REVIEW OF LITERATURE
REVIEW OF AYURVEDIC CONCEPTS

Virtually every country that has a system of traditional medicine, presents a unique configuration designed to be compatible with its own culture and meeting the needs of its own population. (Dahanukar, 1989 : 1-60)

Traditional medicine has been defined by the W.H.O. sponsored meeting at Brazzaville in 1976 as, "The sum of total of all knowledge of practices whether explicable or not used in diagnosis, prevention and elimination of physical, mental or social imbalance and relying exclusively on practical experience and observations handed down from generation to generation verbally or in writing".

Ayurveda, Unani and Siddha all three systems are fundamentally similar in their approach to health and disease. It is therefore not surprising that Ayurvedic thoughts and methods have had a deep impact on the lifestyle of the people of India. The Ayurvedic formulary is rich and diverse and holds a very sound position. This is because it has grown and matured in the soil of India.

Ayurveda as the name implies (Ayu-life, Veda-knowledge) is the knowledge of healthy living and is not confined only to the treatment of illness.

The exact origins of Ayurveda is controversial, it is around 6000 B.C. However the period of ancient Indian Hindu medicine may be divided into three different periods: the prevedic, vedic and Arsha.
PREVEDIC PERIOD: This is the era before the Vedas. The Hindu system of medicine is said to have originated from Lord Brahma, the fountainhead of all learning.

Putting aside all claims of a divine origin of science, one can say that the Pre-vedic period is the long period of development, during which the pre-historic man built the basis of this system of keeping healthy with the help of his accumulated experience. This experience was gained by the primitive man in course of the prolonged struggle for existence, in the face of adverse conditions of nature.

VEDIC PERIOD: The term "vedic period" applies to that period of Indian civilization during which the four Vedas—The Rigveda, Samaveda, Yajurveda and Atharvaveda, the holy writings consisting of religious hymns and dogmatic principles were formulated. Out of these four Vedas, the fourth veda i.e. the Atharvaveda is the first authentic record now available of the state of medical knowledge during the vedic period. The science of Ayurveda is an supplement of the Atharvaveda. The eight branches of Ayurveda are mentioned in Atharvaveda. The growth and development of Ayurveda occurred mainly during Arsha period.

ARSHA PERIOD: This is the period of Rishis. This period extended over several centuries and is characterized by the appearance of many systematized treatises, Bharadwaja and Dhanvantri received the knowledge of life, in this period.
Ayurveda suffered a setback when modern medicine evolved as a method of subjecting all assumptions to experimental verification and statistical validation.

Earlier to the entry of Modern medicine in India, Ayurvedic Medicine had already become a stagnant system for want of experimentation and records of observations on patients. This was partly due to the repeated waves of foreign invasions which displaced the Vedic and Aryan cultures rapidly after the 7th or 8th century A.D.

The ayurvedic Samhitas (texts) are divided into sections, each section dealing with different aspects of Ayurveda, encompassing instructions for preservation of health, cure of disease and maintenance of life in a state of happiness. e.g. The Charaksamhita is divided into eight sections, each section called as Sthana.

1. **Sutrasthana**: deals with the origin, general principles and philosophy of Ayurveda.
2. **Sharirasthana**: deals with anatomy and embryology.
3. **Indriyasthana**: deals with prognosis.
4. **Vimanasthana**: discusses factors affecting drug administration.
5. **Nidanasthana**: deals with cause of diseases.
6. **Chikitsasthana**: deals with diagnosis and treatment of diseases.
7. **Kalpasthana**: discusses pharmacy.
8. **Siddhisthana**: deals with the cure of disease.
Another fact which deserves mention is, the approach to
discription of the disease entities. Diseases have been described
under the headings of Vyakhya (definition), Vyutpatti
(Etymology), Nidana (Etiology), Poorva Rupa (Prodrome), Roopa
(Clinical pictures), Samprapti (Pathophysiology), Sadhyasadhaya
(Prognosis), Chikitsa (Principles of management), Aushadha
(Drugs), Ahara (Diet), Vihara (Practices, lifestyle). This is not
in any way different from the approach that is followed in
contemporary medicine.

Ayurveda expanded through the ages and incorporated
different materials in the therapeutic armamentarium as knowledge
in satellite fields expanded.

The last decade has seen a dramatic resurgence of interest
in alternative systems of medicine, particularly Ayurveda, not
only in India but all over the world.

The revival of interest in the traditional preparations
among the developed countries is based on diverse reasons and has
many facets. For the patient and the physician dis-satisfaction
with modern medicine is a factor of prime importance.

The advent of different types of synthetic drugs, like
corticosteriods, NSAIDS, and antibiotics etc, of modern era has
added a number of useful weapons to the therapeutic
armamentarium. The patient and physicians have realized however,
that they are powerful yet potentially toxic weapons. The high
incidence of side-effects associated with the use of these drugs
invariably results in attempts to correct the side-effects by
prescribing a few more drugs adding further to the risks and cost.

The reductionist approach of modern medicine of treating the symptoms or treating a "finding" revealed by "high-tech-high cost" investigations as against the holistic approach of traditional medicine has lured the patient towards the safety and therefore such alternative modalities of the treatment of the latter.

The renewed interest in traditional medicine in many countries stems also from a desire to be self sufficient in health care or to minimize imports of items which can only be brought with valuable foreign exchange earnings.

The allopathic physician of India is aware of the limitations of modern medicines and is simultaneously conscious of the strength of traditional medicines in certain areas. He may not turn to Ayurveda for treatment of acute infections, but gets tempted to try out therapies for chronic recurrent diseases, metabolic disorders or degenerative diseases.

Research efforts in Ayurveda have increased many fold during the last couple of decades, intention is to discover new drugs from plants and other crude materials.
Presently some of the drugs used from plants in modern system of medicine are

**PLANT DERIVED DRUGS**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Plant source</th>
</tr>
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<tbody>
<tr>
<td>1. Codeine</td>
<td>Papaver somniferum</td>
</tr>
<tr>
<td>2. Atropine</td>
<td>Hyoscyamus muticus</td>
</tr>
<tr>
<td>* 3. Ephedrine</td>
<td>Ephedra spp.</td>
</tr>
<tr>
<td>4. Hyoscyamine</td>
<td>Hyoscyamus muticus</td>
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<tr>
<td>5. Digoxin</td>
<td>Digitalis lanata</td>
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<tr>
<td>6. Hyoscine</td>
<td>Datura metel</td>
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<tr>
<td>7. Digitoxine</td>
<td>Digitalis purpurea</td>
</tr>
<tr>
<td>8. Pilocarpine</td>
<td>Piloporus jaborandi</td>
</tr>
<tr>
<td>9. Quinine</td>
<td>Cinchona spp.</td>
</tr>
<tr>
<td>10. Quinidine</td>
<td>Cinchona spp.</td>
</tr>
<tr>
<td>* 11. Emetine</td>
<td>Cephalis spp.</td>
</tr>
<tr>
<td>* 12. Caffeine</td>
<td>Thea sinensis</td>
</tr>
<tr>
<td>13. Theobromine</td>
<td>Theobroma cacao</td>
</tr>
<tr>
<td>14. Theophylline</td>
<td>Coffea arabica</td>
</tr>
<tr>
<td>* 15. Papaverine</td>
<td>Papavera spp.</td>
</tr>
<tr>
<td>16. Colchicine</td>
<td>Colchinum actumnale</td>
</tr>
<tr>
<td>17. Morphine</td>
<td>Papaver Somniferum</td>
</tr>
<tr>
<td>18. Resrepine</td>
<td>Rauwolfia Serpentina</td>
</tr>
</tbody>
</table>

* = Also synthesized.

In view of this fact, it was natural for researchers to concentrate on the potential of this source for future drug development.
The Chinese have been successful in isolating gossypol from Gossypium and introducing it as a male contraceptive in China. (Stevens, G. De 1978) They have also introduced Quinghaosu a compound from Artemisia annua, especially useful against chloroquin resistant malaria. (Monahan, J. J. 1982)

In India too, phytochemists are exploring the possibility of identifying novel structures from plants, while pharmacologists are looking for novel mechanisms of action of plant products. Guggulipid and Coleonol (Satyavati G. V. 1988, Dahanukar S. A., Tatte U. M.) are two important examples which are results of the efforts of Indian scientists at the Central Drug Research Institute in this respect.

Information sources

As a result of greater interest in the use of medicinal plants, research efforts and the resultant literature in this field have increased manifold during the last two decades. In this context, the importance of services for systematic collection, processing, storage and dissemination of information on medicinal plants cannot be overstated. There are some 500 significant periodicals published in the world covering Medicinal plants. The bulk of these are devoted to disciplines such as Phytochemistry, Pharmacology, Medicine and Botany.

Basic principles of Ayurveda

Ayurveda the science of life elucidated over the last 6000 to 8000 years ago has described the practice of medicine in great detail. Several basic principles have been laid down, which
guide the Vaidya or Ayurvedic physician to arrive at a diagnosis before deciding the mode of therapy. The aim of Ayurveda is to improve the quality and span of life. However, the approach of different founders of Ayurveda to various problems is different.

Ayurveda is essentially composed of several hypotheses based upon astute observation, speculative deductions, inferences and generalizations. As against this, virtually all modern concepts have been based on observations that have been proved only after rigorous scientific experimentation. These methods of research and verification have overshadowed the concepts laid down in Ayurveda.

Ayurveda is a holistic science and lays emphasis on preserving and promoting the fitness of healthy individuals besides giving methods for treatment of diseases. Health is defined in Ayurveda as "soundness of body, organs and mind".

Thus sharira (The body), manas (The mind), and atma (The Soul), "the tripod of life" receive equal attention for achievement of sound health. The objective of preserving and promoting health in Ayurveda is achieved through different modalities based on principles within its own conceptual framework. Ayurveda is not a science dealing only with drugs. It is more a "way of life" and describes methods for the promotion, prolongation and maintenance of positive health. It emphasizes the importance of a specific daily routine, "dinacharya", and seasonal regimen "ritucharya" along with diet, drugs, physical exercise and good personal hygiene.
The conceptual framework of Ayurveda is based on certain basic doctrines: the Darshana. These visualize the fundamental functional units of the body to be formed by three doshas (humours) seven dhatu (tissues) and mala (metabolic end products) which are in equilibrium during health.

Ancient Ayurvedic physicians describe disease as a disequilibrium of functional units. The objective of any therapeutic measure is therefore primarily to re-achieve a state of equilibrium.

Dosha: The dhatu or cell is perceived to be under the influence of the three doshas the pitta, kapha and vata. The doshas are composed of different basic elements, Panchamahabutha. The pitta dosha is composed of apa (water) and teja (energy).

Kapha dosha is derived from apa (water) and Prithivi (earth) while vata dosha has its origin from vayu (air) and akasha (space).

The ancient Ayurvedic texts have characterized the types, sites, sources and qualities of each of the doshas. Attempt has been made in the past to interpret and verify the concepts of tridoshas by different scholars. (Athavale V.B. 1980, Dahanukar, 1987 : 102-109, Shiv Sharma, 1929 : 162-193)

Though there is no conclusive direct proof for the theories, every one is in agreement that the doshas control and affect cellular functions by altering the milieu interior in a very subtle but intricate manner. Thus these three doshas- vata,
Pitta and kapha control all functions of the body, viz., motion, energy, and inertia. They are produced and regulated endogenously. The vata dosha controls the utilization of energy by cells as well as other two doshas, while the pitta dosha gives energy and is responsible for cellular, enzymatic, and metabolic functions. Kapha dosha helps in synthesis and preservation of cellular components.

The doshas are subject to qualitative and quantitative change due to the influence of factors within or outside the body. These doshas in turn influence the function of the dhatu.

Dhatus are of seven different types. They are:

1. Rasa : body fluids
2. Rakta : blood
3. Mamsa : muscular tissue
4. Meda : adipose tissue
5. Asthi : bone
6. Majja : nervous tissue/bone marrow
7. Shukra : generative tissue

All the dhatus receive nutrients from the rasa dhatu and specific nutrients are picked up by the respective dhatu depending on their requirement.

During the process of assimilation of these nutrients constitutional changes occur inside the tissues and three sets of products are formed. One of these sets contributes to the growth of that dhatu itself. The second contributes to the formation and growth of the tissue and third in the formation of the dhatu 'mala' that is metabolic end products.
Hence, the quality of the previous dhatu influences the health of the next dhatu.

Shukra is the only dhatu which does not produce 'mala' if it is in healthy condition.

At all levels tissue enzymes are important functional units.

Thirteen different kinds of Agni which exist in the sapta dhatu and their sukshmavayava (cell) have been described in Ayurvedic texts 'Mala' of different dhatus produced after metabolism have their own characteristics.

Mala: conventionally interpreted as excretory products. These are products of cellular metabolism, are not always harmful and often play a protective role. There are two types of 'Mala's' in Ayurveda. Prasadakhya (protective) or malakhya (harmful). Therefore mala is more appropriately interpreted as metabolic end products rather than excretory products.

In health, a sort of organic equilibrium exists between cellular elements of the different tissues, their secretions and their metabolic turnover. The alterations which affect certain elementary cells in the body can affect other cells correspondingly, the constitution and function of one or more structures. The intrinsic interchange between the cellular groups is the crux of the doctrine "body is nothing but dosha, dhatu and mala".

Srota: Srota are considered to be the internal transport system of the cells. Ayurveda describes Srota as "Channels" of
transport which is literal translation of Sanskrit word. This literal translation does not do justice to the meaning of srota.

Srota by Charak and Sushruta, are similar to the dhatu to which they transport materials either sthula or anu i.e. gross or microscopic respectively. Dirgha or prathana i.e. reticulated.

No structure in the body can grow and develop independent of srota. When the integrity of the srota is impaired, the dhatus are impaired and vice-versa: Morbidity spreads from one dhatu to the other and one srota to the other, because of their interdependence.

The srota are innumerable and diverse, srota spread throughout the body and nourish the different dhatus.

Dosha - humours: The concept of Humours has existed in western medicine since the time of Galen. Empedocles of Agrigentum in Sicily regarded the Universe to be composed of four elements-fire, earth, air and water. Aristotle followed Hippocrates and others in believing that the human body possessed four fundamental qualities: hot and cold, dry and moist, and that it was composed of four humours - blood, phlegm, yellow bile and black bile. Disturbance of the relative predominance of humours caused disease. Later in medieval times the humours were change to "temperament". sanguine, phlegmatic, melancholy and choleric.

Diseases occurred because of unnatural predominance and interaction of these elements and treatment was directed at altering this abnormality.
The concept of humour has slowly disappeared from western medicine after the description of the body processes and structure in terms of anatomy, physiology, and biochemistry with the help of modern physics, chemistry and biology from 17th century onwards.

**Dhatus - cells:** dhatu is the smallest functional unit in the body. Ayurveda describes seven dhatus. Modern medicine, has understood that the functional unit of the body is the cell. Many cells together forms tissue, organs and finally the whole organism. It is amazing that the seven most important tissues in the body were described in Ayurveda for so many years ago.

Ayurveda describes that the rasa dhatu carries nutrients to the different tissues for processing. In fact rasa dhatu is essential for normal growth of other tissues particularly for that of blood. Today, with the advent of cell culture techniques, we know that serum is highly beneficial, if not essential for most cells to multiply and propagate in vitro. Platelet derived growth factor (PDGF) is a growth factor isolated from serum. PDGF is secreted by platelet and other cells, such as monocytes, macrophages, endothelial cells, arterial smooth muscles, embryonic cells. Normal amount of PDGF is required for healthy functioning, while an abnormal amount is implicated in diseases like atherosclerosis and cancer.

This is similar to the Ayurvedic concepts of disease occurring due to excess or imbalance of normally occurring
endogenous substances. An interdependence of different dhatus exemplified by the knowledge obtained due to the availability of clonal assay systems and sophisticated cell separation technology. In terms of ayurvedic hierarchy of dhatu, it can be envisaged as,

Rasa dhatu Secretory majja dhatu mature to form (serum) \(\rightarrow\) (bone marrow) \(\rightarrow\) Rakta dhatu product

(Cellular elements of blood)

**Metabolic end products**

The evidence that many cells produce metabolic end products which are useful if not essential, for their normal functioning, strengthens the belief in the concept of "Mala" as described in Ayurveda.

The knowledge about oxygen derived free radicals best exemplifies this concept. Free radicals are chemical species with one or more unpaired electrons in their outer orbit. Their production is essential for normal metabolism, but they are destructive unless controlled. Free radicals are produced by leucocytes during the respiratory burst to kill micro organisms a necessary defence mechanism. However, if this occurs excessively, it can cause vast amounts of tissue damage as in inflammatory diseases like rheumatoid synovitis and gout.

Another extremely intricate example which amply illustrates the complex phenomenon of rasa - rakta dhatu interaction and formation of dosha and mala is the coagulation and fibrinolytic system in blood.
Much simpler examples from basic physiology or enzymology can be quoted as parallel to the 'mala' metabolic concept in Ayurveda e.g.

Cell Respiration $\rightarrow$ CO₂ production

CO₂ is stimulant of respiration and excess of CO₂ can also act as toxic substance.

Energy + substrate $\leftrightarrow$ Es $\rightarrow$ Product + Enzyme

Excessive accumulation of product can lead to enzyme activity.

Srota: Receptors

The srotas, when translated as channels are restricted to blood vessels and lymphatics which carry nutrients to different tissues. However this only explains anatomic specificity. If one interprets srota as "receptor channel" mechanisms, the area of molecular biology opens up exciting possibilities to explain how a srota can be specific for a tissue, how it can be modified by physiological and pathological processes and how it can be influenced by other tissues.

There is a very interesting concept in Ayurveda, where it is described that a lack of exercise, excessive sleep during daytime and over-eating of fatty substances causes disturbances of the medavahi srota. The derangement of medovaha srota leads to accumulation of meda dhatu (adipose tissue), disturbs nutrition of other tissues and causes srotadushti (obstruction of srota) which finally results in altered metabolic states.

On the basis of such a concept a srota can also be interpreted as a metabolic or biochemical pathway. As the
visible end product of such a pathway was the particular type of tissue (dhatu) the srota was probably named after that tissue e.g. medarhi srota. This is in keeping with the practice of naming a micro-constituent after a make (or visible to the naked eye) constituent or product of the body e.g. pitta, vata, meda, etc.

In a nutshell, the dhatu along with its srota is an important unit. Its integrity and functioning is dependent on its primary qualities and on dosha. The quality of dhatu and dosha are subjected to qualitative and quantitative changes under the influence of factors within or outside the body. (Dahanukar S.A. and Tatte U.M.)

Nature And Ayurvedic Medicine
(Roberte Svoboda, 1992 : 1-100)

In Ayurveda, we have a medical system which still trusts the human sense organs. Ayur means "life" and veda means "knowledge". The knowledge of how to live would be a ready translation. However for the benefit of people living in our hi-tech civilization we should expand this to "the knowledge of how to live naturally".

Ayurveda is a knowledge of natural harmony and a method of removing disharmony. Ayurveda is a form of treatment by natural remedies, which makes use of the power of nature to restore living beings to a state of balance. The human body is one such living being.
The heat of sun, light, air, water and minerals, vegetable and animal substances, are employed in therapy and as remedies. In addition, Ayurveda has something to say on health education and health presentation. Ayurveda helps us to recognize the correct way to live at a given moment in order to overcome special problems, it helps us, in fact, to analyse our habits and our environment and to see where we are going wrong.

Ayurveda, is a holistic medical system and it has no room for that split between spiritual and the physical that is part and parcel of our Western archetype. At the heart of Ayurvedic thinking, is the insight that the universe as macrocosm and man as microcosm are in direct relationship; that they reflect one another, and that the one is always present in the other.

The direct link between man and his surroundings is experienced throughout our lives by the use of our senses, which are the instruments of communications.

Ayurveda sees an immediate connection between the use of senses and the origin of disease. Every wrong use or no use of senses leads to a disharmony in man, and to disharmonies between man and nature. The sameness between nature (prakriti) and the self i.e. purusha is the foundation upon which all the principles of Ayurveda are built.

The balance of well being, and the good health of an individual are dependent on the equilibrium of the three forces that control all bodily and mental activities. These three forces or principles, the Tridoshas, are a reflection of cosmic forces in microcosmic man.
The classical Ayurveda text describe them in an analogy expressing the original human experience of the forces acting on life. Man is standing on the earth. And there is the Sun, there is the Moon, and there is the Wind.

The Sun radiates heat and bestows the energy necessary for all physio-chemical and biochemical processes. Its representative in man is the force Pitta, which controls, all the biochemical processes in the body, all reactions resulting in heat e.g. digestion of food and cellular metabolism. Pitta warms colours red, and produces the glow of energy in us.

The moon stands in a relationship of tension to the earth, it acts on biological rhythms, rules the tides, and has an affinity with the element water, which is cooling and gives the cells of the body form and firmness. Its representative in man is the force Kapha which has a visible effect in altering the equilibrium of fluids in the tissues and organs. It also lubricates the joints. Kapha gives the cells and with them the whole the body a form and firmness. It has a cooling effect.

The wind not only moves other objects it is the movement itself of the atmosphere. It blows the clouds along, dries wet places and sets fires blazing. The motive force in man is the principle Vata, the principle of movement. It is the complex nervous system with its impulses. Vata is the will to live.

A drought, a forest fire, a flood or a hurricane bring about a transitory disturbance in the harmony of nature. An increase
or decrease in Pitta or some disturbance in one of the other principles will upset the harmony of these three Doshas in a living being. Their unstable equilibrium has then to be resolved.

The Tridoshas are at work in all living creatures. All matters, both animate and inanimate, is taken to be a composite whole made out of five basic elements, the Panchabhutas. This essential frame of reference in Indian thought permits a wide variety of relationship between man and his surroundings. It serves to make the relationship, between man and plant understandable, since both have been made from the same building blocks of existence and have the same forces working in them i.e. the same Tridosha.

The relationship was self evident and important to all the early civilizations, it even seemed to be important for their survival.

The essential frame of reference, built up on the basic of Indian philosophy at that time, has not been revised. The modern acquisition of knowledge about the illiterate nature of matters and energy has changed many concepts in Western Medicine. A similar change has not yet occurred in Ayurvedic thinking.

Medicines could be found in the wild, free and without a prescription, and indications for the given remedies rested on the experience of many generations. Knowledge consisted of the transmission of this experience. It seems that Ayurveda is the oldest medical system that is still in use at the present time.
In our highly industrialized and technological society, the pharmaceutical industry has disrupted the ancient relationship between man and plants. However, people are not beginning to take a greater interest in herbs and the so-called grandmothers' remedies are coming into their own use again. There is a gradual recognition that it is more in keeping with causal therapy, i.e., with the treatment of causes to clear up a viral infection such as influence by perspiring in bed and drinking lime-flower tea than fighting it with antibiotics, or to cure constipation with a change of diet rather than palliating it with laxatives. In the logic of our industrialized world, health is a product, and illness is a reduction in productivity.

Charaka, a classical Ayurvedic writer, defines disease and health as "A disharmony in the constituents which support the body, is known as disease; their harmony is called health, the state of normality. A sense of well-being is characteristic of the absence of disease, for disease is always associated with discomfort."

According to this, Ayurvedic therapy aims not only healing but also at harmonization; it combats not the illness but the lack of equilibrium. 'Normality' it is taken to be the balance of all the constituents of man in harmony with his surroundings. According to a more recent definition, an individual can be called healthy "when his metabolism is in equilibrium, he feels mentally well and his sense organs and motor organs are functioning normally. On the other hand, an individual is unhealthy 'when he experiences physical or mental discomfort.'
The term Dukha samyoga 'contact with unpleasant things' means both physical pain and suffering and exposure to such emotional disorders as jealousy, anger, fear, avarice, envy, hate, over excitement, and sorrow. 'All these things are afflictions of the mind and body.' Nowadays, we have to add the Dukha samyoga list such factors as physical and chemical pollution of environment.

The symbol of Ayurveda is the lotus blossom, the eight petals of which represent the eight Ayurvedic disciplines.

**AYURVEDIC DISCIPLINES.**

1. **Kaya chikitsa**
   Internal medicine

2. **Shalya tantra**
   Surgery

3. **Salakya tantra**
   Treatment of the ears, nose, throat, eyes, jaws and teeth

4. **Agada tantra**
   Toxicology, the study of poisons

5. **Bhuta vidya**
   Psychiatry, roughly equivalent to the treatment of mental diseases by mantras and tantras to please the evil spirit.

6. **Bala tantra**
   Gynecology and pediatrics

7. **Rasayana tantra**
   Geriatrics, the study of diseases of old age

8. **Vajikarana tantra**
   Sexology

Ayurveda, for most part, employs fresh or dried plants and their natural extracts. This is clearly different from modern medicine which, although it still uses plants, relies mainly on
the active principles isolated from them and standardized preparations. Homeopathy employs highly attenuated potentized plant tinctures.

Behind the development of every specialized branch of applied science, whether it be Ayurveda, Allopathy or Homeopathy, there is a whole philosophical thought structure with an individual view of humanity and of what goes to make up human life. The core of Ayurveda is the relationship between man and plant, in other words, 'green power'.

The stored experience of the Ayurved races the distillation of historical, religious, philosophical and medical knowledge, is found in four classical vedas. Rigveda, Yajurveda, Samaveda, and Atharvaveda, the origins, and date of which remain uncertain because they were transmitted orally for an unknown period of time before they were written down. The four thousand five years old Rigveda, and the younger three thousand two hundred years old Atharva veda are important in medical history.

The Rigveda, a collection of one thousand and twenty eight hymns gives amazing informations of the state of medical knowledge in the ancient world, and mentions operations, the use of prosthesis and sixty seven medical plants. The Atharva veda documents a further development of the healing art, although it rested earlier on magico-religious basis it offered a whole range of therapies for the most varied diseases, with the help of an impressive armament of herbal remedies.
Immediate perception through the senses

The five building blocks of existence are the point of conflict between philosophy and Ayurveda - the practical application of knowledge. In tracing the origins of the primary elements, philosophy is supreme, but in their development the supremacy belongs to science.

Earth, water, fire, air and ether in spectrum of all their shades of meaning they are quite simply the universe. Each of our five senses is understood to be the main organ of perception for a specific basic element.

The element air is perceived by the sense of touch. We feel the pressure of the air flow on our skin, it is also true that we can perceive any other element form by means of sense of touch.

The classical writer of Ayurveda Charaka, offers this discipline of building blocks of existence.

The main characteristic of earth is roughness, that of water fluidity, fire heat, air expansion and a complete absence of resistance.

Water is perceived by the sense of taste. Taste is very important in Ayurveda because it is the means to make an accurate estimation of properties of medicinal plants and other substances.

Hearing is the main sense associated with the element ether. Any impression of a given substance is the sum of its specific qualities.
The technical substitutes of our sense see with better and sharper vision than our eyes do. They bear finer, nuances than our ears do and they analyse each substance better than it is possible for our tongues. Therefore it is necessary in modern times to reclassify the basic elements.

All matter is built on the basis of Mahabhutas, but only living matter has the tridoshas, the three forces regulating all biological processes. They arise out of the basic elements and are treated in Ayurvedic literature as substances, although they are not. What they are in dynamic principles, three different forms of energy which govern the whole economy in living organisms. From the simplest activity in a cell to the most complicated the bodily functions the tridosha permits and controls, everything that is going on. They always work as a team and one never appears without the other.

Their harmony or disharmony decides the objective conditions of the living being. A harmonious relationship of the three bio-energetic principles is the mark of good health. Any imbalance of the equilibrium which is very unstable reveals itself in a wide variety of symptoms.

The tridoshas, the inner principle of living body are influenced by external factors by sensory influences and by food-stuffs. Therefore, Ayurvedic therapy can restore the physical balance of the three forces by means of "nourishment" in the widest sense of the word.
The tridoshas have acquired a specific character from the elements that rule them. The elements earth and water rule the formation of kapha, fire rules that of pitta, air and ether that of vata. With a little practice it is not difficult to recognize ways in which the three doshas express themselves. Kapha has the firmness and stability of earth, plus a fluid plasticity, pitta displays the energy of fire and vata possesses the mobility of air and ether. It must again be reminded, that these associations have been developed out of the observation of properties with the help of sense corpous only.

The principle Vata is responsible for all the body's sensations and activities. Perception, assimilation and reaction are all properties of Vata which channels perception through the sense organs, transforms them into psychic events, and produces the appropriate reaction via executive organs. Vata converts everything experienced by the senses into psychosomatic reactions. Vata animates the psyche, regulates the breathing and creates activity. In fact it is initiator and promoter of all biological actions. Even the two other doshas receive their motive energy from vata.

The principle 'pitta' belongs to every reaction in which heat is generated. Its main function is all kinds of transformation of food in the body, all metabolic processes. Pitta has the energy of the element fire, and the nature of fire is to alter substances to 'metamorphosize' combustible materials, to create warmth, and to be vivid and restless. Pitta is known as the impetus of life. It stimulates the intellect and the capacity of enthusiasm and encourages singleness of mind.
The principle 'kapha' is formative. It structures everything from individual cells to skeletal frame, it lends strength and stability and also makes the body supple. Mental and physical strength and endurance, a well knit frame and putting on weight are all results of Kapha. This principle can accelerate the healing process and can build up resistance against disease. Generally speaking, whenever the effects of Vata and Pitta are seen in the body, Kapha keeps these two forces confined within their natural limits.

If we try to understand the above three groups of functions, it becomes evident that they are intimately interlinked. They cannot be separated in terms of modern biochemical processes. The classification thus appears to be outdated. However, it was the only available explanation in ancient times.

Sri Sivatatvaratnakara describes the different qualities of plants in terms of ruling doshas as follows:

If a tree is tall and slender or if on the other hand, it is stunted, if it shows signs of dryness or reduced sensitivity, its blossoms and fruits are poorly formed, then it belongs to the Vata type. A tree that will not tolerate the blazing sun a tree with a light coloured barks and pale leaves bearing premature fruits and arrangement of its branches and belonging to the Pitta type. A fully developed plant having a sturdy trunk and strong branches, bearing plenty of flowers and fruits, and with a spreading top and twining creepers belongs to the Kpha type.
Attempts have been made to find the up to date medical equivalents for the traditional doctrine of the Tridosha. Thus it is postulated that Vata performs most of its functions through the release of acetylcholine, both in the central brain and throughout the body at the ending of the parasympathetic nerves and in the peripheral nerves of the voluntary muscles. The function of pitta are equated with the system which have mainly to do with energy discharge and catabolism. The action of kapha is linked to that of the histamine which influences the fluid balance in the tissues and organs and increase the permeability of the capillaries for fluid exchange.

The Tridoshas working in man determine, according to their relative strengths, the constructional types and temperaments.

They mold the basic character of individuals. People from each of the three psychosomatic character types differ from one another in state of health, in susceptibility to certain diseases, and in their response to medications. They also differ in their emotions, in the way their mind work and in their handling of external conditions.

This is where Ayurvedic diagnosis, which looks at the whole man is able to score, based as it is on the tridoshas and their disturbances.

It is necessary for the Ayurvedic physician to be able to recognize deviations from the norm in the tridosha, relationship and for him to know how to restore the balance by means of healing herbs, diet and mental and physical exercise. He has to find the cause of loss of balance.
Of the three forces, Vata is the most important since it sets the two other doshas in motion. Most of the functions described of Vata in the classical text have something to do with movement, with activity, with breathing, animation and inspiration. Air and Ether (Akasha), the dominant element in vata lend in their characteristics.

The principle known as Pitta is the energy released by chemical and biochemical processes. Its main carriers in the body are the harmones, enzymes and co-enzymes. Its activities are similar to the function of the sympathetic nervous system, which are concerned with the breaking down of complex molecules in the body and with the liberation of energy.

The most important function of this power of the inner fire are given as digestion, combustion, metabolic transformation, oxidation, visual faculty, regulation of the body temperature and the colour of blood and skin. The normal appearance and ambience of a person depend on pitta.

The effects of this life principle on the mind are chiefly to help development of intelligence, a sound memory, enthusiasm that can border on ecstasy, a living awareness, boldness and high ideals.

Without the power of Kapha the material universe would be formless as wind and fire would have no cohesion. The basic elements of which it is composed are earth and water, which when mixed give mud and clay.
The creation of the elements was followed by the formation of living things. Earth and water are the building blocks of force kapha, an old Sanskrit name for which is 'slesma' meaning cementing or cohering, in keeping with the properties of its components. Kapha imparts to the body stability, firmness, flexibility, resilience and coolness.

The kapha principle is involved in the construction of the smallest cell and of the largest bone in the body and in the formation of the joints, as well as in mental strength and endurance and in resistance to disease. These are its most important effects, to which we must add its ability to accelerate the healing processes. One essential function of Kapha is to ensure the permeability of the cells. Intra and extracellular fluid transport is assisted by Kapha through the whole body. Kapha aids anabolism and to build up the tissue.

Tissue, vessels and secretions

According to the classical definition of Charaka, an individual may be regarded as healthy, only when the tridoshas are in equilibrium, and the seven Dhatus are normal, Agni for its proper functioning, the thirteen large srotas and the innumerable small ones are open and the three malas are correctly balanced.

The Ayurvedic physician relies on the keenness of his senses when he wants to appraise the leading properties of a medication. He recognizes the quality of an item of food and of a medicinal plant and drug by his sense of taste. The effect of the different flavors on the tridoshas and consequently on the tissues, vessels and waste products, belong to the basics of
medical knowledge. Armed with these one can detect the therapeutic value of any fruit, herbs, drugs or article of diet.

The astringent taste (Kasaya), for instance has an action on the Tridoshas that strengthens the vata and tones down pitta and kapha.

The dhatus are the fundamental tissues of the body. They are formed and nourished by Ahara-rasa, (the so called Chyle, a milky juice, found in the intestine and in the lymph vessels of the intestines). Progressive change between the tissue is the outcome of complex and involves metabolic processes in the microscopic region.
Agni - the fire of life

Agni is responsible for the whole life process, for a person's appearance, for his strength, his energy, his health, his weight increase, for his life essence, glow, for his body, temperature and life breath.

The 'power of fire' working in the body is known as the principle Pitta. Pitta is the energy of fire, while the Agni is the fire itself or biological fire. When this fire extinguishes, life ends. Charaka says of Agni, Food is digested and broken down with the help of Agni. What we eat cannot nourish our body except by Agni, Life and death depends on functioning of Agni.

For the maintenance and improvement of health it is extremely important that Agni should be working properly. When its function is reduced, digestion is incomplete and metabolism is impaired.

The resulting substances consisting of imperfectly converted food are called as Ama or Unripe. The formation of Ama is followed by fermentation and by putrefaction in the stomach and intestines. Most endogenous disease called Amajanya are caused by the absorption of Ama. Ama is the sworn enemy of the Tridosha and plays a significant part in upsetting their balance.

There are thirteen kinds of Agni, of which the most important is Jathara_Agni, since this influences the functioning of all the other Agni. It operates in the region of stomach and intestines and is responsible for the digestion of food, for the absorption of nutrients and for the formation of waste products.
Jathara Agni is also a catalyst in the production of the digestive juices. Its role is in the formation of Ahara Rasa, from which all the dhatus arise and by which they are maintained.

Five more forms of Agni take care of further decomposition of substances. They are known as Bhutagni, and their number is derived from the basic elements. Their chief site is the liver. A certain set of factors undermines the functioning of Agni. One such factor is the undernourishment and another is overnourishment.
SUVARNAMAKSHIKA (ferri sulphuratum)
San: suvarnamakshic, Eng: Iron pyrites Hindi & Bom. Sonamukhi
(Nadkarni, 1982: 60-61)

It is formed by a combination of iron with sulphur. It is met within many parts of India and has been used in the Hindu medicine from a very remote period. Iron pyrites (FeS₂) are brass yellow in colour and their diamorphous form in marcasite is pale bronze yellow, and there are other pyrite-like minerals which are silvery white for instance cobaltite (CoS₂ CoAs₂), smaltite (CoAs₂), Lollingite (FeAs₂ with S) and Leucopyrite (Fe₃As₄). Iron pyrites roasted in air would give a red residue or Fe₂O₃. But it seems more likely that the golden yellow variety is copper pyrite which has a deep yellow colour and besides which when iron pyrites freshly fractured would appear almost silver in colour. In that case the 'essence of the appearance of copper' might be the metal itself.

Iron pyrites occur in two forms, namely in dark yellow nodules with golden metallic lustre (brass yellow colour) and in silvery radiated crystals. The former is a native of Kanauj, and is called Suvarnamakshika and the later Taramakshika is associated with stones and is of inferior quality. Chemically iron pyrites consists of bisulphide iron. Sulphide of iron is contained in preparations like lauhaprapati, sidha and other tantric medicines along with the sulphide of mercury and other vegetable substances. It is thus prepared by taking 2 parts each of mercury and sulphur and one part killed iron. Grinded well
together in an iron ladle and this powder is melted with clarified butter over a gentle fire. It is then poured over plantain, leaves and gently pyrite is purified by boiling in lemon juice with one third of its weight of rocksalt in an iron vessel till the pot turns red hot. It is reduced to powder by mixing with oil or goat's urine and then roasted in a closed crucible. Iron pyrites thus prepared has a sweetish bitter taste. It is a tonic, alterative and useful in anaemia, leucorrhoea, urinary diseases, ascites, prurigo, eye diseases etc.

FERRUM  San.. Lauha, Eng.. Iron,
Mah.. Lokhand
(Nadakarni, 1982 : 54-60)

Rarely met with free form in nature, though very widely distributed in both the organic and inorganic kingdoms. Found in nearly all rocks, soils etc. variously combined with oxygen as haematite, magnetic iron ore etc., with sulphur as iron pyrites, and as carbonate of iron, in spathi iron, in the ashes of plants and also the blood of animals, also in the bile, chyle, gastric juice, lymph, milk, pigment of the eye and urine.
Classification

According to Rasaratna Samuchchaya there are three varieties of iron

1. Cast or wrought iron (mundam), subdivided into three varieties
   a) Mridu- is that variety of iron which easily melts, does not break and is glossy.
   b) Kuntham- which expands with difficulty when struck with the hammer.
   c) Kadaram- which breaks when struck with hammer and has a black fracture.

2. Steel i.e. properly cast iron - six varieties
   a) Khara- rough, free from hair like lines and on breaking shows the lustre of quick silver and breaks easily by bending.
   b) Sara- a variety which breaks in the sides by hammering, it has hair like lines and is a product of brown soil.
   c) Hrinnala- It is black in colour, shows seed or beak like lines and very difficult to cut.
   d) Bajir Lauha - it is of sky colour and shown thin lines.
   e) Torabatta -
   f) kala or Kalayasa- blue black colour brilliant, plain, heavy and does not break even by striking with an iron hammer.

3. Wrought iron (Kantama): It possesses 1, 2, 3, 4, or 5 faces and often many more faces. Yellow black red colour- subdivided in to five varieties:
   a) Bhramaka b) Chumbaka c) Karshaka d) Dravaka e) Romakanta.
Purification: there are three methods.

1. It is first of all beaten into thin plates, which are then heated in fire and when redhot, plunged into the following liquids one at a time, oil, canjee, cow's urine and decoction of Dolichos uniflorus. This is repeated three times in succession.

2. To get rid of impurities: Boil one and a half seer (measure) of water reducing to quarter and then soaking in it half a seer of thin plates of cast iron which have been previously heated. Repeat the process seven times.

3. Powdered iron is to be macerated for a while in the decoction of the three myrobalans triphala in cows urine and then to be mixed up with clarified butter and fried in the earthen vessel and stirred with an iron rod until a blade of straw thrown over it catches fire. The iron powder is to be pounded and the above process is repeated five times.

Preparation of Loha Bhasma: The most easy method of reduction of iron is by soaking it for seven successive days in the juice of pomegranate or jam leaves and drying it in the sun. Then the iron is roasted by putas as usual. By this method 6 to 10 putas are sufficient for efficient reduction of iron.
Action: Iron improves the quality of blood. Iron produces constipation and this is why it was recommended to be administered with triphala powder. Iron stimulates the functional activity of all the organs of the body and is therefore a valuable general tonic. Loha bhasma is a powerful alternative, astringent, tonic and restorative.

Uses: Iron and its preparations are generally given with certain selected vehicles. In consumation it is given with black pepper. In hectic fever loha bhasma is given with honey and dry ginger. In gonorrhoea it is given with guggula. As a haematinic it is used in many diseases. Anaemia and chlorosis. Iron is of great value in both simple and secondary anaemias. The benefit is specially marked in cases of chlorosis and in anaemia caused by malaria, kala azar, chronic discharges or repeated passive haemorrhage. Among the various preparations Navayasalauha is very useful and is very commonly used in all forms of anaemia. A varieties of iron preparations are in use. Besides the preparations, commonly used, other combinations of mercury, iron and talc with the addition of gold, silver, copper etc. in varying proportions and combinations are described under different names. Infact, mercury, iron and talc constitute the basis of the great majority of pills used by Kavirajas. Iron also forms an ingredient of hair dyes. e.g.a paste made up of powdered iron, chebulic and eemblec myrobalans 2 tolas rubbed together with water in an iron vesseland kept for one night. This paste is applied to grey hairs for turning them into black. (Bhavaprakasha).
TALCUM PURIFICATION (Creta Gallica Purificata)

(Nadakarni, 1982 : 123-130)

Sans.-Abhra. Eng.-Powered Talc; Hind.- Avrak. Mah.-Hingool. There are four varieties.—White (pinaka), red(naga), yellow(manauka), and black(vajra), of these, the black variety (Vajrabhra,Krishnabhra or Sheabhra) is used in medicine.

Source : Chiefly found in mountains. In India it is found chiefly in the districts of Nellore and Hazaribagh and in the hills of the Central Provinces and Rajputana. It occurs in a natural state either as an essential constituents of igneous rocks or as a product of mineral silicates by weathering or contact.

Characters : A kind of crystalline mineral, of a foliated texture capable of being divided into extremely thin flakes or leaves, having a sensible elasticity and a metallic lustre. The flakes are transparent, soft and can easily be scratched. When divided across, the plates seem rather to tear than break.

Constituents : Mica is a rock forming mineral. It is a silicate of aluminium together with alkalies and basic hydrogen—(jour.of Ayur.July 1924). It contains 4 to 6 p.c. of water existing as basic hydrogen or as hydroxyl replacing fluorine.

Purification & Preparations : "Mica, the layers of which can be easily separated (by knife) is preferred" (Rasaratna Samucchaya). It is purified by boiling it in the decoction of Triphala or of dried plums for a long time and roasting or calcining it over a fire alternately, soaking it in the juice of lemons till the scales are separated. The calcined scales are ultimately mixed
with the paste of Amaranthus polygamus and finally dried. Or it is first heated and washed in milk. The plates are then separated and soaked in the juice of Amaranthus polygamus and Kanjika for eight days. Talc thus purified is reduced to powder by being rubbed with paddy within a thick piece of cloth, when the powdered talc passes through the pores of cloth in fine particles and is collected for use. Talc thus reduced to powder is called Dhanyabhrak. It is hard, heavy, very fine, black and of saline earthy taste. It is prepared for medicinal use by being mixed with cow’s urine and exposed to a high degree of heat within a closed crucible, repeated for a hundred times. Sometimes the process is repeated a thousand times. When this is the case the preparation is called Sahasraputi Abhrak and sold at high price. Some soak it in the juice of Calotropis gigantea instead of cow’s urine, before calcining. It is of superior efficacy. Ayurvedists believe that burning and pulverising repeatedly of the minerals produce a "potency" or peculiar molecular change in these and add to the therapeutic value of the product. Dhanyabhrak or Talc powder consists of Silicate of magnesia with iron in excess. Abhrak bhasma is prepared by heating together Dhanyabhrak 1 part and borax 2 parts and triturating the whole in milk and evaporating. It is generally given with Lohabhasma. Dose is 2 to 5 grains. Abhrak Kalka (emulsion) is prepared by mixing together Abhrak bhasma, emeblic myrobalan, ginger, pepper, long pepper and Vavadinga in equal parts, reducing the whole to a uniform mass and then adding honey. Dose is 10 to 40 grains. Action: Mica is a disinfectant to some extent, but is seldom used as such. According to Rosenheim and Ehramann (Deut. Med. Woch, 20,
Jan.1910), aluminium silicate when taken into the stomach, reacts with the excess of hydrochloric acid in the gastric juice to form silicic acid and aluminium chloride, the latter acting as a protective to the gastric mucosa in a manner similar to bismuth. It will be interesting to see whether prepared mica which is also a silicate has any such reaction in the stomach as it has always been used in acid dyspepsia and gastric ulcer, e.g., Vidyadharabhra-(Jour. of Ayur., July 1924). Silicic acid is present in various percentages from 0.81 p.c. down to a trace, in the muscle, liver, spleen, lymph and intercellular fluid and also found in the various excreta-Urine, feces and sweat. Mica being a silicate its action as a therapeutic agent can thus be surmised. Reduced mica is described in Ayurveda as a general tonic and alterative. It is said to stimulate metabolic activity of tissue cells. It is also used as aphrodisiac. Reduced mica removes the derangement of the tridoshas and establishes their equipoise. Dhanyabhraw is tonic and aphrodisiac. Generally the preparations of Mica are astringent, tonic aphrodisiac and alterative. Abhrak Kalka is alterative and restorative.

Uses: Abhrak Bhasma is given in anaemia, jaundice, chronic diarrhoea and dysentery, nervous debility, chronic fever enlarged spleen, urinary diseases, impotence, dyspepsia, asthma, hectic fever and in cachexia due to long continued discharges from fistulae, abscesses, gonorrhoea, leucorrhoea etc. It may be given with honey and pipali with benefit. As an astringent it is largely used in diarrhoea, especially of nervous origin. As an alterative it is used in enlargement of glands. Dose is 2 to 6
grains generally with honey, twice a day. In phthisis or tuberculosis it is given in doses of 2 to 3 grains twice daily either with little honey or with honey and some vehicle as the fresh juice of Vasaka or with the fresh juice of the ripe fruits of Cactus grandiflora. The mica supplies silica to the connective tissue cells and thus helps them to form defensive barrier around the tubercles or the pus-forming bacteria. In asthma, reduced mica is given with the juice of Vasaka. In intestinal worms, reduced mica is given with seeds of Embellia ribes and a teaspoonful of clarified butter. In cases of biliousness and jaundice, it is prescribed with sugar and milk. In gonorrhoea, it is given with honey and powdered peepul and turmeric 12 grains per dose. In chronic spermatorrhoea, it is given with the juice of gulaanca and cane sugar. In anemia and chlorosis, it is given in combination with iron (Loha bhasma); in scurvy it is administered with honey and lemon juice. In rheumatism, reduced mica is given with a decoction prepared from ginger, root-bark of Aplotaxis auriculata. In farunculosis and cancer, reduced mica is prescribed with Senevieria Zeylanica. In piles, reduced mica is given with the peduncles of the ripe fruit of Semicarpus anacardium. In leprosy with ulceration of the toes and fingers, Galith kasthuri Rasa described in Bhavaprakash is given. The following additional remedies containing talc are useful in various complaints:- (1) Diabetic abscess- Abhraka Bhasma 2 grains and Triphala churnam 20 grains mixed together, and divided into 12 doses, and each powder-dose given every 4 hours with plenty of honey, to patients suffering from diabetic abscess, have found great relief. (2) Rheumatism-Take Abhraka
bhasma 2 parts, Para Kajali 2 parts, Balsamodendron mukul 4 parts, fecula of Cocculus cordifolia 8, and Tribulus terrestris 5 parts. Mix, then add the juice of Vitex negundo and Cocculus cordifolia. Macerate well, and dry. Dose is 2 to 4 grains with the decoction of long-pepper; used in rheumatism. (3) Intestinal worms and colic - Take Abhrak bhasma 3 parts, sulphur 2 parts, Croton seeds 2 parts, borax 2 parts. Mix and triturate in the juice of Citrus limonum. Make a pill mass. Dose is 3 to 5 grains with rice canjee; used in intestinal worms, colic, etc.
ASPHALTUM (SHILAJIT)
(Nadkarni, 1982 : 23-32)

Sans.-silajit; Silaras. Eng.-Asphalt; Mah.. silajita Hind -Ral-yahudi.

Source: Ejected out of rocks during hot weather in the lower Himalayas, Vindhya and other mountain tracts and Nepal where iron abounds, naturally flowing out from between the fissures in the rocks; or it may be a tar formed in the earth from the decomposition of vegetable substances. "Large quantities are imported into India from Khatmandu (Nepal). A white variety is said to be collected from rocks in Mount Abu (Rajasthan)".- (Chopra).

Remarks: "Alum earth of Nepal which is sold in Calcutta as white shilajit is quite a different substance from the Shilajit used in the Hindu Materia Medica. A product called 'Momia' resembling Silajit, is obtained from some of the mountains in Arabia and Persia".- (Chopra).

Varieties and their Characters: "Four varieties of silajit are described by the Hindu writers:- (1) the gold silajit which is red. (2) the silver shilajit which is white; (3) the copper silajit which is blue coloured; and (4) iron silajit which is blackish brown. Blue and red silajit are not found commonly and the variety most available is the iron variety which, from the therapeutic point of view, is considered to be active".- (Chopra's "I.D.of I" P.433). "Shilajit is a bituminous substance, which is a compact mass of vegetable organic matter composed of dark-red gummy (sticky and unctuous) matrix interspersed with vegetable fibres, sand and earthy matter."- (Chopra's). Shilajit
is of a bitter taste and of a smell resembling cow's stale urine. This is known as gomuthra shilajit. The other variety found in the market is called Karpoora Shilajit which occurs in white plates. On igniting, it leaves a large quantity of ash consisting of lime, magnesia, silica and oxides of iron. The black variety is the one mostly used in medicine, after purifying it by specific process. "Purified Shilajit' (Shodhita) is just like the concentrated watery extract of the crude stuff. Both the crude and purified samples have a urinous odour and slightly bitter, saline, somewhat pungent and astringent taste. The purified substance is nearly completely soluble in water and has an acid reaction" (Chopra).

Constituents: "The gummy substance of silajit dissolves in water and when washed away leaves an earthy matter, vegetable fibres and a few black round button-like masses (1/8th inch in diam.) resembling peas. The insoluble matter is removed by straining through a thick cloth or flannel. The fluid is allowed to stand in the Sun when a creamy substance rises to the top".

"Shilajit contains an oil which when distilled is known as ichothyol. Benzoic acid and benzoates which are present in shilajit in large quantities are considered by Chopra to be the main active principles. Ray (1930) is of opinion that there must be some other active principle or some unknown body or a pyridin derivative, in shilajit" (Chopra).

It contains 65 p.c. of urea. Analysed by Hooper it yielded:--water 8.85 p.c. organic matter 56.20 p.c. and mineral matter 34.95 p.c. containing nitrogen 1.03, lime 7.80, potash
9.07, phosphoric acid 0.16 and Silica 1.35 p.c. It dissolves in water and is neutral in reaction. "The organic matter yields in to spirit a small percentage of brownish coloured wax-like substance which melted on heating and burnt away with a smoky flame. It retained the peculiar odour of the drug and had no marked taste. It was neutral in reaction and did not assume a crystalline structure when carefully evaporated from alcoholic solution. The tests would indicate the presence of a mineral hydrocarbon of a bituminous nature. The bulk of the dark brown organic matter had the properties of humic acid. The drug, from a chemical point of view, should have some valuable manurial properties" (Chopra's "Indian Drugs of I." p. 434).

The result of samples analysed by Chopra and his coworkers are as follows :-

White Shilajit :- A sample of white shilajit, which is considered to be more effective than the black variety, was also examined by Chopra. It was a cream-coloured crystalline with a strong nauseous odour. It was apparently of animal origin and afforded gaseous ammonia when mixed with slaked lime. It yielded 64 percent of pure urea when determined from the amount of nitrogen given off by means of hypobromite of sodium. It appeared to be crude urea or evaporated urine in a solid state.

A careful analysis of the ordinary shilajit was carried out by the author and his co-workers. It does not contain any compound of the nature of an alkaloid.

Both the alcoholic extracts crystallised after several days and were found to contain benzoic acid; and the ash left after ignition showed the presence of a larger quantity of lime. The
crystals had a melting point of 187°C and were identified by further examination to be those of hippuric acid.

The mineral constituents, as obtained from the ash by incineration of the substance at a dull red heat, are also appended as in the table :-
CONSTITUENTS OF SHILAJIT

<table>
<thead>
<tr>
<th></th>
<th>Crude silajit Percent</th>
<th>Pure silajit Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>12.54</td>
<td>29.03</td>
</tr>
<tr>
<td>Loss on ignition</td>
<td>64.58</td>
<td>52.63</td>
</tr>
<tr>
<td>Ash</td>
<td>22.88</td>
<td>18.34</td>
</tr>
<tr>
<td>Silica</td>
<td>4.60</td>
<td>2.69</td>
</tr>
<tr>
<td>Iron (Fe₂O₃)</td>
<td>0.51</td>
<td>0.64</td>
</tr>
<tr>
<td>Alumina (Al₂O₃)</td>
<td>2.26</td>
<td>2.61</td>
</tr>
<tr>
<td>Lime (CaO)</td>
<td>6.83</td>
<td>4.82</td>
</tr>
<tr>
<td>Magnesia (MgO)</td>
<td>1.29</td>
<td>1.20</td>
</tr>
<tr>
<td>Potash (K₂O)</td>
<td>4.60</td>
<td>3.81</td>
</tr>
<tr>
<td>Sulphuric acid (SO₃)</td>
<td>0.64</td>
<td>0.97</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>3.64</td>
<td>3.36</td>
</tr>
</tbody>
</table>

From a comparison of the above results, it appears that there is not much difference between the crude and the purified shilajit. The crude stuff leaves a residue after extraction with water which amounts to about 30 percent, whereas the residue in the purified drug is only about 2/3 per cent. One may interpret that the purified shilajit contains more extractives than the crude form. This would have been the case were it not for the fact that the high percentage of moisture in the purified substance counter-balanced the insoluble matter in the crude stuff. The main point of difference between the varieties is that the chloroform and ethyl acetate extracts of the purified
substance deposit—crystals of benzoic and hippuric acids, but there are none in similar extracts made from the crude shilajit.

The presence of gum and resin is also a point in favour of its vegetable origin. The other possibility is that shilajit may be composed of the excrements of some animals which have been washed off by the rains from the hill-side and have been deposited in the crevices and low-lying rocks. During the summer, the heat of the sun removes the moisture and leaves the residue like an exudation on the rock. The subject of the production of shilajit requires further investigation. (Chopra "I.D. of I" pp434 to 436).

Action: Locally antiseptic, anodyne, parasiticide and antiphlogistic. Internally alterative, tonic, slightly laxative, cholagogue, respiratory stimulant, disinfectant and expectorant intestinal antiseptic, and diuretic.

Uses: Charaka says "There is hardly any curable disease which cannot be controlled or cured with the aid of Shilajit". It is used by Kavirajas and Hakims in a great variety of diseases. It is specially employed in genito-urinary diseases and in diabetes; in gall stones, jaundice, enlarged spleen, fermentative dyspepsia, worms, digestive troubles, piles adiposity, anasarca, renal and bladder calculi, anuria etc. hysteria, neurasthenia, epilepsy and insanity, nervous diseases; amenorrhoea, dysmenorrhoea and menorrhagia; tuberculosis, phthisis and leprosy; eczema, elephantiasis, anaemia, anorexia, biliary congestion, chronic bronchitis, asthma, fracture of bones etc. In diabetes in which it reduces the quantity of sugar and urine. But it increases the quantity of urea; therefore, it should never be given in uric
acid calculus. It diminishes phosphaturia and is useful in phosphatic concretions. It is also useful in ascites, uraemia, cholaemia and the like. It is valuable in cases of diabetic albuminuria, where both casts and albumin diminish; it is said to be a cure for diabetic aurosis. "Under the influence of shilajit, thirst, polyuria, burning sensation and exhaustion disappear quickly. It markedly helps the assimilation of sugar. Kavirajas use shilajit in combination with milk or grape juice." - (Chopra). An extract is made from crude Shilajit by making an emulsion with hot water and repeatedly exposing the emulsion to the sun. A cream floating on the surface is removed and collected. The process is continued as long as any cream rises. The extract of silajit thus collected is sun-dried and then purified by being soaked in a decoction of triphala and dashamula. "Purified shilajit is also recommended to be soaked in the decoctions of one or more of the following plants as this is said to increase its efficacy: - Shorea robusta; Buchanania latifolia; Terminalia tomentosa; Acacia farnesiana; Catchu nigrum; Terminalia chebula; and Sida cordifolia". - (Chopra). It is a powerful tonic and alterative, useful in a variety of diseases. "It is also used as an antiseptic in parasitic diseases of the skin and as an antiphlogistic. Unani physicians used it as an antidote to poisons and in the treatment of other diseases. Hakims use 'Momia' as an external application for inflammatory swellings, arthritis, etc." - (Chopra).

Paste is locally applied to relieve rheumatic pain in joints, used as an embrocation in paralysis, confusions etc; also
in sprains and bruises. When applied externally, shilajit has been credited with antiseptic, parasiticidal, anodyne and antiphlogistic properties by Kavirajas; these are in all probability due to the free benzoic acid which it contains. Internally shilajit is very useful in chronic dyspepsia, and dyspeptic diarrhoea, given with the decoction of emeblic myrobalans; in biliary colic and jaundice with the decoction of the three myrobalans (triphala) or of dasamula. In dyspepsia due to hepatic derangement, shilajit is used in combination with other cholagogues. In the first stage of ascites it is used with iron together with milk diet; salt and water is stopped altogether. Rice and milk boiled together into gruel is a good diet in commencing cirrhosis of the liver of adults. In the first stage of infantile cirrhosis, shilajit is used with other cholagogues like the juice of the leaves of Andrographis paniculata, of Cajanus indicus or of Nyctanthes arbor-tristic. In false angina pectoris even during the absence of paroxysms it is recommended. It is very useful in acute and chronic bronchitis and in bronchiectasis, in asthma with bad liver and indigestion, in the asthma of gouty people, in pulmonary phthisis, in diabetic phthisis and in intestinal tuberculosis. In sexual weakness it is generally administered with Asvagandha, in spermatorrhoea with grape juice or infusion of the three myrobalance (triphala); in chronic gonorrhoea and gleet, with prepared oxides of tin, lead, silver etc. It can also be used alone with much benefit. In functional menorrhagia complicated with biliousness and hepatic derangement it is commonly given with the decoction of emblic myrobalans, or combined with astringent drugs like catechu,
flowers of Woodfordia floribunda or syrup of the corn of red lily.

**BAMBU**SA ARUNDINACEA (Nadkarni, 1982: 93-95, Kirtikar Basu, 1964)

Sansk-vansa, Eng: Bomboo, Hindi: Bans

Tavakshiri or Tugakshiri (Silicious concretion or the milky bark of bamboo)

**Habitat**: Common in Central and South India. Cultivated in Bengal and North Western India.

**Varieties**: 2 varieties are available in the market, the blue and the white, both having sweet taste.

**Parts used**: The interior stalk or stems (Bamboo hallows) of female plant containing silicious concentration (deposit) called tabashir (bamboo manna) in the interior of the stem of Bombusa arundinacca, young shoots of leaves, articulations, seeds and roots.

**Const ituent s**: Tabashir (bangsolochan) contains Silica 90% or Silicam as hydrate of Silicic acid, peroxide of iron, potash, lime, alumina, vegetable matter, cholin betain, urease proteolytic enzymes, diastatic and emulsifying enzyme, cyanogenetic glycoside.

**Action**: Leaves are emmonagogue and Anthelmintic, Tabshir or bangsolochan is stimulant, astringent, febrifuge, tonic, cooling antispdmodic and aphrodisiac.

**Action and uses in Ayurvedic and Siddha**

Mathura Rasam, Kashaya-anurasam, seetha-veeryam, brahmanam, balyam, vrisham, in trishna, kasam, swasam, jwaram, raktapittam etc.
Leaves: mathura kashayarasaam, seeth veeryam kaphapittaharam, saram, chedanm, in kushtam, raktavoraram, soddhum or shodhanam.
Shoots: Katu rasaam, kashaya anurasam, katuvipaka guru, ruksham, Kaphaharam, vatapitta karam, guru vidhahi saram.
Seeds: kashoga rasam, katuvipakam ushna, veergam, ruksham, saram.

Action and uses in Unani

Cold, tonic for heart and liver, sedative or irritation of body in thirst, vomiting, palpitation

Preparations:
Decoction of leaves and bamboo joints (1:20) dose 1-2 ounce.
Compound powder, dose 1 drachm. pickles and poultice.

Uses: Young shoot of the bamboo made into poultice is the most efficacious application for dislodgement of worms from ulcers.

Juice is poured on the vermin and the liquid mass applied and secured by a bandage. Leaf bud:-- is administered as decoction to encourage the free discharge of the menses or lochia after delivery, when it is scanty, used in leprosy, fevers and hoemoptysis, also for thread worms. Leaves are used in hematemesis in veterinary practice.

Pickles or curry prepared out of the tender shoots give much benefit to persons suffering from lack of digestion as it promotes appetite and digestion. The silicious concretion (bamboo manna) found in joints of the female bamboo, it is useful in fever, cough. Paralytic complaints, debilitating diseases, asthma, snake bite etc. A compound powder containing long pepper, cardamoms, cinnamon, sugar in 1/2, 1/4, 1/8 and 2 parts respectively, and the Tabshir as an alternative in phthisis and cachexia, dose is 1 drachm. Root is given as a specific in eruptive affections a older dried stems make very efficient
splints for fractures etc. seeds, resemble rice and eaten by the poor. Tender shoots are also eaten like asparagus.

**SILICUM (Eng; silican) (Nadkarni K.M. 1982)**

*Source:* Very common non-metallic element, obtainable in 3 different forms, i.e. the amorhous the graphitoid and the crystalline, from silica or pure flint, found in nature as Silicon dioxide in rocks, crystal, and flint quarts agate and various other stones and in earths and clay, also as silicates in baysalt, felspar, granite, mica, porphyry i.e. mineral and metallic oxide etc.

*Manufacture:* Heat together fluoride of patassium and silicon with its equal weights of metallic potassium. Put the fused mass into cold water, when silicon will be left behind.

*Characters:* Crystalline or amorphous, dry dark brown powder, non-fusible, insoluble and non-volatile. If heated in the air it becomes converted into Silica.

*Uses:* Used both externally and internally in the form of an alkaline silicate, chiefly in some forms in dentifrices and other in pharmacy.

**SHRINGA BHASMA (SAMBAR BHASMA)**

(Nadkarni, 1982 : 152)

This bhasma is prepared by sabmar or Deer horn (shinga).

*Procedure:* Make small pieces of shinga. Sambar shinga is very hard. The deer shinga is softer than samber shinga. Burn these pieces in a pot. Initially if we could get excellent odour, then immediately this becomes super white. If not then it turns out to black coat. Then make a fine powder and apply it for Korphada.
black coat. Then make a fine powder and apply it for Korphada bhavana, like this about 7-8 bhavanas are given and subject it for 7-8 Gaja puta. Then Rui milk extract is used for Bhavana and at the end, Gaja puta is given, then it becomes a white and good quality bhasma.

Properties: Used in fever, tonic respiratory disorders tuberculosis effective as bacteriostatic action. This is very effective in T.B. in combination, with praval bhasma, to start in a dose of 1 Gunja increase up to 6 Gunja. Also effective in productive cough.

In Vata it is contraindicated, because it produces dryness of bronchial tree. But it is effective in pertussis.

It is effective in heart trouble, provided there is major pathological change, it is effective in strengthening the heart, and it reduces tachycardia.

In Rickets, in combination with praval bhasma-good results are obtained.

In kidney disease this is effective.

TERMINALIA ARJUNA


A large tree, with huge often buttressed trunk and horizontal spreading branches, bark is smooth gray, falling off in large flat pieces.

Leaves usually sub-opposite, 10-15 x 4-7 cms, Oblong or elliptical oblong, obtuse or subacute, pale dull green above, pale brown beneath, shallowly crenate- serrate in the upper part or sometimes throughout, base rounded or cordate, often unequal
sided main nerves orcuate, 10-15 pairs of veins reticulate the pellucid, petioles 6-10 mm long with one or usually two prominent glands at the top immediately below the leaves.

Flowers sessile, in short axillary spikes or in terminal panicles; bracteoles linear lanceolate, shorter than the flowers caducous. Calyx glabrous, teeth triangular, ovary quite glabrous, disk clothed with yellowish or reddish hairs. Drupe 2.5-5.0 cm. ovoid or projecting wings striated with numerous curved veins. Bark-smooth, pinkish gray. Sapwood reddish white, heartwood brown, variegated, with darker coloured streaks.

Distribution

Distributed throughout the greater part of India. In the sub Himalayan tract, Chota Nagpur central India, parts of Mumbai and Madras and Ceylon.

Properties

The bark is acrid and sweet, cooling and heating alexiteric stypic, tonic antidysenteric, useful in fractures, ulcers, blood diseases, intoxications, urinary discharges, "K apha" biliousness, strangury, diseases of the heart, anemia, excessive perspiration, asthma, tremors, leukoderma and false presentation of the foetus, allays thirst and relieves fatigue (Ayurveda)

The bark is bitter is claimed to be expectorant, aphrodisiac, tonic, diuretic, useful in biliousness, externally in wound and fractures both externally and internally in gout and urinary discharges (Yunani)
In fractures and contusion with excessive ecchymosis, powdered Arjun bark is recommended internally with milk. A decoction of the bark is used as a wash in ulcers and cancers.

The bark is astringent and febrifuge. The fruits are used as tonic and deobstruent, the juice of fresh leaves is a remedy for ear ache. The bark is useful in bilious infections and as an antidote to poisons. In Kangara, the bark is used for sores. The ashes of the plants are prescribed for snake bite (Sushruta) and the bark for scorpion sting (Vagabhata)

Although so many ash uses are described in Ayurveda, there are no modern clinical reports to confirm these. Charaka in his samhita distinguishes between Arjuna and Asna. He credits both with diuretic and astringent properties but does not prescribe either in cardiac trouble.

Sushruta gives a complete survey of heart diseases and their treatment without any mention of Arjuna or Asna.

Vagabhata was the first to prescribe the bark of "Arjuna" in heart diseases. The practice was then recommended by Chakradutta, Bhava, Misra.

Ainslie (1826) speaks of Arjuna as possessing antifebrile qualities and being used when powdered and mixed with oil, for aphthae of adults and infants.

Dymock informs about chemical composition of the bark of Arjuna, the ash amounts to 34% of almost pure calcium carbonate,
the watery extract is 23% with 16% tannin, very little colouring matter besides the tannins is extracted by alcohol.

Ghoshal in 1909 reported the presence of sugar, tannin a colouring matter, a body glucosidal in nature and carbonates of calcium and sodium with traces of chlorides of alkali metals. From experiments on frogs, rabbits and man he concluded that the drug is very valuable remedy in heart disease, especially where a combined tonic and stimulant action is necessary.

Komon (1919-20) reported unfavourably about the efficacy of the drug. A decoction of bark was administered to a case of valvular disease of the heart with palpitation and pain over the region of the heart. He did not derive any benefit from the administration. He concluded that the drug is "not useful" in study carried out in 20 patients.

In 1923 Chopra reported that i.v. injection in animals produced a small but persistent rise in BP.

In 1924 Chopra found that alcoholic extracts produce beneficial effects in number of cases of cardiac failure, who did not react well to the digitalis bolus dose. The therapeutic properties seemed to be associated with a glucoside obtained in a state of hydrolysis. Finally in 1929, Chopra and Ghosh published the results of their investigations about the chemistry.
Pharmacology and therapeutic action of Arjuna.

1. About 12% tannin consisting mainly of pyrocatechol tannins.
2. Some colouring matters.
3. An organic acid with a high melting point and a phytosterol.
5. Large amounts of calcium salts with smaller amounts of aluminium and magnesium salts.
6. Sugar etc.

Meanwhile (1926) the results of material from local bazaar showed that except for diuretic properties the drug was pharmacologically inert.

Mhaskar and Caius, found that the ashes are not an antidote to snake venom and bark is useless in prescription for scorpion sting.
PROCESSING OF DIFFERENT INGREDIENTS (SHODHANA PROCEDURES)

Suvarnamakshika Bhasma

Freshly powered 1kg Suvarnamakshika was taken, 20 ml of citric acid (10 gm in 20 ml of water) was added to it. This mixture was subjected for frying till it becomes red after adding 1 litre of castor oil. This procedure was carried out over a period of seven days. On 7th day powder was weighed, it became lighter and weight of the powder was around 700 gms.

Such 15 kg powder was taken, to it 7 kg Korphad (Aloe Barbadensis) pulp was added. This was thoroughly mixed together and kept in earthen pots. Pots were covered with cloth and a seal of wet mud was applied over it. Pots were subjected to heating in natural furnace. (Heat was produced by cow or buffalo dung). After cooling it was sieved by Bhasma seive (having very fine mesh) and again mixed with Korphad pulp. Such procedure was carried out for seventeen times. Then this fine powder of Suvarnamakshika Bhasma was used to prepare Suvarnamakshika tablets.

Abharak Bhasma

26 kg of Abhrak was taken, to this 10 litre of oil of seasame, 8 litre of urine of cow, 8 litre of hulga (Dolichos Biflorus) extract, 8 litre of rice water and 8 litre of butter milk were used one by one separately for its purification. After such procedure of Shodhana Abharak was powdered, to this, extract of Nagarmotha (prepared by adding 500 gms of Nagarmotha powder in 40 liter of water.) Subjected to boiling till only 8 litre extract
remains) 8 litre was added, mixed together, kept in earthen pot, covered with cloth and sealed with wet mud. Then subjected to heat in furnace. This procedure was repeated about 20 to 22 times and then it was powdered and used for preparation of Suvarnamakhsika tablets.

Loha Bhasma

Shodhana of original native Loha was carried out by using oil of Seasame, butter milk and cow urine. Same procedure was followed as for other ingredients.

One kg powder of Triphala was taken, to this 16 litre of water was added. This mixture was subjected to boiling till it remains 4 litre. This purified Loha was heated and added immedialety in the extract of Triphala. Same procedure was repeated for 7 times.

After Triphala Bhavana, to it Mercuric chloride and Korphad pulp was added (amount added was half the quantity of Loha powder). All the ingredients were mixed together and small pieces were prepared and dried. Dried pieces were mixed with Korphad pulp two times. Then Triphala Bhavana was carried out, it was kept in earthen pot. Pots were subjected to heat in furnace. This procedure was repeated for 20/22 times. Then Loha bhasma was powdered and used for preparation of Suvarnamakshika tablets.
Pure Vanshalochan

Vanshalochan, was used as fine powder and there is no need of purification as Bhasmas.

Shilajit

Stones of Shilajit were taken, to it Triphala Powder was added (for 10 kg Shilajit add 3 kg Triphala powder). Add 40 litre water and 20 litre cow urine. Whole mixture was subjected to boiling. During boiling, solid material coming on the top of it was taken. This was evaporated till it became completely dry. This was used for preparation of tablets of Suvarnamakshika. Prior to mixing with other ingredients it was soaked in water for two days.

Shringa Bhasma

Sambar Shrings were broken down into pieces and fried. Powder was prepared. To it Korphas pulp was added and mixed together, kept in earthen pot, closed by cloth and wet mud, kept in furnace. Same procedure was repeated for three times. Powdered Bhasma was used for preparation of Suvarnamakshikadi wati.

Arjuna Sal

For 20 kg of total mixture one kg of Arjuna Sal was required. Extract of Arjuna Sal was prepared by taking 10 kg original substance, adding to it 20 litre of water, boiled till it remains 5 litre.

preparation of Suvarnamashikadi wati

All ingredients in specified quantity were taken. Suvarnamakshik Bhasma, Abharak Bhasma and Loha Bhasma were mixed together, then pure Vanshalochan and Shringa Bhasma were mixed. In the extract of Arjuna Sal, Silajit was dissolved. These all
above powders were grinded over a period of 8 hours. Bhanava of Arjuna Sal was given and then tablets were prepared by hand. Each tablet was of 200 mg weight.

**Indications claimed by vaidya**
- Heart attack
- Pain in Chest
- Hypertension
- Anaemia
- Rajayakshma
- Breathlessness due to vata-pitta
- Tachycardia
- Psycosis
- Dryness of mouth
- Mental disorders
- Faults in heart valves

Daily Doses administered - 2 tablets, twice in a day taken with milk or water on empty stomach. The duration of therapy varies in different conditions
TOXICITY STUDIES

Principles of Testing for Acute Toxic Effects.

(Brayan, 1993 : 50-70, 235-250)

The collection of data on the acute toxic effects of substances has for many years been a focus for research in academic, industrial, contract and government research laboratories. It is commonly done for large number of substances from natural origin to synthetic chemicals. It is also carried out for products of animal and plant origins. Investigation of acute toxicity has led to the identification of selective toxic action and the beneficial use of substances as pesticides in controlling the environment and as drugs for therapeutic use in domesticated animals and man (Albert 1985)

Natural agonist (Reuse 1948) are selective molecules which contribute to the functioning of cells. Antagonists are inhibitors of natural agonists and are inevitably toxic in certain concentrations.

Several definitions of acute toxicity have been formulated.
1) The adverse change(s) occurring immediately or a short time following a single or short period of exposure to a substance or substances.
2) Adverse effects occurring within a short time of administration of a single dose of a substance or multiple doses given within 24 hrs. An Adverse effect (toxicity) can be defined as, any effect that results in functional impairment and/or biochemical lesions that may affect the performance of the whole organism or
that reduce the organ's ability to respond to an additional change (WHO task Group, 1978; vouk al, 1985.)

Toxicity can be considered to be the capacity to cause injury, hazard, is the probability of injury occurring (Barnes 1963 oser 1971) and safety the improbability of injury.

Acute exposure to toxins may result in death or sublethal responses and the signs and symptom may be non-specific reflecting a mass action effect, specific inhibition of vital cellular receptors (antagonists) and enzymes (inhibitors) mimicking endogenous receptors (by peters 1963).

White Mc-clean(1971) and Brown postulate (1980) that response may occur as a consequence of biotransformation to reactive intermediates with subsequent modifications of biochemical and physiological processes. Smyth and Carpenter in 1944 described preliminary data on the oral, dermal, and inhalation toxicity of a chemical. The study of acute toxic effects can be extremely broad. Objectives include the characterization of the acute biological effects of chemicals defining clinical signs, describing a chemicals acute toxic syndrome so that intoxicated patients can be diagnosed and treated and identifying target organs( (OECD) 1981 Zbinden 1984.)

Acute systemic toxicity assessments are carried out to evaluate acute systemic effects in mammals leading to adverse changes in target organs which results is acute ill health and death. Toxicity studies are carried out by using different routes such as peroral, subcutaneous, inhalation, opthalmic and cutaneous.

Development of acute Toxicity (assessment Classification.)
Many principles are generally applied to acute and chronic toxicity evaluation. Primitive man undoubtedly observed previously healthy animals becoming ill and occasionally dying following their ingestion of or contact with various natural materials. Indo-European Hindus (Vedas, 1500-1200 B.C.) are considered to be one who were milestones of references to poisonous substances and material.

Dose response and animal experimentation.

Before the twentieth century the art of poisoning of man was the predecessor of acute toxicology testing in animals. In early Greek and Roman times, the prevalence of assassination initiated efforts to discover antidotes. Zopyras, physician to Mithridates VI, King of Pontus (200 B.C.) adhering to the adage that "the best model for man is man." is believed to have used prisoners to identify materials which acted as antidotes against ingested poisons. The Marquise de Brinvilliers (c AD 1650) is claimed to have administered preparation of toxins to the sick and needy to evaluate potency, speed of onset, specificity, site of action clinical signs and symptoms prior to their use in assassinations.

Paracelsus (c AD 1500) insighted into the relationship of dose and effect resulted in his often paraphrased quotation that "the dose or amount of chemical determines whether a substance is a remedy or a poison."

Orfila in 1817 attempted a systematic correlation of chemically induced biological responses based on his observations of the effects of poisons on dogs, autopsy material for detecting and of confirming accidental or intentional poisonings. In 1813-
1878 further advances were made by the physiologist Claude Bernard. Bernard replaced Magendie’s empirical method of experimentation with one of confirmation or repeatation of a predefined hypothesis.

Anti-vivisection and safety, evaluation: During period of AD 1644 i.e. animals were considered machines without consciousness, were challenged as being morally wrong. Lobbies arose against using animals in medical experimentation. In 1876 in UK, 'The cruelty to animals Act' was passed, by which only licenced practitioners (under specified conditions), medical experimentation can be performed. The animal Act was formed in 1986 and in 1987, the Animal welfare Act was updated.

The increasing legislation for animal’s welfare has been paralleled by the increase in legislation for chemical safety.

Quinine and digitalis were first tested in man before being given to animals (Baker and Davey 1970), since those early days the use of surrogate species prior to exposure of the man to chemicals has become the norm rather than the exception from both ethical and liability point of view.

International Hazard Labelling and Toxicity Ranking.

Trevan (1927) proposed an experimental design to define more accurately the lethal concentration or dose for biologically prepared therapeutic material which were of a variable and inconsistent potency, and introduced the median Lethal dose LD 50 or LC 50, terms consistant with the median effective dose ED50 used in pharmacology.

Deaths following the use of the antibiotic sulphanilamide which had been formulated in ethylene glycol (1937) led to U.S
congress passing the Federal food Drug and cosmetic act (1938) requiring the obligatory testing of drugs for safety using experimental animals.

Acute systemic classification by different countries is either very toxic, Toxic, harmful or class I, class II, class III IV etc or Hazardous, extremely hazardous, slightly hazardous, oral LD5.

Principles and Procedures of Acute Toxicity Studies

The most frequently performed test is acute systemic toxicity assessment. In this study, fewer of animals are used than for chronic studies. In acute effects study 20-60 animals are used. The use of laboratory animals has been an integral part of the screening for toxicity of chemicals. These chemicals may damage one or more of the major organ systems with varying severity. In acute toxicity studies there is need to compare animal species to aid extrapolation of effects in man (Parke 1983). Traditionally, the emphasis in acute toxicity studies was to determine the LD 50, time of death, the slope of the lethality curve, Acute lethality testing designed to determine the amount of chemical that causes death.

A primary focus has been the LD 50 test. Generally this study deals with the adverse effects of single doses, as delayed effects may occur due to accumulation of the chemical in tissue or other mechanism, and it is important to identify any potential for these by repeated dose testing. Dosing period distributed between the single dose and 10 percent of life span dosage are often called subacute.
They may be described as short terms repeated dose studies this applies to 14, 21 and 28 day studies.

The term supra acute, to describe dosing periods of a week or less and subacute per single doses below acute dose levels have been recommended by God and Chengelis (1988).

The main repeat dose study protocols have usually employed durations 14, 28 and 90 days respectively.

The term sub-chronic' has been used to embrace the toxic effects associated with repeated doses of a chemical over greater than a 10 per cent part of an average life span of experimentals animals.

**Parameters studied in Acute Systemic Toxicity Assessments.**

* Establishing a dose response relationship for exposures at which LD 50 under study probability of a known fraction of population of a species under study.

* Dose ranges for subsequent studies.

* Potency, ranking from extreme to non-toxic

* Identifying probable physiological systems/target organs being affected.

* Extent or degree of effects e.g. subdued behaviour, coma, death

**Minimal regulatory guideline requirements.**

**Additional data**

**Signs, time of onset, duration and recovery-time**

**Morbidity:** agonal changes, reflexes, pharmacological effects, dose response curves (ED 50)

**Lethality:** Dose response (LD 50 with confidence limit, estimation of median lethal dose LD 50 with estimation of minimum
lethal dose LD 1 estimation of certain lethal dose LD 100.

**Body weight:** Decreased body weight, gain, reduced food consumption.

**Target organ identification:** Necropsy and gross tissue examinations, Histological examinations, Blood clinical chemistry, Haematology. Physiological function, immunology, neuromuscular reflexes, behavioural screening, electrocardiogram, Electroencephalogram.

**Pharmacokinetic:** Therapeutic index, bioavailability

**Pharmacodynamics:** Relationship between plasma and tissue and occurrence of clinical signs.

**Protocol design:**

The exact protocol depends upon the type of chemical substance and the country in which it will be registered for use. (Zbinder and Flury-Rover si 1981, Oliver et al 1985) There is a considerable amount of hormony in the requirements of acute oral, dermal, inhalation and parenteral toxicity. Often both sexes of two species, employing a route of exposure which is anticipated to be the most probable route of exposure for man is necessary for regulatory purposes. Rats and mice are the species typically selected. The use of parenteral and oral route comparisons was developed primarily in order to gain information about the bioavailability of the chemical.

The experimental design for acute systemic toxicity assessment has for many years been modified, i.e. interval dose levels applied to groups of experimental animals such that an incidence of response can be achieved varying from zero incidence.
to 100% response and the median lethal dose can be derived.

In systemic toxicity testing experimental design should allow broad group classifications of extremely toxic, very toxic, and practically non-toxic.

For above classification one can make use of the induced experimental signs along with LD 50 .

A wide variety of intrinsic and extrinsic factors can influence the outcome of a test (morrison et al 1968, Balaze 1976, Aulette 1988). In order to establish a dose response relationship, the same species, strain, sex and age should be divided randomly into equivalent size groups with the different groups treated at the same time of day with different dosages by the same route and observed for a set and consistent period of time.

All protocols should state the ceiling or limit dosage. Small differences in protocols are probably the major cause of considerable laboratory to laboratory variations in results achieved (Lorke 1983). Protocols include necropsies on all animals found dead and those killed following the 2 weeks post dosing observation period. Body weights are determined on the day i.e. prior to dosing, 7th day and 14th day. Animals should not differ in age by more than 15%.

The assessment of acute systemic toxicity, is the assessment of the potential for severe health effects which results from the major systems of the body, i.e. cardiovascular, respiratory, CNS excretory and locomotor system being compromised by adverse change. The aim of qualitative extrapolation of toxicity data from animals would be to predict potential signs of toxicity in
man. Clinical signs can be reversible or irreversible. Reversible are those that disappear as chemical is cleared from the body. Irreversible are those that do not disappear and are accompanied by organic damage.

Signs also represent pharmacological response which may be adverse. The kidney and liver are most frequently the target organs of acute toxicity.

In Vivo studies:

A small number of animals per dose are administered fixed dose levels. The highest top dose approximates to the limit dose specified by regulatory guidelines. The set dose method works best if the doses are separated by constant multiples (Schutz and fuchs 1982, Lorke 1983 British toxicology society, 1984) Lorké's protocol design consists of 3 animals per dose at 10, 100 and 1000 per Kg. Animals were observed for 14 days post dosing. The dose range was defined as 1.5 x a multiplication factor. The approximate lethal dose is the highest dose that did not cause death.

Sequential up and down dosing

The format for this type of procedure requires single animal to be exposed, with subsequent doses adjusted up or down by some constant factor depending on outcome of the previous dose (Bruce 1985). In Bruce's method, an individual animal is dosed, if an animal dies the dose is decreased by constant factor, and another animal is dosed at this level.
Factors affecting acute systemic toxicity studies

The various factors that influence the outcome of acute systemic toxicity studies are in many respect equivalent to those that modulate all biological studies.

FACTORS AFFECTING THE ASSESSMENT OF ACUTE TOXIC HAZARDS

<table>
<thead>
<tr>
<th>Factor</th>
<th>Type</th>
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<tbody>
<tr>
<td>1. Route of exposure</td>
<td>Ingestion, inhalation, dermal, parenteral.</td>
</tr>
<tr>
<td>2. Severity of effect</td>
<td>Harmless, Harmful, toxic, very toxic, extremely toxic, irritant corrosive</td>
</tr>
<tr>
<td>3. Speed of onset</td>
<td>Immediate, delayed</td>
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<tr>
<td>4. Duration of effect</td>
<td>Acute, persistent, irreversible, reversible</td>
</tr>
<tr>
<td>5. Duration of exposure</td>
<td>Single short duration (acute) short duration (sub acute) multiple long duration (sub chronic) continuous (chronic)</td>
</tr>
</tbody>
</table>

There are many factors which act as variable. e. g.

1. Interspecies differences relating to heterogeneity of populations, specific physiology, basal metabolic rate & size.

2. Intraspecies variation regarding strain, sex, age.

3. Environmental, including route of exposure, amount, physical form, formulation, exposure, intervals, observation intervals.
4. Statistical, including experimental bias, group size, size of population.

All the above factors contribute to the difficulties in achieving reproducible accurate and precise data.

A Testing for acute toxicity.

The objectives of an acute toxicity study are, to define the intrinsic toxicity of a compound, to assess to
1. Susceptible species
2. Identify target organ of toxicity
3. To provide information about risk of using the compound.
4 To provide information for the design and selection of dose levels for prolong studies.

From a regulatory view point, acute toxicity data are essential in the classification, labelling and transportation of chemical. From Academic stand point a carefully designed study can produce information about structure activity relationship.
CONSENSUS BASIC PROCEDURE COMPRISING ACUTE, SUBCHRONIC AND CHRONIC ORAL TOXICITY TESTS

<table>
<thead>
<tr>
<th></th>
<th>Acute oral</th>
<th>Subchronic oral</th>
<th>Chronic oral</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal</td>
<td>Rats</td>
<td>Rodent</td>
<td>Rodent &amp; Nonrodent</td>
</tr>
<tr>
<td>Sex</td>
<td>male</td>
<td>female</td>
<td>equal</td>
</tr>
<tr>
<td>Age and weight</td>
<td>Young adult weight within 20% of mean</td>
<td>Rodent 6 wks; dogs 4-6 months old</td>
<td></td>
</tr>
<tr>
<td>No. per dose level</td>
<td>10 animals</td>
<td>20 Rodents</td>
<td>50 animals</td>
</tr>
<tr>
<td>Vehicle control</td>
<td>if suspending agent of unknown toxicity is used</td>
<td>yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Fasting</td>
<td>Rat overnight</td>
<td>Not appropriate</td>
<td>Not appropriate</td>
</tr>
<tr>
<td>Observation to be made</td>
<td>Nature, onset, severity of effect, and its duration are observed</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Selection of dose levels: the purpose of an acute toxicity study is to establish the degree of toxicity of a new chemical entity. Dose level should be sufficient in number. At least three doses should be taken to get a clear dose-response relationship.
Principles of acute oral tests.

The test substance is dissolved in water or in appropriate solvent, if it is insoluble in aqueous media. If all attempts to dissolve the material fail, then prepare a suspension with appropriate suspending agent to get homogeneous dosing solution. The dosing solution is administered by gastric intubation. Animals in the control groups should receive the same volume of vehicle.

There are two methods of dosing the test material. 1. By varying the dosing volume which means that, animals are given different volumes of the same dosing solution. 2. By varying the concentration of the dosing solutions so that animals are given the same volume of vehicle.

The different methods of dosing can result in different toxicities for the same compound, for instance, when a large volume of corn oil is given orally, it will increase gastrointestinal mobility and have a laxative effect which may decrease the time of the test substance for absorption in gut, conversely irritation of the gut will be decreased when the test substance is given in a diluted form. The volume being kept as small as possible and certainly not to exceed 10 ml/1 kg body weight. The choice between constant concentration versus constant dose volume should be based on scientific judgement. If dose is too large, it can be administered 2 equal doses by keeping interval of 3-4 hrs.

Choice of animal species

Any common laboratory animal may be selected. It is recommended that repeated dose studies be conducted on at least
two species, one being rodent and another nonrodent.

The animal of choice should have a similar response to the test substance to that of humans. If this information is lacking as is often the case, it is good practice to select the most sensitive species to evaluate the safety of the substance. Repeated dose response studies should be carried out by the route, by which man is most likely to come into contact with the substance. In practice, dogs are most commonly used nonrodent species, as multiple blood samples can be taken without sacrificing the animal, the most common route employed in repeated dose studies are oral.

Young and still growing animals are preferred, commonly rats and mice of 6-8 weeks of age are used. Variation in responses due to sex differences may be important. Therefore each group should consist of equal numbers of male and female animals, usually 10-20 animals per sex dose group are taken. The number of animals will need to be increased if interim kills are to be made and if reversibility of effects is to be examined.

Testing for chronic toxicity

Nowadays, the term 'Chronic toxicity' include multigeneration reproduction studies and carcinogenicity test. However only classical chronic toxicity studies which are undertaken to define a safety factor between proposed use or exposure levels. In this study three treatment groups are taken along with control. The xenobiotic is administered 7 days a week for 2 yrs to rats or for 18 months to mice. Dose levels are usually selected after a three months range finding study. This dose defined as the
maximum tolerated dose (MTD) and this is selected as the highest
dose. The two other dose levels are usually 1/4 MTD and 1/8 MTD.

Alternatively, with a relatively non-toxic compound the dose
may be chosen as not exceeding 100-200 times the anticipated
human dose.

Interpretation of results.

All the sub-acute, sub-chronic toxicity studies are
expensive to perform. Observations should be made frequently.
Onset, severity and duration of any observed effect should be
recorded.

Animals found dead should be refrigerated. If necropsy can
be performed immediately it will be excellent. Expression of the
food consumed per unit weight gain is an useful way for
assessing the effect of the treatment on eating habits and food
utilization. Fluid consumption is useful way of identifying an
agent with diuretic action.

The life of modern man has been greatly improved by the
development of chemicals employed in all spheres of human
activity. However, in doing so we must ensure that our own
existance is not endangered by their uncontrolled use, about
which we know so little but are begining to learn more. What we
do know is all that obtained from animal studies conducted under
controlled conditions on a short intermediate or long term basis.
All of these studies are looking for dose-response relationships
so that we may extrapolate them to the human situation. Which is
often uncontrollable. Animal studies can tell us about
observable effect levels, which are used to derive acceptable
daily intakes for environmental contaminants and other chemicals.
The toxicological studies described are applicable to most xenobiotics and, when coupled with tests for reproductive toxicity, mutagenicity/genotoxicity, and carcinogenicity, will usually satisfy the regulatory requirements of most countries. Because of this over-riding ambition, many toxicological experiments have become rather stereo typed. However, a toxicological experiment should not be different from an experiment in any other scientific discipline. Thus, the experimental design should be logical and hence, there is no valid reason to adhere to a standard protocol if this is inappropriate for the specific test material. Regulatory guidelines are only a useful index of what is required and should not be used as a rigid checklist, because there are virtually no findings in experimental toxicology that can be simply extrapolated to man and his ecosystem without careful thought. When it is considered appropriate to modify an approach recommended by a particular regulatory agency, it is wise to discuss this with and get agreement from the agency in order to avoid subsequent administrative complications.

Furthermore, by performing experiments with a standardized protocol, toxicologists are missing real opportunities to unravel some of the most fascinating problems of biology; by identifying chemical tools to probe life processes; There may often be too much emphasis on the generation of data with very little time available for scientific interpretation. It is this aspect of toxicological research that must be encouraged, to survive or else the science will become as stereotyped as the studies.
conventionally performed for economic regulatory purpose. Therefore, one should consider abandoning a standard protocol if it is inappropriate and feel free to increase the number of animals employed, particularly with regard to reversibility studies because the best way of reducing the number of experiments performed on living animals is to conduct them properly. However, as noted above, studies carried out for regulatory agencies should be discussed with the agency before proceeding with extensive modifications.

It is essential to randomize the animals at the beginning of the study, as well as during intermediate and terminal investigations. One should always look for dose-response relationships and compare the data obtained with the controls as well as appropriate historical values for animals of the same age, sex and physiological condition. Above all, ensure that all the observations are recorded as the purpose of a toxicological experiment is the same as that of any other scientific study and that is it can be repeated, blemishes and all.
Screening and evaluative programme
(Laurance, 1964 : 45-55, Nodine, 1963)

Drug screening is essentially a screening procedure designed to distinguish useful from non useful drugs as rapidly, comprehensively and inexpensively as possible. To screen effectively, the investigator is forced to impose numerous limitations as to the species, route of administration, and procedures employed, and as a consequence he must accept the possibility that, he will overlook a useful drug.

One of the most important aspect of drug screening and evaluation is the problem of decision making the "yes" or "no" with respect to interest in the compound. It is important to obtain in earliest phase of screening the information essential to making this decision. It seems, best to proceed from the general observation to the specific and from comprehensive observational techniques, wherever they are applicable, to the use of instrumentations.

Ideally, the pharmacologist should be able to predict, within reasonable limits, the effective dosage, actions, side effects, and potential toxicity, as well as the patients population most likely to benefit from the drug.

An important concept, in drug screening is that drugs may act similarly in animals and human subjects and that
laboratory animals can therefore serve as "model analogues of man." However all biologists are aware that this relationship is tenuous and that vast differences can exist not only between human and animals but also between various animal species.

It is generally accepted in pharmacology that interspecies differences in response to drugs are least likely to occur in closely related phylogenetic form. However, the probability of carry over of drug effects from animals to man seems to be greatest, when diverse species show similar responses to a given drug and least when their responses vary widely. It is important, therefore, that new drugs be evaluated for their effects on several animal species, particularly in species from different orders.

Second important concept in screening is that, the drugs are more readily differentiated or classified from multiple measures of their activity (from the overall profile of their action) than from any single measure. This is true because observed actions frequently arise through different mechanisms and may be shared by different drugs. Houde et al. have demonstrated in spinal dogs that, although barbiturates, muscle relaxant and narcotic analgesic, all depress the flexor reflex, the drugs can be differentiated and classified, if one also considers their differentiated effects on the other reflexes: e.p.silateral extensor trust knee jerk and the e.p.silateral etensor trust.

The most efficient primary screen would seem to be a comprehensive battery of tests which reveals the dose
response profile, the toxicity and the pattern of specificity action of a new drug, a multi-dimensional approach.

**Lacunae in drug screening**

The hazards in drug screening are those which lead to a failure to discern the important action of a drug. They are derived from the limitations necessarily imposed on the process on the species, or strain selected, dosage, route or duration of administration, time of recording or from the nature and range of the observations made.

As long as we do not have suitable analogues of human disease states when studying drug effects in animals, it will be impossible for the pharmacologist to predict the effect of drugs on such states with certainty. Once these effects have been discovered, the pharmacologist could establish a host of laboratory measures to reveal agents with similar activity. An analysis of the major weaknesses in drug screening and evaluation faced by present difficulties in drug selection and clinical prediction follows.

**Standardized multidimensional observation**

The two approaches—those of subjective observation and objective recording are complementary and furnish different kinds of information. The pharmacologist performs a series of laboratory tests at different times, under different conditions, in different species by different routes of administration, integrates the information and may complete his analysis. The clinician observes symptomatic changes within a direct clinical contest. The physicians have been looking more and more to the laboratory for establishment or confirmation of a
diagnosis, conversely the pharmacologist may benefit from a more clinical approach in his evaluations.

The use of observation technique has several major weaknesses, but these are not intrinsic or unsurmountable. It would be pointless to deny that individuals differ in their ability to observe. It requires greater attentiveness and skill. It also requires commensurate training. Other weaknesses are subjective bias. Rating scales used may be non linear and non addition and a tendency to use insufficient number of steps in the rating scale.

**General activity and acute toxicity screening**

This is a comprehensive procedure for use in small animals (Rats, mice) which makes it possible to quantify & correlate in each animal a wide variety of grossly observable changes produced by drugs. e.g. behavioral, neurologic, autonomic and toxic. The sole equipment used in obtaining information is a pair of forceps and hypodermic syringe and a trained observer. In this procedure, 3 animals per dose level are given drug & are housed together in a round glass observation jar, control animal is used for comparison. After treatment, the animals are systematically observed to measure the onset, peak, duration, character and intensity of drug action. Toxicity is observed over a 5 days period. For the purpose of screening, only peak drug effects are recorded. Drug effects are quantified by scoring techniques e.g 0-4 degree of impairment of the righting reflex or diminution.
of struggle response elicited by holding animals in unusual position.

The information obtained is multidimensional. Since one can derive from the data of the nature of the drug actions, the lowest effective dose, the specificity of the effect obtained e.g. ratio between the 'effective' and 'side-effect' dose and the lethal dose. About six doses of drug can be evaluated in a single day. From the profile of the responses obtained, many different classes of behavioral, neurologic or autonomic drugs can be differentiated and/or identified.

Dosage

It is generally believed that human beings are more sensitive to drug effect than laboratory animals and thus require greatly reduced dosage.

This observation assumption particularly in neuro- and psycho pharmacology, probably derives from the fact that in most evaluation procedures with animals, the responses measured, e.g. marked motor incordination, locomotor stimulation, depression are quantitatively and often qualitatively removed from the effects sought clinically the responses measured. When more sensitive techniques are employed to measure in animals the same indices of change that are observed in humans, we find that an unusual degree of correlation exists between man and such species as cat and dog.

Such predictions, of course cannot always be made, species difference in response to drug do exist.
**Experimental Work:**

**Experimental evaluation of Drug activities**


Pharmacological and toxicological studies in animals should allow prediction as whether or not use may be made of a substance in medicine.

The object of any experiment is to reach specific conclusions from which generalisation may then be made. In experimental pharmacology, how particular animals in fact respond to a drug is almost never the only need, or even the main reason for carrying out a test. It may be done to get information, may be about the action of the test substances in general or to determine what is the best design for a particular experiment or to establish the optimum composition of a bath solution or any one or more of many other objectives.

The pharmacologist moreover, must always have in mind that the general applicability of the conclusions he draws from his experiment, may be restricted sometimes within remarkably narrow limits.

**Factors affecting sensitivity - Inter species difference**

It is well known, that many substances have different effects on animals of different species. Thus it has long been established that the cerebral effects of morphine are almost purely depressant in dogs, rabbits, Guinea pigs, rat and birds, whereas its deliriant action is specially prominent in cats, but also operates in horses, cattle, sheep and goats. In the
second group of animals the deliriant action is associated with various degrees with lethargic and analgesic effects.

It is also possible for a response shown unequivocally by one species of test animals to be entirely absent from another e.g. oxytocin-lowering B.P. in hens, but it has no effect on B.P. no effect on male or dioestrous female rats. (Lloyd and Pickford 1961).

The pharmacologist, in generalising from his experimental findings must remain constantly alert for the fact that great quantitative difference may exist between the reactions of different species to the same drug and that qualitative differences in effect also frequently occur. In forming conclusions from experiments on some particular species of animal, the question always arises whether the findings so establishing will apply to animals of different species. Experience and experience alone, has made it clear that in fact they often do.

**Intra species difference**

Different strains of the same species may show marked differences in sensitivity to the same drug. This has become especially apparent since increased attention has been paid to the breeding of laboratory animals with an emphasis on in-breeding and uniformity, so that the characteristics of different inbred strains can be compared.

**Inter Sex Difference**

Males and females of the same strain can differ considerably in sensitivity to the same drug.
It must further be born in mind that such a sex difference may exist in one animal species and not in another. The effect of sex on the therapeutic action of several drugs (hurst 1988.) is of slightly different nature.

Age Difference

The age of the animals must also be taken into account, when generalising from experimental results about sex difference.

It has not always been realized that the use of simple commonly employed procedures can sometimes influence the results. e.g. mating, feeding, fasting, water adlibidum, day and night rhythm and handling.

Extrapolating the conclusions from animal experiments to other animals can thus be limited by factors whose importance is often under-estimated. Such factors, among others are caging arrangement before test, method of immobilisation during test and certain environmental and physiological considerations.

Extrapolation from animals to man

The ultimate purpose of most, if not all pharmacological and toxicological research is to find therapeutic agents suitable for use in man. Few of the experiments considered useful by the pharmacologist can be carried out in man. Fortunately experience has shown that animal experiments do indeed frequently lead to correct predictions of the human response. If this were not so, much of the pharmacological research done on animals would be of little value. The usefulness, and the relative safety of a substance designed for use in human therapy can never
be finally or conclusively demonstrated by animal experimentation alone. On the first trial of a new drug in man, the investigator may indeed meet some surprises. This is one reason why it is so important to lay down as clearly as and as completely as possible the information useful and essential about substance of possible therapeutic value before preliminary test on human subjects.
Experimental Techniques

**Experimental evaluation of analgesics in animals**

There are several controversies in the evaluation of analgesics in animals. One is, whether the animal can interpret the stimulus as painful as the investigator considers it painful. Therefore results may not be real to guide the clinical use of drugs, according to some.

Tests for Analgesic Evaluation Devoid of variable like

1) Subjective variation in the reporting of pain, as pain is a subjective phenomenon.

2) Similarly placebo reactions leading to false positive results.

3) Subject and observer interactions leading to bias. Animal experiments preliminary guide as to whether any work on healthy subjects and patient should be undertaken.

4) Subject and observer interactions leading to bias. An ideal method in the laboratory set up should satisfy some requirements, such as-
   - The stimulus should cause as little tissue damage as possible.
   - Its entering should be parallel to that of the pain it produces.
   - Reaction should be accurately reproducible.
   - It is to be induced at that part of body where individual variations are minimum.
   - Response should be a clearcut phenomenon and its occurrence in time and intensity should be proportional to the stimulus.
   - It should be sensitive enough to detect analgesics of low potency.
The actual tests employed in analgesic evaluation are perhaps as numerous as the number of investigators in this field (Collier, 1964; Sheth and Sethy, 1967; Beecher, 1957).

The following are the most commonly employed analgesic tests in animals.

I. Tests based on mechanical stimuli

The most commonly employed tests in this category are based on pressure on the tails of mice and rats using various means like artery forceps, bulldog clips, pinch cock, (Bianchi and Franceschini, 1954) metal rods (Eagle and Carlson, 1950) plunger of a syringe (Green, 1951). The end points are the animal's responses such as squeak or struggle. Repeated use of the same animals leads to conditioning and lowering the threshold. This can be avoided by making just one or two observations before and after the drug.

Allied methods include applications of artery clips to the toe of the Guinea pig or rat (Collier et al., 1961), Pressure on inflamed foot of the rat after injecting a suspension of brewer's yeast (Randall and Selitto, 1957).

II. Test based on chemical stimuli

The writhing induced by intra peritoneal injection of acetic acid, 2 phenyl 1, 4 benzoquihè (Vander-wende and Morgadia, 1956) or bradykinin (Sheth and Shethy, 1967) is the basis of analgesic evaluation. Bhalla et al. (1969) employed aconitine to induce writhing. There are great variations in the frequency of the writhing and many agents including those possessing no analgesic effect block the writhings. These drawbacks apart,
the relationship of writhing to pain as such is doubtful. However the writhing test is very sensitive when performed in mice, though not on rats (Vonder-Wende and Margalia, 1956) The "Squeaking flight" produced in Rabbits by intracisternal administration of bradykinin is also of questionable value (Sicuteri, 1968)

III. Tests based on Thermal Stimuli

Heat as a painful stimulus can be employed either by radiation or by conduction. The commonly used radiant heat methods are -

1) Focussing light rays on the tip of rats tail.(D Armour and Smith 1941) -
   -( Ercoli and Lewis 1946) focusing a strong beam of light on to a shaved area of the back of a rat. The response is lifting of the tail, or skin muscle twitch, if the back is irradiated
   
   Test employing conducted heat are -

1) Dipping the tail of mouse or rat in hot water.
   (Ben Bassant 1959, and Janansen 1963)

2) Using the "mouse hot plate" the temperature of which may be maintained by a light bulb (Wolff and mac Donald 1944) (Eddy 1950) or a by boiling mixture of ethyl formate and acetone (Cheh and Beaman, 1951). The response is lifting of the tail from hot water or kicking with hind paw as in the hot plate method.

IV. Tests based on electrical stimuli

Ever since (Von Helmholt 1951) used faradic current to produce pain, many electrical methods are employed to study
analgesics. The commonly used ones today are those of (Goetzel, 1943) using tooth pulp stimulation in dog (Thorpl, 1946), stimulating the scrotal sac of the rat (Grewal, 1952), stimulating the tail of the mouse and (Charles, 1961) using the dolorimeters to stimulate the foot or the rectum of rodents.

V. Miscellaneous tests

1) Behavioral tests on rats involving Skinnerian operant behavior to note the "aversive threshold" (Sheth and Sethy, 1967).

2) Oxytocin cramping test in rats (Turner, 1956) this is more applicable to the narcotic analgesics.

3) Functional impairment after pain being studied after injection of formalin in the hind limb of the dog (Pardo and Rodriguez, 1968)

In Vitro test, Blocking the stimulation of guinea pig ileum by arachidonic acid peroxide (Jaques, 1965) However the validity of this approach is doubtful.

The choice of the test for new analgesic depends on the type of activity one looks for. In the case of narcotic type of analgesics, tests depending on superficial stimulation (e.g. thermal and electrical methods) will be preferred and for non-narcotic analgesics tests like mouse peritoneal tests and tooth pulp stimulation are better. This is apart from other criteria like economy in time and animals etc. The ultimate utility of any analgesic drug, however is to be tested on patients with pathological pain (Collier, 1964).
Hepatotoxicity

In review of available literature regarding medicinal plants with hepatoprotective activity, it is revealed that different hepatotoxins were used by different workers to evaluate the activity in invitro + vivo models. In some studies more than one hepatotoxin was used to screen the same plant. The most commonly used hepatotoxin was carbon-tetra-chloride. Out of 104 studies CCl₄ was used in 78 studies. Irrespective of the route administered, was in the range of 0.2 - 2 ml/kg in acute liver damage with one day treatment and in the range of 1.5 - 5 ml/kg in divided doses over a period of 1 week.

Chronic reversible damage was produced with doses ranging from 12 to 28 ml/kg in divided doses over the period of 5-12 weeks.

Next to CCl₄, galactosamine and paracetamol was used in 14 and 7 studies respectively. Rest of the hepatotoxins used were monocrotoline, country made liquor, paraquat phalloidin, allyl alcohol, fat rich diet, aluminium sulphate, Lead nitrate, acetaminophen and alpha-naphthyl-iso-thiocynate in one study each.

The most commonly used parameters to assess the hepatoprotective activity were morphological e.g. liver weight, and volume, biochemical estimations, such as measurement of transaminase activity, SGPT, SGOT, alkaline phosphatase, Lactic dehydrogenase, Serum bilirubin, total serum proteins, albumin and globulin and prothrombin time.
Functional parameters:

Pentobarbitone and hexobarbitone sleeping time and oxalamine induced paralysis and finally histopathological study regarding presence of necrosis, fatty degeneration, cirrhosis.

Apart from the routine biochemical tests used, few workers have estimated RNA, DNA, cytochrome P-450, Neucleolidase, acid phosphatase glycogin, glutethione.

Lipid peroxidation, serum alanine aminotransferase malon-aldehyde formation as a parameter of lipid peroxidation and measurement of free radical formation.

In all these studies, alteration or changes i.e. raised or decreased levels caused by a particular hepatotoxin and its reversal in these changes was taken as a criteria for hepatoprotective effect.

Out of 104 studies, 15 studies (used in vitro method for screening) medicinal plants were used, where in increase in percentage of viability of cells and enhancement of the rate of viability of cells and enhancement of the rate of consumption of oxygen and reversal of the enzymatic values such as SGPT, SGOT, ALT in primary cultured hepatocytes was noted. These methods have most commonly used by Japanese investigators. They have standardised in-vitro screening procedure, employing primary cultured hepatocytes. In India, this in-vitro method of screening medicinal plants for their hepatoprotective activity is not used extensively, may probably due to technical difficulties and non-availability of the facilities to culture & maintain hepatocytes. Large Scale primary screening is possible with this method and further detail study can be done afterwards.
Vivo methods are rather time consuming and costly since no. of animals (rats or mice) required are more, and study of various parameters, such as biochemical, and histopathological studies adds to its cost, with all these only one plant can be screened at a time.

Liver injury may follow inhalation, ingestion or parenteral administration of a number of pharmacological and chemical agents. A variety of therapeutic agents can produce hepatic injury, most of these are hepatotoxic in therapeutic range. A high incidence is with C17 substituted anabolic steroids like testosterone (1.1%), with phenothiazine (0.6%), with sulfonamides antituberculars drugs and many other.
TYPES OF HEPATOTOXICITY

Hepatocellular injury seems to be the primary event in various hepatic drug reactions. Two major types of chemical hepatotoxicity have been recognised. (Sherlock, 1985: 304-329)

1. Direct toxic type
2. Idiosyncratic type

Direct metabolite related hepatic injury.

Drug

\[ \text{Metabolite} \]

\[ \text{Free Radical} \]

\[ \text{Electrophilic metabolite} \]

\[ \text{Lipid Peroxidation} \]

\[ \text{Covalent binding} \]

\[ \text{to liver} \]

\[ \text{macromolecules} \]

\[ \text{Membrane Damage} \]

\[ \text{Cell Death} \]
The drug metabolising enzymes in the liver activate the chemically stable drugs to produce alkylating, arylating or acetylation agents, these electrophilic metabolites bind covalently to liver molecules which are essential to the life of a hepatocyte. This binding exhausts of intracellular substances, such as glutathione which are capable of preferentially conjugating with the toxic metabolite.

Secondly, metabolites with unpaired electrons may be produced by oxidative reaction of cytochrome P-450. These free radicals can also bind covalently to proteins and to unsaturated fatty acids of membrane. This results in lipid peroxidation and membrane damage. The end result is hepatocellular death. (Kaplowitz, 1986: 826)

The morphologic pattern of hepatic injury of direct toxic type is reasonably characteristic and reproducible for each toxin. Light microscopy shows clearcut necrosis, with or without fatty change in the parenchyma of liver. (Dienstag, 1991: 1333-1337)

Idiosyncratic reactions lead to a morphologic pattern, that is more variable than those produced by direct toxins; a single agent is often capable of causing a variety of lesions; Hence there are no animal models for such reactions. The morphologic picture may be indistinguishable from that of viral hepatitis. Morphologic alterations may also include bridging hepatic necrosis.
<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Name of the Hepatotoxin</th>
<th>No. of Studies Conducted</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Carbon Tetrachloride</td>
<td>83</td>
</tr>
<tr>
<td>2</td>
<td>Galactosamine</td>
<td>18</td>
</tr>
<tr>
<td>3</td>
<td>Paracetamol</td>
<td>9</td>
</tr>
<tr>
<td>4</td>
<td>Thio-acetamide</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>Tertiary Butyl Hydroperoxide</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>Monocrutaline</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>Country Made Licquor</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>Paraquat</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>Phalloidin</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td>Allyl-Alcohol+CCL₄</td>
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</tr>
<tr>
<td>11</td>
<td>Fat-Rich Diet</td>
<td>1</td>
</tr>
<tr>
<td>12</td>
<td>Lead Nitrate</td>
<td>1</td>
</tr>
<tr>
<td>13</td>
<td>Aluminium-Sulphate</td>
<td>1</td>
</tr>
<tr>
<td>14</td>
<td>Alpha-Nepthyl-ISO-Thiocyanate</td>
<td>1</td>
</tr>
<tr>
<td>15</td>
<td>Cortisone Acetate</td>
<td>1</td>
</tr>
<tr>
<td>16</td>
<td>Isoniazid</td>
<td>1</td>
</tr>
</tbody>
</table>
Carbon tetrachloride

The mechanism of CCl\textsubscript{4} induced hepatotoxicity has been extensively studied. Several reviews have been appeared on this topic. The leading theory for the mechanism of cellular damage caused by CCl\textsubscript{4} is that the compound is bioactivated by cytochrome P-450 mediated reactions to CCl\textsubscript{3}O\textsubscript{2}, free radical which is further converted to as peroxy radical CCl\textsubscript{3}O\textsubscript{2}. There is evidence for covalent-binding of CCl\textsubscript{4} upon bioactivation, CCl\textsubscript{3}O\textsubscript{2} radical is also thought to decompose to phosgene and electrophilic Cl\textsuperscript{-},Which can react with other macromolecules. The free radicals CCl\textsubscript{3} and CCl\textsubscript{3}O\textsubscript{2} readily react with polyunsaturated fatty acids of the endoplasmic reticulum and other hepatocellular membranes to initiate the formation of organic lipid peroxides. (Farber, 1984 : 715-755)

In the presence of cellular O\textsubscript{2} these organic peroxy radicals in turn can react with other polyunsaturated fatty acids to perpetuate a series of self-propagating chain reactions, a process referred to as propagation of lipid peroxidation. The coactivation of CCl\textsubscript{4} and initiation of self-propagating lipid peroxidation working in random destroy the cellular membrane leading to cell death. The principle hepatic lesion is characterised by centirubular necrosis. The extent of injury depends upon dose. The demonstration of metabolism of CCl\textsubscript{4} to CH\textsubscript{3}Cl\textsubscript{3} to CO\textsubscript{2} and covalent binding of CCl\textsubscript{4} to liver proteins and lipid, lend experimental support to bioactivation of CCl\textsubscript{4} injury.
Carbon tetrachloride is a simple molecule which when administered causes centritubular hepatic necrosis and fatty liver. It is lipid soluble and well distributed throughout the body. It is toxic to the liver irrespective of route of administration.

Low dose causes only fatty liver destruction of cytochrome P-450, while chronic administration leads to liver cirrhosis. Carbon tetrachloride is metabolised by the microsomal enzymes via a free radical. Hepatotoxicity of carbon tetrachloride is increased by microsomal enzyme inducers and decreases by enzyme inhibitors, indicating that metabolic activation is required for hepatotoxicity. The production of necrosis particularly in the centritubular region may probably be due to presence of higher levels of cytochrome P-450 to this region.

Microosomal enzyme - mediated activation of CCl₄

CCl₄ \[\rightarrow\] e⁻ \[\rightarrow\] CCl₃ + Cl⁻

Carbon tetrachloride Trichloromethyl radical

CCl₃⁺ Rh \[\rightarrow\] CHCl₃ + R

The role of microsomal enzymes is as an electron donor. The bichloromethyl radical so produced react with cellular constituents and abstraction of hydrogen atom leads to the production of chloroform. Chloroform is produced in microsomes in the absence of air, indicates that metabolic transformation although dependent upon cytochrome P-450 probably involves some form of electron transfer independant of oxygen.
Trichloromethyl radical and peroxy radical, the reactive intermediates produced, will have a small radius of action. The immediate damage caused by CCl$_4$ is in the smooth endoplasmic reticulum, the site of metabolic activation and cytochrome P-450 is destroyed.

Small doses of CCl$_4$ destroy hepatic cytochrome P-450 decreasing activity by 75% and thereby protect liver against further large doses of the compound.

Formation of trichloromethyl radical is not the major cause of damage. A cascade of events initiated by radical reactions is responsible.

As already mentioned, metabolism of carbon tetrachloride by the microsomal monooxygenase is necessary for hepatotoxicity and the product is certainly a radical such as Trichloromethyl radical. This radical reacts particularly with SH-groups and methylene bridges on polyunsaturated fatty acids. Because of its short half life, its radius of action is small and probably confined to the immediate vicinity of cytochrome p-450. Production of this radical and its covalent interaction with proteins occur in the absence of oxygen, but destruction of cytochrome p-450 and damage to microsomes measured in vitro as less of glucose-6-phosphatase activity occurs in the presence of oxygen. Hence covalent binding of trichloromethyl radical to cellular constituents may not necessarily be of major importance to the toxicity.

The more important event is the production of lipid radicals which on reaction with oxygen form peroxidase and eventually
short-chain aldehydes and hydroxy alkenates. It seems now clear that although the trichloromethyl radical may be important in damaging cytochrome p-450 and its immediate vicinity, distant cellular targets are damaged by products of lipid peroxidation in a cascade system. Damage to structural lipids particularly of the smooth and rough endoplasmic reticulum by lipid radicals and their peroxidase plays a major role.

Inhibition of protein synthesis occurs and is partially responsible for the fatty liver and not for necrosis.

Altered membrane function due to the direct attack by radicals or from peroxidation is a major event.

It is clear that both the covalent binding of carbon tetrachloride metabolites to proteins and lipids and lipid peroxidation result from the metabolic production of the trichlororomethyl radical. The reactions of bichloromethyl radical or peroxy derivates may be responsible for the damage of cytochrome P-450 and its immediate vicinity. The major cellular damage is however probably the result of lipid radicals produced by the interaction of trichloromethyl radical with unsaturated lipids and fatty acids. The cascade of events set in train by these lipid radicals includes membrane damage which may lead to cellular necrosis.

Histopathological patterns of Hepatic Injury

The histopathological pattern of hepatic injury varies with different hepatotoxic agents. Principal alterations of hepatic morphology produced by some commonly used may be listed as follows. (Diestag, 1991)
# Drug-Induced Hepatotoxicity

<table>
<thead>
<tr>
<th>Principal morphologic change</th>
<th>Class of agent</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Necrosis</td>
<td>Analgesic</td>
<td>Paracetamol</td>
</tr>
<tr>
<td>2) Fatty Liver</td>
<td>Chemotherapeutic</td>
<td>Tetracycline</td>
</tr>
<tr>
<td></td>
<td>Anticonvulsant</td>
<td>Sod. valproate</td>
</tr>
<tr>
<td></td>
<td>Antiarrhythmic</td>
<td>Amiodarone</td>
</tr>
<tr>
<td>3) Hepatitis</td>
<td>Anaesthetic</td>
<td>Halothane</td>
</tr>
<tr>
<td></td>
<td>Anticonvulsant</td>
<td>Phenytoin</td>
</tr>
<tr>
<td></td>
<td>Antihypertensive</td>
<td>methyldopa</td>
</tr>
<tr>
<td></td>
<td>Chemotherapeutic</td>
<td>Isoniazide</td>
</tr>
<tr>
<td></td>
<td>Diuretic</td>
<td>Chlorthiazide</td>
</tr>
<tr>
<td>4) Cholestasis</td>
<td>Anabolic steroid</td>
<td>Methyltestosterone</td>
</tr>
<tr>
<td></td>
<td>Antithyroid</td>
<td>methimazole</td>
</tr>
<tr>
<td></td>
<td>Chemotherapeutic</td>
<td>erythromycin estolate</td>
</tr>
<tr>
<td></td>
<td>Oral Contraceptive</td>
<td>Norethynodrel mestranol</td>
</tr>
<tr>
<td></td>
<td>Oral antidibetics</td>
<td>Chlorpropamide</td>
</tr>
<tr>
<td></td>
<td>Tranquilizer</td>
<td>Chlorpromazine</td>
</tr>
<tr>
<td>5) Granulomas</td>
<td>Anti inflammatory</td>
<td>Sulfonamides</td>
</tr>
<tr>
<td></td>
<td>Chemotherapeutic</td>
<td>Allopurinol</td>
</tr>
<tr>
<td></td>
<td>Xanthine Oxidase inhibitor</td>
<td>Allopurinol</td>
</tr>
</tbody>
</table>

(Kaplwitz, 1986: 826)
The Structural changes can be graded arbitrarily. A method of scoring of the structural changes has been described by National health services.

- Degeneration,
- Necrosis,
- Fibrosis
- Regeneration,

**Experimental models of Hepatotoxicity**

To test the efficacy of proposed therapeutic remedy for the treatment of liver diseases, initially different models of hepatic damage set up in experimental animals are used and then studies are carried out in clinical conditions. Effect of an agent claimed to have an hepatoprotective action is studied either by its pretreatment, or by its co-administration along with the hepatotoxin. Protection against development of hepatic damage is considered as the criterion for efficacy of the test agent. Reversal of hepatic damage is another method, which is used to assess efficacy of the test agent, where, treatment with it is carried out after hepatic damage is induced. (Rubin, 1967 : 145-208)

Liver injury may be of an acute type or a chronic type depending on the duration of exposure to the hepatotoxic agent. Accordingly, the model for acute liver injury and those for chronic liver injury, differ in the duration of treatment with the hepatotoxin.
Ideally an experimental model should have the following features.

1) The lesion should be reproducible.
2) The mortality rate should be zero. (Rubin, 1987: 224-242)

Among the various hepatotoxins used in the animal model, carbon tetra chloride is the most frequently used one. It causes a hepatocellular fatty degeneration. The toxic effects are mediated through formation of free radical of trichlormethyl radical (\(\text{CCI}_3\)) carbon tetra chloride is claimed to induce experimentally conditions simulating viral hepatitis, fatty infiltration and liver cirrhosis. (Rege, 1984: 544-555)

Apart from carbon tetrachloride, chemical hepatotoxins such as d-galactosamine and ANIT (alfa naphthyl isothiocyanate) have been used in some studies: Galacto-samine causes hepatic necrosis by depletion of hepatic uridine stones. It has been suggested that galactosamine hepatitis may be a potential model for generalised inflammatory liver injury. ANIT causes intrahepatic cholestasis by cholangitis. (Maeda, 1985: 347-353)

Hepatotoxicity can also be induced experimentally, by drugs such as ethylalcohol, thioactamide, paracetamol, antituberculous drugs like pyrazinamide, rifampin etc. (Decker, 1972: 183-190)

ASSESSMENT OF LIVER DAMAGE

In the experimental models of liver injury, the severity of damage produced by the insult and the ability of a given
therapeutic is to be assessed. This assessment can be obtained with the help of following parameters.

1) Liver Structure- gross and microscopic
2) Metabolising capacity of the liver
3) Bio-chemical investigation

1) Assessment of damage to Liver Structure

Can be done by the gross and histopathological examination of the liver specimens.

Gross examination gives an idea about the change in liver weight and volume, its consistancy and colour.

- Liver weight is in constant proportion with the body weight. Hence changes in liver weight changes in body weight e.g. it decreases in catabolic states. A shrunken liver is also indicative of an insult of long duration e.g. in Cirrhosis. An increase in liver weight may be due to an excessive regenerative activity, secondly it may be secondary to an increase in the volume of Liver as occurs in liver congestion.

Liver Volume

Congestion of the liver due to internal haemorrhage or conditions associated with obstruction to venous out flow, thereby increasing back pressure in the venous system may lead to increased liver volume.

Purplish brown discoloration of the liver may indicate an internal damage, while yellow discoloration is associated with hyperbilirubinaemia.
In cirrhotic condition the liver become nodular and hard.

**Histopathological examination**: Provides sufficient clue to the nature of injury. Various stains can be employed to detect the changes in liver structure, e.g. haematoxylin eosine stain gives an idea about any change in parenchyma. The reticulin stain gives an idea about liver architecture by staining the reticulin fibres. Massontrichoma stain is a specific stain for collagen fibres, Similarly Serin red stain can also be used to detect the deposition of collagen.

The structural changes can be graded as, Degeneration, Necrosis, fibrosis and Regeneration.

2. Assessment of Metabolising capacity of the liver can be achieved by employing tests like pentobarbitone sleeping time. The barbiturates gets metabolised in the liver. Therefore duration of sleeping time after their administration gives a measure of hepatic function, Viz. metabolism of drugs. Liver damage associated with decrease in number of functioning cells or reduced microsomal enzyme activity will increase the pentobarbitone sleeping time. Thus prolonged sleeping time indicates functional derangement of hepatic cells. In animals treated with hepatoprotective agents along with the hepatotoxin, prolongation of sleeping time may be reduced or the sleeping time may be normalised.
Induction of hepatic microsomal enzymes by the test drug will be reflected as reduced sleeping time after pentobabitone, indicating enhanced metabolism of the barbiturate. (Chaudhari, 1978: 830-832)

Increased metabolising capacity of the liver may also indicate an increase in number of functional hepatic cells, as in case of hepatoma or any other neoplastic condition of the liver. It also indicates excessive hepatic regeneration.

3) Biochemical investigations

Liver function tests are the biochemical determinants of hepatic activity. Liver is the largest and most important biosynthetic catabolic and detoxifying organ in the body. Conventional liver function tests are routine tests which are commonly used and may represent the initial step playing a decisive role in assessing the presence or absence of disease. These tests are based on the assessment of endogenous substances viz. bilirubin, enzymes which are liberated in the serum after injury to liver cells. Basically they reflect the essential pathological features of the disease.
Cardiovascular Activity

(Laurence, 1964: 382-450)

The technique used for testing sympathomimetic amines were among the first to be introduced into modern pharmacology. As a result they have become universally accepted in both academic and industrial areas of drug investigation and have been retained in use with relatively little change. These procedures are in many ways as useful today as when they were first introduced. The advent in recent years of modern electronic equipment has however made possible, technical variations in the procedures that have brought new insights in the study of different drugs.

The lowering of blood pressure that accompanies sympathetic blockade is readily followed in anesthetised animals. Blood pressure is usually recorded from the femoral or carotid artery in dogs and from the carotid in cats, rabbits and smaller species. If the cat is used, the blood pressure and nictitating membrane responses can be recorded at the same time. It is convenient to use dog for testing a new compound to carry out a series of tests before and after administration of different doses of the compound. Dosage should be began at a very low level. A three fold step up in dosage is reasonable. The procedure is more appropriate for blocking agents with a long duration of action.

Valuable, extensive reviews of the many aspects of hypertensive disease and its treatment have been prepared by Pickering in 1955 to 1961. In 1962 Hoobler reviewed the use of drugs in the treatment of hypertension.
Hypertension is a multifactorial disease. It is classified as primary (or essential) and secondary hypertension. Primary hypertension is associated with increased peripheral vascular resistance, the cardiac output usually being within normal limits. The disease can remain benign but is often progressive, arterial walls becoming thickened with a consequent further increase in resistance. Secondary hypertension is distinct, in so far as the raised blood pressure is a consequence of some pathological condition such as phaeochromocytoma or renal damage.

Many factors are believed to be involved in the pathogenesis of essential or malignant hypertension; important among them are the genotype, psychological stress and diet. The setting of baroreceptor mechanism in the body and derangement of metabolism, especially in the kidney and adrenal medulla and cortex probably also play major parts. The disease may be progressive because of a vicious cycle that develops. A temporary high production of angiotensin from a diseased kidney might be a precipitating humoral factor. Wilson and Byrom (1939) have shown that damage to one kidney in the rat can cause an elevation of blood pressure resulting in damage to the other, this in turn leads to exaggeration of release of angiotensin released from ischemic kidney and may stimulate the production of aldosterone by the adrenal cortex.

Most of the drugs used for the treatment of hypertension act by blocking sympathetic mechanisms so that peripheral vasoconstrictor reflexes are abolished and vascular resistance decreased.
Major advances in the drug treatment of hypertension are likely to depend upon the agents that modify the action of endogenous factors peculiar to hypertensive disease. This is expected to require the use of hypertensive laboratory animals, both for extending aetiological studies of the disease and for testing new compounds. The methods required are more expensive, cumbersome, inaccurate and time consuming than those needed for the finding of sympathetic blocking agents. It is very difficult to select a method of tests which will evaluate effectiveness of various types of antihypertensive agents in animals. It will have to correlate with the effects of same compounds in the different forms of hypertension in man. Such a correlation can not be attempted, since practically all drugs lower blood pressure in normotensive animals and in man they show limited specificity.

Repeated Measurement of Arterial Blood Pressure.

In acute experiments, the animals are usually anaesthetised, the blood pressure being recorded for the duration of the experiment by exposing an easily accessible artery and inserting in it a cannula connected to a mercury manometer. If a fine needle is used instead of a cannula, the animal may be allowed to recover and be used repeatedly. On ethical grounds alone, most experimenters would agree that frequent repetition of the operative procedure of arterial exposure under anaesthesia is undesirable. Further, sepsis may occur, and then the animal suffers considerable stress, which itself leads to a rise in the blood pressure.
Methods are classified into
A. Direct or intravascular method.
B. Indirect (bloodless) method.

Whichever may be the method, there are several difficulties.

Use of anaesthesia (additional variable) which is essential to immobilise the animal, makes arterial blood pressure measurement easier and more accurate.

In the absence of anaesthesia, there is a danger of inaccuracies (Byrom (1947) - "referring to indirect methods - the tail of rats wrote"). The decision to avoid anaesthesia should not be lightly taken. It is true that after preliminary training many rats will give consistent readings, but meticulous care must be taken to avoid rough handling, injections, and unusual noises and smells. All these may excite the animal and raise pressure by 40 mm Hg. (Kampf and Page 1942)

Many workers have found that errors may be introduced if the experimental conditions for dogs are not kept absolutely constant and (Goldblatt 1948) has emphasised the need for training the animals.

Verney and Vogt (1938) reported that it was neccessary to accostom some dogs to the experimental procedure several days before reliable readings could be obtained. Every effort should be made to avoid the emotional stress of the animals and maintain quiet, uniform environmental conditions. Ideally blood pressure is recorded continuously. Maintenance of uniform temperature is
particularly important in those species which include the rat and rabbit in which body temperature varies with the environment.

A. Direct Methods.

1) In the Dog and the Rabbit

The arterial blood pressure can be measured in some unanaesthetised co-operative animals simply by inserting into a superficial artery a fine hypodermic syringe needle connected by flexible tubing to a mercury manometer, preferably of low displacement or to a pressure transducer, the system being filled with an anticoagulant solution. Goldbatt in 1948, 1960 has been described a suitable technique involving femoral artery in dogs and big rabbits. In rabbits median artery of the ear may be used.

A pressure transducer or electronic physiological recorder is preferred for small displacement.

Disadvantages
- Frequent insertion and withdrawal of needle
- Animal Excitement.

Advantages
- Repeated measurements could be carried out.

2) In the Rat (Still and Whitecomb (1956))

A small polythene tube may be inserted into the lumen of the abdominal aorta without major disturbance to blood flow and led beneath the skin to an exit at the back of the neck.
Modifications by Weeks and Jones (1960), use of pressure transducers and electronic recorders, Nash and Taylor (1961) showed that catheter can be tied into the carotid artery in the rat and the animal allowed to recover for blood pressure measurements, but we can record with this for a few days only whereas aortic intubated rats can be used for up to 6 months. A capacitor transducer has been designed for measuring blood pressure in small experimental animals with an indwelling aortic catheter.

Indirect Methods

The indirect methods of recording the blood pressure in animals involve the occlusion of the arterial supply to a limb or a tail by an inflatable cuff. In dogs, rabbit or cat, we can measure B.P., by raising pressure in the cuff above that in the artery and then slowly releasing it. The systolic blood pressure is taken to be equal to minimum cuff pressure required to arrest the arterial circulation. Inaccuracies may result due to elasticity of tissue surrounding the artery and spontaneous alteration in vasomotor tone, in the conscious state.

1) Methods used mainly on Dogs

Blood pressure can be measured in hind limb of large dogs by a simple modification of the auscultatory method in man. A condenser microphone can be used for registering arterial pulsation, which determines systolic and diastolic blood pressure in the caudal artery of Dog.
Leersum (1911) and Gorman and Yeary (1958) described a surgical technique for bringing an artery to the exterior enclosed in a fold of skin.

Carotid loops have been used for the measurements of arterial pressure in dogs by Goldblatt. The procedure can be criticised on the grounds that except in an animal whose carotid sinus has been denervated, the pressure reflex elicited by occlusion of the carotid artery with the cuff may lead to an erroneously high estimate of the basal blood pressure. To avoid this, femoral artery loops were used in cats and dogs.

Using an instrument O'connor found that observations could be made every 2 min. and any single estimate was probably within 10 mm of Hg. of the true value. Condensed microphone may also be used to record the arterial pulsations.

Arterial loops have been used in several laboratory species for dogs and to a limited extent in rabbits. They can be conveniently handled and have been successfully used for longer periods by several investigators. It is important to select and train docile co-operative animals and to provide good quality accommodation combined with ample nursing care by experienced handlers.

ii) Mainly on Rabbits

Grant and Rathschild (1934) have described a method for determining the blood pressure in the median artery of the ear of the unanesthetised rabbit. The artery is compressed by a transparent membrane and the systolic blood pressure is taken as the minimal pressure required to arrest the blood flow.
Advantages
1. No surgical intervention is required.
2. Measurements can be made rapidly and at frequent interval.
3. The apparatus is cheap and simple to use.
4. Rabbits, when made hypertensive develop arteriolar necroses resembling the malignant hypertensive.

Disadvantages
1. Blood pressure in rabbits is highly labile.
2. The pressure observed in the median artery is lower than that found in the carotid artery by direct puncture.
3. Pressure measurements depend on tone of the artery.

iii) Methods mainly used in Rats

In determination of blood pressure of rats in the feet or tail with specially designed hindlimb apparatus, minimise the difficulties inherent in determining the systolic end point in this species.

The Advantage of use of tail method is the relative ease of applying an occluding cuff and immobilising the tail.

The pressure in the occluding cuff is lowered below systolic pressure, by means of photoelectric cell.

More sophisticated apparatus has been developed to measure blood pressure. These instruments produce accurate determination of tail blood pressure in unanaesthetised rats.

Friedman and Ireed used a sensitive microphone, while Berger (1961) used a tail cuff linked to an infratron pick up and this in turn to direct writing electronic recorder.
Genetic Hypertension

The possible existence of inherited factors influencing development of hypertensive disease has been suspected for many years. Smirk and Hall (1958) showed that it is possible to develop a colony of hypertensive rats by selective breeding. This can be achieved by cross breeding selected animals. There was a difference of 14 to 20 mm of Hg. in blood pressure as compared to blood pressure of control colony rats.

Hexamethonium has also been used to induce chemical sympathectomy, (Laverty and Smirk 1961).

Psychogenic Hypertension

Several investigations have reported that repeated exposure of rats to noxious stimuli caused a persistent elevation in blood pressure e.g. rats that had been subjected to air blasts for 5-10 min. of 5 days a week for 33 weeks.

Assembly for recording of the blood pressure: The Mercury monometer consists of a glass 'U' tube (5mm bore) with two vertical limbs about 30 cm in height which is half filled with mercury. Since the mercury is displaced equally up in one limb and down in the other, on the surface of the mercury in one limb is a cylindrical float of vulcanite from which a stiff fine wire rises bearing on its upper end, a stylus that is writing point, which writes on the travelling surface of the smoke paper. The other limb of the manometer has a side tube which is connected through inextensible tube made of thick rubber or polythene to an
arterial cannula. The upper end of this limb is also connected with reservoir bottle containing some anticoagulant fluid, which can be pumped into this limb and through interconnecting tube to arterial cannula.

Respiration Recording

(Ghosh, 1984 : 130-132)

In anaesthetised animal trachea is exposed. Transverse cut is made between two rings so as to make an opening for the introduction of the tracheal cannula. To a cannula a rubber tube is attached which is connected to Merry's Tambur having 22 mm diameter. To the side metal arm of Tambur, stylus is attached. This is used for recording of respiratory changes by adjusting the slit of the tracheal cannula.

Recording of blood pressure

Recording of blood pressure in an anaesthetized animals is one of the standard methods for the pharmacological studies of drugs.

The mercury manometer originally designed by Poissuille in 1828 was modified by Carl Ludwig in 1847 to allow graphic records to be obtained from a float on the mercury column so that progressive changes in blood pressure could be studied. Even after more than century, the assembly virtually remains the same consisting of a mercury manometer with a float supporting a long soft wire which writes on the smoked surface of the kymograph with the help of a stylus. Owing to the inertia of the column of mercury the assembly dose not however register accurately rapid changes of pressure in the artery with each heart beat. They
appear as comparatively small fluctuations. These fluctuations become larger when the heart beat is slow, because the manometer is more nearly capable of keeping up with the changes in B.P. The assembly thus gives only a true and valuable record of the mean arterial pressure.

Procedure in brief
(Ghosh, 1984: 130-131)

A dog, cat or rabbit is anaesthetized with a suitable anaesthetic. A mid line incision is made on the skin of the neck starting from the lower end of larynx up to the upper end of thorax. Muscles are separated along the midline with the help of a pair of scissors by introducing the closed tips and then separating the blades. Trachea is exposed by retracting the pretracheal muscles, and a transverse cut is made in between two rings so as to make an opening for the introduction of the tracheal cannula. The cannula is introduced into the opening pointing towards lungs and held firmly in position with the help of the twin ligature. The purpose of cannulating the trachea as a routine is to allow free breathing without any obstructions by secretions which can be cleared as and when necessary and also to provide artificial respiration with the help of respiratory pump when required. The volume of air per stroke of the pump and the rate are adjusted depending on the species of animals.

Carotid arteries, which lie close to the trachea on either side along with the veins and nerves are easily recognised by their elastic and pulsating nature. One of these arteries is cleaned from the accompanying structures for a sufficient length
with the help of a blunt dissector. It is then tied as near the head end as possible, a bulldog clamp is placed about 3 cm near the heart. A cut is made carefully on the artery close to the first ligature with the help of sharp curved scissors so as to make a small opening through which an arterial cannula already filled with some anticoagulant fluid, is inserted so that it is directed towards the heart and is firmly secured with the help of the ligature already in position.

The three way tap of the manometer is then turned so as to fill the proximal limb, the connecting tubings and the cannula with the anticoagulant fluid from the reservoir bottle. The pinch cock on the short thin tubing attached to the side tube of the artery cannula is opened at the same time so as to allow the anticoagulant fluid to run out, when the whole system is rendered free of air bubbles the pinch cock is closed again. The pressure in proximal limb of the manometer is then increased to about 150 mm of Hg. The positive pressure being approximately equal to that of the animal, prevents too much blood from coming out of the animal's artery into the recording system. Before the bulldog clamp is removed, 0.5 ml of 1% heparin solution is injected into the arterial cannula through the thin rubber tubing. The bulldog clamp is then taken off, the column of mercury rises or falls slightly until its pressure counter balances that of the blood. The writing point remains at a constant level except for slight oscillations due to heart beats and respiratory movements. The height of mercury column midway between the top and bottom of these oscillations is taken as the mean arterial Blood Pressure. The femoral vein is exposed by a
midline incision on the medial surface of the upper part of the thigh. The venous cannula is inserted in to the vein, which is connected to burette filled with saline. Drugs are injected through the rubber tubing close to the cannula. After administration of every dose a constant volume of saline is allowed to run each time.

**Recording of blood pressure in Anaesthetized Rat**

A rat, suitably anaesthetized, is placed supine on a small animal operating board, and secured by tying the limbs. A midline incision is made on the neck, the trachea exposed and cannulated to ensure a free airway. The common carotid artery on one side is then exposed and ligated at the superior end, then clamped at the inferior end. A polythene cannula is inserted in the artery through a small incision, attached to a 23 gauge needle, and connected via a three-way stop cock to a Condon mercury manometer, and to the pressure bottle of the mercury manometer system filled with 0.9% Nacl solution, containing heparin 1000 units per ml. The system is flushed briefly with heparin saline solution and blood pressure recorded by releasing the arterial clamp. The external jugular vein is exposed on the otherside and cannulated with a polyethylene tubing attached to a 23 gauge needle and connected via three way stopcock for injecting drugs and for flushing with saline heparin solution after each injection.
Experimental models for Epilepsy

A number of animal models are available to evaluate the compounds likely to have an antiepileptic activity. The recently introduced models have been discussed by David J (1985).

In general, it is evident that different types of stimuli like electrical or chemical or sensory stimuli have been used to produce seizures in susceptible animals. The parameters which have been evaluated in animals vary from recording electrical activities in central nervous system. It appears that the specificity of experimental models of epilepsy used in the evaluation of potential antiepileptic drugs is primarily due to the intensity rather than the nature of the stimulus used or the kind of seizure component evoked. (Tomon et al 1946; Goodman et al 1953 chan et al 1954).

The data of Pivedda and his coworker (1985) suggest that for the routine evaluation of antiepileptic drugs, two models are most reliable.

1) Supramaximal seizures by electroshock
2) Minimal seizures induced by pentylenetetrazole.

Supramaximal Seizures by electroshock

The maximal Electroshock seizures (MES) test is one of the commonly employed procedure for the evaluation of drugs (Tedeschi 1956 ; Thoman 1964 krali 1980) The preliminary experimental observations made by Thomon and his colleague (1946) indicated that seizures elicited with a stimulus having intensity of 150 M.A. 60 cycles A.C., 0.2 sec duration, applied through corneal electrodes are maximal, and that in general increments in current
intensity do not alter the pattern or the duration of seizures. Since then a stimulus of 12 to 150 M. A. at 50-60 hz. a.c. current and 60 to 250 M.A. applied for 0.2 to 0.3 sec. through either corneal electrodes or earclip electrodes has been used in mice maximal or supra maximal convulsions.

Depending upon the strength of the electro stimulus convulsions are observed.

Pentylenetetrazol (Leptazol, metrazol, PTZ) seizures.

Pentylenetetrazol induced seizures in mice and rats is another commonly used (employed) animal model to evaluate compounds having anticonvulsant action. In mice, PTZ has been used in the doses of 0.85 - 8.5 mg/ 100 gms subcutaneously (Karall, 1980 Pivedda 1985) 10.5 mg /100 gms intraperitoneally (Chambon 1985) or by slow intravenous infusion 0.25 mg/10 sec (Bernard 1985) to induce minimal seizures.

Rats have also been employed, where in, PTZ has been used in dose of 10 mg/ 100 gms intraperitoneally or 2 - 3 mg/100 gms intravenous to induce minimal seizures (Bernard 1985)

The severity of the seizures induced by PTZ can be graded.

The possible mechanism of induction of seizures have been summarised by Woodbary (1980). At present there is some evidence to accept the following three mechanisms.

1. Blockade of GABA mediated inhibition either by direct receptor effect or by an action on the chloride ion channel (olsen, 1982; Heinemann and Lux 1983 nonald 1985)
2. Increase in neuronal excitability by a direct effect on neuronal membranes most probably through interference with K-conductance (Heinemann and Lux, 1983)

3. Inhibiting acetylcholinesterase and thereby causing an accumulation of Ach, a excitatory central neurotransmitter. A single (Nutt et al, 1981, Bowdler and Green 1982, Green et al 1988) or repeated (Bernard et al 1985) electroshock seizures have been observed to alter the GABA turn-over differentially in various brain regions. The repeated electroshock has also been observed to enhance the behavioural responses to 5-HT and dopamine in rats and these effects appear to be dependent on central non-adrenergic function (Goodwin and Heal 1985).
Pharmacological Studies for Iron detection

(Laurence, 1964 : 265-367)

Whatever the other pharmacological properties it may display, a haematinic will elicit a response determined partly by the presence of free ionic iron or the readiness with which the iron dissociate from the chelate or complex and partly by the tendancy of the haematinic to precipitate in blood stream. Ferrous ion is said to have adrenergic blocking, neuromuscular blocking and even ganglionic blocking properties (Reviewed by Rajparkar, Sachade and Panjwani 1962).

Majue and Buez 1955 showed that ferric ions introduced intravenously produce a vasodepressor effect, attributable to a short lived suppression of adrenergic vasomotoar tone.

Tests for oral iron preparations:

1) Determining iron Absorption

It is known that the degree of absorption is related to the dose of iron administered, the size of the iron stores, the degree of anaemia and activity of bone marrow (Beutler 1961).

Three main methods can be used to determine the absorption of iron after oral administration. The oldest is the balance study in which a known dose of iron is given orally to an animal, usually in rat, and the amount appearing in the faeces is determined by chemical analysis. If chemical methods are used to determine the unabsorbed iron, great care must be taken to reduce the iron content of food and water.

Secondly, oral absorption studies may be simplified and made more accurate if the haematinic can be labelled with
radioactivity. The amount of iron absorbed by the mouse or rat that has received \( ^{57}\text{Fe} \) may be measured by whole body counting of the living animal.

The third method of measuring iron absorption involves the use of isotope of iron. Iron preparation is labelled with \( ^{55}\text{Fe} \) and given orally. Fe is given intravenously in trace amounts bound to plasma transferrin. It is assumed that the distribution of iron entering the body by G.I. tract is identical with that of iron administered parenterally. Accordingly, by drawing blood samples at 10-14 days the percentage of each dose incorporated into circulating red cell mass is determined.

Iron utilisation

Determination of the percentage utilisation of an iron preparation is simple in principle. Animals are rendered iron-deficient and on the basis of their haemoglobin concentration and blood volume a dose of an haematinic is given that is calculated to be below the amount required to restore the haemoglobin level to its normal value. The resulting increase in circulating haemoglobin is determined at various times after medication so that the amount of iron incorporated into haemoglobin may be calculated. In order to arrive at an accurate assessment of iron utilisation, it is essential to measure the blood volume accurately at the times when the haemoglobin measurements are made.

Iron Deficiency in the rat

The rats are housed in aluminium cages and supplied with iron free drinking water. The pregnant rats, preferably a few
days before parturition are transferred from the breeding cage to a large aluminium main cage. In this cage instead of food, plentiful supply of iron free drinking water is provided. After parturition, the feeding routine of the mother is maintained until the young rats are removed at 21 days. Only 8 offsprings are taken for further study.

The young rats live on their mother's milk until they are 12 days old. After this, they open their eyes and consume the fortified cow's milk in place of water. By about 17 days the offsprings should be drinking appreciable amount of the milk. At the end of 21 days the young rats are distributed individually in the small aluminium cages, they are supplied with milk fortified with copper.

A particular attention must be paid to ensure lack of contact of iron to rats. The rats are weaned and Haemoglobin concentration is determined. When the Haemoglobin concentration has reached about 4-6 gm/100 ml, it can be taken as iron deficiency anaemia. Then iron preparation is administered by either the oral or the parenteral route, at a suboptimal dose and the measurements enumerated above are repeated at weekly intervals over the next four weeks.

2) The anaemic piglets

The pig weighs about 1-2 kg at birth and contains only about 50 mg iron (McCance and Kiddosm 1977). The suckling piglet doubles its weight at 1 week and again at 3-4 wks. Cow's milk has a low iron content. In consequence, of this poor provision of
iron, the piglets haemoglobin level begins to fall during first week of life. Haemoglobin level as low as 3-4 g/100 ml may be reached by the second to third week. Piglets thus develop severe iron deficiency anaemia by 7-10 days. The haematinic is administered parenterally or by stomach tube and Iron utilisation is determined.

Anaemia of the other types:

a) Other neonatal anaemias

The calf also is a suitable animal for the study of hemoglobin regeneration (matrone, conley wise and klaugh 1957). The chick is an exceptionally convenient species, in which iron deficiency was studied by Elvehjem and Hart (1929). Hill and Matrone (1961) describe a basal diet with which both iron and copper deficiencies were induced.

b) Blood loss

The most convenient animal for this purpose is the rabbit, which tolerates stoically the loss of large volume of blood. Golberg, matrin and smith (1960) withdrew on an average about 1.5 L blood from each rabbit by repeated phlebotomy. Cardiac puncture has been employed to induce haemorrhagic anaemia in mice (Krantz, Goldwasser and Jacobson, 1959), but it would appear simpler to bleed rats by immersing the cut tails in warm citrate solutions (Givin and Klong 1961).

c) Accelerated rate of erythropoiesis
Here, instead of depleting the body iron, the bone marrow is stimulated to increase its erythropoietic activity. This may be brought about by administering erythropoietin (Mendel, 1961) or cobalt (Saikkonen, 1962) or by inducing anoxic hypoxia (Vassor and Taylor, 1956). The procedure, most commonly used is the induction of haemolytic anaemia with freshly prepared solutions of phenylhydrazine or its acetyl derivative in mice and rabbits. (Lokly, Keighley et al. 1959 or Rats, Kadlor 1954).
Atomic absorption spectro-photometry - Heavy Metal Analysis

Principles of atomic absorption

Atomic absorption is a physical process involving the absorption by free atoms of an element, of light at a wave length specific to that element. (Bruce, 1984 : 69-83)

If we consider a sample or sample solution burn in a flame or heated in a tube, the individual atoms of the sample are released to form a cloud inside the flame or tube. Each atom consists of a positively charged nucleus surrounded by a number of electrons in rapid motion around the nucleus. For each electron in each atom there is a discrete set of energy levels that the electron can occupy. The spacing of the energy levels is different for each electron in the atom, but for similar atoms corresponding electrons have identical spacing. Energy levels are labelled from E0, the ground state to E alfa, at which stage the electron has enough energy to break away from the atom, i.e. ionisation occurs.

The wavelength at which an atom with its valency electrons in the ground state can absorb radiation is called resonance wave length.

* Atomic absorption, emission and fluorescence.

1) Atomic absorption
2) Atomic Emission
3) Atomic fluorescence

Transition diagramme shows three types of transition involving, in this example if we consider the ground state and excite state, absorbing a proportion of the impinging photons
illustrates the principle of atomic absorption. In Second case, where the electrons are raised to the first excited state by heat energy and then fall back to the ground state, emitting a photon illustrates atomic emission. The third case, where the electrons are raised to the first excited state by photons from a spectral Source and then fall back to the ground state, emitting a photon illustrates atomic fluorescence.

Sample Preparation

Any sample which is analysed by atomic absorption Spectroscopy has to be in the form of a nonviscous solution when it is aspirated into the spray chamber via the capillary tube and nebuliser. Thus solids must be dissolved by some suitable procedure giving a complete dissolution of the entire sample. Organic liquids such as lubricating oils also need to be diluted with a suitable organic solvents, white spirit for example, to make the solution sufficiently non-viscous to achieve an uptake rate of 3-5 ml/min.

Sample treatment considerations

I) Generally for solid samples, simple possible procedure should be employed to ensure complete dissolution of the sample.

II) For trace elements excessive dilutions must be avoided.

III) Care should be taken to avoid loss of element.

IV) Apparatus used should be scrupulously clean.

V) Analar reagents should be used

VI) Accuracy should be maintained in dilution.

VII) Viscosity of Sample and calibration solutions must be similar.
Sample Preparation
Solid

The dissolution procedure will depend on the nature of the sample. For water soluble salts, distilled water can be used.

A) Aqueous dissolution

Salts of the alkali metals, first row transition metal salts.

B) Dilute acid dissolution (HCl or HNO₃)

Carbonates, some metals or metallic ores in powder or granular form

C) Mixed acid / concentrated acid

D) Fusion Technique

Siliceous material, ores, refractory materials etc. The sample is fused with an alkali carbonate or hydroxide. The temperatures are in the order of 1000°C.

E) Dry ashing

Food, biological and clinical specimens, plants. The organic materials are volatilised or oxidised at 450 - 550°C and inorganic residues can be extracted with either HCl or HNO₃. This is not suitable for volatile elements, As, Se and Hg.

F) Wet ashing

Foods, biological and clinical specimens plants. The sample is digested, preferably in a kjeldahl flask with mixture of
HN_3/\text{H}_2\text{SO}_4$ or $\text{H}_2\text{SO}_4/\text{HClO}_4$. The volatile elements are usually retained by this procedure.

G) Plasma ashing

Non moist foods, hair, dried serum etc. This is relatively recent procedure and involves the use of low temperature, low pressure oxidising plasma. However, the sample has to be dried initially. Finger nails, plants etc can take several hours.
Anaesthesia of Animals for Biomedical research. (Plecknell, 1993 : 885-594)

Anaesthetists trained to deal with human patients may be required an anaesthetist for animal species as part of their involvement in biochemical research. Although the skills developed in clinical anaesthesia are of obvious relevance, several problems may be encountered when attempting to anaesthetise unfamiliar species.

Ethical and Legal considerations

The use of animals in biochemical research attracts considerable criticism from animal welfare groups. It is important that all those involved in such research projects are aware of the sensitive nature of this issue. It is helpful to become familiar with the moral and the ethical arguments which have been advanced on both sides of this debate.

In U.K. "The Animal Scientific Procedures Act", 1986 (A(SP)) is administered by home office. The protected animals are all vertebrates. Other than man and Act also includes protection for immature forms, foetuses and embryo.

The home office definition of pain, suffering, distress or lasting harm is very broad and includes physiological and psychological stress, disease injury, disturbances to normal health and any significant discomfort.

Personal Licences: The Act controls the competence of individual research workers by means of personal licences. It lists the techniques that may be carried out by the individual research worker, states species of animal and whether anaesthesia
is to be used for all. Personal Licences are very specific and it is important not to carry out any procedure which is not specifically listed. These licences are granted to people who are believed to be competent to perform the procedures.

Project Licences: Before a research worker who holds a personal licence can begin work which involves the use of animals, the research project must also have been authorized by means of a project's licence. Project Licensing is central to the operation of the act; the philosophical basis of the act is to perform a cost benefit analysis of research work, the cost to the animals, in terms of adverse effect is weighed against the likely benefits to man or other animals.

The animal welfare act: In north America the methods of regulation of research involving live animals are somewhat different, but are based on similar principle. In the U.S.A. the Animal Welfare Act sets out national standards. Within each research institute a Local Ethics committee reviews individual research proposals and determines if they comply with Local and national requirements.

Comparative Anesthesia: The most obvious practical problem that may be encountered by an anaesthetist who routinely deals with human subjects arises as a result of the small body size of some of the animals that are used in research laboratories. Body size influences the type of equipment and the anaesthetic techniques that may be used and the overall management of the animal during the perioperative period.
## Equipment Required for Tracheal Intubation in Laboratory Animals

<table>
<thead>
<tr>
<th>Species</th>
<th>Body weight</th>
<th>Tube diameter (o.d.) (mm)</th>
<th>Laryngoscope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>25-35 g</td>
<td>1.0</td>
<td>Purpose made</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>laryngoscope 14J</td>
</tr>
<tr>
<td>Hamster</td>
<td>120 g</td>
<td>1.5</td>
<td>Purpose made</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>laryngoscope 1.4 J</td>
</tr>
<tr>
<td>Rat</td>
<td>200-400 g</td>
<td>1.8</td>
<td>Purpose made</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(12-16 gauge plastic cannula)</td>
</tr>
<tr>
<td>Guineapig</td>
<td>100-400 g</td>
<td>1.5-2.5</td>
<td>Purpose made</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>laryngoscope 14J</td>
</tr>
<tr>
<td>Rabbit</td>
<td>1-3 kg</td>
<td>2-3</td>
<td>Wisconsin size 0-1</td>
</tr>
<tr>
<td></td>
<td>3-7 kg</td>
<td>3-6</td>
<td></td>
</tr>
<tr>
<td>Cat</td>
<td>0.5-1.5 kg</td>
<td>2.0-3</td>
<td>MacIntosh size 1</td>
</tr>
<tr>
<td></td>
<td>&gt;1.5 kg</td>
<td>3-4.5</td>
<td></td>
</tr>
<tr>
<td>Dog</td>
<td>0.5-5 kg</td>
<td>2-5</td>
<td>MacIntosh size 1-4</td>
</tr>
<tr>
<td></td>
<td>&gt;5 kg</td>
<td>4.0-15</td>
<td></td>
</tr>
<tr>
<td>Primate</td>
<td>&lt;0.5 kg</td>
<td>--</td>
<td>Not reported</td>
</tr>
<tr>
<td></td>
<td>0.5-20 kg</td>
<td>2-8</td>
<td></td>
</tr>
<tr>
<td>Sheep</td>
<td>10-90 kg</td>
<td>5-15</td>
<td>MacIntosh size 1-3, 2-4</td>
</tr>
<tr>
<td>Pig</td>
<td>1-10 kg</td>
<td>2-6</td>
<td>Soper or Wisconsin size 1-4</td>
</tr>
<tr>
<td></td>
<td>10-200 kg</td>
<td>6-15</td>
<td></td>
</tr>
</tbody>
</table>
Anesthetic Equipment: When dealing with animals of sizes comparable to those of adult humans and infants, standard anesthetic apparatus can be utilized. Human paediatric breathing systems are particularly useful for animals weighing 1-10 kg and a Bain's circuit or Ayre's T pieces is the apparatus used most frequently. Species with body weight > 1 kg, the majority of anaesthetic breathing circuits, tracheal tubes and laryngoscopes designed for use in humans are unsuitable. Table - Lists the approximate dimensions of tracheal tubes and laryngoscope suitable for intubating the trachea of common laboratory animals.

Because of the technical difficulties associated with intubation, volatile anesthetics are administered to small rodents via a face mask. The concentric face mask system described (Hunter, Allen and Butcher) is preferred.

Anesthetic induction with volatile anaesthetics in small rodents is best carried using a purpose made induction chamber connected to a standard anaesthetic vaporizer and gas scavenging equipment.

Species variation in Drug Responses: In addition to the variation in drug response between individuals, different species varies in their responses to some of the commonly used anaesthetic, Particularly for ketamine and other dissociative anesthetics. In man ketamine is reported to produce good analgesia and in the majority of non-human primates a cataleptic state with analgesia and immobilization is attained. The efficacy of this agent decreases in species with less cortical development. In cat moderately effective but ketamine rarely
immobilize pigs and sheep completely. In Rodents it is remarkably ineffective.

The depth of anaesthesia that can be provided by an anaesthetic before significant respiratory or cardiac depression ensues also varies in different species.

Propofol is an effective anesthetic in rats, mice, dogs, cats, pigs and sheep, but in rabbit it causes severe respiratory depression.

Anesthetic management
Animal health status: Laboratory species are susceptible to a wide range of diseases and a pre-anesthetic clinical examination should be carried out to ensure that the subject is free from clinical signs of any disease. Before major surgical procedures routine haematological and biochemical evaluations should be undertaken, working with animals that are free from disease is often essential if meaningful research data are to be obtained. It is also important to appreciate that some infectious disease are zoonoses and may represent a health hazard to personnel. Again advice from veterinary staff should be obtained and local safety codes of practice should be adhered to.

Preoperative Preparation
In order to allow time for preanaesthetic clinical evaluation to be undertaken, it is essential that animals are obtained 1-2 weeks before commencing any study; transport of animals from a supplier to the research laboratory is stressful and animals require a period of acclimatization to adapt to their new surrounding. This acclimatization period also enables
recording of body weight and food and water consumption -
information, which is extremely useful for monitoring post
operative recovery.

Food should be withheld for 12-16 house before anaesthesia
of dogs, cats, primates ferrets pigs and sheep. Fasting is
unnecessary in small rodents and rabbits, unless upper G I T
surgery is to be undertaken.

Sheep and goats presents particular problems with regard to
anesthetic management. As these species have a stomach that
consists of several sections, one of which, the rumen acts as a
fermentation chamber for the digestion of cellulose. During
anaesthesia normal oesophageal activity is suppressed and the gas
which is produced, accumulates in the rumen causing massive
distension of the abdomen. It is also essential to pass a tracheal
tube in these species, to prevent inhalation of liquid rumen
contents, which are regurgitated during anaesthesia.