In the year 2012, INSA initiated a program called "INSA-100 Lectures" to enable INSA Fellows to visit remote institutions, schools, colleges and Universities and deliver popular lectures that will not only deal with contemporary developments in the field but also inspire the students and teachers who are deprived of exposure to higher institutions of learning.

Frontier Lectures In Biology

By INSA Fellows

Editor S. K. Saidapur FNA

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Foreword

In the year 2012, INSA initiated a program called "INSA-100 Lectures" to enable INSA Fellows to visit remote instiutions, schools, colleges and Universities and deliver popular lectures that will not only deal with contemporary developments in the field but also inspire the students and teachers who are deprived of exposure to higher institutions of learning. The INSA generously met the travel of its fellows and also provided honorarium. All those who delivered lectures under the said scheme were also requested to submit summary or full length articles along with a video CD to INSA. The lectures are held in different areas of science by various fellows since then.

In one of the Officers' meeting of INSA an idea was generated to compile such lectures in the form of an e-book and make it available free on the INSA website to serve as supplementary reading material for students and teachers all ove the country. The President of INSA, Prof. R. Gadagkar desired that l compile the lectures held in the field of biology. I gladly agreed to do so. Then I wrote to all the speakers seeking their writeup, expanded summary or full length article in a popular format vis-a-vis the actual lecture delivered and indicate a few papers/books for additional reading in the form of "Suggested Reading". I finally received 22 articles of lectures delivered during the financial year 2012-2013 and 2013-2014 which are compiled here. I wish others also responded by submitting their articles. However, if the writeups are received in future they could be compiled in the subsequent volumes in this series.

These articles are very useful to biology students/teachers as well as to nonspecialist readers outside the realms of biological sciences. They are intended to generate interest in the subject and also provide basic information. I am thankful to INSA for giving me an opportunity of compiling and editing this e-book. I have greatly enjoyed reading the articles presented in this book. I am sure you too will enjoy reading them.

May 25, 2015 Dharwad **S. K. Saidapur** ***FNA***

Section 1: General Biology

Biology: Its Past, Present & Future

S. K. Saidapur
Former Vice Chancellor & Diamond Jubilee Professor for life
Karnatak University, Dharwad-580003
Email: saidapur@gmail.com

Scientific thinking enables man to understand the nature or universe to a great extent. The terms 'natural sciences' [1]are used to the study of natural world around us. In contrast, the 'social sciences' deal with the study of social patterns, human behaviour and so on. The natural sciences include the study of astronomy, biology, chemistry, physics, materials science, earth science (including atmospheric science and oceanography). Prior to 17th century these subjects came under '*natural philosophy*'.

The various branches of sciences except the biological sciences developed around the 17th century and great progresses were made especially in physics, and subsequently in chemical and earth sciences. However, biology could not progress much for want of instruments and technology to probe the interior of the cells and organisms. It depended on the other sciences to develop and contribute to the needed technology. This included for instance, construction of lenses, microscopes (light and electron), spectrophotometers, x-ray diffraction machine etc., to name a few. Once the technology became available, biology began making a great progress by the turn of 19th century that culminated in the birth of molecular biology by the late 20th century. In view of many expected breakthroughs in the field of basic biology and biotechnology, it is said that the 21st century belongs to biology.

Study of biology includes all forms of organisms; microbes, plants and animals – single celled to complex multi-cellular forms. All prokaryotes lack nucleus and other cell organelles. The world of *protists* is vast and diverse. These are eukaryotes but do not qualify to be called animals, plants or fungi. They live as single unicellular forms or sometimes form colonies with cells showing some differentiation and division of labour. Some protists such as *Choanoflagellates* live in colonies. Such cells are also found to surround the feeding apparatus of sponges. These are considered cousins of animals. The eukaryotes possess well organized cell organelles with division of labour. The unicellular forms are also complex; these carry out all functions in a very effective way. However, a multi-cellular organization is much more complex. A primitive form of multi-cellular organization is depicted in some unicellular forms by way of colony formation (e.g. Volvox). The total biodiversity is very vast with large number of forms in each category of microbes, plants and animals; thousands of species are still waiting to be discovered!

Lecture delivered on November 15, 2013 at University of Sikkim, Gangtok, Sikkim

The biology stands on four major pillars:

- The Cell Theory:
- Theory of Evolution
- Mendel's principles of Inheritance
- Blueprint of Life – the structure of DNA

A complex multi-cellular organization involves differentiation of cells in to specific tissues and organs. This is achieved by selective gene activation / inactivation during development. Yet all cells in a body have the same DNA, the blue print. It is very important that in animals the differentiated cells, tissues and organs remember their new role and function faithfully. When these cells divide, they pass on the memory to their progeny. Thus 'cell memory' is an important phenomenon. During the production of various organs a few cells remain undifferentiated. These are called 'stem cells' which are capable of differentiating into any kind cell/tissue and can be used to produce desired organs. This is called 'tissue engineering'

It should be noted that the DNA embedded safely in the form of chromosomes in eukaryotes represents the blueprint of a given organism. The various genes located on the DNA determine form and function of a given individual. In other words, we can distinguish '*developmental genes and housekeeping genes*'. The former regulate developmental processes (tissue differentiation and development of organs), and the latter, regulate routine activities of the cells. Through transcription and translation processes the structural and functional proteins are produced in tissue specific manner. Beyond transcription, the processes are essentially under epigenetic control. However, these control mechanisms are not well known. It is also to be noted that nature and nurture play a key role in the life of organisms. This is evident from the experiments on agouti mice which show that exposure to high methyl donors before conception can switch off certain genes and prevent their expression. Many foods have methyl donors and therefore have great implications in humans as well. Hence, in recent years interest in the epigenetic control mechanisms have attracted considerable interest of researchers i.e. importance of *nature and nurture*!

A multi-cellular organization calls for coordination of all bodily functions i.e. *physiological coordination*. This is chiefly accomplished by two means; chemical integration and nervous integration. Both these processes are mutually inclusive and work for the common good of the individual and constitute components of physiological integration.

In the life of all living beings, microbes to man, environment plays a key role in shaping them. All organisms are affected by both abiotic and biotic factors of the environment in which they live. Furthermore, the environment rarely remains constant. Therefore, changing environmental conditions pose challenges to organisms. In this process, organisms that can deal with the new challenges because of certain useful traits (genes) survive and, others die. Over a period of time, this leads to changes in the structure of populations. The process is called '*natural selection*' and this leads to evolution, speciation and sometimes extinction. Darwin and Wallace (1958) suggested that *natural selection* is the main mechanism of evolution. They did not know genetics or molecular biology. Today we know that meiosis

(crossing over), mutation, genetic drift produce variations upon which natural selection acts. The famous evolutionary geneticist, Theodosius Dobzhansky summarized the importance of evolution by stating "Nothing in biology makes sense except in the light of evolution". Today, all biological phenomena are being studied in light of evolutionary biology. The ideas derived from the principles of evolution have helped us understand not only the impact of evolution on organisms but also its impact on the health and diseases of man.

In summary, development of biological sciences was slow and took time compared to other branches of sciences; made rapid progresses from the turn of 19th century and more so by the end of the last century. The elucidation of DNA structure laid foundation for the birth of molecular biology culminating in the successful completion of Human Genome Project. The *in vitro* fertilization, cloning, genetic engineering and many biotechnological innovations reflect rapid progress of biology in the last century. The 21st century belongs to biology and great breakthroughs are expected especially in the area of tissue engineering (stem cell biology), neurobiology and so on. At the same time many challenges are also expected. Human longevity will increase. This will bring problems of the old and ethical concerns. Healthcare facilities will rise. Microbes and pest will offer more challenges by evolving more and more virulence as well as drug resistance. Biodiversity loss and pollution will pose serious problems. In view of the many expected breakthroughs, challenges and opportunities, a career in biology is certain to be both exciting and rewarding.

Suggested Readings:

1. Life Evolving by C. de Duve 2002.

2. Biology by N. Campbell and J, Reece, 7th Edition, 2004.

Discoveries leading to Innovations For Mankind

V P.Kamboj
Central Drug Research Institute and Biotech Park, Lucknow
Biotech Consortium India Ltd., New Delhi
Email: kambojvp@yahoo.com

Discoveries in science and technology and the consequential innovations have guided the path of evolution and development of humanity and every facet of our life. These have led to food security, health security, energy security, shelter security and environment security. The developments in communication namely road, rail, air, space and information technology have been revolutionary. In a wink, messages can be exchanged; as is believed in mythology that the saints after closing their eyes used to reach from one place to the other.

In essence, discoveries play a significant role in the excitement of science. These have led to many innovations for mankind. The innovations have been seen both in basic and applied sciences. The innovations have contributed immensely to human welfare and societal development in all spheres of life. The discoveries in each area have many facets.

Intuition plays a great role in discovering something new and innovative. The great scientist Albert Einstein said: "The only real valuable thing is intuition". He further added: "The intellect has little to do on the road to discovery. There comes a leap in consciousness, call it intuition or what you will, the solution comes to you and you don't know how or why" is not known. There is no scientific theory that can explain or predict the characteristics of intuition. Nevertheless, many great scientific discoveries relied heavily on intuitive insights. The connection between intellect and intuition is one of the great mysteries of life. Albert Einstein believed that intuition played a dominant role in his life.

The discoveries come through love for science, devotion and dedication to science, dreaming science, hard work, keen sense of observation and looking for the dissimilarities or outliers in your data rather than the similarities. Chance has played a major role in discoveries and development of science. Louis Pasteur has rightly said that "Chance favours the prepared mind". A well-known physicist Joseph Henry has said that "The seeds of great discoveries are constantly floating around us but they only take root in minds well prepared to receive them". The discoveries have led to innovations useful for human and have come primarily through serendipity or accidentally with keen sense of observation, visualized through dreams, 'jugaad' or hit and trial to meet human needs and lastly well planned, designed and targeted; the last one in the area of drug development with which I am more familiar. I will give a few examples of each type to generate excitement.

Lecture delivered on March 8, 2013 at Pilingkata High School, Basistha at border of Assam and Meghalaya

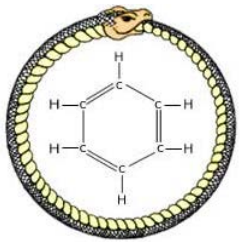
Discovery through dreams

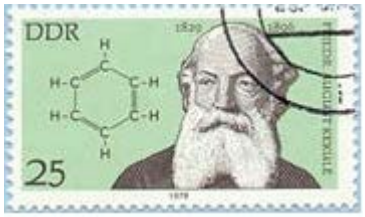
Sigmund Freud has said that "The interpretation of dreams is the royal road to a knowledge of the unconscious activities of the mind" and to discover the unknown. The undermentioned discoveries are through interpretation of dreams.

All of us know that the great Indian mathematician Srinivasa Ramanujan Iyengar (popularly known as Ramanujan) was a genius in numbers and a dreamer with no formal training in pure mathematics. He could not pass high school because of his passion for mathematics; he passed with distinction in maths but failed in other subjects. He got a clerks position in accounts department to support him. His work, at that time, was not understood and/or appreciated by mathematicians of that time in India. He had faith and self-confidence in what he was doing. So he didn't lose heart but sent some of his equations and their solution to Prof. G.H. Hardy of Cambridge University in U.K. and two other mathematicians in UK. Dr Hardy understood the great thinking behind these equations. He invited Ramanujan to his University in 1914. During his stay in Cambridge, he produced startling results, proved over 3000 theorems in 5 years, earned Ph.D. and was elected FRS for these contributions to mathematics. Ramanujan said that inspiration and insight for his work many times came to him in dreams—A Hindu goddess Mahalakshmi, of Namakkal/Namagiri would appear and present mathematical formulae which he would verify after waking up. The dreams often repeated and the connection with the dream world as a source for his work was constant throughout his life.

Ramanujan describes one of his dreams of mathematical discovery: "While asleep I had an unusual experience. There was a red screen formed by flowing blood as it were. I was observing it. Suddenly a hand began to write on the screen. I became all attention. That hand wrote a number of results in elliptic integrals. They stuck to my mind. As soon as I woke up, I committed them to writing." This mathematical wizard developed tuberculosis in UK and returned to India in 1919 to recover. But died young at the age of 32 years, in April, 1920.

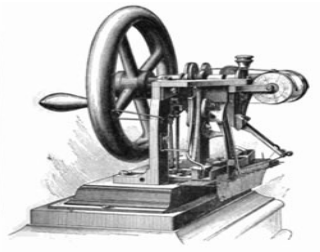
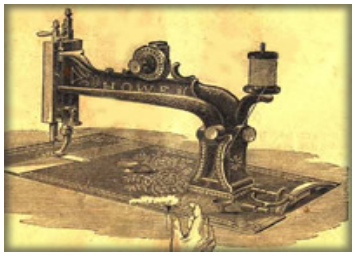
Discovery of the structure of benzene that has revolutionized organic chemistry is another discovery through dream. Dr. Frederick Kekule (1865) saw in his dream a number of



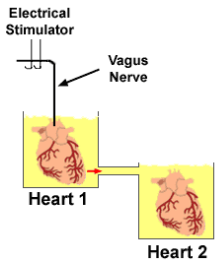


atoms were moving back and forth and sometimes appeared as a coiled snake biting its tail. The structure of benzene molecule was discovered after an intuitive dream, lots of analytical thinking on the understanding of molecules and that the molecular structure was characterized by a ring of carbon atoms. He thus realized that the molecular structure of benzene was a 6-membered ring of carbon atoms and 6 hydrogen atoms with the carbon-carbon bonds arranged alternatively as single and double. This could happen to a mind which is deeply engrossed in a problem, thinks differently and has the ability to understand and decipher a dream.

The discovery of sewing machine by Elias Howe (1845) is another great discovery through a dream which has revolutionized the cloth industry. Howe had the idea of a machine with a needle which would go through a piece of cloth but how it would work to pick up the thread was not working. He designed a needle with two narrow ends and a whole in the middle thick region but it didn't work. He saw a dream of a cruel native king who will kill him if does not develop the sewing machine. He saw the king has sent native soldiers who were dancing with spears in their hands and each spear had a whole near the tip. He woke up and realized that the dream had provided solution to his problem. He changed the design of needle with a whole near the tip and it worked to pick a thread from underneath after it went through cloth thus making his machine operable.

A German physiologist Otte Leewi had an idea in 1903 that there may be chemical transmission of the nervous impulse rather than the hereto believed to be an electric one. Seventeen years later he had a dream, jotted it but could not decipher. The next night hesaw the design of an experiment to determine whether or not the hypothesis of chemical transmission was true. He got up, went to his lab and performed an experiment on frog's heart as per his nocturnal dream. He used vagus nerve to stimulate frog's heart in saline solution. He took another ten years to conclusively prove it. The result of his dream-induced experiment became the foundation of chemical transmission of the nervous impulse and for which he was awarded the noble prize in medicine in 1938.

Discoveries by chance

Accidental discoveries or serendipity has played a major role in the development of science but we should not forget the famous quote of Louis Pasteur "Chance favours the prepared mind" or as Frans Johansson writes in book "Capitalizing the click moment" that you should have sense of interconnectivity of events and of thinking differently to create opportunities for serendipitous or accidental discovery

Edward Jenner in 1798 observed that milkmaids who have suffered from cow pox never had small pox in their life. An idea struck to him that if he could inoculate patients suffering from small pox with cow pox and whether they would be saved from the deadly small pox. Thus he inoculated small pox patients with scabs or pus taken from cow pox lesions His observation, idea and intuition proved to be true and the incidence of tuberculosis was reduced from 20% to 2%. Thus he accidentally

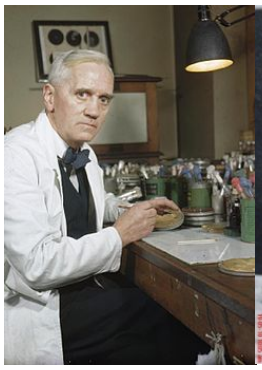
discovered the concept to design the small pox vaccine.

It was in the nineteenth century that cow pox virus was replaced with vaccinia virus for vaccination. However the stability of this type of vaccine at room temperature was around 1-2 days and thus only fresh material had to be used every time. In 1940s, Leslie Collier added a heat stable protein, peptone, which led to the production of stable small pox vaccine. The use of this stable vaccine, under the aegies of WHO from 1967 to 1977, resulted in the eradication of small pox globally.

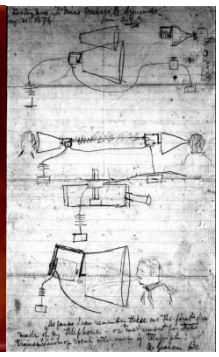
The discovery of attenuation by Louis Pasteur (1882, 1885) led to the discovery of live vaccines. He was working on chicken cholera and in disgust left a culture of chicken cholera causing *Pasteurela multicida* on bench while going for summer vacation. On return he injected

dried culture into chickens and found that it did not cause disease. Fresh cultureinjection to these chickens also did not cause disease whereas to another batch did. Pasteur thought that heat exposure has probably killed the bacteria and that is why instead of causing the disease they protected it from the disease. Thus he hypothesized that pathogen could be attenuated on exposure to insults such as high temperature, oxygen and chemicals and confirmed it by studies on anthrax and rabies. This chance observation led to live attenuated vaccines for the management/control of a number of diseases.

The discovery of Penicillin by Alexander Fleming in 1928 is another exciting example of chance discovery. Fleming in 1922 was suffering from cold and he made a culture from his own nasal secretions. The culture dish was filled with yellow bacteria and a tear fell from his



eye into the dish. Next day he found a clear space in the culture where the tear hadfallen. His insight led him to conclude that tear contained a substance that did not permit the bacteria to grow. Fleming in 1928 was conducting research on influenza and noticed that one dish had an unusual clear area where a bit of mould was placed. Remembering the tear experience, he concluded that mould did produce a substance that was deadly to the staphylococcus bacteria in the dish. Penicillin was isolated from this mould. Several years later in 1939, Ernest Chain and Howard Florey developed a way to isolate penicillin and determined its structure. It was used to treat bacterial infections during the Second World War. The new drug discovered by chance came into clinical usage in 1946 and made a huge impact on public health. Fleming, Chain and

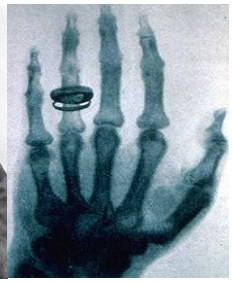


Florey were awarded the noble prize in 1945 for this discovery. John Sheehan synthesized penicillin in 1957 and since 1982 it is produced by recombinant technology.

Alexander Graham Bell was interested in developing a speaking telegraph and designed a number of experiments to achieve this objective. His aim was transmission of sound by

electricity and had made many devices using multi-reeds. In 1875 he along with his assistant Watson built an electro-mechanical ear for creating a speaking telegraph.Watson was transmitting telegraph signals when one metallic reed got struck. He plucked to break it free. Bell came rushing to his room to see what Watson was doing because he had heard a strong signal from the receiving reed in the other room. The signal was so strong that he could use this simple device to transmit speech and thus saw the birth of telephone. Thus this simple observation of noise from the receiving reed led by chance to the discovery of telephone and its latest version mobile has revolutionized communication and thereby world appears to be a small village.

The discovery of X-Rays by Wilhelm Roentgen in 1895 has revolutionized diagnosis in medical sciences. Before this discovery he was working for quite some time on cathode rays before discovery of X-rays. He was passing electric current through gases at extremely low temperature. Roentgen in 1895 experimenting with electric current flowing through gas filled tube, called a cathode ray tube noticed a strong glow coming from a little fluorescent screen (barium platinocyanide screen), which had been lying on a bench more than a meter away. He



speculated that a new kind of ray may be responsible and called it X-rays; some called them Roentgen rays. He put his hand between the tube and the screen and wassurprised to see a clear image of the bones of his hand. They appeared a dark shadow within the shadowy image of the hand itself. Excited with this he asked his wife to place her hand between the tube and screen and took a picture on a photographic plate using his photographic skills. He was excited to see the hand bones and they were not seen at the place where marriage ring was present. He was awarded the first ever noble prize in physics in 1901.

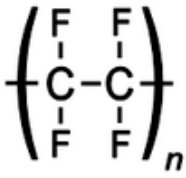
Alfred Nobel was working to develop dynamite (an explosive material). At that time, a highly explosive and unsafe material, nitro-glycerine was used for this purpose. He lost some family members because of unanticipated explosions while experimenting. One day sitting outside his lab in a chair he noticed some amount of nitro-glycerine leaking from a truck and falling on the ground. He found that so formed mixture of clay and nitro-glycerine had explosive properties and was safe to handle. First he called it "Noble's safety powder" and later

on dynamite. Thus he developed dynamite i.e. nitro-glycerine absorbed in keiselguhr mud (siliceous or diatomaceous earth; specifically porous infusorial earth). Dynamite is three parts glycerine and one part keiselguhr mud and he patented this discovery in 1867. Thus the unsafe and difficult to handle explosive nitro-glycerine was replaced with, by chance discovered, safe explosive dynamite. Later on, he combined nitro-glycerine with a number of other nitrocellulose substances but finally settled with potassium nitrate to obtain a transparent jelly-like substance, more powerful explosive than dynamite, and named it "Gelginitemor blasting jelly"; patented in 1876. He was an armaments manufacturer.

"On 27 November 1895, at the Swedish-Norwegian Club in Paris, Nobel signed his last will and testament and set aside the bulk of his estate to establish the Nobel Prizes, to be awarded annually without distinction of nationality". The first four prizes are in physical science, chemistry, medical science, literary work "in an ideal direction" and the fifth is to be

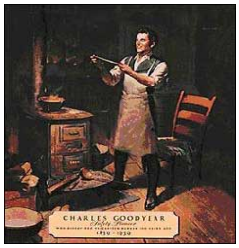
given to a person or society who has done greatest service to the cause of international fraternity. He died on 10th December, 1896. The first Noble prizes were awarded posthumously in 1901 from the interest of his estate and are being continued till now.

Roy Plunckett was just 27 years of age and for only 2 years as a chemist at Du Pont was trying to make a new type of Freon or CFCs refrigerant used as a gas in refrigerators and air conditioners; this gas is the main culprit in depleting atmosphere's ozone layer. So he started working on tetrafluorodichloroethane which was the commonly used refrigerant when Plunckett started working. He hypothesized to work with tetrafluoroethylene or tfe which on reaction with hydrochloric acid will yield the desired compound. He wanted to make about 100 lbs and store in metal cans so that he can do his experiments. He placed can on dry ice so that tfe will liquefy at low pressure inside the can. He was surprised to see that liquefied gas did not come out as happened previously. He thought that something has gone wrong. On cutting open the container found that instead of gas there were white flakes on the side of the cans. He thought and concluded the next day that tetrafluoroethylene against his expectations had polymerized when carried in an iron pressurized container. In this reaction, iron from inside the container acted as a catalyst. Thus in 1938 developed accidentally polytetrafluoroethylene, PTFE or Teflon, the most slippery material known. In contrast, a few months earlier in the same year Nylon was developed as a well-planned material by the same company.



In 1954, Marc Gregoire a French engineer was discussing the properties of Teflon with his wife and she suggested to him to make a pan using this highly slippery material and which he did. Thus the first Tefal (Teflon on aluminium) coated non-resin stick pan was made. Now a number of cookery material coated with Teflon are available.

Percy LaBaron Spencer was working as usual in his lab in 1945. He walked past a radar tube (magnetron) and noticed that the chocolate bar in his pocket melted. He realizedthat he might be on to a hot new product. Then he placed a small bowel of corn in front of the tube and popcorns quickly popped all over the room. Then he tried a number of foods for heating. Thus by accident was discovered microwave which has now made life comfortable for millions of people.



Charles Goodyear was experimenting to make rubber a useful product that would not melt on heat or become brittle and stiff in cold. He treated it with a number of substances but none worked. In one winter night in 1839, while playing with rubber in his drawing room he accidentally dropped a piece of rubber sprinkled with sulphur onto a red hot stove. He observed that instead of melting rubber flattened out into a small disk. He lifted it from the stove and found it was still flexible and strong. He then hung this rubber disk on his door post overnight in a cold winter night. On the next morning the disk still had rubber-like

qualities. Thus Goodyear accidentally invented vulcanization i.e. the process of heating rubber with sulphur to give it elasticity, hardness and strength.

William Perkins in 1856 at the age of 18 years was trying to synthesize quinine which is isolated from the bark of Cinchona tree and is used to treat malaria, as per the challenge of his teacher in a class lecture. He built a small lab in his house during summer vacation and started synthesis of quinine. He got a brown sludge on oxidizing toluidine and a black one on oxidizing aniline instead of the expected product. He washed the flask containing black sludge with water and was surprised to see purple coloured water. He found it to dye cloth. Then he developed a method to extract the purple dye from black product.

Perkins patented the product, opened a factory and became a millionaire. This is how accidently the dye and cloth printing industry developed.

It is not only that the luck smiled only on scientists who were deeply involved in a problem. Earlier it had been mentioned that intuition, deep sense of observation, thinking unusual and the will to achieve something does play a role in discovering something new and useful. The next two innovations come in this category.

Frank Epperson in 1905 at 11 years of age invented Popsicle. He kept it as a secret for 18 years and then patented it as Popsicle. Epperson made a fruit flavoured soda drink from powder (a concoction used at that time) and water. People drinking left some of it in a glass. And he placed a glass containing liquid with stir stick outside on porch. The night temperature reached a record low. Next morning, he discovered that it had frozen to the stir stick creating a fruit flavoured ice treat and named it eppsicle. He took it out of the glass and licked and tasted superbly. He sold it as frozen lollipops to public. It was an instant success and made more flavoured ones. His children didn't like this name and they agreed to name it popsicle. Thus this accidental discovery was made by an observant mind.

Another exciting million dollar discovery was made by a restaurant cook cum waiter name George Crum. He was selling fried cut pieces of potatoes for eating. These potato pieces were too thick and soggy and the customers used to complain constantly. Having fed up with these complaints, one day he decided to slice potatoes so thin that they could not be eaten with fork. The chips turned out to be crisp and the customers against expectations were ecstatic about the new chips. These chips became highly popular and he in 1853 named them as "Sartoga chips". This is how by accident and sheer determination and to meet customer needs came potato chips which are enjoyed by millions of children globally.

Innovations by 'Jugaad (Hit & Trial)

These inventions have primarily been made by ordinary people who were thinkers and designed things to meet community needs. Over 10,000 inventions have been recorded and there could be many more and I will give a few examples of Indian ones. Remya Jones from,

Kerala, India developed a pedal powered washing machine as there was no electricity in her village. Her mother fell sick and she had to wash the entire laundry of her home by hand. This affected her studies. Thus by creativity she developed a pedal driven washing machine so that she could study by sitting on a cycle to paddle it and simultaneously wash her house laundry.

Dr. Sathya Jaganathan, a paediatrician in a rural hospital, Chengalpattu Government Medical College, in South India was concerned by high infant mortality in her hospital. She thought and created a minimalist incubator from a wooden table made of locally harvested wood, a Plexiglas top and a standard 100 watt light bulb to provide heat. She saved 50% of the babies by this device costing Rs. 5,000 as compared to Rs. 10 lakhs of imported machine.

Indra Nooyi of PepsiCo India due to scarcity of labour and water introduced "Direct Seeding" of rice paddies in Punjab in 2004 for water use efficiency instead of the traditionally used method of sowing rice seed in small nursery where they germinate into seedlings. These seedlings are then transplanted into main field and grown in 4-5 inches of water at the base of crop for 6-8 weeks mainly to prevent weed growing. The direct seeding avoids the water-intensive operation of pudding and pedalling, transplanting and growing in standing water thereby saving about 30% water in paddy cultivation of say 900kl (2,38,000 gallons) of water per acre. This method also cuts green gas emissions by 70%, improves product output and provides access to safe water. The direct seeding therefore helps farmers to increase/ maintain yield and quality, reduce water input and save time. PepsiCo India redesigned peanut planter for direct seeding of rice seeds. In 2010, through direct seeding in three states of India had saved more than 700 crore litres of water; over 12,000 acres of land had been planted by direct seeding in 2012.

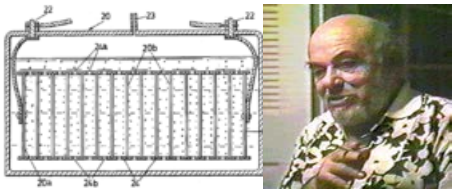
Discoveries by Planning & Experimentation

Drug discovery

The new drug discovery has been on the decline since the use of molecular modelling, QSAR, bio informatics, genomics, metabolomics, combichem and high throughput screening and these techniques have not so far helped in tailor-made drug discovery. This could be due to the fact that drugs for simpler diseases have already been discovered and better understanding of the pathology of the complicated diseases such as metabolic, degenerative and genetic disorders will help discover more new drugs each year. Earlier in the decade from 1980-1990, on an average about 50 new chemical entities were introduced each year which were reduced to 45 from 1990-2000, about 25 from 2001 to 2006 and 21 thereafter; luckily over 35 drugs each were introduced during 2012 & 2013. Serendipity has played a major role in drug discovery and about 64% of the leads have come from plant sources. Thus far only 2 drugs have been discovered by drug designing. They include Captopril, an ACE inhibitor for cardiovascular disorders and, Saquinavir, a protease inhibitor for HIV. However, I am confident that with the better understanding of pathology of diseases, drug targets and ligand target interaction, more new drugs will be developed.

Most exciting discovery in Energy

The most exciting discovery of the century in my opinion was made by Yull Brown in 1974. It was believed that you can burn water and he proved it to be true. Water is made up of hydrogen and oxygen and can easily be separated into its constituents. However, when recombined releases tremendous amount of energy and controlling it is the key. Brown developed a machine to convert plain water to a stoichiometric mixture of hydrogen and oxygen (i.e. 2:1) without causing an explosion. Thus he discovered another state of water besides ice, water or steam and named it Brown's gas; one litre of water makes 1,860 litres of Brown's gas. It does not explode if spark is added but Brown's gas implodes. When it explodes it makes a unique welder which can weld glass to metal, metal to brick or say just about anything to anything. The unique property of Brown's gas relates to the fact that it reacts molecularly with whatever material it approaches and temperature of the flame adjusts accordingly. The temperature of the flame when not in use is 259-279^{o} F. This machine welds aluminium at 900^{0} F, braises copper at 1800^{0} F, sublimates tungsten at 13, 000 ^{o} F and can achieve the same temperature as sun i.e. 8, 000 ^{o} C. In short, Brown's gas welder converts ordinary water into fuel for welding not by explosion but by series of implosions and the end product is water and thus no pollution. This gas can be used to draw water by causing instant vacuum, pump water without mechanical pump and power internal combustion engines. This discovery when put to use after optimization will solve the much wanted problem of energy.



Raman Scatter – Multi-area utility discovery

I will conclude this write up with the superbly planned, well designed and experimentally proved discovery of the first Indian Noble Laureate in Science, Sir C.V. Raman. He observed diffraction bands using a lab spectrometer in 1904 while studying for his BA degree. After completing his MA degree he joined Indian Finance service at Kolkata. After office hours he will do experiments at the Indian Association for the cultivation of Science. In 1919 he resigned from Accounts service and joined Kolkata University at the invitation of its Vice-Chancellor Prof. Ashutosh Mukherjee at half the salary. Raman wanted to do science in India and was against going abroad. However, Prof. Mukherjee motivated him to attend the University Science Congress in Oxford, UK, in 1921. On his return voyage to India, he conducted experiments on the ship and demonstrated that blueness of the sea water is due to molecular scattering by the water of the ocean. In 1922, he began studies on scattering of light by liquids. He demonstrated that ray of incident light excites molecules in the object which then scatter the light. Most of the scattered light is of the same wave length as incident light but some is scattered at a different wave length. This inelastically scattered light is called "Raman Scatter" which results from molecule changing its molecular motion. He continued these studies and announced his discovery "change in frequency of monochromatic light after scattering through a medium" known as "Raman Effect" on 28th February, 1928 which earned him the Noble Prize in 1930. He was so confident that this discovery will get him a Noble prize that he along with his wife reached Sweden before the announcement of Noble Prize. The Raman spectrometer which he designed has been upgraded and miniaturized and the Mass

Micro-beam Raman Spectrometer excels at identifying minerals, trace amounts of organic substances or even identify biological substances such as proteins, DNA and amino acids.

I have not covered discoveries in other areas of science because of ignorance, lack of knowledge and understanding. My aim has been to cover well known discoveries in different areas primarily by unusual methods to excite you in science and to know that some of them have been discovered by not well read persons. It is evident that intuition and observation have played a great role in all of them.

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Growth in Childhood and Adolescence

K. N. Agarwal
President Heath Care and Research Association for Adolescents & Formerly
Professor Pediatrics, University College of Medical Sciences&GTBH, Delhi
E-mail: adolcare@hotmail.com

Firstly let us discuss how we grow and methods to assess that the growth progress corresponds to our age.

Definition

Growth is a continuous process commencing at conception and progressing at a varying pace till its completion about 2 decades later, with closure of epiphysis. The process of 'Growth' is accompanied with increase in body size and or mass at varying rates. It is multi factorial and complex, still remarkably predictable. Boys and girls grow differently and each child has his or her distinct growth pattern.

To summarize:

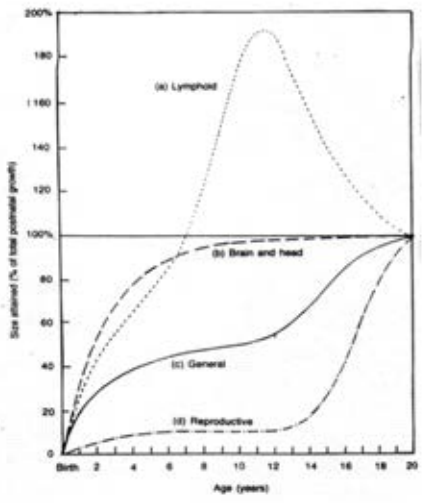
- Growth is a fundamental characteristic of childhood
- Despite being influenced by many factors, it remains remarkably predictable
- Normal growth is an indicator of optimum health
- Deviation from the normal pattern is indicative of a pathological process
- Periodic assessment facilitates early detection of growth faltering which may be the first manifestation of under/over nutrition, infection /disease.

Our body grows with varying velocities and period for different systems see the figure and description below.

In Fig 1 below depicts four important growth components a) **General Body** Growth; b)**Brain** and head; c) **Lymphoid** (immunity GENERAL , and d)**Reproductive** are shown in relation to age.

Lecture delivered on September 1-3, 2013 at Govt. School, Pilibhit and Barielly

Human Growth Pattern



Growth pattern of different body systems

- **General body growth-** *During 1st year of life 25 cm; 2nd year = 12.5* cm; 3rd year = 7.5-10 cm.
- 7cm/year at 3-4 yrs. 5 yr onwards= 5cm/yr until puberty.
- **Brain growth**-At birth, the brain of the infant is 25% of the adult size. At the age of one year, the brain has grown to 75% of its adult size and to 80% by age three, reaching 90% by age seven.
- **Lymphoid tissue-** during middle childhood maximum 8-9 yr. reaching > 180%.
- **Reproductive organs-** puberty by 9-11 yr in girls and 11-14 yr in boys..

The importance of growth monitoring

- **Measurement of Height and Head circumference**
  - Height is the best index of measuring linear growth (stature) as height reflects growth over a longer period than does weight.- *During 1st year of life 25 cm; 2nd year = 12.5* cm; 3rd year = 7.5-10 cm; and 7cm/year at 3-4 yrs, and 5 yr onwards= 5cm/yr until puberty.
  - Head circumference can be used to assess brain growth in children mainly under 2 years, as during this period brain growth is very rapid (*at birth, the brain of the infant is 25% of the adult size. At the age of one year, the brain has grown to 75% of its adult size and to 80% by age three, reaching 90% by age seven).*

B. Weight in growth monitoring

Weight-for-age is usually used to monitor growth. It is particularly useful in small infants who normally gain weight fast. Normal weight gain suggests that the infant is healthy and growing normally. Failure to gain weight normally is often the earliest sign of illness or malnutrition (i.e. under nutrition). Normally weight doubles by 5-6 months, trebles on first birth day and becomes four times of the birth weight at 2 year of age.

Growth monitoring is the regular measurement of a child's size in terms of length, weight and brain size as head circumference in order to document growth with age. The child's size measurements must then be plotted on a growth chart. This is extremely important as it can detect early changes in a child's growth. Both growing too slowly or too fast may indicate a health problem.

Growth charts (curves) are used to measure growth. The distance curve is a measure of size over time; it records height /weight as a function of age and gets higher with age (see growth curves for head circumference, height, weight and BMI). Growth charts consist of a series of percentile curves that illustrate the distribution of selected body measurements in children. Pediatricians, nurses, and parents use these to track the growth of infants, children, and adolescents.

Percentiles (centile) describe the frequency distribution of anthropometric parameters like weight height, skull circumference, BMI etc. 50^{th} percentile is the average (median) line for the given population. Describes the % of children expected to be on or below that line e.g. 50^{th} centile means that 49% of the observations are below & 50% above that observation. A child's growth parameters may be on the centile line or between two centile lines. **Conventionally, for all parameters, 3^{rd} and 97^{th} percentiles are the lowest and highest 94% of observations.**

- *Any child with parameters below or above these limits or those who cross percentiles after 2 years of age needs careful evaluation.*
- **Examples**: **a)** If height and weight consistently are on the 60th percentile line until a child is 5 years old, then the height has dropped to the 30^{th} percentile at age 6, that might indicate that there's a growth problem(catch down – retardation of growth) because the child is not following his or her previous growth pattern- this indicates disease.

  b) Boy with height in 40th percentile and weight in the 85th percentile. (he is taller than 40% of kids his age, but weighs more than 85% of kids his age.) There might be a health problem (overwight/obesity). On the other hand, if he's in the 85th percentile for height and weight and follows that pattern consistently over time, that usually means that he is a normal child, just larger than average.

  The growth curves developed by Agarwal et al 1992, 1994, 2001 are to be followed for regular growth assessment.

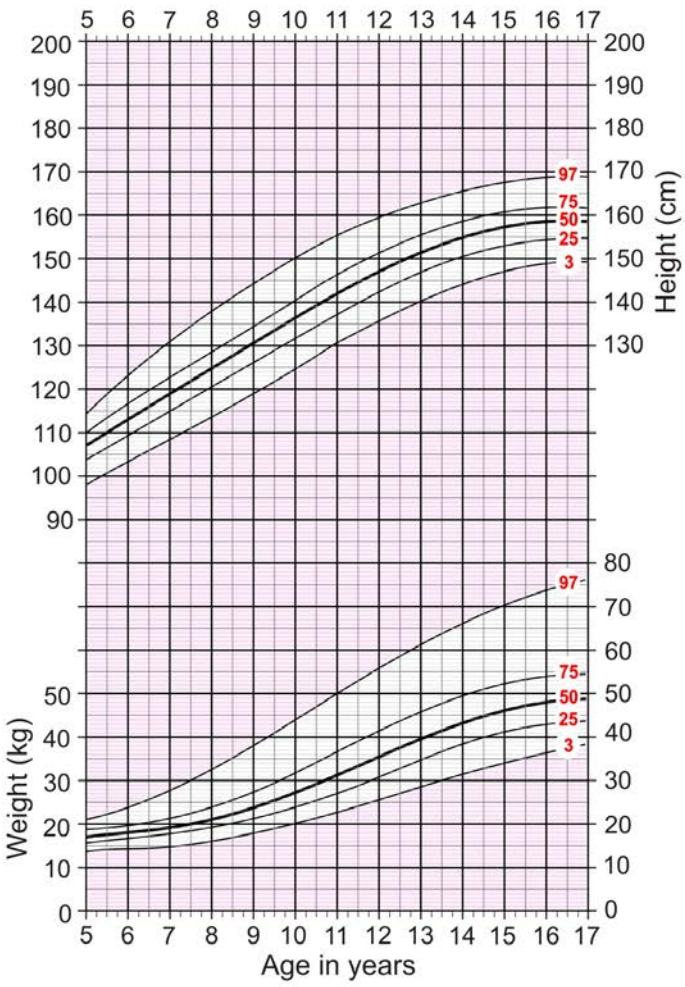
	Height, Weight & Head Circumference Percentile Curves								
	(Boys 0-5 years)								
	Name: _____ DOB:_____ Record No.:_____								
									Sequence
									97th
									75th
									50th
									25th
Source: Agarwal & Agarwal, Ind Pediat Vol 31, Apr 1994									3rd

Growth Chart For Indian Children

Weight-for-age and Height-for-age Percentiles

Girls (5-17 Years)

Name ..... Date of birth ..... Record No. .....



Adapted from : Agrawal DK, et al. Indian Pediatr. 1992; 29:1203
(with permission from Prof. K.N. Agarwal)

Monitoring Height-Weight by Parents/family members.

- Growth monitoring by a trainable family member who has learnt from a health worker is an ideal approach- *for weight recordings* with increasing age. It will help to assess that child is gaining weight- **good health** OR if there is loss in weight on monthly recordings – **warning sign poor feeding or illness**.
- Length/height measurements in infancy and early childhood are very difficult however may serve as an approximate/ close estimates. Parents can measure on a flat hard smooth surface, mark the length head to foot then measure with fibre glass tape.

Limitations: wrong measurement of the weight or height by untrained or half trained hands – can miss growth faltering? Still we must train family to record weight on monthly intervals in first 12 months; on alternate months in 2nd year and 3 monthly up to the age of 5-6 years.

Fortunately infants and children are visiting the health centers for immunizations, this is the unique opportunity to record- Length, head circumference and weight by trained hands and calibrated tools. These values be plotted on a growth curve to compare with the early date - if increment is following the growth percentile curve *Good* --if not – worry.

Adolescence

Growth process in puberty

- Both primary and secondary sexual characteristics appear- between 8 and 14 years of age in girls and 9 and 15 years of age in boys.
- Girls-Breast enlargement, occasionally initially unilateral, is the first obvious sign of puberty and occurs between 10 and 11 years of age.
- Boys- Penile and scrotal enlargement occur typically about a year after testicular volume increase -from 2.0 ml to>4 ml or testes length from 2 cm to 3.2 cm.

Adolescent Growth

•Adolescence Growth-Period extends from 2.5 to 3 years; to cross SMR stages 2-5(American kids 4-5 yr).

•Height gain is 27-29cm in boys & 24-26cm in girls; Weight gain in both 25-30 kg.

•Most rapid period of growth in the postnatal life (average gain being 19g/day in boys & 16g/day in girls).This velocity is around 3 month age.

•During this period Skeletal growth is completed (50% of adult bone mass and 20% of the body stature). Mainly "Cortical Bone Growth". 1 cm ht gain needs 20 g Ca. In adolescence 145g Ca/yr is assimilated.

6

Puberty -GIRLS

1. **First sign of ovarian estradiol secretion is breast development "Thelarche".SMR-B-2 (Breast budding)-GROWTH IN HEIGHT.**

Estradiol is a good stimulator of "GH" it doubles the growth velocity "PEAK HEIGHT VELOCITY'(9-10 cm / yr). Coincides with B-3. Follows B-2 by 1 yr.

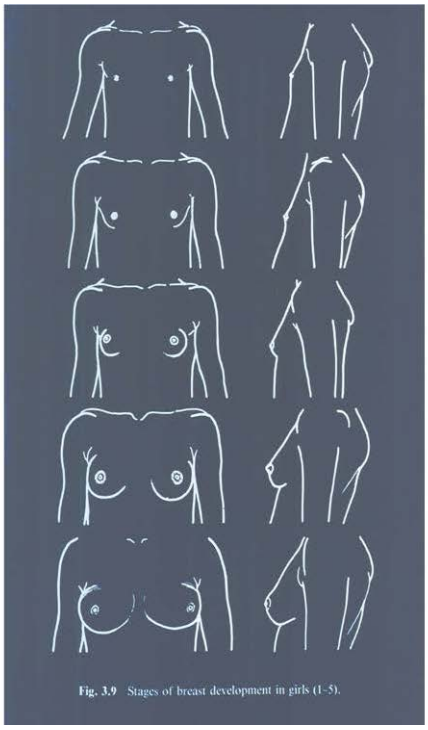


Fig. 3.9 Stages of breast development in girls (1–5).

Figure shows breast development in sexual maturity grades 1-5.

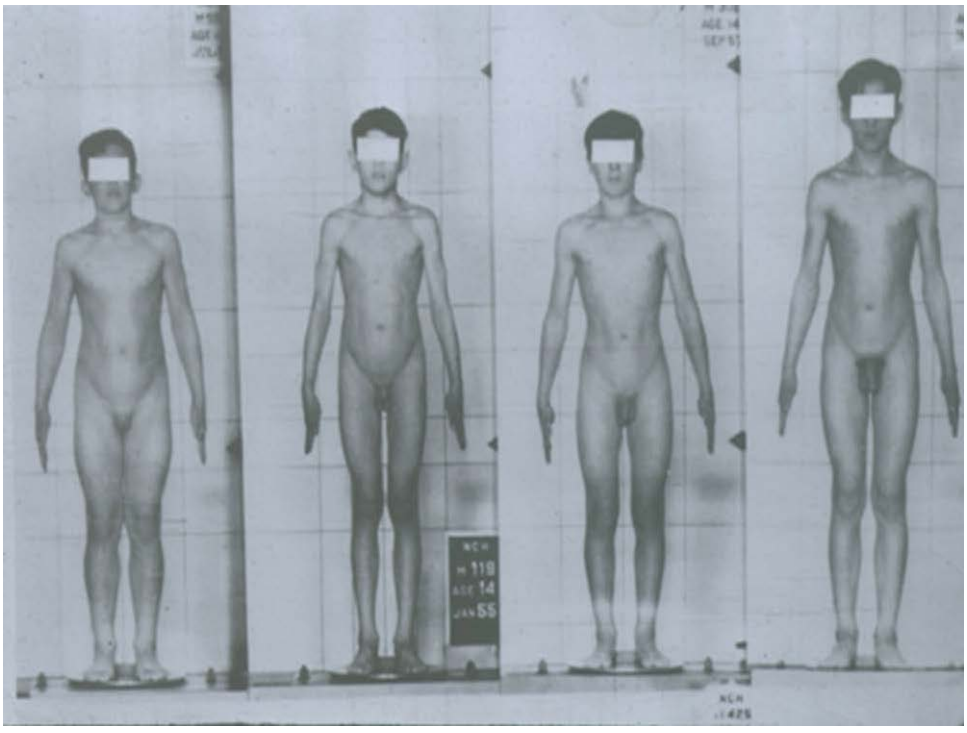
Menarche & linear growth

The growth in the post menarche period is limited as girls can gain 5-6 cm in linear growth, only.

Thus the maximum gain in height is pre-menarche in SMR- stages –B-2 & B-3.

Puberty- BOYS

1. **Adrenarche is the ONSET & CONTINUITY of male PUBERTY**
2. **Testosterone/dihydrotestosterone are needed in large concentration to initiate "GH" via the androgen receptors. (Thus later than girls by 1-2 yr).**
1. **As early as 10 years of age- enlargement of seminiferous tubules, epididymis, seminal vesicles and prostate, occur.**
3. **Initiation testicular volume > 4 ml; maximum growth "PHV" (10-11 cm /year) attained at Testicular volume 10-12 ml. (During SMR- G 3-4).**



Genital development in sexual grades 2-5.

Children's Brain- grows very rapidly from 22nd week of intrauterine life.At birth, the brain of

the infant is 25% of the adult size. At the age of one year, the brain has grown to 75% of its adult size and to 80% by age three, reaching 90% by age seven.

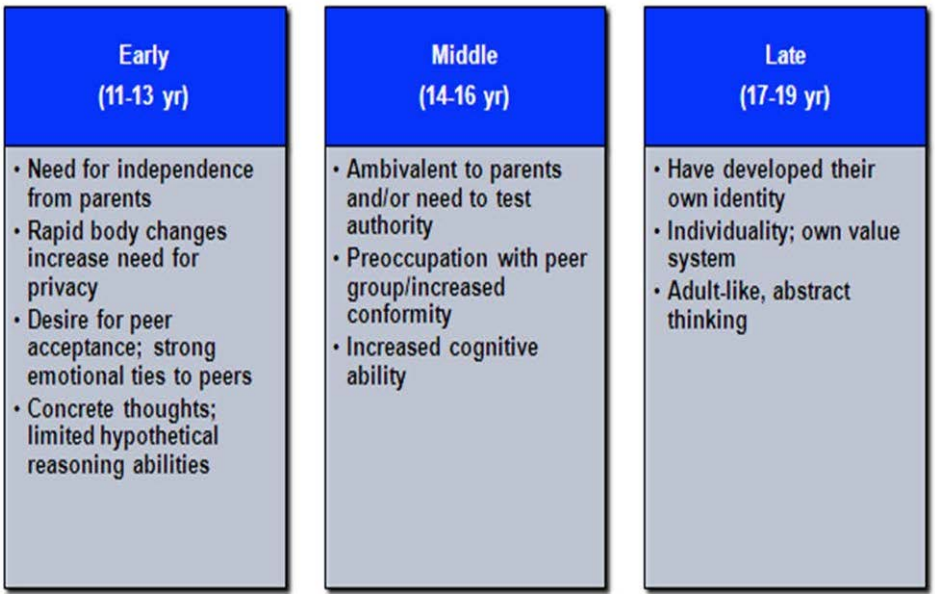
Children's brains are much busier than an adult's.

- **Gray matter** is made up of the cell bodies of neurons, the nerve fibers that project from them, and support cells
- At **birth** each neuron has 2,500 synapses.
- By **2 years**, there are 15,000 synapses per neuron.
- At **3 years** the first period of consolidation begins. This period tends to be characterized by children asking the question "why?"
- It is estimated that a **four year** old asks a "why" question every two and a half minutes!
- Around the age of **six**, there is a second surge as the brain starts to use language in increasingly complex ways.
- Up to the age of **nine** a child's brain continues to **be twice as active as an adult's brain.**

Adolescent Brain

- Brain adopts a **"use-it-or-lose-it" pruning system,** sloughing unused connections and increasing the speed of others. **30,000 synapses may be lost per second in the early adolescent brain leading to an ultimate loss of almost one half of the synapses.**
- Areas of the brain responsible for **executive functioning** (such as **strategic thinking, weighing risks and benefits and impulse control**) continue to develop and refine connections through adolescence and into the mid-twenties.

Events in ADOLESCENCE

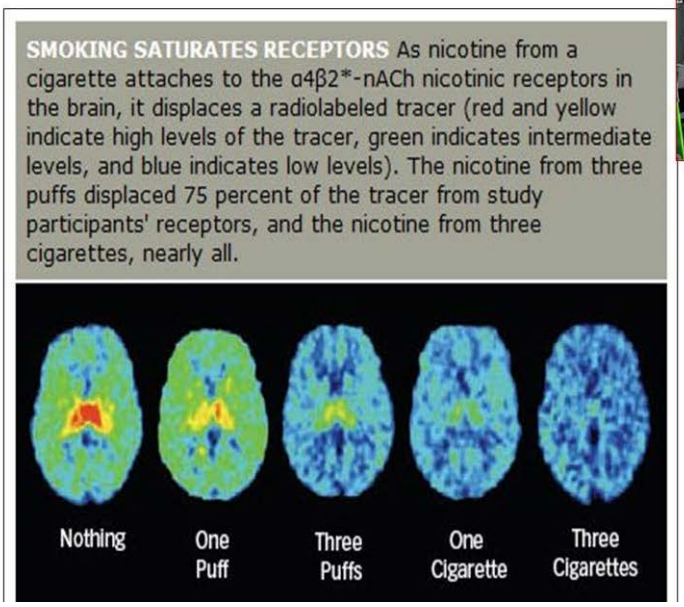


The affects of alcohol consumption or smoking tobacco are shown below. It is advised not to indulge in either.

Alcohol and the Adolescent Brain

- Delays in normal brain development over time
- More vulnerable to long term damage to memory and other systems
  - Prevents changes in neural circuits involved in learning and attention
  - Prone to seizures after binge drinking
  - Less brain activity overall
- Less vulnerable to perceived negative effects: motor coordination and sedation
- **Reduced hippocampus volumes in alcohol abuse indicates permanent brain damage.**
- Causes reduced testosterone in adulthood

Tobacco and Brain



Deprived children

- Children who had been in an **orphanage** at any time in their lives had much **smaller gray matter volume** in the cortex of the brain and had **smaller white matter volume** than those who had never been in an orphanage. Even if children were placed in loving foster homes, the formerly institutionalized children's **gray matter didn't catch up.**

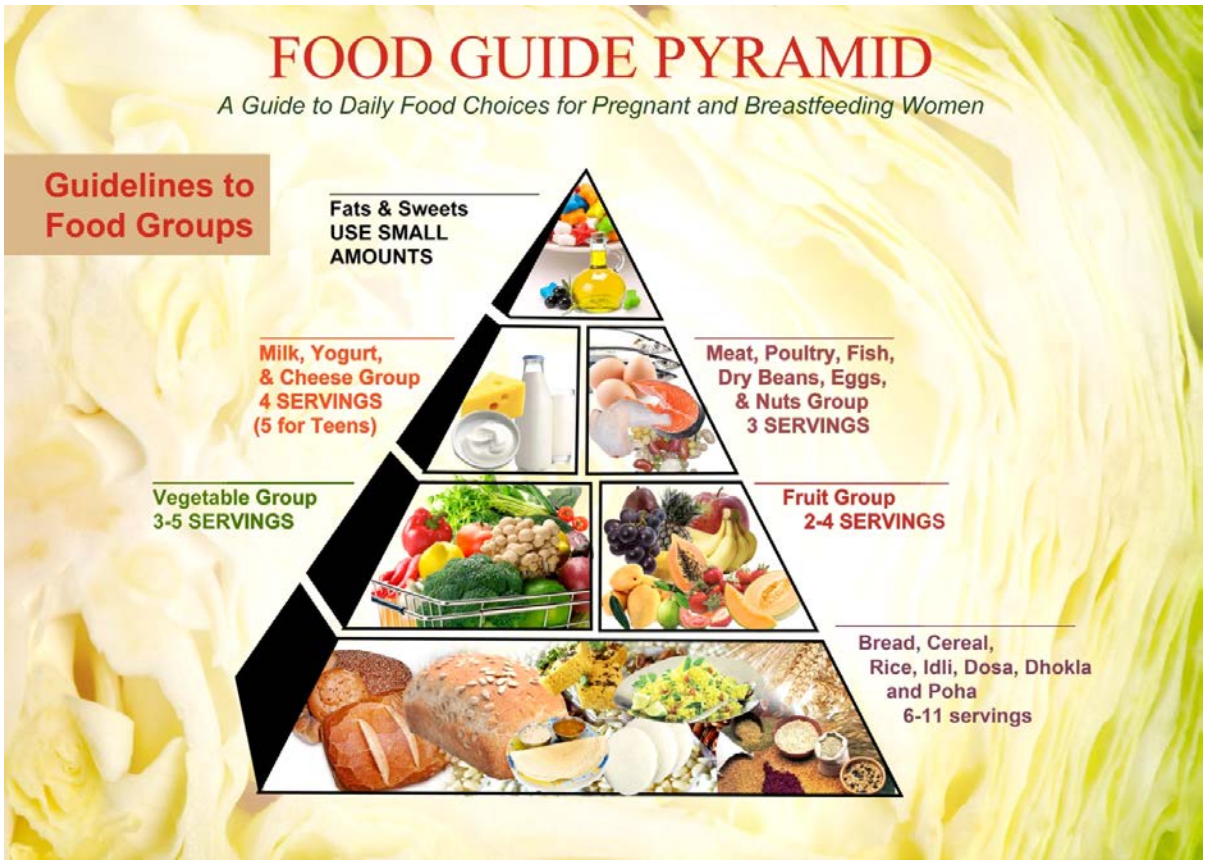
- **White matter**, however, seemed to be more resilient. As orphaned children placed in high-quality foster care had the **same white matter volume as those who were never in an orphanage.**

Dietary Recommendations in School Children

Age yr	Protein g/day	CHO g/day	Calcium mg
4-8 Boys-1200-2000 Girls 1,200 to 1,800 Cal	19	130	1000 mg (Vit D =600 IU)
Boys 9-11- 1600-2600 Girls 9-11 1,400 to 2,200 cal	34 34	130 130	1300 mg (Vit D =600 IU)
Boys 14-18- 2000-3200 Girls 14-18 1800-2400 cal Sexual development- **Boys** (>12 years); **Girls**	52 46 60 46.0	130 130 3000 Kcal/d 2500 kcal/d	**-Same-1300/600** **Boys-** **have lean body mass 2 times of girls—need more Fe, Ca and Zn.**

Why NUTRITION for 5 to 10 yr growth Is very important ?

- **Middle childhood, is characterized by a slow, steady rate of physical growth. However, cognitive, emotional, and social development occur at a tremendous rate.**
- To achieve **optimal growth and development, children** need a variety of healthy **foods that provide sufficient energy, protein, carbohydrates, fat, vitamins, and minerals. They need three meals per day, plus snacks.(Working mother with school kids)**
- **Preparatory period for Adolescence. Growth failure of this period**(under nutrition) will delay onset of PUBESCENCE; No catch up growth; further the PHV will not be observed.
- **Approximate Growth –Ht 25 cm girls and 30 cm in boys; Wt 25-30 kg.**
- **Lymphoid Growth is maximum in this period.(Immunity)**



These five FOOD groups in daily diet(Food guide pyramid) will get you all the nutrients you need.

Vegetables & Fruits –provide Vitamins/Minerals/Antioxidants

Oral Hygiene

- One thing is clear: the body and mouth are not separate. "Your body can affect your mouth and likewise, your mouth can affect your body,". "Taking good care of your teeth and gums can really help you live well longer." This means-
- 1.**brushing twice a day**,
- **2.flossing once a day,** and
- **3.going for regular dental cleanings and check-ups.**

What is a healthy life style

- **Eating the right food**
- **Physical activity(40-60 min daily)**
- **Maintaining a healthy body weight**
- **No alcohol intake**
- **No smoking**
- **Oral Hygiene**

Nature of Science and Biology Education

Kambadur Muralidhar

Hormone Research Laboratory
Department of Zoology, University of Delhi
Delhi-110007
E-mail: kambadur@hotmail.com

Abstract

Human knowledge is vast and all of it is not verifiable. Science is one part of human knowledge acquired systematically through application of scientific method. Science or more correctly Natural science wishes to understand the structure and functioning of Nature. Physics addresses some questions about Nature and Chemistry likewise addresses some other questions about Nature. Biology, the third component of Natural science deals with living organisms. Science is all about experimentation to get answers to questions raised about Nature. Biology progressed in three phases. The first phase of growth of biology was based on description of biological structures and biological phenomena by observation and not by experimentation. In the second phase, experimentation was introduced. Tools and techniques of Physics and Chemistry were applied for this purpose. The result was the vast body of knowledge called Reductionist Biology. It laid more emphasis on mechanisms underlying physiological processes in living organisms than on description of living structures. Though the professed aim of Reductionist Biology was 'understanding biological processes', the resulting knowledge did not satisfy many. Biological phenomena are emergent properties of living systems. This means that the properties of a system like an organism or a cell are not merely sum of the properties of the components of that system like tissues of an organism or sub-cellular organelles of a cell. The whole system exhibits emergent properties which are not found in any of the parts of the system. Hence to understand these aspects of Biological systems, in the third phase, a new approach called 'Systems biology' has been recommended. What one must never forget is that nothing in Biology makes sense unless viewed in the evolutionary context. The greatest concept in Biology, indeed, in the whole of human history and knowledge, is the concept and phenomenon of organic evolution as enunciated initially by Darwin and Wallace. To understand Biology, one should first observe and learn what organisms do. Knowing classification and names of organisms is necessary but only to explain and understand biological processes and phenomena. Biology for a long time remained a body of information unable to answer any question. Today this is not the case. Biology can be conceptually discussed. Further, it poses many conceptual problems not encountered in Physics or Chemistry. While Physics and Chemistry through their inventions and discoveries led to technologies that have enriched human lives, Biological knowledge is closest to human

Lecture delivered on March 7, 2013, at Kiang Nangbha College, Jowai, Near Shillong, NEHU

beings. Human Biology is of immense interest to scientist as well as the common man. Human Biology interfaces with Sociology, Psychology and indeed Philosophy. Many areas of biology in general and human biology in particular cannot be understood or explained by Chemistry and Physics alone. Mathematics and computational techniques have also to be utilized. Indeed Neurobiology is the frontier area of all sciences. The most mysterious part of Natural phenomena is biological phenomena exhibited by living organisms. While we are close to understanding material world and phenomena, we are far from understanding and explaining biological phenomena more so human activities, affairs and indeed 'Life' itself. The consequence of all that has been said above is that we should teach and learn Biology as such and not as fragments like Botany, Zoology and Microbiology or as Biochemistry or Physiology or Genetics. This spirit should be maintained at School and undergraduate levels. Only post-graduate education including research can be on a small part of Biology like cell biology or physiology etc. In addition to answering some questions about Natural Phenomena, Science also offers opportunities to build successful careers. Pursuit of science in teaching and research is a passion for many in addition to providing a career. Such scientists, through dedicated work make discoveries and sometimes invent things for human welfare. Pursuit of Biology is no exception. It is a passion and a source of joy and understanding of the phenomenon of 'Living'. A philosophical understanding of human life is possible, in near future, only through studying Human Biology.

What is Science?

Awareness about surroundings is a hall mark of all living organisms. In human beings, more than in any other living organism, this awareness is also a result of curiosity driven activity. In all organized societies, there are few who pursue this curiosity driven mental and physical activity more than others. They are called thinkers or philosophers. Philosophers are obsessed about knowing or realizing 'TRUTH'. Since pre-historic times, philosophers in various countries have gained knowledge about many things and made pronouncements in the form of general truths. Most of them deal with cosmic significance of human lives and are also the frame work for the founding and propagation of religions and cults of this world. Very few of them dealt with material world or 'Nature' as we call. There was no uniform method by which these 'truths' were realized. Further, most of them are not verifiable by others. All this changed in andaround 14^{th} century. A cultural and intellectual revolution occurred in Europe and is usually termed 'Renaissance' in History. A new method of inquiry called 'Scientific Method' was established. The aim of this new inquiry was clearly spelt out as understanding the structure and functioning of this material world called 'Nature'. Francis Bacon was the best of these new philosophers of this type. This new natural philosophy was aptly named 'Natural Science'. It used the well-defined scientific method to raise and answer questions about 'Nature'. Physicists, Chemists and Biologists are the three new breed of philosophers or more aptly natural scientists. The body of knowledge that they established and practiced was respectively named Physics, Chemistry and Biology. All of them employed mathematical tools to different degrees to describe their respective domain knowledge. Mathematics, by conventional wisdom, isnot included under Natural Science. A major reason for this is that pure mathematics can be created without reference to the real world. It is built by pure abstract thinking. Natural Science deals with the real Nature consisting of material world including living organisms. However there are other reasons also. Mathematical conclusions are infallible or more correctly non-falsifiable. According to Karl Popper, all scientific claims

should also provide for experimental/observational falsifiability. However mathematics and science do interact in many areas. In a way, no truth has stood the test of time like mathematical truth. We cannot, unfortunately, say this about science derived truths about Nature. As an example a statement like "All swans are white in appearance" is a relative truth. This is because, until someone sights a black swan, it holds good. A scientist will not say that there is no probability of a black swan being sighted somewhere at some time in future. In the words of Richard Feynman, all scientific truths are stated with different degrees of certainty/uncertainty. No scientific truth/law is hundred percent certain. The phenomenal world can be stated and understood in terms of probability only. Later in this lecture we will realize that in Biology also, Chance and Necessity have dictated living activities/processes/phenomena. We live in a probable world whose future activity cannot be predicted by any algebraic expression. That is the mystery of this Universe and indeed of human life! We will explain this later in the lecture.

Around 17^{th} and 18^{th} centuries of the post-Christ era, another objective of Natural Science was introduced by Rene des Cartes of France. This was that science should be useful to Man in making creature comforts. It means that scientific knowledge should be applied to achieve a specified goal of human comfort or growth and development of society. Here we are referring to Engineering, Technology and Medicine. Over a period of the next few centuries, this utilitarian view of Science and Technology has dominated over the earlier aim of understanding Nature. Indeed, civilizational growth or advance has been defined in terms of technological competence of a particular society. Science got linked to economic strength of nations. Today we talk of 'Knowledge economies' precisely for this reason. Science and Technology driven countries are called advanced countries/developed countries/first world.

In summary, Science is one of the approaches to realize Truth. It restricts itself to understanding the Truth of this real world or Nature. The activity of doing science is pursued strictly through application of the scientific method. Scientific method defines science as well as forms its limitation. Scientific method largely refers to doing experiments whose results are reproducible. Experimental protocol becomes very important and has to be strictly followed. All experiments in Science essentially measure something. Hence measurement is the essence of data collection in science. Science does not deal with anything that is not measurable.

Physics, dealing with the real world of material objects, measures properties of Space and Time like volume, temperature, electricity, magnetic force etc. Physics reveals the relationship among these measured operational concepts like that between volume and pressure of a gas etc. Ultimately Physics constructs the nature of this Physical world and its truth and reveals these truths in the form of well-defined Laws of Nature, for example, the Laws of Thermodynamics or Laws of Mechanics etc. These laws are also stated in the form of an algebraic expression, the most precise, concise and elegant form of stating truth. As you remember, the most famous of these expressions is $E= MC^2$ attributed to Albert Einstein.

Chemistry, the second component of Natural Science also measures either transition state temperatures like melting point or boiling point or it measures concentration of solutions. The concepts and tools of Physics are applied in the form of techniques to understand the nature of matter. It also seeks the Truth about Nature. However it believes this truth in the form of a universal substance, which by some mechanism, manifests as diversity of objects in

nature. We understand however that the truth of the material world of objects is limited to the level of Elements. Mendeleev was the first one to state these elemental truths in the form of the now famous Periodic Table of Elements! The periodic table tells us that there are more than one hundred elements in this world, each of which is true to itself and not convertible, by spontaneous process, into another element. There are exceptions, of course, in the case of radioactive elements. While Chemistry deals with forms of Matter only, we realize that Energy and Matter are two sides of the same coin! Chemistry does not do justice to this equivalence. It only writes somewhat balanced equations including energy terms while describing chemical reactions. Only Physics takes into consideration all aspects of Space and Time. When it comes to living organisms, both of them arestruggling in understanding biological phenomena. Indeed they have raised more questions than answers to questions with regard to biology.

One important part of nature of Science is that science is *amoral* i.e. neither moral nor immoral in the words of Vannewar Bush, once a scientific advisor to the President of USA. Science does not discuss values. It does not address questions about purpose of all physical, chemical and biological processes. It does not seek meaning of values like ethics or beauty in life. Lest you may misunderstand, let me clearly state that the pursuit of science strictly follows ethical standards and principles. Scientists are highly ethical in practicing and communicating science. They do not discuss ethics per say as a subject of investigation! We have to look to Biology to get some partial answers to these vexing questions. We have to understand the nature of Biology. I will attempt that in the next few minutes.

Nature of Biology

Biology, simply stated, deals with living organisms. It is more complex than either Physics or Chemistry. Indeed it poses more conceptual problems than the other two. It reveals certain unique features of living organisms which may not be understood or explained through Physics and/or Chemistry. It also suffers from historical mistakes in its pursuit. We know more about history of biology than biology itself. In one way Biology is the history of life on earth. It is also taught in class rooms more as history than as domain knowledge *per se*. Why is it so? We mentioned that measurement is the key to experimentation. Unfortunately in the first few centuries, biologists did not measure anything!. Indeed they did not think of getting answers to biological questions, if any, by experimentation. To exaggerate a little bit, they did not raise any questions! It was pure description of what was observed. Initially, till seventeenth century, it was a pair of eyes that was used to observe and describe. Later it was the microscope that became a tool for observation and making notes. For a long time no question was asked nor any experiment conducted on a scale that we witnessed in the history of Physics or even Chemistry. We can also designate this phase as the 1st phase of Biology. In the first phase, as description led to information load, scientists divided their tasks, for convenience sake, into Botany, Zoology and later Microbiology. Broadly these three disciplines dealt with information about plants, animals and microorganisms. What did we get out of these efforts? Areas of Biology like Ecology, Evolutionary Biology, Taxonomy etc fully developed. It does not mean that we did not learn anything in the first seventeen centuries of this and the preceding millenniums. A huge body of organized knowledge in the form of Taxonomy and Systematic resulted during this era. Highly scholarly tomes were written in this area of Biology. Carolus Linnaeus was the foremost of these scholars. *Hortus malabaricus* was one such tome about plants in Kerala but written in Latin. The extent of Biodiversity was fully recognized and

appreciated. Curiosity prompted them to raise, uncomfortable though, questions of origin of life and indeed of this huge biodiversity. No satisfactory answers came. But scientists like Lamarck made exciting statements about this phenomenon. The culminating product of this type of descriptive inquiry was the revolutionary idea developed by Wallace and Charles Darwin. It is generally referred to as 'the theory of organic evolution by Natural Selection'. While I will not discuss this in detail, suffice it to say that it is the greatest concept in the history of human knowledge, not just in conceptual biology.

Since the days of Aristotle (approximately 350 BC) till seventeenth century AD, there was no serious experimentation in the true sense. Later, under the influence of Rene des Cartes, experimentation was undertaken using the then available physical and chemical techniques. When this type of description increased, the body of information was sufficiently large and interesting to raise questions. As a consequence investigative experimentation started in Biology. When investigative experimentation began in Biology, many discoveries were made about living organisms. Physiology in all its glory was the most dominant area of Biology. There was more emphasis on living processes than living forms. Biochemical and Biophysical description of processes inside living organisms revealed many insights. Mechanism of living was to be understood in physical and chemical language. Scientists essentially established the relationship between the proximate causes and their effects in the form of physiological processes and even ecological phenomena was established. This phase/face of Biology is popularly called 'Reductionist Biology'. One attempts to reduce Biology to Chemistry and Physics. The information gathered during the first phase on biological structures and their organization into a hierarchy was also reduced to a problem of relating structure to function, but in molecular terms.

While the professed aim of Reductionist Biology was 'understanding living phenomenon' as against the merely descriptive nature of phase I Biology, major part of this body of knowledge still remained as description, although in molecular language (biochemical and biophysical). By over emphasizing Structure-Function relationship, it led to some mischievous scientists to talk of 'most suitable Design and hence Designer' type of hypotheses. This was most unfortunate. There is another reason for the delay in conceptualizing Biology and the dominance of reductionist biology not withstanding its inability to explain biology. One should briefly look at the history of Chemistry and its interface with Biology. Physicists and Chemists in the beginning did not investigate Biology with their techniques. Chemistry, in particular, started as inorganic chemistry/general chemistry. When some of them asked questions about chemical composition of living organisms, it was a very courageous question given the hold of religious belief on the question of origin of life. When they, after elemental analysis realized that a sample of top soil and a sample of a tissue from a organism had the same elemental composition except for the higher proportion of carbon, hydrogen and oxygen, they merely asserted what was told by all religions that man was made by God from a ball of soil or earth and hence we go back to earth after death. However chemical analysis revealed that an amazing range of carbon compounds are found in living/dead tissues from plants and animals. All these carbon compounds were isolated from nature i.e. plants and animals. Inorganic chemistry gave rise to Natural products chemistry. Still they did not attempt synthesis of natural products. Wohler's synthesis of urea, found in living organisms from ammonium cyanate (actually silver cyanate), a mental barrier was broken down. Scientists synthesized increasingly complex natural products, and organic chemistry with emphasis on synthesis

replaced natural products chemistry. When chemists asked an embarrassing question about what these organic compounds were doing inside living organisms, the answer came in the form of discovery of metabolism. Further studies revealed the bewildering range of enzymatic activities and the nature of biocatalysis. Biochemistry replaced organic chemistry as the research front of Chemistry. When metabolic basis of all physiological and behavioral processes were established, metabolism became the basis of defining Health and Disease. Hundreds of genetic disorders, essentially defects in one or two metabolic steps in the body, were reported. Reductionist biology came to dead end, unable to explain disease symptoms through biochemical reaction defects. The chapter on Vitamins and what they do in our body faced similar end. After all most of the vitamins are biosynthesized in plants and the biochemical role ascribed to them exists in plants also. What is the link between biochemical reactions involving vitamins as cofactors and the symptoms of diseases appearing in their absence (dietary deficiency)?

By focusing on establishing the immediate cause and effect relationship, reductionist biologists forgot ultimate causes. What causes both physiological processes and ecological and evolutionary phenomena? Biologists had to go back to Darwinian ideas of evolution to understand reductionist biology derived information. Biology required explanation more than 'understanding'. When such attempts were made, what is now popularly called 'Organismic Biology' resulted. Evolutionary and ecological phenomena were examined under new light. Information from every area of Biology including population ecology, Genetics, individual physiology, Biochemistry and Biophysics were understood and applied to explain ecology and evolution. True biology has arrived. This is the most exciting period in the history of biology.

Three aspects of biology are still not explained satisfactorily. One was the observation of emergent properties in biological systems. Properties of a cell are not found in the sub cellular organelles. Whole is not simply summation of its components. Any amount of description of the component parts of a system does not explain appearance of a new (the emergent) property at the higher level of organization. For example, describing the molecular components of ribosome in great detail does not enable the scientist to put them together and reconstitute functional ribosome in the test tube. A partial success has been registered in reconstituting 'respiration linked oxidative phosphorylation'- a property of intact mitochondria. A unique way of organizing the components in space and more importantly interactions among these components appears to result in the new function of ribosome. Systems Biology is the third phase of biology and attempts to use computer aided simulation studies and modeling to understand/explain this emergent phenomenon.

A second problem is in areas like behavior. Reductionist biology cannot satisfactorily make us understand, leave alone explain, animal and much of human behavior. Human Biology especially neurobiology, is grappling with this problem. Many of the methods employed are not clearly understood nor are they highly reproducible. Hence the resulting sociobiology is already being criticized as a soft science. The borders between science and social science and that between social anthropology and humanities appear to become porous and in some cases altogether disappearing.

The third problem arises when we examine Biology from de cartean view point that science has to be useful to man. As told earlier, Rene des Cartes emphasized the utilitarian

aspect of Science & Technology. Physics and Chemistry covered themselves with glory when it comes making human life more comfortable. Our daily life, including cultural life, would not be possible without the benefits of applied physics, applied Chemistry and the respective technologies that came. Think of Electricity, automobiles, airplanes, transistors, house hold appliances, railways, paved roads, medicines, textiles, fertilizers and how much we are dependent on them. Luckily some developments in molecular biology, Polymer chemistry, Biophysics etc gave birth to biotechnology in the modern sense. Biotechnology can solve many of our problems in personal, community and public health sectors. How does this affect biology education at school, college and post-graduate levels of education? Let us briefly look at this problem.

Biology Education

Education in its purest sense has only one aim of increasing the consciousness of every student to its highest level. In ancient times, individuals sought teachers (guru) and learnt at their feet. It was for individual emancipation or empowerment in some cases. Empowerment guarantees employment. In later years, especially during colonial rule in India, English education aimed at producing administrators for running the country. Post-independence, Jawaharlal Nehru dreamed of creating the new middle class of good engineers, doctors, lawyers etc. Post-globalization in trade and commerce, a heightened perception of nationhood has been inculcated among students as every nation has to develop its own technology to solve all sorts of problems connected with development and human welfare. Education in science aims at producing fully empowered scientists in basic and applied sciences required to man the huge empire of research institutions and educational institutions. Science in general and Biology in particular poses a number of problems in teaching-learning process. Let me first deal with some of the perceived problems and solutions to those problems.

There is a complaint that all teaching has become examination centric with no emphasis laid on learning. We must divert our focus from exams to understanding and hence learning. Exams should be designed to test understanding than as a mechanism to filter students.

There is a complaint that biology we teach has no relevance to student's daily life. 'Black board science' especially biology sounds more and more like fiction, esoteric and impracticable. That is where Faraday Lectures of the Royal Society come to our mind. The student has to first see and experience the phenomena characteristic of the domain knowledge. For biology, one must get an idea of what organisms do. After that, the teacher can construct the theory. In other words empirical experience has to precede theoretical construction.

There is a complaint that most teachers teach history of biology in terms of who did what as biology. One forgets that constructing domain knowledge from a historical perspective is a hind sight driven attempt. Teaching of biology is to relate the process of arriving at the concepts. Let students realize the concepts constituting Biology by a fast but guided track.

There is a complaint that standards of teaching biology are declining. 'Standard' in teaching has many dimensions. Scholarship, communicating skill, hard work and enthusiasm, conviction in the philosophy of a particular science domain and last but not the least,

compassion for the average child and many other qualities comprise the phrase 'standard of teaching'. Teaching without personal research will create quiz masters and not great teachers. There is a complaint that we teach too much and students do not learn anything. Let us learn without burden.

There is a complaint that people have not thought carefully about what to teach and how much to teach. We should teach valid knowledge. As an example, it is understood that the child must first be made of her immediate environment through a suitable vocabulary build up. Questions immediately arise with regard to medium of instruction, about nature of the environment that the child must recognize (material world of objects, cultural world of relations and attitudes, mental world of values and habits etc.) There is no consensus on this. There is consensus that in the second stage the student should learn 'Human Knowledge'. We have no idea whether human knowledge should be presented in conventional categories (like science, humanities, social sciences, performing arts, language and literature etc.) or as a series of participatory activities. The latter is pure skill building and hence empowerment of the student. Physical, mental and emotional skills would be built. How are values in life to be imparted and built in the child? Should education be pure empowerment or should it also include inculcation of values? How do we avoid bias in a pluralistic society?

There is a complaint that students are not opting for science, more so biology, because career options are few and even if they are available, they are not as remunerative as careers in management or information technology. This is not today's problem. This is a typical middle class reaction to the career concept. Philistine attitude that career is meant to get us rich and that the best job is that where is no work to do, is atypical middle class mind set. Our problem is bright students are going away from science. The solution lies in bringing about a paradigm shift in value system around career. Intellectual challenge posed by science, personal pride of discovery in science and the sheer joy of knowing something hitherto unknown have to be marketed as new values. Even from a philosophical perspective, all thinking men and women have asked the same question i.e. what is the cosmic significance of our lives? What is the nature of 'TRUTH' or 'REALITY?' If knowing the truth is the aim of life, knowing something new in daily increments should be the life-long ambition of all men and women. Science is the only profession which guarantees this discovery and this joy of knowing.

Perhaps the biggest complaint that we have is that biology is not being taught as it should be. For centuries, biology was not taught but biological forms and structures were listed with names. Biological phenomena and processes were talked about as a matter of fact and as hearsay information. Biological processes were never experienced in the laboratory or even in the field. Excessive emphasis was laid on memorizing names and information. The growth of biological knowledge and the process of biological discoveries, the nature of biological questions and the validity of biological experimentation are never told or revealed or discussed. The truth is very few teachers of biology understand it. Most of the time is being engaged in sectarian battles about zoology or botany, basic or applied etc. Majority of teachers miss conveying the excitement in biology. They leave students bored. They make all bright students run away from biology. What we need to do urgently is discuss the nature of biology, understand biology and train first the teachers in understanding biology so that they. In their turn, convey the spirit to students. I will not discuss today anything on problems of pedagogy, examinations or co-curricular activities for want of time.

A brief account of Indian research in Biology

Biology in India during pre-historic and historical periods comprised pure observations and recording, mythology, domestication (of animals & agriculture) and even 'culture'. In European value dominated India, of course, Natural History blossomed. .It was purely camp following and trained work. Experts flourished on each of the taxonomic group. As there was nothing else in Biology, respect from peers and general society depended on scholarship and personal integrity. No originality marked the research work. Some discoveries of medical importance were made possible both in Pharmocognacy and from studies in Surgery and Medicine (for example in the areas of Medical Entomology, Infectious diseases, Vaccines etc.). There was no record of experimental Biology. In the twentieth century, investigative Biology started peaking with Biochemistry as the dominant area of Biology. Reductionist Biology became popular, respected and feared by classical Biologists. Funding pattern and policies and priorities followed suit. Classical areas of Biology were looked down. Lip sympathy, however, was paid to Darwinian ideas. Evolution was not taught in modern Biology departments and taxonomists did not cover themselves with glory either by teaching Evolutionary Biology. In due course of time (by 1990s) the type of research work done by reductionists fell into a pattern and soon became repetitive to a large extent. They commanded little respect just like the classical taxonomists. Thrust areas were identified. They became, instead, thrusted areas. These biologists practiced reductionist Biology empirically without investigative component relevant to real time biology. Techniques became end in themselves without a viable real time biological research question or problem.

In recent years Biotechnology has become the face of reductionist biology. It is ironic that creativity and innovation, characteristic of technology, is conspicuous by its absence in Indian Biotechnology. In the twenty first century, a kind of backlash occurred from type of research work. Both became more rigorous with lot of Physics and Mathes thrown in. Both apparently started addressing real time Biology problems. We are witnessing this today in the form of good Evolutionary Biologists including those in the areas of Behavior, Wild Life and Population Ecology. Similarly, more Physics, Maths, Statistics, Engineering and Chemistry were applied into research work in reductionist Biology. In both the cases, innovation in conceptual analysis of the research problem can be noticed. Only true Biologists who always deal with real time research problems of Biology will ultimately survive. The empirical scientists will get washed out both from classical biology and in reductionist biology. I wish to say something on Ethics in Science especially Biology/Animal Science. Regarding use of animals in teaching and research in zoological sciences, let me say that Science and Technology have influenced all developmental activities of nations, if not the entire civilizational growth. A major part of this development and progress is in the sphere of individual human health and public hygiene including social and preventive medicine in the context of communicable diseases. Germ theory of Pasteur and Koch heralded a new era in public health policies of many governments. In India too, the role of Haffkin institute in the production and use of vaccines as a prophylactic measure in the control of infectious diseases and thus ensuring national health cannot be underestimated. This progress in the sphere of health of the nation depended on medical research which for most part of it, involved work on non-human animal models. Therefore animal houses have always been part of medical colleges and research institutions. The contribution to medical progress by the use of animals in research is too well known to every educated layman.

In summary, research in Biology in India has always over emphasized applications to medical and agricultural practices at the cost of Fundamental Biology. No great discovery in biology in general and animal science in particular has come from Indian research. India has a bright future in the areas of Evolutionary Biology/Behavior and in true Biophysics/Mathematical Biology.

Biological Sciences and Society

Discoveries and inventions in biological sciences like in the areas of Health, Contraception, personal medicine, bioremediation, biopharmaceuticals, vaccines against diseases, and many more such useful products and processes have become realities. In addition, developments in sociobiology and even in developmental biology have influenced human society, certain socio-cultural attitudes and socio-legal issues. On the other hand changes in our cultural values and political views (expressed by powerful people through government legislation and upheld by legal procedures) are also influencing how biology is transacted in the class rooms and in the field. How biology has influenced human society and how human society has influenced biology will be briefly discussed in the following pages.

In the case of the physical sciences, discoveries and inventions have been translated into 'Creature comforts' for humans. In some cases 'market demands' or perceived demands have influenced technological developments (for example in telecommunications, automobiles etc). Biological sciences did not influence human life for a very long time. However domestication of wild animals, use of animal hide, agriculture and other such civilizational developments did take place during pre-historic times even without any knowledge of formal Biology! Similarly the influence of biological material on socio-cultural practices (e.g.: sacred groves, construction material etc) was also not due to application of formal knowledge of biology. Only after the nineteenth century, developments in biological sciences have started influencing human lives. This has been accelerated during the later part of the 20^{th} century in the areas of agriculture, medicine, conservation, management of environment etc. One should also remember that society and social issues have also influenced progress in biology.

Human beings are divided on the basis of language, culture, caste and community. Developments in the area of genome analysis have shown that there is no biological or genetic basis of these divisions. The progressive ideas of secularism, democracy, socialism individual freedom are being understood in the light of revelations in biological research. Modern biological findings do not support superiority or inferiority or social discrimination on the basis of language, religion, race, cultural hatred etc. There is no genetic basis of even 'merit' which is not innate but a learnt behavior.

Human beings are conscious of ethics in public and private lives. It is logical to be good as society will not survive if it is otherwise. Is there a biological basis of morals? Yes, studies in animal behavior have brought out extraordinary sense of morals. Parental behavior, grazing behavior and even reproductive behavior can be easily interpreted from human ethical perspective. In animal communities, be it ants or antelopes, cats or dogs, these moral rules can be discerned. However, it is certainly not clear whether these behavioral patterns in animals are innate (instincts) or learnt behavior. Modern sociobiology is based on certain paradigms and is influenced by Darwinian ideas of evolution. This means there is a genetic

component in behavior and not everything is learnt behavior Instinct is one of innate behavioral pattern strongly influenced and determined by genes inherited. This includes nest building by birds, web-weaving by spiders, courtship and mating behavior etc. Such innate behaviors cannot be subject to natural selection as a deviant behavior does not result in successful reproduction.

Living in groups or societies appears to have evolved in spite of certain disadvantages to living in social groups. Of course there are advantages like finding a mate, defense against predators etc. The advantages are tradeoff for disadvantages.

Cooperation and conflict is the theme to be learnt from watching animal groups like social insects, pelicans etc. Human life is full of conflict. Should we not learn that if we cooperate with each other survival in more than when we fight among ourselves? This applies to institutions of family, community, university and even nation.

Altruistic behavior is often observed in human beings. In evolutionary biology altruism is defined as behavior which decreases the fitness of the performer while it increases the fitness of another individual. In animal social groups this is explained by Hamilton's inclusive fitness theory. Altruistic behavior would result in certain reduction in fitness or 'cost' to the altruist. A corresponding gain in fitness or 'benefit' occurs to another individual who shares a certain fraction of the altruist's genotype. It is reasoned that whenever gain in the inclusive fitness to the altruist's genotype exceeds the cost, natural selection would favor altruism. This means that because of benefit to relatives (those sharing a fraction of the altruist's genotype) exceeding the cost, the probability of representation of the particular genotype increases in future generations (hence called inclusive fitness). Kin selection is also explained by this. Human behavior loosely termed 'casteist' or 'communal' or 'parochial' can be understood form this theory. In simple words, when one helps a cousin at the risk of one's own survival, one is actually ensuring conservation and expansion of one's gene pool.

Deviant human behavior like child abuse or rape has been classified under 'aggressive behavior' and thus be should be treated as crimes of violence than as crimes of sex. Many socio biologists do not agree with this. They argue that these are sex related crimes and hence have to be interpreted by application of Darwinian ideas on learnt behavior.

Human societies all over the world have debated crime and punishment from various angles. One such angle is how much is an individual responsible for his crimes? If genes are everything then how is accountability fixed? If deviant behavior is due genetic makeup, how do we sustain civilized societies? As against initial euphoria when the structure and function of DNA was unraveled, knowledge of genomes and epigenetics have given us insights that help solve many such vexed questions. It is now believed that a genome is only your potential. The actual phenotype that you exhibit is a result of epigenetic changes during and after gene expression. These epigenetic changes start right during embryonic life. These epigenetic changes lead to introduction of variation right from embryonic stage through adult life till death. Each phenotype is different from other phenotypes in the same species. There is no preprogramming of our life in our genes. While the genome is potential, epigenome is what is expressed and each epigenome is different in different tissues of the same individual and in individuals of same population. Hence biology does not support determinism. Our lives are

what we make of it and not simply what we inherit? Hence individual deviant behavior is punishable if it leads to survival advantage of the society at large.

Similarly knowledge of genomics of diseases has given enormous insights into prophylactic and therapeutic approaches to handle diseases. Survival strategies have been designed even in situation where one is genetically predisposed to morbidity. Biology has certainly allowed us to manage our lives better. One can only observe the increasing longevity of people in countries the India to certify the benefits of applying biological knowledge in human health problems.

The correct knowledge of genetics, genomics and epigenetics will also result in eradication of social evils of female feticide, eugenics etc. and uphold the individual human dignity and assert that everyone has a right to live with dignity. Everyone is a potential Einstein, Rabindranath Tagore, Aurobindo, Socrates, Akbar or Alexander. Nobody, much less the government, has any right to deny this potential to any fetus or child. Modern genomic knowledge has also taught us that every drug will not have the same action in intensity and secondary effects in all patients. Each of us has a certain genetic predisposition not just for phenotypic feature but even for drug efficacy.

Study of one's genes can lead to gene therapy for cancer. Regulation of physiological processes by hormones and neurotransmitters has given clues about drug targets in many diseases. Many are psycho stimulants, anti depressants, antidotes to drug addiction etc.

Biology is a historical science and population genetics has revealed the story of human migration and evolution and formation of societies. Man by nature in not only curious about environment but is also pedigree conscious. At a much higher plane, man is obsessed with history, mythology and indeed of origins of mankind. Population genetic studies involving gene transfer across language barriers has revealed the story of human migration, anthropological relatedness and mythological legacies. It will also lead to origins and spread of religious thoughts and societies. Will biology ultimately lead us to discover God?

Both food security and nutrition security are linked to biological knowledge. Genetic engineering has revolutionized agricultural practices and is close to solving food problems. For example GM-tomato has increased shelf life.

Increased taxonomic knowledge has revealed many new fruits, vegetables, spices, ornamental and avenue trees, exotic garden plants etc. Diversification of agriculturally viable food crops and cash crops has assured food security and nutrition security. Developments in genetic engineering have led to disease resistant food and non – foodcrops like Bt – brinjal or Bt – cotton. Fortified foods (Iodinated salt for sample) are here to stay. Permanent preventive measures against many nutritional disorders are in place (e.g. thyroid disorders, anemia etc.). Without biological knowledge agricultural growth called 'green revolution' would not have been possible. Biology has made a major contribution in transgenic crop development both in non edible and edible crops. Thousands of plant derived natural products are used as medicines, fragrances, pesticides, food additives etc. Cultures of different human societies with regard to dietary and culinary habits are highly influenced by agricultural knowledge of that society.

Adaptation has taught us that while survival is dependent on adaptability, adaptability

appears to be backed by a design in body or behavior. However one should realize that the design is only apparent. It is only after survival that 'designs' become visible. There is no design. In fact survival reveals successful variations are inherent.

Natural methods of population size control have been revealed through analysis of reproductive behavior of animals. But in humans where reproductive behavior is a cultural behavior, reproductive biology has revealed contraceptive protocols including even vaccines. In the field of contraceptive technology, discoveries in reproductive biology have led to many developments contraceptive techniques like surgical methods, hormonal methods (e.g. the 'pill'), IUCDs, morning after pill which uses estrogen, androgen injection as male contraceptive method and vaccination against human chorionic gonadotropins (hCG) as an immunocontraceptive technology. It is also note worthy that in this field social groups (feminist movement groups) have influenced reproductive biologists to develop contraceptive technologies based on sound scientific principles. The 'combination pill' was developed by Gregory Pincus of Harvard University in response to demands from women leaders of the society in USA. The same group sensitized scientists to develop male contraceptives so that man also takes some contraceptive burden. The possibility of vaccinating men against pituitary follicle stimulating hormone (FSH) in near future is real and was also influenced by the same progressive idea. A perceptible change in developing countries as a result of awareness through compulsory education in the increasing respect for girl – child and adult women an independent decision making citizens. This is a good sign for society.

While man was always aware of unhealth and the desirability of possessing good health, it is progress in medical field and human biology that has resulted in better health for today's citizen, man or woman, boy or girl. Nutritional science has given us the idea of vitamins and trace elements, calorific value of foods and about life-style diseases etc. This has definitely influenced society's health – consciousness and access to good food. Governments have framed rules that food security and nutrition security is guaranteed for every citizen especially for those below poverty line (bpl). Nutritional science also has revealed to us the ingredients of health food (fiber, vitamins, proteins etc.) and consequences of eating junk food. But for biology this awareness would have not come.

Similarly it is knowledge of microbes causing carbohydrate dependent tooth decay, or of excess fluorine in drinking water causing dental caries that has brought to the notice of the public the necessity of using potable drinking water, of brushing are teeth immediately after meals etc. It is the same nutritional science that has given us criteria like BMI as indicators of health or unhealth. Body mass index is simply obtained by applying the equation w/ h^2 where w is body weight in kilograms, h is height in meters. A BMI of 25 or about is overweight and a BMI of 30 or above is obesity. Increased awareness of these biological concepts at least in educated middle class has led to altered life styles and more attention to food and body exercise.

Parasitic, viral and bacterial diseases have been studied in great detail. Many new drug targets have been identified. Novel strategies of disease control have been revealed. More important, biology has taught us how to deal with disease burden and disease surveillance. Novel prophylactic and therapeutic measures have resulted.

Concept of public and personal hygiene has been clearly established and has resulted in development in social and preventive medicine. The idea of vector control is one such idea.

Many organisms arising out of a large number of phylogenic groups cause diseases. Antibiotic concept is well entrenched in society largely due to studies in microbiology, medicine and pharmacology.

Earlier insulin was extracted from pancreas of cattle and pig. Eli Lily Company has been artificially manufacturing insulin using recombinant DNA technology. Orally active Insulin preparations are also going to be in the market.

Developments in biomedical research have led to so many benefits to mankind. Mention may be made of vaccines against infectious diseases including such dreaded ones like T.B. or AIDS.

Development of nations in terms of material progress is not at all bad. However many a time a developmental project may be at the cost of another sector of human activity like agriculture or at the cost of environment (example forest cover etc.). In such situations, cost – benefit analysis is done and decisions made. In general ecological knowledge has given us ideas that if man and development result in degradation of environment, the human race itself is likely to disappear.

Acknowledgements: I am grateful to INSA for sponsoring these lectures addressing undergraduate and school children. I am grateful to Prof BBP Gupta of NEHU who could make arrangements for the lectures. I am also grateful to Prof Saidapur who initiated this activity of getting the lectures printed and published. They will surely serve as supplementary reading material at school level and in the process provoking them to think independently. Einstein once remarked that "it is a miracle that curiosity survives formal education". Hopefully these INSA lectures keep the curiosity and scientific spirit among school going children.

Suggested Readings:

1. E.O.Wilson (1975) Sociobiology: The New Synthesis. The twenty-fifth anniversary edition published in 2000 by the Belknap Press of Harvard University Press.

2. Arvind Kumar (1996) Chaos, Fractals and Self-organization, NBT, Delhi

3. Muralidhar, K. (2009) What Organisms Do. In "Project on History of Indian Science, Philosophy & Culture" Ed. D.P. Chattopadhyay, Centre for Studies in Civilizations. Volume XII, Part 6 on 'Life and Organicism'. Ed NS Rangaswamy pp. 117-157.

4. Biology Text Book for Class XI, 2006 NCERT, New Delhi-110016

5. Biology Text Book for Class XII, 2006 NCERT, New Delhi-110016

Careers in Science: shaping human lives

Narinder K Mehra
Dean (Research)
All India Institute of Medical Sciences
New Delhi – 110029
Email: narin98@hotmail.com

As part of the science promotion activities particularly among high school children and those in the age group of 10 – 30 years, the Indian National Science Academy decided to hold a series of popular lectures for young students and teachers from schools/colleges of rural areas. The idea was to inspire young minds to take up careers in science by citing compelling evidence, through personal examples and debate on the opportunities that science offers in shaping human lives and in a way the country's future. It is imperative that the young minds understand that science embodies the paradigms, skills, knowledge and techniques by which we relate with and understand the many mysteries of nature. It is important for them to appreciate that everything in nature follows logic and no field except science accepts truth or lie logically whether it be a simple thing like an apple falling from a tree or how we breathe, what determines the species survival or development of disease. Science and scientists have shaped the human life as we live today owing to the inquisitiveness and enquiry that forms the fundamental basis of human mind. Science has an answer to every question only if asked correctly. Einstein developed the theory of general relativity and changed the way we see our world as part of the larger universe. Thinkers like him began by asking questions that may have betrayed conventional belief or may not have been asked at all. The continuous quest to ask impertinent questions and find pertinent answers is therefore the essence of science.

I opted to be counted for this cause and chose to address a few such schools in the rural belt of Amritsar. I thought it was a great opportunity to discuss with the students of class 10 or 12 the challenges and excitement that science offers in general and in the 21st century in particular, be it environmental consciousness, march over infectious diseases like tuberculosis, AIDS and other deadly viruses, food and health security, waste disposal, nuclear energy, invasive medical procedures, organ donation and the list is endless. Since it is obligatory for them to opt for careers at that stage, they should be made aware that advancements in science and technology are not merely development of skills anyone can learn, nor are they formulas and facts which one can learn by rote and recite. Science is not merely books and journals that one can read, but a factual understanding of the world we live in based on creative thinking, logic and evidence. Today the focus is shifting from theories and laws to the translational aspects of scientific knowledge as it relates to human health, social welfare and economic development. Science is also becoming more holistic in nature without compartmentalization into basic or strategic components. Major scientific discoveries have contributed to the world in immeasurable ways and some of these achievements are

Lecture delivered on October 27, 2012 at Guru Nanak Dev University, Amritsar

extraordinary. Careers in science provide the perfect forum where knowledge is built and organized in the form of testable predictions about the ways nature functions, discuss plausible explanations and rationalize testable hypotheses.

Since the objective was to motivate students to take up science as careers, I thought that the best was to start by presenting personal example. My belonging to the Amritsar city and upbringing in that environment in the 50's, 60's and 70's could be appealing and how despite all odds of yesteryears, I chose the biological sciences as my career path. The Vice Chancellor of the Guru Nanak Dev university helped collect students from three schools belonging to the rural belt of Chhehrata, a small Tehsil about 15 kms away from the main city of Amritsar. The experience was exhilarating, the students a good mix of boys and girls were disciplined, participatory and knowledgeable. Many a shining faces reminded of the untapped talent that must exist in rural India and that requires nurturing. The sharp minds among them spoke of the inadequacies that exist in the system compared to their urban brethren, lack of library and internet facilities, and exposure in general.

I chose to speak to them on the '*biological mysteries and the changing face of biomedical science*'. The idea was to let them appreciate the Why, How and What aspect of each observation that science offers as compared to any other career and this is indeed most desirable for the bright inquisitive mind. Why could only Newton discover the laws of gravity? Didn't people before him ever see an apple falling from a tree? He not only observed but also asked the why and how; reasoned the observation and went on to finding the reason(s). Didn't this one single observation change the way we live today?

Turn of the Century: Landmark years

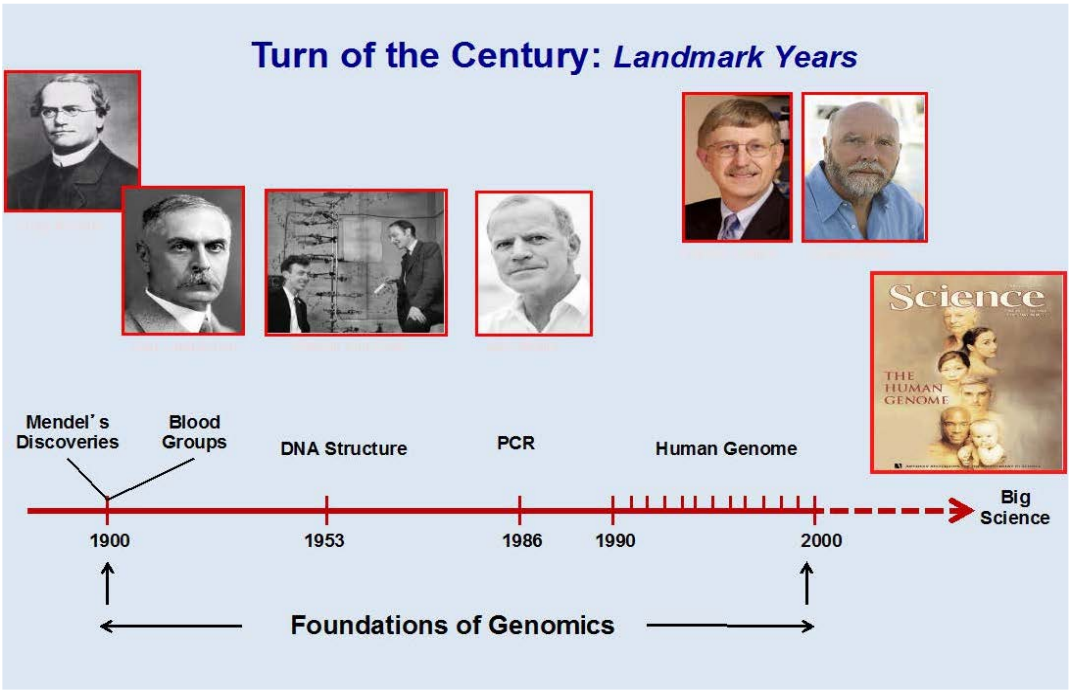
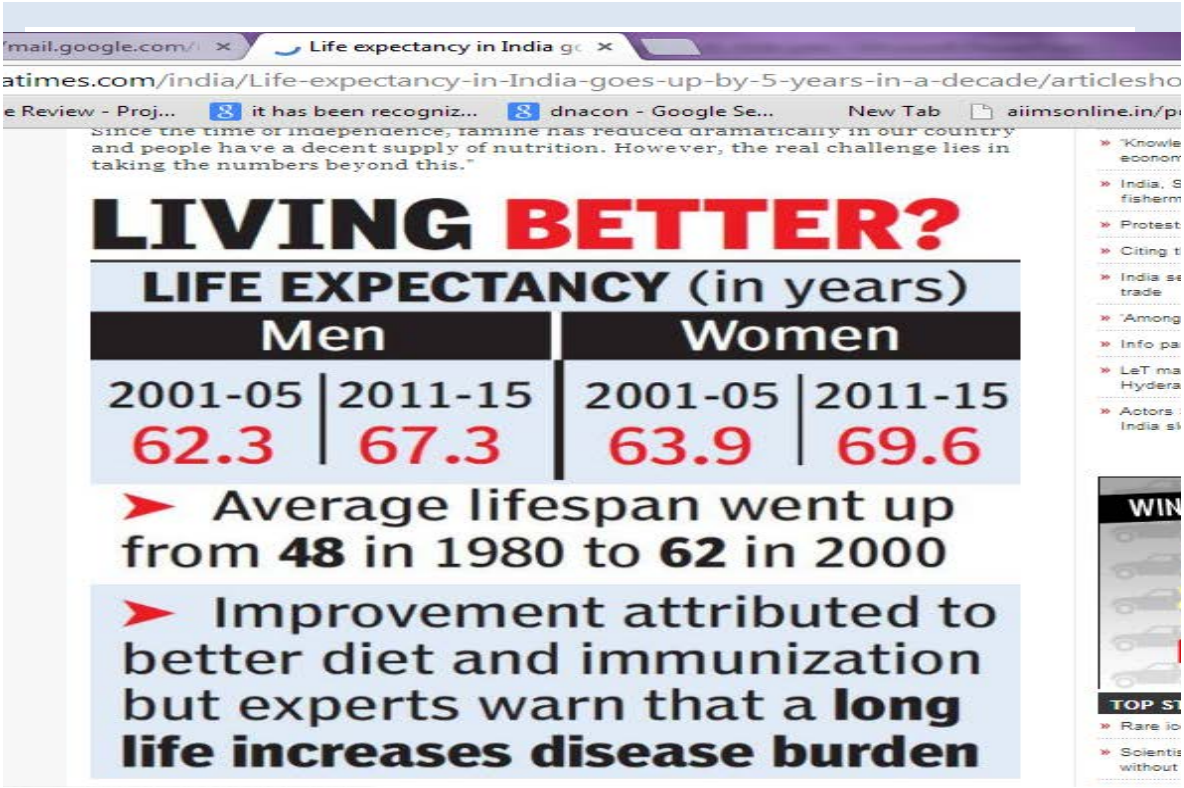


Fig 1 : The turn of a century has generally been a landmark year.

I decided to begin by starting a debate about the numerous excitements that science offers and how does one get fascinated with a new result each day and to tell them that anyone amongst you can become a doctor or a biomedical scientist. It just needs inquisitiveness, humane approach and a keen sense of observation to stand apart from the crowd and be appreciated. Generally speaking, the turn of a century has been a landmark year (Fig 1). For example, 1901 saw two epoch making discoveries that changed our lives - Mendel's work on inheritance getting recognized a good 35 years after he first reported, while the blood groups were also discovered the same year, and again by an Austrian scientist by the name, Karl Landsteiner. Similarly turn of the 21st century with discovery of the human genome has been the landmark year, providing us with the '*book of genes*' in hand. Francis Collins, one of the discoverers' of the human genome had commented, "it is my hope and expectation that over the next one to two decades ……or however long it takes…genomic discoveries will lead to an increasingly long list of health benefits for all the world's peoples". Several ground breaking discoveries made in the last few decades have impacted the way Medicine is practiced today. It may be noted that the top ten causes of death today have undergone a dramatic change from infections alone in the 1900s to cancer, cardiovascular disease and stroke today. At the same time, the average life span of the humans has seen a remarkable jump upwards and it is not uncommon to see more and more centenarians today. As per the US census Bureau, there will be over 834,000 centenarians in US alone by the year 2050. Population ageing is becoming a global phenomenon and it is estimated that India will soon become home to the second largest number of older people with females surpassing the male elderly. The average life span of an Indian has gone up from a mere 44 years in 1960 to over 68 years in 2011 and increasing further (see box). All this will pose new challenges for India in the area of human health, thus opening up new opportunities for careers in medical and life sciences.



The Big Three Killers:

Conquering infectious disease remains a big challenge even today with HIV/AIDS, tuberculosis and malaria constituting the big three killers despite billions of dollars having been spent in understanding their causation, pathogenesis and presentation. But where are we with regard to the vaccines for them? The hidden mysteries behind their control must be unraveled. Medical scenario is full of very successful examples, be it in the area of polio eradication, maternal and childhood mortality, organ and tissue transplantation, inherited disorders and many others.

DECLINING INTEREST IN SCIENCE IN INDIA

With considerable improvements in resources, trained manpower and research infrastructure, the contributions of India towards science have been remarkably noteworthy. Rapid economic growth witnessed in the last two decades makes it imperative that an ambient ecosystem for scientific research is built to keep pace with the country's development. A recent survey (Nature 2004) of impact-making scientific publications reveals that India ranks 22nd globally – below China, South Korea and Poland. The fraction of GDP that is spent on R&D has remained stagnant in India for more than two decades now whereas more dynamic Asian countries have surpassed us during the same period (Fig 2). Hence a policy of vigorous pursuit and popularization of science early at the school level is necessary and this alone can lead to renewed and rejuvenated interest in sciences in the country not only as mere subjects for passing the examination but as a career choice as well.

Less than 20 years ago, the brightest and the most talented students always opted for science as a preferred career; however the current science students often take it as a last resort and even those who graduate in science end up taking up commerce and/or related subjects for higher studies. It is important to understand that origin of the problem is not only at the decision making stage of the adolescents pursuing under graduate courses, but must be at the level of primary and secondary education imparted in schools since our system does not encourage young minds towards scientific enquiry and research. A close look at the pattern of school, undergraduate and post-graduate science education in India reveals a number of interesting facts that need to be seriously addressed. .

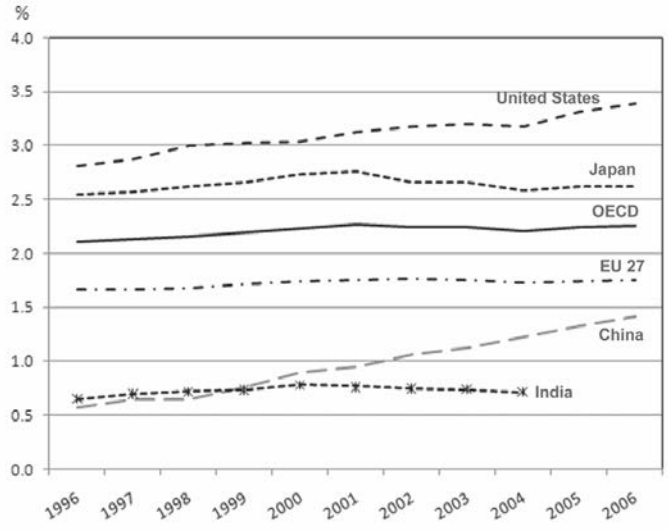


Fig2: Gross domestic expenditure on R & D by various countries during the period 1996 – 2006 as a percentage of GDP (*Ref: Organization for Economic Co-operation and Development (OECD) Science, Technology and Industry Outlook 2008)*

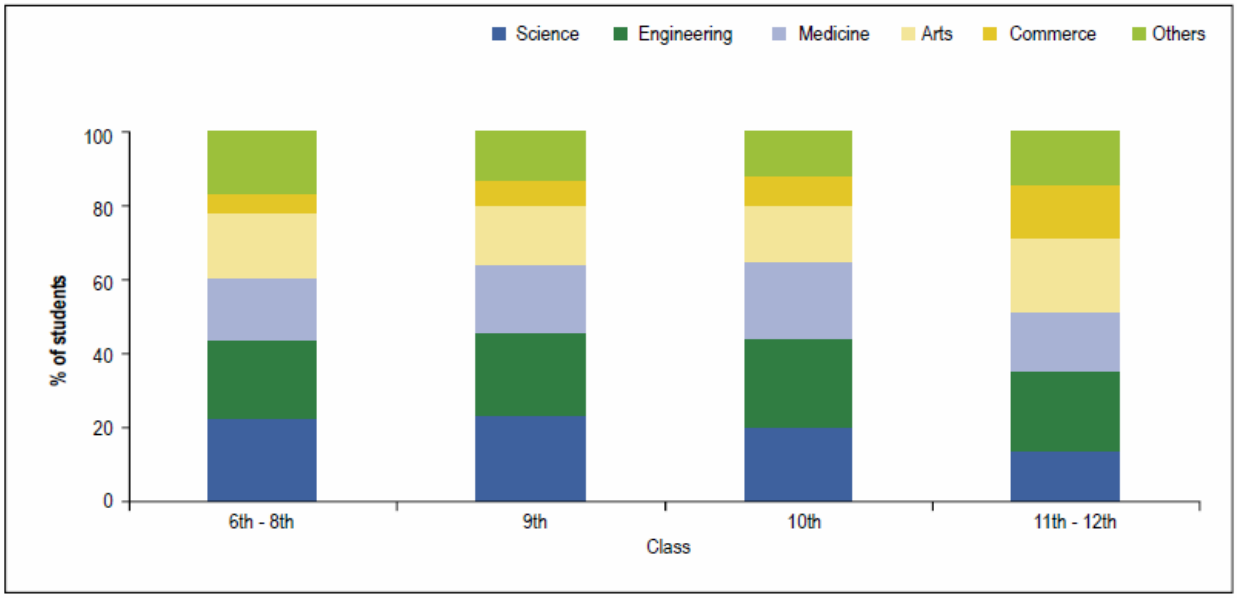
Following is a summary of some of the figures and statistics drawn from several sources and those provided by the Indian Academy of Science. It enables us to reflect upon the magnitude of the issues pertaining to science as a career amongst today's Indian youth:

i) Although in sheer numbers, the number of students attending school has been continuously increasing at the rate of ~3% annually, the infrastructure of education in terms of well equipped schools, laboratories, trained and competent staff haven't been able to keep pace with this inflation. During my own interaction with the rural school children, this fact was clearly brought out and the kids lamented that such facilities available to them were way below as compared to their peers in the urban, elite schools.

ii) Interestingly, bulk of the funds allocated by the government is spent on salaries and infrastructure maintenance of schools and only a meager 15% of the total annual school education budget of Rs.2500 crores which works out to Rs.375 crores and approximately Rs.30 per student is spent to bridge these gaps to be able to provide quality education to one and all. As a result, the vast majority of schools are ill equipped to impart a reasonable exposure to science education; yet the few that do so barely manage to maintain a high level of enthusiasm among the teachers and students.

iii) Even though the absolute number of undergraduate science students has increased from 1,28,000 in 1950 to 7,25,000 in the late 80's, the total number choosing to pursue science for higher studies has actually decreased during the same period from 32% to approximately 19% today.

iv)The commonly held and expressed perception among the academicians involved in higher education and research about the present state of science education in Indian colleges and universities is that there is a definite downward trend in interest among the youth. While pure science is the preferred choice of subject at mid class levels; this interest gradually wanes off at higher secondary levels. According to the India Science Report published by the National Council of Applied Economic Research (NCAER), only 22% of the mid class level students said that they preferred to study science in the future. However this dropped to a dismal 13.4% at 10+2 level.



Source: India Science Report, National Council of Applied Economic Research

Fig 3: Preferred subject for higher education by level of students (2004)

v) Only 11% of students at the undergraduate level opt for pure sciences whereas a whopping 47% opt for arts, which clearly reflect the trend of higher education in India as well as the propensity towards non-science subjects for further studies (Fig 3).

vi) While interacting with the students, I felt that one factor that must be keeping them away from choosing science as a career is the general apathy towards such careers that they see around. Perhaps we don't put in enough efforts towards highlighting the indigenous scientific contributions and giving due recognition to our own scientists from various fields including vital sectors such as the health sciences, atomic energy, defense, space, agriculture and related fields. Perhaps we have not debated enough on reasons for this apathy at the national level and what must be done to create a critical mass so as to avoid and prevent a crisis for the future. Well-planned efforts are needed to attract and retain dedicated young scientists, to pursue a professional career in science with dignity in the country.

Interaction with the students made me feel that although they viewed careers in science as rewarding and challenging, or even offering a satisfying professional life, but many of them definitely lacked motivation and perhaps guidance. For them, science as a career was perceived as painstakingly prolonged and arduous journey requiring intense hard work and devotion without the due recognition and rewards as this noble profession truly deserves. This is more so true in India where science is incomparable materially with other professions where within a lesser time frame and much less effort invested, one is able to gain much better prospects. Here I cannot resist but mention the role of today's media for the downfall of science as a career in India. To a large extent the media is a true reflection of the society and its role in glorifying professions other than science has contributed immensely to the downtrend in the student interest in the field of science. How many popular television channels in India carry science related programs and debates? Do we ever see scientists being felicitated the way our artists, film stars and business entrepreneurs are? There has also been negligible initiative or participation by private non-governmental organizations towards encouraging higher levels of education and research. This is particularly unfortunate since private enterprises depend on products of the educational system for their own needs.

It is widely perceived that the sorry state of affairs outlined above is aggravated by the government's policy of the past half century of establishing chains of specialized research institutions and national laboratories outside the university system, without proper and healthy linkages to the latter. This policy, especially the disproportionate funding of these institutions, has deprived universities of both talent and material support. Further the transition of young motivated students to become future leaders in various fields of science, which is so very smooth in a university setting in developed countries and so essential to promoting creative work at a young age, has been virtually missing in the Indian set up. Thus the critical period when the young minds can be nurtured and scientists of the future be groomed is not effectively made use of. It is important that an environment where students idolize and get inspired by talented scientists and imbibe and inculcate their ideals is nurtured. Without this, it may not be possible to rekindle the interest towards science as a career among our young minds.

Why take up Medical science as a career?

Before I started the lecture, merely 10-12 out of the nearly 120 students put up their hands for a career in Science. I thought this must be because they were not being expressive enough and needed time to open up. At the middle of our session, the number had improved a bit, but most among those who expressed taking up science wanted to be engineers with a degree in business administration afterwards. Less than 10% of this lot wanted to take up biology and Medicine, but none wanted to be a scientist. I reasoned with them that with the population explsion, diseases are increasing at an alarming rate and those with medicine or related degrees will not only be in demand but will also have a more satisfying career path. After all it is highly humanitarian to be able to serve the daily needs of the society. It is very exciting to discover new and safe therapies for deadly diseases for which no cure has been found as yet. Over a hundred job options are available in biomedical, pharmaceutical and related sciences which is far greater than any other profession. Isn't that remarkable? Organ transplantation is a hugely success story and there are numerous possibilities for a career in this one single branch alone. Stem cells and regenerative medicine are opening up new avenues for research and development leading to new and more exciting therapeutic strategies for several diseases. Custom made bioengineered organs could be the answer to shortage of organs for transplantation on one hand and overcoming biological hurdles posed

by toxicity due to lifelong immunosuppression on the other. Shinya Yamanaka, a Japanese medical scientist who won the 2012 Nobel prize for discovering the inducible pluripotent stem cell technology (iPS) commented, "*My goals over the decade include to develop new drugs to treat intractable diseases by using the iPS cell technology and to conduct clinical trials using it on a few patients with Parkinson's disease, diabetes or blood diseases".*

I ended up my lecture by telling them about what David Baltimore, the famous Nobel Laureate from USA commented during his recent visit to India in 2007, "*Doing science at the highest possible level is one of the greatest forms of fun as there can be in this world. You get paid for it, people appreciate you for it, you contribute to society and in my opinion, you just can't beat it. I'll be 70 years old next year and I still love a new result as I never did, It is the greatest high I know*". In the post-genomic era, there are new opportunities and challenges for the scientist of tomorrow. The four 'P's of Medicine – preventive, predictive, participatory and pre-emptive will soon lead to the establishment of 'personalized medicine' for the future and rightly so. Health information technology will reach the doors of individual patients forcing the doctors to change the way they practice medicine and one will get to see ipad doctors and scientists. Targeted drug therapy will become a boon for several diseases including cancers, cardiovascular and autoimmune diseases. Similarly great advancements are expected in surgical procedures through imaging, minimally invasive and robotic techniques. Finally conquering the big three and other emerging infections will be a challenge through discovering new vaccines or immunotherapeutic interventions.

I thought that I had done a good job by convincing the students about the many virtues of science and motivating them to take up medicine and biology and thus aim to be good biomedical scientists. Most looked happy and were grateful for the opportunity provided and I thought the exercise was well begun by INSA, but it must continue rather than get drowned as a one-time

Acknowledgements: The author would like to thank Prof Jai Rup Singh, Former Vice Chancellor of Guru Nanak Dev University Amritsar and Prof Vanita Kumar, Head Department of Human Genetics, GNDU for their help in getting students from the rural area schools of Amritsar District.

Excitement and Joy of doing Science as Career

T.J.Pandian
9 Old Natham Road,
Madurai – 625014
Email: tjpandian16@gmail.com

This presentation is general, elementary and is meant +2 children. Assisted by gesturer, it was made to inspire deaf and dumb children of Florance Swainson School, Palayamkottai and Shanthi School, Sivasailam at Florance Swainson, Thirunelveli, Tamil nadu. Sponsored by INSA, I made an "agnipariksha" to reach these children. After the presentation, many deaf and dumb children posed interesting questions, clearly indicating that they did understood the presentation.

This presentation begins with story from the Tamil poem Thiruvilaiyadal Puranam; science provides a solution to the dispute between Lord Siva claiming that the odour arising from tuft of hair and body of an individual is natural and Nakkiran, the President of the Tamil Academy, Madurai opposing it. A cross section through the human skin is characterized by the presence of sweat and sebaceous glands, producing individual specific odour; indeed the odour arising from a criminal or animal to be hunted guides a sniffer dog to locate them. With suitable examples, it was explained how scientific discoveries and technological developments have (i) minimized sufferings from diseases by man and his domesticated animals and plants, (ii) reduced drudgery of labour and brought (iii) social equality between (a) man and woman as well as (b) poor and rich.

The rich heritage of scientific and technological developments of India is explained by (i) Aryabhata's contributions to cosmic science (ii) the discovery of zero in mathematics, (Romans could not distinguish numerals from alphabets; they used I, V, X, L, C, D, M and so on) (iii) the marvel of rust-free iron pillar (near Quipt Minar, Delhi) in metallurgy (iv) the origin of taxonomy in biology and (v) hundreds of surgical tools developed by Sus'ruta Samhita. Representing the sacred symbol of 'Science Sangamam', the Hindu temples tell us the need for holistic scientific approach; the idea of staking one stone upon another without the binding paste in between them but holding them in position by the inner ball and socket joint in human anatomy (Fig. 1) ; in the absence of windows, the temple remains cool and pleasant during summer, as water in the temple pond absorbs heat and the external heat is not conducted beyond 5o % diameter of the stones characterized by high particle density; thus temples remain strong and 'air conditioned', thanks to the physics and biological principles adopted by the then Indian architects. The dancing Siva statue seems to represent

Lecture delivered on January 9, 2013 at Gram Gandhi Trust School and Florence School, Palayamkottai, Tamil Nadu

the combination of the mass (Siva) and energy (Sakti); with the combination of matter and energy, there is action in the form of His celestial dance.

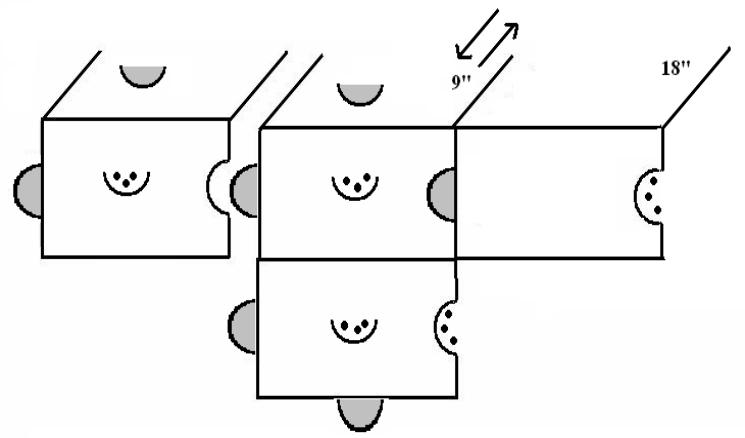


Fig. 1 Diagrammatic representation of the structural organization of stones staked one over the other in a typical South Indian temple. Note the ball and socket joints

Until today, Sir Isac Newton remains the tallest scientist, as his finding of gravitational force is applicable to atom as well as the universe. From Newton one learns to be inquisitive. The findings of Archemedis teach us to cultivate the habit of sustained thinking, even while bathing. Ramunujam, who has gone to school without breakfast, has foregone also lunch in order to solve a problem, which could not be solved by his own teacher. We learn from Ramunujam that if one wants to be a famous scientist, he must be ready to be occupied by sustained thinking, even by foregoing food, sleep and so on. The patriotic innovations of Homi Bhba, Vikram Sarabhai, M. S. Swaminathan, and Abdul Kalam are thankfully explained.

A series of simple experiments on skin transplantation of differently coloured rabbits by the Zoologist and Nobel Laureate Sir Peter Brian Medawar has laid the foundation for two great sciences: (i) Plastic Surgery and (ii) Forensic Science. With hilarious illustrations, the hair colour of humans is shown to be characterized by (i) colour: black, brown, golden yellow and silver white, (ii) short or long, (iii) straight or curled (iv) direction, (v) distribution and so on. A white or coloured skin graft from one area of rabbit, on transplantation, to another characterized by differently coloured hairs, continues to grow the hair colour of the same original donor graft. Hence some characters of the hair, as known from Medawar's experiments, are used in plastic surgery and forensic science. Incidentally, another interesting area of biological research may be the revival life of sleep in seeds and spores. For example, paddy plant has been revived from seeds 'sleeping' for more than 3000 years from mummified Pharoh. Likewise, a fungus has also been revived from its gonidium 'sleeping' for longer than 18 million years from sediment buried at, 5700 m depth of Indian Ocean. An M. Sc student at California Institute of Technology has revived the life of a bacterium from its spore 'sleeping' for longer than 30 million years from the gut of an insect stuck in amber.

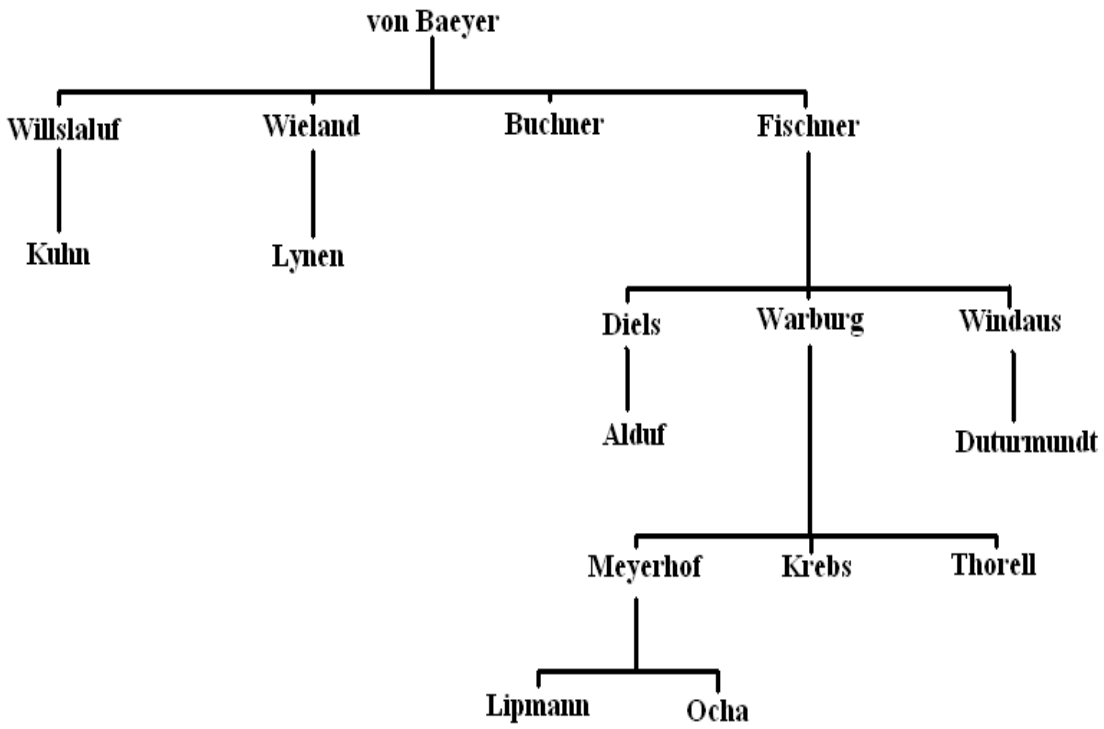


Fig. 2 Genealogy of the von Baeyer's family of Nobel Laureates

India was politically colonized by England. Thanks to the sacrifices of millions of freedom fighters under the great leadership of Mahatama Gandhi, we got our independence in 1947. With the establishment of UNESCO, today it may not be possible for a country to politically colonize another country but it is possible to scientifically colonize a country. We import high tech-products ranging from cell phone, say, Nokia from Finland to Boeing aero plane from America. An example for scientific colonization can be cell phone. Nokia, the Finnish company, entered into a Memorandum of Understanding with by the Government of Tamil nadu to establish a factory at Chennai in 2005. Accordingly, the government incurred a loss of Rs. 7.4 crores on leasing one hectare of its SIPCOT land; further it provided uninterrupted power supply, even when the entire Tamil nadu was reeling under power cut for 6-16 hours a day. From 2006-2013, the company manufactured 1,30,00,000 cell phones and sold them in India and abroad. By exempting exercise, customs, income and sale taxes, both the State and Central government incurred a loss of Rs. 30, 000 crore. Cell phones and Boeings and high tech-products and can be manufactured only with scientific discoveries and technological developments. It requires talented and motivated scientific man power. In 2011, 15 millions students were graduated from the portals of colleges in India, 12% of them pursued post-graduate degree courses and less than 1% of them undertook research. The number of engineers entering the post-graduate course is less than 3.4%, compared to 3.9% in China. The number of Ph.D degree holding engineers in India is far less than those in China and America. Not surprisingly, India has 5 patents for every million people but this number is 289 for USA, 779 for South Korea and 994 for Japan. Therefore, Government of India has come forward in a big way to support talented + two students to enter into science

courses by massive funding through programmers like INSPIRE by the Department of Science and Technology and Science Awareness Camp by the Indian National Science Academy. Science is exciting and offers an enjoyable learning career. By doing science, you will be patriotic scientific innovators and avoid/ minimize scientific colonization of our country by high tech countries. The less fortunate and physically challenged students also can do science, especially mathematics, which does not require physical movement and/or speaking and hearing. All it requires is deep and sustained thinking, as many scientists have done.

Uniquely, as many as 27 scientists from Gottingen, a small town in Germany, have been decorated with Nobel Prize. Germany has also the longest lineage of 5 generations of Nobel Laureates (Fig. 2). All these Nobel Laureates have stated that they have got the prize, because they took their respective teacher as role model; many state first rank holding + two students also say the same. Hence select a good teacher and best institution to bloom into a leading science. Incidentally, jaundice, the viral disease can be contained by *Phyllanthus*, a medicinal herb discovered by Indian "grandmas"; however, the same herb grown in Thailand does not possess this medicinal property. Transplantation experiments have shown that the microbial populations in the soil of Tamilnadu (but not in Thailand) stimulate the synthesis of a secondary plant product that contains jaundice. That seems to hold true for the Nobel Laureates too. Poet Ravidranath Tagore was born in Kalkutta; Prof.C.V.Raman and Mother Thresha, who came to serve in Kalkutta, also received Nobel Prize. Let the sprit of Kalkutta be extended through the length on breadth of India so that you are all blessed to receive Nobel Prize.

Communications in Living Organisms- Paradigms and Puzzles

Kambadur Muralidhar
Hormone Research Laboratory
Department of Zoology, University of Delhi
Delhi-110007
Email: kambadur@hotmail.com

Abstract

Living organisms are self-replicating, self-referring, autopoietic and evolving energy systems. One of the most important observations made on them is that their physiological and behavioral activities are highly regulated. Regulation ultimately is achieved at the molecular level by modulation of the velocity of catalytic events, so that the system moves from one steady state to another. The crucial elements are the mechanism of sensing the environmental cues and accordingly mount the appropriate response. In complex systems such as higher animals and plants, an additional complication is the demand for coordination among the internal tissues in the response limb of the 'consciousness'. This coordination necessitates communication among the tissues. Unlike the communications among organisms, communications within an organism among the tissues brings benefit to the organism. This communication is without exception chemical in nature. Hormones and neurotransmitters are the chemicals. We will try to learn about the nature of this communications. However the more important thing to realize is the delay in understanding these phenomena. The history of Biology and the nature of science itself have to be examined to get satisfactory answers. We are at the crucial stage in the history of biological research where there are no conceptual, technological or societal constraints. In such a golden age of biology, we should be making many discoveries. If Indian share of discoveries are not matching with the aspirations of the Indian society, we have to blame the educational and research institutions in not nurturing the scientific spirit and not respecting innovation and creativity.

What is a living organism?

What is the difference between an inanimate thing and a living organism? How do we recognize any thing as living? Let us take some examples. In the case of humans, if we are suspecting somebody is not alive, we usually check his/her breath for signs of breathing. Alternately a doctor would be checking the pulse. Does this technique work for every

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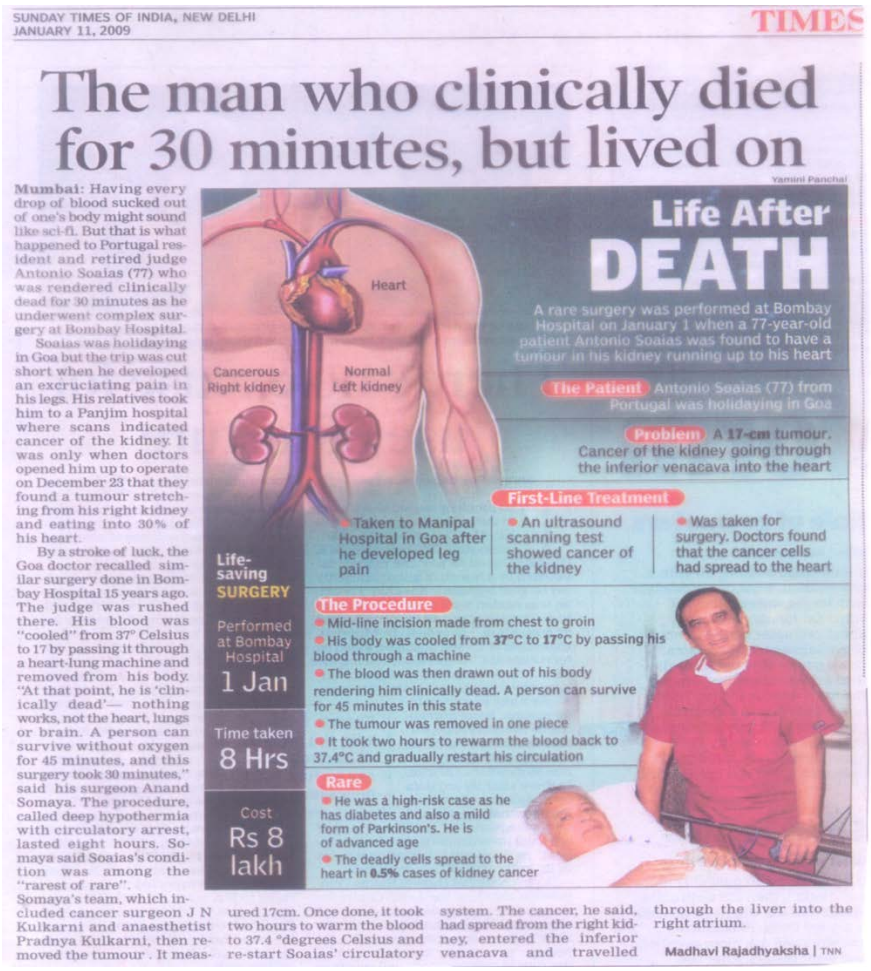
organism? Obviously it does not apply. Let us look at it from common sense perspective. We notice that if one provokes a living organism (say a dog or a cockroach or a house fly), it responds by some behavioral change. However this does not apply to every case. For example if one kicks a big tree, one feels pain in the leg but the tree would not have responded. Can we call a tree as non-living? We will come back to this later to say the tree does respond but one cannot perceive it with eyes or ears. Let us take another common example. Look at the following figure. Let us discuss it.



SUNDAY TIMES OF INDIA, NEW DELHI
JANUARY 11, 2009

TIMES

The man who clinically died for 30 minutes, but lived on

Yamini Panchal

Mumbai: Having every drop of blood sucked out of one's body might sound like sci-fi. But that is what happened to Portugal resident and retired judge Antonio Soaias (77) who was rendered clinically dead for 30 minutes as he underwent complex surgery at Bombay Hospital.

Soaias was holidaying in Goa but the trip was cut short when he developed an excruciating pain in his legs. His relatives took him to a Panjim hospital where scans indicated cancer of the kidney. It was only when doctors opened him up to operate on December 23 that they found a tumour stretching from his right kidney and eating into 30% of his heart.

By a stroke of luck, the Goa doctor recalled similar surgery done in Bombay Hospital 15 years ago. The judge was rushed there. His blood was "cooled" from 37° Celsius to 17 by passing it through a heart-lung machine and removed from his body. "At that point, he is 'clinically dead'— nothing works, not the heart, lungs or brain. A person can survive without oxygen for 45 minutes, and this surgery took 30 minutes," said his surgeon Anand Somaya. The procedure, called deep hypothermia with circulatory arrest, lasted eight hours. Somaya said Soaias's condition was among the "rarest of rare".

Somaya's team, which included cancer surgeon J N Kulkarni and anaesthetist Pradnya Kulkarni, then removed the tumour . It measured 17cm. Once done, it took two hours to warm the blood to 37.4 °degrees Celsius and re-start Soaias' circulatory system. The cancer, he said, had spread from the right kidney, entered the inferior venacava and travelled through the liver into the right atrium.

Madhavi Rajadhyaksha | TNN

Life After DEATH

A rare surgery was performed at Bombay Hospital on January 1 when a 77-year-old patient Antonio Soaias was found to have a tumour in his kidney running up to his heart

The Patient Antonio Soaias (77) from Portugal was holidaying in Goa

Problem A 17-cm tumour. Cancer of the kidney going through the inferior venacava into the heart

First-Line Treatment

- Taken to Manipal Hospital in Goa after he developed leg pain
- An ultrasound scanning test showed cancer of the kidney
- Was taken for surgery. Doctors found that the cancer cells had spread to the heart

Life-saving SURGERY

Performed at Bombay Hospital: 1 Jan

Time taken: 8 Hrs

Cost: Rs 8 lakh

The Procedure

- Mid-line incision made from chest to groin
- His body was cooled from 37°C to 17°C by passing his blood through a machine
- The blood was then drawn out of his body rendering him clinically dead. A person can survive for 45 minutes in this state
- The tumour was removed in one piece
- It took two hours to rewarm the blood back to 37.4°C and gradually restart his circulation

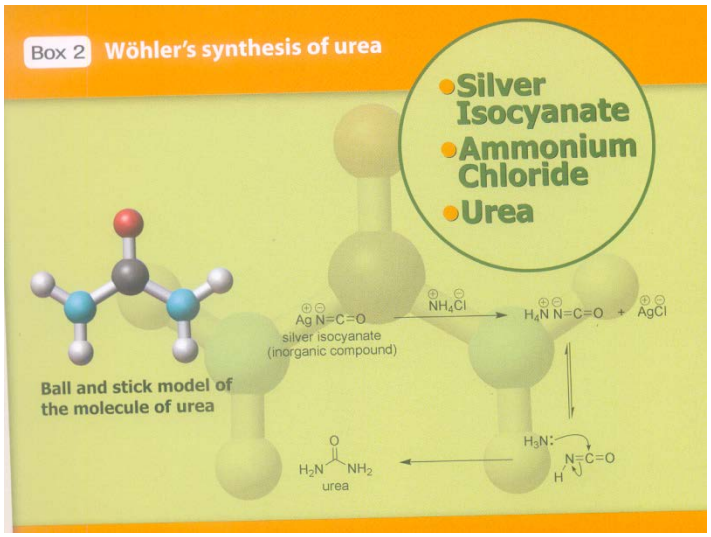
Rare

- He was a high-risk case as he has diabetes and also a mild form of Parkinson's. He is of advanced age
- The deadly cells spread to the heart in 0.5% cases of kidney cancer

A patient in ICU of a hospital is declared 'brain-dead' by the doctor. The vital functions of the patient however, are being maintained with the help of a Heart-Lung machine. The patient does not recognize near and dear people around. Can we call the patient dead? The family would refuse to call the patient dead but the doctor has pronounced the patient dead. We are confused. All the above definitions of living state are deficient in some sense. From the perspective of Biology, you would have been told that anything that grows, reproduces and responds to external stimuli can be pronounced as living. Even here there are exceptions to the rule. There are many humans who do not have children. They are called infertile couples. But we do not consider them as non-living! A hillock also grows over a period of millions of years. Still we do not include a hillock or a mountain under the category of living organisms. The reason is non-living things increase in size by the process of accretion i.e. involuntary

deposition of material on the surface. But a living organism grows by the process of 'intussusception' i.e. from inside. At this point, it looks like ability to respond to external stimuli would stand as a correct definition of living state. All we need is a method to measure the type of response. Trees respond, to sunlight, to many trace elements in soil water, to insect pests etc. Hence they are living objects. If we measure the starch content of leaf from a plant exposed to sunlight and compare it to that kept in dark, one can notice the difference. In other words, one needs a 'scientific method' (a carefully designed and spelt out protocol) to prove what is not obvious to naked eyes or ears.

Let us discuss it at higher level. Biochemists are those scientists who apply the concepts and techniques of Chemistry to analyze and understand Biological structure and physiological processes. They also asked the same question i.e. what is the difference between a living organism (even if it is dead!) from an inanimate object like a sample of top soil. They initially sought to know whether there is any difference in terms of elemental composition. This a typical chemistry question. They have a method of doing 'elemental analyses'. One needs some instruments for this. When they did the experiment, they realized that, qualitatively speaking, all the elements present in a soil sample are also found in a tissue sample from a living organism. However, quantitatively speaking, living organisms have relatively more carbon, hydrogen, oxygen etc. than in inorganic substances like glass or soil sample. They also discovered, upon further analysis, that living organisms have more compounds of carbon than inanimate objects. When F. Wohler demonstrated successful synthesis of urea, an organic compound found in living organism (you and me for example (see figure below). An adult human being excretes 30 gms of urea every day in 24 hrs through urine!), we got another definition of 'living' i.e. living things have organic compounds while non-living (excluding dead) (soil or glass) objects have inorganic compounds. When they asked further questions as to what these organic compounds (called by biochemists as 'Biomolecules') are doing inside the organism, they got the answer. The answer was that all the organic compounds inside the living organism are constantly undergoing chemical reactions. There is a simple method to demonstrate this. Feed a dog a known chemical. After sometime, analyze the urine and fecal matter from the dog and find out whether the fed compound retained its structure or underwent chemical alterations. Scientists discovered thousands of such biochemical reactions.



$$H_2N\text{-}\overset{\overset{O}{\|}}{C}\text{-}NH_2 + H_2O \xrightarrow[\text{Urease}]{} NH_3 + H_2N\text{-}\overset{\overset{O}{\|}}{C}\text{-}OH$$

$$H_2N\text{-}\overset{\overset{O}{\|}}{C}\text{-}OH + H_2O \longrightarrow NH_3 + H_2CO_3$$

Fig; An example of a biochemical reaction.

The sum total of all the biochemical reactions inside our body is called 'Metabolism'. Hence metabolism became an indicator of living state. Further, biochemists, took out a tissue (say apiece of liver or a leaf) from an organism and incubated in test tube with a chemical compound and demonstrated that under some conditions even the tissue can metabolize. Hence intact body is not essential for exhibiting metabolism. Therefore the tissue is living even though the body from which it is taken is dead. They went further and demonstrated that cells from the tissue also exhibit metabolism. Hence cells are living even though the tissue is dead. Further, when they broke the cells and still found metabolism in cell-free extracts, one realized that cellular integrity is not required for metabolism or 'living'. All these biochemical reactions are catalyzed by enzymes or biocatalysts. Hence an enzyme in a test tube is a living object. Therefore one can conclude that "metabolism is a defining property of living state". You will not find an exception. By this definition a virus is definitely a non-living object. Let us go even further to understand the 'Living State' as opposed to non-living state including 'Dead state'. Physicists are those scientists who study the physical properties of 'Nature' and from that discover the 'Laws' which Nature obeys and which explain Natural phenomena like electricity, magnetism, gravity etc. One of the fundamental laws of Nature is the Law (s) of Thermodynamics which discusses the nature of energy in this universe. It asserts that Energy can neither be created nor destroyed. It also says that energy flows from a state of high energy to a state of lower energy. It is similar to the saying water finds its level. Every system in this universe has internal energy. This has two components. One, the useful energy or Free Energy and the second, Entropy which is an indicator of disorder. Any spontaneous process,

physical or chemical occurs in the direction of decrease in energy and increase in entropy. Equilibrium is a state where free energy change is zero (▲G equals 0). This means systems at equilibrium cannot perform work. All living organisms as long as they are living perform work. What type of work we do? We do mechanical work (movement), osmotic work (during absorption at the end of digestion) and biosynthetic work (making more biomass during growth). Any work requires energy. From where do the living organisms get energy? They take food. Food is complex. We digest this food. So does an amoeba or cockroach or an alga. In other words we break it down. This liberates energy. That energy is stored as bond energy(in the form of energy currency called ATP). The day the living organism cannot extract energy from food, and use the stored energy and currency energy to do work, it is dead. Death is therefore similar to thermodynamic equilibrium. Hence living organisms are energy machines, from the perspective of Physics. Living state is therefore a non-equilibrium state in which the organism creates stores and utilizes energy. We got another definition of living organism. Now you notice how difficult it is to define and answer the question 'what is a living organism'.

Order in Living Organisms

Look at any living organism and we notice a degree of order in structure and in function. Symmetry is one aspect of this order. Perfect symmetry is synonymous with 'beauty'. The structure of any living organism exhibits this order in shape and size and this is reproducible generation after generation. Observe 'Nature' and you will observe high degree of order in form. More important than this is the order in living processes. Take any physiological process like movement, digestion, growth, reproduction, development, behavior etc and one notices only order in the sense each of them is a perfectly coordinated set of linked cellular and molecular events in space and time. You will take some more time and deep study of Biology to understand this statement made above. Just watch a galloping horse, a frog catching its prey and gulping it, a honey bee visiting flowers and picking them and making honey out of the flower contents, an eagle flying, a bat scooping its prey, the movements of a great foot ball player or cricket player or a pitcher plant trapping an insect etc and you notice beauty or symmetric movements or order. Metabolic reactions which are underlying all these physiological activities are ceaseless and do not stop even a moment. Moreover they occur in the form of linked reactions looking like metabolic pathways. The metabolite traffic in metabolic pathways is similar to automobile traffic in city roads. But unlike automobile traffic, nobody has heard of accidents. Metabolite traffic in our cells is perfectly controlled in speed and direction at traffic junctions. In a multicellular organism like a horse or human being, there are so many different tissues, in all of which metabolism is going on and there appears to be coordination among them. It is similar to a symphony orchestra or an army marching towards and fighting the enemy or any group activity which pleases the eye or the ear. Any coordinated group activity is not possible unless there is communication among the components of the group. Therefore we come to understand that communications among the components of a group is the basis and explanation for the observed ordered group activity be it a cricket team or a national government or a complex living organism. Even a simple unicellular prokaryote like a bacterium has a high degree of structural and functional complexity. Hence all of such groups have evolved elaborate systems of intra- cellular, inter-cellular-communication, intra-population and inter-population systems of communication. Complex human behavior without a language is unthinkable. Let us briefly look at these

systems of communication.

Communications systems in living organisms

We have realized by now that Energy input and communication among components of an organism as well as between organisms and environment keep the living organism in a state where all physiological and behavioural processes occur like a well orchestrated symphony without a jarring note. Communication is simply a codified message. It takes place, say for example, among organisms. The most evolved of these is human language. But we are not going to discuss this today for many reasons not the least, is the fact that man is clever enough to use language to hide his thoughts and not to convey any message. The evolution of languages over time from a comparative linguistics perspective reveals the story of human migrations. But this evolution is not due to selection pressure and hence we will not discuss this also. Communication in the living world can be classified, for the sake of convenience, into three types. ONE, that between or among organisms of a group (technically called a population) or between species. It could be either intentional or unintentional and results in influence/effect. TWO, that between inanimate and animate world. I do not like to call it even communication. It is influential but not intentional (for example, photo period on living organisms) and THREE, that within an organism between cells/tissues. It is not intentional but results in the regulation of a set point. Let us understand this.

We refer to the systems of **signals** operating within a higher animal or plant and which is essentially inter-cellular communication. The signals are mostly chemical in nature but occasionally physical like light etc. We will discuss the nature of these signals, their origin and transmission, their reception and decoding and how they bring about **control, regulation** and **order** within the living organism. To understand this idea properly, let us start with our day to day experience. We sense many things of the environment. We sense light through eyes, sound through ears, taste through tongue, pressure through touch and smell through nose. These are popularly called five senses. If you think a bit and reflect upon this, you realize we sense many more. In addition to these five, we sense direction (magnetoception), pain (nociception), balance (equilibrioception), acceleration (kinethesioception), time (zeitception), space (volume/size) and quorum (number). In fact, the greatest research problem in Biology is to know how organisms measure space and lapse of time! If we call what we sense as signal, then sensing would be receiving these signals. What enables us to receive the signals is called 'Receptor'. For a human being at the common sense level, the receptor is the special sensing organ like eyes, ears, skin, tongue and nose. At another organization level, these are cells at specific location like the retinal cones and rods as photoreceptors etc. At the molecular level, these are proteins located in some sub-cellular organelle, usually the cell membrane otherwise called plasma membrane. Let us understand these signals and receptors.

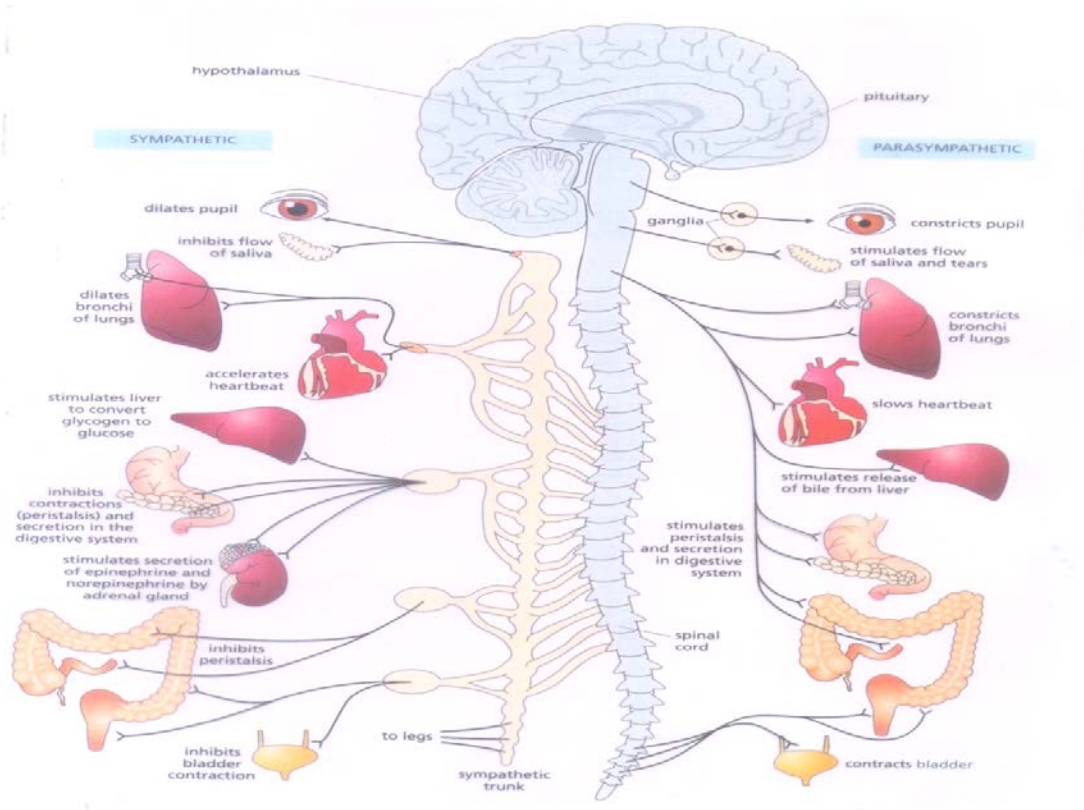
Signals

We have already mentioned that communications are carried out through chemicals and sometimes through physical entities. Light and sound are physical entities. Light and sound travel in wave form and hence two properties are important. One, the wave length and the related frequency (energy content) and two, the amplitude or the intensity. Pressure (sound and touch) is sensed through mechano receptors. Light is sensed through photo

receptors. Chemo receptors sense taste and smell. What is interesting to note is the fact independent of the fact whether the signal is physical or chemical, the receptors are chemical in nature. They are all proteins. Environment can be either external to the corporeal body in the true sense or other cells/tissue but within the body. Accordingly
signals arise from outside the organism (true environment) or from within the organism as in the case of multicellular lower invertebrates and lower plants, advanced organisms like vertebrates, higher plants and many higher invertebrates like arthropods, crustaceans etc. Most of these signals from internal environment are transmitted through diffusion while some are carried by blood. The tissue or cell that carries the receptor is called the target tissue or cell. Based on the cell/tissue of origin and the mode of transport of the signal as well as the proximity of the target cell/tissue, the effects are classified into autocrine, paracrine and endocrine categories. Let us now focus on higher invertebrates, vertebrates, higher plants and discuss signals which originate within the organism and affect some other cell/tissue but within the organism.

Hormones and Neurotransmitters

Neural and Endocrine systems represent two types of signal systems in our body. Neural signals are like telegrams (fast, loud and short distance). Endocrine signals are like postcards (slow, whispering and long distance). There are subdivisions in each type. The endocrine tissues are located in many regions of our body. Try to locate these in the case of a cow or human being or cockroach or earthworm etc by reading and talking to your biology teacher. Pituitary, thyroids, pancreas, gonads etc are some examples for mammalian endocrine tissues. Other vertebrates also have similar endocrine tissues. Comparative endocrinologists and evolutionary biologists study other organisms and try to understand how such sources and their corresponding signals are organized including their location and complexity. These endocrine or comparatively similar signals are named 'Hormones'. In many organisms, there are no typical endocrine tissues or glands. In such cases the hormones are not directly secreted into the blood stream. The neural system in the case of mammals is organized into Central Nervous system and Peripheral Nervous system.



A diagram indicating the peripheral (sympathetic and parasympathetic) neural system of nerves and the functions they control in a mammal.

The signals from neural system are named 'Neurotransmiters'. Neurotransmitters are released from nerve endings in all animals, from simple to complex. Plant systems do not have nerves. Both hormones and neurotransmitters are biomolecules. Biochemists are interested in knowing their chemical nature. You must have already come across the different chemical classes into which biomolecules are classified. Read your class XI and XII biology text books. Accordingly hormones can be simple amino acid derivatives like epinephrine or thyroxine, steroids like estrogens and androgens, polypeptides like insulin and oxytocin or proteins like Growth Hormone or even glycoprotein like Luteinizing Hormone (LH). Similarly neurotransmitters are either amino acid derivatives like serotonin and Dopamine or peptides.

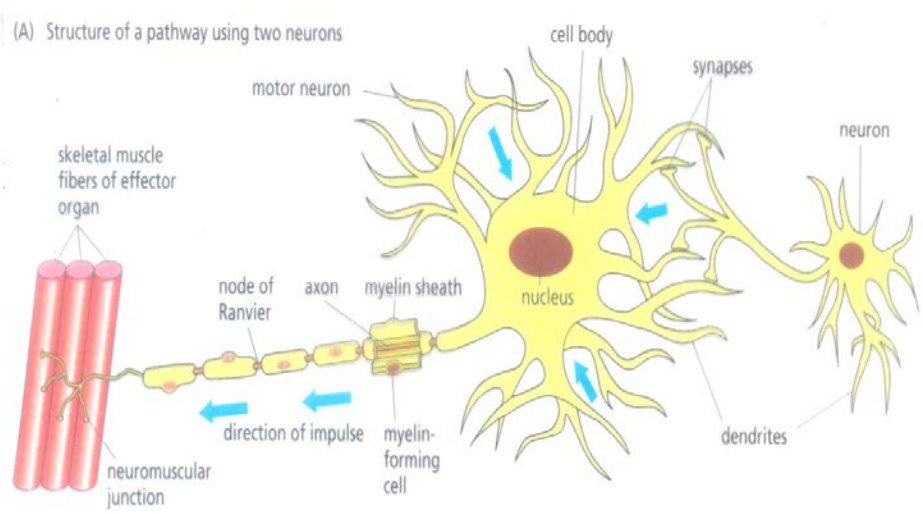


Diagram showing neurons and synaptic junctions and other parts.

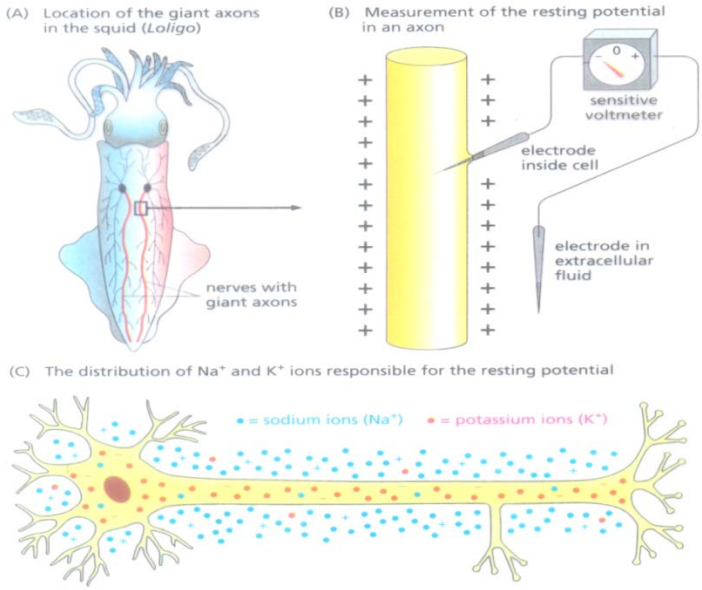


Diagram showing a typical nerve and the nature of signal transmission along the axon.

In prokaryotes and single celled eukaryotes, environmental signals (nutrients, another single cell/ organism secretary product like antibiotic etc) are sensed by the whole cell through membrane receptors and the target cell as such responds in some way through biochemical processes. Thus it is a single cell system. In higher multicellular systems the environmental signal might be sensed by some cell/tissue and responding cell/tissue could be different. In higher vertebrates it could even be three stage processes. Hence elaborate sensing and responding systems have evolved over millions of years resulting in neural, neuroendocrine and endocrine/paracrine cell/tissue systems. Thus in a typical vertebrate (fish to mammals), environmental ques/signals are sensed by neural system of brain and its parts. In turn the central neural system of brain and its parts responds by sending signals to neuroendocrine

cells/tissue which in their turn respond by sending neuroendocrine signals to which another endocrine tissue/cells respond. Finally the signals from the endocrine tissue/cells elicit response from peripheraltarget tissues which themselves could be another type of endocrine/non-endocrine tissue. Let us take an example from a mammal. By now you must be familiar with the names and locations of different endocrine tissues/cells in a typical mammal like human being or a cow or a rat. Typical endocrine tissues are pituitary, thyroid, pancreas and gonads (ovaries and testes).

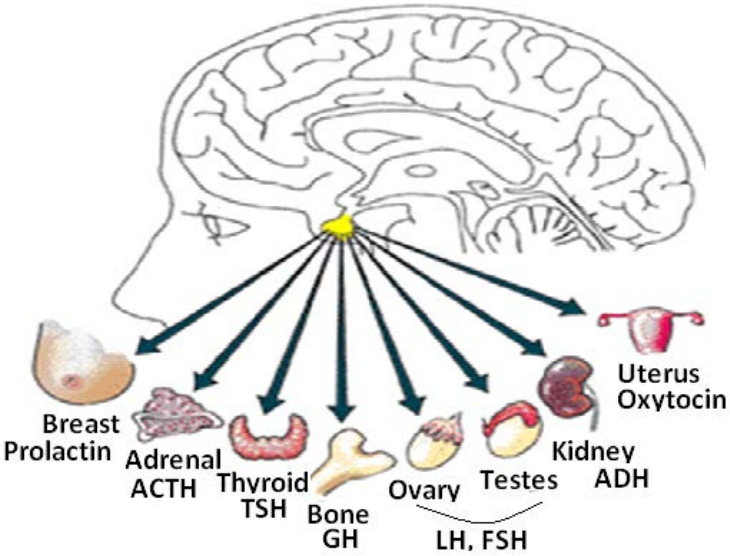


Diagram showing pituitary and how its secretions influence different peripheral target tissues.

In a typical case, the environmental signal like light is received by photoreceptors in neural system (say pineal tissue). The brain parts send out in response, signals to hypothalamus- a neuroendocrine tissue. The hypothalamus, in turn, sends neuroendocrine signals to pituitary. The pituitary in turn sends appropriate signals to peripheral target tissues. Some of the peripheral target tissues themselves might be endocrine tissues like gonads or thyroids. Ultimately the signals regulate target tissue metabolism and through that physiological processes.

We will not discuss here an important aspect of this whole story. This is about mechanism of signal sensing and responding at cellular and molecular level. How does the target tissue sense and respond to the signal from internal environment? More importantly how do we respond to external environmental signals with responses like memory and emotions? This is a highly complex subject and you may have to study at a later stage in your education, a subject like Neurobiology to understand these aspects. Let us understand the basics of signals and their characteristics.

Discovery of Hormones (signals)

How are hormones discovered to begin with? One needs a scientific method. The method used is called 'surgical extirpation followed by replacement therapy'. Look at the

following diagrams carefully and let us understand.

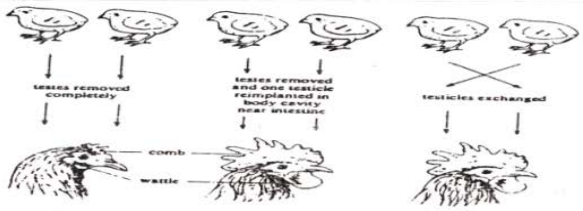
1.2 The discovery of hormones

- Berthold's experiment
  In 1849 a German doctor called Berthold carried out the first experiment that showed how hormones work (Fig. 1.3). He castrated six young cockerels which led to the regression of their combs and wattles. He then implanted one testicle into the body cavity of some of the birds, and exchanged testicles between birds. The castrated birds with testicular implants showed normal sexual behaviour and developed typical male features. Examination of the implanted birds showed that the testicles had regrown connections with the blood but not with the nervous system. Berthold concluded that some substance must be produced by the testes and released into the blood to maintain male sexual behaviour and sexual characteristics.

- Bayliss and Starling's experiment
  The word hormone was proposed some 50 years later by Bayliss and Starling in 1902. They demonstrated the existence of a substance that was produced by the duodenum and that stimulated the secretion of pancreatic juices (Fig. 1.4). They called this hormone secretin.

- Identification of a hormone
  Hormones are generally identified by reducing the function of a tissue (e.g. by removal of the tissue), and then restoring the function by injections of an extract of the tissue. The hormone is the active principle in the extract.

- Hormones and parahormones
  Hormones travel in the blood to distant target organs. There are also parahormones that are secreted into the interstitial fluid surrounding cells and that act locally within the same organ. When cells produce compounds that act on neighbouring cells this is called a paracrine effect. An example is the release of insulin from cells in the pancreas. Insulin is carried in the blood to distant target cells, but also acts locally within the pancreas to inhibit glucagon release from nearby cells.



Berthold's experiment that showed in 1849 how the testes produce a hormone that is carried in the blood to a distant tissue (from Stewart, 1991).

Disclaimer: This diagram and all others are adopted with some modifications from different textbooks and are not original.

In this experiment, a part of the body is surgically removed (for example orchiectomy or removal of testes) and the consequences in the operated animal watched. In this case the adult bird did not grow secondary sexual characteristics like crown and wattle. However when the extirpated testes is put back into the chick anywhere in its body cavity, the adult bird from this chick gets back both crown and wattle. It was concluded that a diffusible chemical travelsfrom testes to those sites and induces the formation of wattle and

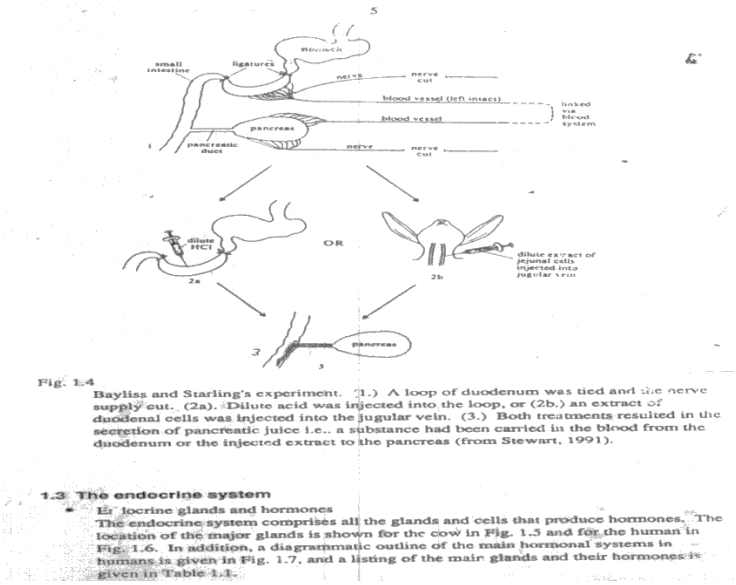


Fig. 1.4
Bayliss and Starling's experiment. (1.) A loop of duodenum was tied and the nerve supply cut. (2a). Dilute acid was injected into the loop, or (2b.) an extract of duodenal cells was injected into the jugular vein. (3.) Both treatments resulted in the secretion of pancreatic juice i.e., a substance had been carried in the blood from the duodenum or the injected extract to the pancreas (from Stewart, 1991).

1.3 The endocrine system

Endocrine glands and hormones

The endocrine system comprises all the glands and cells that produce hormones. The location of the major glands is shown for the cow in Fig. 1.5 and for the human in Fig. 1.6. In addition, a diagrammatic outline of the main hormonal systems in humans is given in Fig. 1.7, and a listing of the main glands and their hormones is given in Table 1.1.

Diagram showing Starling's classical experiment leading to the discovery of Secretin, the first hormone discovered.

crown. This is because the testes cannot move to those places and affect the distant tissue. That diffusible substance was called as a 'Hormone' (means to excite). This came about in 1902 and usually Starling is credited with this discovery. In this case, injection of dilute hydrochloric acid into denervated duodenum or of jejuna cells extract into jugular vein led to bicarbonate secretion from denervated pancreas, indicating that a chemical from one tissue traveled through blood and induced changes in a distant tissue. The proof of this conclusion is completed only when the suspected hormone is purified from its source and the 'pure' chemical elicits the same response in such animals as the tissue extract. In the next hundred years, many other scientists performed similar experiments and discovered new hormones for a variety of functions in different animal groups. In your class XI and XII text books, the list of hormones and their functions is given. Kindly study that carefully after you go back. At this juncture, we should know that many different classes of chemicals (biomolecules) function as hormones. Moreover, they have been evolving also. Comparative endocrinology teaches us that the same hormonal activity in different phylogenic groups is represented by slightly different structures. For example LH from different vertebrates is the same protein in overall architecture but different in amino acid sequence. More interestingly a protein hormone like pituitary 'Prolactin' exhibits more than 100 different physiological activities in vertebrates from fish to mammals. Evolutionary biologists, tell us that responding tissues have adopted preexisting chemicals as hormones and subsequently both the hormones and their corresponding 'Receptors' have co-evolved. Endocrinologists are interested to know what structural features of a hormone determines it hormonal activity in a given phylogenic group. For example Estradiol-17 β is a female sex hormone but estradiol 17 α is not a hormone. Progesterone is a gestational hormone in eutherian mammals but 20 α dihydro progesterone. On the other hand, the latter is the gestational hormone in a marsupial like kangaroo. Let us understand the common significant characteristics of hormonal mode of communication in mammals.

Feedback Inhibition and Feed forward Activation

Hormonal signals from hypothalamus or pituitary are chemicals and belong to the class called peptides and proteins. When a pituitary hormone like Thyroid stimulating hormone (TSH) comes into blood circulation and stimulates thyroid gland to secrete Thyroxin (T_4) hormone. When the level of Thyroxin in the blood reaches a certain value (called threshold), increasing level of T_4 will invariably affect the pituitary and can stop further secretion of TSH from pituitary. This phenomenon is named 'Feedback inhibition'. You may recall electronic circuits where stimulation leads to, after some level, closure of the circuit by similar process. This results in silencing of the stimulatory signal. In this area of Biology, one uses terminology like Hypothalamus-Hypophysis (pituitary)-thyroid/gonad axis. Further analysis revealed that there are three types of feedback loops operating in the animal. One, 'long feedback loop' when a gonadal/thyroid hormone cuts off secretion from the hypothalamus, two, 'short feedback loop' when the same cuts off secretion from the pituitary and three, 'ultra short feedback loop' when the one pituitary secretion cuts off the secretion of another pituitary hormone.Other types of feedback loops also have been demonstrated

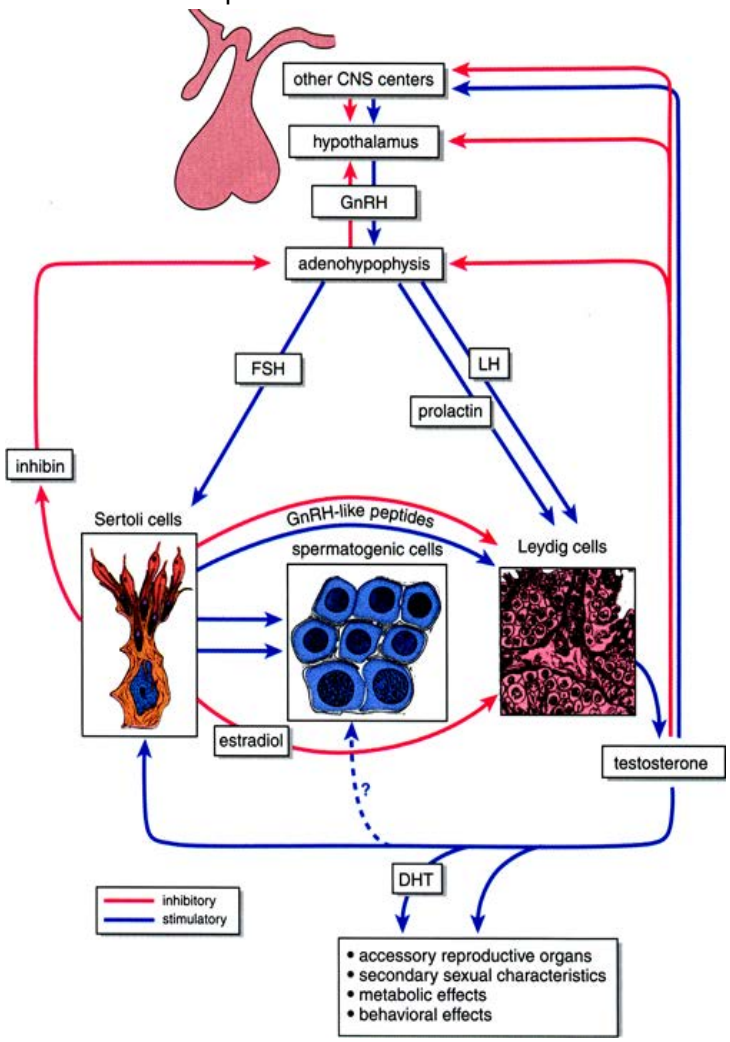


Diagram indicating different feedback loops operating in the case of hypothalamo-hypophysial-gonadal axis in mammals.

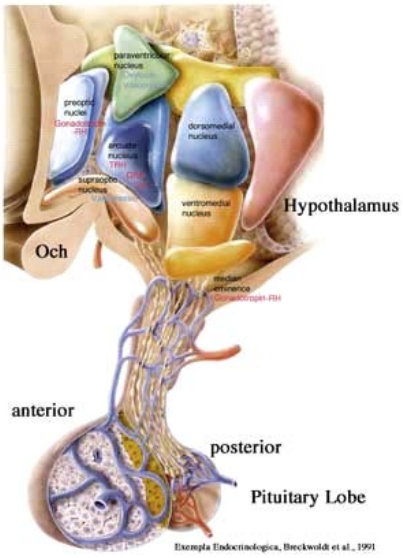


Diagram showing some details of hypothalamo-pituitary region

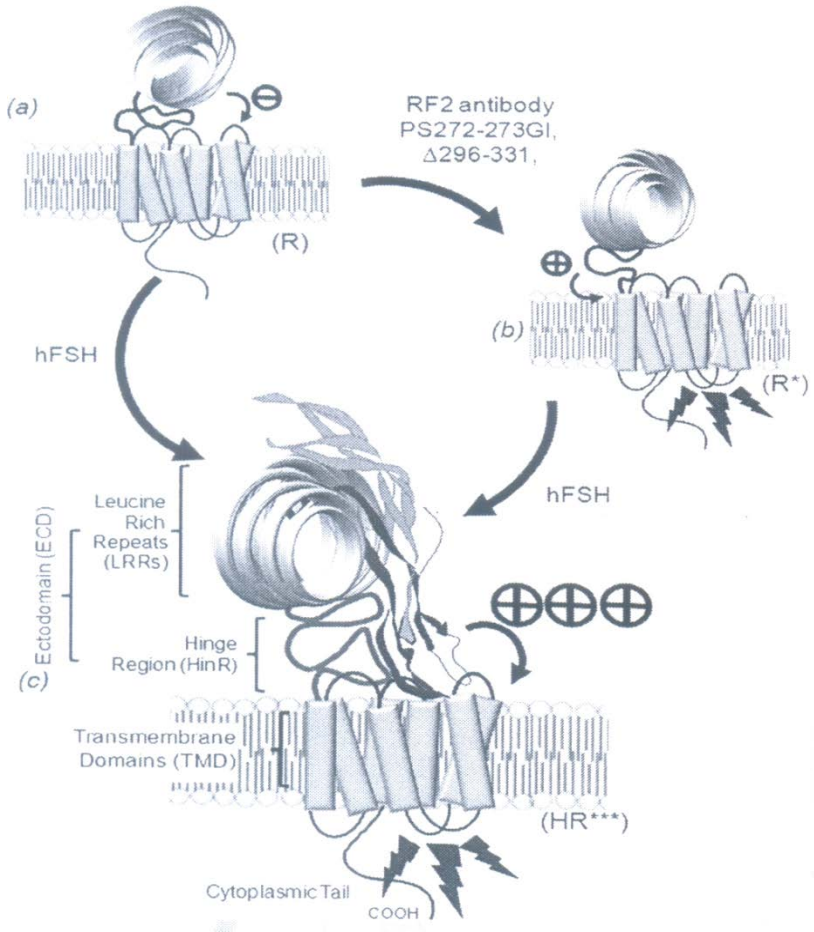
Thus we learn that feedback inhibition and feed forward activation are characteristics of signaling mechanisms in our body. There is a network of signaling and metabolic pathways with built in feedback loops which regulate living processes. Let us now understand how signals are decoded by the target tissues.

Receptors (cells and biomolecules)

Any physiological process like digestion, growth, reproduction, development is a series of linked cellular processes all of which have underlying biochemical reactions including metabolic reactions and molecular events like gene expression. Hormones and neurotransmitters, being message carriers (hence called signals) essentially regulate these molecular events by serving as accelerators or decelerators. When we use the term 'signal' and apply to hormones and neurotransmitters, one must clearly understand that what actually constitutes the signal is not the presence or absence of hormone or neurotransmitter. It is the change in their concentration that constitutes the signal. Further for a hormone to produce its effect, the target tissue must have a mechanism of decoding the signal. In built into this whole activity, the dialogue between the messenger (signal) and the target tissue/cell is highly specific. No other tissue/cell should have the ability to listen to this nor able to decode the particular signal. How is this achieved? Even though blood carrying all sorts of signals moves through all tissues, the response to a particular hormone/signal is seen only in a particular target tissue/cell. After a series of investigations, scientists realized that this is so because, only the target tissue is endowed with the presence of 'listening posts' or Receptors. RECEPTOR is a pharmacological term. It refers to the entity in the target tissue which exhibits 3 functions as follows:

i) Signal discrimination
ii) Signal transduction
iii) Signal amplification

What does this mean? If hormone is the signal, the receptor in the target tissue like the photoreceptors in our retina, has the ability to bind the hormone fairly tightly, retain it against the forces of flowing blood, transduce this signal recognition process into response process. In the process one notices amplification of the signaling molecule as well as the signal effect. Technically speaking, one can say hormonal action is not a stoichiometric process like all chemical reactions but a catalytic process. It is like a loud speaker. A whisper through the microphone is amplified into a loud signal. That is how one notices that the effects of hormones are dramatic like growth of a child into an adult, the development of a fertilized egg into a fetus etc. These receptors are proteins in chemical language. They are usually located on the plasma membrane or nucleus. The receptor protein carries out only the first of the three functions. The other two happen inside the cell in the form of a cascade process akin to blood clotting. Two such processes are shown diagrammatically below.



Cartoon indicating the interaction of Follicle stimulating hormone (FSH, the signal) from the pituitary with the ovarian cell receptor shown as drums embedded in the plasma membrane. The binding is followed by transduction which is an intramembrane event(s) which generates second messengers and leads to, in a cascade fashion through signaling molecular

intermediates, the final event of growth and differentiation of ovarian follicles.

Let us reiterate once again. Hormones or for that matter any signal does not initiate any process but only accelerates or decelerates an existing process. Further it is not one way traffic. The process is regulated so that the response action (process) takes place limited in space and time. In the case all physiological processes, we have a fairly good description of the cellular and molecular events taking place in the target tissue. What we do not understand is what prevents the response of the target tissue during certain periods of ontogeny.

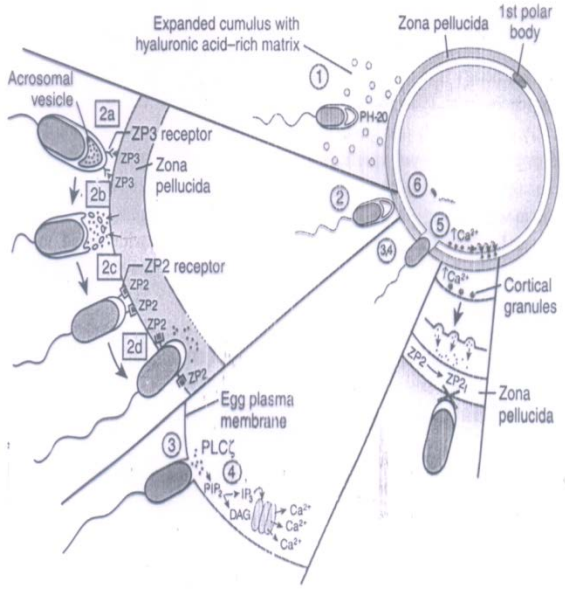


FIGURE 10-2 ■ Events of fertilization: 1, penetration of expanded cumulus; 2, acrosome reaction; 3, fusion of sperm membrane with egg membrane and 4, egg activation; 5, exocytosis of cortical granules and block to polyspermy. DAG, diacylglycerol; IP_3, inositol 1,4,5-triphosphate; PIP_2, phosphatidylinositol 4,5-biphosphate; PLCζ, phospholipase Cζ; ZP2, ZP3, zona pellucida glycoproteins 2 and 3; $ZP2_f$. *(Courtesy of Dr. Lisa Mehlmann, Department of Cell Biology, UConn Health Center, Farmington, Conn.)*

Cartoon showing events during fertilization of an egg by sperm in a mammal.

For example, during pre-puberty age, gonads exist and the concentration of pituitary hormones like FSH and LH also is very high. Paradoxically it is called sexually immature stage. Obviously receptors are undergoing changes during ontogeny as well as evolve during phylogeny. We can conceptualize this by saying that target tissue response is regulated not by extracellular hormones and other factors but also by receptors themselves by undergoing change in number and affinity. You will understand this statement when you go to higher classes in biology.

Communications in behavioral processes

We have said in the beginning that living represents a high degree of ord, we are not clear about many details.er. This is true for any physiological, developmental and behavioral process. We have also understood to some extent that through communications among the participating tissues or individuals, any group activity can be regulated. The biochemical description of this orchestration by hormones and receptors was also clear. We must admit however, that in the case of much part of development (embryogenesis and aging or senescence) we are not clear about many events. Death is the most mysterious of all such

events of which we do not understand anything in any detail. In the case of behavioral events (emotions, memory etc) also, not much progress has been registered. Some inroads have been made but not to complete satisfaction. Let us take two examples to explain this situation.

What is Health and Happiness?

We understand illhealth but not health. Any physical or mental discomfort is unhealthy. Absence of discomfort is not health. Even the World Health Organization defines health peculiarly as "physical, mental and spiritual l well being". Research work in the area of biomedical sciences has come to this tentative conclusion that communications and hence regulated events in the three organ systems of our body i.e. immune, endocrine and neural systems results in health. Any perturbation in any of these with respect to steady state conditions lead to unhealthy! (see the diagram below). In human history, different societies have interpreted health and unhealthy differently. The four humor idea of Hippocrates, the *panchaboota* and *tridosha* ideas in Ayurveda system or the ideas of *ch'i*, *prana* and *pneuma* in Chinese, Hindu and Greek thoughts respectively are some examples.

Mind (Brain), Immune system, Endocrine system

A publication in the prestigious journal *Science* has claimed that the neuro transmitter Serotonin is behind the feeling ' that I am being cheated'. When a person who was witness to a robbery was bribed with 20% of the loot was satisfied and did not report the matter to police. The same person, after one week of being fed on a tryptophan-free (tryptophan is an amino acid and is the biosynthetic precursor to serotonin in our body) diet felt offended by the 20% offer in the same experiment and was satisfied only with a 45% offer. You can see that we are far from understanding complex behavioral processes in biochemical terms as in the case of hormones.

Serotonin Modulates Behavioral Reactions to Unfairness

Molly J. Crockett,[1,2]* Luke Clark,[1,2] Golnaz Tabibnia,[3] Matthew D. Lieberman,[3] Trevor W. Robbins[1,2]

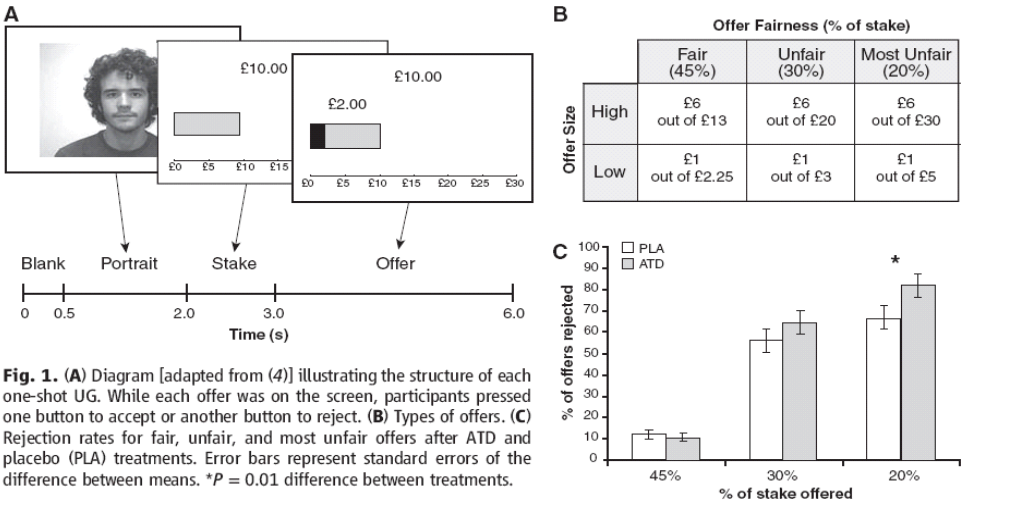


Fig. 1. (**A**) Diagram [adapted from (*4*)] illustrating the structure of each one-shot UG. While each offer was on the screen, participants pressed one button to accept or another button to reject. (**B**) Types of offers. (**C**) Rejection rates for fair, unfair, and most unfair offers after ATD and placebo (PLA) treatments. Error bars represent standard errors of the difference between means. *$P = 0.01$ difference between treatments.

Paradigms and Puzzles

We get the feeling that all physiological processes including development are regulated and orchestrated where nothing goes wrong. It gives the impression of programmed events. We fall into the trap of cause and effect relationship discussion and believe that all living processes follow predetermined pathways. When we observe certain behavioral processes (foraging behavior, developmental behavior etc) at molecular or biochemical level, we get puzzled and dissatisfied with deterministic models. For example how does the chick penguin know that it has to go and swim in freezing waters in due course of time and as though in anticipation of that develop a thick fatty layer on the front to insulate against the biting cold? All of you should start reflecting on what you have studied and learnt. I wish you all the best in your lives.

***Acknowledgements*:** I am grateful to INSA for sponsoring these lectures addressing undergraduate and school children. I am grateful to Prof BBP Gupta of NEHU who could make arrangements for the lectures. I am also grateful to Prof S. K. Saidapur who initiated this activity of getting the lectures printed and published. They will surely serve as supplementary reading material at school level and in the process provoking them to think independently. Einstein once remarked that "it is a miracle that curiosity survives formal education". Hopefully these INSA lectures keep the curiosity and scientific spirit among school going children.

Suggested Readings:

1. Porterfield, SP and White, BA (2007) *Endocrine Physiology*, 3rd Edition, Mosby Elsevier

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Section II: Endocrinology and Reproduction

Reproductive Strategies in Vertebrates

Bhagyashri A. Shanbhag
INSA Senior Scientist
Department of Marine Biology
Karnatak University P.G. Centre, Kodibag, Karwar- 581303
Email: bhagyashrishanbhag@gmail.com

Reproduction is a complex biological phenomenon. Successful reproduction is basic to the fitness of any organism. In evolutionary biology parlance, *fitness* refers to the number of offspring produced by an individual that survive to reproduce. An individual spends certain proportion of the total energy budget for reproduction leaving aside some for the somatic growth and maintenance, mate selection, territory guarding, male-male fight, predator avoidance etc. The amount of energy devoted for reproduction is called reproductive effort (RE) and fitness of an individual depends on RE. We come across distinct but interrelated phenomenon associated with reproduction such as diverse reproductive patterns, modes and strategies. Based on reproductive frequency, the animals may be grouped into two categories, semelparous and iteroparous species. Some vertebrates devote entire reproductive effort to a single event, after which the individual dies. This represents *semelparity*, e.g. Atlantic salmon, eel. Semelparity is also termed a big bang, or explosive reproduction. While most vertebrates, divide reproductive effort into several events and reproduce multiple times (*iteroparity)* throughout their life span. Semelparity does not necessarily mean a shorter life span than iteroparity. For instance, some of the eels reproduce only after attaining the age of 18-25 years.

All vertebrates reproduce sexually and we can distinguish three reproductive patterns. They are (1) Biparental reproduction (involving individuals of opposite sexes and fertilization) and it is most common pattern among vertebrates. A few others reproduce by (2) parthenogenic and (3) hermaphroditic patterns. Parthenogenesis or unisex reproduction (without fertilization) is found in a few species of fishes, amphibians and reptiles. The parthenognetic fishes (e.g. Amazon Molly, *Poecilia formosa*) and the urodela (salamander, *Ambystoma jeffersonianum complex*) consist of only females and mate heterospecific males. Mating triggers the development of eggs without fertilization. Hence, sperm do not contribute any genetic material to the offspring. On the other hand, in the lizard *Cnemidophorus uniparens*, two females mate, one of them acts like male and the other with mature eggs acts as female. The mating facilitates ovulation. The hormonal profile of two mates revealed that the male acting individual has higher levels of progesterone with regressed ovaries while the female acting individual has high levels of estrogen with fully developed eggs.

Hermaphroditic individuals have both types of sex organs. Now, the question is why be a hermaphrodite? If there is diminishing gain in producing egg or sperm, it is beneficial to be

Lecture delivered on November 15, 2013 at Sikkim University, Sikkim

hermaphrodite. In contrast, if there is an increasing gain on investment in male or female, it is beneficial to be single sex. Non-motile and solitary animals and those with sparse distribution exhibit hermaphroditism. Among vertebrates this pattern is found only among some fishes. There are two kinds of hermaphrodites, the simultaneous and the sequential. In simultaneous hermaphrodites, both eggs and sperm are produced simultaneously. Each animal serves as male and female donating and receiving sperm. Every individual is also a potential mate. Many offspring can be produced from single mating than if only one individual's eggs are fertilized. This is found in some deep sea teleosts (e.g. Hamlets: *Hypoplectrus sp)* which occur in low densities, and have reduced opportunity for mating. In sequential hermaphrodites, the individual matures as a member of one sex, reproduces for some time and then changes to second sex. Sequential hermaphroditism is very rare. We come across this pattern in some fish species which start as female first phase, protogyny (Moon wrasse, *Thalassoma lunae*) while others (e.g. clown fish) start as male first phase, protoandry. The direction of sex change can be explained on basis of *size advantage model*. If smaller individuals have higher reproductive fitness as one gender and larger individuals have higher reproductive fitness as the opposite gender, sequential hermaphroditism is beneficial. The species with female first hermaphrodite, large male phase individual defends spawning territories (male-male competition) where the smaller female phase individuals visit to lay eggs (Moon wrasse, *Thalassoma lunare*). Male first hermaphrodites have *scramble competition* for mates (clown fish). Often many male first individuals crowd around one larger female individual to release their sperm while the female releases the eggs.

Among the vertebrates three different modes of reproduction can be distinguished:

Oviparity: The animals lay eggs either unfertilized (external fertilization) or fertilized (internal fertilization) outside the maternal body (e.g. cyclostomes and most selachians, teleosts, amphibians and reptiles, and all birds).

Ovoviviparity: The eggs are retained for longer period in the oviducts until the embryos develop to an advanced stage. The embryonic nutrition is ensured through egg yolk. Examples include several species of teleosts, selachians, some amphibians, a few squamate reptiles.

Viviparity: Complete or almost complete development occurs within maternal body. Here we can distinguish two patterns.

i) Aplacental viviparity as found in several selachians. The developing embryos are nourished through uterine secretions.

ii) Placental viviparity where the developing foetus is connected to the mother by a placenta. Examples include some selachians, several skinks, marsupials, and placental mammals. Thus, viviparity has evolved several times, independently in different groups of vertebrates.

In nature different species respond differently even to similar challenges owing to their evolutionary history. During the breeding season, animals make several important "decisions" concerning their timing to mate, choice of mates, how much time and energy to devote for

rearing offspring, and how to protect their developing young at certain phases anticipating changes in the environment. These decisions constitute aspects of an animal's reproductive strategies, a set of behaviours that presumably evolved to maximize reproductive success. Reproductive strategies have evolved partly in response to the energetic costs of reproduction and the way food resources, nest sites, and members of the opposite sex are spatially distributed in the environment. In short, reproductive strategies reflect how an individual or population responds in a given environment to maximize its fitness or reproductive success. Therefore, one can expect any given species in a given ecological context to reveal series of novel strategies based on their phylogeny. Following are some of the reproductive strategies found among vertebrates.

Mate choice and evolution of sexual selection:

Charles Darwin was perhaps the first person to note that females often do not mate with the first male they encounter and they seem to evaluate a male's quality before deciding to mate. Why? Studies have shown that reproductive/parental investment is high in females. Females, who are higher investors, are the *limiting resource* for males. Therefore, females have to be choosy, and chosen male can provide better parental support or traits to her offspring. In contrast, the males are not a limiting resource for females. This leads to male-biased operational sex ratio, i.e. on a breeding ground there are always more sexually mature males than sexually mature *receptive* females. This results in competition among members of a sex, generally males, for the limited number of receptive females. This competition for mate is called *sexual selection*. Sexual selection involves both *intrasexual* selection, an interactions between members of one sex (male-male competition/combat e.g. deer, bull frog) and *intersexual* selection, essentially mate (female) choice (example, peacock). The intrasexual competitions lead to evolution of characters such as large body size, huge antlers, claws etc needed for winning the combat with competitors, while mate choice leads to evolution of characters such as beautiful plumage in birds, large trail in peacock.

Choosing the right season for reproduction:

To ensure better survival of the offspring, animals time their reproduction in such a way that their offspring are born when the environmental conditions are congenial for their survival. We come across three categories of breeding patterns. They are:

1). Predictable/seasonal: Reproduction becomes a seasonal phenomenon occurring generally during spring/summer. This pattern is seen in temperate and also in some tropical species.

2). Opportunistic: This pattern is generally found in desert fauna. The favorable conditions are unpredictable here. The animals are found to maintain their gonads in a partially ready state for reproduction. When occasional rainfall occurs, and if other environmental conditions are favourable, animals reproduce in a short time (e.g. desert frog).

3) *Potentially continuous*: This pattern is exhibited by species living in tropical areas, especially by those living near equatorial zones, where there are not many variations in environmental conditions. At a given point of time at least some individuals of a population will breed i.e. at population level breeding is throughout the year. However, slight variation in any one of the environmental factors upsets aseasonal reproduction.

Generally, in most cases synchronization of male and female reproductive cycles is observed. However, in some cases gonadal cycles between the sexes are not synchronized. In such cases, the animals adopt other pre-mating and post-mating strategies for successful reproduction. Let me give a few examples in both these strategies.

1. *Pre-mating Mechanisms*:

Male sperm storage: Some of the temperate hibernating bats and colubrid snakes, the sperm are produced in fall and then the testis regress. The sperm are stored in the epididymis. The females ovulate and mate in spring. The sperm stored in epididymis fertilize the eggs and the offspring are born in spring/summer.

2. *Post-mating Mechanisms*:

Female sperm storage: Some vertebrates, like temperate skink species, the females mate during fall and store the sperm in the oviduct when the ovaries are still not fully matured. Upon maturation of ovaries and ovulation during spring, the eggs are fertilized using stored sperm. The Indian agamid lizards such as *Calotes versicolor*, *Psammophilus dorsalis,* the gametogenesis is synchronous and yet oviductal sperm storage is observed. This may be because these lizards are multi-clutched with extended breeding season. The female sperm storage in these lizards helps in avoiding the need for repeated mating, thus reducing their exposure to predators.

Delayed implantation and embryonic diapauses:

This post-mating mechanism is observed in roe deer and badgers. Here mating and fertilization occur in fall. The embryo (blastocyst) does not immediately implant in the uterus, but is maintained in a state of dormancy until onset of spring. This is a case of obligatory embryonic diapause. We also come across delayed implantation and embryonic diapause in marsupials. The females may mate while lactating the young that is in the pouch leading to delayed implantation and embryonic diapause, a case of optional/facultative embryonic diapause.

Prolonged oviductal egg retention in Squamates:

In temperate regions, some species of lizards retain eggs in the oviduct for longer duration. Holding the eggs in the oviduct for longer duration provides thermal benefit to the embryos during unusually cold weather and female can lay eggs in advanced stage of development such that they hatch at an early date to exploit seasonal food. In tropical zones, such as India, agamid lizards that breed during monsoon, the females are known to retain eggs in the oviduct in the absence of soil moisture and humidity due to failure of rains. The oviductal egg retention is viewed as one of the steps in the evolution of viviparity in vertebrates. Indeed studies on Australian skink, *Saiphos equalis* show environmental temperature driven evolution of viviparity. *S. equalis* is found in eastern Australia at different altitudes, exhibits 3 modes of reproduction. The population of *S. equalis* found at lower altitudes are oviparous with long (>15-day) incubation period. The thickness of egg shell is normal like other oviparous skinks. The population found at intermediate altitudes are ovoviviparous with short (~5-day) incubation period. There is progressive reduction in their

egg shell thickness with uterine angiogenesis for maintaining the developing embryo. The populations found at higher altitudes are viviparous (0-day incubation period). The egg shell in these populations is membrane like and there is increased uterine angiogenesis. These findings on *S. equalis* provide insight on variations that occur in the oviparity-viviparity spectrum.

Studies on prolonged oviductal egg retention in Indian agamid lizard, *C. versicolor* provide an insight on the mechanism of egg retention. In normal course, progesterone (P) levels dwindle by two weeks (as the corpora lutea degenerate) following ovulation and eggs are oviposited. However, in egg retained lizards, even beyond 2 weeks, P levels are high. In such lizards, the adrenal glands (the main target for stress) produce progesterone. This may explain promotion of egg retention in the oviducts by prevention of oviductal motility as P is known to be antimyometrial. Further, the egg retention is accompanied by embryonic diapause by arresting the embryonic growth at stage 34 in retained eggs. The embryonic diapause is thus yet another strategy exhibited during egg retention. This arrests embryonic growth under unfavorable condition, so that when favorable conditions return, the eggs can pass through the pelvic aperture that is fixed in adult lizards. Further, if growth of the embryo is not arrested, energy demands would not be met by yolk reserves of the egg. It is interesting to note that the gravid female brings about embryonic diapauses of her eggs by lowering the body temperature by about 3-5° C, a phenomenon rare in ectotherms.

Other post-fertilization strategies:

Many bizarre strategies related to parental care are also found in fishes and amphibians. For instance, in the gastric brooding frog, *Rheobatrachus (extinct recently)*, the eggs are brooded in the stomach and upon hatching, the tadpoles escape out of the mouth of the parent. The Surinam toad, *Pipa pipa* retain eggs in skin pouch until hatching. The frog, *Rhinoderma darwini*, keeps eggs in vocal sacs until hatching. In some teleosts (which lack Mullerian ducts or true oviducts), embryonic development occurs within follicular or ovarian cavities. In all the above cases, egg development occurs in structures that are *not homologous* to oviducts and hence do not represent viviparity. They are in fact *adaptive responses to selection pressures* to improve fitness.

Manipulation of fecundity:

Fecundity is defined as the number of eggs (*clutch size*) or offspring (*litter size*) produced in a given period. The animals may lay eggs/ produce offspring in a fixed number (*fixed clutch/litter*) or vary the clutch/litter size (*variate clutch/litter*) between subsequent reproductive episodes during a breeding season. Clutch patterns are mainly governed by phylogeny. Yet, animals manipulate fecundity by varying their egg/offspring number and also the frequency of reproductive episodes depending upon resource availability during breeding time. For instance, *C. versicolor* is a multi-clutched lizard with variate clutch size. It has an extended breeding season (May to October) in Dharwad, Karnataka. Interestingly, energy invested in egg production (clutch mass) is nearly the same in early and late clutches. But the clutch sizes vary from 7-33 depending upon the maternal body size and breeding time. Clutches laid early in the breeding season are always larger and the eggs are smaller in size than those laid late in the breeding season. The clutches laid in late breeding season though

small have large sized eggs. Now question is why do these lizards manipulate fecundity, laying large and less number of eggs during late breeding season? Is it to produce bigger hatchlings to enable them to compete for food with early born hatchlings? Contrary to this, it is found that large eggs do not produce larger hatchlings but they produce heavier hatchlings. The hatchlings weigh heavier because they *internalize* more yolk (called residual yolk) subcutaneously. In all oviparous vertebrates, some yolk is internalized subcutaneously at hatching and serves as reserve energy for offspring during early post-hatching activities / sustenance. More residual yolk in the late born hatchlings obviously contributes to greater reserve energy possible for competition with those born early in the season.

Reproductive modes are more ancient and conservative while reproductive strategies are recent and often plastic. Plasticity in reproductive strategies can fine tune the reproductive potential in the prevailing environment to ensure fitness. At present not much is known about the age at first reproduction, number of clutches in a breeding season, clutch size, age specific fecundity, survivorship of hatchlings/offspring to sexual maturity, reproductive life expectancy of adults etc in different species of Indian vertebrates. Studies on reproductive cycle of different species living in a habitat or closely related species living in different habitats are essential to generate information about the reproductive strategies. Comparative data on vertebrate species are needed to generate information on the mechanisms governing the evolution of diversity in reproductive patterns and strategies. India with its rich diversity provides an opportunity to undertake such works.

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Current Status of Fertility Control Methods

A. Jagannadha Rao
INSA Senior Scientist, Department of Biochemistry,
Indian Institute of Science, Bangalore, India
Email: ajrao@biochem.iisc.ernet.in; ajrao2006@gmail.com

Considering the explosive growth in the population particularly in the developing countries there is an urgent need to develop methods to control fertility both in the males and females. Before we understand the intricacies involved in development of fertility control methods, it is essential to know basics of reproductive physiology. Reproduction both in the male and female are controlled by hormones produced by the hypothalamus, pituitary and gonads, testis in the case of male and ovary, in the case of female. The important hormone which regulates synthesis and secretion of main hormones involved in reproduction, namely Follicle stimulating hormone (FSH) and Luteinizing hormone (LH) is Gonadotropin Releasing Hormone (GnRH). It is secreted by the hypothalamic neurons and secreted in to the capillary net work of hypothalamus. In the case of females it is released in pulses while in the case of males it is a tonic release (Rao, 2005).

FSH, as the name indicates is necessary for follicular maturation and the target cells for its action are the granulosa cells surrounding the growing follicle. These cells produce estrogen under the influence of FSH and estrogen is a known mitogen and estrogen action results in addition of several layers of granulose cells leading to formation of a mature follicle. This mature follicle ruptures under the influence of LH resulting in ovulation. The oocyte is fertilized by the sperm resulting in zygote which implants in uterus leading to pregnancy. Although several targets in the sequence of events leading to pregnancy have been identified the most successful one has been the blocking the surge of LH which is indispensable for induction of ovulation by utilizing the feedback inhibition of LH surge at the hypothalamic and pituitary level by use of a combination of derivatives of progesterone and estrogen which are taken regularly by women. These are the widely used oral contraceptive pills. Thus it has been relatively easy to develop a simple and effective method for fertility control in the case of female as one has to target only one egg which is released once in a month (Sitruk-Ware, 2013).

In the case of males, FSH is necessary for initiating spermatogenesis and is indispensible for spermatogenesis and the target cell for FSH is the Sertoli cell. LH stimulates testosterone production by Leydig cells and testosterone is indispensible for sperm production (Sriraman and Rao, 2005). Testosterone and FSH together regulate function of Sertoli cells which provides micro environment for the successful development of sperms by creating a blood testes barrier. Although the use of oral contraceptive pill has significantly contributed in curtailing the population increase, there is no need to emphasize the need for wider

Lecture delivered on January 29, 2014 at *the Department of Zoology, Poorna Prajna Pre-University College, Udupi*

participation by the males in the family planning program in the developing countries. The current estimate is a meager 2% of the male population in India in using the available fertility control methods and these include age old methods of barrier and vasectomy. It is distressing to note that in spite of concerted efforts by several organizations, over several decades, we still do not have any new methods other than the two mentioned above namely, vasectomy and barrier method. Why is it so difficult to develop a male contraceptive drug? Unlike in the female where only one egg is targeted each month, in the male continuously several million sperms have to be targeted (either stop production or inactivate them) as theoretically even one sperm is adequate for fertilizing the egg. Firstly, we have to deal with a millions of sperms produced continuously and not a single egg a month as in the case of females. One may ask why one cannot use testosterone as a male pill just as in the case of a combination of progesterone and estrogen derivatives in the case of women. In fact the efficacy of testosterone to block spermatogenesis has been demonstrated in males. However, it has not been possible to implement the use of it. One should note that although testosterone is indispensible for spermatogenesis and inhibition of testosterone can be successfully used as an effective approach to block spermatogenesis, it is also necessary for maintaining the libido and male secondary sexual characteristics also. Thus any male contraceptive method to be practical should not interfere with libido and secondary sexual characters. It is very easy to block spermatogenesis by use of androgens by inhibiting pituitary LH. But this also decreases in testosterone and thus libido which is not acceptable (Chitra Lekha and Rao 2006).

In spite of all the serious problems associated with developing a male contraceptive method, efforts are in progress in this direction. These efforts can be grouped under two heads; those which interfere with testosterone and those which do not. As mentioned earlier one can give long acting testosterone derivatives such as Testosterone undecanoate (TU), Testosterone enanthate (TE), Testosterone buciclate (TB) to block hypothalamo-pituitary axis and block LH. Administration of these testosterone derivatives by injection or as a patch under the skin T. enanthate and T. buciclate have been found to be effective and along with this an androgen is given as a supplement to take care of libido. This approach is very effective and results in oligo to azoospermia. In fact, a clinical study has been carried out in Australia when 55 couples have been given testosterone and 'DMPA' for 12 months, no pregnancy was observed and this was hailed as a possible male pill. While this is very encouraging it has its share of problems. The quantity of androgen used is very high and is known to have side effects on prostate and more importantly the problem of frequency of injection needed. In addition, the most important requirement is that administration of testosterone derivatives the subject should become azoospermic as theoretically even one sperm is adequate to fertilize the egg. Consequently until azoospermia is achieved one has to employ alternate methods of contraception.

Both GnRH agonists and antagonists have been employed to block pituitary FSH and LH and thus testosterone. However, this approach also needs supplementation with androgens. In addition recent studies have shown that GnRH has action on several non-reproductive tissues. Considering this it is unlikely that this will be a usable approach (Behre, 1995).

An important point to be noted that although several of the male contraceptive methods are initially tested in rodent models; ultimately it has to be tested in non-human primate model before it can be tested in human volunteers. At Indian Institute of Science (IISc.), Bangalore, a very successful monkey model has been developed using the locally available bonnet monkey (Macaca radiata). A critical requirement in employing the monkey as a model, it is essential to establish the fertility status of male and female monkeys in the colony where the fertility studies are going to be carried out. This was done by monitoring the menstrual cycles and ovulation status of the female monkeys used in the study, by determining the serum estrogen and progesterone levels during the menstrual cycles and ability of the male monkeys to impregnate the females. Studies carried out over 20 year period at IISC Bangalore have established that an average of 2.3 exposures of the regularly cycling ovulating female to a fertile male is adequate to achieve pregnancy in the range 75-80% of exposed monkeys. This forms the basis for concluding that an herbal product or a vaccine or a synthetic chemical which is being tested has anti-fertility properties (Rao, 2002).

Another approach which has been tried is the use of a combination of NET enathate and estradiol valerate, which was very effective in decreasing sperm count. As mentioned earlier one of the problems in employing androgen to block the LH and thus testosterone is that one has to give a very high dose of testosterone. We have tried a compound called 7-methyl-19-nortestosterone (MENT) which is a synthetic androgen. MENT which originally was developed as a supplement for hypogonadal patients has several interesting features. It does not undergo 5-reduction, thus minimizing the adverse effects on prostate, which is otherwise a major problem with conventional androgens. It should be noted that dihydro testosterone which is the active of form testosterone in exerting its action in prostate and it is produced by the action of 5 alpha reductase. It is 10 times more potent as an anabolic steroid and 4 times more potent as an androgen. More importantly, it is 12 times more potent than other androgens in inhibiting the hypothalamo-pituitary-gonadal (HPG) axis as assessed in male rats. Consequently, the important active feature associated with MENT, is that it is possible to achieve inhibition of the HPG axis using concentrations which are very much lower than those required in the case of conventional androgens or other derivatives (Ramachandra, 2002).

As mentioned earlier, all these approaches which interfere with testosterone production have serious inherent problems such as providing alternate source of minimal requirement of androgen in their utility. In view of this, several attempts were made to develop methods that do not interfere with testosterone production. One of the important methods in this approach is to interfere with the action of FSH, as FSH is essential for spermatogenesis in all the males of the species studied. Blocking the action of FSH was achieved by active immunization of adult male bonnet monkeys against sheep FSH, which resulted in Oligospermia / Azoospermia, loss of fertility and more importantly no effect on serum testosterone levels and secondary sexual characters and these effects were reversible. While the loss of fertility with no decrease in serum testosterone levels and thus no effect on libido was very encouraging, even this approach had some inherent problems in mass application. These include availability of sufficient quantity (which can be several grams or more) of the antigen in this case FSH and need for repeated injection to maintain required titter to neutralize the circulating level of FSH. Another problem is the possibility of production of antibodies that cross react with other glycoprotein hormones because of conserved subunit sequences These hormones namely

FSH, LH hCG and TSH all share a common alpha subunit and thus production of antibodies to FSH can interfere with the action of LH and TSH. Some of these problems were taken care of by using the FSH receptor as an antigen and also in one study small peptides whose specific sequences in the FSH receptor are not common to receptors of other hormones namely LH and TSH for production of antibodies, were employed as antigen to produce antibodies which are capable neutralizing endogenous FSH (Rao, 2004). However, the problem of frequent injections and use of alternate methods still remains, although this approach has the potential as a model to understand the interaction between the receptor and the ligand, so that small non-peptide molecule which can block the action of FSH can be developed (Rao, 2001) in future.

Another approach to interfere with fertility is to interfere with sperm maturation. It is a well established fact that testicular sperms lack forward motility and fertilizing ability. This forward motility and fertilizing ability is achieved by the sperms as they pass through epididymis. Sperm membrane undergoes important changes during its transit through epididymis and epididymal secretory proteins are associated with sperm surface. It has been demonstrated that several new proteins are added on and some existing proteins are removed from sperm membrane during the process of maturation. Some of these proteins have been isolated and antibodies have been raised against these proteins. By using the specific antibodies, to these proteins it should be possible to interfere with this process of maturation and induce infertility (Rao, 2011). One of the proteins namely, epididymal glycoprotein DE 37 is synthesized in the proximal epididymis and active immunization against this protein resulted in infertility in male and female rats. An equivalent in human is the Acidic Epididymal glycoprotein related Product (ARP). Other epididymal proteins tested in rodents are human epididymal protein, HE to HE6. HE 1 accumulates in the cauda and functions as cholesterol transfer protein. HE 4 acts as decapitation factor. Another protein which has been tested is sperm specific Glyceraldehyde 3-phosphate dehydrogenase (GAPDS).

Another epidydimal protein which was tested in monkeys is Eppin. It is an epididymal protease inhibitor. It is a cysteine-rich protein containing both Kunitz-type and WAP type of disulfide core protease inhibitor consensus sequences. Eppin-2 is expressed only in the epididymis and immunochemical studies indicate strong pattern of expression by the ciliated cells of the efferent ducts and strong staining of ejaculated spermatozoa. Eppin has a molecular weight of ~17 kDa consisting of 133 amino acids and Eppin is regulated by 'T' and has an androgen response element in its gene sequence. Fertility tests were carried out in proven fertile male monkeys and significant decrease in fertility was noticed in monkeys actively immunized again Eppin. One mechanism to explain the infertility is that antibodies to Eppin interfere with normal Eppin interaction with the sperm surface and with semenogelin. (O'Rand, 2004). Other sperm proteins tested are SP10, PH 20 FA-1and LDH-4, some of which were tested non-human primates. These may serve as novel targets to interfere with sperm maturation process.

Another potential approach of male contraception is to interfere with the action of estrogen at the epidydimal level and thus with sperm maturation in males; The role of estrogen in sperm maturation was established by results obtained by studies on male reproduction in the estrogen receptor alpha knockout mice (disruption of ERα) which resulted

in a decrease in Na+ transport from lumen to interstitium and thus a decrease in water (H2O) and fluid reabsorption. The inhibition of fluid reabsorption results in the dilution of cauda epididymal sperm, disruption of sperm morphology, and eventual decreased fertility. Based on these results it was concluded that estrogen has an important role in sperm maturation. These results obtained using rodent model were extended to a non-human primate using the adult male bonnet monkey.

These studies consisted of monitoring the effect of chronic administration of estrogen receptor antagonist (ICI 182780) on estrogen dependent parameters in adult male bonnet monkeys. Monkeys weighing 6-8 kg of proven fertility, that exhibited a characteristic nocturnal surge of serum testosterone, were recruited for the study. All animals chosen for the study exhibited sperm counts in the range of 150-500 million/ejaculate. Three animals per group were administered estrogen receptor antagonist ICI 182780 in propylene glycol (250 ug ICI/day/animal) via Alzet pumps, which were changed every 28 days. As controls a set of animals were administered propylene glycol. Results revealed that administration of estrogen receptor antagonist to adult male bonnet monkeys resulted in a significant decrease in forward motility as assessed by CASA (Computer Aided Sperm Analysis) without affecting serum testosterone levels or total sperm count. This established the important role for estrogen in epidydimal sperm maturation (Shayu, 2007).

Recently we have demonstrated the presence of FSH receptors, in cauda region of the epididymis and they respond to FSH by way of increase in proliferation only in immature rats. These results suggests that as in the case of Sertoli cells, which act as nurse cells for the germ cells in response to FSH action, FSH may act in cauda which is a region for the storage of mature sperms until they are ejaculate and FSH action maybe of importance in providing a microenvironment during their storage there. Consequently, this could be a contraceptive target (Dahia and Rao, 2006).

A novel approach to block the transport of sperms from vas deferens was developed recently and is currently it is undergoing phase 2 clinical trials. It consists of injection of styrene maleic anhydride into the lumen of vas deferens. (Guha, 1999). Another approach is injection of polyurethane plug directly into the sperm duct which was reported to be highly effective and easily reversible

Another approach consists of reduced vas deferns contraction and induction of infertility in male mice lacking P2X1 receptor (Mulryan, 2000). P2X1 receptors for ATP are ligand gated ion channels are present on many excitable cells including vas deferns smooth muscle cells. A substantial component of the contractile response of the vas deferns to sympathetic nerve stimulation which propels sperms into the ejaculate is mediated through P2X1 receptors. Male fertility is reduced by ~90% in mice with a targeted deletion of the P2X receptor gene. And it is reported that this method does not cause any sperm dysfunction and had no effect on testosterone levels.

Concluding Remarks

A male contraceptive should be easy to use, effective and effects should be reversible. Main requirement is that it should not interfere with libido and secondary sexual characteristics. In the case of anti fertility vaccine approach, the problems are ethnic differences in response to antigen, availability of purified antigen in sufficient quantity, variability of titter and frequency of injection to maintain an effective titter. More importantly

alternate approach to be employed until the effectiveness the method employed is established.

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Evolution of Viviparity in Vertebrates

Bhagyashri A. Shanbhag
INSA Senior Scientist
Department of Marine Biology
Karnatak Uniiversity P.G. Centre, Kodibag, Karwar- 581 303
Email: bhagyashrishanbhag@gmail.com

Reproduction is an important aspect of animal life. Species perpetuation is possible only through reproduction. Among the vertebrates we come across two main modes of reproduction, oviparity and viviparity. Oviparity refers to egg laying mode of reproduction. Viviparity involves development of the embryo inside the mother's body leading to live birth. Oviparous mode of reproduction is most common, but we come across viviparity in every class of vertebrates except birds. Oviparous mode of reproduction is relatively simple wherein the eggs are laid outside either in water or soil, generally in large numbers and there is no parental care except in a few taxa. Therefore, in oviparous mode of reproduction the eggs and embryos are vulnerable to hostile environmental pressures such as desiccation and predation pressure. The eggs also face the problems of osmoregulation. It is believed that in order to provide protection to the eggs under certain harsh environmental conditions, viviparity might have evolved in different groups. However, viviparity is not a simple phenomenon, and it necessitates evolution of mechanisms (both structural and physiological) to support it. For instance, viviparity requires necessary changes in the oviducts to house the growing embryos and meet their requirements. For example, development of a transient structure such as a placenta to provide nourishment and gaseous exchange for the embryo becomes essential. In addition, mother has to evolve mechanisms to avoid expulsion of embryo/foetus by preventing oviductal/uterine contractions during gestation/pregnancy. Yet, triggering parturition at appropriate time is equally important. Thus, viviparous mode of reproduction is quite complex. Yet, viviparity has evolved in different vertebrate groups more than 150 times. Wonder why? In order to get some insights, let us have a look at the cost *vs* benefits of viviparity.

The costs of viviparity:

- The mother has to provide nourishment to the developing embryo until parturition, and need to develop placenta for overall support of developing embryo.
- The mother also faces increased risk of predation due to lowered motility during gestation.
- Gestation delays subsequent reproductions and thus limits number of offspring / progeny in the lifetime of an individual.

The benefits of viviparity:

- The developing embryos are protected from hostile environmental conditions.
- It promotes developmental rate and survival of embryos thus increasing fitness of the parent.

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Therefore, we can expect viviparity to evolve in a species if benefits of 'live bearing' outweigh the costs involved.

If one surveys the animal kingdom, we come across several examples of animals that are not viviparous but yet they care for their developing embryos in different fashion. For example, we come across many oviparous vertebrates that guard their eggs/nests or carry the eggs on them or brood them in unusual places in the body until hatching e.g. mouth brooding in Cichlid fish, gastric brooding in frog, *Rhinobatrachus* (now extinct), vocal sac brooding in *Rhinoderma darwini* and so on. In some teleost (e.g. some species in *Poecilidae*), the embryonic development occurs in the ovarian or follicular cavity and young ones are released from the ovary. These teleost are sometimes referred as viviparous. Since the development of embryos does not take place in oviducts (teleosts lack oviducts), these are actually the examples of *psuedoviviparity*. Such examples of extraoviductal embryonic development do not represent a stage on way to viviparity; but simply depict responses (in diverse groups of animals) to *same selection pressures* (desiccation, osmoregulation, predation etc.).

In some lizards and snakes (squamate reptiles) and egg laying mammals (monotremes), the eggs are retained in their oviducts for longer periods and embryonic nourishment occurs through the egg shell. The eggs are then laid at advanced stages of development. Such a phenomenon is called ovoviviparity. At the same time, some skinks and snakes are seen to exhibit true viviparity. A skink, *Saiphos equalis* found in eastern Australia, inhabiting different altitude, exhibit oviparous (in coastal areas), ovoviviparous (at lower elevation) and viviparous (at >1000m elevation) mode of reproduction. The oviparous individuals of the species lay the eggs with shell and they hatch after ~15d incubation, while ovoviviparous individuals lay thin shelled eggs that contain embryos in advanced stages of development and need ~ 5 days to hatch. Viviparous forms give birth to fully developed offspring in transparent membrane. This suggests that transition from oviparous to viviparous mode reproduction perhaps involved a stage like ovoviviparous mode of reproduction. A study on Indian garden lizard, *Calotes versicolor* carried out at Karnatak University, Dharwad throws light on the probable mechnism involved in the transition from oviparity to viviparity. The garden lizard is an oviparous lizard. It breeds during south-west monsoon period. It normally retains eggs in the oviduct for ~ 2 weeks. These lizards produce eggs with parchment like shell which need moisture for preventing desiccation of developing embryo. The gravid females dig soil to prepare nests and then lay their eggs in the moist soil nest and close it. During incubation in the soil nest, the eggs imbibe moisture and swell to larger size with the growth of the embryo. But under unfavourable conditions, such as lack of moist soil, the lizards retain eggs in their oviducts as long as 6 months or more. However, egg retention for long periods poses certain problems. The egg retained lizards have to overcome problems of (1) preventing the enlargement of the eggs for their smooth passage through cloaca at egg laying and keeping the embryos in viable condition and (2) inhibition of oviductal contraction to prevent egg expulsion. The *C. versicolor* have evolved strategies of arresting embryonic growth at stage 34 to keep the eggs in viable condition and also preventing the growth of egg size for their smooth passage through cloaca following the return of favourable conditions. The embryonic diapause is achieved in these ectothermic lizards by lowering their body temperature by 3-5^{0}C by some physiological mechanism yet to be identified. One problem is resolved. How about the second problem of preventing expulsion of oviductal eggs? In mammals, it is well known that progesterone produced by the ovarian corpora lutea (CL)

serves as the anti-myometrial hormone and prevents oviductal contractions. In *C. versicolor* also during normal gestation period, ovarian CL are active until mid-gestation and plasma P levels are high until then. During late gestation, CL regress and plasma P levels drop. Interestingly, however, the lizards that retain eggs beyond normal gestation, plasma P level remains high in spite of the dwindling of CL. In vertebrates, only the gonads and adrenal gland are known to produce steroid hormones (besides the placenta during pregnancy). The egg retention beyond normal gestation is also a stressful phenomenon. The adrenal glands are known responders to stress. Indeed, the studies showed that adrenal glands are the source of P during prolonged egg retention in *C. versicolor*. The findings on the garden lizard have shown probable mechanism involved in the evolution of ovoviviparity, an intermediate step between oviparity and viviparity but also provide experimental proof to 'stress induced evolution of viviparity hypothesis' in reptiles.

In summary, it appears that ovoviviparity was the first step in the evolution of viviparity possibly following the steps outlined above for the garden lizard in the process of prolonged egg retention, by evolving appropriate mechanism for the same. Subsequent evolution might have led to mother's nourishing oviductal embryos through thinning of egg shell membrane like that shown in the skink, *S. equalis*. The physiological problems associated with the prolonged egg retention (especially of large eggs that swell) perhaps resulted in egg size reduction and getting rid of egg shell as seen in mammals. Finally, development of a true placenta (from extra-embryonic membranes), an ultimate refinement of viviparity, might have evolved along with the associated endocrine mechanisms.

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Possible significance of expression of hormones and their receptors in cancer tissues

A. Jagannadha Rao
INSA Senior Scientist, Department of Biochemistry,
Indian Institute of Science, Bangalore, India
Email: ajrao@biochem.iisc.ernet.in; ajrao2006@gmail.com

Hormones secreted by the endocrine glands regulate a variety of functions. One of the important glands is pituitary which is located at the base of hypothalamus and produces a variety of hormones which include gonadotropins, ACTH, Growth hormone, etc. The production of hormone is generally restricted to the specific endocrine glands; for example, gonadotrophins, ACTH and GH to pituitary, Insulin to pancreas, steroid hormones to gonads and adrenal etc. However, it is known that several cancers express hormones like FSH, hCG, LH, TSH, etc. and their cognate receptors. For example, the synthesis and secretion of hCG by squamous cell carcinoma of the lung has been demonstrated. It is known that levels of hCG are elevated in lung tumors and hCG is a biochemical marker of malignancy associated with all the major types of cancer. The expression of hCG has been shown to correlate with tumor aggressiveness, i.e., the greater the hCG expression, the more aggressive is the tumor. In fact, the presence of beta sub unit of hCG in semen is considered as a marker for early detection of prostate cancer (Porter, 2001).

To understand the significance of expression of hormones in cancer cells, it is necessary to know some of the important features of cancer cells. Fundamental characteristic of all cancerous cells is the uncontrolled division. Cancer cells have phenotypic traits similar to undifferentiated embryonic cells: So cancer is considered as a disease of differentiation.

Generally trophic hormones (FSH, LH, TSH & hCG) are believed to promote growth and function of target cells or tissues. For example, in the case of Leydig cells which are present in the interstitium of the testis, LH which is produced by pituitary is supposed to promote growth and function. However, several studies including our own revealed that administration of LH to adult male rats did not result in an increase in the number of Leydig cells which should have been the case if LH did indeed promoted growth of Leydig cells . Furthermore, no mitotic Leydig cells were seen in testis sections from adult rats which were administered LH or hCG (Ren-Shan, 1996; Christensen, 1980; Kuopio, 1989; Abney, 1986; Moore, 1992; Sriraman, 2003). In order to explain this observation, that LH did not stimulate the growth of Leydig cells in an adult rat, an attempt was made to check whether there is any dependence on the age of rats at which Leydig cells can be stimulated to divide following administration of LH.

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It is known that Leydig cells go through three stages during their differentiation. These include the progenitor Leydig cells, (PLC) which exhibit minimal expression of LH receptors as well as very minimal capacity to produce testosterone which is considered as a marker for Leydig cells, immature Leydig cells (ILC) stage which have intermediate level of expression of LH receptors and slightly better capacity to produce testosterone than the PLC and finally the adult Leydig cells (ALC) which have maximum expression of LH receptors and maximum capacity to produce testosterone in response to added LH (Ren-Shan,1996). Generally the presence as well as increase in genes such as PCNA (Proliferation Cell Nuclear Antigen), and Cycling are considered as markers for dividing cell. Before undertaking the *in-vivo* experiments which consisted of administering LH to rats, isolating purified Leydig cells and monitoring the level of expression of the proliferation markers mentioned above, namely PCNA and cyclin, their expression level was monitored by RTPCR of the RNA and Western blot analysis of protein extracts isolated from the purified PLC, ILC and ALC. When the three types of cells isolated from testis namely PLC, ILC and ALC were checked for the presence of these markers, their presence was detected only in the PLC and very low level in ILC and total absence in ALC. This explains why there was no increase in Leydig cell number when LH was administered to adult rats. Furthermore, when the action of endogenous LH was blocked in immature male rats by administration of an antibody capable of neutralizing endogenous LH, the expression level of the above proliferation markers was significantly decreased. This indicated that only the undifferentiated PLC respond to LH by way of proliferation. This was further established by the fact that when proliferation i.e. actual increase in Leydig cell number was assessed by monitoring the incorporation of BrDU which is an indicator of DNA synthesis by Leydig cells, incorporation was seen only in PLC and this significantly decreased in rats administered LH antibody for 7 days prior to isolation of Leydig cells (Sriraman, 2000).

A similar study was carried out to investigate the role of FSH in regulation of proliferation of Sertoli cells. It is known that Sertoli cells divide only up to day 18 after birth in rats. Our results based on BrdU incorporation by Sertoli cells revealed that peak incorporation is seen on day 9 after birth. Just as in the case of Leydig cells, checking for the presence of proliferation markers such as Cyclins and PCNA in the Sertoli cells isolated from immature and adult rats revealed that their expression was seen only in Sertoli cells of immature rats and not in Sertoli cells isolated from adult rats. Also the level of expression decreased only in immature rat Sertoli cells when FSH was deprived by administration of a specific antibody capable of neutralizing the endogenous FSH in immature rats. In addition, in the adult rat Sertoli cells, there is no regulation of FSHR following FSH deprival and no mobilization of calcium and increase IL-6 in response to FSH which also supported the conclusion that Sertoli cells isolated from adult rats do not respond to FSH administration by way of proliferation. We have also demonstrated the presence of FSHR in cauda epididymis of rat and monkey and only immature rat caudal cells respond to FSH by way of proliferation independent of testosterone (Chitra and Rao, 2006).

We have observed that GnRH which is known to stimulate the release of LH and FSH from pituitary gonadotrophins also induces differentiation of uninucleated cytotrophoblasts (CT) into multinucleated syncytiotrophoblasts (ST). Similarly we have observed that estrogen

also has role in differentiation CT in to ST although estrogen is known to be a mitogenic hormone. During pregnancy, the feto-placental unit produces large quantity of estrogen and estrogen is a key factor in the regulation of placental function. It is also important to note that ERs have been demonstrated in human trophoblast. Estrogen is an important pregnancy hormone and it serves to regulate placental function by influencing the expression of hCG and LDL-R which is involved in the supply of cholesterol needed for synthesis of steroid hormones during early pregnancy. Interestingly estrogen is generally recognized as a growth-promoting hormone (Rama S, 2004).

Experimental and clinical studies suggest a potential role for estrogen in tumor progression. Over-production of E2 is one of the causative factors for endometrial cancers. E2 is also implicated in the pathogenesis of human breast and ovarian cancers. E2 plays a crucial role in the development of a hormone-dependent phenotype of gynaecological tumors. In the normal cycling endometrium, the estrogenic phase of the cycle is associated with cell proliferation and telomerase activity. Unlike in the above-mentioned systems where in estrogen stimulates proliferation, E2 induces terminal differentiation and cessation of proliferation in human placental trophoblasts and thus estrogen exerts differential action in placental cells. There are several examples of non-traditional roles attributable to Estrogen for example; 17β-Estradiol induces human osteoblast differentiation by up-regulating TGFβ1 production (Ousler, 1991). In cartilage, 17β-Estradiol is known to induce cessation of proliferation and onset of cellular differentiation by increasing TGF 1 production (Nasatzky, 1999).

It is suggested that possible way by which some of the hormones like LH, FSH and hCG exert differential action is by utilizing the alternatively spliced form of their receptors. For example, alternatively spliced form of LH / hCG receptor mRNA is expressed in human breast epithelial cells. In ethylene dimethane sulphonate (EDS) treated rat Leydig cells during early proliferative phase a truncated form of LHR is expressed (Tena-Sempere 1997 & 1994; Dahia and Rao, 2006; Jiang, 2002; Sumathi, 1999). The results presented so far clearly establish that the trophic hormones stimulate the proliferation of cells only when they are in an undifferentiated state and stimulate functional parameters when the cells are in a differentiated state. One may ask a very relevant question as to the significance of the results obtained using these cell systems. An attempt is made to explain the significance of the results obtained suggesting differential action, using the prostate as model system. Prostate is an androgen-dependent tissue that requires testosterone for its growth and differentiation. Abnormal over-growth of the human prostate is common in over 80% of males before the age of 80. This enlargement, called Benign Prostatic Hyperplasia (BPH), causes urinary obstruction. This situation can be corrected by surgery. In some cases, the over-growth becomes cancerous. Carcinoma of prostate is a leading cause of death due to cancer in most aging males. The usual treatment for prostate cancer involves androgen-depletion. The hormone-ablation therapy, however, is effective only for a short period in controlling cancer progression. However, following these treatments consisting of androgen deprival or anti- androgen treatment, some of the carcinoma cells become androgen-independent and are therefore not amenable for therapy by anti-androgen strategy.

What are the mechanisms that underlie these hormone-independent forms of cancer?

In this connection, it is essential to recall that FSH, LH, TSH, hCG, TGF beta belong to the group of cystine knot super family of proteins, many of which have growth promoting activity. Results obtained using Leydig cells, Sertoli cells and Placental cells revealed that the trophic hormones exert growth promoting action when the cells are in an undifferentiated state and stimulate only functional parameters as observed as in the case of Leydig cells by LH and FSH in the case of Sertoli cells. It is possible that the prostatic cells following prolonged androgen deprival in cancer patients revert into an undifferentiated state and these undifferentiated cells can be stimulated to divide by an appropriate facto,. just as in the case of stimulation of proliferation by LH in Progenitor Leydig cells which are undifferentiated cells. In support of this suggestion is the fact that receptors for LH are expressed in rat prostate as assessed by RT-PCR, Northern and Western Blot, binding of iodinated LH to prostatic cells. Also the presence of LH receptors was demonstrated in human prostate by Western blot and stimulation of proliferation by LH in the prostatic cells (Sriraman, 2001) differentiation.

Based on the observation that some of the tropic hormones exert differential action strategies have been developed to block the action of these hormones so that progression of some cancers can be controlled. One such case is the loss of tumorigenic potential by human lung tumor cell in the presence antisense RNA specific to ectopically synthesized alpha subunit of hCG (Rivera, 1989). Targeted destruction of prostate cancer cells and xenografts by lytic peptide-LH conjugates (Hansel, 2001). Lytic peptides Phor-14 or Hectate-conjugated to amino acid 81-95 of the chain of hCG selectively destroyed both androgen sensitive and insensitive human prostate cancer cells. It is possible that there are other mechanisms that underlie the hormone-independent phase of prostatic over-growth. But these results provide evidence for the concept that a single hormone can exert differential effects on certain cell types, depending on the stage of the

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Section III: Genetics and Evolution

Packing and Visualization of DNA

Alok Bhattacharya
Jawaharlal Nehru University, New Delhi
Email:alok0200@mail.jnu.ac.in

All of you here in this audience are either pursuing a career in science or working towards one. However, I wonder, how many of you are clear about "what constitutes science and scientific pursuit". The spirit of enquiry, formulation of questions and the quest to understand natural world around us are the founding principles of scientific pursuit. In this lecture I will illustrate these principles by formulating and solving a scientific problem. The problem chosen has been worked on for decades and we have a reasonable idea about the solution. In the initial part, the problem will be defined and the importance will be discussed in light of our understanding of cellular structure and genome organization.

We know that the size of bacterial cells is on the average one micron in diameter whereas the average size of bacterial genome is about 0.2 centimeter. In human the size of a cell on the average is about 10 μM whereas the size of the genome is about 200 cm. How do we pack such a large DNA molecule into such a small space? Is the problem related to only packing or there are other issues/ boundary conditions that are needed to be considered. One of the major considerations is that we should be able to open up one small segment without disturbing the packing state of other regions. I am sure you all are aware of the fact that in a cell there will be a few genes that are involved in mRNA synthesis (switched on state) and others are inactive, that is switched off state. It is also known that "on" genes are relatively less packed or folded compared to the ones that are in "off" state. Since "on" genes are spread all over the genome, it means that there are less folded regions spread across the genome. Therefore, the major boundary condition for packing of DNA in cells is to provide a mechanism by which some selected regions can be partially opened without unpacking the other regions.

We can consider this as a topological problem and try to solve in different ways. For example, you can take a string and try to fold/pack into a small space. A few examples of different ways of packing are taken from real life objects are shown in Fig. 1.

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Packing of DNA

Fig. 1

I would suggest to the students to try out this exercise and see if they can come out with novel ways of packing. If you analyze all the examples given above, you will realize that none of the solutions meet our boundary condition, ability to selectively unfold a small region in the middle without disturbing the surrounding regions. It is interesting that the solution that nature has come out with fits all our requirements. The packing is done at different layers, with basic folding of DNA with the help of histone complex to generate nucleosomes. These are then successively folded to generate higher order highly packed structure. The principle behind packing is either DNA-protein or protein-protein interactions. Some non-coding RNAs are also thought to be involved in the process. Localized unfolding is done by manipulating these interactions at a specific location allowing for regulation of unfolding.

When the genome is circular (bacteria), the problem is of different nature. Circular DNA can exist as open circle or super helically folded structure. One can think of folding a circle in different ways, and some examples are shown in Fig. 2.

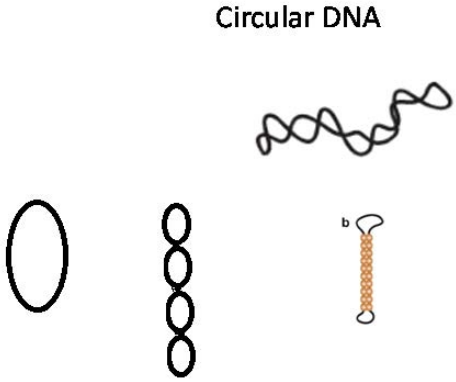

Figure 2

DNA can be folded into different kinds of structures and scientists are using DNA as basic material for making nano-machines and nano-sensors that can work in live cells and systems. Here the imagination is the ultimate limit. I have shown a few examples of different DNA structures in Fig. 3.

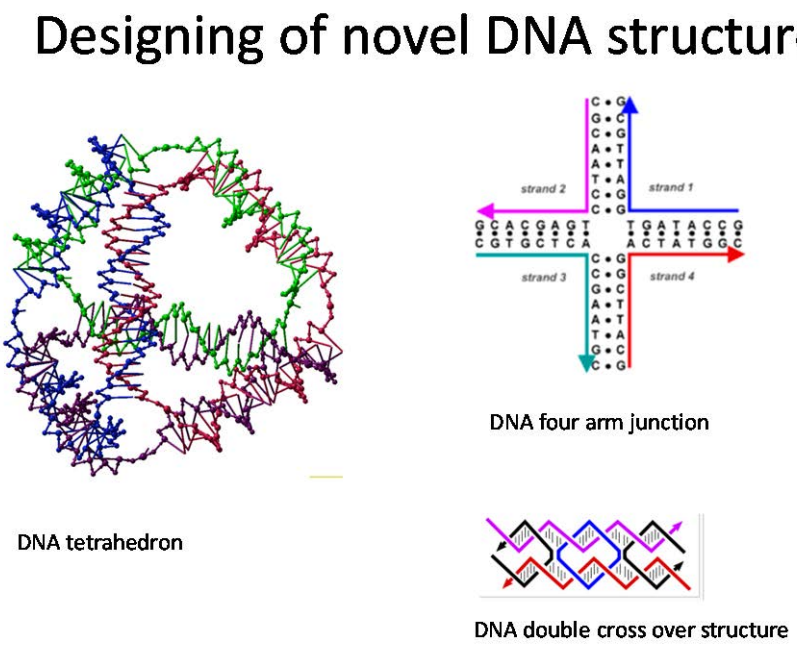

Figure 3 (references 3-5)

It is important that we should be able to visualize DNA molecules so as to appreciate their structure and organization. Most of the students think that one would require fancy equipments for this. However, methods have been developed that will allow anyone to see DNA and chromosomes in their laboratory using fluorescent microscope. For example, optical mapping using a fluorescent microscope allows one to do a physical map of a chromosome and also visualize small chromosomes. I am sure you will go back to your laboratory and use your creativity to solve scientific problems, and not worry about the lack of big machines or sophisticated infrastructure. Sometimes a piece of nylon string can be highly helpful.

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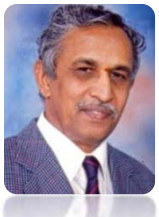
An Introduction to Epigenetics



H. A. Ranganath
Distinguished Professor (for life) of University of Mysore,
Former Vice Chancellor, Bangalore University &
Director, National Assessment & Accreditation Council
Email: haranganath@gmail.com

Evolution of Genetics

The doyen of genetics, Morgan once said "treasure your exceptions" and this has become the guiding principle of genetic analysis of any biological process during 20th century and it is more so today. If one looks at the evolution of the discipline of genetics, since its rediscovery, exceptions and deviations to the then available principles or laws are the ones which have not only enriched but also have contributed to the growth and diversification that is, emergence of new avenues of analysis.

Gregor Johann Mendel (1822-1884) with his elegant experiments with the pea plant was able to document how a few characters in the plant are inherited from parents to off-springs. He realised that each character is controlled by a 'factor' (Genes) and these factors are passed on from parents to offsprings. He was able to recognise a pattern in this, and with this he could make some very important generalisations/laws of inhertiance, such as Law of dominance, Law of Segregation and Law of independent assortment. Carl Correns, Hugo de Vries, Erich von Tschermak (1900) independently rediscovered these Mendelian principles. At that point of time, the pattern of inheritance of any character was deduced from "Ratios"- such as 3:1, 9:3:3:1 or 1:1:1:1. Thus, the Gene was treated as a "Statistical entity". The next question was, where are these genes? Cytologists and Geneticists began to see parallels between the behaviour of chromosomes during cell division and the expected behaviour of Mendel's Factors. Around 1902, Walter S. Sutton, Theodor Boveri and others independently noted these parallels and 'chromosomal theory of inheritance' began to take form, which meant Mendelian genes are located on chromosomes.

Post 1900 investigations revealed many more facets of inheritance other than what Mendel visualized such as interaction of genes, epistasis, hypostasis, polygenic-inheritance, sex-linkage, linkage, etc. as well as epigenetics and genome imprinting. The next issue to be addressed was what are these Genes?

How do they control different characters? The arrival as well the consolidation of modern genetics was witnessed during 1940-1970. To start with the experiments of Griffith, Avery, MacLeod and McCarty as well as of Hershey and Chase, and Fraenkel Conrat

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Lecture delivered on January 21, 2013 at a school in Palamedu

unambiguosly showed the chemical nature of the genetic material. The gene is a chemical substance called Deoxyribo-Nucleic acid (DNA) and it is made up of four units, namely A- Adenine, C- Cytosine, G- Guanine and T- Thymine. This lead to the 'Birth of Genetic Chemistry'. With this, the gene which was an invisible statistical entity, becomes visible in the form of a chemical substance, that is DNA or RNA. Hence forth, the structure and function of genes have to be understood in terms of structure and function of nucleic acid. The structure of DNA was resolved with the contributions from Watson, Crick, Wilkins,Chargaff and Franklin. The famous double helical Model DNA proposed by these scientists in the coming years has changed the face of biological research. Subsequently, a host of investigators have contributed to our understanding of the functions of genes, in terms of transactions of nucleic acids such as replication, transcription, translation, recombination, repair, regulation, mutations etc. Both prokaryotic and eukaryotic models have been adopted for these studies. Contemporary research in genetics centers around genetic engineering, biotech related activities, genomics, proteomics, bioinformatics etc. Chemistry of Nucleic Acids has not only made gene "visible" but also made it possible to "dissect and manipulate" it. This is in brief of the evolution of genetics.

Genesis of the concept of Epigenetics:

Observations that lead to the recognition of Epigenetic phenomena: Historically the word "epigenetics" was used to describe a few genetic events that could not be explained by the then known genetic principles of Mendel. A few examples: (1) Position effect variegation in *Drosophila* – (PEV)– in which the local chromatin environment of a gene determines its expression. For instance, the w+ gene as long as it is away from the centromeric heterochromatin, fly gets red eye. By a process of inversion, the if the w+ gene brought closer to centromeric heterochromatin, its expression on the pigmentation will be different, instead of total red eye, the fly possess white eye with red patches
(PEV - Position Effect Variegation). (2) Mealybugs (coccids): Heterochromatinization of paternal set of chromosomes in males. Males and Females have 2n=10 chromosomes. Of these, 5 are from father and 5 are from mother. In females, both paternal and maternal sets are euchromatic. In males, only the maternal set of chromosomes is euchromatic while the paternal set is herterochromatic - an event related to sex determination. In males, during spermatogenesis, only the euchromatic set (which is inherited from the mother) is included in the haploid sperm. (3) Inactivation of one of the X-chromosomes in female mammals. Only one X-chromosome remains active per diploid set of chromosomes **-** an event concerned with the dosage compensation. In females of Eutherian mammals (for eg. Human), inactivation of either paternal or maternal X- chromosome (random) occurs. On the other hand, in the female Metatherian mammals: (eg. Marsupials - Kangaroo) inactivation of only Paternal X-chromosome occurs in all cells (Non-random; Imprinting). In maize, an interaction between two alleles in which one allele causes heritable changes in the other allele (Paramutation).

The theory of Preformation (homunculus theory) was the doctrine that the organism was contained, in miniature, in the germ cell, that is, that it was completely formed before development began.Development was therefore only a matter of increasing size – there was no increase in complexity, no formation of new parts. Subsequently, it was demonstrated that Germ cell consists of a homogeneous substance. The parts of an organism arise progressively.

Development was thus viewed as a process of "new formation" from which 'epigenesis' is derived. That is the unfolding of the development of an organism that is, the development from an egg or spore through a sequence of steps in which cells differentiate and organs form. Waddington (1942) defined Epigenetics as (literal meaning "above genetics". It is a branch of biology which studies the causal interactions between genes and their products, which bring the phenotype into being. Even today, Waddington's (1957) classical landscape is an excellent metaphor to demonstrate how gene regulation determines development.

Today, Epigenetics is typically defined as the study of heritable changes in gene expression that are not due to DNA sequence. Epigenetics describes heritable, reversible changes in gene function in the absence of changes in primary DNA sequence. The study of mitotically and/or meiotically heritable changes in gene function that cannot be explained by changes in DNA sequence. Structural adaptation of chromosomal regions so as to register, signal or perpetuate altered activity states. More specifically, epigenetics may be defined as the study of any potentially stable and ideally heritable change in gene expression or cellular phenotype that occurs without changes in Watson-Crick base pairing of DNA.

"The sum of the alterations to the chromatic template that collectively establish and propagate different Patterns of gene expression and/or silencing from the same genome" (Goldberg *et. al.*, 2007; Allis *et. al.*, 2007). In principle it means that inheritance of altered gene expression without altering the nucleotide sequence of the gene! Therefore, in epigenetics, inheritance will be there, but not as we know it. Geneticists study the 'Gene', however, for Epigeneticists; there is no obvious 'Epigene'. Hence, there is a profound shift in our understanding of inheritance. Therefore, it means, that inheritance is not only due to the message hidden in the sequence of nucleotides of a gene, but also due to additional features of chromatin. if so, what are the other players/components of this machinery ?

Epigenetics and Chromatin Organisation*:*

Chromatin is a string of Nucleosomes. Nucleosomes contain nucleotide bases such as Adenine, Thymine, Cytosine, Guanine; and two each of Histone proteins namely H2A, H2B, H3 & H4 +Linker Histone H1 and also may be Non-Histone Proteins and non-coding RNA etc.

(1) *DNA Modifications:* DNA Methylation: Methyl groups added to certain DNA bases represses gene activity. Addition of methyl group at 5 position of the cytosine pyrimidine ring does not sterically interfere with GC base pairing. DNA methyltransferases associate covalently with the carbon-6 position during methyhl group transfer. DNA methylation is associated with stable gene silencing (for example on the inactive X-chromosome) either through interference with transcription – factor binding or through recruitment of repressors that specifically bind sites containing methylated CG. On the otherhand, 'Maintenance methyltransferases' add methyl groups to hemimethylated DNA during DNA replication; Example: Dnmt1 *de novo* methyltransferases act after DNA replication. Example: Dnmt2, Dnmt3b, Dnmt3a, Dnmt3B.

(2) *The Histone Code:* Histones are small, basic proteins – extremely conserved in evolution. The first 20 or so amino acids of histones, known as the histone tail, are highly conserved and are accessible to post-translational modifications by enzymes. Example: In the histone

tail, Serine, Threonine, & Tyrosine can undergo Phosphorylation; Lysine & Arginine can be methylated, acetylated, ubiquitinated & sumoylated. Furthermore, Lysine residues have potential to be mono-, di- or tri- methylated. These Histone modifications are known as "MARKS" & provide docking sites for many chromatin-associated proteins. The histone markings are done by different proteins and are commonly referred as 'writers, readers and erasers. For example, writers are histone acetyltransferases, histone kinases, histone ethyltransferases. Similarly, readers are chromodomain for methylation, bromodomain for acetylation while are histone deacetylases, phosphatases and lysine specific demethylases. The histone code hypothesis predicts that the type, location, and combination of histone marks determine the recruitment of specific chromatin associated proteins or transcription factors & subsequently determine whether the gene would be expressed or silenced. Example - histone acetylation is associated with transcriptionally active genes while deacetylation is associated with inactive genes (gene silencing). The coding in the histones may be heritable. Nucleosome Remodeling Machines and Histone variants (Seo et al., 2013). Further the Nucleosome Remodeling complexes such as SW1/SNF family transiently alters the structure of the nucleosome, exposing DNA. Nucleosome Remodeling & deacetylation (NuRD) and chromatin accessibility (CHRAC) can move nucleosomes along DNA. SWR1 complex are involved in exchange of histones and histone variants. Eg: histone 3 has three variants in mammals, H3.1, H3.2 and H3.3, differ from each other by 1 to 4 amino acids in the tail have different affinities to the binding factors.

(3) *Non-coding RNA:* Involvement of non-coding RNA in controlling multiple epigenetic phenomena are recorded. Examples: Role of RNA in dosage compensation mechanisms in Drosophila and mammals; silencing of both genes and repetitive DNA sequences by posttranscriptional (PTGs) and transcriptional (TGS) by RNAi related pathways. Small RNA and other factors such as Dicer and Argonaute are typically associated with RNAi (interference) and targets homologous DNA sequences and recruits factors that modify chromatin. Histone lysine methyl transferases (KMTs) add methyl groups to lysine within the tails of histones- Di or Tri methylation of Lysine 9 of H3 (H3K9me) is a, typical mark of heterochromatin.

Now the key questions regarding chromatin based inheritance are epigenetic modifications inherited through replication, and if so, how does this occur? How do newly incorporated histones "learn" from parental chromatin? Is there an active mechanism for templating modifications during DNA replication? From the findings of Probst (2009) and Gondor and Ohisson, (2009) it is beginning to emerge that at the replication fork, the inheritance of genetic and epigenetic information can be coupled. The components of the DNA machinery potentially cross talk with all aspects of inheritance beyond DNA sequence.
Genetics of Epigenetics: The epigenetic markings are done by different proteins and non coding RNA: Therefore, genome of an individual has to code for these different players and also has to regulate their expression.

Epigenetics and Human disease: Alterations in epigenetic machinery as a leading cause in diseaseinitiation and progression; misregulation of the epigenetic regulators can lead to abnormal gene expression. Today, a wide variety of illnesses, behaviors, and other health indicators already have some level of evidence linking them with epigenetic mechanisms, including cancers of almost all types, cognitive dysfunction, and respiratory, cardiovascular,

reproductive, autoimmune, and neurobehavioral illnesses. Known or suspected drivers behind epigenetic processes include many agents, including heavy metals, pesticides, diesel exhaust, tobacco smoke, polycyclic aromatic hydrocarbons, hormones, radioactivity, viruses, bacteria, and basic nutrients (Weinhold, 2006). In view of these developments, the current interest centers around "Common Disease Genetic and Epigenetic Hypothesis" (CDGH).

Human disease & Epigenetic Therapy: Epigenetic changes are not only heritable but also reversible. More than 100 epigenetic agents are currently under investigation. Some of the approved drugs are: DNA methyltransferase inhibitors 5-azacitidine and Decitabine; Histone deacetylase inhibitors: Vorinostat and Romidepsin (Ho et al., 2013 Popovic et al., 2013).

Epigenomics aims to study of the effects of structural and chemical modifications to chromatin and its components namely protein and DNA. Human Epigenome project aims to identify catalogue and interpret genome-wide DNA methylation patterns and profiles of all human genes in all major tissues and their disease variants (Methylome analysis at the genomic level).

Population epigenetics and evolution are emerging as active fields at the interface of molecular genetics, genomics and population Biol*ogy* (Richards, 2008). Epigenetically acquired marks on DNA and passed on from parents to off-springs, i.e., altered functional status of gene is inherited – it means trans-generational: Lamarkian flavour? (Valena and Moczek, 2012).

Summary

Epigenetics describes heritable but reversible changes in gene function in the absence of changes in primary DNA sequence. Alterations in chromatin organisation caused by modifications in DNA and Histones and noncoding RNA bring about epigenetic change. Epigenetic mechanisms play a critical role in normal development and the disease state. Epigenetics of tomorrow revolves around Epigenomics, Epigenetic drugs, Genome reprogramming, Gene-Environment-Evolution etc., Thus there is a shift in the equation from GENOTYPE → PHENOTYPE to GENOTYPE → EPIGENOTYPE → PHENOTYPE.

Suggested Readings:

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Transgenic animals: need of the country

Subeer Majumdar
National Institute of Immunology, New Delhi
Email:subeer@nii.res.in

In the era of human genome, transgenic animals provide an advanced tool for addressing the issues of gene regulation, development, pathogenesis and treatment of diseases. Since we have information about sequences of several thousand genes but have no information about their functions, transgenic animals are the only option to explore the function of a particular gene. Highthroughput techniques can swiftly identify several genes associated with a disease or an abnormal physiological situation in an individual. Action of genes can be determined partially by in vitro transfection in cells/cell lines. Any molecular biological advancement achieved *in vitro,* needs to be confirmed *in vivo.* We need to have an animal carrying one of such transgene to know its exact role in modulating the physiology. This has generated the need for developing for overcoming deficiencies) is presently generated using recombinant DNA technology in bacteria or other micro organisms. It is noteworthy that such proteins are not modified upon synthesis exactly as that happens in mammalian system, reducing their action . The cost of these proteins is also exorbitant (some times Rs.14000/vial). If such proteins can be generated in milk via udder cells of large animals i.e. cattle, not only they will be modified properly post-translationally but they will also be very cheap and affordable for common people of developing countries like ours.

Microinjection of foreign DNA in male pronucleus by *in-vitro* embryo manipulation, though difficult, is the method of choice for generating transgenic animals (Gordon et al, 1980; Palmiter et al, 1982). Other procedures, including retroviral and embryonic stem cell mediated transgenesis are equally complicated and less popular (Beard et al, 2006; Vander et al, 1985). The widely used technique involves microinjection of the foreign DNA (to be tested) in male pronucleus of a zygote (fertilized oocyte) by embryo manipulation. This is cumbersome, needs expensive equipments as well as skilled manpower. It is time consuming and requires large number of females for super-ovulation. DNA injected embryos are cultured and those which survive this trauma are transferred in the oviduct or uterus of surrogate mother for implantation and development. Due to technical difficulties in obtaining substantial number of embryos from large animals (cattle, primates, etc.) and limitations in repeated surgical intervention and embryo transfers in surrogate mothers, unlike mice, it is not practically possible to make much needed transgenic cattle. All these drawbacks generated a

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-On November 26, 2012 at the Dept. of Biotechnology, Nagaland University, Dimapur, Nagaland
-On November 27, 2012 at National research centre- Mithun, Jharnapani, Nagaland
-On December, 14, 2012 at Women's college, Agartala, and on December, 15, 2012, Belonia Women's college, Belonia, Tripura

need for developing a handy and cost effective technique for producing transgenic rodents, cattle and primates, preferably with minimal number of animal usage. To address this issue, we have chosen testis as a route of gene transmission, instead of oocytes, by appropriate insertion of the foreign gene in spermatogonial stem cells. Linearized fragments of the constructs containing the mammalian genes are injected into one of the testis of mice followed by mild electric pulses. Voltage, duration and frequency of pulses to the testis needs to be varied for obtaining best possible *in vivo* transfection. Such males are mated with wild type females to obtain F1 generation, without requirement of assisted reproductive techniques. We have established successful transmission of the transgene to F2 and F3 generations by PCR, Southern hybridization and also by RT-PCR analyses. Detailed basis of achieving at this approach is described below.

In the peripheral inner side of the seminiferous tubules of testis lie several undifferentiated male germ cells, some of which are spermatogonial stem cells (SSCs). SSCs are the only adult stem cell that has the potential to self-renew and differentiate into committed progenitors that maintain spermatogenesis throughout adult life. SSC lie at the foundation of the highly organized and productive spermatogenic process that generates 10^7 mature sperms per gram of tissue per day in the rat testis (Wing and Christensen, 1982; Russell et al., 1990). Once committed to spermatogenesis, a single rat spermatogonial stem cell can theoretically produce 4096 mature spermatozoa (Russell *et al*., 1990), hence, modification of the spermatogonial stem cell can theoretically produce equal number of genetically modified transgenic sperms. The spermatogonial stem cell is unique among adult tissue stem cells because its genotype is passed through the germline to subsequent generations and in any case, it is destined to make an individual.

The work of Ralph Brinster and his colleague at University of Pennsylvania on spermatogonial stem cell (SSC) of murine testis had generated great interest to manipulate the germ cells. They showed that either total or fractionated germ cell populations can be injected into the tubules of a recipient mice testis which has been devoid of endogenous germ cells (Brinster and Zimmermann, 1994; Brinster and Avarbock, 1994). They found that normal spermatogenesis was restored in 18–36% of the recipient mice (Brinster and Zimmermann, 1994). Following this, attempts were made to develop a method for the long-term culture of stem cells (Nagano *et al.,* 1998), with the aim of eventually transfecting them *in vitro* before re-implanting them into the seminiferous tubules of recipient animal. Nagano *et al.,* (2000) successfully used a retrovirus to transfect male SSC *in vitro* before microinjecting these germ cells into the seminiferous tubules.

The *lacZ* reporter transgene used was expressed for more than 6 months in the testis of recipient mice, strongly suggesting that the transgene had been integrated in the transfected stem cells. Later, they confirmed this hypothesis, when they showed the integration and the transmission of the transgene in 4.5% of the progeny (Nagano *et al.,* 2001). Another study used lentiviruses, a sub-class of retroviruses capable of transducing non-dividing cells, for transfecting male germ line stem cells (Nagano *et al.*, 2002). Later, transgenic rats expressing reporter genes *lac*Z or GFP were produced by *in vitro* lentiviral transduction of male germ cells using different procedures for transduction (Hamra et al.,

2002; Ryu et al., 2007).

Ablation of endogenous spermatogenesis by exposure of testis to chemicals like busulfan, which is a prerequisite for efficient colonization of donor cells (Shinohara *et al.*, 2002), often damages the environment of the recipient testes for donor cell colonization and may be responsible for reduced fertility (Ogawa *et al.*, 1999). The application of spermatogonial transplantation is still limited in most other animal species (other than mice) in which immuno-compatible recipients are not readily available. For these reasons, the efficiency of fertility restoration after spermatogonial transplantation remained limited and prevented the practical application of the technique for transgenesis.

Recently more efficient and less time consuming method of transgenesis via permanent integration of genes into spermatogonial stem cells was established by us (Dhup and Majumdar, 2008). This method exploits the testis of the male as compared to oocyte mediated transgenesis. For this they have surgically exposed the testis under the stereozoom microscope under sterile environment and injected the linearized DNA directly into one of the testis followed by electroporation with square wave pulses and suturing back the animal. Other testis was surgically removed. This study showed the presence of transgene upto F3 generation of pups. It also showed that transfected male mice can sire the transgenic pups even after 395 days of electoporation (one spermatogenic cycle in mice is about 32-35 days), thereby proving the stable and permanent gene integration in the Spermatogonial stem cells.

The technique of *in vivo* testicular electroporation for generation of transgenic animals carrying mammalian genes is most attractive because it does not involve sacrifice of any animal**.** The added advantage of this technique resides in its ability to generate transgenic progenies without assisted reproductive techniques thereby reducing time and the usage of animals for obtaining transgenic individuals. Following this principal, recently, germ cell mediated transgenesis via viral transduction of gene *in vivo* has also been reported (Sehgal etal. 2011, PLoS one).

Although our previously reported testicular transgenesis overcame many limitations, it involved several steps in addition to surgery and hemicastration carrying risk of infection. Inappropriate survival surgery of reproductive organs may also lead to impotency. Moreover, surgical procedure may not be suitable for large animals, like cattle or buffalo under farm condition. Hence, we have developed a non invasive procedure for integration of the foreign DNA in spermatogonial Stem cells of the testis. We improved this technique into a two step non-surgical electroporation procedure, for making transgenic mice. For this purpose, the linearized DNA (20-25µl of 0.5 µg/µl) containing promoter and gene of interest was injected aseptically into the testis of anesthetized animals and modified parameters of electroporation was used for *in-vivo* gene integration in germ cells. The gene integration was achieved without any surgical intervention. The whole procedure can be accomplished in less than 10 minutes. Using variety of constructs, germ cell integration of the gene and its transmission in progeny was confirmed by PCR, slot blot and immunohistochemical analysis. This improved non-surgical technique developed by us is efficient, requires substantially less time and can be easily adopted by various biomedical researchers for generating humanized models of animals at faster pace (Usmani etal., 2013). This technique has substantially increased the feasibility of making transgenic farm animals (cattle etc.) for various purposes.

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Power of Evolution

S. K. Saidapur
Former Vice Chancellor & Diamond Jubilee Professor for life
Karnatak University, Dharwad-580003
Email: saidapur@gmail.com

Today, I wish to talk on the beauty and power of evolution considering the fact that 'evolution' is not taught in many universities despite its central role in biology. Even the basic concepts like, *struggle for existence*, *survival of the fittest*, *process of natural selection* are not properly understood. Actually, evolution is a core theme in biology. It connects all branches of biological sciences and all forms – microbes to man – extinct and extant. Even human diseases are influenced by the evolutionary processes that include host parasite relations, drug resistance and evolution of virulence and so on. Apparently, the power and significance of evolutionary principles are less appreciated than they deserve.

First, let me explain what evolution means. It simply means *change*. In molecular terms it means a change in the gene frequency in a population i.e. changes in certain traits. Such changes are brought about as we know now by mutations, genetic drift etc., followed by their selection or elimination. If a certain trait is useful or at least harmless (until the time of reproduction) in a given environment it will be favored or simply retained. If a changed trait is harmful, especially in early life (before reproduction) it is not favored and not selected. The *selection* process can take place in the hands of nature as well as man i.e. naturally or artificially. Breeding plants, pet and domestic animals (fishes, birds, mammals etc) with certain chosen features by man has produced many varieties that are rarely found in nature. For example, vegetable varieties such as cauliflower, broccoli, kohlrabi, cabbage, are all derived from the wild mustard. Similarly large varieties of dogs, ornamental fishes, cats, pigeons, love birds and the like are produced in the hands of man from time immemorial. This represents *artificial selection* which man accomplished successfully thousands of years ago without any prior knowledge of genetics. Charles Darwin was very much aware of such breeding experiments in plants and animals. He himself was a great breeder of animals and plants. With this background in mind and many careful documentations and analysis, Darwin came to the conclusion that animals/plants evolve following selections taking place in nature also though at a very slow pace (geological time scale – thousands to millions of years). Accordingly, he concluded for instance, that some ancestral form of canines may have given rise to many varieties of canines like dog, wolf, fox, coyote, jackal etc that we see today. Based on his one long argument presented in the "*Origin of Species*" published in 1859 he called this phenomenon as the *natural selection* to distinguish it from *artificial selection* accomplished in the hands of man. Darwin thus visualized the *selection process* and suggested it as a chief mechanism of evolution in nature. However, he had not seen evolution

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in action. One can ask, is it possible to see evolution? We can now answer in affirmative. Yes, we can see and demonstrate the process of natural/artificial selection in action. Let me illustrate these processes and their impacts on evolution of organisms.

First, I shall deal with a classic experiment that lasted over 40 years, involving domestication of farm foxes in Russia by D. Belyaev and Lyudmila N. Trut in 1950s at the Institute of Cytology and Genetics, Russia. The breeding experiments were initiated with 30 males & 100 vixens. They bred the foxes by carefully choosing the most timid individuals each time, over 35 generations producing around 45000 offspring. By 6^{th} generation the foxes showed signs of human friendliness. This trend increased with each new generation. After 10 generations, the foxes lost musky fox-smell, wagged their tail when happy, lost negative emotions towards human approach and were human friendly. Interestingly, the ears became droopy, tail became shortened and curled due to loss of vertebrae. They also started barking like dogs. In short, domestication vis-à-vis selection pressure for tameness, made the foxes dog-like. Therefore, it is quite logical to assume that in nature following millions of years of selection, many varieties may have been produced from an ancestral form; a process that Darwin and Wallace called *Natural selection* (1858). It is important to note that the results of the farm-fox experiment show 1) an interplay between behaviour, genetics and development, 2) evolutionary changes must get incorporated into the developmental programs and pass on the selected genes (traits) to subsequent generations. They also elegantly demonstrate the power of evolution.

The above study is a classic experiment involving artificial selection. Do such changes occur in nature? Can one see natural selection in action? Yes. Now let me describe two other classic studies; Darwin's finches and, Endler's guppies.

In 1830s Darwin briefly visited the Galapagos Islands where he collected few finches assuming that they all belonged to the same species. Later, a careful study by ornithologists it was found that there were indeed 14 species that appeared very similar but their beaks differed greatly. The beaks of birds are shaped as per their food habits. Interestingly, the nearby mainland, the South America harbors only one species of finch. Darwin guessed that all the 14 varieties of finches seen on Galapagos Islands were derived from the same ancestor and that the islands are living laboratories for speciation. Recent molecular studies have supported the idea that these Galapagos finches are closely related to the single mainland finch sp. Peter and Rosemary Grants studied Galapagos finches for over two decades in the past century. They chose Daphne Major, a small island belonging to Galapagos group of islands. It has three main species of finches; the medium ground finch (*Geospiza fortis*), the cactus finch and, the small ground finch (*G. fulginosa*). Seeds of the *tribulus* plant constitute the main food for the ground finches. *Tribulus* is a weed which produces seeds having thick cover and spines. It is hard to open. For the ground finches, only a strong beak that is big in length, width and depth can cut open these seeds. An initial survey showed that there were about 1400 finches in March 1976; the bird number was reduced to <300 by December 1977 following a severe drought. The Grants made a detailed analysis of the effect of drought on finches. In the year 1977 following the drought the seed mass of *tribulus* also went down and down. Further, the available seeds were bigger in size and harder, making it difficult for the birds to open them. Between January 1976 and December of 1977, the ground finches were reduced from 1200 to 180 (85% loss) or so; the cactus finches from 208 to 110 (60% loss)

and the small ground finch from about a dozen to one! Apparently, drought affected the life of birds severely. Peter Grant and his coworkers found that average size of beaks (height, length and thickness) of the survivors was significantly greater than those who died and that of the population before the drought. The changes in the beak size were very minute and difficult to see with naked eyes, but they made difference between life and death to finches. There were also other consequences of the drought on the finches. The sex ratio that was almost 1:1 in the beginning of the drought now became 6:1. There were more males than the females. The females could therefore choose a male before settling with him. The females happily took part in the sex chase. In effect all females could pair of but only a few males had the chance of mating. What were the females choosing? They actually picked up males that were largest of the large. The largest males were the blackest with most mature plumage and deepest beaks. The males that became fathers were biggest of the big birds. The offspring were also big in body and beak sizes (~5% bigger than their ancestors). The survivors and their offspring were thus the largest in weight, wing length, tarsus length, and overall beak size. However, these changes were hardly noticeable to the naked eyes. Yet, it made a difference between life and death to the birds. In short, selection pressure following a severe drought favored bigger body size and deeper beaks in the medium ground finch, to enable opening of *tribulus* seeds. In short, environmental challenge following a severe drought and the process of natural selection resulted in a change in the population structure. These findings reveal intense episode of *natural selection* seen in action.

Now, let us turn to Endler's guppies. John Endler and coworkers studied guppies in the mountain streams and basins of Trinidad and Venezuela. They found that guppies up in the mountain are brightly colored but drab in the basin. They also observed that guppies experienced a differential predator pressures; up in the mountain, the guppies suffered a *low* predation pressure that was mainly due to the killifishes (*Rivulus hartii*), while at the basin they suffered *high* predation pressure due to the cichlids (*Crenichichla alta*). Thus, the colorfulness of guppies appeared to be inversely related to predation pressure. They hypothesized that 1) predators are selectively consuming brightly colored guppies, sparing the drab ones, 2) the guppies are evolving to match or stand out from the substratum/environment and 3) the females are choosing brightly colored males giving them a chance to pass on their genes to next generation forcing them to retain bright color in spite of predator risk. They studied the guppies for over a decade and also conducted several experiments in nature. In one set of experiments, they reared 10 different populations of guppies under high, low and no predation pressures in natural large field ponds for 10 generations. In another set of experiments, they relocated guppies from the basin to mountain top there by relaxing predation pressure on them. They were reared over 15 generations. The results of these experiments showed many interesting phenomena including a change in the life history strategies in relation to low or high predation pressures. Under a low predation pressure, guppy populations evolved gaudy color with large spots, adults were larger in size, and they matured slowly, mated at a later age and less often, and had fewer offspring. On the other hand, under a high predation pressure, the guppies evolved into a drab color forms, adults were smaller in size, and they matured quickly, mated at an early age and more often. Thus, these reproduced at an younger age and also produced more offspring. The guppies thus revealed how selection pressures in nature influence evolution by natural selection.

My last example is the rise and fall of peppered moths in England following the industrial revolution in the 19th century. The peppered moths occur in two forms; peppered form and a mutant form that is black color. Before the industrialization in the UK, the peppered moths were in plenty. The black variety was rarely available to the collector. It was indeed a prize item. However, after the industrial revolution, peppered moths became a rarity and black forms were in plenty. The rise and fall in peppered moth is explained as follows: before the industrial revolution, the plant barks had plenty of lichens growth and peppered moths could be rarely spotted by the birds and other predators and hence escaped predation to a large extent. Whereas, the black variety of moths were easily seen over the lichens cover and succumbed to predation easily. Soon after the industrial revolution, that brought carbon deposition over the plants and reduced or prevented lichens growth. Consequently, under the changed scenario, the peppered moths became victims of predation more easily than the black forms of moths. In short, before industrialization the peppered forms were abundant and after the industrialization the black forms. The rise and fall of peppered moths also highlights how predation pressure operates in nature and how natural selection takes place.

In nutshell, life arose some 4.5 billion years ago by the assembly of self-replicating molecules. Eventually, countless forms of reproducing cells were formed. Much more were subject to elimination. Those that could survive, continued to face challenges of nature and evolve to give rise to multi-cellular organization, increasing complexities each time. Each new form was chosen or eliminated by the force of natural selection. The great biodiversity we encounter today, microbes, plants, and animals are indeed the products of evolution by a process called *descent with modification* and *natural selection*. Obviously, the evolutionary principles have far reaching implications in biology; human health and diseases included. For instance, drug resistance and evolution of virulence in pathogens is a global problem and a serious one. Selection of harmful genes also has evolutionary origin. The antibiotic resistance, pesticide resistance and evolution of virulence are now explained on the basis of natural selection. They are not due to gradual tolerance. Evolutionary principles have now opened up new branches like, Darwinian Medicine, Darwinian Agriculture, Darwinian Fisheries, and Evolutionary Psychiatry and so on.

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Section IV: Plant Sciences

Our biodiversity – our heritage

Floristic diversity in India: Inventorization, Conservation and Bioprospection

Dr. R.R. Rao
INSA Honorary Scientist
No. 328, B-4, Kendriya Vihar, Yelahanka, Bangalore -560064
Email: raocimap@gmail.com

Introduction

Diversity simply means to be different or unlike. Biological diversity or biodiversity refers to the variety and variability among living organisms from all sources including inter alia, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystem for food, clothing, medicines, housing and major industries. It is of scientific importance for protecting and maintaining soil and regulating climate). Biological diversity includes many species of wild plants and animals that have significant economic potential but currently undiscovered. The importance of genetic diversity for sustaining and increasing agricultural production is well acknowledged. Floristic diversity provides a strong and broad genetic base for plant breeding and development of high yielding and disease resistant crop varieties.

Biodiversity in India

India, a mega diverse country with only 2.4% of the world's land area, harbours 7-8% of all recorded species, including over 45,000 species of plants and 91,000 species of animals. It is also amongst the few countries that have developed a biogeographic classification for conservation planning, and has mapped biodiversity-rich areas in the country. Of the 34 global biodiversity hotspots, four are present in India, represented by the Himalaya, the Western Ghats, the North-east, and the Nicobar Islands. Considering the outstanding universal values and exceptionally high levels of endemism in the Western Ghats, 39 sites in the States of Kerala, Karnataka, Tamil Nadu and Maharashtra have been inscribed on the United Nations Education Scientific and Cultural Organization (UNESCO) World Heritage List in 2012. The

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richness of the vegetation cover of India is well known. The Indian region (600 45' to 370 6'N and 680 7' to 970 25' E) with a total area of about 3029 million hectares is considered to be one of the twelve centers of origin and diversity of several plant species in the world and supports an enormous biodiversity of ancient lineage. According to the Russian botanist N. I. Vavilov (1926) the Indian region forms the 'Hindustan centre of origin of cultivated plants'. The great British botanist, Sir J. D. Hooker (1 904) remarks at one place "The Indian Flora is more varied than that of any other country of equal area in the eastern hemisphere, if not on the globe". It is estimated that about 19,200 vascular plants (Flowering plants, Gymnosperms and Pteridophytes) are accounted for in this region (Table 1).

Table 1 Sowing floristic diversity in various groups of plants in India (Source BSI)

Group	**No. of Species**	**Endemic**	**%**
Angiosperms	17,817	5,725	32.71
Gymnosperms	74(137)	10	07.50
Pteridophytes	1,265	193	16.08
Bryophytes	2,479	629	33.90
Lichens	2333	520	22.50
Fungi	14,698	3,500	24.13
Algae	7,192	1,924	27.88
Virus & Bacteria	966	-	-

A significant feature of the Indian flora is the confluence of floras from the surrounding countries like Malaya, Tibet, China, Japan, Europe and even from wide separated continents like America, Africa and Australia. This fact had prompted Hooker (1904) to arrive at the erroneous conclusion that India has no flora as a separate entity but is an admixture of the floras from the adjacent countries. The subsequent phyto geographers after critical analysis of flora have convincingly concluded that India has a flora of its own, and in fact as many as 5500 species are endemic to this region (1962; Nayar, 1980). In fact, India is said to harbour more endemic species of plants than any other region of the world except Australia. There are about 150 endemic genera distributed over 47 families. The total endemic genera in India represent 6.5% of the 2252 genera occurring in India. The largest endemic genera are

Pteracanthus (20 spp.) and *Nilgirianthus* (20 spp.) of the family Acanthaceae. Areas rich in endemism are the northeast India, followed by southern parts of Peninsular India and northwestern Himalaya. The northwest Himalaya is estimated to harbour about 1000 endemics, out of about 4000 total species, while the Eastern Himalaya has about 1600 endemic species out of a total of about **5**500 species. Peninsular India with an estimated total of 6000 species contains about 2000 endemic species. The Andaman & Nicobar Islands contribute 185 species to the endemic flora of India. Monotypic genera which have no closely related genomes anywhere in the world are also well represented in India. *ca*. 236 genera (dicots 176 genera and monocots 60 genera) are monotypic. Over 60 families are believed to be represented so far by just one species in India, e.g. Coriariaceae, Turneraceae, lliciaceae, Ruppiaceae, Philydraceae, Tetracentraceae and Siphonodontaceae, etc. Poaceae with 32 monotypic genera is most dominant followed by Leguminosae (15) Asteraceae (12) Rubiaceae (11) and Orchidaceae (6).

This enormous richness of floristic diversity is due to the immense variety of the climatic and altitudinal variations coupled with varied ecological habitats. There are almost rainless areas to the highest rainfall area in the world. The altitude varies from the sea level to the highest mountain ranges of the world. The habitat types vary from the humid tropical Western Ghats to the hot desert of Rajasthan; from cold desert of Ladakh and icy mountains of the Himalayas to the long, warm coast line stretches of Peninsular India. Further, the strategic position of the Himalaya has not only drawn numerous elements from the adjacent countries and regions, but also through active speciation, has added many Neo-endemics.

Some monotypic and endemic plants of Western Ghats

Species	Family
Acrotrema arnotttianum Wight	Dilleniaceae
Adenoon indicum Dalz.	Asteraceae
Chandrasekharania keralensis Nair,Ramachandran & Sree Kumar	Poaceae
Hubbardia heptaneuron Bor	Poaceae
Indobanalia thyrsiflora (Moq.) Henry & B. Roy	Amaranthaceae
Indopoa paupercula (Stapf) Bor	Poaceae
Janakia arayalpathra Joseph & Chandrasekaran	Asclepiadaceae
Kanjarum palghatense Ramamurthy	Acanthaceae
Kingiodendron pinnatum (Roxb. ex Dc.)	Leguminosae
Kunstleria keralensis Mohanan & Nair	"do"
Lamprachaenium microcephalum (Dalz.)Benth.	Asteraceae
Limnopoa meeboldii (Fischer) Hubb.	Poaceae
Moullava spicata (Dalz.)Nicolson	Leguminosae
Nanothamnus sericeus Thoms.	Asteraceae
Otonephelium stipulaceum (Bedd.) Radlk.	Sapindaceae
Paracautleya bhatii R.M.Smith	Zingiberaceae
Polyzygus tuberosus Dalz.	Apiaceae
Proteroceras holtumii Joseph & Vajravelu	Orchidaceae
Pseudodichanthium serrafalcoides (Coke & Stapf) Bor	Poaceae
Santapaua madurensis Balak. ex Subramanyan	Acanthaceae
Seshagiria sahyadrica Ansari & Hemadri	Asclepiadaceae
Silentvaleya nairii Nair & Bhargavan	Poaceae
Solenocarpus indica Wight & Arn.	Anacardiaceae
Trilobanche cookie (Stapf) Sch. ex Henr.	Poaceae
Triplopogon romasissimus (Hack.) Bor	Poaceae

The extreme diversity of the habitats has resulted in almost all types of forests, ranging from scrub forest to the tropical evergreen rain forest, and coastal mangrove to the temperate and alpine floras. The richness and diversity of flora of India can be further appreciated by the fact that as many as 10 biogeographic regions representing 3 basic biomes and 2 natural realms as identified by Udvardy (1 975) are recognized within the territory of the Indian Republic. The flowering plants of India comprise about 18500 species which are placed under

ca 320 families and 2300 genera. Several families as listed below show great floristic diversity and are represented by more than 100 species.

Ranunculaceae	160	Lauraceae	163
Brassicaceae	164	Euphorbiaceae	419
Caryophyllaceae	110	Moraceae	105
Malvaceae	100	Urticaceae	115
Fabaceae	775	Ericaceae	150
Rosaceae	217	Lamiaceae	393
Aplaceae	202	Scrophulariaceae	356
Rubiaceae	495	Acanhaceae	379
Asteraceae	1150	Orchidaceae	990
Primulaceae	165	Zingiberaceae	133
Asclepiadaceae	209	Liliaceae	159
Gentianaceae	147	Arecaceae	90
Boraginaceae	138	Araceae	138
Convolvulaceae	161	Cyperaceae	449
Myrsinaceae	115	Poaceae	1225

Several taxa such as *Berberis* (ca. 52 species with ca. 30 infra specific categories) have in the Himalayan region a primary center of diversification. These taxa reveal maximum diversity not only in different ecological zones but also in one and the same habitat, often posing immense taxonomical problems.

The Indian region has approximately world's half of the aquatic flowering plants. The aquatic families in the Indian flora are Alismataceae (5 genera, 8 spp.), Aponogetonaceae (6 spp.), Azollaceae (1 sp.), Barclayaceae (2 spp.), Butomaceae (1 sp.), Cabombaceae (2 genera, 2 spp.), Callitrichaceae (2 spp.), Ceratophyllaceae (3 spp.), Hydrocharitaceae (8 genera, 13 spp.), lsoetaceae (10 spp.), Lemnaceae (4 genera, 14 spp.), Marsileaceae (10 spp.), Najadaceae (7 spp.), Nelumbonaceae (1 sp.), Nymphaeaceae (2 genera, 7 spp.),

Podostemaceae (11 genera, 24 spp.), Pontederiaceae (2 genera, 3 spp.), Potamogetonaceae (6 spp.), Ruppiaceae (1 sp.), Salviniaceae (3 spp.), Trapaceae (2 spp.), Typhaceae (4 spp.), Zanichelliaceae (1 sp.). The species of the families Podostemaceae and Tristichaceae grow on rocks firmly attached in swift flowing mountain streams and rivers.

On the other end of the spectrum, there are many biologically curious plants like insectivorous plants, parasites, saprophytes, etc. in the Indian region. The families having characteristic insectivorous plants are Droseraceae (3 spp.), Nepenthaceae (1 sp.), and Lentibulariaceae (36 spp.). The parasitic families are represented by Loranthaceae (46 spp.), Santalaceae (10 spp.), Balanophoraceae (6 spp.), Rafflesiaceae (1 sp.), Cuscutaceae (12 spp.) and Orobanchaceae (54 spp.). Metal tolerant species are found in the families Caryophyllaceae, Ceratophyllaceae, Portulacaceae, Tamaricaceae, Salvadoraceae, Thymelaeaceae and Fabaceae while species of the families Chenopodiaceae, Basellaceae, Amaranthaceae and Phytolaccaceae are salt tolerant. Several plants e.g. species of *Arenaria, Thylacospermum, Acantholimon, Festuca* and *Juniperus* that occur especially in the high alpine meadows survive the extreme adverse ecological conditions by special adaptations. The woolly species of *Saussurea* form an interesting plant of the West Himalaya. *Sapria himalayana* and *Mitrastemon yamamotoi* of the family Rafflesiaceae, with only the flower (representing the whole plant) projecting from the roots of the host plant form an unusual case of botanical interest. Similarly *Balanophora dioica*, *Boschiniaekia himalaica, Aeginitia indica* are other root parasites of great morphological interest. Genera like *Galeola, Epipogium* and *Monotropa* are the best examples of saprophytes. Among the insectivorous plants *Nepenthes khasiana*, *Drosera burmannii, D. peltata*, *Utricularia* spp. and *Aldrovanda* spp. are of significant interest. *Poa litorosa* (Poaceae), the Angiosperm with highest chromosome number, also occurs in India.

The presence of a large number of **primitive flowering plants** in India, particularly northeast India renders the region 'a cradle of flowering plants' (Takhtajan, 1969). In this region as many as 131 species are stated to be primitive (table -1).

Primitive Angiosperms of N. E. India

Name of species	Family	Distribution
Alcimandra cathcartii	Magnoliaceae	E. Himalaya, Assam
Magnolia campbellii	-do-	E. Himalaya, Manipur
M. gustavii	-do-	Assam
M. pealiana	-do-	Assam
Manglietia insignis	-do-	E. Himalaya, Meghalaya
Michelia champaca	-do-	E. Himalaya, Assam
Pachylarnax pleiocarpa	-do-	Assam, Nagaland
Paramichelia baillonii	-do-	Assam
Talauma hodgsoni	-do-	Sikkim, Assam
T. rabaniana	-do-	Assam
Ilicium griffithii	Illiciaceae	Meghalaya

Kadsura heteroclita	Schisandraceae	E. Himalaya, Assam
Schisandra axillaris	-do-	Meghalaya
Tetracentron sinense var. himalayana	Tetracentraceae	E. Himalaya
Euptelea pleiosperma	Eupteleaceae	Assam
Brasenia schreberi	Cabombaceae	Meghalaya
Aspidocarya uvifera	Menispermaceae	E. Himalaya
Haematocarpus thomsonii	-do-	Sikkim, Meghalaya
Pycnarrhena pleniflora	-do-	Assam
Decaisnea insignis	Lardizabalaceae	E. Himalaya, Assam
Holboellia latifolia	-do-	Meghalaya
Parvatia brunoniana	-do-	Assam
Stauntonia brunoniana	-do-	Assam
Altingia excelsa	Hamelidaceae	Assam
Corylopsis himalayana	-do-	Meghalaya
Distylium indicum	-do-	Meghalaya
Exbuclandia populnea	-do-	E. Himalaya, Meghalaya
Houttuynia cordata	Piperaceae	Meghalaya
Myrica esculenta	Myricaceae	Assam
Alnus nepalensis	Betulaceae	Assam

The Himalayan bio geographic zone is the richest and unique botanical region in India and encompasses a broad range of ecological habitats varying from grassy meadows to dense humid evergreen forests; disturbed secondary formations to almost virgin and relict types as in `Sacred Forests'. A mixture of tropical, temperate and alpine forests each type depicting its own characteristic biodiversity is represented in this region. About 50% of the total number of higher plant species of India occur in this region which includes nearly 35% of endemic taxa. This region being the `Sanctuary of Primitive Angiosperms' is considered as the *cradle of Flowering Plants* where some groups of angiosperms have originated and diversified (Takhtajan, 1969; Rao, 1994). Several families in the Himalaya show great floristic diversity both at species and infra specific level and are represented by more than 100 species. Some plants e.g. species of *Christolea, Arenaria, Thylacospermum, Acantholimon, Festuca* and *Juniperus* that occur especially in the high alpine meadows in cold desert regions of Trans-Himalaya survive the extreme adverse ecological conditions by special adaptations. Cushion forming plants include species of *Androsace* (Primulaceae), *Saxifraga* (Saxifragaceae), *Rhodiola* (Crassulaceae), *Thylacospermum* and *Arenaria* (Caryophyllaceae).Several hundred plants aggregate together to form a dense, spherical or globose cushion-like habit. Certainly this is an adaptation against severe cold and heavy snowfall during winters. One cushion of *Arenaria* or *Thylacospermum* measuring *ca* 30cm diam takes as much as 100-150 years. Among the insectivorous plants *Nepenthes khasiana*, *Drosera burmannii, D. peltata*, *Utricularia* spp. are of significant interest. *Christolea himalayensis* is reported to be growing at 6300 m, which is the highest altitude known for a flowering plant in the world. On the other end of the spectrum there are some plants with unusual forms in the high alpine areas. These may be

'cushion forming' or 'snow ball' plants or the 'hot house' plants. The 'snow ball' plants, *Saussurea gossypiphora* and *S. graminifolia* (Asteraceae) look like a snow ball due to the dense, white woolly hairs which cover the entire plant and protect from cold wind and snow and keeps warm in day time even if outside temperature suddenly falls. Bees or flies take shelter in the warmth and at the same time pollinate the flowers. The dense woolly hairs that cover the apical meristem act as a sort of thermal insulation. There is yet, another interesting group of 'hot house' plants like the *Rheum nobile* and *Saussurea obvallata* which have their inflorescence sheltered by leafy bracts that can be compared to glasses of a 'hot house'. The flowers open inside the bracts, where the insects also take shelter for warmth and at the same time pollinate the flowers.

A rich concentration of medicinal and aromatic plants, canes and bamboos, orchids, rhododendrons and other groups of horticultural value is also observed in the region. High value medicinal plant species like *Aquilaria malaccensis*, *Dioscorea deltoidea*, *Podophyllum hexandrum*, *Rauvolfia serpentina*, *Saussurea lappa*, *Taxus wallichiana* *Picrorrhiza kurrooa*, *Nardostachys jatamanasi*, *Aconitum* spp., *Berberis* spp., *Coptis teeta*, and numerous others are well represented in the Himalayan region and many have already become endangered because of their selective removal. Further, the Himalayan region is a storehouse of wild plant genetic resources of a number of our cultivated crops including citrus, mango, banana, plums, pears, cucurbits, grain legumes, areca nut, wild date palm and many wild varieties of rice. The present day Himalayan flora is related to the flora of northwestern and western China in the eastern sector while the Mediterranean elements become more numerous westward from Sikkim to Kashmir through Nepal, Kumaon and Garhwal region including Nanda Devi Biosphere Reserve. Although the Chinese mountains being much older in age compared to Himalaya and have contributed significantly to the Himalayan flora, the Himalayas have added quite significantly to the endemic flora of the country through speciation.

Western Ghats is another major floristic diversity center in India. About 12000 species from lower groups to flowering plants are estimated to occur here. About 2100 endemic flowering plants have been reported from out of 5800 flowering plant species in this mega endemic area (Rao, 1994). This constitutes approximately 27 % of the total Indian flora. Agasthyamalai (200 km^2) support 2000 species; The Nilagiris support *ca* 2611 species while Silent Valley (90 Sq Km) supports 1300 species. Most of the District floras published in recent years also reveal that most of them have more than 1500 species. Several species of *Piper*, *Oryza*, *Myristica*, *Elettaria*, *Amomum*, *Zingiber*, *Phaseolus*, *Vigna*, *Atylosia*, *Costus*, *Cinnamomum*, *Curcuma* show great variability in areas of Wynad, Silent Valley, Parambikulum, Anaimudi, Devikulam, Agastyamalai, Palni hills, Mudumalai, Bolurampatti, Megamalai, Kalakad, Keenparai, Shevoroy, Nagarhole, Talakaveri, Brahmagiri, Kudremukh, Biligirirangan hill's and Gopalaswamy hills. Evidently, these areas also happen to be rich storehouses of floristic diversity but have come under great human influences. Of the five locations in India identified by the IUCN's Threatened plants unit as priority sites for conservation, three locations belong to Western Ghats alone namely, (i) Agastyamalai hills (ii) Silent valley and New Amarambalam Reserve, and (iii) Periyar National park. Agastyamalai Hills are located at the southern extremity of Western Ghats. Climax evergreen forests, tropical deciduous forests and tropical montane forests that cover this zone include at least 150 localized endemic species including trees like *Diospyros barberi*, *Elaeocarpus venustus*, *Eugenia flaccosa*,

Garcinia travancorica, etc. The Silent valley- New Amarambalam basin contain some of the relict undisturbed tropical rain forests in India. The unique and dominant tree species are *Bischofia javanica, Calophyllum clatum, Canarium strictum, Cinnamomum verum, Cullenia exarillata, C. excelsa, Dysoxylum malabaricum, Elaeocarpus tuberculatus, Hopea parviflora, Knema attenuata, Palanquium ellipticum, Persea macrantha, Poeciloneuron indicum* and *Vateria indica.* Several wild species of commercial crops like pepper, cardamom, banana, cinnamon etc., have much variability here, apart from numerous endemic species of Western Ghats. The Periyar National park till recent years was much disturbed for Eucalyptus plantations. The dense evergreen and deciduous forests cover ca 60% of the park. The riparian vegetation along the Periyar River includes species like *Cinnamomum riparium*, *Homonia riparia, Syzygium occidentale* and several bamboos. Although much of these hot spots are now brought under the Nilgiri Biosphere Reserve, their future lies in proper understanding and management of the biosphere reserve.

The floristic diversity of the country is spread in various biogeographic regions such as Trans Himalaya, West Himalaya, East Himalaya, North-East India, Deccan region, Western Ghats, Arid and Semi arid zone, Indian desert, Indian coasts and Andaman and Nicobar Islands as shown below. It is worthwhile to appreciate the floristic diversity in some taxa/groups in India. Orchids are well known for their showy and long lasting flowers. The group exhibits remarkable diversity both in the Himalaya and Western Ghats and forms the second largest family of flowering plants in India. Roughly, there are 1200 species of orchids in India with a high concentration of about 700 species in northeast India alone. *Dendrobium* (80), *Bulbophyllum* 58, *Liparis* 45, *Coelogyne* (38) *Habenaria* (105) are some of the dominant genera in India. About 300 species of orchids have already become endangered in India. Similarly, the *Rhododendron* group is also highly diverse. The genus *Rhododendron* of the family Ericaceae is also a highly ornamental group having varied colours of flowers. The genus has about 96 species in India (Himalaya) and only one species, *R. arboretum* var. *nilagiricum* extends to Western Ghats. Eastern Himalaya particularly, the Arunachal Pradesh has more that 75 species of *Rhododendron.* Apart from the number of species, the genus has life form diversity as herbs, shrubs and trees. *Rhododendron nivale* is the smallest of the rhododendrons in India. Some of the common species are *R. anthopogon, R. campanulatum, R. arboretum, R. hodgsonii* and *R. thomsonii.*

The genus *Hedychium* of the family Zingiberaceae is another group of ornamental plants that can be directly introduced into our gardens. The fragrant flowers on terminal spikes have attractive white, yellow, orange, and red colours. There are ca 40 species in India, of which 35 species occur in east Himalaya alone. From Arunachal Pradesh itself 18 species are reported. Some of the common species of the genus are *H. aurantiacum, H. coronarium, H. densiflorum*, *H. thyrsiforme and H. villosum*; and the rare ones are *H. luteum*, *H. greenii*, *H. aureum*, *H. longipedunculatum*, *H. radiatum*, *H. dekianum* and *H. wardii*

The diversity of bamboos in India is also very high. Bamboos play an important role in the economy of a county and are associated with the humankind since ancient times. Out of 20 genera and 135 species so far known in India 15 genera and 65 species are represented in eastern Himalaya. Eastern Himalaya forms the center of diversity for the genera *Bambusa, Dendrocalaumus, Arundinaria and Cephalostachyum.* Some dominant genera are *Arundinaria*

(11species), *Bambusa* (22 species), *Cephalostachyum* (7 species), *Chimanobambusa* (9 species), *Dendrocalamus* (15 species), *Thamnocalamus* (4 species), *Dinochloa* (2 species) and *Oxytenanthera* (2 species).

Major 'hotspots' of endemic and genetic diversity distributed in various biogeographic zones of India

Hot spots	**Biogeographic Zone**
Karakoram & Ladakh	Trans Himalaya
Kumaon-Garhwal Himalaya	W. Himalaya
Siwaliks	-do-
Sikkim Himalaya	E. Himalaya
Arunachal Pradesh	N.E India
Lushai hills	-do-
Tura, Balphakram, Khasi Hills	-do-
Aravallis	Semi-arid zone
Bundelkhand	Gangetic plain
Chotanagpur plateau	Deccan
Panchmarhi-Satpura ranges	-do-
Simlipal & Jeypore hills of Orissa	Eastern Ghats
Bastar & koraput hills	Deccan
Vishakhapatnam hills & Araku Valley	-do-
Tirupati-Cuddappa hills	Eastern Ghats
Marathwada hills	Deccan
Saurashtra-Kutch	-do-
Mahabaleshwar-Khandala ranges	W. Ghats
Agumbe-Phonda ranges	-do-
Ratnagiri & Kolaba ranges	-do-
Nilgiris	-do-
Silent Valley & Wynaad	-do-
Anaamalai	-do-
Idduki-Sabarigiri	-do-
Kalakad & Agastaimalai hills	-do-

Diversity of Medicinal & Aromatic plants:

The use of plants to alleviate human suffering is as old as the evolution of human civilization itself. Mention of the medicinal virtues of plants in India has been made even in the great epics like the Ramayana and Mahabharatha. The most ancient and celebrated treatises on Hindu medicine are no doubt the Ayurveda. The authoritative works like *Charaka samhita, Susruta samhita, Rigveda* and *Astanga Hridaya* marks the early base of

herbal science in India. As many as 4000 plants are collectively mentioned in these early works. Medicinal Plants Diversity in India is also very high. Nearly 550 ethnic tribes dwelling in different forest regions have vast amount of traditional knowledge about plants, particularly medicinal plants. Over 8000 species reported to be medicinal are found in different ecosystems in the country. According to FRLHT's report Ayurveda-1689; Folk medical system-4775; Homeopathy-491; Modern medicine-200; Siddha-1563; Tibetan-343 and Unani-843 species are medicinal plants. Again, these species are represented by numerous subtypes or populations depending upon the climatic and edaphic conditions. While on one side we have not been able to evaluate the infra specific diversity in most of these medicinal plants for identifying the elite types, many species and populations are facing the threat of extinction due to several anthropogenic reasons. Already a number of reputed medicinal species such as *Aquilaria malaccensis*, *Dioscorea deltoidea*, *Podophyllum hexandrum*, *Pterocarpus santalinus*, *Rauvolfia serpentina*, *Saussurea lappa* and *Taxus wallichiana* have become endangered. Western Ghats is a store house of many medicinal plants like *Rauvolfia serpentina*, *Gloriosa superba*, *Cassia angustifolia*, *Withania somnifera*, *Chlorophytum sps*., *Catharanthus roseus*, *Andrographis paniculata*, *Phyllanthus amarus*, *Trichopus zeylanicus*, *Janakia arayalpathra*, *Utleria salicifolia*, *Aristolochia tagala*, *piper barberi*, *Adenia hondala*, *Garcinia sps*., *Thottea siliquosa*, *Caryota urens*, *Adhatoda beddomei*, *Myristica malabarica*, *Coscinium fenestratum*, etc. and offers immense prospects for bioprospection of medicinal flora. As a step in this direction, it is necessary to shortlist the species for Bioprospection following cross cultural ethnobotanical studies. The Himalayan region has always been considered as a store house of many life saving drug plants. Based on several ethnobotanical and other publications, the medicinal plants in this region cover almost 50% of the total medicinal plants of India, i.e, ca 4000 species. It is roughly estimated that West Himalaya harbors 1500 species of medicinal plants, East Himalaya 3000, Western Ghats 3500, and Eastern Ghats 1500, Andaman & Nicobar Islands 750 species. Further, it is shown that Karnataka hosts for about 1495 medicinal species while Tamil Nadu, 1574; Kerala, 1500; Andhra Pradesh, 1100 species. Further, the varying habitats, from tropical to alpine flora support the growth of numerous medicinal plants, many of which have not been critically evaluated. Some of the important medicinal plants of the region are Coptis teeta, (Mishmiteeta) Paederia foetida (Gandhali), Podophyllum *hexandrum* (Papra) *Nardostachys grandiflora* (Jatamansi), *Panax pseudo-ginseng* (ginseng), *Picrorhiza kurroa* (Kutki) *Alpinia galanga* (Bara kulapjan), *Dactylorhiza hatagirea*, *Rheum emodi*, *Berberis* spp, *Aconitum heterophyllum*, *Elaeocarpus sphaericus*, *Acorus calamus*, *Atropa acuminata*, *Costus speciosus*, *Mucuna prurita*, *Rauwolfia serpentina*, *Swertia chirayata*, *S. hookeri*, *Valeriana hardwickii*, *Berginia ciliata*, *Mahonia nepalaensis*, *Saussurea obovallata*, *S. graminifolia*, *Solanum khasianum*, *Ephedra girardiana* and many others (Rao, 1994)

The medicinal plants diversity in Northeast India is quite enormous. The numerous adivasi tribes occupying the different forested areas in northeast region depend on the surrounding vegetation for all their ailments and they have known the use of medicinal plants for all major diseases like malaria, leprosy, pneumonia, tuberculosis, Typhoid, night blindness, ulcers, cancer, skin diseases, hypertension, jaundice, eye diseases, lever disorder, kidney troubles, gynecological disorders etc. Although no data is available on the exact number of medicinal plants occurring in Northeast India, a recent publication by the Indian Institute of Remote Sensing (Anonymous 2002) has listed 419 species of medicinal plants from Arunachal

Pradesh, 228 species from Assam, 86 species from Manipur, 74 species from Meghalaya, 83 species from Mizoram, 86 species from Nagaland, 73 species from Tripura. Some important such species are *Aconitum chasmanthum, Aconitum deinorrhizum, Aconitum ferox, Aconitum heterophyllum, Coptis teeta, Swertia chirayita, Swertia hookeri, Swertia ciliata, Nardostachys grandiflora, Picrorhiza kurrooa, Podophyllum hexandrum, Rheum australe, Rheum nobile, Valeriana hardwickii, Valeriana jatamansi* (alpine & subalpine zone) *Berberis asiatica, Berberis wallichiana, Bergenia ciliata, Brugmansia suaveolens, Daphne cannabina, Datura stramonium, Ephedra gerardiana, Habenaria commelinifolia, Hoya globulosa, Gaultheria fragrantissima, Gentiana kurroo, Illicium griffithii, Mahonia nepalensis, Mahonia pycnophylla, Myrica esculenta, Panax pseudo-ginseng, Plantago major, Sarcandra glabra, Saussurea lappa, Taxus wallichiana* (Temperate zone) and *Abroma augusta, Abrus precatorius, Acorus calamus, Adhatoda zeylanica, Adiantum lunulatum, Allium chinense, Alpinia bracteata, Alpinia galanga, Alysicarpus monilifer, Amomum sublatum, Anaphalis adnata, Aristolochia tagala, Asparagus racemosus, Atropa acuminata, Begonia palmata, Boehmeria malabarica, Bonnaya reptans, Borreria articularis, Brassica campestris, Careya arborea, Centella asiatica, Centranthera grandiflora, Citrus medica, Cinnamomum tamala, Colocasia esculenta, Costus speciosus, Curcuma angustifolium, Curcuma domestica, Curcuma Montana, Curcuma zeodaria, Cyclea bicristata, Dendrocalamus hamilonii, Dichrocephala bicolor, Dioscorea deltoidea, Dysoxylum procerum, Embelica officinalis, Entada purseatha, Garcinia cowa, Garcinia lancifolia, Gerbera macrophylla, Glochidion khasicum, Gloriosa superba, Habenaria acuifera, Hedyotis scandens, Hodgsonia heteroclita, Hydrocotyle javanica, Iphigenia indica, Ipomoea aquatica, Kaempferia rotunda, Leucosceptrum canum, Hydnocarpus kurzii, Hyoscyamus niger, Lycopodium clavatum, Mesua ferrea, Mimosa pudica, Mucuna prurita, Nerium indicum, Nepenthes khasiana, Ocimum sanctum, Oxalis corniculata, Paederia foetida, Panicum maximum, Piper brachystachyum, Piper griffithii, Piper betel, Plectranthus coetsa, Plumbago zeylanica, Polygonum capitatum, Polygonum perfoliatum, Pouzolzia hirta, Psidium guajava, Punica granatum, Rhus semialata, Rauvolfia serpentina, Rubia cordifolia* and *Sarcandra glabra* (tropical & subtropical zone)

The medicinal plant diversity in Western Ghats is of a very high order both in terms of species diversity as well as infra specific diversity. Apart from the well established medicinal plants like *Rauvolfia serpentina*, *Gloriosa superba*, *Cassia angustifolia*, *Withania somnifera*, *Chlorophytum sps*., *Catharanthus roseus*, *Andrographis paniculata*, *Phyllanthus amarus*, etc. the region particularly the Southern Western Ghats harbours many ethnobotanically important species like *Trichopus zeylanicus*, *Janakia arayalpathra*, *Utleria salicifolia*, *Aristolochia tagala*, *Piper barberi*, *Adenia hondala*, *Garcinia sps*., *Thottea siliquosa*, *Caryota urens*, *Adhatoda beddomei*, *Myristica malabarica*, *Coscinium fenestratum*, etc with many curative properties. Roughly, 1800 species of medicinal plants from out of the total of 6000 species of Western Ghats are reported (Yoganarasimhan, 1996, 2000). The floristic diversity of wild aromatic plants in Western Ghats is also incompletely known. While medicinal plants have received some attention, other groups such as the essential oil yielding plants (which are also of medicinal value) of the region are least studied. There are more than 200 such aromatic species in different ecosystems of Western Ghats and are predominantly spread among Lamiaceae, Asteraceae, Rutaceae, Zingiberaceae, Lauraceae, Oleaceae and Poaceae. While species diversity is assessed to some extent, infra specific diversity in these aromatic species is least known. Nevertheless, many species like *Hyptis sauveolens*, *Blumea lacera*, *B. hieracifolia*, *B. membranacea*, *Cymbopogon flexuosus, Ocimum basilicum*, *Plectranthus mollis*

exhibit remarkable morphological variations in the region. Western Ghats with a wide variety of ecological habitats certainly provides for numerous ecotypes / chemotypes in some of these medicinal and aromatic plants. Investigations on infra specific diversity and genetic diversity of at least a few commercially important medicinal plants like *Berberis asiatica*, *Bergenia ciliata*, *Illicium griffithii*, *Myrica esculenta*, *Panax pseudo-ginseng*, *Plantago major, Saussurea lappa, Taxus wallichiana*, *Aconitum chasmanthum*, *Aconitum heterophyllum*, *Coptis teeta*, *Swertia chirayita*, *Swertia ciliata*, *Nardostachys grandiflora*, *Picrorhiza kurrooa*, *Podophyllum hexandrum*, *Rheum australe*, *Rheum nobile*, *Valeriana jatamansi* are needed, so as to identify the 'elite' types for large scale commercial cultivation. Authentication and development of value added products from these drug plants after critical scientific evaluation and pharmacological trials can certainly boost the regional economy. Pharmaceutical companies must take a lead in R & D activities on these medicinal plants and establish scientifically sound pharmacopoeias for the drugs developed from these plants.

Diversity in wild relatives of cultivated plants

According to Vavilov the northeastern region of India, forms the 'Hindustani Centre of Origin of Cultivated Plants' and is very important for tropical and subtropical fruits, cereals, etc. The northeastern region forms the richest reservoir of genetic variability of many groups of crop plants. Over 50 species of economic plants have their genetic diversity in this Hindustani Centre of Diversity (Zeven and Zhukovsky, 1975). Assam- Burma-Siam-Indo-China region is considered as the centre of origin of *Musa.* This is the area where large numbers of species, some of which are endemic, have been recorded. Banana in northeast India grows wild along the hill slopes of Arunachal Pradesh, Meghalaya and Assam, and Nagaland. North-East India is also very rich in *Citrus* wealth. There are 64 varieties of *Citrus* which grow wild in this region. Vavilov (l.c.) reported the presence of *Citrus sinensis*, *C. reticulata*, *C. media*, *C. aurantium*, *C. aurantifolia* and *C. limon*, both as wild and as cultivated forms and hence treated the Himalayan region (particularly North-East region) as the center of origin of Citrus. Similarly, a rich diversity has been observed in the wild species of temperate fruits, particularly of the family Rosaceae. *Malus baccata* and *Pyrus pashia* which are used as rootstocks of apple and pear respectively occur in Khasi Hills. Some of the other wild fruit types in this family are *Prunus napaulensis*, *P. undulata*, *P. cerasoides*, *P. jenkinsii, P. wallichii*, *P. persica*, and *Sorbus khasiana*. Most of these species occur in Khasi hills and this area perhaps forms the primary/secondary centre of distribution of the genus *Prunus* and *Pyrus*.

Recent surveys by I.A.R.I. also have shown that N.E. India is the richest reservoir for genetic variability in many crop plants. Out of 6730 collections of different types of rice cultivars 5000 come from the hilly regions of northeast India. India is considered to be the primary centre of origin of rice. Many land races of jute and mesta have been found in this region. Native types of cotton trees are located in the sub-temperate climate of Arunachal Pradesh. There are also fiber yielding plants with many variant forms belonging to genera like *Sesbania, Crotalaria, Sida* and *Cannabis.* A total number of 811 cultivated plants and 902 of their wild relatives have been documented so far.

Cucurbits, for which E. Himalaya is one of the major centers of origin also exhibits

enormous variability in this region. Some of the common cucurbits in this region are: *Lagenaria siceraria* (bottle gourd), *Luffa cylindrica*. (sponge gourd), *L. acutangula* (ridge gourd), *Momordica charantia* (bitter gourd), *Trichosanthes anguina* (snake gourd), *T. dioica* (pointed gourd), *Citrullus* sp., *Cucumis melo* (long rnelon), *Benincasa hispida* ((ash gourd), *Sechium edule* (chayote) and *Cucurbita maxima*, *C. pepo*, *C. moschata* (pumpkin). The genus *Cucurbita* has maximum diversity in this region as evident by a number of fruit types that are sold in tribal markets. The local tribals and villagers are also the custodians of the germplasm of several native types of fruits and vegetables including some chillies. Diversity in legume crops and their wild related types in East Himalaya are also of a high order. Maximum variability can be observed in *Cajanus cajan* (Pigeon pea), *Vigna umbellata* (rice bean), V*icia faba* (broad bean), *Vigna aconitifolia* (moth bean*), V. mungo* (black gram), *Lathyrus sativus* (khesari) and *Glycine max* (soya bean). In addition, there are about 200 non conventional legumes, of which about 50 species are used as vegetables by the various ethnic groups in the region.

Inventorization & Documentation of National biodiversity: some strategies

While the flora of the country is fairly well known mainly through the efforts of Botanical Survey of India and some other agencies, still considerable survey work remains to be accomplished particularly in northeast India, Andaman & Nicobar group of islands and even is some parts of Western Ghats. Several new taxa are being discovered every year indicating the assessment and documentation of the floristic diversity in the country is incomplete. Some of the priority actions suggested for survey and documentation of the national biodiversity are Development of coordinated programmes on all major groups for stock taking and identifying gaps, Avoiding duplication of efforts, Developing expertise for all groups through training programmes, Strengthening biodiversity collection centers (Herbaria), Identifying areas needing further exploration following coordinated multi disciplinary programmes, Attempting infra specific diversity in at least a few selected economic groups, Development of distribution maps for all species, and Developing a consolidated National Biodiversity database under central supervision with networking of information among different regional centers. However there are also many constraints coming in the way of achieving the complete documentation of flora and fauna in the country. Lack of trained manpower, Lack of zeal and enthusiasm for hard fieldwork among the current generation, Birth of more sophisticated and fashionable experimental sciences, Lack of adequate encouragement and funds, Less job opportunity for taxonomists, Lack of coordination among taxonomists and taxonomic institutions, Lack of strong leaders with vision, dedication and will to accomplish required tasks are some such impediments for completion of documentation of biodiversity.

Threats to floristic diversity and India's concerns for Conservation

The country's biodiversity faces a variety of threats, ranging from land use changes in natural habitats to over exploitation of natural resources, proliferation of invasive species and climate change. While we are proud of our rich biodiversity, at the same time, the rich biodiversity in India is under considerable threat from a variety of human generated factors

like (a) Ever increasing population growth. (b) Selective removal of specific groups of plants such as the orchids, medicinal plants, etc. (c) Extensive practice of shifting agriculture by local people. (d) Extension of townships. (e) Road construction on Hills creating accessibility of remote areas. (f) 'Modernization' leading to change of life style and cultural values of local tribals. (g) Free access and unregulated exploitation of bio resources in some areas. (h) Tourists influx and their greed for collection of specific groups of ornamental plants (orchids, Rhododendrons etc.). (i) Dependence of plant based industries solely on wild resources of biodiversity. (j) Wrong policies of the government that allow unregulated export of timber, Bamboos and other forest products impoverishing the biodiversity sink of the region and lastly (h) Spread of certain alien weeds such as *Eupatorium*, *Mikania*, *Parthenium*, *Lantana* and others endangering the native flora. Alien weeds particularly the weeds from the Neotropics have posed the second major threat to diversity of Indian flora after habitat destruction. But, India has shown keen interest and concern for the conservation of nature and natural resources. India happens to be a signatory for the major International Conventions on Biodiversity including the recent Convention on Biological Diversity (CBD) during the earth summit at Rio de Janeiro, Brazil in 1992. The CBD, which came in to force on 29 December 1993, has become almost an International law as regards the biodiversity and its conservation is concerned. The enactment of the Indian Wildlife Protection act of 1972 and the UNESCO's Man and Biosphere Programme have resulted in the declaration of large forest areas as protected for *in situ* conservation of Flora and Fauna. Today, there are 89 National Parks, 496 Wildlife Sanctuaries and 16 Biosphere Reserves representing the major ecosystems in different biogeographic zones of the country. These cover almost three fourth region of the country. Five of the existing protected areas in India have also been recognized as World Heritage Sites. These sites represent moist alpine, montane forests, inland and coastal wetlands. Of the 27 global hot spots, four rich floristic zones in India, namely Eastern Himalaya, northeast India, Western Ghats and Andaman & Nicobar Islands form the 'hottest hotspots'. Keeping in view of the fact that the entire region cannot be kept protected, the author suggests to identify smaller, most severely affected pockets of hotspots within these zones for effective conservation. Twenty six such pockets in East Himalaya and Northeast India and 19 in Western Ghats are identified by the author as pockets of hotspots. Some priority actions suggested for conservation include (a) Inventorization and documentation of all flora and fauna including endemic and monotypic taxa, (b) identification of specific causal factors/threats to biodiversity, (c) monitoring of flora and fauna for checking any invasive alien species, (d) assessment of rare and endangered taxa and development of distribution maps of these, (e) possibilities of establishment of biological corridors for linking the protected pockets of hot spots, (f) assessment of the extent of loss of original forest cover in these hot spot pockets, (g) rehabilitation and restoration of degraded ecosystems and promoting the recovery of threatened and endemic species, (h) strengthening research capabilities relevant to taxonomic studies of local flora, biodiversity conservation and restoration of degraded habitats, (i) establishment of international co-operation in conserving and managing the hot spots spread across different nations, and (j) ensuring local people participation at all stages of conservation, management and sustainable development of the bioresources. Sacred forests, particularly in East Himalaya and Northeast India, which act as both 'refuge' of relict elements and 'Death Traps' for flora and fauna are also suggested to be considered as hot spots for conservation. Identifying the habitats of certain unique species like *Nepenthes khasiana*, *Sapria himalayana*, *Drosera spp.*, etc. for creating species-oriented conservation

sites, attempting reproductive biology studies on those critically endangered species for locating the 'bottlenecks', if any, in their life cycles to overcome these, establishing gene-banks/ seed-banks of all wild plants for long term preservation, identifying medicinal plants in trade for large scale cultivation, lastly and most importantly, training and generation of a band of devoted field botanists and taxonomists for complete Inventorization of flora and for close interaction with biotechnologists for successful bioprospection programmes are certain other priority agenda suggested.

In addition to declaring some forested areas and wetland ecosystems as protected, numerous *ex situ* conservation programmes have also been launched through the research programmes financed by the Department of Biotechnology, Department of Science and Technology, Ministry of Environment & Forests, etc. under the Government of India. The Department of Biotechnology has extensively funded the programmes on *ex situ* conservation of flora making use of the biotechnological tools. Several hundred rare and endangered and endemic species of the country are being multiplied through Tissue Culture and Micropropagation and are rehabilitated in their natural or near natural habitats. Establishment of gene banks at CIMAP, TBGRI, NBPGR are other noteworthy steps towards conservation of biodiversity in the country. The efforts of Botanical Survey of India and other organizations have identified more than 3,000 species of flowering plants falling under one or the other Threat categories of IUCN. Red Data Books of India list 620 extremely rare species of which 550 species are endemic and some critically endangered. Programme on conservation of the rare and endemic species in the Botanical Gardens and Arboreta is another significant attempt by the Ministry of Environment & Forests for the conservation of the rare flora of our country. As part of the country's commitment under UN convention on biological diversity, the Ministry of Environment & Forests, Government of India has recently finalized the National Biodiversity Strategy and Action Plan (NBSAP). Some Recommendations of NBSAP on India's extant Biodiversity are (a) Strengthening and increasing the effectiveness of present Protected Areas, (b) Survey, catalogue and study the threatened ecosystems and develop conservation strategies, (c) Identify and map large forest fragments and develop methodology for management of biodiversity, (d) Identify, catalogue and study the hyper-diversity areas and develop strategies for their conservation, (e) Identify overexploited species and reduce anthropogenic pressure by cultivating them, (f) Develop strategies that involve indigenous people and in benefit sharing, (g) Develop regional and national biodiversity database, (h) Incorporate biodiversity concerns in Environmental Impact Assessments and in Forest Working Plans, (i) Identify and map grassland/savanna areas and develop management strategies, (h) Mount a multi-tier education system for public awareness. This consolidated report outlines the directions that India should take for conservation and sustainable use of biodiversity and biological resources. The efforts towards biological conservation and protection of habitats in the country though laudable, efforts towards checking the deterioration of the forests, fragmentation of species and habitats is rather increasing due to the anthropogenic disturbances. What we know of our biodiversity is far less than what we still do not know. Several species having high economic potential may likely to be lost for ever if we fail to create effective checks and remove the threats facing some of these species.

Bioprospection

Bioprospection and sustainable utilization of medicinal plants is much neglected in India. Biodiversity prospecting, particularly on medicinal plants, aromatic plants, wild food plants, cold tolerant high altitude species of Himalaya etc., can certainly result in some lead/novel molecules of great economic significance. Bioprospection for metal tolerant genes in families like Caryophyllaceae, Ceratophyllaceae, Portulaccaceae, Tamaricaceae, Salvadoraceae, Thymealeaceae and Fabaceae could lead to discovery of genes/molecules tolerant to heavy metals and thus helpful in environmental bioremediation. The diversity in wild aromatic plants in India is also of a very high order. Chemical prospecting of the wild aromatic flora of India particularly belonging to Asteraceae, Lamiaceae, Apiaceae, Rosaceae, Lauraceae, Poaceae can provide some clues for newer potential aromatic crops for the future. As ecological diversity in India is very high, a greater genetic diversity in the widely distributed taxa is also expected. Scanning of the entire biodiversity in some short listed species (through cross cultural ethnobotanical investigation) particularly at the population level making use of the modern biotechnological tools can be highly rewarding. Bioprospection of tree flora, particularly of Western Ghats, where important antitumor plants like *Aphanamixis polystachya*, *Nothopodytes nimmoniana*, *Mesua nagassarium*, *Semecarpus anacardium*, etc exist, would be rewarding. *Nothopodytes foetida* is shown to contain 0.1% camptothecine, an antitumor/anticancer drug. Camptothecine (0.005%) was earlier found only in *Camptotheca acuminata* (Nyssaceae) occuring in China, whereas our species contains 0.1%, highly promising for treatment of cancer. Another urgent task in this direction relates to the evaluation of infra specific diversity with in a given species. Indian region with a varied topography climate, rainfall and soil types offers scope for extreme variations within a species, particularly a wide spread species. Some of these habitat specific populations could be elite-types needing cultivation and commercialization. As the Tropical trees are well known for their variability, Bioprospection/chemoprospection of such medicinally important trees like *Nothapodytes foetida*, *Mesua nagassarium*, *Aphanamixis polystachya*, *Semecarpus anacardium*, *Butea monosperma*, *Hymenodictyon orixense* for commercial isolation of biochemicals and novel molecules could be rewarding. Investigations on infra specific diversity and genetic diversity of at least a few commercially important medicinal plants like *Berberis asiatica*, *Bergenia ciliata*, *Illicium griffithii*, *Myrica esculenta*, *Panax pseudo-ginseng*, *Plantago major*, *Saussurea lappa*, *Taxus wallichiana*, *Aconitum chasmanthum*, *Aconitum heterophyllum*, *Coptis teeta*, *Swertia chirayita*, *Swertia ciliata*, *Nardostachys grandiflora*, *Picrorhiza kurrooa*, *Podophyllum hexandrum*, *Rheum australe*, *Rheum nobile*, *Valeriana jatamansi* are needed, so as to identify the 'elite' types for popularization and commercial cultivation. Bioprospection of some of the high valued medicinal plants like *pseudo-ginseng*, *Coptis teeta*, *Dactylorrhiza sp*., *Swertia chirayita*, *Nepenthes khasiana*, *Aconitum spp*., etc. is urgently called for. The opportunity for Bioprospection of medicinal flora in India is quite enormous because of the enormous diversity in medicinal plants, enormous habitat variation resulting in vast infra-specific diversity in medicinal and aromatic plants. Added to this, we have excellent biotechnologists, field taxonomists and well equipped laboratory facilities. The Field botanists or ethnobotanists have a great role in Bioprospection at the species level. Ethnobotanists can scan the entire biodiversity and shortlist medicinal species for Bioprospection at molecular level (anti-cancer, anti-diabetic, anti-malarial, and neutraceutical). Field botanists can also help in correct identification and in collection of required plant material, Field botanists can

suggest species for Bioprospection based on field knowledge about species. But the constraints for Bioprospection are also too many. Lack of much required cooperation between taxonomists and molecular biologists; (some of the excellent field botanists are poor in biotechnology and good biotechnologists are poor in field knowledge), shortage of required number of good taxonomists / field botanists, vast array of flora with enormous infra-specific variation in taxa spread over vast extension of the geographical boundaries of the country, incomplete knowledge of our medicinal flora, lack of comprehensive ethnobotanical databases among biodiversity rich developing nations for comparative ethnobotanical study and huge cost involved in Bioprospection work are some of them. The author feels that serious and meaningful efforts should be initiated to overcome these constraints so that the plant wealth of the country is properly and profitably utilized at least in the 21st century. Further that in view of the possibilities of gene transfer from one organism to the other, the author alerts the scientific community to safeguard not only the rich and unique biodiversity but also the total gene pool of the Indian region against any unauthorized foreign agencies.

Concluding remarks:

In the preceding pages it has been clearly pointed out that the Indian subcontinent forms one of the richest plant diversity centers but still a complete knowledge of the flora of the region is lacking as much of the areas still remain under explored. After the Rio Convention on Biodiversity, no country can freely access the genetic diversity of another country without a proper memorandum of understanding for sharing of benefits resulting from out of the genetic resources. The biotechnology rich nations are looking towards biodiversity rich nations like India for biological materials and therefore, every one must try to safeguard the biodiversity of our country so that these are utilized for the welfare of the human good and for the economic development of the country at large. Some developed nations have exploited the rich genetic diversity of developing countries as a free resource for research and development. The products of such research are then patented and sold back to the developing countries at excessively high prices. We must therefore formulate mechanisms for effective co-operation with reciprocal benefits between biotechnologically - rich developed countries and gene-rich developing countries. The author has outlined the immense opportunities for Bioprospection of the Himalayan and Western Ghats flora. Recent developments in molecular biology and biotechnology have made it possible to scan the biodiversity for molecules with potential for commercial application. Also, now it is possible to transfer the useful genes from any plant or animal to the required organism. Therefore it is high time that we convert our bio wealth into monetary wealth by intense Bioprospection programmes involving both the traditional botanists and biotechnologists. But before we are able to accomplish this task, the rich diversity of plant wealth is also getting diminished due to various anthropogenic factors and therefore equally important to take all possible steps to protect our biological diversity of this high magnitude.

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Role of Plant taxonomy and Ethno- botany in advancing Research on Medicinal plants in India

Dr. R.R. Rao
INSA Honorary Scientist
No. 328, B-4, Kendriya Vihar, Yelahanka, Bangalore -560064
Email: raocimap@gmail.com

Introduction

Plant taxonomy is one of the earliest of all botanical disciplines which primarily deals with the collection, preservation, identification, naming and classification of all plant species of the globe. The subject started as a "folk taxonomy" in the early 15th century has undergone revolutionary changes during the last five hundred years with regard to both concept and scope. Yet, regrettably, taxonomy has not secured the place it ought to have. At the global level, in spite of the serious efforts to explore the flora of the earth during the last 4 centuries, modern taxonomists are still to face the challenge of completing the inventories of the floras, particularly of the tropics. The situation has now become highly critical because the tropical countries which hold rich floras are now under great threat. So far 4,50,000 plant species are identified, of which 2,90,000 are flowering plants. Approximately, 2 million plant species are still expected to be discovered in the tropics. The same holds good for India. While the need for intensive exploration and the need for holistic information on all plant species have become urgent, the number taxonomist throughout the world is drastically declining. Added to this, birth of many dynamic, experimental and fashionable areas including the latest Cell and Molecular biology has rendered the conservative classical taxonomy unfashionable and unattractive to the students. In India, the decline in taxonomic activities and expertise has now reached a crucial point where the other disciplines which rely on adequate levels of taxonomic information are being hampered. Taxonomy teaching (and research) in most Universities is much[8] neglected. Instead of trained taxonomists handling the course, the subject is taught by non-specialists who just narrate the characters of families without actual field work. A sort of aversion has developed among the students towards this subject. Added to this, taxonomic literature and herbaria which are fundamental for taxonomy teaching and research do not exist in most universities. Some Universities even think that Herbaria are redundant and holding large bundles of specimens of a particular species do not serve any purpose. The utility or functions of a herbarium are not even understood by some of our senior scientists and policy makers in Universities. In fact, taxonomy is basic to many other investigations including medicinal plants research like identification/delimitation and determination of taxa, correct naming of taxa, utilitarian Flora writing, determination of

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relationship, position, rank for new and old taxa, basic information for botanical and future research, biosystematics and modern biology, biodiversity assessment, monitoring of flora, biomonitoring of fragile ecosystem, monographic study, conservation programmes – (i) survey of wild genetic resources (ii) seed banks (iii) gene Banks, environmental impact assessments, ecosystem management, agricultural development, forest management programme, forensic science – identification of plant material in solving crime, evolutionary studies, establishment and development of botanic gardens/parks, discovery of newer drug plants for pharmaceutical companies, nurseries/seed companies, village, town and city planners, horticulture, architecture and landscape designers, customs for CITES operation, bioprospecting, protected area management, ethno-botany and discovery of newer economic plants, development of data bases for Bioinformatics sectors and I P R – Patent regimes. In the medicinal plants sector too, taxonomy is essential for survey, collection and preservation of medicinal plants, for gathering information on distribution, abundance and ecology of medicinal plants, for discovery of newer medicinal plants through ethno-botanical investigations, for correct identification and for fixing correct nomenclature of medicinal plants, determining the correct names, segregation of closely related medicinal plants, correct author citation, assessment of rare, endangered or endemic medicinal plants, for Bioprospection of medicinal plants and lastly for development of computer data bases on medicinal plants. The Paramount role of taxonomy in advancing medicinal plants researches is highlighted in this discussion.

Ethnobotany

Ethno botany, which is again an offshoot of plant taxonomy is equally important in the discovery of newer medicinal plants and even in drug development. The term 'Ethno botany' was coined by Harshberger in 1876 to denote 'the study of plants used by aboriginal people'. Today the term denotes the entire realm of direct relationship between plants and human population. Ethno botany is a synthetic discipline involving the study of Botany (Taxonomy, Ecology, Conservation) Anthropology, Sociology & culture, Language & linguistics, Religion & Social customs, Archaeology, Medicine, Mythology, Geography, Economics and a few other allied subjects. Methods and techniques of Ethno-botanical study includes primarily the field work in tribal areas, scrutiny of the herbarium specimens in large national and International Herbaria, scrutiny of ancient literature including the unpublished dairies and travelogues, scrutiny of ancient sculptures on temples, forts and paintings, analysis of different tribal folklore, scrutiny of plants and plant parts from the archaeological sites and excavations, and Study of fossilized plant materials and remains (paleo-ethno-botany, archaeo-ethno-botany). Discussing the development of ethnobiology in India, it is said that although the subject is as old as human civilization, the organized and systematic ethno botanical studies started with reorganization of the Botanical Survey of India under the guidance of Dr. E.K. Janaki Ammal and later by Dr. S.K. Jain. The All India Co-ordinated Research Programme on Ethnobiology by the Ministry of Environment & Forests, Government of India, establishment of a Society of Ethno botanists at Lucknow, followed by the regular publication of the Journal of Ethnobotany , training courses in Ethnobotany , establishment of an Institute of Ethno botany are all the major milestones in the development of ethnobiology in India.

The heritage of medicinal plant use in India is very ancient and dates back to pre - Vedic culture, at least 4000 years. India's vast resource base of medicinal plants is well known the world over and is uniquely positioned to exploit her riches in medicinal plants. About 50 million

people in India spread among 550 ethnic tribes live in and around forest areas and rely on wild medicinal plants for all ailments. Although *ca*.8000 species are traditionally used (6198 according to FRLHT's report; Ayurveda-1689; Folk medical system- 4775; Homeopathy-491; Modern medicine-200; Sidha-1563; Tibetan-343 and Unani-843), the survey and documentation of medicinal plants in the country is itself incomplete. Despite these statistics, India's effort to mobilize on the abundant medicinal plant resources is unsatisfactory. Further, botanical identity of several important ayurvedic drugs *like soma, bala, shankhapushpi, punarnava, chirayita, sanjivani, kalpavriksha,* etc is still not correctly established and some remain unknown.

Role of plant taxonomy in medicinal plants research:

The Role of taxonomy in promoting Medicinal & Aromatic plants research is immense. Taxonomy is basic to all kinds of medicinal plants researches and helps in survey, collection and preservation of Medicinal plants, provides Information on distribution, abundance and ecology of medicinal plants, helps in assessment of diversity (species & infra-specific diversity) in medicinal plants, helps in correct identity of medicinal plants, helpful in fixing correct nomenclature for medicinal plants, determining the correct names, segregation of closely related medicinal Plants, correct author citation, in bio prospection of medicinal plants, in ethno botanical studies, in assessment of rare, endangered or endemic medicinal plants, and in development of computer data bases on medicinal plants.

Survey & documentation of medicinal plants

Roughly 8000 species in India are said to be medicinal; yet the botanical surveys are incomplete. Many tribal pockets, particularly in East Himalaya, North-East India & Andaman & Nicobar islands are only marginally surveyed. Intensive ethnobotanical surveys by taxonomists are urgently needed to document all medicinal plants and ethnic knowledge connected with these plants. State wise data base on medicinal plants need to be established with parameters like correct names, distribution, abundance, photographs/illustrations, local names/vernacular names, uses, dosage, ethnic tribes, and names of the tribal resource persons and their photographs. Taxonomy also helps in assessment of infra-specific diversity in medicinal plants too.

Plant taxonomy & correct identity of medicinal plants

Scrutiny of literature on medicinal plants invariably reveals a number of mistaken identity or wrong nomenclature and even wrong author citation. Taxonomic researches only can solve such nomenclatural or identity problems. A few such cases where taxonomists have helped in solving the correct identity and nomenclature of medicinal plants (which were wrongly used in literature) are discussed. Correct identification of medicinal plants is a major problem; often very valuable research findings go under wrong identification of plants. Even in some of the established herbaria, there are innumerable cases of wrong identification of the plants; these could be cases of mistaking a plant similar to another reported from other part of the world or identification mistakes or cases of conspecificity – two names given for the same species and such other cases. Only a few such selected cases are discussed below.

Phyllanthus niruri treated in most of our earlier floras is an effective anti-jaundice plant. Chaudhury & Rao (2002) have clearly established that true *P.niruri* does not occur in India; instead 3 distinct species namely *P. amurus*, *P. debilis* and *P. fraternus* are wrongly treated as *P. niruri*, which is actually endemic to America and does not occur in New World. A botanical key for correct identification of the three closely related and morphologically similar looking species is provided below.

1a. Calyx lobes five - *P. amurus*

1b. Calyx lobes Six.

2a. Male disc segments cupular with glandular margin; disc cup-shaped with laciniate margins; style minutely bifid - *P. fraternus*

2b. Male disc segments roundish; disc saucer- shaped, with entire to obtusely and shallowly lobed; style bifid up to the middle - *P. debilis*

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As vast amount of literature on medicinal aspects has already accumulated on *Phyllanthus niruri* in India (which is a wrong name), it is necessary to correct the taxonomic history of the species as below:

Phyllanthus amarus Schum. Beskriv.Guin.Pl.421.1827; Chaudhury & Rao, in Phytotaxonomy 2: 148.2002.

P. niruri auct. *non* L. 1753; Fl. Brit. India, Gamble, Cook and all other regional floras

Another case is that of *Tribulus terrestris* which is an important medicinal plant. *Tribulus terrestris* is known in Sanskrit as Gokshura. It is believed to contribute to overall physical, as well as sexual, strength by building all the tissues, especially shukra dhatu (reproductive tissue). It is known to be useful in BHP, and believed to be useful in kidney, bladder, urinary tract and uro-genital related conditions, where it is said to act as a diuretic. *T. terrestris* has long been a constituent in tonics in Indian Ayurveda practice. The genus *Tribulus* (Zygophyllaceae) has two closely resembling medicinally important species which can be segregated based on the number of spines on the mericarps as below. Also, the species has two distinct ecotypes- one green type and the other ash coloured, both occurring sympatrically.

1a. Fruit mericarps with two major divergent spines and 20-30 minor spines - *T. rajasthanensis*

1b. Fruit mericarps dorsally only tuberculate with 4 spines - *T. terrestris*

The genus *Berberis* (Tree turmeric)is an important drug used in various Ayurvedic preparations and also in traditional medicines. As an antibacterial, the herb is administered in eye disorders, indolent ulcers and hemorrhoids. Tree Turmeric is also a potent antiseptic, which promotes wound healing. As an anti-inflammatory, it alleviates joint aches and pains

associated with rheumatism, arthritis and osteoporosis. The genus *Berberis* in the Himalaya is again most confusing not only for botanists but even for experienced taxonomists. Most species morphologically look similar. *Berberis aristata* (*Daru haridra, Indian Barbery*) and *Berberis asiatica* (*Asiatic Barbery*) are morphologically similar and often confused. Taxonomically they can be separated as follows:

Leaves glossy green, not glaucous beneath; flowers in simple or paniculate racemes; petals entire - *B. aristata*

Leaves pale green, glaucous beneath; flowers in corymbose or umbellate racemes; petals emarginated - *B. asiatica*

There has been a persisting Taxonomic confusion in identification of *Picrorhiza kurrooa and Neopicrorhiza scrophulriaefolia* both are high value medicinal plants of the Himalayan region having export potential. *P. kurrooa* is a reputed medicinal herb, valuable bitter tonic effective in liver and stomach diseases and as appetizer and as ingredient of many traditional medicines. Often this species is confused with *Neopicrorhiza scrophul*ariaefolia (syn. *P. scrophulariaefolia)* which is considered endemic to central and eastern Himalaya. In the vegetative condition, both of them more or less look alike. But *N. scrophul*ariaefolia is easily distinguished by smaller habit, few flowered, moderately dense inflorescence, zygomorphic, bilabiate, exserted corolla with 4 unequal lobes; upper lip 3 lobed , lower lip simple, much smaller; stamens slightly exceeding corolla, upper part of stamens attached half way to corolla tube and shorter than lower pair. *P. kurrooa* on the other hand is comparatively larger, inflorescence densely many flowered; corolla weekly zygomorphic or sub actinomorphic with equal lobes, included within calyx; stamens equal, long exserted.

The two species of *Calotropis* of the family Asclepiadaceae, *C. gigantea and C. procera* are highly medicinal; latex is purgative, leaves applied as fomentations on swellings; pounded leaves applied on burns; both species are very similar, often confusing having overlapping characters. But the two can be separated as below

Buds ovoid; Corolla lobes spreading, bluish pink or pale lavender; corona scales narrow, truncate shorter than staminal column; corona lobes auricled at apex; large shrubs up to 4.5 m - *C. gigantea*

Buds hemispheric; Corolla lobes erect, pink or white or purple spotted; corona scales acute, longer than stamina column; corona lobes not auricled at apex; smaller shrubs up to 1.5 m high - *C. procera*

The two species of *Valeriana, V. hardwickii* (*Shammia, Heeverum, Tagar, Sugandhwala*) and *V. jatamasii (Balchari, Heeverum, Indian valerian)* are very similar and have more or less the same use – urinary disorders, for joint pains and dried roots as incense and insecticide. The two species can be separated as below.

Erect herbs; leaves radical & cauline; flowers pale white, in terminal or axillary, lax corymbose panicles - *V. hardwickii*

Decumbent herbs; leaves mainly radical; flowers pinkish white in dense terminal corymbs - *V. jatamansii*

Heracleum equilegifolium and *Puecedanum grande* (both of Apiaceae) morphologically appear very similar. In the Blatter Herbarium, Bombay and in Botanical Survey of India, Pune some sheets actually belonging to *Heracleum* were wrongly identified *as Puecedanum grande*. Almeida (1983) correctly identified the material as below

1a. Inflorescence racemose umbels; fruit ellipsoidal, narrowed at base - *Heracleum equilegifolium*

1b. Inflorescence simple or compound umbels; fruit obvoate, grooved at base - *Puecedanum grande*

Taxonomy of Ginseng, the root of immortality has been a perennial confusion. Ginseng is prized for its roots, which are used as medicine for curing many ailments. It is said to be a sovereign remedy for almost all diseases. Ginseng is used as drug for a variety of purposes, boost energy levels and alertness. A tonic herb that helps to improve overall health and used for centuries to boost energy, sharpen mind, reduce stress, treat impotency and extend life, Enhances the resiliency and balance metabolism, remove stress, strengthens the sexual inadequacy, improves athletic performance and endurance, gently stimulate the nervous system, Helps in regulation of blood pressure, balance blood sugar levels and support the adrenal glands, thymus and spleen, and Practitioners of traditional Chinese medicine prescribe Ginseng to strengthen immunity in the elderly. Botanically Ginseng refers to a species of *Panax* (Family Araliaceae) (*Pan* meaning all; *akos* meaning cure) .True Ginseng refers to *Panax ginseng* occurring in China and Korea; also known as Korean Ginseng. This represents the original Ginseng with highest potency, highly prized in America and western countries. American Ginseng (*Panax quinquefolium*) smaller than Asian Ginseng, largely cultivated in Canada and US, but majority exported to Asian countries including China. 'Japanese Ginseng' or 'Chikusetsu Ginseng' is the *Panax japonicus* 'and has much less potency (less ginsenosides) 'Sanchi' Ginseng is *Panax notoginseng* and is distinct from *Panax ginseng* and is used for haemostatic and pain relief purpose in China, largely cultivated in S.W. China and Vietnam. Himalayan Ginseng or the Indian Ginseng refers to the *P. pseudoginseng* subsp. *himalaicus*. Medicinal potency is lower than the true *Panax ginseng*. Indian ginseng is a conglomerate of 5 different taxa. Therefore even today, the Taxonomy of Indian *Panax* is confusing. Hara (1966) merges all the species under *P. pseudoginseng* Wall. subsp. *himalaicus* with two varieties var. *angustifolius* and var. *bipinnatifidus.* Benerjee, (1968) recognizes 4 species (*P. fruticosus*, *P. pseudoginseng*, *P. sikkimensis* and *P. assamicus*). Cannon, (1979) treats *P. sikkimensis* and *P. assamicus* as synonyms of *P. pseudoginseng* var. *angustifolius.* Morphologically *P. assamicus* is similar to *P. wangiuanus* of China . Bennet & Vishwanathan (1984) treat *P. pseudoginseng* var. *angustifolius* as *P. burkillianus.* Changkija & Kumar (1992) also record *P. schinseng* from Nagaland, the identity of which needs verification. Kapil *et al.* (1997) believe the Indian Ginseng is a conglomerate of all 5-6 species. Taxonomic confusion is due to sympatry of morphologically distinct taxa and the existence of occasional morphological intermediates however, this needs urgent taxonomic revision.

Another case is that o*f Garcinia* (Family Clusiaceae). *Garcinia spicata* and *G. talboti*

are so similar that it is very difficult to segregate them. Some authors even suggest that the two should be merged and G. spicata having priority over the other species should be the correct name.

There are innumerable such cases of either misidentification or confusion over closely allied species. The medicinally important genus *Hedychium* (Zingiberaceae) includes 38 species in India distributed in northeast India and Himalaya, and peninsular India. Most of the species are alike morphologically. Even microscopic characters like stomatal pattern and pollen grains are also similar in many species. Therefore, critical identification of the material both living and herbarium specimens are necessary, that too by an expert taxonomist. Further, several closely allied genera of *Hedychium*, like *Hitchenia*, *Alpinia*, *Amomum*, *Cautleya and Globba* are difficult to segregate even by taxonomists in the absence of flowers.

Nomenclatural changes on medicinal plants species due to strict application of the International Rules of nomenclature is also discussed. It is said that taxonomic revisions/monographs can only provide the correct identity and nomenclature of any medicinal plant.

Herbaria aid in Ethno-botanical study of Medicinal plants ?

Herbarium is a place where dried and pressed collection of plant specimens collected from far and wide are arranged in some accepted system of classification. Herbaria are a sort of Data bank on plants where information of all aspects of plants including correct identity, place of collection, name of collector, date of collection, uses of the plant including medicinal uses are recorded. Herbarium of the Royal Botanic Garden, Kew houses ca. 10 million specimens; Komorov Botanic Garden houses about 8 million specimens, collected practically from all over the world. If such large herbaria are scrutinized sheet by sheet for ethno-botanical and medicinal information, one can gather vast amount of valuable data pertaining to medicinal plants sitting at one place. With the help of well established Herbarium, three main kinds of data can be collected – i. record all the uses of plants of a particular locality or tribe ii. record the use of a particular plant species among several tribes iii. To record all species of the globe used for a particular ailment, say cancer, jaundice, etc. Outstanding ethno-botanical work using Herbarium is that of Altschul (1968, 70) who scrutinized several thousand specimens of Harvard University Herbarium and recorded ethno-botanical uses of 5178 species. In India, great opportunity exists as not much work in this direction has been done. Well established herbaria greatly help in the correct identification of medicinal plants, fixing correct nomenclature, and even act as the custodians of voucher specimens of medicinal plants. Herbaria also hold the Type specimens of medicinal plants which also help in revisionary/monographic work.

Taxonomy in assessment of rare and endangered medicinal plants

The role of taxonomy in assessment of endangered medicinal plant species is also significant. Endangered species are those which are under threat of extinction and whose survival is unlikely if the causal factors continue operating. While on one side we have not been able to evaluate species diversity and infra- specific diversity in most of these medicinal plants for identifying the elite types, many species and populations are facing the threat of

extinction due to several anthropogenic reasons. Already a number of reputed medicinal species such as *Aquilaria malaccensis, Dioscorea deltoidea, Podophyllum hexandrum, Pterocarpus santalinus, Rauvolfia serpentina, Saussurea lappa, Taxus wallichiana, Picrorhiza kurrooa, Dactylorhiza hatagirea, Valeriana jatamansi* and numerous others have become endangered. Actual field surveys and population analysis by taxonomists and scrutiny of specimens in large national and international herbaria can throw light on the status of medicinal plant species for sustainable utilization. Scrutiny of specimens in a large herbarium also helps in listing all medicinal species of a given region or all species used for a particular ailment or all medicinal species among a particular tribe.

Role of taxonomy in Ethno botany & search for newer medicinal plants

As already stated above, ethno botany, which is again an offshoot of plant taxonomy is equally important in the discovery o newer medicinal plants and even in drug development. All aspects of field taxonomy are involved in ethno- botanical investigations. In addition to all taxonomic procedures, the ethno-botanists during field work also studies the tribal culture and brings out hidden tribal knowledge on the plant species used for various ailments in addition to collection of plants for the herbarium. Cross -cultural ethno-botany is the Comparative study of different ethnic societies in relation to interrelationship between plants and folks and greatly help in short listing species for further intense Bioprospection. Use of a number of diverse, unrelated medicinal plant species for a particular ailment by different ethnic tribes in India (may be more than 500 anti diabetic species in India alone) is another major issue observed with regard to the use of traditional medicinal plants. The same is observed even in authoritative treatises like *Charka samhita.* The author stresses the need for Short listing and prioritizing the leads for a specific ailment by cris-crossing of information **through cross-cultural studies a**mong different ethnic tribes within a country and then compare with other developing countries for intense bio prospecting and product development. Not many species are common for a particular ailment among different ethnic tribes in India. Therefore short listing and prioritizing the leads for a specific ailment by cris-crossing of information through cross-cultural studies among different ethnic tribes within a country and then comparing with other developing countries is very essential and should be the first step in any drug development programme. Cross cultural ethnobotany (again by taxonomists) helps in short listing species for further intense Bioprospection. Certainly, use of a particular species for the same ailment by different unrelated ethnic groups indicates the efficacy and potential of these plants for drug development. Cross cultural ethnobotany also helps in establishing similarities and dissimilarities among different the ethnic communities, helps in assessment of diversity of species employed by the tribes for a specific ailment, use of same plant for the same ailment by different ethnic groups, often totally unrelated and in assessing the prevalence and dominance of ailments in the region. Cross-cultural studies particularly involving developing countries are very meager and need urgent attention. Collective and co-operative efforts of all developing nations is a must for the study of cross-cultural ethnobotany for ultimately establishing safer, cheaper and acceptable drugs for most of the ailments rampart among ethnic communities in the region.

Role of Taxonomy & Ethno botany in Bioprospection

Bioprospection is nothing but the scanning of the biodiversity for genes which can yield better crops/new medicines. Cross-cultural ethnobotany as discussed above, is in fact the Bioprospection at species level. Scanning of entire biodiversity by taxonomists and ethnobotanists for short listing of species for a particular ailment, by comparative ethnobotanical study of different tribes within a nation or among different nations is the first step in any Bioprospection programme. Such short listed species/leads can be subjected to scientific scrutiny and further bioprospection at molecular and chemical levels. Recently, based on clues from traditional knowledge, scientists from Wisconsin University, USA have isolated a protein (brazzein) from the berry of *Pentadiplandra brazzeana* (Pentadiplandraceae), a wild plant that grows in Gabon (West Africa). This protein is reported to be 2000 times sweeter than sugar& the discovery is believed to make inroads into US $ 100 billion a year world wide market for sweeteners (RAFI, 1995). Why not such discoveries from India? World over high percentage of useful plant –derived modern drugs are discovered as a result of scientific follow up of traditional medicine (119 plant based drugs 74% from traditional herbs (Farnsworth, 1990). About 50 pharmaceutical drugs from Ethnobotanical leads (Cox, 1994). In contrast, other approaches like systematic phytochemical screening, massive biological screening of randomly collected plants, and phytochemical examination of plants with the aim of identifying new chemical compounds have not proved satisfactory (Farnsworth et al., 1985)

Indian region is a store house of many life saving medicinal plants like *Rauvolfia serpentina, Gloriosa superba, Cassia angustifolia, Withania somnifera, Chlorophytum sp., Catharanthus roseus, Andrographis paniculata, Phyllanthus amarus, Trichopus zeylanicus, Janakia arayalpathra, Utleria salicifolia, Aristolochia tagala, piper barberi, Adenia hondala, Garcinia spp., Thottea siliquosa, Caryota urens, Adhatoda beddomei, Myristica malabarica, Coscinium fenestratum*, etc. in Western Ghats; *Inula racemosa (poshkar root), Aconitum heterophyllum (atees root), Picrorhiza kurrooa (kutki root), Dactylorhiza hatagirea (salampanja root), Valeriana jatamansi (sugandhbala root), Ephedra gerardiana (aasmani booti, somlata stem), Taxus wallichiana (birmi leaf), Angelica glauca (Chora root), Artemisia maritima (seski herb), Mucuna pruriens (kaunchbeej), Cinnamomum tamala (tejpat leaf) and Saussurea costus (kuth root*) in the Himalayan region. These traditional medicinal species certainly offer immense prospects for Bioprospection. Role of taxonomists and ethno-Botanists in Bioprospection and drug development is indispensable. Ethno botanists can scan the entire biodiversity and shortlist medicinal species for bio prospection at molecular level (anti-cancer, anti-diabetic, anti-malarial, nutraceutical). Field botanists can help in correct identification and collection of required material. Field botanists can suggest species for bio prospection based on field knowledge about species. Future responsibilities for Taxonomists and Ethno biologists include inventorying the traditionally used biological resources and development of data bases, conservation and revitalization of the traditional cultures, safeguarding the traditional knowledge against misuse or over use by 'modern societies', acting as custodians of the traditional knowledge and on behalf of the ethnic tribes decide and distribute the benefits that may accrue for their traditional knowledge, and finally identify the knowledgeable resource persons in each region for providing some subsidy for pursuing their unique profession.

Concluding remarks

Taxonomy, a field discipline is indispensible for all kinds of research on medicinal plants, including bio-prospecting of medicinal plants. Invariably, most of the closely related medicinal plants are wrongly treated in literature on medicinal plants as shown above. Critical taxonomic studies can only correctly identify such closely related or complex species. Taxonomic knowledge is also necessary in checking the nomenclature of the species and in their correct author citation. Indian region is a treasure house of medicinal plant resources yet, not a single noteworthy global level product from India has emerged. The vast adivasi tribes dwelling in remote forest areas in parts of North-east region, Central India, Andaman & Nicobar Islands have a wealth of information hidden among their tribal cultures. Such valuable, time tested information on medicinal plants, particularly for such serious ailments as cancer, jaundice, diabetes, fever, etc need to be extracted on priority basis and verified through cross cultural ethobotanical studies for further intensive bio-prospection and product development. This is possible only through Collaborative research programmes involving taxonomists, ethnobotanists, phyto-chemists, molecular biologists and pharmacologists, etc. But Taxonomists who are equipped to do such an ethno-botanical surveys are dwindling. In addition to survey and documentation of medicinal plants, taxonomists are to play a major role not only in bioprospection but in all other spheres of medicinal plants research as discussed above. Therefore, in order to boost the medicinal plants research in the country, there is a need to generate a band of good taxonomists and field botanists to shoulder the, the University Grants Commission's efforts are needed to resurrect and rejuvenate the subject through planned research and intensive training programmes.

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Section V: Diet, Nutrition, Health and Disease

Diet, Nutrition and Health: Challenges and Opportunities

Mahtab S. Bamji
INSA Hon. Scientist, Dangoria Charitable Trust, 1-7-1074,
Musheerabad, Hyderabad, 500020
msbamji@gmail.com

Health Is the Most Important Determinant of Development

For national development people should be healthy, and educated. For good health, there has to be Awareness and Access at Affordable cost to good nutrition (balanced diet), clean disease- free environment, safe drinking water, primary health care outreach and physically active life style.

Definition of Nutrition Security

Nutrition security is defined as "Physical, economic and social access to, and utilisation of, an appropriate, balanced diet, safe drinking water, environmental hygiene, and primary health care for all. (Appropriate diets are based on age, gender, physiological status, and activity levels.) Thus Nutrition security goes beyond food security.

India being a country in developmental transition is facing the double burden of pre-transition diseases like under nutrition and communicable diseases, as well as post-transition diseases like obesity, diabetes, blood pressure, heart disease etc. Undernutrition contributes to both the situations.

Undernutrition and Other Areas of Concern

Under nutrition is of two types. Energy- protein malnutrition which results in growth retardation (under weight and stunting) and micronutrient deficiencies- deficiencies of vitamins and minerals. India has the highest incidence of undernutrition in the world. Almost 50% of children and 30% adults are underweight and stunted. Over 70 % women and children suffer from anaemia whose major cause is iron deficiency. Deficiencies of micronutrients like, calcium, zinc, vitamins A, B-complex and C are rampant. At one time we used to think that India cannot have vitamin D deficiency, since vitamin D can be synthesised in the skin from its precursor molecule 7-Dehydrocholesterol on exposure to sunlight. However, recent studies show that vitamin D deficiency is also rampant in India.

Lecture delivered on September 4, 2012 at Elanki Degree College, Don Bosco High School, Village Narsapur, Medak District

Though vitamin B-12 is needed in very small amounts (1 microgram per day) its deficiency is also emerging, because vitamin B- 12 is derived only through animal foods or bacteria. Unlike macronutrients (carbohydrates, proteins and fats) which are needed in gram quantities, micronutrients are needed in milligram or microgram quantities. While macronutrients provide energy and building blocks for making muscle, bones, blood, organs etc, micronutrients facilitate chemical reactions in the body to ensure proper metabolism and utilisation of the macronutrients.

Major cause of nutrient deficiency is poor diet due to poverty and/or lack of awareness. Infections can also lead to nutrient deficiencies due to loss of appetite and loss of nutrients from the body due to tissue breakdown. Thus for preventing undernutrition, good diet as well as healthy environment and clean drinking water is necessary.

Other Areas of Concern in India are:

- High infant, child, and maternal mortality
- Low sex ratio, (number of females to 1000 males) suggesting gender bias
- Early marriage, motherhood
- Low literacy-particularly female literacy
- Poor access to sanitation – use of latrines only 31%
- Low birth weight (<2.5 Kg)

Causes and Consequences of Low Birth Weight (LBW)

Low birth weight (LBW, <2.5Kg) is due to impaired development of the foetus for a variety of reasons, maternal malnutrition being the most important one. Other causes are:

- Maternal age (under 18 years or over 30 years)
- Premature delivery- < 37 weeks gestation
- Lack of antenatal care
- Infections
- Physical work till the last days of pregnancy

Some of the consequences of LBW are:

- High morbidity – greater neonatal and infant mortality. 6-10 times higher perinatal (within one week after birth) mortality.
- Adverse future pregnancy outcome of the daughters.
- Slower growth rate –stunting
- Impaired physical and mental performance.
- Greater susceptibility to age-onset diseases -Foetal origins of adult diseases

Foetal Origins of Adult Diseases- Barker hypothesis

A British doctor, Barker and colleagues in UK first demonstrated that Individuals who have suffered malnutrition in foetal life and are born with low birth weight (<2.5Kg), due to

intra-uterine growth retardation (IUGR) have higher body fat and are more susceptible to diseases like obesity, diabetes, blood pressure, cardiovascular diseases (CVD), and raised serum lipids in later life, particularly if there is change in their diet and life-style (sedentary lifestyle) due to affluence. The effect is multi generational. First 1000 days after conception are most critical for physical and mental development. The window of opportunity for correction is within the first year after birth. India is witnessing growing incidence of post – transition, age- related diseases like obesity, diabetes and CVD. India is believed to be the diabetes capital of the world. This, to some extent can be attributed to the phenomenon of developmental origins of adult diseases. In a country in developmental transition, many move from rags to riches and fall prey to wrong life styles. Shift from consumption of natural and unrefined foods rich in fibre and nutrients, to highly refined and processed junk foods rich in salt, sugar and saturated fat typical of modernisation is not good for health.

Food Taboos during Pregnancy and Faulty Feeding and Cooking Habits

In India there are many superstitious beliefs which are not good for health. Pregnant women are advised to eat less when they need more. They are told not to eat nutritious fruits like papaya, fearing abortion. Some people feel that if pregnant women eat banana they will have only one child since banana tree fruits only once. Early (first three days) mothers' milk (colostrum) is discarded thinking it is bad milk. Realty is: colostrum is rich in protective antibodies, vitamin A and other nutrients. The current WHO guidelines are: start breast feeding from the first hour after birth, do exclusive breast feeding (not even water) for six months and start feeding complementary food from the seventh month after birth. None of these are followed. There are also faulty cooking habits like cooking rice in excess water and discarding the water (ganji), which is rich in B-vitamins and minerals. Vegetables are cut and washed where as they should be first washed and then cut to prevent loss of water soluble nutrients. There is tremendous scope for behavioural change in our society and school children can be agents of change.

What Is a Balanced Diet?

Balanced diet should have the right amount of cereals and millets, pulses (dal), vegetables and fruits and animal products like milk, eggs, meat, fish. In Andhra Pradesh, thanks to the cheap rice scheme, consumption of nutritious millets (jowar, bajra, ragi etc) has come down. Rice has become a prestigious and convenient food. We need to revert to what our forefathers used to eat and bring back millets in our diet. Green leafy vegetables (GLV) are cheap and easy to grow, yet their consumption is less than 20% of what is needed. Table 1 gives the balanced diet for infants, children and adolescents. Adolescent children should consume almost 100 g of GLV and same quantity of other vegetables. Actual consumption is less than half this amount. A balanced diet will provide adequate amounts of all nutrients: carbohydrates, proteins, fats, minerals and vitamins. Animal proteins are of better quality than plant proteins because they have all the 10 essential amino acids, (building block of proteins), in adequate quantity. Cereals and millets are generally low in amino acid called lysine and pulses are low in a sulphur- containing amino acid called methionine. A proper combination of cereals and pulses (3-4 parts of cereals to one part of pulse) can give balanced protein. Due to high cost of pulses, Indian diets are high in cereals and low in pulses besides vegetables, fruits and animal products.

Diet surveys done in India, show that Indian diets are qualitatively poor in vitamins and minerals, leading to hidden hunger. This is because of low intake of vegetables, fruits and animal products. In rural areas unlike the urban areas, families can grow vegetables and fruits in their back-yards. They can also keep backyard poultry and milch cattle for milk for their families. Diet surveys also show that within a family, diet of young children (Under 2 years of age) is more deficient. This is due to mother's ignorance. Small children cannot demand food if they are hungry.

Recent studies show that plant foods contain chemicals (phytochemicals) other than known nutrients, which protect against diseases. They function as anti-oxidants (destroy harmful oxygen species), stimulate immunity; detoxify harmful substances in food (protect against cancer) etc. Plant foods are also rich in fibre, which helps bowel movement and reduces blood cholesterol. Thus plant foods are healthy.

Table 1 Balanced Diet for Infants, Children and Adolescents

(Number of Portions)

Food Groups	g / ml per portion	Infants 6-12 months	Years						
			1-3	4-6	7-9	10-12		13-18	
						Girls	Boys	Girls	Boys
Cereals & Millets	30	1.5	4	7	9	9	11	10	14
Pulses & Legumes	30	0.5	1	1.5	2	2	2	2	2
Milk & Milk Products[a]	100	5[a]	5	5	5	5	5	5	5
Roots & Tubers	100	0.5	0.5	1	1	1	1	1	2
Green Leafy Vegetables	100	0.25	0.5	0.5	1	1	1	1	1
Other Vegetables	100	0.25	0.5	0.5	1	1	1	1	1
Fruits	100	1	1	1	1	1	1	1	1
Sugar & Jaggery	5	5	5	6	6	6	7	6	7
Fats & Oils (Visible)	5	2	4	5	5	5	5	5	5

[a] Quantity indicates top milk. For breastfed infants, 200 ml top milk is required.
For Non-vegetarians substitute one pulse portion with one portion of egg/meat/chicken/fish

For infants egg/meat/chicken/fish maybe introduced around 9 months

Source: Radha MS in Text Book of Human Nutrition, Bamji MS, Krishnaswamy K and Brahmam GNV editors. 3rd Edition, Oxford IBH, 2009.

The Problem of Hidden Hunger

In recent years severe forms of micronutrient deficiency diseases have come down, but marginal malnutrition does exist. Consequences of micronutrient deficiencies like iron deficiency anaemia, iodine deficiency, and vitamin A deficiency are mentioned below.

Iron deficiency anaemia

Adverse effects on:

6. Growth
7. Immunity
8. Mental and motor development
9. Reproductive performance.
10. Almost 20% of pregnancy-related deaths are caused by iron deficiency.

Iodine deficiency

11. Goitre
12. Permanent brain damage, (cretinism, -mental retardation, and deaf mutism).
13. Reproductive failure
14. Decreased child survival

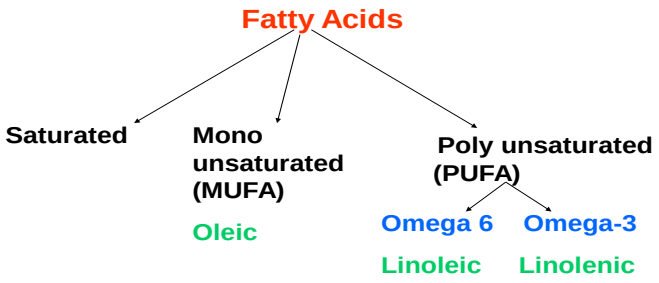
Vitamin A deficiency

15. Severe deficiency can cause blindness due to corneal ulceration(Keratomalacia)
16. Decreased resistance to infections
17. Growth retardation and even death.

For every frank case of nutrition deficiency, there are dozens of others who suffer from sub-clinical malnutrition. The cumulative burden of marginal malnutrition on productivity and medical expenditure can be substantial.

Healthy Fat

Fat is a combination of fatty acids and glycerol. Quality of fat is determined by the type of fatty acid as indicated in the following figure.



MUFA and PUFA are good for health. Lower cholesterol. Omega -3 needed for brain development and membrane stability.

Table 2 gives the composition of fatty acids in some common oils consumed in India

Oil	Satd.	Oleic	N-6	N-3
Coconut	89.5	7.8	2.0	-
Ground nut	20.9	47.9	29.9	-
Mustard/Rape seed	10.7	8.9	18.1	14.5
Olive	14.8	74.5	10.0	-
Palmolein	47.7	41.0	10.3	0.3
Rice bran	22.1	41.0	34.3	1.4
Safflower	10.7	16.7	73.5	-
Soya bean	13.1	28.9	50.7	6.5
Sunflower	9.1	25.1	66.2	-

It is obvious that most Indian oils are deficient in N-3 (Omega- 3) fatty acids and therefore it is advisable to mix oils so that all types of fatty acids can be derived.

Omega -3 fatty acids are derived from foods like nuts, and vegetables. Animal fats like butter and ghee are fully saturated and hence should be consumed in moderation. While carbohydrates and proteins give 4 K cals per gram, fats give 9 K cals per gram. Excess consumption of fats can lead to obesity. Daily consumption of visible fat should be only about 30 -50 g. Hydrogenated fats (Vanaspati) contain Trans fatty acids which have adverse impact on serum lipids and hence are injurious to health. They should be avoided.

Strategies for Combating Malnutrition- the three `A' approach

Awareness

There should be awareness about the importance of foods &, nutrition and feeding practices not only in the community but also among planners and policy makers, health and agriculture professionals, teachers and students, NGOs and media. Print as well as visual, media can play an important role. Science lessons in school curriculum should have information on health and nutrition. Medical and agriculture education should emphasize nutrition.

Access at Affordable cost to food

There are government programmes like the feeding programme in ICDS and the Mid Day Meal programme in schools that improve access to at least one meal to the resource-poor pregnant women and poor children. But they have had little impact on nutrition for a variety of reasons. The content of vegetables and fruits and animal products like milk and eggs is missing or insufficient, though some states have tried to add eggs and milk in the feeding programmes. The public distribution system and now the food security act (FSA) attempt to give cereals to the poor at highly subsidised rate. Unfortunately the FSA does not include protective foods like pulses, vegetables and fruits and animal products. But people should be educated to divert the money saved in buying cereals to purchasing protective foods that go to make a balanced diet.

Prevention of Micronutrient Deficiencies

The two important programmes for combating micronutrient deficiencies are: the anaemia prophylaxis programme and the massive dose vitamin A supplementation programme.

Anaemia prophylaxis programme

As mentioned earlier, anaemia is a micronutrient deficiency disease of great concern due to associated morbidity and even mortality. It is primarily due to deficiency of iron in our food, particularly vegetarian food. Intestinal parasites also add to the problem. Plants have non-haem iron which is poorly absorbed because of inhibitors like phytate in plants. Haem iron present in animal foods is better absorbed. In the anaemia prophylaxis programme, tablets of iron and folic acid are given to pregnant and lactating women, children 6 months to 5 years and adolescents. Coverage of children and adolescents is poor and where the tablets are available, the compliance is poor due to lack of understanding of the importance of this programme. These programmes have failed to reduce the incidence of anaemia. An important intervention should be improved supplies and education of the public.

Massive dose vitamin A programme

In this programme, preschool children aged 9-36 months are given bi-annual dose of 100,000-200,000 iu of vitamin A at six monthly intervals. It was started to prevent blindness due to vitamin A deficiency (keratomalacea). However, despite poor implementation of the

programme, now blindness due to vitamin A deficiency has come down due to better environment and prevention of measles. But in view of dietary deficiency of vitamin A and other adverse effects of vitamin A deficiency on growth and development, the programme is being continued.

Food fortification

Foods commonly consumed can be chemically fortified with the limiting micronutrient like iron, zinc, vitamin A, folic acid etc**.** In India iodised salt is used to prevent iodine deficiency. This has been a successful programme. Children must make sure that only iodised salt is used in their homes. Now salt fortified with both iodine and iron has been developed and this approach may help to prevent anaemia as well. Many foods can be fortified, but for a country like India, such foods should be consumed by large number of poor. Fortification of expensive processed foods will not help the poor. Attempt is being made to sell iron- fortified wheat flour in some states. Its acceptability has to be studied.

Biofortification

In this approach attempt is made to enrich seeds with nutrients like provitamin A (beta carotene) and some minerals like iron and zinc, through variety of plant breeding technologies like conventional breeding, marker driven molecular breeding and genetic engineering. In the last method, gene from one species which produces the required nutrient is transferred to a common crop (cereal/ vegetable) to enrich it through genetic engineering. There is apprehension in the minds of people about such foods called genetically modified foods (GMO). While all efforts to ensure their safety to health and environment is needed through proper testing, false fears should not hamper the progress of good science which can help to ensure micronutrient security. Genetic engineering can also be used to develop pest, drought and heat resistant varieties, which can help to mitigate the adverse impact of climate change.

Dietary diversification

A balanced diet can provide all the necessary nutrients. Unfortunately due to ignorance and poor purchasing power, protective foods like vegetables, pulses, and foods of animal origin like milk, eggs, meat, fish etc are not consumed in adequate amounts. Green leafy vegetables are rich in all micronutrients. India has hundreds of varieties of GLV. They are easy to grow and cheap. Yet not consumed. In villages, people should be motivated to grow vegetables in their homestead gardens and consume them. As it is, most of the vegetables grown are sold. Besides homestead gardens, back-yard dairy, poultry, fish farm (where possible) can increase access to precious foods without needing to purchase them. All villages should strive to grow enough food for their consumption and make sure that vulnerable groups like pregnant and lactating women, children and adolescents whose nutrition needs are special eat those foods.

Your health is in your hands. With proper knowledge of food and nutrition children can be agents of behavioural change and contribute to nation's health.

Suggested Readings:

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Emergence and Reemergence of Infectious Diseases: How and why?

Amit Ghosh
National Institute of Cholera and Enteric Diseases, Kolkata
(Lecture delivered at S.S. College, Hailakandi, Assam)
Email: amitghosh24@yahoo.com

In the recent years the problem of emergence and and Re-emergence of Infectious Diseases have attracted a lot of attention for obvious reasons. First it was AIDS, then SARS then Bird Flu. Dengue has re-appeared in more virulent form; cholera re-emerged in many parts of the world from where it was thought to have disappeared long time ago and sometimes in more severe form. Every now and then we hear about a new infectious disease emerging or an old one re-emerging. This was not the case however, in the yester years, not at least in some parts of the world. In a very thought provoking book -"The Natural History of Viruses", Sir McFarlane Burnett, who won Nobel Prize in 1962 in Medicine for his clonal selection theory, wrote "One can think of the 20th century as the end of the one of the most important social transitions in history – virtual elimination of infectious diseases as a significant factor in social life." (Fig 1)

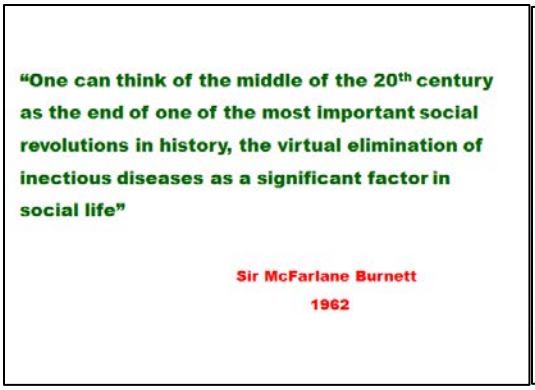


Figure 1

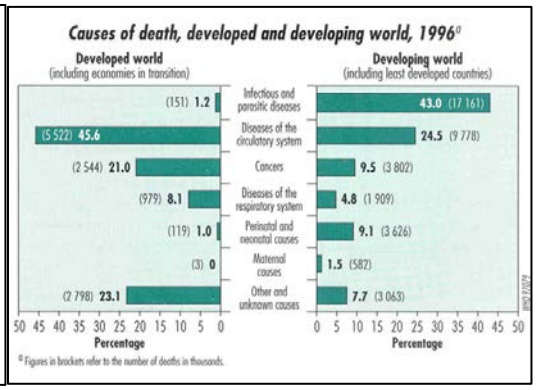


Figure 2

In the USA, at least at that time, "infectious diseases" was considered to be a dead end speciality and the total membership of the Infectious Diseases Society of America was less than 300. Even though the situation was never quite like this in India and other third world

Lecture delivered on December 8, 2012 at Hailakandi S.S. College, Hailakandi

countries, we too were not unduly concerned as it seemed that some diseases were always present in the tropical countries. However, this complacency about the infectious diseases, particularly in the developed countries, was shaken rudely by the appearance of AIDS and the whole world appeared to be jolted. Even before this happened, however, infectious diseases were the number one killer in the developing countries and it continues to be so even now (Fig 2). In 1996, for example, while the death due to infectious diseases was only 1.2% in the developed countries it was 43% in the developing countries. It has been estimated, for example, that in 1990 infectious diseases caused 59% of all deaths among the world's poorest 20%. In the developed countries, however, by the third quarter of the 20th century mortality index due to infectious diseases fell from 500 in the year 1900 to 30 in 1950 and stayed there for the next 30 years till the AIDS struck. Since then as the slide shows a large number of old and new diseases have emerged and reemerged all over the world (Fig. 3)

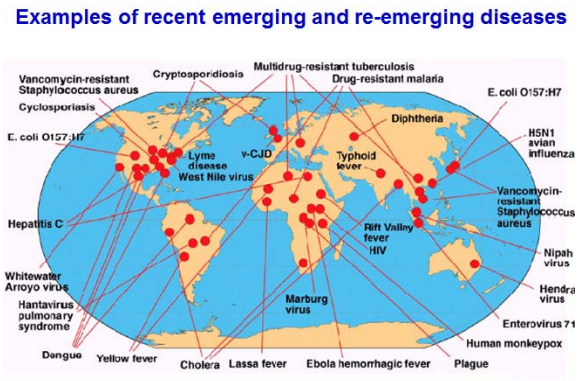


Fig. 3

Fig. 4

Now, what constitutes an emerging or a re-emerging disease? In 1992, US Institute of Medicine defined emerging diseases as subsuming three things: (Fig. 4)

- Established infectious diseases undergoing increased incidence. For example, cholera
- Newly discovered infectious diseases, For example, Nipah viral disease
- Newly evolving infectious diseases, For example, Bird Flu

Further, worried by the emergence of new diseases and the re-appearance of the old ones, Institute of Medicine under the leadership of Joshua Lederberg came to the conclusion that it is the convergence of four broad factors that ultimately promote disease emergence - old or new: (Fig. 5)

- Genetic and Biological factors
- Physical and Environmental factors
- Ecological factors and
- Social, Political and Economic factors

It is to be realized though that these are not distinctly seperable, because the concept of environment in the context of disease emergence is essentially anthropocentric- i.e. the concept deals with the idea of interaction of humans with their surroundings (Fig. 6) and hence physical and biological environment cannot be treated separately from economic and socio-cultural factors without distortion. This will become clear when the phenomenon of re-emergence of cholera in South America after a gap of 100 years is discussed. Even though interlinked, each of these factors nonetheless can be envisioned to be composed of many more sub factors, which can be grouped under a few broad heads: (Fig. 7)

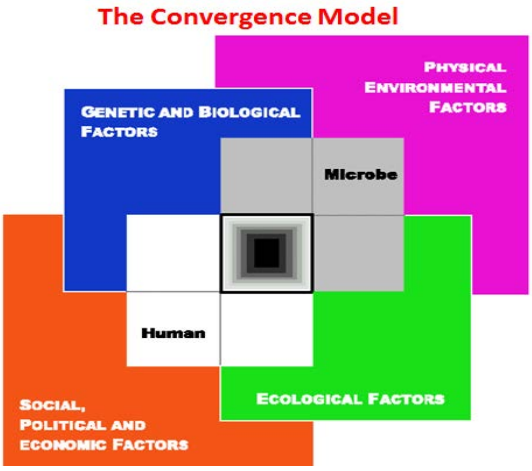


Fig. 5

Environment

- The concept of environment is anthropocentric.
- It carries the idea of interaction between the human beings and their surroundings.
- The physical and biological environment can not be treated separately from the economic and socio-cultural environment without distortion.

Fig. 6

1. Factors directly linked to human activities
    - Change in demographics and behavior
    - Environmental change and land use
    - Breakdown in public health measures
    - International trade and travel

2. Factors influenced by human activities
    - Climate change
    - Microbial adaptation and change

    Let these now be considered one by one:

One of the primary factors behind disease emergence is the change in demographic patterns. These occur due to a variety of reasons - population growth, mass migration, war, changes in societal norms - so on and so forth.

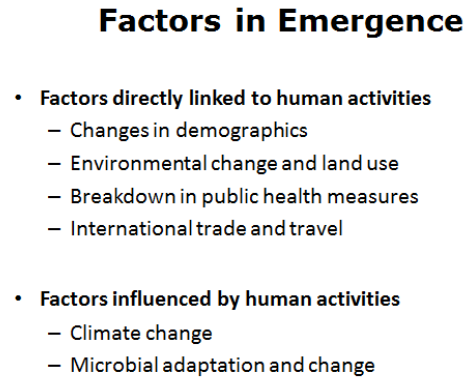


Fig.7

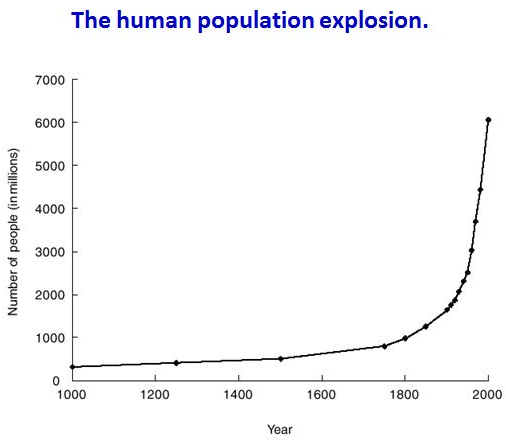


Fig. 8

At the beginning of the 20th century world population was around 1.5 billion (Fig. 8). Today the population of our country alone is around 1.2 billion. This population explosion has radically altered the way we live. It has not only led to denser living conditions but also has forced us to move into areas where we had never lived before. It is also forcing people to move into urban area - slums and thus live under most unhygienic conditions.

Burgeoning population pressure is also compelling people to destroy forests and set up habitation there. It is estimated that every year around 4.7 million hectares of rain forest are being destroyed. Setting up human habitations in forest areas bring humans in contact with potential pathogens with which it had no contact before resulting in the emergence of new diseases. Three typical examples may be considered: (Fig. 9)

- Bolivian haemoregic fever
- Nipah Viral fever
- Lyme Disease

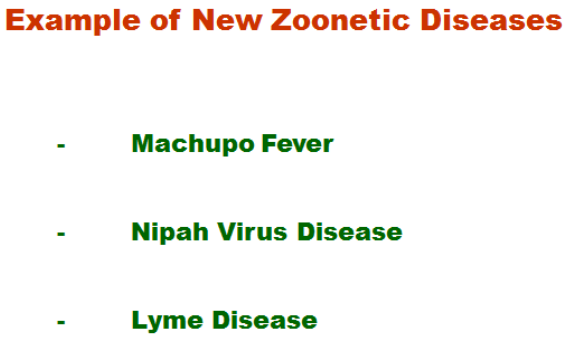


Fig. 9

Fig. 10

Clearing of forest land in Bolivia in the 1960s for the creation of new farmlands allowed the common mouse Calomys calosus to increase in number as the food availability increased. This proliferation in turn, led to greater contact of humans with them and through them with a virus harboured by these mice. The virus jumped species barrier and caused a disease unknown to humanity till then. The disease subsequently named Bolivian Haemoregic Fever, eventually killed almost a third of the infected individuals. The second example is that of Nipah

Virus. Nipah emerged for the first time in the recorded history in 1998, in Malaysia. Orchards built on reclaimed forest land attracted fruit bats whose droppings infected pigs in pig-pens located in the orchards. Infected pigs in turn infected their handlers (Fig. 10) and an outbreak of a new disease, which eventually killed around 100 people in its first appearance occurred. Quite ominously the disease emerged in Bangladesh in 2001, 2002 and 2004 pointing to its ability to move to newer areas. Furthermore, in case of Bangladesh outbreaks no intermediate animal hosts could be identified suggesting that the virus probably acquired the ability to move directly from bat to humans. Nipah is now known to have established a foot-hold in our country as well. It ought to be pointed out here though that this kind of human contact with unknown microbes due to human migration into re-claimed forest lands is not always due to economic compulsions. It can also happen due to life style changes arising out of affluence. Lyme disease provides a good example. This disease named after a small town in Connecticut, U.S.A., emerged for the first time in 1975. Since then it has continued to expand both in terms of the cases reported and the geographical spread. Its emergence has been linked to the re-forestation of farm lands. Reforestation led to a dramatic increase in the number of white-tailed deers horbouring ticks which are reservoirs for the pathogen *Borrelia burgdorferi*, the causative agent of Lyme disease in these areas. As more and more people started building homes and Golf courses closer to these "re-created" forests in order live closer to nature, they also came in greater contact with the deers harbouring these ticks whose bites transmitted *B. burgdorferi* to them.

At this stage a question may be asked - what is the possibility of the emergence of more such pathogens?

Studies conducted in 1990 and 2001 showed that a gram of soil may contain upto 4000 different microbial species and that out of these staggering number of microbes, only 1415 species belonging to 472 different genera are associated with human diseases (Fig. 11). It is therefore not unlikely that many more pathogens are lurking in the shadows waiting to emerge at an opportune moment. Indeed every year some new pathogens are emerging (Fig. 12).

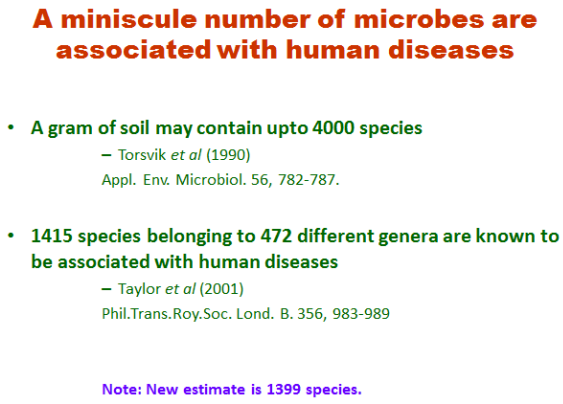


Fig. 11

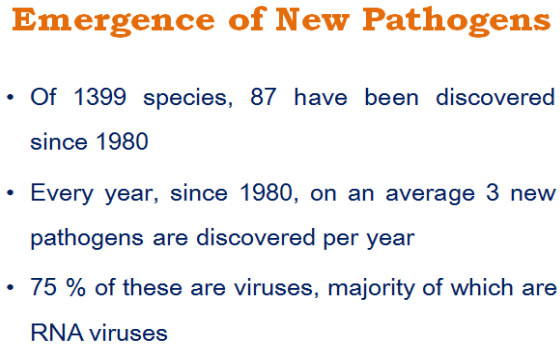


Fig. 12

It was pointed out earlier, that one of the factors that the Institute of Medicine considered important as a cause for the re-emergence of a disease, is the break down of public health measures. Two examples, may be considered – Re - emergence of Plague, which

all but disappeared from the erstwhile Soviet union, in Russian Federation after the collapse of the Soviet Union, and the re-emergence of Malaria in Afghanistan, a country which till 1979 was almost free from this disease, after the civil strife began.

Another factor which plays an extremely important role in spreading a disease is travel. Every year an enormous number of people cross international boundaries. In the year 2000 the number was around 600 million (Fig. 13). In the 19th Century, most diseases and infections that travelers carried manifested themselves during the long sea voyages that were the primary means of travel at the time for covering great distances. And hence, recognizing the symptoms the authorities at the ports of entry could quarantine contagious individuals. In the age of Jet travel, however, a person incubating a disease such as Ebola, can board a plane, travel 12000 miles, pass unnoticed through Customs, take a domestic carrier to a remote destination and still not develop symptoms for several days, and before his condition becomes noticeable, possibly infect many individuals.

When a "new" disease arrives in a country the population at greatest risk is the aging population because of their weakening immune systems. Once a "new" disease finds such a niche it becomes easier for it to "settle down" in the new place and spread. Thus ageing population is also an important factor in the emergence and re-emergence of infectious diseases. Just how important this factor is, highlighted by the fact that by 2020 the number of people above 65 years of age is going to be uncomfortably high in most countries of the world (Fig. 14).

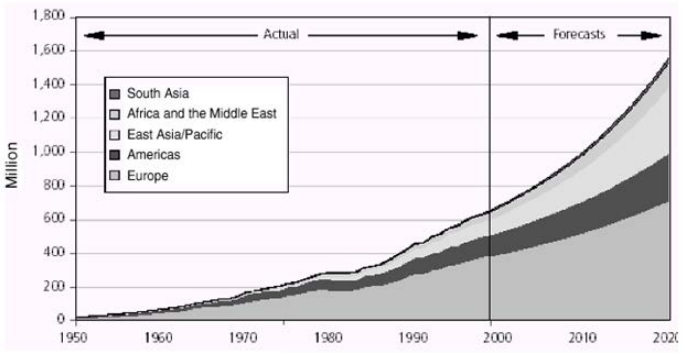


Fig. 13

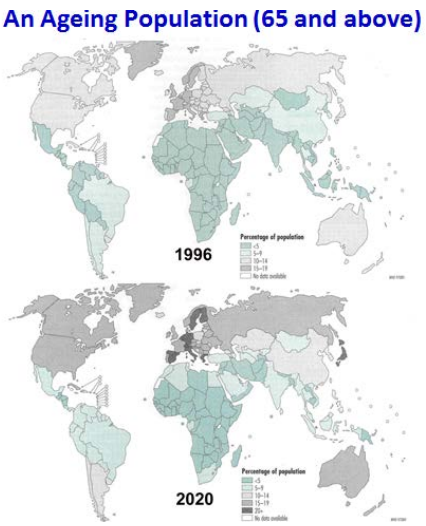


Fig. 14

The factors that have been discussed so far are the direct consequences of human behavior. Besides these, however, there are other factors which are not linked directly to the kind of activities described above. Primary among these are climate change and the ability of the microbes to adapt to the changing environments resulting from man made activities.

Global temperature is rising and has increased by about 1° on the average . It is likely to go up considerably higher unless some drastic measures are taken. This average rise

in temperature actually conceals the fact that this rise in reality can be quite uneven (Fig. 15). This is because environmental changes are the results of human activities that are distributed unevenly over the world. Actually an average rise in global temperature hides the fact that the rise is greater at higher altitudes. For example, a 1° rise in temperature in equator could mean a 12° rise in the poles. Average temperature rise also hides the fact that rise is greater over land than over the sea and that since each 1° rise in temperature enables the atmosphere to hold 6 % more moisture, the rain fall pattern would be vastly different in different places. To appreciate how such an apparently innocuous change in temperature impacts disease emergence, it would be worthwhile to discuss in brief, the modes of disease transmission. Whether anthropocentric i.e. transmitted from man to man or Zoonetic i.e. transmitted from animals to man, disease transmission follows two paths - direct or indirect (Fig. 16). In direct transmission, the pathogens spend very little time outside the host and hence are unlikely to be influenced by climatic changes. In indirect transmission, the disease is transmitted by physical vehicles e.g. soil or a vectors like mosquitoes. None of these have temperature regulatory mechanisms. Therefore, external temperature influences the life cycle of the pathogens harboured by them. The period that a pathogen spends outside the host is called "Extrinsic incubation Period" (EIP). It is defined as the "number of days" between the vector's ingestion of the blood meal and the time at which the vector becomes capable of transmitting the pathogen. Replication rate of a microbe also is dependent on the ambient temperature. When the ambient temperature drops well below a critical threshold, replication ceases. At that time, bite by the vector does not lead to disease transmission even though it harbours the pathogen. Furthermore, below a particular temperature the vector itself cannot breed. The consequence of temperature rise due to global warming is seen most dramatically in the case of diseases like dengue or malaria. Dengue is numerically the most important viral disease in the world today. Around 80 million cases are reported every year with more than 20, 000 deaths. For this disease shortening of EIP increases the proportion of mosquitoes that can become infectious at a certain time. For example, EIP of type 2 dengue virus is 12 days at 30° which shortens to 7 days at 32°. This shortening of EIP by 5 days translates into a 3 fold increase in the transmission rate of the disease (Fig. 17). Further, the vector *Aedes agepti* itself can not breed if the environmental temperature falls below a certain value. It has been shown by Epstein of U.Washington, USA, that Dengue, which (transmitted by the mosquito by the mosquito *A. agepti*) was once confined to the low altitudes areas of the Himalayas has now moved 1 km up due to global warming.

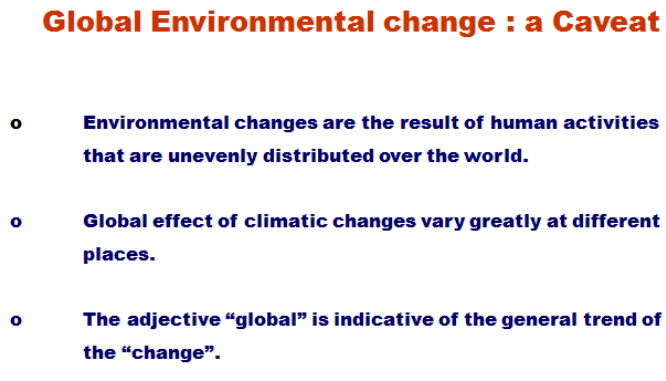


Fig. 15

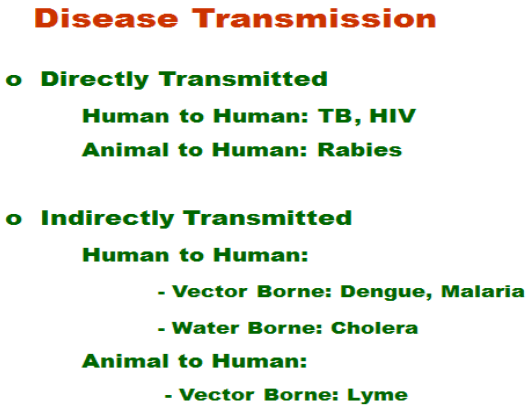


Fig. 16

It may be recalled that microbes ability to adapt and change was listed earlier as an important factor behind the emergence and re-emergence of infectious diseases. As Stanley Falkow, one of the top microbiologists of the world pointed out, underlying disease emergence there is evolutionary conflict between the rapidly evolving infectious pathogens and their slowly evolving hosts and that adaptation is a must for the pathogen to be able to survive. Microbes can adapt fast because their genomes are inherently dynamic. They can change through a variety of means over time scales miniscule compared to the evolutionary time scales of their hosts. A good example is provided by the emergence of drug resistant microbes or new clones of a microbial species.

As one considers all these factors, the question that naturally comes to ones mind is what are the factors that contribute the most. In a milestone paper published in Feb 2008, in Nature , scientists from three institutes - two in the USA and one in UK have shown after a thorough analysis of all the available data, that 60% of all diseases "travelled" to humans from animals and 20% of the disease re-emergence is due to the emergence of drug resistant pathogens (Fig. 18). Based on this and other supporting data, they came out with a "Prediction map" highlighting the risk areas in which unfortunately India (particularly the North-East) occupies a prominent place (Fig. 19).

Dependence of Extrinsic Incubation Period on Temperature: Dengue Example

Temp (°C)	EIP (days)
30 °	12
32 °	7

2° C rise in temperature translates into a 3 fold Increase in the disease transmission

Patz et al (1996); JAMA ***275***: 217- 223

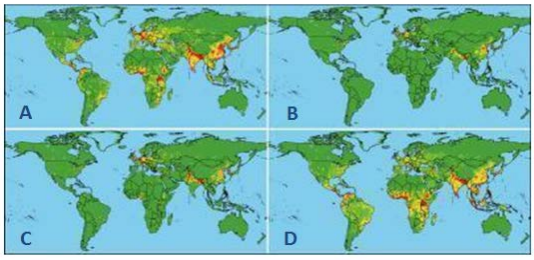
Fig. 17

Origins

- **60% of the diseases traveled from animals to humans.**
- **About 20% of known emergences are multidrug-resistant strains of previously known pathogens.**

» Jones et. al, (2008), Nature, *451*, 990-993

Fig. 18



Global distribution of relative risk of an EID event

- A: Zoonotic Pathogens from wild life
- B: Zoonotic Pathogens from non wild life
- C: Drug resistant pathogens
- D: Vector borne pathogens

Highest risk

Lowest risk

Fig. 19

Recrudescence of cholera

- **Epidemic in Peru, 1991**
- **Advent of *V. cholerae* O139; 1992**
- **Epidemic in Zaire; 1994**
- **Emergence of Hybrid Strains**

Fig. 20

So far our discussion remained confined to factors influencing disease emergence in the context of new diseases. At this juncture it would interesting to see how these same factors help the re-emergence of the old diseases in areas from where they disappeared long time ago. Re-emergence of cholera, an ancient disease, in areas of the world from where it disappeared long long ago provides a good example. Cholera is a terrifying disease - it attacks suddenly and if the patient is left untreated it can cause death within hours of onset. It is an acutely dehydrating diarrhoeal disease caused by a bacterium called *Vibrio cholerae.*

Cholera originated in India and then some 200 years ago moved out of the subcontinent. However, aggressive public health measures caused it to disappear from many countries of the world including South America, more than 100 years ago. In the early 1990s cholera, however, joined the ranks of re-emerging diseases because of the four events (Fig. 20)

- Epidemic in Peru in South America
- Advent of a new strain of *Vibrio cholerae* - *Vibrio cholerae* O139
- Epidemic in Zaire due to drug resistant strains of *V. cholerae*
- Emergence of more virulent hybrid strains of *V. cholerae*

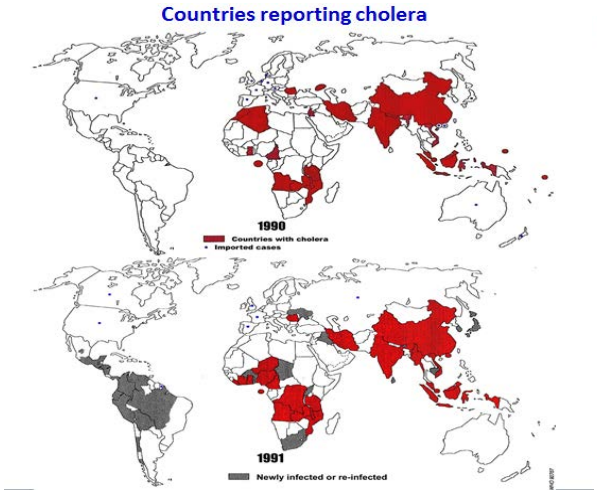


Fig. 21

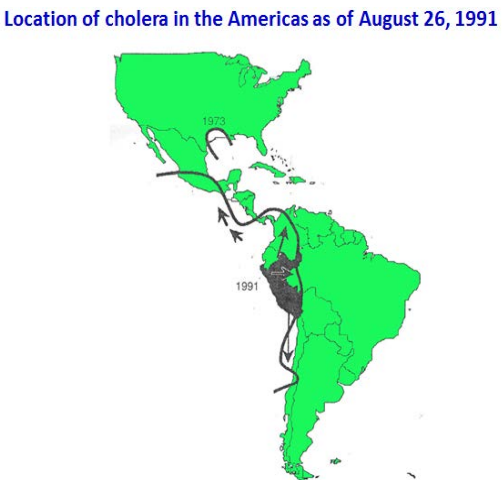


Fig. 22

We will consider only the first two of these. First is the case of epidemic in Peru. Prior to 1991 cholera was present in many countries of the world but South America was free from it (Fig. 21). But in Jan. 1991, cholera broke out in Peru - First in Chancay, the harbor city near Lima and then on the very next day in Chimbote, a city 400 km up north of Lima (Fig. 22). And soon it engulfed the whole country. An Analysis of what caused this re-emergence revealed that many of the factors I have mentioned in this lecture, played a direct role. In the beginning it was thought that global trade was responsible and that the affecting strain reached Peru through the bilge water of a Chinese cargo ship. This hypothesis, however, was questioned on the ground that Peru strains did not match any other strain isolated anywhere else in the world. Moreover, it was realized that this hypothesis could not explain how cholera could appear nearly simultaneously all along Peru's 2000km coast line. It was suggested therefore, that a more plausible explanation could be sought in *El Nino*, which is essentially the

warming of the sea-surface water in central pacific. *El Nino* brings in its wake rain and influx of nutrients into coastal waters from inland areas triggering algal bloom. It is now known that *V.cholerae* can survive for a long time in a viable but non-culturable state in association with zoo planktons. An algal bloom can lead to heavy accumulation (high concentration) of vibrios in water. In fact in a year round study carried out in Bangladesh, a co-relation between cholera outbreak and sea-surface temperature rise has been recorded.

Whatever could have been the reason for the appearance of *V.cholerae* in the harbor waters of Lima and its eventual migration to the Peru's drinking water supply system, one reason for its gaining foothold was the erroneous policies pursued by the Peruvian government. Peruvian government under pressure from IMF was forced to enforce the so-called structural adjustment, the fall out of which was a sharp drop in the per capita health expenditure and wide spread malnutrition. By the time cholera reached the shores of Peru, according to the WHO, more than 60% of Peruvians were suffering from malnutrition. Another bad decision was to stop chlorination of the drinking water supply based on the fear that chlorination increases the risk of cancer. In conclusion, it can be said that the phenomenon of the re-emergence of cholera in Peru illustrated beautifully the concept that physical and biological factors can not be treated separately from the economic and socioeconomic factors without distortion.

Now we turn to the second event in the list. The advent of a new strains of *V.cholerae*, *V.cholerae* O139 in 1991. Strains of *V.cholerae* are differentiated on the basis of their O-antigen (Fig. 23). Until 1991, only the *V.cholerae* strains belonging to serogroup O1 were known to cause cholera. In 1991, however, when a large epidemic broke out in the Indian subcontinent, detailed characterization of the causative agent showed that it was not agglutinable by the O1 antisera or by any other anti-sera raised against any of the 138 the then known serogroup *V.cholerae* strains. The Strain was therefore assigned a new serogroup-serogroup O139. Cholera caused by this strain very quickly spread all over India (Fig. 24) indicating that it was a new strain. Evolution of a new strain to cause an old disease pointed to the importance of the role played by microbial adaptability in disease emergence. A thorough analysis of the new O139 strain revealed that it evolved from the O1 El Tor strains through the alteration of its genome structure by a piece of DNA it acquired from somewhere.

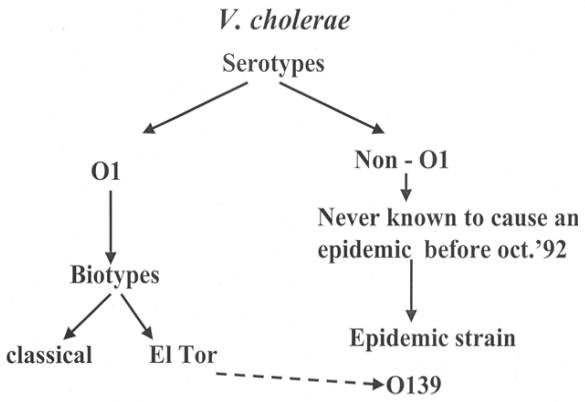
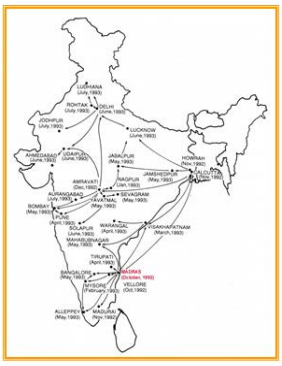


Fig. 23

Spread of *V. cholerae* O139 Bengal During 1992-93



Courtesy: Dr. G. B. Nair

Fig. 24

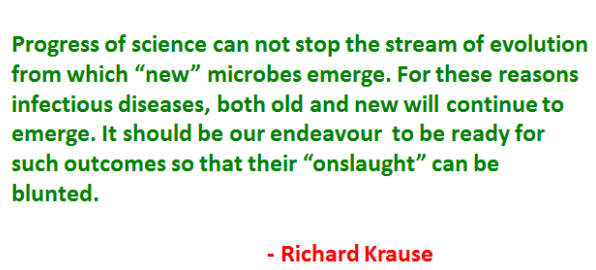


Fig. 25

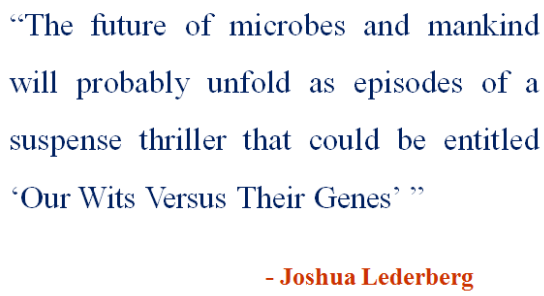


Fig. 26

Though it is possible to give many more examples of how the microbes' ability to change in combination with the other factors can aid disease emergence, it is perhaps not necessary. What has been attempted in this lecture is to provide an overview of how a complex interplay of a variety of factors contribute to the emergence of new diseases and some times re-emergence of the old ones. As the human civilization progresses and more technological advances take place mankind will perhaps be able to find newer ways to tackle infectious diseases. However, as has been aptly stated by Richard Krause, a foremost expert on infectious diseases and a former director of NIAID (USA), "Progress of science can not stop the stream of evolution from which new microbes emerge. For these reasons infectious diseases both old and new will continue to emerge. It should be our endeavor to be ready for such outcomes through increased surveillance so that their onslaught can be blunted and epidemics can be forestalled" (Fig. 25). But will we be successful? We do not know. Because as stated by Joshua Lederberg (Fig. 26) "The future of mankind and microbes will probably unfold as episodes of a suspense thriller that could be entitled 'Our Wits versus Their Genes' ".

Parasites are more clever than man

Hemanta K. Majumder
J.C. Bose National Fellow
Raja Ramanna Fellow
CSIR- Indian Institute of Chemical Biology
Jadavpur, Kolkata - 700032
Email: hkmajumder@iicb.res.in

Parasites are organisms that live within a host and they obtain nourishment from the host without benefitting or killing the host. Parasites are sneaky little creatures that infiltrate our bodies and start camping out. They cause diseases in humans and animals. Some of the disease can be treated easily and some are treated with difficulty. They can live in a human host without being detected for months or even years before becoming infectious to the host. The burden of the diseases often rests on communities in the tropics and subtropics, but infection can also affect people in the developed countries.

There are all sorts of parasites. Some estimates claim that there are over five hundred thousand different types of parasites. Parasites can be classified in three basic groups: First is the protozoa, second is the helminths and the third is arthropods. There are over 30000 species of protozoa that we are aware of and many of these are parasitic. Protozoa usually have flagella and thus can actively move. Protozoa are microscopic, single celled organisms that can be free living or parasitic in nature. They multiply in humans and other animals, which contribute to their survival. They also permit serious infections to develop from just a single organism.

The protozoa that are infectious to humans can be classified in to four groups.

1. Sarcodina : the amoeba, e.g. *Entamoeba*
2. Mastigophora : the flagellates, e.g. *Giardia, Leishmania*
3. Ciliophora : the ciliates, e.g. *Balantidinum*
4. Sporozoa : organisms whose adult stage is not motile e.g. *Plasmodium*

Of all the parasitic diseases, malaria causes most deaths globally. Approximately one million people are killed each year, most of them being young children in Sub-Saharan Africa. Malaria is caused by protozoan parasites called *Plasmodia*, which belongs to the parasitic phylum *Apicomplexa*. More than 200 species of the genus *Plasmodium* have been identified that are parasitic to reptiles, birds, and mammals. Four *Plasmodium* species have been well known to cause human malaria, namely, *P. falciparum, P. vivax, P. ovale, and P. Malariae.* All malaria parasites infecting humans probably jumped from the great apes (in case of *P. knowlesi*, macaques) to man.

Some of the tropical diseases belong to the group of Neglected Tropical Diseases (NTDs). The NTDs have been suffering from a lack of attention by the public health community and

Lecture delivered on December 10, 2012 at Gandhi Bhawan, Silchar, Assam

include parasitic diseases like filariasis, trypanosomiasis, leishmaniasis onchocerciasis and Guinea worm diseases. These NTDs affect more than one billion people i.e. approximately one sixth of the total global population. The populations are largely from the rural areas of low income countries. These diseases cause havoc and take large toll on endemic populations including lost abilities to attend school, retardation of growth in children, impairment of cognitive skills and serious economic burden in the affected countries.

Leishmaniasis, a neglected tropical disease is one of the most serious forms of parasitic diseases caused by the protozoan flagellates of the genus *Leishmania.* The schematic diagram is shown in **Fig. 1**.

Leishmania Parasites : Schematic Diagram

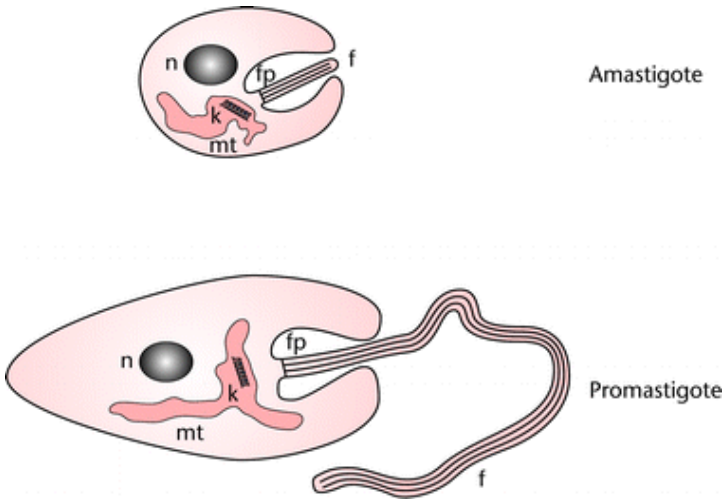


Fig. 1: The two forms of *Leishmania* Parasites; Amastigotes and Promastigotes. N, Nucleus; mt, Mitochondrion; k, Kinetoplast DNA; fp, Flagellar Pocket and f, Flagellum. Picture taken from internet.

The genus *Leishmania* presents a complex set of clinical features ranging from a self healing cutaneous lesion through the most destructive mucosal inflammation to often fatal visceralizing form **(Fig. 2).**

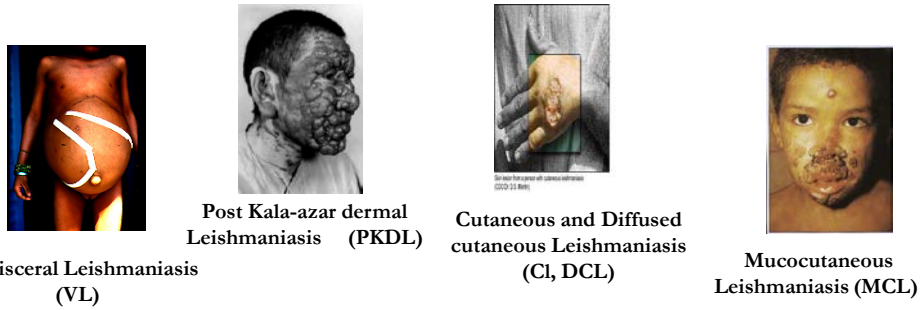


Fig. 2: Different types of Leishmaniasis. (Pictures taken from internet)

The disease leishmaniasis has been and still is one of the most major health problems facing mankind. The initial evidence was in first century AD. In the early and late nineties 100000 deaths have been reported in Sudan and 200000 cases have been reported in Kabul. In the global scenario it is understood that 12 million people are affected and 350 million are at risk in 88 countries. The disease causes 70000 deaths each year globally. The worst affected countries are Afghanistan, Bangladesh, Bolivia, Brazil, India, Iran, Peru, Saudi Arabia and Syria. India is one of the most endemic regions in the world and visceral leishmaniasis is prevalent in eastern, central and southern India. It is estimated that 50 million people in Eastern India alone are at risk.

The *Leishmania* parasites have a dimorphic life cycle. It is carried by the vector sand fly. Two different genera of sand fly transmit *Leishmania* and these are *Phlebotomus* (old world) and *Lutzomyia* (New World). Out of 500 Phlebotomus only 30 species have been identified as the carrier of the parasites. The dimorphic life cycle is shown in **Fig. 3**. In brief, the intracellular form of the parasite, the amastigotes are ingested by the sandfly during taking a blood meal from infected vertebrate hosts and migrate to the midgut of the sand fly vector where they get transformed in the promastigotes approximately with in three days. With in the midgut the promastigotes replicate by means of binary fission. When the infected sandfly takes second blood meal, it injects the infectious promastigotes from its pharynx in to the blood stream of the vertebrates. Then the promastigotes enter in the mononuclear phagocytic cells of the host, where they are transformed in to amastigotes again and replicate within the host cells, thereby completing the life cycle.

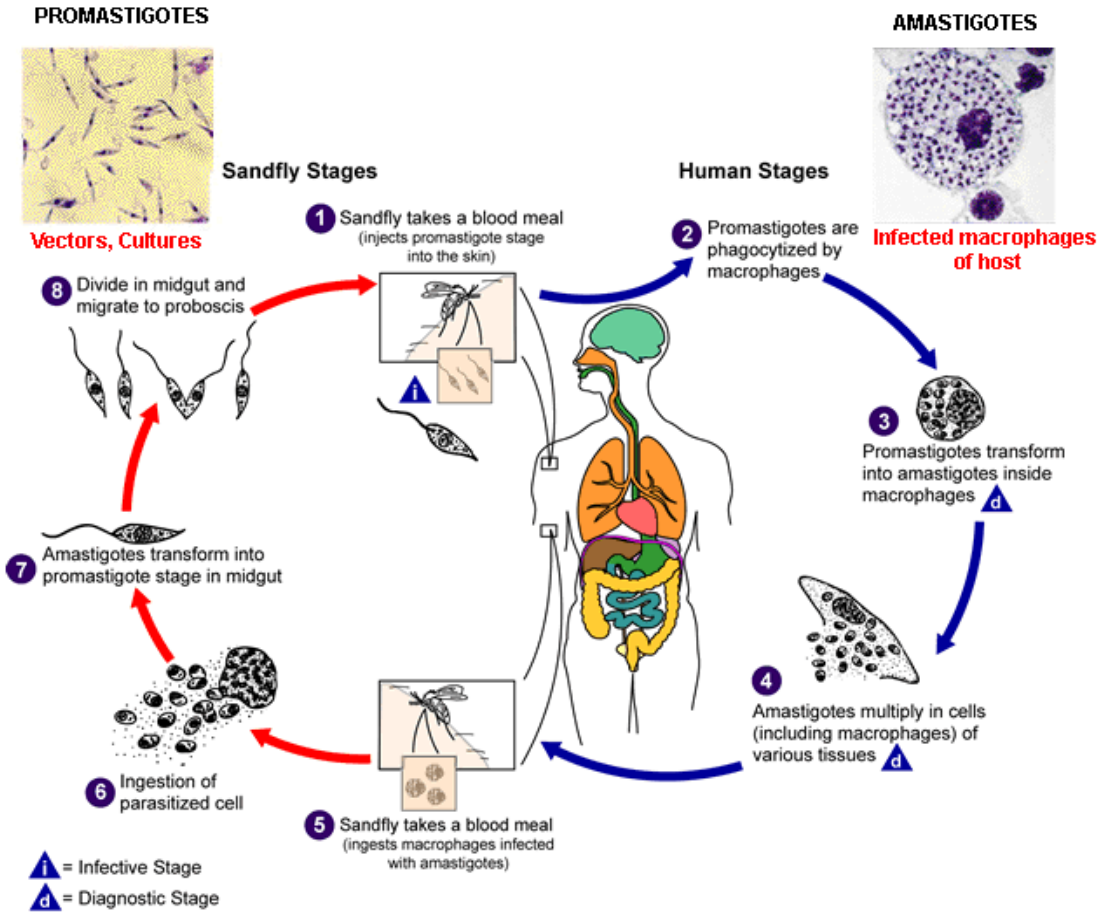


Fig. 3: The di-morphic life cycle of *Leishmania donovani* parasites. The two stages are coloured differently (arrow). Red = Life cycle in Sand fly gut and Blue = Life cycle in Human. (Figure taken from http://www.uni-tuebingen.de/modeling/Mod_Leish_Cycle_en.html)

Measures to control leishmaniasis have not been totally successful and attempts to develop a useful vaccine against visceral leishmaniasis or kala-azar are still not successful. Therefore, chemotherapy remains the only practical means of combating the disease. Pentavalent antimonials (SbV discovered by Sir U.N. Brahmachari in 1922 made a remarkable advance in the fight against kala-azar and reduced the mortality substantially. But emergence of clinically resistant strains to these drugs pose a serious threat for control and treatment of the disease. In India visceral leishmaniasis or kala-azar (black or fatal fever) was reported as early as 1872 in Burdwan district. The disease established itself in the fertile alluvial plains of Bengal and later moved to northern part of Bangladesh, Assam and Bihar. A historic report submitted by Kala- azar Commission in 1926 established the transmission of the disease through sand fly vectors. Since the parasites are vector borne, one of the ways of combating these parasitic diseases is to control the vector population. Vector control strategies are being developed in many countries.

In 1900 the parasite *Leishmania donovani* was discovered by William Leishman in the post mortem spleen smear of a soldier of Royal Irish Rifles at Dum Dum. But it was confused with trypanosomiasis till 1903. In 1903 Charles Donovan working on Indian patients and viewing the report of Leishman in British Medical Journal identified the new organism. Sir Ronald Ross later coined the name as *Leishmania donovani.*

Indian Institute of Chemical Biology (IICB) has a long association with the disease kala-azar. Dr. J.C. Ray, the founder Director of the of the institute initiated Leishmania research at the institute. Although lots of research is going on in the country and abroad, the disease kala-azar is still problem till date, because the diagnosis is difficult and treatment is unsatisfactory as the parasites cleverly evade the immune systems of the host. Trypanosomes, the causative organism for the disease sleeping sickness in humans and cattle in African and South American countries and the parasite Plasmodium which causes devastating malaria in human evade the immune system of the host by changing their immunogenic surface coat. The rapid change in the surface coat is called antigenic variation. Because of the antigenic variation in these two parasites, vaccine development is a real problem. Antigenic variation has not been reported in *Leishmania.* However it has different survival strategy by which it can affect human. Leishmania infection induces immunosuppressive effect and there is no host response. No effective vaccine is available till date.

Therefore chemotherapy is the only choice. Urea stibamine and sodium stibogluconate, the conventional drugs are effective but have increasing toxicity and serious side effects. The second line of drugs pentamidine and amphotericin B, although have high cure rate are very toxic and very expensive. Recently a phosphocholine anticancer compound miltefosine is being used for treatment of visceral leishmaniasis. Miltefosine also has high cure rate but there are reports of emergence of resistant parasites.

Leishmania and *Trypanosoma* belong to the order kinetoplastidae. They differ from other parasites with respect to their mitochondrial DNA, called the kinetoplast DNA (kDNA).

This kDNA is present within the specialized region of the single mitochondrion of the parasite. The kDNA is composed of several thousands of small circular DNA molecules (approximately 1 kb), the minicircles and a handful of larger circular DNA molecules (20-25 kb), the maxicircles **(Fig. 4).** Maxicircles code for some of the mitochondrial biogenesis enzymes e.g cytochrome oxidases, NADH dehydrogenases, ATP ases etc. required for oxidative phosphorylation but minicircles apparently do not have any protein coding functions. So the question remains, why nature has provided such an enormous amount of DNA in the parasite. Further research on the function of kDNA minicircles has established that minicircles are functional and they are involved in RNA processing and maturation in the parasite. Maxicircles are mitochondrial equivalent and are involved in energy generation in the parasite. Therefore unless the complicated nature of the DNAs present in bothe the nucleus and in the mitochondria and their replication is understood, it will be difficult to get away from the parasitic menace.

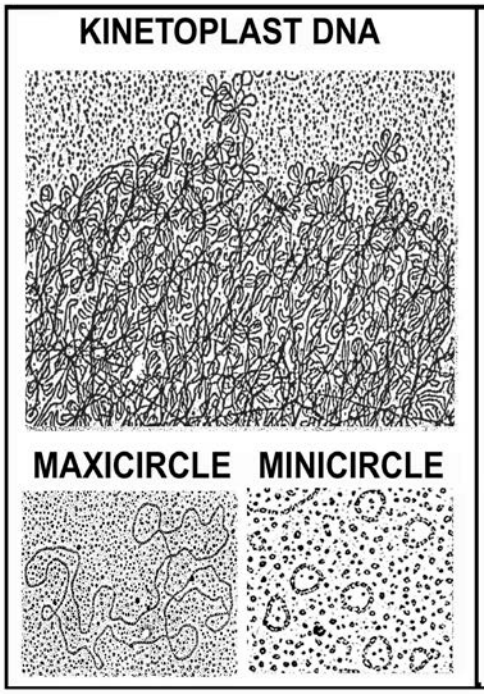


Fig. 4: Electron micrograph of Kinetoplast DNA network of Leishmania donovani. Upper panel shows kDNA network. Decatenated maxicircles and minicircles are shown in bottom left and right panel.

Indian Institute of Chemical Biology is an established and globally recognized center for *Leishmania* research in the country. Dr. J.C. Ray, the founder member and the second director of this institute pioneered Leishmania research in the institute. A number of scientists have been working on this parasite and the disease for last several years. Major research activities have been centered to (i) development of diagnostics (ii) development of vaccines (iii) host pathogen interaction (iv) drug development and chemotherapy of leishmaniasis (v) cell biology and immunology of Leishmania parasites (vi) molecular biology and enzymology of the parasite. All these research activities are targeted to development of intervention strategies against the deadly pathogen. It is hoped that the concerted effortsof the scientists at Indian Institute of Chemical Biology and in different othr laboratories in the country and abroad will certainly be successful in overcoming this menace in the near future.

Targeted Drug Delivery: Magic Bullet Approach

Dr. Pijush K. Das
Co-ordinator & Head,
Infectious Diseases and Immunology Division
CSIR-Indian Institute of Chemical Biology
4 Raja S.C. Mullick Road, Jadavpur
Kolkata - 700032
Email: pijush52@gmail.com

Drugs are chemical substances used in treatment, cure, prevention or diagnosis of a disease. In simplest term drug delivery or drug targeting means delivery or transport of a drug to the appropriate diseased site to bring in maximum action against the disease. This approach of targeting drug is known as "magic bullet" approach where the drug is the "bullet" having potency to strike and the drug carrier is the "magic" which delivers the bullet/drug to its specific target or diseased site. Each and every drug has certain amount of toxicity by which it kills the causative germ. A drug is usually taken either orally or parenteraly. Through blood circulation drug is distributed throughout the body. In addition to the diseased site, the drug reaches all other places comprising of normal cells where it is not needed. By the toxic effect of the drug, the normal cells get affected and this is the main reason for side effect of a drug. If by any means drug could be directed only to the diseased site, then the amount of drug needed would be very less. Naturally the side effects would be very less.

It was Paul Ehrlich who first coined the term "magic bullet" for targeted drug delivery in 1903 and Bangham's observation on phospholipid bilayers led to the discovery of liposome, the first carrier for drug delivery.

The rationale behind drug delivery is to tag the drug with a carrier molecule as free drug has limited access for the target site whereas drug with carrier molecule supposedly will have facilitated transport of drug to target site. Hence ideal characteristics of a drug delivery system should be – specificity of the drug to targeted areas ensuring minimal drug leakage and metabolism during transit to target (Fig. 1). It should be able to protect the drug from premature clearance, should ultimately be bio-degradable and non-antigenic. The drug carriers should be able to cross anatomical barriers, should be non-toxic, non-immunogenic and biodegradable entity. Most importantly it should be recognized by the target and it should be able to release the active moiety or drug inside the target.

There are various types of carrier systems for drug delivery such as a) colloidal carriers like liposomes, niosomes and nanoparticles, b) cellular carriers like resealed erythrocytes, c) supra molecular delivery like micelles, d) polymer based delivery like soluble synthetic carrier

Lecture delivered on December 09, 2012 at Karimgunj College, Karimgunj, Barak valley, Assam

and e) macromolecular carriers like proteins and polysaccharides.

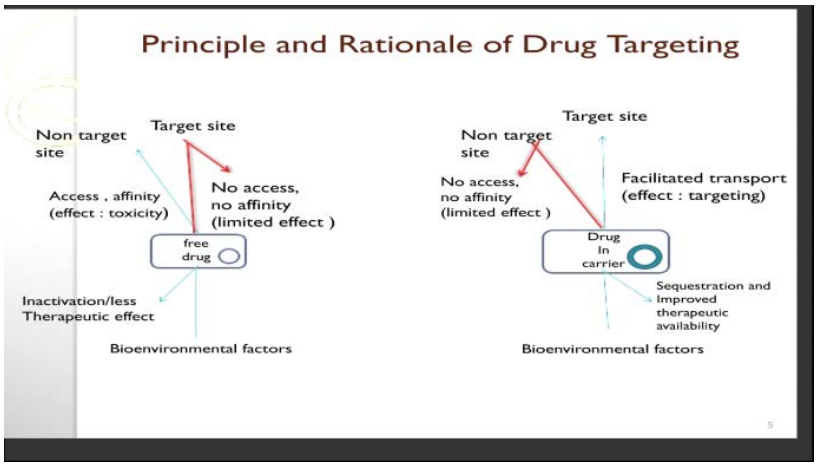


Fig. 1. Ideal characteristics of a drug delivery system

But, though drug delivery seemed exigent, there are various problems associated with it. Mostly the delivery system gets rapidly cleared and body shows immune reactions against intravenously administered carriers. Moreover, target tissue heterogeneity is a problem for the carrier system and diffusion of drug delivery system, however efficient, may still lead to non-specific accumulation.

I will now talk about our work on drug delivery. We tried to develop a macrophage-specific drug delivery system. For this we used visceral leishmaniasis as model macrophage disease. Macrophages are the primary defense cell line of our body. Many different types of macrophages are there like spleen macrophages, lung or alveolar macrophages, liver macrophages or Kupffer cells, peritoneal macrophages, circulating macrophages etc. These are very strong cells. They do many vital works in our body. They do housekeeping job. Just like we clean our daily wastes as part of household work, macrophages also do similar kind of work in our body. The cells in our body are always in a dynamic equilibrium – being synthesized and destroyed. Macrophages clear the dead cells of our body. In addition, macrophages play a very important role in the immune system of our body. All the time we are confronted with various microbes and macrophages are the cells which try to digest and destroy these incoming pathogens or microbes by various defense arsenals like oxidative radicals, lysosomal enzymes etc. But the unusual thing is that like other microbes macrophages engulf *Leishmania* parasites also, but they cannot digest. In converse, *Leishmania* parasites neutralize the defensive machinery of macrophages and reside and replicate within macrophages. *Leishmania* parasite, the causative agent of Kala-azar or visceral leishmaniasis, is a unicellular parasite prevalent in many tropical and subtropical countries. Every year 2 million people are affected by this disease. In India, the disease is prevalent in Eastern part mainly West Bengal and Bihar.

Although leishmaniasis is not a household name like malaria, the disease is somewhat similar to malaria in the sense that it is also a parasitic disease and just like malaria parasites

are carried by mosquitoes, here also the parasites are carried by a kind of fly called sand fly. The disease is of three types. Cutaneous - characterized by single lesion of the skin which develops at the site of the bite of the insect vector. Mucocutaneous - characterized by diffused lesion of the skin and the parasite disseminates only to distant cutaneous site. Visceral - affects liver and spleen, mainly infects the macrophages of liver, spleen and bone marrow and is very dangerous and fatal if not diagnosed early and treated properly. Typical symptoms are irregular fever, anemia and enlargement of liver and spleen. This fatal form is prevalent in India.

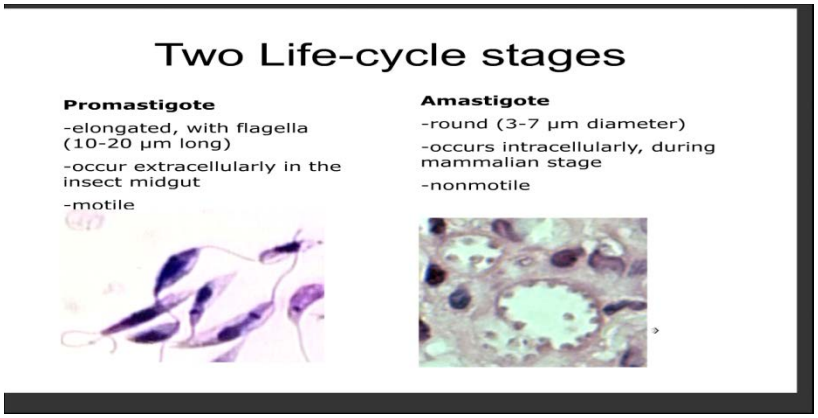


Fig. 2. Different forms of *Leishmania* parasites

Although all three disease types are of strikingly different pathological response, the causative parasite of the genus *Leishmania* are very similar morphologically. Thus, cutaneous is caused by *L. major* and *L. tropica*, mucocutaneous is caused by *L. braziliensis* and *L. mexicana* and visceral is caused by *L. donovani*. The protozoan is transmitted from infected dogs or other reservoirs like man or wild canids by the insect *Phlebotomus argentipes*, *P. papatasi* or *P. sergentis*, commonly known as sand fly. *Leishmania* parasites exist in two forms: Promastigote - long tubular-shaped with a long flagella, 10-20 µm in length and resides in the gut of the sandfly vector. Amastigotes - aflagellar, ovoid-shaped, contains no flagella, 1-2 µm in diameter and resides in macrophages of mammalian host (Fig. 2). *Leishmania* has a digenetic life cycle. After inoculation by sand fly bite, promastigotes enter host cells and change their shape, shed the flagella and transform into amastigotes. Amastigotes then replicate in the macrophages and are presumably released into the blood stream by macrophage lysis. They must then rapidly gain entry into the adjacent host cells. This way the disease progresses. When the sand fly takes a blood meal of the infected host, amastigotes are taken up and transformed into flagellated promastigotes in the sand fly gut. When this fly bites fresh individual, it injects the promastigotes. This way the disease propagates (Fig. 3).

In earlier days many people used to get killed by this fatal disease. In early part of 20th century, one Bengali medical practitioner's name got spread throughout the world. This famous doctor's name is Upendra Nath Brahmachari. He discovered urea stibamine, the first drug against Kala-azar which saved the lives of millions of patients at that time. Although many modifications have been made for the drug, but till now the major component of the drug is the heavy metal antimony.

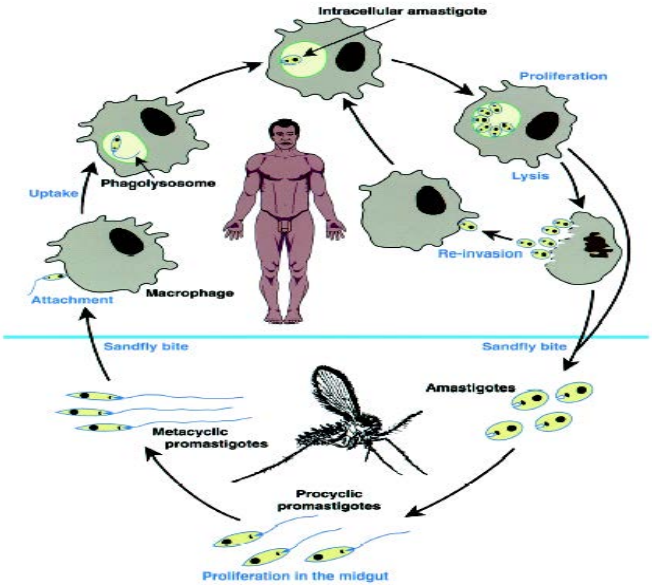


Fig. 3. Life cycle of *Leishmania* parasites

By extensive research it is now known that macrophages express cell surface receptors that specifically bind and internalize mannose-terminated glycoproteins. We tried to exploit the presence of mannose receptors on macrophage surface for designing a drug delivery approach for macrophage-associated diseases like Kala-azar (Fig. 4). We took a simple protein, human serum albumin and chemically conjugated mannose to make the neoglycoprotein mannosyl human serum albumin. We then observed that if we inject this neoglycoprotein into blood, more than 90% reach liver and spleen within 5 min of injection. Further experimentation showed that this 90% reaches only macrophages of liver and spleen. This result is not obtained if we inject only albumin. What did we do after getting this promising result? We then conjugated a drug doxorubicin onto the neoglycoproten. Doxorubicin is an anti-cancer drug which kills all types of cells non-specifically. Our main aim of this work is not to discover drug but to identify the proper carrier of drug. The main theme of drug delivery is that drug should be toxic for all the cells but it should be allowed to reach only the designated target. That is why doxorubicin is our drug of choice.

Let us discuss our results. First of all we tasted whether doxorubicin has got anti-leishmanial activity or not. We found that yes it is very active and 1 microgram of doxorubicin can kill 2 million parasites. We did lot of experimentation on carrier-bound drug, but I will discuss only the salient points. We divided our experiments in two parts. One is to check the ant-parasite activity in *Leishmania*-infected macrophage culture and the other is to check the activity in *Leishmania*-infected animals (Fig. 5). It was found that in comparison to free drug the activity of neoglycoprotein-conjugated drug was almost few hundred times higher in both the macrophage culture and animal model. The most striking part of this entire study with a model macrophage disease is that, since the delivery system targets macrophages, it could be efficiently used against any macrophage disease like tuberculosis, leprosy, AIDS etc.

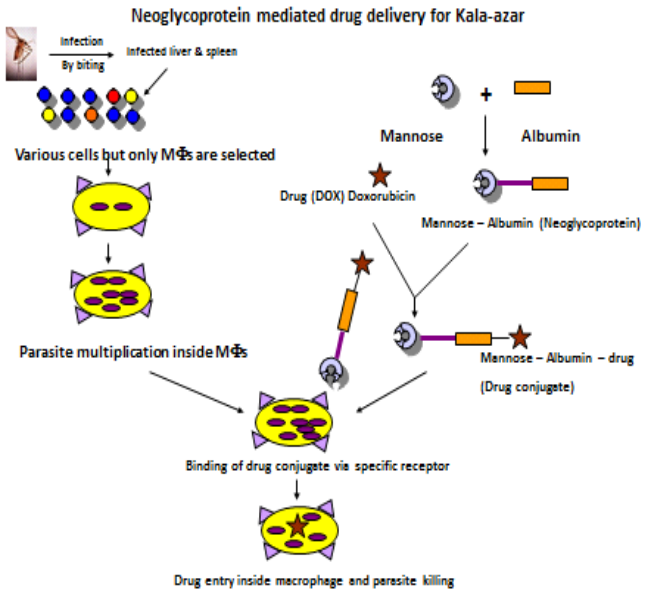


Fig. 4. Schematic representation of neoglycoprotein mediated doxorubicin delivery for killing of *Leishmania* parasites

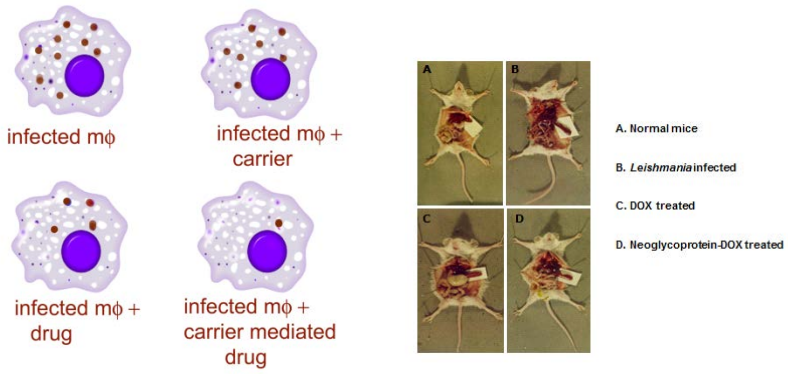


Fig. 5. Parasite killing activity of neoglycoprotein-conjugated doxorubicin in infected macrophages and BALB/c mice.

But to bring out a drug or a drug delivery system commercially, clinical trials are an essential part. Drugs under development must undergo extensive studies in animals and humans to establish safety and efficacy at a particular dose prior to marketing. Clinical trials verify the pharmacodynamics and pharmakokinetics of the drug.

Clinical trials in a nutshell include various steps (Fig. 6). An approved protocol is chosen and a process is designed and approved. Patients are recruited for participation and after applying the approved process, all data are entered and statistically analyzed. Such data are reported and if found suitable for human use, registration is obtained. The entire clinical trial has several phases - (I), (II), (III) and (IV) and ultimate marketing of a drug needs passing of all the phases efficiently (Fig. 7).

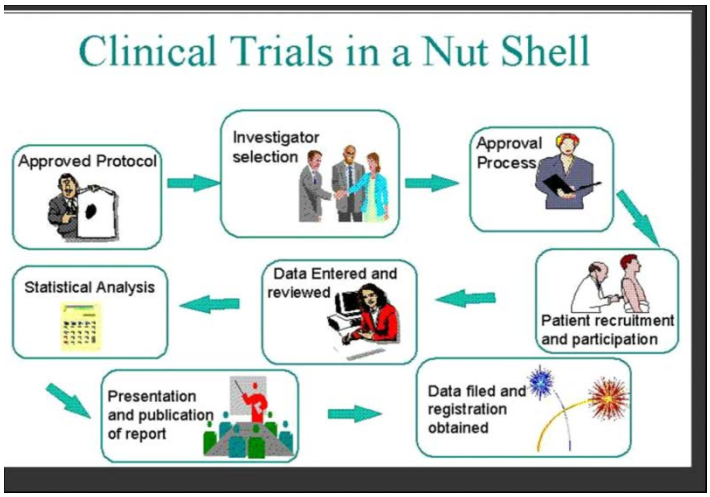


Fig. 6. Various steps of clinical trial of a drug

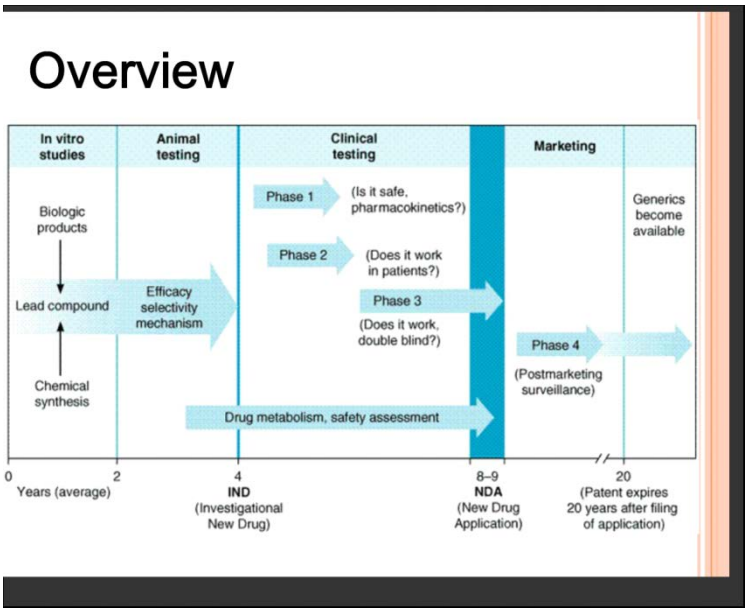


Fig. 7. Overview and total time span of a drug development

Finally, by discovering appropriate drug or efficient drug delivery systems we aspire for disease-free world.

Evolution and Human Health

S. K. Saidapur
Former Vice Chancellor & Diamond Jubilee Professor for Life
Karnatak University, Dharwad-580003
Email: saidapur@gmail.com

Evolution is a core theme of biology. It affects all organisms, microbes to man. Natural selection favours traits (genes) that are useful in the survival and or reproduction of organisms in their current environment. Individuals that do not possess useful traits do not survive up to the age of reproduction to leave behind progeny. Hence, such individuals and their genes get eliminated in the population. Ideas from evolutionary biology have contributed to a greater understanding of human health and medicine giving rise to a new approach called 'Darwinian Medicine' or 'Evolutionary Medicine'. The present talk provides an insight into how evolution affects the human health.

According to evolutionary biology, human diseases arise due to any of the following:

1. Selection of harmful genes

2. Defence strategies

3. Resistance to drugs & evolution of Virulence

4. Living in novel environment

5. Design compromises

6. Diseases of civilization (Modern life style related)

1. Selection of Harmful Genes: Obviously, selection of harmful or bad genes will produce disease in an individual. For instance, sickle gene, is responsible for making RBCs sickle shaped and weak oxygen carriers. Presence of sickle cell genes in homozygous condition is lethal but in heterozygous condition, the person suffers from sickle cell anaemia. Such people do survive and reproduce spreading the sickle cell gene in the population. Interestingly, persons with this disease are resistant to malaria and, the disease is prevalent in regions where death due to malaria is common. Another example of a rare genetic disease is Progeria. Persons suffering from Progeria die before reaching the age of reproduction; hence are unable to pass on the gene into the population. On the other hand, Huntington's disease sets in middle ages and therefore genes of this disease get into the population following reproduction. Therefore, let us ask why natural selection does not get rid of such bad genes and retain only good genes during the course of evolution? The above three examples suggest the following:

Lecture delivered on November 15,2013 at Sikkim University

i) A heterozygous advantage can lead to selection of a harmful gene (e.g.sickle cell genes offering resistance against malaria)

ii) Genes that are harmful in early part of the life are eliminated (e.g. Progeria genes)

iii) Genes that are not harmful in early part of the life are favoured even if they are harmful in later life (e.g. Huntington's genes).

2. Defence Strategies: In the course of evolution, hominids have developed many defence strategies, which are often mistaken for disease. For instance, defences such as coughing, sneezing, vomiting, morning sickness diarrhoea, dysentery (all expulsion-based defences), fever and iron withholding during infection etc. Evolutionary biology offers explanation to these strategies and suggests that these are indeed defence mechanisms. The expulsion-based strategies are meant to get rid of toxic substances. Fever is an adaptation to suppress infection. Likewise, iron withholding is a strategy to prevent microbial growth. If so, excessive suppression of these defences may actually harm rather than help the patients. Administration of iron supplements during infections can actually help multiplication of the germs and thereby harm the patient!

3. Resistance to drugs & evolution of virulence: Evolution of resistance to antibiotics in the microbes and, pesticide resistance in pest insects is a serious problem. This necessitates production of new drugs every now and then. In fact, antibiotic resistance is a biggest threat to man today. Ideas from evolutionary biology provide clues regarding the evolution of virulence. Let us not forget that microbes and insects also have right to live and succeed in their struggle for existence. Obviously, a few individuals who possess the ability/mechanisms to overcome the toxic effects of drugs manage to survive and reproduce and pass on the useful traits/genes to their progeny. The drugs are ineffective against the new progeny, which is capable of overcoming the effects of the drug (antibiotic or pesticide as the case may be). Repeated challenges lead to drug resistance in target organisms. In addition, virulence evolves with repeated challenges with drugs, toxic substances, and polluted water bodies. Providing opportunities for evolution of virulence is dangerous. We cannot win battle over microbes, the sophisticated opponents. What is the option then? We need to mange pathogens. Fortunately, virulence is reversible. Good sanitary conditions and avoidance of repeated use of antibiotics can lower virulence. Let the pathogens cause us minor discomfort but not floor us. It is in our interest that we let the pathogens and pests remain less virulent. This calls for management of pathogens based on the knowledge of how virulence evolves.

4. Living in novel environment: The life of any organism is closely linked with the environment in which it lives. Man is no exception. The prehistoric man was necessarily a hunter and gatherer living in the wild. From 20,000 B.C. to modern times threat to man was mainly from accidents, starvation, predation and, infectious diseases. In technologically advanced countries, common threats to man are obesity, atherosclerosis, heart attack, cancer, chronic diseases associated with lifestyle and longevity, non-insulin dependent diabetes and, new infectious diseases. Possibly a mismatch between design and the environment has led to many new diseases. Evolution needs time to revise our body design to cope up with fatty diet, automobiles, drugs, and artificial lights, the major ingredients of novel environment.

5. Design compromises: In the course of evolution, man became bipedal from the quadruped

ancestors. This resulted in an erect posture. It also gave man hands that helped him carry babies, food and so on. The erect posture is prone to many orthopaedic problems and varicose veins. Other examples of defects include human eye, crossing of air and food passages, and a narrow birth canal and so on. Briefly speaking, the human eye is inferior to that of the squid eye. The optic nerve pierces through the retina making it vulnerable to infections, glaucoma and blind spot. The crossing of food and air passages with each other is a serious problem. Each time food passes from the mouth to stomach it has a chance of going into the trachea and lungs. Thanks to the elegant closure and opening of the glottis sitting over the larynx, death due to choking is a rare phenomenon. The use of larynx for speech in humans is an additional problem because it increases the chances of food particles entering the respiratory tract. Women often require assistance during delivery because the birth canal is so narrow that skull of the baby finds it difficult to pass. Therefore, human babies are born with a small skull and poorly developed brain. Thus, evolutionary legacies of have left numerous design defects in human bodies that cause diseases at some point of time in life. Evolution is neither perfect nor progressive. The above examples are only representative. There exist many more!

6. Diseases of civilization (Modern life style related): The modern life style has resulted in many diseases: orthopaedic, ophthalmic, geriatric and stress - induced diabetes etc. These are indeed diseases of civilization.

Thus, Darwinian medicine attempts to explain origin of many human diseases. It also attempts to explain the significance of various kinds of emotions often considered as psychological problems. In short, evolution and human health are closely related. We need to relook at the human diseases from an evolutionary perspective. Only then, we can understand the origin of diseases better and evolve appropriate measures to alleviate human sufferings.

Suggested Readings:

1. Evolution and Healing by R. M. Nesse and G. C. Williams. 1995, Phoenix publishers.

2. Why We Get Sick? By R. M. Nesse and G. C. Williams. 1995, Vintage Books, New York, USA.

3. Survival of the Sickest by S. Moalem and J. Prince. 2007, Harper Collins publishers.