6%. None of the foreign workers were found positive for leishmaniasis or trypanosomiasias.

FOREIGN WORKERS STUDY: BLOOD PARASITES

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Lymphatic filariasis is endemic in Asia. The infections persist as a major cause of clinical morbidity and a significant impediment to socioeconomic development. Its prevalence is increasing world wide, largely because of rapid unplanned urbanization in many endemic areas. It is estimated that at least 120 million people are infected. In our study on foreign workers, a total of 241 day time blood samples were collected. The countries represented were Bangladesh (134), Indonesia (103), Pakistan (3) and Myanmar(l). The tests conducted on blood samples were thick blood film for microfilaria and thin blood film for malaria and quantitation of eosinophiles using the Giemsa stain. Out of the 241 blood samples tested, one was positive for *Wuchereria bancrofti* and one other was positive for malaria (*Plasmodium falciparum*) each from Bangladesh and Indonesia respectively.As for the blood eosinophiles, 39 (16.18%) blood samples showed high eosinophilia. Fifteen (6.22%) were from Banglandesh and 24 (9.96%) were from Indonesia. The Bangladeshi male who was positive for *Wuchereria bancrofti* also showed eosinophilia of 22%. We believe that some of these cases with high eosinophilia, may be positive for microfilaria. We may have missed some cases because of the methodology we chose. Lymphatic filariasis is endemic in Bangladesh and Indonesia. In Malaysia *W. brancrofti*, especially in the cities have been eliminated. However their vectors for the transmission of W. bancrofti is rampant in the cities. With the influx of immigrants with *W. bancrofti* and in relation to their occupational nature, *W. bancrofti* may eventually be introduced into the community and change the whole facet of the disease in Malaysia.

SCREENING FOR ABUSED DRUGS AMONG IMMIGRANT WORKERS

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Screening for drugs of abused were conducted by using Gas chromatography-Mass spectrometry (GCMS) fitted with capillary column. Urine samples supplied by Centre of Immigrant Studies, University of Malaya were processed upon arrival and screened for the following drugs. Amphetamines, methamphetamines, aphedrine, hydroxy-amphetamines, ecstasy, benzamphetamines, codeine, mor-

phine, heroine, cocaine and diazepam. The analysis was done using Shimadzu QP5000 Gas chromatograph-mass spectrometer by selected ion monitoring with BPX35 column, 15 m length, 0.32 ID, helium gas as carrier and quadropole mass detector at 1.60 kV electron gain.

Analysis were performed using selected ion monitoring (SIM) mode and quantitations were based on area under the curve of individual standards at various concentrations. The method is sensitive to nanogram levels and are able to detect the presence of these drugs in both urine and plasma. From this study (n=100), none of the urine were found positive for the drugs screened. The major problem encountered were adulterated with water based on the clearness and the colour of the urine. Based on this suspicion we would like to suggest that the testing be done on blood samples as this would be more confirmative and quantitative.

THE CHEST RADIOGRAPHIC CHANGES IN AN IMMIGRANT POPULATION-A PILOT STUDY

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With the increasing ease of travel and the passage of peoples between countries there is a need to ensure that the recipient country is not burdened by the need for care of immigrant with health problems as well as the increased risk posed to the local population from exposure to communicable disease. To assess the chest radiographs of a selected group of immigrants to ascertain the presence of abnormalities especially the presence of tuberculosis. A total of 250 immigrants were prospectively evaluated by a PA chest radiograph. The chest radiograph was evaluated by two radiologists for the presence of abnormalities of the heart, lungs, mediastinum and bony rib cage. There were 112 Indonesians, 133 Bangladeshis, one Myanmar, three Pakistanis and one others. Males made-up 222 while there were 28 females. The chest radiograph was diagnostic in all cases. There were 13 cases with enlarged hearts but with no evidence of heart failure. There was only a single immigrant who had evidence of active TB though there were 6 others who had evidence of old disease. There was evidence of other infections in five. With regard to the mediastinum there was a single case with enlarged hila probably secondary to increased cardiac output. There were 21 patients with scoliosis of the spine and two with abnormalities of the ribs. Even though there was a single case with evidence of TB from this pilot study, from unreported data from the UMMC, there were 15, 16 and 23 immigrants treated for TB for 1994, 1995 and 1996 respectively. This was mainly seen in the Indonesians followed by the Bangladeshis and Myanmar. We attribute this discrepancy to the biased sample in this study where probably only the healthy were seen while those who were not well did not want to participate in this study. In addition, this may also be due to the small sample used in this study. We feel that screening of the immigrants out in the field may be able to detect cases of active TB.

As for the large hearts we feel that in the absence of any cardiac symptoms and other radiological changes these are probably due to the increased workload on the heart from physical activities. This is a recognised presentation. The changes in the mediastinum and bony rib cage are probably not very significant.

PILOT DATA - WHAT DOES IT TELL?

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A pilot study on 250 foreign workers was undertaken during a 7-month period beginning December 1996. The sample of subjects mainly males (88.8%) was selected on a non-probability basis from two sources i.e. from University of Malaya (72.8%) and PEREMBA group (27.2%).

The study was a clinic-based and a face-to-face interview was carried out to elicit information on social, demographic, environmental, medical and recent illness using a structured questionnaire. Physical examinations were also performed on the same day of the interview. Subjects were also required to give their stool, venous blood, and urine specimens for microbiological, parasitological and clinical laboratory investigations. Chest X-Rays was done on all subjects.

The other investigators had already reported findings on the various specific areas of the study. In this part of the report attempt was made to relate the infectious diseases to some of the socio-environmental variables on the 112 Indonesian, 133 Bangladeshi workers. Some aspects of health seeking behaviour of these foreign workers were also presented.

Most of the Indonesian workers (84%) were from Jawa Timur and Jambi, Sumatra, while majority of the Bangladeshis (67.7%) were from two neighboring administrative districts of Dhaka and Chittagong. Majorities of the Indonesians (50.0%) were working in service industry, while 53.5% Bangladeshis were in the manufacturing. One-fifth of the workers lived in squatter areas, and nearly half of them were working for the service industry.

About 70% of the workers had at least one infection. The proportion was slightly higher among the Indonesians (72.3%) compared to the Bangladeshis (67.7%). It is of interest to point out that 40.0% had multiple infections. Thirteen had five or more infections (details for the two of the 13 cases are presented as case studies). However, the findings did not indicate any association between sanitation and infections. Risk for transmission was developed based on the number of infections in the person. The Indonesian workers carried a higher risk of transmitting the diseases (33.9%) compared to 19.5% among the Bangladeshi workers. Those working in the construction industry were at a higher risk of transmitting the diseases compared to other industries.

Slightly more than half of the workers experienced some form of minor illness or injury during the two-week period preceding the interview. Majority sought private care (43.1%), while 42.3% either self-medicate or did nothing at all. Nearly two-thirds paid out of their own pocket. Among the employers, construction sector made negligible contribution (2.9%) to the payment. It is interesting to find that 41.0% of the workers took some form of health supplements, and majority (48.4%) got it from the pharmacy or traditional sources. Nearly all (88.5%) paid on their own for the health supplements.

The findings from this pilot project need to be interpreted with some caution. However, it appears that the foreign workers carry sizeable amount of health problems. If these are not addressed quickly it may endanger the health of a nation, while we readily acknowledge their contribution towards our national development.

REVIEW OF QUESTIONNAIRES: THE SOCIO-DEMOGRAPHY AND GEOGRAPHICAL ASPECTS

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A review of the questionnaire was carried out basically to assess the relevance of the questions to the objectives of the study, to identify weaknesses of the questions particularly in terms of the wording in order to make them as clear as possible to the respondents and to minimize ambiguity and thus the problems of getting the questions across to the respondents. Based on the review a new set of questionnaire would be proposed.

The review thus focuses on two major aspects namely the structure and the content of the questionnaire. From the structural aspects each question was reviewed in terms of the language, wording, sequencing and continuity between one another. Basically, not much problems have been identified except in certain cases of ambiguity largely due to language and words used and some cases lack of continuity due to improper sequencing of the questions In terms of the content, for each questions, the purpose of asking, and what is expected of the questions was thoroughly examined and then the relevance assessed. Based on the analysis, three group of questions were identified i.e., the irrelevant questions, the partially relevant and most important non-existence of many relevant questions. It is recommended that the irrelevant questions be omitted, those partially relevant to be modified and new questions added.

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