CHAPTER II

REVIEW OF LITERATURE
THE AMPHIBIANS
SECTION 1

AMPHIBIANS
Amphibians are intermediate in some way between the fully aquatic fishes and terrestrial amniotes. However, they are not simply terrestrial in their morphology, life history, ecology and behaviour. In the successful attainment of independence from water and colonization, land amphibians have undergone a remarkable adaptatation and this living groups exhibit a greater diversity of modes of life history than any other groups of vertebrates.

Amphibia, the first vertebrates to become adapted to life on land, may be distinguished from the fishes, their predecessor, chiefly by their pentadactyle limbs and the absence of fin rays in the unpaired fins. The term amphibians can be interpreted in two ways - either as an animal spending part of its life in water and then changing to adult or as an animal that alternates life in and out of water such as so called pond frogs. Actually both interpretations are valid in part but neither applies to all amphibians. Some of which are aquatic throughout their lives but others of which neither enter water nor have aquatic stages in their life history (Duellman and Trube, 1986).

Internally, also, the structure of living amphibians is intermediate between that of fishes and amniotes. The heart has two atria, a single ventricle (which may be partially divided) and distinct conus arteriosus with several valves. The aortic arches are symmetrical. Typically, amphibians have two lungs but the lung are reduced in some salamanders. The left lung is greatly reduced in most of the elongated caecilians (as in snakes). Living amphibians also have some unique characters. They all have pedicellate teeth and specialized papillae in the inner ear and salamanders and anurans have green rods in the retina of their eye. Essentially, amphibians can be defined as quadrupedal vertebrates having two occipital
condyles on the skull and no more than one sacral vertebra. The skin is granular and lacks
the epidermal structures (scales, feathers, hair), characteristic of other groups of tetrapods.

Generally living amphibians are classified in three orders-

(1) Apoda or Gymnophiona or Caecilians.

(2) Caudata or Urodela or Salamander.

(3) Salientia or Anura.

The largest salamanders attains a total length of about 1500 mm, whereas largest frog
is about 300 mm. Caecilians reach a length of about 1500 mm (Duellman and Trube, 1986).
Caecilians and some salamanders lack limbs and girdles and in some other salamanders these
structures are reduced. In frogs (Anura) the post sacral vertebrae are fused into a single rod
like element, the coccyx, the tail is absent and the hind limbs are elongated and modified for
jumping. Although epidermal scales are absent in amphibians, dermal scales are present in the
skin of most caecilians. The skin is highly glandular and contains both mucous and granular
(poison) glands. True claws are absent but horny tips are present on the toes of some frogs
and salamanders.

At a first glance, the three lineages of amphibians (Caecilians, Caudata and Anura)
appear to be very different kinds of animals. Frogs (Anura) have long hindlimbs and short,
stiff bodies that don’t bend when they walk. Salamanders (Caudata) have foel limbs and hind
limbs of equal size and move with lateral undulations. Caecilians are limbless and employ
serpentine locomotion. These obvious differences are all related to locomotor specialization,
however closer examinations showed that amphibians have many characters in common. Some of these shared character play important roles in the functional biology of amphibians. The moist, permeable skin in particular creates both limits and opportunities in terms of the things amphibians can do.

All living adult amphibians are carnivorous and relatively little morphological specialization is associated with different dietary habits within each group. Amphibians eat almost anything they are able to catch and swallow. The tongue of aquatic frog is broad, flat and relatively immobile but some terrestrial amphians can protrude the tongue from the mouth to capture prey. The size of the head is an important determinant of the maximum size of the prey that can be taken and, sympatric species of salamanders frequently have markedly different head sizes, suggesting that this is a feature that reduces competition in prey selection (Pough, Heiser and McFarland, 1996).

The anuran body form probably evolved from a salamander like starting point. Both jumping and swimming have been suggested as the mode of locomotion that made the change advantageous. Salamanders and caecilians swim as fish do by passing a sine wave down the body. Anurans have inflexible bodies and swim with simultaneous thrusts of the hind legs. Some paleontologists have proposed that the anuran body form evolved because of the advantages of that mode of swimming.

The life history of amphibians are highly diversified. Most species of anurans have external fertilization, whereas internal fertilization occur in the majority of salamanders and
presumably in all caecilians. The classic amphibians life history of aquatic eggs and larvae, although typical of many frogs and some salamanders, is only one of many modes of reproduction, which include direct development of terrestrial eggs (no aquatic larval stage), ovoviviparity, and even viviparity. The eggs lack a shell and the embryonic membranes (amnion, allantosis and chorion) of higher vertebrates. In those amphibians that have aquatic larvae, undergo metamorphosis into adult form, which is an especially dramatic change in frog (Duellman and Trube, 1986).

Anurans

In contrast to the limited number of species of salamanders and caecilians and their restricted geographic distribution, the anurans (an = without, uro = tail) include 27 families, nearly 3750 species occur on all of the continents except Antartica. Specialization of the body for jumping is the most conspicuous skeletal feature of anurans. The hind limbs and muscles form a lever system that catapult an anuran into the air and numerous morphological specilization are associated with this type of locomotion. The hind legs are elongated and the tibia and fibula are fused. A powerful pelvis strongly fastened to the vertebral column. The posterior vertebrae are fused into a solid rod like urostyle. The pelvis and urostyle render the posterior or half of the trunk rigid. The vertebral column is short, with only five to nine presacral vertebrae and these are strongly braced by zygapophyses that restrict lateral bending. The strong forelimbs and flexible pectoral girdle absorb the impact of landing. The eyes are large and are placed well forward on the head, giving binocular vision. The ilium is elongated and reaches far anterioly (Pough, Heiser and McFarland, 1996).
Specialization of the locomotor system can be used to distinguish different kinds of anurans. The difficulty is finding names for them—the diversity of anurans exceeds the number of common names that can be used to distinguish various ecological specialities. Animals called frogs usually have long legs and move by jumping. Many species of ranids (superfamily of anurans) have this body form and very similar jumping frogs are found in other lineages as well. Semiaquatic forms are moderately streamlined and have webbed feet. Snout bodied terrestrial anurans that make short hops instead of long leaps are often called toads. They usually have blunt heads, heavy bodies, short legs and little webbing between the toes. This body form is represented by members of the Bufonidae and very similar body forms are found in other families, including the spadefoot toads of Western North America and the horned frogs of South America. Spadefoot toads take their name from a Keratinized structure of the hind foot that use for digging backward into the with rapid movements of their hind legs. The horned frogs have extremely large heads and mouths. They feed on small vertebrates, including birds and mammals but particularly on other frogs. The tadpoles of horned frogs also are carnivorous and feed on other tadpoles. Many frogs that burrow head-first have pointed heads, snout bodies and short legs (Duellman and Trube, 1986).

Several aspects of the natural history of anurans appear to be related to their different modes of locomotion. In particular, short legged species that move by hopping cover large areas as they search for food. This behaviour exposes them to predators and their short legs prevent them from fleeing rapidly to escape. Many of these anurans have potent defensive chemicals that are released from glands in the skin when they are attacked.
Species of frogs that move by jumping, in contrast to those that hop, are usually sedentary predators that wait in ambush for prey that passes their hiding places. These species are usually cryptically coloured and they often lack chemical defenses. If they are discovered by a predator, they rely on a series of rapid leaps to get away. Anurans look for, widely encounter different kind of prey from those that wait in one spot and differences in dietary habits may be associated with differences in locomotor mode. Aquatic species of anurans use suction feeding to engulf food in the water; semiaquatic and terrestrial species have highly specialized sticky tongues that can be flipped out to trap prey and carry it back to the mouth. The toad (*Bufo melanostictus*) represented this group. Other examples are Xenopus, Bufo, Hyla, Rana, Dendrobates, Ascaphus. There are 4,780 species of amphibians exist around the world, among them 234 species are Indian.

**SOME COMMON INDIAN TOADS / FROGS (Deuti and Goswami, 1995)**

i)  **Marbled Toad (*Bufo stomaticus*)**: Medium-sized toad. Head is broader than long and without any bony ridges. Snout is rounded. Nostril is nearer to the tip of the snout than the eye. Ear-drum is district, circular or oval. Parotid glands are large, flat and elliptical but not bean-shaped. Upper part of the body is brownish or grey, sometimes marbled. Belly and upper lip is dull whitish. The breeding season lasts from June to September. They breed in shallow rainwater after sunset, during the monsoons. They are terrestrial and nocturnal but may be seen roaming about during day time in the breeding season. They prefer dry semi-arid areas. They are more agile than the common Indian toad (*Bufo melanostictus*) and burrow in sandy or wet soil. They generally feed on ants, termites, earwings, spring-tails, crickets,
grashoppers, flies etc. Besides India, this species can be found in Nepal, Burma, Pakistan, Afganisthan, Eastern Iran and Arabian Peninsula.

ii) **Chinese Frog** (*Microhyla ornate*): Small sized, slender, active frogs with bulging eyes. Head is broader than long. Snout is pointed and projects beyond the narrow mouth. Tongue is elliptical. Nostril is nearer to the tip of the snout. Eardrum is indistinct. Tips of the fingers are flattened. Rudiment of webs present between the toes. The upper side of the body is light brown diamond shaped marking over the back. A dark streak extends along the sides from behind the eye to the shoulder. Limbs are marked with dark cross bars. The belly is dull white and the throat and chest are brown. This species is common and is terrestrial and nocturnal. They are normally found in the grasses and bushes growing on moist soil and under dry fallen bamboo leaves on the banks of ponds and tanks. Generally they feed on ants, wingless termites and small beetles. Besides India they are found in Burma, South China, Malay Peninsula, Taiwan and Japan.

iii) **Painted Frog** (*Kaloula poulchar*): The medium-sized toad-like colourful borrowing frog is perhaps the prettiest of all Indian amphibians. Head is broader than long. Snout is rounded. Nostril is nearer to the tip of the snout than the eye. Eardrum is not exposed. Tongue is elliptical. Tips of the fingers bear short well developed truncate discs. Back is black-brown or grayish-brown with brown or light red spots and patches margined with black. A dark reddish lateral band runs on each side from the eyes to the groin of the hind feet. The belly is light brownish or white, spotted with grey. This species is terrestrial burrowing and nocturnal. They can also climb trees about one and a half meters about the ground and have been found in holes of trees trunk, infested with termites,. On being distribute, they inhale air and swell
like a balloon, keeping the head down and secrete at milky sticky fluid from epidermal pores of the skin. They have a deep guttural call. They can hop well and are good swimmers too. They mainly feed on termites and various small beetles and other insects. Beside India they can be found in Bangladesh, Burma, Thailand, China, Sumatra and Borneo.

iv) Balloon Frog (*Uperodon globulosus*): Medium-sized, round, toad-like burrowing frogs with small beady eyes. Head is small and more broader than long. Snout is rounded. Nostril is equidistant from the tip of the snout and the eyes. Eardrum is not exposed. Tongue is oval. Fingers are without webs. The hind legs are short. Toes are with rudiment of webs. Two well developed, large and shovelshaped (inner and outer) pedal tubercles are present. This species is very rare. They are nocturnal and subterranean in habit. They prefer loose sandy soil and are excellent burrowers using the strong pedal tubercles. In clay soil, however, an opening is left for breathing while the frog may remain at a depth of almost two meters. They often dig into termataria and feed on the eggs and adults of termites. Emergence of these frogs coincides with the emergence of the winged termites following heavy rains. They are rarely seen on the surface or near water except during the breeding season. They breeding activities commence with the beginning of the rainy season in June and extended upto July. They feed voraciously on winged termites and also consume ants and small beetles. Besides India they can be found in Bangladesh.

v) Indian Bull Frog (*Rana tigrina*): Large frogs with smooth skin and longitudinal glandular folds on the back. Head is as long as broad. The snout is pointed and projects beyond the mouth. Nostril is almost equidistant from the tip of the snout and the eye. Eardrum
is distinct, nearly equal to the diameter of the eye. Fingers are without webs. Tips of the fingers and toes are not sharply pointed. Toes are fully webbed but the web does not reach the tip of the third toe. The fifth toe is with an outer fringe of web. Upper side of the body is yellowish or olive green or brownish green with darker leopard like spots. A yellowish median strip runs from the tip of the snout to the vent, sometimes bifurcating over both of the things. Belly is whitish. Limbs are black barred or spotted. Males are smaller, slimmer and darker than the females. Males possess external vocal sacs which are bright blue in colour. This species is very common. They are nocturnal, semiaquatic and good swimmers and hide among grasses, bushes and hollows at the edge of ponds, ditches and canals. Bull frogs are carnivorous and sometimes cannibalistic and consume various kinds of insects. Beside India they can be found in Pakistan, Burma, Thailand, South China and Taiwan.

vi) **Green Pond Frog** (*Rana hexadactyla*): A giant leaf-green frog, perhaps the most aquatic of all amphibians of India. Head is as long as broad. Snout is flat and rounded. Nostril is nearer to the tip of the snout than the eye. Eardrum is distinct. Fingers are without webs. Tips of the fingers and toes are pointed. Toes are fully webbed. There is a strong dermal frings on the outer toes. Skin is smooth above. Longitudinal glandular folds are absent on the back but a skin fold runs from behind the eye to the shoulder. Granules and warts are present on the back, throat, belly and under the things. Two rows of large porous warts are present along the flanks. They back is olive-green or bright grass green with or without a yellow median streak. Under parts and flanks are white or yellowish white. The male has external vocal sacs and during breeding season the throat becomes deep orange. This species is found floating in old ponds and fishing bheries where dense green floating vegetation grow
abundantly. The colour of the body merges with the vegetation so that they can easily camouflage and protect themselves from predators. This species feeds mainly on weeds but also on various insects especially dragonflies as well as their larvae, small fishes and smaller frogs. Beside India they can also be found in Sri Lanka and Bangladesh.

vii) Skipping Frog (*Rana cyanophlyctis*): Medium-sized pond frog. Head is broader than long. Nostril is equidistant from the tip of the snout and the eye. Eardrum is distinct. Fingers are without webs. Tips of the fingers are pointed. Toes are fully webbed. Tips of the toes are swollen and rounded. Small warts are present on the upper side. Glandular longitudinal folds are absent on the back but a prominent skin fold runs from behind the eye to the shoulder. Belly is smooth with a single row of porous warts on each flank. The colour of the body is either gray, grayish-brown or grayish-black with dark rounded spots on the back and stripes on the limbs. Their belly is white with black spots. This species spend most of their life in water or in the vicinity of water but may migrate onto land in the summer months when the temporary pools of water dry up. They are highly vocal during the monsoons. They can tolerate a certain degree of salinity and thrive in dirty drains and ditches contaminated with organic pollutants. They feed on earthworms, beetles, mosquito-larva and other aquatic insects. Beside India they can also be found in Sri Lanka, Afganisthan, Pakistan, Iran, Saudi Arabia, Burma, Thailand.

viii) Cricket Frog (*Rana limnocharis*): Small to medium-sized frogs. Head is almost as long as broad. Snout is pointed and projects beyond the mouth. Nostril is nearer to the tip of the snout than the eye. Eardrum is distinct. Fingers are without webs. Tips of the fingers and toes are swollen but not disc-like. Toes are half-webbed. Some short and interrupted
longitudinal glandular folds are present on the back. A prominent skin fold runs from behind
the eye to the shoulder. Belly is smooth. Back is greyish or olive-brownish, usually with
distinct darker marking. There is a ‘V’-shaped black band in between they eyes. Lips and
limbs are barred. A yellowish or whitish median streak of varying width is often present but
sometimes it is restricted to the trunk region only. These species are found near the bushes
growing at the edges of paddy fields or on the banks of ponds, ditches and canals. Sometimes
they reside on the moist forest beds, under logs and stones, usually near water. They feed
mainly on small beetles, ants, termites and insects. Beside India they can be found in Sumatra,
Borneo, China, Japan and Phillipines.

ix) Leaping Frog (*Rana erythraea*) : Medium-sized, elongated, slender frogs. Head is
narrow and is longer than broad. Snout is pointed and projects beyond the mouth. Nostril is
nearer to the tip of the snout tan the eyes. Eardrum is very distinct. Tongue is long and deeply
notched behind. Fingers are slender with rudimentary web. Tips of the finger and toes are
dilated into prominent discs. Toes are partly webbed. The upper side of the body is smooth
with two broad dorsolateral longitudinal skin folds running from hear the eyelid to the hip of
both sides. Another ventrolateral longitudinal skin fold stretches from upper jaw to the
eardrum and than extends upto things. Belly is smooth but the skin is glandular around the
vent and below the things. Their colouration is cryptic, camouflaging the frog against the
surrounding vegetation. They are totally yellowish during juvenile stage, yellowish-brown to
leaf green during mature stage. This species is generally found in thick floating aquatic
vegetation, on lily ponds, bushes growing at the edge of ponds and marshlands. This species
mainly feed on grasshoppers, spiders, mosquitoes and small beetles. Beside India they can also be found in Burma, South China, Taiwan, Java, Sumatra and Malaysia.

x) Common Indian Tree Frog (*Polypedates maculates*): Medium-sized, slim narrow-waisted tree-frogs with slender elongated limbs and goggling eyes. Head is broader than long. Snout is pointed and projects a little beyond the mouth. Nostril is nearer to the tip of the snout than the eye. Eardrum is distinct. Fingers are with rudimentary webs. Toes are almost half-webbed. Tips of the fingers and toes are dilated into flattened, spherical or horse-shoe shaped adhesive discs. It has a smooth skin and granular belly and underside of the things. On its back are a pair of distinct elevations, which is observed only when it is at rest. The colouration of the body is highly variable and depends on the colour of the habitat. It has the ability to change colour readily from grayish-yellow to brown and deep yellow on the upper side. Their underparts are white. The limbs are cross-barred. This species is nocturnal and arboreal. They avoid daylight and hide among the green vegetation. The breeding season commences with the onset of the rains in the middle of May and lasts till the end of August. Pairing occur among grasses and bushes by the side of ponds, ditches and canals. These frogs are opportunistic feeder. They mainly consume spider and large insect including beetles grasshopper, cockroaches, ants and termites. Beside India they can also be found in Sri Lanka, Nepal and Bangladesh.

xi) Bamboo Tree-Frog (*Polypedates leucomystax*): Large-sized tree frogs with elongated limbs and bulging eyes. Head is broader than long. Snout is not pointed and projects slightly beyond the mouth. Nostril is nearer to the tip of the snout than the eye.
Eardrum is distinct. Fingers are without webs. Toes are almost fully webbed. Tips of the fingers and toes are dilated into horse-shoe shaped adhesive discs. It has a smooth skin and granular belly. This species is quite common, found in agricultural lands and light forests. They are nocturnal and arboreal, hiding during day time under the logs and stones are sit immovably on branches of bushes and especially among bamboo shoots by tucking in all the times under the body. Beside India they can also be found in South China, Thailand, Java, Sumatra, Singapore, Phillipines and Japan.

A few selected photograph of different toads/frogs have been shown in Fig.2.1.1a & 2.2.2b.
Marbled Toad (*Bufo stomaticus*)  
Chinese Frog (*Microhyla ornata*)

Painted Frog (*Kaloula poulchra*)  
Indian Bull Frog (*Rana tigerina*)

Leaping Frog (*Rana erythraea*)  
Green Pond Frog (*Rana hexadactyla*)

Fig. 2.1.1a. A few selected photographs of different toads/frogs
Green tree frog (*Hyla Cinerea*)

Chinese fire bellied toad
(*Bombina orientalis*)

Indian toad (*Bufo melanostictus*)

Grass frog (*Limnonectes cancrivora*)

Fig. 2.1.1b. A few selected photograph of different toads / frogs.
NEED FOR CONSERVATION OF TOAD & FROGS

The first vertebrate animal to which virtually everyone is introduced to in their high school practical classes in India is undoubtedly the common Indian toad (*Bufo melanostictus*). Every year millions of amphibians are killed throughout the world to acquaint the young biologist with the intricacies of vertebrate anatomy. Perhaps still greater is the number that are sacrificed in research and clinical laboratories of the world over for experiments on physiology, pharmacology and medicine.

Amphibians render incalculable services in agriculture. By sowing vast areas with food and other crops, we create the most suitable condition for the life and reproduction of insect pests that feed on plants. And they in turn take advantage of these ideal living conditions to inflict considerable and sometimes catastrophic losses on our agriculture. This is where the significance of amphibians is the greatest. Wherever we have not sharply reduced their numbers they become active defenders of our crops and help us in saving the harvest.

The overall importance of the amphibians in the biological control of pest has not yet been fully documented. Investigation in the amphibian diet in our country and worldwide has revealed that the insect they feed on are mostly destructive. Frogs and toads favour whatever small living things they see more often and owing to the act that insect pests are more numerous in our agricultural fields than other insects, they make up 80-85% of food consumed by them. For instance, a single toad can eat 22,500 destructive pest during the growing season from May till September at the rate of 150/day. Therefore, toads are valuable additions to the fauna of the agricultural areas despite their warts and unappealing appearance. Every year amphibians thus protect crops worth crores of rupees (Deuti and Goswami, 1995). One major feature of the hunting habits of frogs and toads has made them the most versatile
protectors of our crops. Insectivorous birds feed only during the day time. Hence their diet comprises only of pests that are active during day time. But amphibians hunt round the clock – mostly at twilight and by night and even sometimes during the morning. Therefore, they render great service to mankind by exterminating the nocturnal insects, that are not taken by the birds. They also feed on poisonous insects and to not abstain from taking certain caterpillars that are brightly coloured and re avoided by the majority of the birds. They help in reducing the number of many blood-sucking insects which are vectors of deadly diseases. Adults actively feed on mosquitoes and flies while the tadpoles and young froglets consume their larvae and pupae. Thus in every respect they may be considered as our true friends.

The most important function of amphibians in nature has been gross underestimated. While on the one hand they are active predators, on the other hand they constitute a vital link in the food chain of life by serving as prey base for apex predators in the ecosystem. Being extremely voracious they are naturally population regulators of the numerous invertebrate species that they feed on. In their turn, amphibians are extremely prolific breeders capable of rapid growth and intensive utilization of available food resources. Thus they are able to increase their number and biomass very rapidly and so govern the population of secondary predators who feed on them.

Amphibians are also utilized as food by human in many parts of the world. Frog’s legs are considered a delicacy in Europe and the United states. Therefore, since 1959 the export of ‘frozen frog legs’ worth lakhs of rupees had steadily caught up. The species involved were the Indian Bull Frog (Rana tigerina), and the Green Pond Frog (Rana hexadactyla) and occasionally the Skipping Frog (Rana cyanophlyctis). It has been estimated that 15-20 edible frogs are required to make a decent meal for a single person and in favourable weather a frog-
catcher could collect 50-60 frogs in a single night. This sort of wanton destruction of the entire frog population resulted in depletion of the large-sized frogs, affecting the status of the species as well as its commercial importance. Due to this merciless removal of these common frogs from nature, villagers in many areas felt a disturbance in the balance of nature. Therefore, the Government of India has now banned the processing and exporting of frog-legs, but these beneficial creatures cannot be protected by framing Wildlife Protection Law only, the creation of public awareness is required too.

But apart from the direct assaults on these useful animas, the indirect effects of human activities are far more devastating to the amphibians. Disturbance of their habitat and breeding grounds by clearing of land for human habitation, draining of swamps and marshes, filling up of wetlands, felling of natural forest to claim agricultural land and polluting of rivers, lakes and ponds with pesticides, detergents and toxic industrial effluents are all taking a heavy toll on the population of amphibia all over the countryside. In case of some species, not even a solitary individual can be seen any longer in areas where they were quite common even a few years before. Habit destruction is the single most factor responsible for the present decline of the amphibian fauna.

Conservation of amphibian fauna also desirable for further research on newer drug clue from amphibian organs mainly from skin. In addition to the previously discussed therapeutic potentiality against bacterial, viral, fungal infection, immunomodulation, anticancer, antidiabetic etc., it is warranted to discover the newer drug clue against HIV, neurodegenerative disease for mankind and better living.
SECTION 2

THE BUFO MELANOSTICTUS
PHYLOGENETIC STATUS OF THE COMMON INDIAN TOAD (DUBOIS, 1983)

PHYLUM - CHORDATA
SUBPHYLUM - VERTEBRATE
SUPERCLASS - GNATHOSTOMATA
CLASS - AMPHIBIA
SUCLASS - LISSAMPHIBIA
SUPERORDER - SALIENTIA
ORDER - ANURA
SUBORDER - RANOIDEI
SUPERFAMILY - HYLOIDAE
FAMILY - BUFONIDAE
GENUS - BUFO
SPECIES - MELANOSTICTUS
SCIENTIFIC NAME - *Bufo melanostictus*, Schneider (1799)
LOCAL NAME - ‘KUNO BANG’ implying that it resides in the corners of rooms.
Bufo melanostictus is distributed worldwide mainly in India, Pakistan, Sri Lanka, Nepal, Bhutan, Bangladesh, Burma, South China, Thailand, Malay Peninsula and Philippines.
**Characteristics features** — Large-sized toads, head is broader than long with cornified bony ridges. Snout is rounded. Nostril is a little nearer to the tip of the snout that the eye. Ear-drum is distinct, circular or oval, early two-thirds the diameter of the eye. Parotid glands are large and bean shaped. Fingers are without webs, The first finger is equal to or a little longer than the second. Tips of the fingers and toes are swollen. Toes are partly webbed with more than three segments are swollen. Toes are partly webbed with more than three segments and the fourth toe is free. Two oval (inner and outer) pedal tubercles are present. Skin is rough with several black-tipped spiny warts on the upper side of the body. Lower side is also rough in texture.

**Colour** — Uniformly grayish or dark brownish with a few yellow coloured spots on the back. Lower parts are dull white with a yellowish tinge. Juveniles are black or dark grey on the upper side and the lower side is uniform white or speckled with black.

**Sexual characters** — Females are larger than the males. During the breeding season the males develop nuptial thumbpads on the inner side of the first or second finger. The throat of the breeding male becomes yellow or light orange in colour and the vocal sacs turn black.

**Habit and Habitat** — The species is very common. They are terrestrial and nocturnal, found in roadsides and gardens. During the day time, they hide under logs, stones, piles of bricks, in moist holes and cervices of tree-trunk or even in the dark corners of village huts. They seem to be well acquainted with their habitat and often return to the same site again and again. They are solitary but congregate during the breeding season, when they are seen near water. They
are highly terrestrial at this time and their call consist of a series of identical low pitch notes. They are neither good swimmers nor leapers but do breast strokes while crossing a pool of water. During rainy season when insects are abundant, the toads locate them by vision and capture them by flibbing the tongue.

**Life History** – Toads lay their eggs inside translucent gelatinous string in stagnant water, on weeds or around the steam of water plants. In the absence of such plants, the eggs are laid in long strings at the bottom of the pond. Hatching take place in about four days. Larvae are black with strong mandibles and the anal opening is in the midline of the body. They usually swim at the surface and feed at the edge of the water. They are gregarious and consume algae, leaves of aquatic plants. Development is rapid being complete in 34-52 days. They metamorphosed toad lets range from 8-10 mm in length. After metamorphosis they migrate from the breeding sites in large numbers. In juveniles, the bony ridges on the head do not appear until they attain a snout to vent length of over 20 mm.

**Food** – They feed on termites, ants, mosquito, cockroaches, small beetles, earthworm but do not try to catch honey bees and wasps once they find out that these can sting.
SECTION 3

AMPHIBIAN SKIN CONSTITUENTS
The integument is the structural and functional interface between the organism and its environment. The skin or amphibians generally is described as being naked, that is, lacking the covering of scales, feathers or hair characteristic of most other classes of vertebrates. Furthermore, amphibian skin is permeable to water and as such is important in respiration, osmoregulation and to a limited degree, thermoregulation. Also, the general appearance of amphibians is the result of integumentary structure; colour and pattern are determined by the chromatophore and texture is the result of integumentary modifications (Whitear, 1977).

Amphibian skin
As in all vertebrates, the integument consists of outer layer, the epidermis of ectodermal origin and an underline layer, the dermis. Most of the latter is of mesodermal origin. The pigment cells are derived from the neural crest and these are ectodermal, also the glands embedded in the dermis are derived from the ectoderm (Whitear, 1977).

The outermost layer of epidermis, the stratum corneum, consists of a single layer of flattened cells. The stratum corneum is keratinized in most adult amphibians, but it is not keratinized in obligated neotenic salamanders, such as Necturus. The keratinized stratum corneum is separated from underlying stratum germinativum by irregular intercellular spaces that are interrupted by interconnecting filaments (desmosomes). The fibers of these keratinized cells form a double horizontal network reinforced by vertical bundles of filaments (tonofilaments) (LE Quan-Trong and Bouligand, 1976). Underlying the stratum corneum is the stratum germinativum which normally is 4-8 cell thick, the innermost cells are coloumar and the outer ones are progressively shorter. Lying within the stratum germinativum are
specialized mitochondria-rich cells and flask cells of unknown function. The epidermis is separate from the dermis by a basement membrane of collagenous fibers.

The stratum corneum is sloughed (shed or molted) periodically. The duration of intermolt period varies from 4-5 days in Ambystoma to 3-19 days in Bufo (Ling, 1972). In both salamanders and anurans, the stratum corneum splits middorsally beginning in the head, the splitting of the dorsal skin progress posteriorly. Most of the amphibians use their limbs to loosen and remove the slough either in patches or in one large piece, usually the slough is eaten. During the sloughing cycle, the intercellular subcorneal space between the stratum corneum and underlying stratum germinativum is filled with mucus thought to be secreted by the mitochondria rich cells (Deullman and Trube, 1986).

The dermis also consists of two layers. The outer stratum spongiosum is made up of areolar connective tissue with intertwining fibers and various types of cells, including the pigment bearing chromatophores. The underlying stratum compactum is composed of compactly arranged collagenous fibers. Mucous and granular (poison) glands of epidermal origin are embedded in stratum spongiosum. Other structures in the dermis include capillaries, nerve fibers and smooth muscles (Whitear, 1977).

In salamanders and especially caecilians, there is a practically imperceptible transition from the collagenous fibers of the stratum compactum of the dermis to the connective tissues covering the underlying bones and muscles. However, anurans are unique in having a loose skin attached to the body wall only at discrete places is one of the following ways: (1) by lymphatic septa which are thin, transparent sheets of connective tissue that divide the space between the skin and the muscles into separate compartment, the lymphatic sacs, (2) by fibers of transparent connective tissue commonly aggregate to hold a particular part of the skin close
to the body wall, (3) by coossification of the skin with underlying dermal bones, (4) by direct attachment of the skin to muscles, as in vocal sacs of some hylids and (5) by cutaneous muscles that insert on the skin (Deullman and Trube, 1986).

**Integumentary Glands**

Some earlier worker (Muhse, 1909) believed that only one kind of integumentary gland was present in amphibians, even in toads. It is now known that all amphibians have both mucous ad granular (poison) glands. In 1979, Neuwirth *et al.* first conclude that granular (poison) glands shared primitive characters among amphibians and their original function probably was other than poison synthesis; the glands were a preadaptation for producing the diverse toxins that evolved separately in some groups of amphibians.

All of the glands are alveolar. Typical mucous glands are smaller than granular glands and enclosed completely in the stratum spongiosum. The glands have one or two types of myoepithelial cells and at least in some dendrobatids there is a layer of melanophores around the lateral and superficial surfaces of the glands (Neuwirth *et al.*, 1979).

The number of mucos and granular glands vary throughout the body. Generally mucous glands are more abundant in the dorsal skin than ventrally. Also, mucous glands are more numerous and widely distributed throughout the integument than are granular glands, which tend to be aggregated at specific sites in many species, e.g., head and neck of many anurans and some salamanders and dorsal surface of tail in other salamanders (LE Quang-Trong, 1975).

Mucopolysaccharides secreted spontaneously and continuously serve to keep the skin moist. Granular glands secrete on sympathetic nervous or humoral stimulation. Various
substances (e.g., peptides ad alkaloids) in these secretion commonly are noxious and in some cases highly toxic. These secretions are important defense mechanism (discussed earlier). Three other kinds of integumentary glands are known in amphibians. Rather large, elongated glands are present in the skin in the dermal folds of caecilians. Taylor (1968) noted that these glands are associated with dermal scales and suggested that secretion from these glands may form these sales. Blaylock et al. (1976) discovered lipid glands in the skin of phyllomedusa, hylid frogs that secrete an impervious coating that protects them from desiccation. Lipid glands are slightly larger granular glands, usually are in contact with the stratum corneum basally and have a distinct myoepithelium. Breeding glands in the skin of the chest region of the microhylid *Gastrophyryne carolinensis* were described by Conaway and Meter (1967). Similar have also been noted in other microhylids. The breeding glands are about the same size as the granular glands. The secretion is released by the fragmentation of the superficial part of the gland and the sticky secretion adheres the male to the dorsum of the female. Histochemically, the secretion is similar to that of mucous glands, but it lacks the sulfate groups characteristic of mucus (Holloway and Dapson, 1971).

Clusters of mucous or granular glands form obvious integumentary structures (macroglans) in many anurans and in some salamanders. Many of these structures develop only in males in response to testicular hormones and these structures are present only in the breeding season. The most widespread of these are nuptial excrescences, which are highly keratinized clusters of mucous glands, on the thumbs of many kinds of anurans and on the limbs of some salamanders. Clusters of granular glands may be present only in males (e.g., mental glands in plethodontid salamanders) and although these glands may become enlarged during the breeding season and therefore, be affected by testicular hormones, they are not
strictly seasonal in their presence. Other clusters of granular glands, such as the dorsal warts and parotid glands of bufonids and some salamandrids, the lumber glands of several genera of leptodactylids, the tibial glands of some myobatrachids and bufonids and dorsolateral and dorsal ridges of ranids are permanent structures and present in both male and female. The secretion of many of these glands are known to be important in defense against predator (Deullman and Trube, 1986).

The secretions produced by the integumentary glands include numerous complex biogenic amines and active polypeptides, Erspamer (1971) noted the presence of three groups of aromatic amines and five groups of polypeptides. The amines are:

1. **Indolealkylamines**, including 5-hydroxytryptamine (5-HT), which is present in most families and genera of amphibians and N-methylated derivatives such as bufotenin and bufotenidine, which are found in pipids, leptodactyllids, bufonids, hylis, ranids and some salamanders.

2. **Imidazolealkylamines** known from *Leptodactylus labyrinthicus* and *L. pentadactylus*. Related histamines occur in several unrelated genera (e.g., Leptodactylus, Taudactylus and Litoria).

3. **Hydroxyphenylalkylamines**, including leptodactylin known from various leptodactylids and epinephrine and norepinephrine known from Bufo.

The active polypeptides include numerous toxins and other less toxic substances –

a) **Eledosine** – like polypeptides, such as physalaemin isolated from physalaemus, phyllomedusin from phyllo-medusa and uperolein from uperoleid.

b) **Bradykinin ad bradykinin** – like polypeptides: bradykinin isolated from *Rana temporaria* and phyllokinin from phyllomedusa.
c) Caerulein and caerulein-like polypeptides: Caerulein isolated from *Xenopus laevis* and various species of Litoria and Leptodactylus and Phyllocaerulein from Phyllomedusa.

d) Three types of alytesin and alytesin-like polypeptides: I from *Alytes obstetricans*; II from *Bambina bombina* and *B. Variegata* and III from *Rana pipsiens*.

e) Miscellaneous polypeptides, including several other kinds, the chemical nature of which is not yet known.

**AMPHIBIAN SKIN CONSTITUENTS**

**Basic constituents**

The secretion of the skin glands of toad contains two principal classes of pharmacologically active constituent. One class is formed by substances belonging to the steroids, the bufogenin and their derivatives, the bufotoxin. To the other class belong different basic compounds. In some cases these compounds have been isolated from excised parotid glands, in other cases the whole skin, including adhering parotid and other small glands, has been extracted.

From the studies on the distribution, characterization, isolation and determination of the chemical structure of the basic constituent present in the toad skin where carried out in several laboratories. The skin constituents of the genus *bufo* contain bases of two different chemical types – (a) derived from phenylethylamine (b) derived from tryptamine (Deulofeu and Ruveda, 1971).
The Phenylethylamine Bases - The following bases derived from phenylethylamine have been identified in toad venom and/or skin. Dopamine, N-methyldopamine, noradrenaline and adrenaline all belong to the class of the catecholamines. The catecholamines have been isolated from the skin of different species of toad and frog.

Only adrenaline has actually been isolated. It was found to be the same stereoisomers obtained from the adrenal of higher animals: R(−) adrenaline (Pratesi et al., 1958). Noradrenaline has been detected pharmacologically, chemically by paper chromatography by Lasagana (1951) from *B. marinus*. It has been detected in the extract from excised parotid glands and from the secretion of the same species (Marki et al., 1962). N-methyldopamine and dopamine have been detected) by paper chromatography from *B. marinus* (Marki et al., 1962), the former being the first time from animals. This catecholamine now has been isolated from different species of toad/frog. Adrenaline and noradrenaline have been identified from *B. marinus* (Marki et al., 1962). *B. regularis* (Chen and Chen, 1933) and from many others species of toad/frog. Adrenaline has been first isolated from *B. marinus* (Abel and Macht, 1911).

The tryptamine bases and derivatives - The following bases derived from tryptamine have been isolated or identified from toad venoms, skin or excised parotid glands – (a) 5-hydroxytryptamine (serotonin), (b) N-methyl-5-hydroxytryptamine, (c) N-methyl-5-methoxytryptamine, (d) bufotenine and it’s sulfuric acid ester bufoviridine, (e) O-methylbufotenine, (f) bufotenidine, (g) dehydrobufotenine and its sulfuric acid ester bufothionine (Deulofeu and Ruveda, 1971).
a) **5-hydroxytryptamine (Serotonin)**: The identification of 5-hydroxytryptamine in different animal tissues and its physiological activities was well established. 5-hydroxytryptamine has been identified from *B. marinus* (Udenfriend *et al.*, 1952), *B. bufo bufo* (Spandrio, 1961), *B. viridis* (Erspamer, 1961) and in other genera: Xenopus, Bombinator, Rana, Salmander (Erspamer, 1961).

b) **N-methyl-5-hydroxytryptamine**: This amine was characterized for the first time by Erspamer and Viali (1915) in the extracts from skin and parotid gland from several species of bufo (*B. americanus*, *B. calamita*, *B. marinus*, *B. viridis* etc.) and was further characterized by paper chromatography (Erspamer *et al.*, 1966). It is absent in other genera of amphibia.

c) **N-methyl-5-methoxytryptamine**: In animals this base has been found only in extracts of the skin of *B. alvarius* (Erspamer *et al.*, 1966).

d) **N,N-dimethyl-5-hydroxytryptamine (Bufotenine)**: Bufotenin is the indolic base isolated from toad skin. This compound was identified from the skin of *B. americanus*, *B. calamita*, *B. fowleri* (Erspamer, 1954). Erspamer (1959) isolated from the skin secretion of *B. viridis* and *B. calamita* its sulfuric acid conjugate, which has been named as bufoviridine. The formation of bufoviridine is an indication of the capacity for the sulfoconjugation of 5-hydroxyindole derivatives which was found in toad skin.

e) **N,N-dimethyl-5-methoxytryptamine (O-methylbufotenine)**: O-methylbufotenin has been isolated for the first time from an animal source from the skin and glands of *B. alvarius* (Erspamer *et al.*, 1965). They also calculated the amount obtained from this species, which was 1.0-3.5 mg/gm dried skin.
f) **N,N,N-Trimethyl-5-hydroxytryptamine (Bufotenidine, Cinobufagine):**
Cinobufagine was the name given to a base isolated as flavinated from Chan’su by Jensen and Chen (1930). Wieland et al. (1931) isolated it from the same source and found that it was also present in the skin gland secretion of *B. vulgaris* and named the base bufotenidins (Wieland et al., 1931). Latter this compound was also identified from *B. americanus, B. calamita, B. paracuemis, B. viridie* (Erspamer, 1954) and *Xenopus laevis* (Jensen, 1935). Wieland et al. (1934) also identified this compound from *B. bufo bufo* (170 μg/animal).

g) **Dehydrobufotenin and Bufothionine:** Bufothionine was the first isolated by Wieland and Vocke (1930) from B. gama. By acid hydrolysis this compound produced a sulfuric acid base, named dehydrobufotenin. Dehydrobufotenin was also isolated from several species of toad skin (Jensen and Chen, 1930; Weiland et al. 1934), e.g., *B. marinus, B. bufo bufo*, *B. americanus, B. fowleri* etc.

**Steroids**

Secretion of parotid gland and other skin glands contain two groups of toxic substances. They are steroid derivatives known as (i) befogenin of systematically as bufodienolides and (ii) bufotoxin, which are primarily responsible for the pharmacological effect of poisonous secretion (Meyer and Linde, 1971).

**Bufogenin:** The bufogenins are C₂₄ steroids. The stereochemistry of the six asymmetric centers of the ring junction at C-5, C-8, C-9, C-10, C-14 and C-14 making possible in principle 64 isomers, is the same of all naturally occurring bufogenins. Befogenins and it’s
different isomer had been isolated from different species of toad and frog, e.g., *Bufo alvarius* (Barbier et al. 1961), *Bufo arenarum* (Rees et al., 1959), *Bufo usper* (Iseli et al., 1964), *B bufo bufo* (Zelnik and Ziti, 1963), *Bufo fowleri* (Linds and Meyer, 1958).

The bufogeninis differs primarily in the number and position of the hydroxyl groups, which are scattered all over the skeleton. So far known, a primary hydroxyl group had been found in cinobufoginol and in hellebrigenol. Each bufogenin had at least at C-3 a secondary hydroxyl group. Additional hydroxyl groups were found at C-11 (gamabufotalin, arenobufogin), at C-12 (buforenogin) and at C-16 (cinobufagin and cinobufotalin). Most bufogenins has an integral part a tertiary hydroxyl group located at C-14 and sometimes and additional one at C-5. A separate group was made up of those bufogenins carrying an oxido group at C-14/C-15 (resibufogenin, marinobufogin, cinobufogin, cinobufotalin). A keto group was detected at C-12 (arenobufogin) and C-11 (buforenogin), an □-diketo group at C-11, C-12 (argentinogeinin and an aldehyde group at C-11 (bufotalinin) (Meyer and Linde, 1971).

Arenobufogin (C_{24}H_{32}O_{6}) was first isolated by Chen and Co-worker (1932) from the South American toad *B. arenarum* (Chen et al., 1932) argentinogenin (C_{24}H_{30}O_{6}) was first by Rees and associates (1959) from *B. arenarum*. It was also observed that on prolonged contact with alumina column, arenobufogin undergoes a change : an isomer bufarogin and a dehydration product of arenobufogin, called argentinogenin, are formed (Rees et al., 1959). Epibufolin, another bufogenin was obtained from an alcoholic extract of fresh skin of *B. formosus* (Iseli et al. 1965). The ketol bufogenin (C_{24}H_{32}O_{6}) was first isolated by Rees and associated (1959) from *B. arenarum* is isometric with arenobufagin (Ress et al., 1959). Bufotalin was first isolated in crystalline from *B. bufo bufo* by Weil and co-worker in 1913.
This has been described by them as an amorphous substance and found strongly
cardiostimulator. For three decades bufotalin has remained the most investigated bufogenin.
The formula was worked out and was found to be \( \text{C}_{26}\text{C}_{36}\text{O}_6 \) (Wieland and Weyland, 1920).
Bufotalinin \( \text{C}_{26}\text{C}_{36}\text{O}_6 \) was also isolated from \textit{B. bufo bufo} (Wieland and Hess, 1935) and the
substance does not possess an acetoxyl group and readily decompose. Bufotalin was obtained
by \( \text{CrO}_3 \) oxidation of bufotalin (Wieland and Weyland, 1920). Later on it was isolated from
\textit{B. formsus} and described in detail by Kotake (Kotake et al., 1928).

**Bufotoxin**- Toad skin contains beside the bufogenin, another type of cardioactive substance,
called bufotoxin. The represented conjugates of bufogenin with suberylarginine were isolated
and investigated in a number of laboratories in 1920’s. In 1922, Wieland and Alles first
isolated a nitrogen containing substance from the venom of \textit{B. bufo bufo}, which they named
bufotoxin (Weiland and Alles, 1922). The bufotoxin was also isolated from Japanese toad \textit{B. formosus} (Weiland and Vocke, 1930), \textit{B. marinus} (Jensen and Chen, 1930) and many other
species.

**Polypeptides**

The active polypeptides so far detected in the amphibian skin may be divided into five
groups characterized by distinctive features: eledoisin-like polypeptides or techykinins,
bradykinin-like polypeptides or bradykinin, aerulein-like polypeptides, alytensin-like
polypeptides and finally miscellaneous polypeptides (Erspamer, 1971).

**Eledoisin-like polypeptides**- The members of this polypeptide group display
pharmacological actions mimicking those of eledoisin, an endacapeptide first found in the
The most important eledoisin-like polypeptide detected in amphibian skin is physalaemin, which has been isolated in a pure form methanolic extract of the skin of Physalaemus bigilonigerus and which is present in the skin extract of other physalaemus species as well. The content of physalaemin ranges from 370 to 700 µg/g dry skin (Anastasi et al., 1964).

Like eledoisin, physalaemin displayed a tremendously intense hypotensive action in the dog, a potent stimulant action on isolated large intestine of the rabbit and the isolated ileum and large intestine of the guineapig, while eliciting a poor response in the rat uterus and rat colon (Bertaccini et al., 1965). Moreover, the polypeptide produced a powerful stimulation of the salivary and lacrimal secretion of the chicken, rat, dog and strikingly increased capillary permeability in the guineapig rat and man (Erspamer, 1971).

It has been known for several years that skin extract of the South American hylid frogs of the genus *Phyllomedusa* displayed physalaemin-like activities. The polypeptides or more likely, one of the polypeptides responsible for this action, has been isolated from the skin of *Phyllomedusa bicolor* and its structure has been fully elucidated (Anastasi et al., 1970). From the formulae it can be seen that its amino acid composition and sequence is strictly related both to eledoisin and to physalaemin.

Physalaemin and phyllomedusin are representatives of eledoisin-like polypeptides occurring in nature. Another highly active peptide belonging to this group is uperolein, isolated from the skin of the Australin amphibia *Uperolein regosa* (Erspamer et al., 1966). Still other physalaemin-like peptides probably occur in the skin of Australian amphbiians (Taudactylus and Pseudophryne) and in the skin of African amphbiians (Erspamer, 1971).
Bradykinin and bradykinin-like polypeptides - Polypeptides of this group were characterized by a remarkable but not exceptionally intense hypotensive action in the dog, rabbit, cat; by a potent stimulant action of the isolated guineapig ileum and cat large intestine; by a formidable stimulant action on the estrous rat uterus. They have a poor stimulant action on the rabbit and rat colon and display an inhibitory action on the rat deodenum. Bradykinin-like polypeptides effectively increase capillary permeability in man and experimental animals and cause pain when administered inter-arterially or intra-peritoneally (Erspamer, 1971). In addition to authentic bradykinin, 7-natural bradykinin-like polypeptides have been so far isolated: (i) Kallidin or Lysylbradykinin, (ii) methionyl-kallidin or methionyl-lysyl-bradykinn, (iii) glycyl-bradykinin, (iv) phyllokinin or bradykinyl-isoleucyl-tyrosine-o-sulphate, (v) polysteskinin and finally (vi) colostrokinin (Erspamer, 1971).

Authentic bradykinin has been isolated from the skin of common European brown frog *Rana temporaria*, which contained as high as 200 to 250 μg/g bradykinin in fresh tissues (Anastasi et al., 1964). Phyllokinin has been prepared in a pure state from the skin extracts of the Brazilian *Phyllomedusa rohdei* and also in *Phyllomedusa bicolor* and in other phyllomedusa species as well (Erspamer, 1971). Polysteskinin has been isolated from skin extracts of the Japanese frog *Rana nigromaculosa*, together with authentic bradykinin (Nakajima et al., 1968). Bradykinin or bradykinin-like peptides are present in the skin of several other species of the genus Rana and outside the genera *Phyllomedusa* and *Rana*, in the skin of other amphibian species as well (Nakajima et al., 1968).

Caerulein and caerulein-like peptides- Caerulein is a decapeptide first isolated from methanol extracts of the skin of the Australian hylid frog *Hyla caerulein*, where it was present
in the concentration of 100-1000 μg/g fresh skin (Anastasi et al., 1968). Later on, authentic caerulein was prepared from the skin of the South American leptodactylid frog *Leptodactylus pentadactylus labyrinthicus* and of the South African amphibian *Xenopus leavis* (Anastasi et al., 1970). Caerulein is also present in the skin of a number of other Australian hylid frog, *Hyla infrasrenata* and *Hyla moorei* (3500-3000 μg/g dry tissue) and South American leptodactylid frog, *Leptodactylus laticeps* (1300 μg/g fresh tissue) (Erspamer, 1971). Caerulein soon revealed a tremendous stimulant action on the musculature of the gall bladder and small intestine and a formidable stimulant action on the pancreatic secretion, together with a conspicuous stimulant action on gastric secretion (Erspamer, 1971).

**Alytensin and alytensin-like polypeptides** - Three polypeptides belonging to this family had been isolated in a pure form. Alytensin was a tetradecapeptide obtained from methanol extracts of the skin of the European frog *Alytes obstetricans*. Bombesin was again a tetradecapeptide obtained from the skin extracts of two European frogs *Bombina bombina* and *Bombina variegata* (Anastasi et al., 1970). Ranatensin is an endecapeptide prepared from skin extract of American frog *Rana pipiens* (Nakajima et al., 1970). It produced hypertensive action in the dog presenting tachyphylaxis, potent stimulant action on the estrous uterus of the rat, guineapig colon and cat ileum. Beside these, it produced moderate hyperglycemic effect on rats and dogs (Erspamer, 1971).

**Miscellaneous polypeptides** - A new class of antimicrobial peptides called maginin has been isolated from the skin of African clawed from *Xenopus leavis*. By acting as sterilizing agent of the skin, they may be responsible for the extra-ordinary freedom from infection
characteristic of wound healing in these frogs, even in a microbially contaminated habitat. At a low concentration, these water soluble peptides inhibit the growth of numerous strains of bacteria and fungi and induce osmotic lysis of protozoa (Zasloff, 1987).

Another novel antimicrobial peptide, designated dermaseptin b, was isolated from the skin of the arboreal frog *Phyllomedusa bicolor* (Mor et al., 1994). A 34-residue cationic and amphiphatic peptide, designated dermaseptin 1, was also isolated from the skin of arboreal frog *Phyllomedusa sauvagii* and was shown to exhibit antimicrobial activity against various pathogenic microorganisms (Mor and Nicolas, 1994). Two unique antimicrobial peptides named brevinin-1 and brevinin-2 were isolated from the skin of the frog *Rana brevipada porsa*. Both of these peptides have structural similarity with any known antimicrobial peptide of amphibian and nonamphibia origin (Morikawa et al., 1992). The mechanism of action for frog skin antimicrobial peptide, has been proposed, based on amphipathic nature of the peptides when they contact bacterial surfaces. This results in anion channel formation and penetration of the membrane which allows efflux of OH⁻ and uncoupling of respiration in the bacteria (Spencer, 1992).

Beside these antimicrobial peptides, a new active polypeptide, named Saugagine, was isolated from the skin of *Phyllomedusa sauvagii*, a frog of Central and South America. Sauvagine possesses a number of pharmacological actions on diuresis, the cardiovascular system and endocrine glands. It can be considered the prototype of a new family of amphibian peptide, in addition to the tachykinin, bradykinin, deromorphins, caerulin-like and bombes in-like peptides (Montecucchi and Henschen, 1981). Sauvagine also acts on brain to increase plasma level of catecholamine and glucose and to elevate mean arterial pressure. Outside the
brain sauvagine increases superior mesenteric artery flow and plasma glucose concentration (Brown et al., 1982). Sauvagine also increased the release of adrenocorticotropin, betaendrophin and corticosterone in rat after subcutaneous (5 μg/kg) injection (Negri et al., 1983). A new antibiotic caerin 1 peptide has been isolated from the skin secretion of the Australian tree from *Litoria chloria* (Steinborner et al., 1998).
SECTION 4

THERAPEUTIC POTENTIAL OF AMPHIBIAN SKIN CONSTITUENTS
TRADITIONAL AND FOLK INFORMATION

In Shakespeare's famous play Macbeth, the three witches were shown to boil their poisonous "hell-broth" in a charmed pot by stirring in "Eye of newt, and toe of frog". Medieval European traditions correlated everything ugly and repulsive - starting from warts to witchcraft, to the toads and their cousins.

In 18th century Swedish taxonomist Carolus Linnaeus described frogs and toads as "These foul and loathsome animals are abhorrent because of their cold body, pale color, cartilaginous skeleton, filthy skin, fierce aspect, calculating eye, offensive smell, harsh voice, squalid habitation, and terrible venom...." Actually, the amphibians are rather defenseless creatures that are consumed readily by a great variety of predators. In order to protect themselves from these predators, the amphibians have evolved different morphological, physiological and behavioural features. All frogs and toads have two types of skin glands - the mucous and the granular. It is evident to note that amphibians like frog and toad possess different bioactive substances in their skin (Duellman and Trueb, 1986). Certain Amazonian tribal hunters rub the skin secretions of the giant monkey frog (*Phyllomedusa bicolor*) into self inflicted burns or wounds so that the toxins would induce nausea and hallucination, which they believe will increase their awareness and hunting success. Many tribes of South and Central America have used compounds from frogs and toads as poisons and hallucinogenic drugs for religious rituals. In Chinese folklore it was found that they used to dipped the toad skin in wine and the next day the wine was administered in leukemic patient. The science behind it was not clear at that time. But they experienced the possibly the positive effect of this type of therapeutic wine. A popular medieval Europe superstition was the "toad-stone" - a jewel that was supposed to be found inside the toad's head. The "toad-stone" was actually a
hard lump cut out of the head of the largest, old, warty European toads and placed in a ring or necklace. It was believed that it could reduce pain or swelling of a skin wound caused by the bite of some poisonous creature. In the Indian tribal culture (Santhals, Lodhas etc.), amphibians were believed to possess medicinal properties (Bodding, 1934). Frog poisons were used as aphrodisiacs, impotent and infertility preventions, contraceptives and in many other illnesses. Today, the skins, bodies and body parts of salamanders are used in traditional medicines. Torched newts are sometimes sold in Asia as aphrodisiacs and the skin of certain species are said to cure illnesses. Superstitions and folklore as these may be, they were actually the stepping-stones to modern biological sciences.

Amphibian skin is a morphologically, biochemically and physiologically complex organ, which fulfils a wide range of functions necessary for the amphibian survival, including respiration, water regulation, anti-predator, antimicrobial defense, excretion, temperature control etc. (Clarke, 1997). Toads, particularly members of the genus Bufo, are identified as a particularly convenient and useful source of granular gland secretions, which commonly contain biogenic amines, bufodienolides, alkaloids and steroids, peptides and proteins (Cei et al., 1967; Clarke, 1997; Daly et al., 2004; Maciel et al., 2003; Steyn and Van Heerden, 1998). In the Chinese traditional medicine, amphibians skin extract had been used for the alleviation of human sufferings (Clarke, 1997; Ko et al., 2005). Chan Sue, the Chinese toad (Bufo Bufo gargarizans) skin extract preparation was used in the treatment of various diseases, like cancer, arrhythmia and other heart diseases (Datta and Dasgupta, 2000; Bhuiyan et al., 2003; Nogawa et al., 2001).

Chan Su is a traditional Chinese medicine prepared from dried white secretion of the skin glands of the Chinese toad and has been used as an oriental drug for treating heart
diseases and other systemic illnesses. Scientific research has confirmed the presence of bufalin in Chan Su, which is responsible for digoxin-like action. A Chinese flu remedy when analysed was also found to contain Bufo toxins. Scientists have also found that Chan Su possesses antineoplastic properties and components of Chan Su were capable of potentiating immune responses in experimental animals. Scientists throughout the world are now exploring the therapeutic potential of various toad and frog skin extracts and secretions. Some of the most toxic frogs and toads hold some of the best pharmaceutical promises.

MODERN INFORMATION

Chemicals from nature have been a part of human civilization ever since our early ancestors began exploiting natural compounds to improve and enrich their own lives (Agosta, 1996). A major part of these chemicals come from the animals. Animal-based medicines have been elaborated from parts of the animal body, from products of its metabolism (secretion and excretion). The investigation of tradition medicines has proven a valuable tool in the developing art of bioprospecting for pharmaceutical compounds. Of the 252 essential chemicals that have been selected by the World Health Organization, 11.1% come from plants, and 8.7% from animals (Marques, 1997). The therapeutic potentiality of amphibian skin components are presented here based on its modern scientific information.

Antibacterial, Antifungal & Antiprotzoal - Amphibian skin is a rich resource of antimicrobial peptides like maximins and maximins H from the toad Bombina maxima. The Maximin 3 and Maximin H5 were isolated from a skin cDNA library of Bombina maxima. The predicted primary structure of maximin H5 was ILGPVLGLVSDTLDDVLGIL-NH2.
Different from cationic maximin H5 the present anionic maximin H5 only sensitive to Gram positive bacteria eg. Staphylococcus aureus (Lai et al., 2002). Amphibian peptides often have no mammalian counterpart, and display varying degrees of specificity for both bacterial and eukaryotic cells. For example, some exhibit broad-spectrum antibiotic activity while others are active against only selected micro-organisms (Erspamer et al., 1994). Magainins, a new class of peptides, isolated from the skin of the African clawed toad, *Xenopus laevis* (Zasloff, 1987). Magainin I and II differ by two amino acid residue. Magainins exhibits wide-spectrum antibiotic activity, inhibiting the growth of both Gram positive and Gram negative bacteria, fungal species such as *Candida albicans*, *Cryptococcus neoformans* and *Saccharomyces cerevisiae* and also have been demonstrated to induce lysis in several protozoan species, e.g. *Paramecium caudatum*, *Amoeba proteus* and *Euglena gracilis* (Zasloff, 1987). Citropin 1.1 (GLFDVIIKVASVIGGL-NH₂, MW 1613) is the major wide-spectrum antibiotic peptide in the secretion of the skin glands of *Litoria citropa* (Wegener et al., 1999). Interestingly citropin 1.1 are active against a number of pathogens that show resistance towards currently used antibiotics (Hancock et al., 1997). Regarding the antibacterial activity of these peptides, a number of mechanisms have been proposed to rationalize membrane penetration by the peptide, the simplest of which include the barrel-stave and carpet mechanisms. In the barrel-stave model, peptides aggregate at the membrane surface in α-helical form, driven by electrostatic attraction between charged residues and ionic sites on the bilayer. Subsequent insertion into the membrane then occurs via formation of a trans-membrane barrel-like pore, in which peptides are oriented perpendicular to the plane of the bilayer (Apponyi et al., 2004). The largest group of antibacterial amphibian peptides isolated to date is that of the caerin peptides, with over 30 identified from more than six Australian frog species of the *Litoria*
genus. These can be further divided into four subgroups, with the caerin 1 broad-spectrum peptides the most common. All caerin 1 peptides have similar primary structures based on that of caerin 1.1 (GLLSVLGSVAKHVLPHVVPVIAEHL-NH2, MW 2582), and are active mainly against Gram-positive bacteria (Steinborner et al., 1998; Apponyi et al., 2004). Tigerinins, a novel antimicrobial peptide isolated from Indian frog Rana tigerina (Sai et al., 2001). Proteomic analysis of electrically stimulated skin secretions from the crawfish frog, Rana areolata enabled the identification and characterization of eight peptides with antimicrobial and hemolytic activity belonging to the brevinin-1, temporin-1, palustrin-2, palustrin-3, esculentin-1 (two peptides), and ranatuerin-2 (two peptides) families. The primary structures of the peptides were consistent with a close phylogenetic relationship between R. areolata and the pickerel frog, Rana palustris. (Ali et al., 2002). PS-1 (phylloseptins) isolated from Phyllomedusa skin secretions also showed antimicrobial activity (Leite et al., 2005). Very recently, a bombesin-like peptide PR-bombesin derived from the skin of the Chinese red belly toad, Bombina maxima, showed antimicrobial activity, which was likely due to the proline rich sequence (Li et al., 2006). Six brevinin family antimicrobial peptides were also identified from the Tsushima brown frog Rana tsushimensis Stejneger (Conlon et al., 2006). Syphaxin 1.5 (1577.83D peptide) from Leptodactylus syphax showed antimicrobial activity on Staphylococcus aureus and Escherichia coli (Dourado et al., 2006). Temporins A and B secreted from the skin of European red frog Rana temporaria showed anti-leishmania activity at micromolar concentrations, with no cytolytic activity against human erythrocytes. They cause severe membranalysis of the parasite, which is likely to make it difficult for the pathogen to develop resistance (Mangoni et al., 2005).
Anti Leishmaniasis - Leishmaniasis encompasses a wide range of infections caused by the human parasitic protozoan species belonging to the Leishmania genus. It appears frequently as an opportunistic disease, especially in virus-infected immunodepressed people. Similarly to other pathogens, parasites became resistant to most of the first-line drugs. Therefore, there is an urgent need to develop antiparasitic agents with new modes of action. Gene-encoded antimicrobial peptides are promising candidates, but so far only a few of them have shown anti-protozoa activities. Temporins A and B, 13-amino acid antimicrobial peptides secreted from the skin of the European red frog *Rana temporaria*, display anti-Leishmania activity at micromolar concentrations, with no cytolytic activity against human erythrocytes. Temporins represent the shortest natural peptides having the highest leishmanicidal activity and the lowest number of positively charged amino acids (a single lysine/arginine) and maintain biological function in serum. Their lethal mechanism involves plasma membrane permeation based on the following data. (i) They induce a rapid collapse of the plasma membrane potential. (ii) They reduce intracellular ATP levels. (iii) They severely damage the membrane of the parasite, as shown by transmission electron microscopy. The unique properties of temporins, as well as their membranolytic effect, which should make it difficult for the pathogen to develop resistance, suggest them as potential candidates for the future design of antiparasitic drugs with a new mode of action (Mangoni et al., 2005).

Antimalarial, Antiviral & Anti HIV activity- *Plasmodium falciparum*, a harmful and life threatening parasite produces malignant malaria. There is no alternative other than Quinine groups to combat the fever. Only chloroquine is substituted by other quinine derivative primaquine. But interestingly certain amphibian peptides kill the malaria parasite (*P.*
falciparum). Very recently Apponyi et al., (2004) first observed that the caerin 1 peptides of the amphibian skin secretion are active against this parasite at micromolar concentration. Among them caerin 1.8 (amino acid sequence: GLFKVLGSVAKHLLPHVVPVIAEKLNH₂, MW 2662) is the most potent.

Brevinin 1, a frog skin defensive peptide was found to be potent antiviral activity on Herpes simplex virus 1 and 2. The antiviral activity of brevinin-1 was maintained after reduction and carboxamidomethylation, procedures that abolished its otherwise prominent hemolytic and cytotoxic effects (Yasin et al., 2000). Esculentin-2P (E2P) and ranatuerin-2P (R2P), two antimicrobial peptides isolated from Rana pipiens, inactivate frog virus 3, a potentially pathogenic iridovirus infecting anurans, and channel catfish herpes virus. In contrast to mammalian antimicrobial peptides, E2P and R2P act within minutes, at temperatures as low as 0°C, to inhibit viral infectivity. Moreover, these compounds appear to inactivate the virus directly and do not act by inhibiting replication in infected cells (Chinchar et al., 2001).

Two groups of antimicrobial peptides have been isolated from skin secretions of Bombina maxima. Peptides in the first group, named maximins 1, 2, 3, 4 and 5, are structurally related to bombinin-like peptides (BLPs). Among the maximin peptides, maximin 3 possessed a significant anti-HIV activity. Maximins 1 and 3 were toxic to mice with LD(50) values of 8.2 and 4.3 mg/kg, respectively (Lai et al., 2002). Recently, a novel 63kD heme-containing protein BAS-AH isolated from skin secretions of Bufo andrewsi displayed dose dependent inhibition on HIV-1 infection and replication (Zhao et al., 2005).
Anticancer activity- There are several reports on the anticancer activity of toad and frog skin bioactive compounds. Magainins are an ionophoric class of vertebrate peptides with antibiotic activity against various micro-organisms. Cruciani et al., (1991) reported that magainin 2 and synthetic analogues can rapidly and irreversibly lyses hematopoietic tumor and solid tumor target cells with a relative cytotoxic potency that parallels their antibacterial efficacy and at concentrations that are relatively nontoxic to well-differentiated cells. The cytotoxicity is prevented by cell depolarization. Magainins represent a natural cytolytic agent in vertebrates and may provide another therapeutic strategy for certain tumors. Already mentioned above that Citropin 1.1 has potent wide-spectrum antibacterial activity within $10^{-6}$ M concentration range which also include the anticancer activity by inhibiting nNOS (Doyle et al., 2003). This concentration is significantly less than that required to cause lysis of red blood cells. Synthetic modification (A4K14-ctropin) of citropin 1.1 can achieve a 10 fold increase in antibacterial and anticancer activities (Doyle et al., 2003). The naturally occurring bufadienolides were purified from Chan Sue and tested for differentiation induction and antileukemic activities on K562 cell by the nitroblue tetrazolium reduction assay (Numazawa et al., 1994). The same test was carried out with the synthetic derivatives of bufalin. Interestingly bufalin showed the strongest activity among all the bufadienolides tested in this study. The degree of the induction of nitro blue diformazan positive cells by the bufadienolides correlated well with their inhibitory activities against Na⁺-K⁺-ATPase prepared from K562 cell in vitro. Na⁺-K⁺-ATPases from a variant K562 clone (ouabain resistant, OuaR) and murine leukemia cell line M1-T22, which were insensitive to the bufadienolides in terms of growth inhibition and cell differentiation, appeared to be refractory to bufalin in vitro. A binding study of 3H-bufaline and 3H-ouabain revealed that saturated levels of both ligands associated with K562 cells were
virtually similar; however, affinity of 3H-bufalin was considerably higher than 3H-ouabain. These studies suggested that bufalin acts on the cells by binding to sites on the cell membrane which also bind ouabain and proposed that Na⁺-K⁺-ATPase inhibition is closely related to the initiation for the induction of K562 cell differentiation induced by bufalin. In another study endogenous bufalin like factor was found which also found to inhibits the growth of several human derived leukemia cell line and interestingly this property of the factor retained after protease and heat treatment (Numazawa et al., 1995). They proposed from this studies that an endogenous Na⁺-K⁺-ATPase inhibitor in human plasma may play a role in cell differentiation. Since bufalin specifically inhibits the Na⁺-K⁺-ATPase of human but not murine tumor cells, and since this inhibition leads to a change in intracellular concentration of Na⁺ ions, the findings of Kawazoe et al., (1999) suggest that bufalin induces apoptosis in human tumor cells selectively via inhibition of the Na⁺-K⁺-ATPase, which acts upstream of the bcl-2 protein. Bufalin is a potent inducer of differentiation in human erythroleukemia K562 cells and other human leukemia derived cell lines (human promyelocytic HL60, monoblastic U937 and myeloblastic ML1). Treatment of K562 cells with toad and frog skin derived other cardiotonic steroids, such as cinobufagin, oubain and digitoxigenin, at same dose of bufalin showed weak or no effect on the cells (Zhang et al., 1991). Bufalin inhibited the proliferation of bovine aortic endothelial (BAE) cells and tube formation in three dimensional type I collagen matrix (Lee et al., 1997). As a potent inhibitor of endothelial cell proliferation, bufalin specifically prevented the entry of BAE cells into the G0/G1 phase of a cell cycle. These finding suggest that in vitro angioinhibitory action of bufalin may be induced by the proliferation inhibition of endothelial cells through the arrest at the G2/M phase of a cell cycle. Bufalin and cinobufagin may inhibit the proliferation of prostate cancer cell lines
associated with sustained elevation of \([\text{Ca}(2+)](i)\) and that of apoptosis (Yeh et al., 2003). Very recently Ko et al., (2005) reported that Chan Su induced apoptosis in a human bladder carcinoma cell line, T24 in a concentration dependent manner, which was associated with a down-regulation of anti-apoptotic Bcl-2 and Bcl-X(S/L) expression and an up-regulation of pro-apoptotic Bax expression. Chan Su treatment induced the proteolytic activation of caspase-3 and caspase-9, and a concomitant degradation of poly(ADP-ribose)-polymerase and beta-catenin protein. Chan Su also decreased the levels of COX-2 mRNA and protein expression without significant changes in the levels of COX-1, which was correlated with an inhibition in prostaglandin E(2) synthesis (Ko et al., 2005). Bufalin significantly inhibited the cell proliferation and DNA synthesis of cultured ovarian endometriotic cyst stromal cells and induced apoptosis and the G0/G1 phase cell cycle arrest of these cells by down-regulation of the cyclin A, Bcl-2, and Bcl-X(L) expression with the simultaneous up-regulation of the p21 and Bax expression, and caspase-9 activation (Nasu et al., 2005).

**Antidiabetic activity** - Recently, skin secretion of the toad *Bombina variegata* were evaluated for the isolation and characterization of insulinotropic peptides (Marenah et al., 2004). Crude secretions by the mild electrical stimulation of the dorsal skin surface were purified by reverse phase HPLC yielding 44 peaks. In acute incubations with glucose responsive BRIN-BD11 cells, peaks 21, 22, 23, 24 and 25 showed a 1.5-3.5- fold increase in insulin release compared with 5.6 mM glucose alone. Structural analysis of the purified insulin-releasing peaks were performed by automated Edman degradation and mass spectrometry. Peptide represented by peaks 21, 22 and 23 had molecular masses of 1641.7 Da, 1662.6 Da, and 1619.8 Da respectively. Peak 21 yielded a primary structure of Pyr-
QRLGHQWAVGHLM, which is analogue of bombesin (His6 bombesin), while peak 23 was an exact match of bombesin (Pyr-QRLGNQWAVGHLM) originally isolated from Bombina bombina. Peak 22 indicated a primary structure of Pyr-DSFGNQWARGHFM (72% homology with bombesin). Peaks 24 and 25 revealed entirely novel insulinotropic peptides with molecular masses and primary structure of 1650.5 Da and 2300.0 Da and GKPFPYPPYPEDM (GM-14) and IYNAICPKHCNKCCKPGLLAN (IN-21) respectively. The mechanism underlying the insulinotropic actions of peaks 21, 22, 23 and 24 suggest possible involvement of a cAMP dependent, G-protein insensitive pathway. These observations indicate that Bombina variegata skin secretions contain peptides with insulin-releasing activity, which may have mammalian counterparts and prove useful for possible exploitation as anti-diabetic agents from amphibian skin. Insulinotropic peptides were also secreted by the skin of Agalychnis litodryas and Agalychnis calcarifer frogs (Marenah et al., 2004; Abdel-Waheb et al., 2005). An insulin-releasing peptide isolated from Rana pipiens had 100% sequence homology with an antimicrobial peptide pipinin-1 (Marenah et al., 2005). Skin secretions of Rana saharica possessed nontoxic insulinotropic peptides (Marenah et al., 2006).

Immunomodulatory activity - Immunomodulatory action is mostly concerned with cellular involvement of haemopoetic-lymphoid tissue. The water-soluble, non-dialyzable fraction from crude Chan Su showed lymphocyte proliferating activity. It increased IL-2 and IL-12 level in the supernatant of spleen cell culture, and increased the natural killer activity of the C3H/HeN mice. These results show that Chan Su contains immunopotentiating substances that may serve as an immunomodulator in an organism (Shimuzu et al., 2004). Indian
common toad (*Bufo melanostictus*) skin extract (TSE) showed significant rise of blood lymphocyte, splenic lymphocyte and macrophage count in experimental animals, which strongly suggested the possible involvement of toad skin component as a first line of defense through immunomodulation of lymphoid cell. It was assumed that TSE contained a variety of immunomodulators, which might be involved for immunopotentiation as judged by its positive chemotactic property, negative T-cell rosette formation and macrophage migration inhibition capacity (Das et al., 1998b). On the basis of histamine release from rat peritoneal mast cells, an octadecapeptide was isolated from the skin extract of the Northern Leopard frog (*Rana pipiens*). This peptide was purified to homogeneity using reversed-phase high performance liquid chromatography and found to have the following primary structure by Edman degradation and pyridylethylatation: LVRGCWTKSYPPKPCFVR, in which Cys(5) and Cys(15) are disulfide bridged. This peptide inhibited the early development of granulocyte macrophage colonies from bone marrow stem cells but did not induce apoptosis of the end stage granulocytes, i.e. mature neutrophils. This peptide therefore displays biological activity with both granulopoietic progenitor cells and mast cells and thus represents a novel bioactive peptide from frog skin (Salmon et al., 2001). An octadecapeptide pYR (YLKGCWTKSYPPKPCFSR) from the skin secretions of the dusky gopher frog (*Rana sevosa*), shared 77.8% homology with pLR, could release histamine from rat peritoneal mast cells and inhibited the early development of granulocyte macrophage colonies from bone marrow stem cells but did not induce apoptosis of the end stage granulocytes (Graham et al., 2005).
Cardioactive & Anti-arrhythmic

The Chinese medicine Chan’sue prepared from Chinese toad skin is used in treating arrhythmia and other heart diseases (Datta et al., 2002). In the pharmacological effects of the toad skin venom containing drug “kyushin” (KY) on aconitine and thyroxine induced arrhythmia in guinea pigs, on the conduction system in Langendorff preparations of rabbit hearts and on the autonomic nervous system in cats, it was found that KY significantly inhibited the aconitine induced arrhythmia after intraduodenal administration (i.d.) with 80 mg/Kg, and the thyroxine induced arrhythmia with 40mg/Kg, i.d.. The decreased in heart rate induced by electrical stimulation to the vagus nerve was potentiated by KY at 30 mg/Kg, i.d. (Morishita et al, 1993). It has been reported that KY increased aortic pressure, peak positive first derivative of left ventricular pressure, stroke work index, percent segment shortening in left ventricular myocardium and myocardial oxygen consumption, and decreased heart rate and total peripheral vascular resistance. KY (Japanese equivalent- Senso) induced positive inotropic and vasodilating actions possibly originating from both digitalis and adrenaline like action of a Senso (Ojiri et al, 1991). They again reported that beta adrenergic action may be involved in the vasodilating effect of KY and partly in the positive ionotropic action (Ojiri et al 1992). Several reports suggest the presence of sodium-potassium pump inhibitor in plasma and various tissues, particularly during volume-expanded state and low-renin hypertension. One such endogenous inhibitor, the bufodienolide derivative, resibufogenin from toad skin have been chemically characterized. Based on the research on this compound, Yuan et. al.,(1993) hypothesized that, resibufogenin by inhibiting the cardiovascular muscle cell Na+-K+-pump, this inhibitor can constrict blood vessels, enhance vasoconstriction and increase cardiac contractility, thereby raising blood pressure. Bufogenines and bufotoxins have
cardioacceloratory properties, increasing the strength of the heart beat and decreasing heart rate. Dried toad skin preparation Chan’su or Senso have been used in oriental medicine for the past 3000 years and were introduced into Europe in the 1600s until they were replaced by digitalis some 200 years later (Clarke, 1997).

Sleep inducing activity - Amphibian glandular gland produced large amounts of small peptides related to the tryptophyllin (tryptophan containing peptide), first discovered in the South American hylid frog *Phyllomedusa rohdei* (Montecucchi et al. 1984). One of the tryptophyllin (FPPWM-NH$_2$), induces sedation and behavioral sleep in birds, and is also immunoreactive to a set of cells in the rat adenohypophysis (Renda et al., 1985). From our laboratory, a nonprotein sleep inducing factor, SIF (conjugated aromatic compound with a hydroxyl and carbonyl functional group, MW 880 Dalton) isolated from Indian toad (*Bufo melanostictus*) skin extract (Das et al., 2000). Biological study showed that SIF produced no lethality in male albino mice up to 8 mg/kg, i.v. dose. Cyproheptadine antagonised SIF induced contraction of isolated smooth muscle indicating histamine/serotonin receptor mediated action of SIF. EEG studies showed that SIF increased sleep and decreased awakening condition of freely moving rats. Biochemical studies showed that SIF produced significant alteration of brain biogenic amine levels, monoamine oxidase (MAO) and tryptophan hydroxylase (TH) activity (Das et al., 2000).

Analgesic & Local Anesthetic activity - Amphibian skin secretions are a potential source of new and powerful anaesthetics. Many of the bufogenines and bufotoxins also exert remarkable effect as a local anesthetics (Clarke, 1997). The majority of frogs of the genus
Litoria contain at least one neuropeptide of the caerulein group. Caerulein 1.1 \([\text{pEQDY (SO}_3\text{)} \text{TGWMDF-NH}_2]\) is a common neuropeptide found in many frog species world-wide. This peptide is several thousand times more potent analgesic than the well-known morphine and has been used during gall bladder operation (Apponyi et al. 2004). The skin of the South American frogs Phyllomedusa secretes, in addition to numerous mammalian-like hormones and neuropeptides, several gene-encoded opioid peptides that contain a D-amino acid in position 2 of their sequence. Dermorphin, Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH\(_2\), dermenkephalin/deltorphin A, Tyr-D-Met-Phe-His-Leu-Met-Asp-NH\(_2\) and the deltorphins, Tyr-D-Ala-Phe-Xaa-Val-Val-Gly-NH\(_2\) (where Xaa is either Asp or Glu) are highly potent at, and exquisitely selective, for the mu- and delta-opioid receptors. D-Ala and D-Met present in dermorphin and related peptides are coded for by the usual codons in the corresponding messenger RNAs. Prepro-dermorphin/dermenkephalin and prepro-deltorphins have considerable sequence identities to precursors encoding 10-46-residue-long antimicrobial peptides—dermaseptins, brevinins, temporins, esculentins and gaegurins—originating from various amphibian species (Amiche et al., 1998). Dermorphin is the representative of a new class of potent opioid peptides occurring in amphibian skin and possesses the unique feature of having a D-Ala residue incorporated in the peptide molecule. The effect of dermorphin on the spontaneous and evoked neuronal activity by a nociceptive stimulus was studied in the nucleus lateralis anterior and ventrobasal complex of the rat thalamus. The high firing frequency induced by nociceptive stimuli was blocked when dermorphin was injected intraperitoneally at the dose of 1.5 mg/kg. The action starts about 10 min after injection and lasts on average for 120 min. Naloxone, a specific opioid antagonist, injected i.p. at a dose of 1 mg/kg antagonized the effect of dermorphin. The dermorphin time-course is about twice that of morphine (1.5 mg/kg
i.p.) under the same experimental conditions (Braga et al., 1984). Dermorphin, exerted a depressive effect on locomotor activity of C57B1/6 mice and an analgesic effect when injected intravenously. Intracerebroventricular injections of dermorphin enhanced locomotor activity and resulted in analgesia. It was suggested that dermorphin acts on central receptor populations activated by morphine and enkephalins (Puglisi-Allegra et al., 1982). Dermorphin and Hyp6-dermorphin are the first representatives of a new class of potent opioid peptides occurring in amphibian skin. They present the unique feature of having a D-Ala residue incorporated in the peptide molecule. Dermorphin displayed a potent depressive action on electrically stimulated contractions of the guinea-pig ileum and mouse vas deferens preparations. Dermorphin was respectively 57,294, 18 and 39 times more potent than Met-enkephalin, Leu-enkephalin, beta-endorphin, and morphine on the guinea-pig ileum opiate receptors. On the vas deferens receptors, dermorphin was about as potent as the enkephalins and 40 times more potent than morphine. Naloxone was a powerful antagonist to dermorphin in both preparations (Broccardo et al., 1981).

Contraceptive, wound healing & in Plastic Surgery - Magainin-II-amide possesses a significant embryotoxicity, to the point that its potential use as a contraceptive agent was suggested (Mystkowska et al., 2001). Skin secretion of Chinese red belly frog Bombina maxima contains maximin group of peptides which are structurally related to bombinin-like peptides. The maximin peptides possess significant spermicidal action indicating the contraceptive drug development in the near future (Lai et al., 2002).
Studies were carried out on the Indian frog (*Rana tigerina*) skin on experimental wounding of albino rats skin. The wounds were created in the experimental and control groups. The experimental group was dressed with dorsal skin of freshly sacrificed frogs (*Rana tigerina*) while the control group was dressed with cotton gauze. A faster healing was observed in the experimental group over the control group. Biochemical estimations of the wound granulation tissue were carried out every 2 days till the complete healing of the wound in both the groups (Sai et al., 1995). Another study on the skin collagen of the frog (*Rana tigerina*) suggested and proposed a hypothesis that part of the healing efficacy of frog skin may be due to the collagen since proliferation, migration, and differentiation of epithelial cells are prime requisites for a normal healing mechanism (Kumar et al., 2002). There is interesting differences in the frog skin collagen when compared to the hitherto known vertebrate collagens in experimental wound healing process. This could probably be attributed to the position of the amphibians in the vertebrate hierarchy. Detailed investigations on the various physico-chemical properties, such as reconstitution, redissolution, viscosity and denaturation confirms the structural relationship of collagen to habitat and function (Sai et al., 2001). During wound-healing in cultured frog skin fragments, fibronectin (FN) was detected in the dermal-epidermal junction. Intracellular fibronectin was stained using permeabilization and DAB immunoperoxidase. With electron microscopy intracytoplasmic FN granules were localized in the epidermal processes of the stratum germinativum cells protruding towards the dermis and in their marginal regions. Anti-fibronectin serum inhibits the disorganization of the dermal-epidermal junction in cultured wounded skin (Denefle et al., 1989). Fibronectin (FN) in the larval and adult skin of the frog (*Rana esculenta*) both either in in vivo or in in vitro conditions revealed that the FN distribution in the larval skin is related to the cell
rearrangement during the metamorphic climax, and, in the adult skin to the cell migration during the wound healing process and the pigment cell patterning (Denefle et al., 1993).

Some microhylid frogs, e.g. *Breviceps* spp., produce a natural ‘glue’ to bond the sexes together during mating (Visser et al., 1982); further research is needed to establish whether subsequent separation is achieved by skin shedding or the production of a solvent for this glue. It may be possible to use such naturally occurring bioadhesives (Abbott, 1990) as biocompatible glues which are of potential use in plastic surgery and wound healing.

From the above traditional and modern information on the therapeutic potentiality of different frog and toad skin components (Table 2.4.1.), it is clear that amphibian skin is a treasure house of pharmacological agents which may be utilized for future drug development. Although considerable research is going on in other parts of the world, very few scientific report are available from India on the therapeutic potentiality of Indian toads and frog skin. Earlier studies, from the present laboratory isolated a sleep inducing factor from the Indian common toad (*Bufo melanostictus*, Schneider) skin extract (Das et al., 2000). Das et al., (1998a & 1998b) also reported the pharmacological properties of *Bufo melanostictus* skin extract, which was also active against Ehrlich ascites carcinoma (EAC) cells of EAC bearing mice and had immunomodulatory activities. But no detail work was carried out on the antineoplastic activity of Indian toad (*Bufo melanostictus*, Schneider) skin extract and its component. Therefore, the aims of the present study was to explore the antineoplastic activity of Indian toad (*Bufo melanostictus*, Schneider) skin extract and its active constituents.
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<td>4. Caerin 1.8 (<em>Litoria genus</em>)</td>
<td>4. Apponyi et al., 2004</td>
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<td>5. Dermaseptin DS01 (<em>Phyllomedusa genus</em>)</td>
<td>5. Brand et al., 2002</td>
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<td>1. Brevinin1</td>
<td>1. Yasin et al., 2000</td>
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<td>2. E2P (<em>Rana pipiens</em>)</td>
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<td>4. Dermaseptins (S1-S5) (synthetic)</td>
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<td>6. BAS-AH(<em>Bufo andrewsi</em>)</td>
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<td><strong>Antineoplastic</strong></td>
<td>1. Magainins</td>
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<td>8. TSE (<em>Bufo melanostictus</em>)</td>
<td>8. Ding et al., 1994</td>
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<td>9. BM-ANF1 (<em>Bufo melanostictus</em>)</td>
<td>9. Giri et al., 2006</td>
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<td><strong>Sleep inducing</strong></td>
<td>1. Tryptophyllin peptides (<em>Phyllomedusa rhodei</em>)</td>
<td>1. Montecucchi et al., 1984</td>
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<td>2. SIF in TSE (<em>Bufo melanostictus</em>)</td>
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<td><strong>Analgesic</strong></td>
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<td>4. Bufalin from Chan Su</td>
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<td>5. Caerulein 1.1 (Litoria)</td>
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<td>4. Wang et al., 1994</td>
<td>5. Doyle et al., 2004</td>
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<td>2. Bv8 (Bombina variegata)</td>
<td>2. Negri et al., 2004</td>
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<td>3. Skin extracts (Phyllomedusa rhodei)</td>
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CANCER BIOLOGY
SECTION 5

WHAT IS CANCER.
Cancer is characterized by (1) uncontrolled cell growth, (2) invasion to the local tissues and (3) spread or metastasis to other parts of the body. Cells of benign tumors also show diminished control of growth but do not invade local tissue or spread to other parts of the body. The term ‘neoplasia’ means new growth, the new growth produced is called neoplasm or tumor. However, all new growth are not neoplasm since examples of new growth of tissue and cells also exist in the process of embryogenesis, regeneration and repair, hyperplasia and hormonal stimulation. Therefore, satisfactory definition of a neoplasm or tumour is a mass of tissue formed as a result of abnormal, excessive, uncoordinated, autonomous and purposeless proliferation of cells. Neoplasm may be ‘benign’ when they are slow-growing and localized without causing much difficulty to the host, or ‘malignant’ when they proliferate rapidly, spread throughout the body and may eventually cause death of the host. The common term used for all malignant tumours is cancer.

All tumours, benign as well as malignant, have two basic components: (a) ‘Parenchyma’ comprised by proliferating tumour cells; parenchyma determines the nature and evolution of the tumour. (b) ‘Supportive stroma’ composed of fibrous connective tissue and blood vessels; it provides the framework on which the parenchymal tumour cells grow.

The tumours derive their nomenclature on the basis of the parenchymal component comprising them. The suffix ‘-oma’ is added to denote benign tumours. Malignant tumours of epithelial origin are called carcinomas, while malignnt mesenchymal tumours are named sarcomas (sarcos = fleshy). However, some cancers are composed of highly undifferentiated cells and are referred to and are referred to as undifferentiated malignant tumours.

Some examples contrary to this concepts are: melanoma for carcinoma of the melanocytes, hepatoma for carcinoma of the hepatocytes, lymphoma for malignant tumour of
the lymphoid tissue, and seminoma for malignant tumour of the testis. Leukaemia is the term used for cancer of blood forming cells.

CHARACTERISTICS OF TUMOURS (Mohan S, 2005)

I. Rate of Growth

The tumour cells generally proliferate more rapidly than the normal cells. In general, benign tumours grow slowly and malignant tumours rapidly. The rate at which the tumour enlarges depends upon two main factors:

(a) Rate of division and destruction of tumour cells. In general, malignant tumour cells have increased mitotic rate and slower death rate i.e. the cancer cells do not follow normal controls in cell cycle and are immortal. If the rate of cell division is high, it is likely that tumour cells in the centre of the tumour do not receive adequate nourishment and undergo ischaemic necrosis.

(b) Degree of differentiation. Secondly, the rate of growth of malignant tumour is directly proportionate to the degree of differentiation. Poorly differentiated tumours show aggressive growth pattern as compared to better differentiated tumours. Some tumours, after a period of slow growth, may suddenly show spurt in their growth due to development of an aggressive clone of malignant cells. On the other hand, some tumours may cease to grow after sometime.

The regulation of tumour growth is under the control of growth factors secreted by the tumour cells: (i) Epidermal growth factor (EGF), (ii) Fibroblast growth factor (FGF), (iii) Platelet-derived growth factor (PDGF), (iv) Colony stimulating factor (CSF), (v) Transforming growth factor-β (TGF-β), (vi) Interleukins (IL).
II. Clinical and gross features

Clinically, benign tumours are generally slow growing, and depending upon the location, may remain asymptomatic (e.g. subcutaneous lipoma), or may produce serious symptoms (e.g. meningioma in the nervous system). On the other hand, malignant tumours grow rapidly, may ulcerate on the surface, invade locally into deeper tissue, may spread to distant site (metastasis), and also produce systemic features such as weight loss, anorexia and anaemia. In fact, two of the cardinal clinical features of malignant tumours are: invasiveness and metastasis.

The gross appearance of benign and malignant tumours may be quite variable. However, certain distinctive features characterize almost all tumours- they have a different colour, texture and consistency as compared to the surrounding tissue of origin. Gross terms such as papillary, fungating, infiltrating, haemorrhagic, ulcerative and cystic are used to describe the macroscopic appearance of the tumours.

Benign tumours are generally spherical or ovoid in shape. They are encapsulated or well-circumscribed, freely movable, more often firm and uniform, unless secondary changes like haemorrhage or infarction supervene. Malignant tumours, on the other hand, are usually irregular in shape, poorly-circumscribed and extend into the adjacent tissue. Secondary changes like haemorrhage, infarction and ulceration are seen more often. Sarcomas typically have fish-flesh like consistency, while carcinomas are generally firm.

III. Microscopic features

For recognizing and classifying the tumours, the microscopic characteristics of tumour cells are very important.
(a) Microscopic Pattern

*The epithelial tumours* - generally consist of acini, sheets, columns or cords of epithelial tumour cells that may be arranged in solid or papillary pattern. *The mesenchymal tumours* - have mesenchymal tumour cells arranged as interlacing bundles, fasicles or whorls, lying separated from each other usually by the intercellular matrix substance. Certain tumours have mixed patterns e.g. teratoma arising from totipotent cells, pleomorphic adenoma of salivary gland (mixed salivary tumour), fibroadenoma of breast, carcinoma of the uterus and various other combinations of tumour types. *Haematopoietic tumours* - such as leukaemias and lymphomas often have none or little stromal support.

Generally, most benign tumours and low grade malignant tumours reduplicate the normal structure of origin more closely so that there is little difficulty in identifying and classifying such tumours. However, anaplastic tumours differ greatly from the arrangement in normal tissue of origin of the tumour and may occasionally pose problems in classifying the tumour.

(b) Cytomorphology of Neoplastic Cells

The neoplastic cell is characterized by morphologic and functional alterations, the most significant of which are ‘differentiation’ and ‘anaplasia’. Differentiation is defined as the extent of morphological and functional resemblance of parenchymal tumour cells to corresponding normal cells. If the deviation of neoplastic cells in structure and function is minimal as compared to normal cell, the tumour is described as well differentiated such as most benign and low grade malignant tumours. Poorly differentiated, undifferentiated or dedifferenciated are synonymous term for poor structural and functional resemblance to...
corresponding normal cell. Anaplasia is lack of differentiation and is a characteristic feature of most malignant tumors. Depending upon the degree of differentiation, the extent of anaplasia is also variable i.e. poorly differentiated malignant tumors have high degree of anaplasia. As a result of anaplasia, noticeable morphological and functional alterations in the neoplastic cells are observed.

(c) Tumour angiogenesis and stroma

Tumour angiogenesis: In order to provide nourishment to growing tumor, new blood vessels are formed from pre-existing ones (angiogenesis).

Micovascular density: The new capillaries add to the vascular density of the tumor which has been used as a marker to assess the rate of growth of tumors and hence grade the tumors. This is done by counting microvascular density in the section of the tumor.

Central necrosis: However, if the tumor outgrows its blood supply as occurs in rapidly growing tumors or tumor angiogenesis fails, its core undergoes ischaemic necrosis.

Tumour stroma: The connective tissue in the stroma may be scanty or excessive. In the former case, the tumor is soft and fleshy (e.g. in sarcomas, lymphomas), while in the latter case the tumor is hard and gritty (e.g. infiltrating duct carcinoma breast). Growth of fibrous tissue in tumor is stimulated by basic fibroblast growth factor (bFGF) elaborated by tumor cells.

If the epithelial tumor is almost entirely composed of parenchymal cells, it is called medulillary e.g. medullary carcinoma of the breast, medullary carcinoma of the thyroid. If there is excessive connective tissue stroma in the epithelial tumor, it is referred to as desmoplasia.
and the tumour is hard or scirrhous e.g. infiltrating duct carcinoma breast, linitis plastica of the stomach.

(d) Inflammatory Reaction

At times, prominent inflammatory reaction is present in and around the tumours. It could be the result of ulceration in the cancer when there is secondary infection. The inflammatory reaction in such instances may be acute or chronic. However, some tumours show chronic inflammatory reaction, chiefly of lymphocytes, plasma cells and macrophages, and in some instances granulomatous reaction, in the absence of ulceration. This is due to cell-mediated immunologic response by the host in an attempt to destroy the tumour. In some cases, such an immune response improves the prognosis. The examples of such reaction are: seminoma testis, malignant melanoma of the skin, lymphoepithelioma of the throat, medulary carcinoma of the breast, choriocarcinoma, Warthin’s tumour of salivary glands etc.

IV. Local invasion (Direct spread)

Benign tumours: Most benign tumours from encapsulated or circumscribed masses that expand and push aside the surrounding normal tissues without actually invading, infiltrating or metastasizing.

Malignant tumours: Malignant tumours also enlarge by expansion and some well-differentiated tumours may be partially encapsulated as well e.g. follicular carcinoma of thyroid. But characteristically, they are distinguished from benign tumours by invasion, infiltration and destruction of the surrounding tissue, besides distant metastasis. Often, cancers extend through tissue spaces, permeate lymphatics, blood vessels, perineural spaces and may penetrate a bone by growing through nutrient foramina.
V. Metastasis (Distance spread)

Metastasis is defined as spread of tumour by invasion in such a way that discontinuous secondary tumour mass/masses are formed at the site of lodgement. Metastasis and invasiveness are the two most important features to distinguish malignant from the benign tumours. Benign tumours do not metastasise while all the malignant tumours with a few exceptions like gliomas of the central nervous system and basal cell carcinoma of the skin, can metastasise. Generally, larger, more aggressive and rapidly-growing tumours are more likely to metastasise but there are numerous exceptions.

Cancer may spread to distant sites by following process-

Lymphatic spread- In general, carcinomas metastasise by lymphatic route while sarcomas favour haematogenous route. However, sarcomas may also spread by lymphatic pathway.

Haematogenous spread- Blood-borne metastasis is the common route for sarcomas but certain carcinomas also frequently metastasise by this mode, especially those of lungs, breast, thyroid, kidney, liver, prostate and ovary. The common sites for blood-borne metastasis are: the liver, lungs, brain, bones, kidney and adrenals. However, a few organs such as spleen, heart, skeletal muscle do not allow tumour metastasis to grow. Spleen is unfavourable site due to open sinusoidal pattern, which does not permit tumour cells to stay there long enough to produce metastasis.

Spread along body cavities and natural passages- Uncommonly, some cancers may spread by seeding at other surfaces. These routes of distant spread are- transcoelomic spread, spread along epithelium-lined surfaces, spread via cerebrospinal fluid, implantation etc.
**Biology of cancer cell invasion and metastasis** - The process of local invasion and distant spread (by lymphatic and haematogenous routes) discussed above's passage through barriers before gaining access to the vascular lumen. This includes making the passage by the cancer cells by dissolution of extracellular matrix (ECM) at three levels - at the basement membrane of tumour itself, at the level of interstitial connective tissue, and at the basement membrane of microvasculature. The following steps are involved at the molecular level:

(i) Aggressive clonal proliferation and angiogenesis.

(ii) Tumour cell loosening.

(iii) Tumour cell- extracellular matrix interaction.

(iv) Degradation of extracellular matrix.

(v) Entry of tumour cells into capillary lumen.

(vi) Thrombus formation.

(vii) Extravasation of tumour cells.

(viii) Survival and growth of metastatic deposit.

**EPIDEMIOLOGY OF CANCER (Mohan S, 2005)**

**Cancer incidence**: Worldwide, it has been estimated that about 20% of all deaths are cancer-related. There have been changing patterns in incidence of cancers in both the sexes and in different locations as outlined below. Table 2.5.1. shows worldwide incidence (in descending order) and mortality (Fig. 2.5.1.) of different forms of cancer in men, women and/or children. As evident from the Table, some types of cancers are common in India while others are more common in the Western populations since etiologic factors are different.
**Epidemiologic factors**: The pattern and incidence of cancer depends upon the following: (A) A large number of predisposing epidemiologic factors or cofactors, (B) Chronic non-neoplastic (premalignant) conditions, and (C) Hormones

(A) **Predisposing factors**-

1. **Familial and genetical factors**- In general, the risk of developing cancer in relatives of a known cancer patient is almost three times higher as compared to control subjects. The overall estimates suggest that genetic cancers comprise not greater than 5% of all cancers. Some of the common examples are as under:

   (i) **Retinoblastoma**- About 40% of retinoblastomas are familial and show an autosomal dominant inheritance. Carriers of such genetic composition have 10,000 times higher risk of developing retinoblastoma which is often bilateral. Such patients are predisposed to develop another primary malignant tumour, notably osteogenic sarcoma. Retinoblastoma susceptibility gene, $RB$ gene, located on chromosome 13 was the first cancer-predisposing gene identified.

   (ii) **Familial polyposis coli**- This condition has autosomal dominant inheritance. The polyposoid adenomas may be seen at birth or in early age. By the age of 50 years, almost 100% cases of familial polyposis coli develop cancer in the colon.

   (iii) **Multiple endocrine neoplasia (MEN)**- A combination of adenomas of pituitary, parathyroid and pancreatic islets (MEN-I) or syndrome of medullary carcinoma of thyroid, pheochromocytomas and parathyroid tumour (MEN-II) are encountered in families.
Table 2.5.1. Worldwide incidence of different forms of Cancer (in decending order).

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<tr>
<th>MEN</th>
<th>WOMEN</th>
<th>CHILDREN</th>
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<tr>
<td>1. Lung (oral cavity in India)</td>
<td>Breast (cervix in India)</td>
<td>Acute leukaemia</td>
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<tr>
<td>2. Prostate</td>
<td>Lung</td>
<td>CNS tumours</td>
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<td>3. Colon-rectum</td>
<td>Colon-rectum</td>
<td>Lymphomas</td>
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<td>4. Leukaemia-lymphoma</td>
<td>Leukaemia-lymphoma</td>
<td>Neuroblastoma</td>
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<tr>
<td>5. Liver</td>
<td>Ovary</td>
<td>Bone sarcoma</td>
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Fig. 2.5.1. The world wide mortality due to different cancers

Source: CBS
(iv) **Neurofibromatosis or von Recklinghausen's disease**- This condition is characterized by multiple neurofibromas and pigmented skin spots.

(v) **Cancer of the breast**- Female relatives of breast cancer patients have 2 to 3 times higher risk of developing breast cancer.

(vi) **DNA-chromosomal instability syndromes**- These are a group of pre-neoplastic conditions having defect in DNA repair mechanism. A classical example is xeroderma pigmentosum, an autosomal recessive disorder, characterized by extreme sensitivity to ultraviolet radiation. The patients may develop various types of skin cancers such as basal cell carcinoma, squamous cell carcinoma and malignant melanoma.

2. **Racial and geographic factor**- Differences in racial incidence of some cancers may be partly attributed to the role of genetic composition but are largely due to influence of the environment and geographic differences affecting the whole population such as climate, soil, water, diet, habits, customs etc.

   (i) White Europians and Americans develop most common malignancies of lung, breast, and colon. Liver cancer is uncommon in these races. Breast cancer is uncommon in Japanese women but is more common in American women.

   (ii) Black Africans, on the other hand, have more commonly cancers of the skin, penis, cervix and liver.

   (iii) Japanees have five times higher incidence of carcinoma of the stomach than the Americans.

   (iv) South-East Asians, especially of Chinese origin have more commonly nasopharyngeal cancer.
(v) Indians of both sexes have higher incidence of carcinoma of oral cavity and upper aerodigestive tract, while in females carcinoma of uterine cervix and breast run parallel in incidence. Cancer of the liver in India is more due to viral hepatitis (HBV and HCV) and subsequent cirrhosis than due to alcoholism.

3. Environmental and cultural factors- We are surrounded by an environment of carcinogens which we eat, drink, inhale and touch. Some of the examples are given below:

(i) Cigarette smoking is the single most important environmental factor implicated in the etiology of cancer of the oral cavity, pharynx, larynx, oesophagus, lungs, pancreas and urinary bladder.

(ii) Alcohol abuse predisposes to the development of cancer of oropharynx, larynx, oesophagus and liver.

(iii) Alcohol and tobacco together further accentuate the risk of developing cancer of the upper aerodigestive tract.

(iv) Cancer of the cervix is linked to a number of factors such as age at first coitus, frequency of coitus, multiplicity of partners, parity etc. Sexual partners of circumcised males have lower incidence of cervical cancer than the partners of uncircumcised males.

(v) Penile cancer is rare in the Jews and Muslims as they are customarily circumcised.

(vi) Betel nut cancer of the cheek and tongue is quite common in some parts of India due to habitual practice of keeping the bolus of paan in particular place in mouth for a long time.

(vii) A large number of industrial and environmental substances are carcinogenic and occupational hazard for some populations. These include exposure to substances like arsenic, asbestos, benzene, vinyl chloride, naphthylamine etc.
Certain constituents of the diet have also been implicated in the causation of cancer. Overweight individuals, deficiency of vitamin A and people consuming a diet rich in animal fats and low in fibre content are more at risk of developing colonic cancer. Diet rich in vitamin E, on the other hand, possibly has some protective influence by its antioxidant action.

4. **Age**—Generally, cancers occur in older individuals past 5th decade of life. Some tumours have two peaks of incidence e.g. acute leukaemias occur in children and in older age group. Besides acute leukaemias, other tumours in infancy and childhood are: neuroblastoma, nephroblastoma (Wilms’ tumour), retinoblastoma, hepatoblastoma, rhabdomyosarcoma, Ewing’s sarcoma, teratoma and CNS tumours.

5. **Sex**—Apart from the malignant tumours of organs peculiar to each sex, most tumours are generally more common in men than in women except cancer of the breast, gall bladder, thyroid and hypopharynx. Although there are geographic and racial variations, cancer of the breast is the commonest cancer in women throughout the world while lung cancer is the commonest cancer in men.

**(B) Chronic Non-neoplastic (Premalignant) conditions**—
Premalignant lesions are a group of conditions which predispose to the subsequent development of cancer. Some examples of premalignant lesions are given below:

1. **Carcinoma in situ (intraepithelial neoplasia)**—When the cytological features of malignancy are present but the malignant cells are confined to epithelium without invasion
across the basement membrane, it is called as carcinoma in situ or intraepithelial neoplasia (CIN). The common sites are:

(i) Uterine cervix at the junction of ecto and endocervix

(ii) Bowen’s disease of the skin

(iii) Actinic or solar keratosis

(iv) Oral leukoplakia

(v) Intralobular and intraductal carcinoma of the breast

The area involved in carcinoma in situ may be single and small, or multifocal. As regards the behaviour of CIN, it may regress and return to normal or may develop into invasive cancer.

2. Some benign tumours- Commonly, benign tumours do not become malignant. However, there are some exceptions e.g.

(i) Multiple villous adenomas of the large intestine have high incidence of developing adenocarcinoma.

(ii) Neurofibromatosis (von Recklinghausen’s disease) may develop into sarcoma

3. Miscellaneous conditions- Certain inflammatory and hyperplastic conditions are prone to development of cancer, e.g.

(i) Patients of long-standing ulcerative colitis are predisposed to develop colorectal cancer.

(ii) Cirrhosis of the liver has predisposition to develop hepatocellular carcinoma.

(iii) Chronic bronchitis in heavy cigarette smokers may develop cancer of the bronchus.
(iv) Chronic irritation from jagged tooth or ill-fitting denture may lead to cancer of the oral cavity.

(v) Squamous cell carcinoma developing in an old burn scar (Marjolin’s ulcer).

(C) Hormones and Cancer-

Cancer is more likely to develop in organs and tissues which undergo proliferation under the influence of excessive hormonal stimulation. On cessation of hormonal stimulation, such tissues become atrophic. Hormone sensitive tissues developing tumours are the breast, endometrium, myometrium, vagina, thyroid, liver, prostate and testis. Some examples of hormones influencing carcinogenesis in experimental animals and humans are given below:

1. *Oestrogen*- Women receiving oestrogen therapy and women with oestrogen-secreting granulose cell tumour of the ovary have increased risk of developing endometrial carcinoma. Adenocarcinoma of the vagina is seen with increased frequency in adolescent daughters of mothers who had received oestrogen-therapy during pregnancy.

2. *Contraceptive hormones*- The sequential types of oral contraceptives increase the risk of developing breast cancer. Other tumours showing slightly increased frequency in women receiving contraceptive pills for long durations are benogn tumours of the liver.

3. *Anabolic steroid*- Consumption of anabolic steroids by athelets to increase the muscle mass, increases the risk of developing benign and malignant tumours of the liver.
4. **Hormone-dependent tumours**- There is tumour regression on removal of the stimulus for excessive hormonal secretion e.g.

(i) Prostate cancer usually responds to the administration of oestrogens.

(ii) Breast cancer may regress with oophorectomy, hypophysectomy or on administration of male hormones.

(iii) Thyroid cancer may slow down in growth with administration of thyroxine that suppresses the secretion of TSH by the pituitary.

**TYPE AND CAUSES OF CANCER**

**Lung Cancer**

Lung cancer is the leading cause of cancer death among men and women. The most common risk factor for lung cancer is smoking, due to the harmful carcinogens found in tobacco smoke. This inhaled smoke damages the cells that line the bronchi, or air passages, a process many doctors believe represents an early stage of cancer. The first symptoms are often coughing or wheezing. Blood-stained mucus may also be expelled. Other symptoms may include weight loss and chest or back pain, and in the more advanced stages, hoarseness and shortness of breath. The best way to avoid lung cancer is to not smoke, since 85% of lung cancer is associated with tobacco use. Other risk factors include exposure to industrial substances, such as asbestos and coal gas, and the involuntary breathing in of tobacco smoke by non-smokers.
Thyroid Cancer

There are two types of cells in the thyroid gland, each of which can develop a specific type of cancer. Ninety-five percent of all tumors found in the thyroid gland are benign, meaning they pose no threat. About one-third of all people will develop a tumor in the thyroid at some point during their lives. These can usually be felt as lumps on the throat since the thyroid is so close to the surface of the skin. About seventeen-thousand cases of thyroid cancer are discovered each year, about two-thirds of which are in women. Most forms of malignant thyroid tumors are curable. The primary treatments for lung cancer are surgery, radiation, and chemotherapy, depending on the type of cancer, the location of the tumor, and the stage of the disease.

Bladder Cancer

Cancer of the bladder is the most frequently occurring malignant tumor in the urinary tract, affecting men more often than women. Although it can strike at any age, it usually occurs between the ages of fifty and seventy. One of the earliest signs of bladder cancer is the appearance of blood in the urine. It usually appears suddenly, without pain, and is often associated with the increased need to urinate. The exact cause of bladder cancer is not known, but some associations have been made. Because the urinary tract carries waste product from the body, it comes in contact with a number of foreign substances, some of which are cancer-causing agents. The greatest risk factor is the tar from cigarettes, which places smokers at twice the risk of developing bladder cancer. The key to curing bladder cancer is detecting it early. Treatment often involves a combination of surgery and other therapies. If the tumor is
small enough, it may be cauterize or burned. Larger tumors may require more intense surgery, with chemotherapy or radiation therapy used to kill any remaining cells.

**Hodgkin's Disease**

Hodgkin's disease is a cancer of the lymph nodes. Although it can strike at any age, it is most common among children and young adults. Hodgkin's disease often begins as a swelling of a lymph node in the neck area or just under the collar bone. It tends to spread downward, involving the lymph tissue in the chest, and then the spleen and lymph nodes in the abdomen and pelvis. If the spleen is involved, the liver may also be affected. In adults, the disease often spreads to the lungs, bones, and bone marrow. Dramatic improvements have been made in recent years in treating both Hodgkin's disease and other forms of lymphomas.

**Bone Cancer**

Most bone tumors are considered secondary tumors because they have developed from cancer cells spread from a primary malignant tumor to the bone. There are two main exceptions; they include a blood cancer that begins in the bone marrow and primary bone malignancy. Both of these are much less common than cancer that travels to a bone. Generally, bone tumors are benign. They are found most often in the arms and legs, but can occur in any bone in the body. Children and young people are more likely than adults to have bone cancers. Signs and symptoms of bone cancer include a hard lump on the surface of a bone, accompanying pain, and fractures of the bone.
Bone Marrow Cancer

Most bone cancers are called secondary tumors because they arise from other cancers which have spread from other parts of the body. Cancers that actually arise in the bones are relatively uncommon. However, there are two major types of bone cancer: Osteosarcoma and Ewing's sarcoma. Ewing's sarcoma, or bone marrow cancer, is usually most common in people under the age of twenty. Bone marrow cancer most commonly occurs in the shafts of long bones. Although the first signs of bone marrow cancer varies from patient to patient, symptoms may include fever, fatigue, poor appetite, and weight loss. However, the early symptoms may be so sporadic and subtle, the patient may not see a doctor until the cancer has spread to other parts of the body. In the past, when people were diagnosed with bone marrow cancer, the treatment typically involved the removal of the cancerous marrow through extensive surgery. However, a combination of high does of chemotherapy, radiation therapy, and conservative surgery is now being used. Generally, during the surgery, the healthy bone marrow is removed through two very small incisions in the back of the hip while the patient is under anesthesia. The bone marrow is processed and stored in a freezer. While this is being done, the patient will receive chemotherapy and radiation. Usually one or two days later, they will then receive their own marrow back.

Brain Cancer

More commonly, tumors are secondary, which means that they have spread to the brain from other locations. In adults, the highest rates of brain cancers occur between the ages of sixty-five and seventy-four. In children, primary brain tumors are the second most common form of cancer. As a tumor grows, it presses against the brain, resulting in headaches,
dizziness, blurred vision, and seizures. Depending on where the tumor is located, a person may also experience a loss of memory or a change in personality. If you experience any one, or a combination of these symptoms, it is recommended that you see your doctor immediately. Though surgery is the standard treatment for a brain tumor, a malignant tumor may require further treatment, including chemotherapy or radiation therapy.

Gallbladder Cancer

According to the American Cancer Society, cancer of the gallbladder is the fourth most common cancer arising from the gastrointestinal tract, representing approximately two to three percent of all cancers in the United States. This cancer is most prevalent in people in their late sixties and early seventies, with a slight female predominance. Although some individuals may not have any symptoms, others may experience significant pain, weight loss, an abdominal mass on the right side below the rib cage, or jaundice, a yellowish discoloration of the skin. Your health care provider's examination can determine if additional tests or surgery is required. Surgical removal depends on whether the cancer has spread to local tissues. When the growth is not removable, chemotherapy and radiation therapy may slow or arrest the growth of the cancer and improve the symptoms associated with the disease.

Colon cancer

The large intestine consists of the colon and rectum, which make up the final portion of the digestive tract. Cancer of the colon is the third most common type of cancer in men and the second most common cancer in women. It is a leading cause of death by cancer. It is also one of the most curable forms if found and treated early. Colon cancer occurs most commonly
in people over the age of forty. Symptoms include rectal bleeding, cramping pain in the abdomen, and a change in bowel habits. Some colon cancers may cause obstruction of the intestines, discomfort, weight loss, and anemia. Because this cancer develops over a period of time, it can be detected before symptoms appear. Early detection offers the best chance for cure. The American Cancer Society recommends that a digital rectal examination be performed yearly after age forty; a stool blood test every year after age fifty; and a procto test with a lighted instrument every three to five years after fifty, following two annual negative examinations. If cancer is found, treatment may include surgery, radiation, or chemotherapy. On the preventive side, a diet high in fiber is believed to protect against colon cancer. Fiber-rich foods includes breads made with whole-grain flours, fresh fruits, and vegetables.

Leukaemia

Leukaemia is a cancer of the white blood cells, the word leukaemia comes from the Greek and means “white blood”. As there are various types of bone marrow cells, various types of leukaemia can develop each requiring different treatments. The main types of leukaemia are as follows:

(i) *Acute lymphoblastic leukaemia (ALL)* - this is a cancer of immature lymphocyte cells, known as lymphoblasts. This disease is the most common type of leukaemia in young children, usually between the ages of 1 and 7 and is quite rare in adults.

(ii) *Acute myeloid leukaemia (AML)* - this is a cancer of the immature myeloid cells. This disease occurs mainly in adults but can also affect children.
(iii) *Chronic lymphocytic leukaemia (CLL)* - this is a cancer of the lymphocyte cells. This disease is the most common type of leukaemia affecting adults, and is very rare in children.

(iv) *Chronic myeloid leukaemia (CML)* - this is a cancer of the neutrophils cells. This type of leukaemia is rare in children and commonly affects male adults more than females.

**Causes of leukaemia**: The cause of most cases of leukaemia is not known, although there are some risk factors that increase the chance of developing the disease. These include:

(i) a weakened immune system - this may be a result of drugs that suppress the immune system (such as those used for organ transplants), high doses of radiation (such as in radiotherapy for another cancer), or diseases that affect the immune system (such as HIV)

(ii) age - chronic leukaemias are more common over the age of 40

(iii) smoking

(iv) certain genetic conditions, such as Down's syndrome

(v) previous chemotherapy for another cancer

(vi) other blood disorders, such as aplastic anaemia, a rare condition where the bone marrow fails to produce blood cells correctly

(vii) contact with a chemical called benzene, one of the chemicals in petrol and a solvent used in the rubber and plastics industry

**Symptoms of leukaemia**: The symptoms of leukaemia vary greatly, depending on the exact type of disease and how advanced it is. Few or no symptoms may occur in the early
stages, especially in people with chronic leukaemia. Many symptoms are vague, such as fever, headaches, weight loss and night sweats.

*Symptoms of leukaemia may include-*

(i) tiredness, breathlessness and pale skin (due to anaemia, a reduction in number of red cells in the blood)

(ii) frequent infections that do not get better (due to reduction in white blood cells, which fight infection)

(iii) abnormal bleeding from gums and cuts (due to a reduction in platelets which are important for normal blood clotting)

(iv) increased bruising (due to platelet reduction)

(v) heavier periods in women (due to platelet reduction)

(vi) nosebleeds (due to platelet reduction)

(vii) abdominal pain, due to an enlarged spleen or liver

(viii) swollen lymph glands (glands in the neck, groin and under the arms)

(ix) bone pain, due to the pressure of cell build-up

(x) swollen gums, and occasionally, swollen testicles

Some of the major cause of different cancer discussed above has been shown in Fig.2.5.2.
Fig. 2.5.2. The major causative agents responsible for cancer incidence
ETIOLOGY AND PATHOGENESISIS OF CANCER

There has been ever-increasing list of agents implicated in etiology of cancer. There has been still greater knowledge on the pathogenesis of cancer, especially due to tremendous strides made in the field of molecular biology and genetics in the last decade. The etiology and pathogenesis of cancer may be discussed as follows (Mohan S, 2005)-

A. Molecular pathogenesis of cancer

The mechanism as to how a normal cell is transformed to a cancer cell is complex. In the last decade, there has been vast accumulation of literature to explain the pathogenesis of cancer at molecular level.

1. Monoclonality of tumours- There is strong evidence that most human cancers arise from a single clone of cells by genetic transformation or mutation. For example-
   (i) In a case of multiple myeloma (a malignant disorder of plasma cells), there is production of a single type of immunoglobulin or its chain as seen by monoclonal spike in serum electrophoresis.
   (ii) Due to inactivation of one of the two X-chromosomes in females (paternal or maternal derived), women are mosaics with two types of cell populations e.g. for glucose-6-phosphate dehydrogenase (G6PD) isozymes A and B. It is observed that all the tumour cells in benign uterine tumours (leiomyoma) contain either A or B genotype of G6PD (i.e. the tumour cells are derived from a single progenitor clone of cell), while the normal myometrium is mosaic of both types of cells derived from A as well as B isozyme.

2. Genetic theory of cancer- Cell growth of normal as well as abnormal types is under genetic control. In cancer, there is either abnormality in the genes of the cell, or there are
normal genes with abnormal expression. The abnormality in genetic composition may be from an inherited or induced mutation (induced by etiologic carcinogenic agents namely: chemicals, viruses, radiation). The mutated cells transmit their characters to the next progeny of cells and result in cancer.

3. Genetic regulators of normal and abnormal mitosis- In normal cell growth, regulatory genes control mitosis as well as cell aging, terminating in cell death by apoptosis. In normal cell growth, there are four regulatory genes:

(i) Proto-oncogenes are growth-promoting genes.
(ii) Anti-oncogenes are growth-inhibiting or growth suppressor genes.
(iii) Apoptosis regulatory genes control the programmed cell death.
(iv) DNA repair genes are those normal genes which regulate the repair of DNA damage that has occurred during mitosis.

In cancer, the transformed cells are produced by abnormal cell growth due to genetic damage to these normal controlling genes. Thus, corresponding abnormalities are genetic, as under:

(i) Activation of growth-promoting oncogenes causing transformation of cell (mutant form of normal proto-oncogenes in cancer is termed oncogene). Gene products of oncogenes are called oncoproteins. Oncogenes are considered dominant since they appear inspite of presence of normal protooncogenes.

(ii) Inactivation of cancer-suppressor genes (i.e. inactivation of anti-oncogenes) permitting the cellular proliferation of transformed cells. Anti-oncogenes are active in recessive form which mean that they are active only if both alleles are damaged.
(iii) *Abnormal apoptosis regulatory genes* which may act as oncogenes or anti-oncogenes. Accordingly, these genes may be active in dominant or recessive form.

(iv) *Failure of DNA repair genes* and thus inability to repair the DNA damage resulting in mutations.

4. *Multi-step process of cancer growth and progression*- Carcinogenesis is a gradual multi-step process involving many generations of cells. The various causes may act on the cell one after another (multi-hit process). The same process is also involved in further progression of tumour. Ultimately, the cell so formed are genetically and phenotypically transformed cells having phenotypic features of malignancy- excessive growth, invasiveness and distant metastasis.

**B. Cancer related genes and cell growth**

It is apparent from the above discussion that genes control the normal cellular growth, while in cancer these controlling genes are altered, typically by mutation. A large number of such cancer-associated genes have been described, each with a specific function in cell growth. Some of these genes are commonly associated in many tumours (e.g. p53 or TP53), while others are specific to particular tumours. Therefore, it is considered appropriate to discuss the role of cancer-related genes with regard to their functions in cellular growth.

Following are the major genetic properties or hallmarks of cancer.

1. Excessive and autonomous growth: Growth-promoting oncogenes.
3. Escaping cell death by apoptosis: Genes regulating apoptosis and cancer.
4. Avoiding cellular aging: Telomeres and telomerase in cancer
1. Excessive and autonomous growth: Growth promoting oncogenes

Mutated form of normal proto-oncogenes in cancer is called oncogenes. Oncogenes differ from normal genes in following respect: Mutation in the structure of the gene, lacking the normal growth-promoting signals of proto-oncogenes; and they act by over-expression to promote autonomous and excessive cellular proliferation.

In general, activation of oncogenes in human tumours can occur by following mechanisms:

(i)  *Point mutations and deletion*- The most important example is ras oncogene carried in many human tumours such as bladder cancer, pancreatic adenocarcinoma, cholangiocarcinoma.

(ii) *Chromosomal translocation*- Mechanism of transfer of a portion of one chromosome to another is implicated in the pathogenesis of leukaemias and lymphomas.

(iii) *Gene amplification*- Chromosomal alterations that result in increase in the number of copies of gene is found in some examples of solid human tumours e.g. neuroblastoma having n-MYC HSR region, ERB-B in breast and ovarian cancer.
Table 2.5.2. Oncogenes and associated human tumours

<table>
<thead>
<tr>
<th>Type</th>
<th>Oncogene</th>
<th>Associated human tumours</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Growth factors (GFs)</td>
<td>i) PDGF</td>
<td>Glioblastoma</td>
</tr>
<tr>
<td></td>
<td>ii) TGF-α</td>
<td>Sarcomas</td>
</tr>
<tr>
<td></td>
<td>iii) FGF</td>
<td>Cancer of bowel, breast</td>
</tr>
<tr>
<td>2. Receptors for GFs</td>
<td>i) ERB B1</td>
<td>Squamous cell carcinoma</td>
</tr>
<tr>
<td></td>
<td>ii) HER2</td>
<td>Cancer breast, ovary, stomach, lungs (αERB2)</td>
</tr>
<tr>
<td></td>
<td>BCR-ABL</td>
<td>CML, acute leukaemias</td>
</tr>
<tr>
<td>4. Nuclear regulatory molecules</td>
<td>MYC (translocated)-</td>
<td>Burkitt’s lymphoma</td>
</tr>
<tr>
<td>(Transcription proteins)</td>
<td>MYC (amplified)-</td>
<td>Cancer of lung, breast, colon</td>
</tr>
<tr>
<td>5. Cell cycle regulatory proteins</td>
<td>Cyclin D</td>
<td>Cancer of breast, liver, mantle cell lymphoma</td>
</tr>
<tr>
<td></td>
<td>CDK4</td>
<td>Glioblastoma, melanoma, Sarcomas</td>
</tr>
</tbody>
</table>
The steps in signal transduction for cell proliferation by oncogenes shown in Table 2.5.2. have been discussed below (Mohan S, 2005)-

(a) *Growth factors (GFs)*- These are polypeptides elaborated by many cells and normally act on another cell than the one which synthesized it to stimulate its proliferation i.e. paracrine action. However, a cancer cell may synthesise a GF are — PDGF in glioblastoma, TGF-α in sarcomas and FGF in cancer of bowel and breast.

(b) *Receptors for GFs*- Many oncogenes encoding for GF receptors have been described which act more commonly by overexpression of normal GF than by mutation. For example: i) ERB1 is an EGF receptors which acts by overexpression of normal GF receptor in squamous cell carcinoma. ii) HER2, also called ERB2, is another EGF receptor which is overexpressed in breast cancer and carcinoma of lungs, ovary, stomach.

(c) *Signal transduction proteins*- The normal signal transduction proteins which transducer signal from the GF receptors on the cell surface to the nucleus of the cell is mutated in some cancers e.g. i) *Mutated ras gene*- This is the most common form of oncogenes in human tumours. About a third of all human tumours carry mutated ras gene (*ras* for rat sarcoma gene where it is first described), seen in particular in carcinoma of colon, lung and pancreas. Normally, the inactive form of ras protein is GDP (guanosine diphosphate)- bound, while the activated form is bound to GTP. GDP/GTP are homologous to G proteins and take part in signal transduction in a similar way just as G proteins act as ‘on-off switch’ for signal transduction. Normally, active ras protein is inactivated by GTPase activity, while mutated *ras* gene remains unaffected by GTPase and therefore, continues to signal the cell proliferation.
Common mode of activation of RAS gene is by point mutation. ii) *BCR-ABL hybrid gene*- *ABL* gene is a non-GF receptor proto-oncogene having tyrosine kinase activity. *ABL* gene from its normal location on chromosome 9 is translocated to chromosome 22 where it fuses with *BCR* (breakpoint cluster region) gene and forms an *ABL-BCR* hybrid gene which is more potent in signal transduction pathway. *ABL-BCR* hybrid gene is seen in chronic myeloid leukaemia and some acute leukaemias.

(d) *Nuclear regulatory molecules*- The signal transduction pathway that started with GFs ultimately reaches the nucleas where it regulates DNA transcription. Out of various nuclear regulatory transcription proteins described, the most important is MYC gene, seen most commonly in human cancers. Normally MYC protein binds to the DNA and regulates the cell cycle by transcriptional activation and its levels fall immediately after cell enters the cell cycle. MYC oncogene, on the otherhand, is associated with persistent or overexpression of MYC oncoproteins which, in turn, causes autonomous cell proliferation. The examples of tumours carrying MYC oncogene are: i) Burkitt's lymphoma in which mutation in MYC gene is due to translocation t(8;14). ii) In cancer of lung, breast and colon, MYC gene is mutated due to amplification.

(e) *Cell cycle regulatory proteins*- Normally the cell cycle is under regulatory control of cyclins and cyclin-dependent kinases (CDKs) A, B, E and D. Cyclins are so named since they are cyclically synthesized during different phases of the cell cycle and their degradation is also cyclic. Cyclins activate as well as work together with CDKs, while many inhibitors of CDKs (CDKIs) are also known. Although all steps in the cell cycle are under regulatory controls, G1→S phase is the most important checkpoint for regulation by oncogene as well as anti-oncogenes. Mutations in cyclins (in particular
cyclin D) and CDKs (in particular CDK4) are most important growth promoting signals in cancers. The examples of tumours having such oncogenes are:

i) Overexpression of cyclin D in cancers of breast, liver and mantle cell lymphoma.

ii) Amplification of CDK4 in malignant melanoma, glioblastoma and sarcomas.

2. Refractoriness to growth inhibition: Growth suppressing anti-oncogenes-

The mutation of normal growth suppressor anti-oncogenes results in removal of the breaks for growth; thus the inhibitory effect of cell growth is removed and the abnormal growth is continues unchecked. In other words, mutated anti-oncogenes behaves like growth promoting oncogenes.

As compared to the signals and signal transduction pathways for oncogenes, the mechanism of action by growth suppressors are not well understood. In general the point of action of anti-oncogenes is also G1→S phase transition and probably act either by inducing the dividing cell cycle to enter into G0 (resting) phase, or by acting in a way that the cell lies in the post-mitotic pool losing its dividing capability.

Major anti-oncogenes implicated in human cancers are shown below (Table 2.5.3.).
Table 2.5.3. Tumour-suppressor genes and associated human tumours

<table>
<thead>
<tr>
<th>Gene</th>
<th>Location</th>
<th>Associated human tumours</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. RB</td>
<td>Nucleus (q13)</td>
<td>Retinoblastoma</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Osteosarcoma</td>
</tr>
<tr>
<td>2. TP53 (p53)</td>
<td>Nucleus (p17)</td>
<td>Most human cancers, common in cancer of the lung, neck, colon and breast</td>
</tr>
<tr>
<td>3. TGF-β</td>
<td>Extracellular GF</td>
<td>Cancer of pancreas, colon and stomach</td>
</tr>
<tr>
<td>4. APC</td>
<td>Cytosol</td>
<td>Cancer of colon</td>
</tr>
<tr>
<td>5. Others</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(i) WT1 and 2</td>
<td>Nucleus</td>
<td>Willms, tumour</td>
</tr>
<tr>
<td>(ii) NF 1 and 2</td>
<td>Plasma membrane</td>
<td>Neurofibromatosis type 1 and 2</td>
</tr>
<tr>
<td>(iii) BRCA 1 and 2</td>
<td>Nucleus</td>
<td>Cancer of breast, ovary</td>
</tr>
</tbody>
</table>

(i) **RB gene** - RB gene located on long arm (q) of chromosome 13. This is the first ever tumour suppressor gene identified and thus has been amply studied. Normally, RB gene product is a nuclear transcription protein which is virtually present in every human cell. It exists in both an active and an inactive form. Active RB gene protein acts to inhibit the cell cycle at G1→S phase.

The mutant form of RB gene is involved in retinoblastoma, the most common intraocular tumour in young children. The tumour occurs in two forms: sporadic
and inherited. Besides retinoblastoma, children inheriting mutant RB gene have 200 times greater risk of development of other cancers in early adult life, most notably osteosarcoma; others are cancers of breast, colon and lungs.

(ii) **TP53 gene (p53)** - Located on the short arm of chromosome 17, p53 gene (currently termed TP53) is normally a growth suppressor anti-oncogene. The two major functions of TP53 in the normal cell cycle are as under: 

a) *In blocking mitotic activity* - TP53 inhibits the cyclins and CDKs and prevents the cell to enter G1 phase transiently. This breathing time in the cell cycle is utilized by the cell to repair the DNA damage.

b) *In promoting apoptosis* - TP53 acts together with another anti-oncogene, RB gene, and identifies the genes that have damaged DNA which cannot be repaired by inbuilt system. TP53 directs such cells to apoptosis by activating apoptosis inducing BAX gene, and thus bringing the defective cells to an end.

In its mutated form, TP53 stops to act as growth suppressor and instead acts like an oncogene, c-onc. Majority of human cancers have either a mutation in TP53 or its expression is upregulated. Some common examples of human cancers having defective TP53 is also seen in the sequential development stages of cancer from hyperplasia to carcinoma in situ and into invasive carcinoma.

(iii) **Transforming growth factor-β (TGF-β)** - Normally, TGF-β is significant inhibitor of cell proliferation, mainly by its action on G1 phase of cell cycle. Its mutant form impairs the growth inhibiting effect and thus permits cell proliferation. Examples of mutated form of TGF-β are seen in cancers of pancreas, colon, stomach etc.

(iv) **Adenomatous polyposis coli (APC) gene** - The APC gene is normally inhibitory to
mitosis, which is done by a cytoplasmic protein, β-catenin. β-catenin normally blocks the signal to the nucleus for activating mitosis. In colon cancer, APC gene is lost and thus the cancer cells continue to undergo mitosis without the inhibitory influence of β-catenin.

(iv) Other anti-oncogenes- A few other tumour-suppressor genes having mutated germline in various tumours are: a) Wilms' tumour (WT) gene- WT gene normally prevents neoplastic proliferation of cells in embryonic kidney. Mutant form of WT-1 and 2 are seen in hereditary Wilms' tumour. b) Neurofibroma (NF) gene- NF genes normally prevents proliferation of Schwann cells. Two mutant forms are NF1 and NF2 seen in neurofibromatosis type 1 and type 2. c) BRCA 1 and BRCA 2 genes: These are breast (BR) cancer (CA) susceptibility genes, especially in inherited cases of breast cancer.

3. Escaping cell death by apoptosis: Genes regulating apoptosis and cancer

Apoptosis in normal cell is guided by cell death receptor, CD95, resulting in DNA damage. Besides, there role of some other pro-apoptotic factors (BAD, BAX, BID and TP53) and apoptosis-inhibitors (BCL2, BCL-X).

In cancer cells, the function of apoptosis is interfered due to mutations in the above genes which regulate apoptosis is interfered due to mutations in the above genes which regulate apoptosis in the normal cell. The examples of tumours by this mechanism are: a) BCL2 gene is seen in normal lymphocytes, but its mutant form with characteristic translocation was first described in B-cell lymphoma and hence the name. It is also seen in many other human cancers such as that of breast, thyroid and prostate. Mutation in BCL2
gene removes the apoptosis-inhibitory control on cancer cells, thus more live cells undergoing mitosis contributing to tumour growth. b) CD95 receptors are depleted in hepatocellular carcinoma and hence the tumour cells escape apoptosis.

4. Avoiding cellular aging: Telomeres and telomerase in cancer

After each mitosis (cell doubling) there is progressive shortening of telomeres which are the terminal tips of chromosomes. Telomerase is the RNA enzyme that helps in repair of such damage to DNA and maintains normal telomere length in successive cell divisions. However, it has been seen that after repetitive mitosis for a maximum of 60 to 70 times, telomeres are lost in normal cells and the cells cease to undergo mitosis. Telomerase is active in normal stem cells but not in normal somatic cells.

Cancer cells in most malignancies have markedly upregulated telomerase enzyme, and hence telomere length is maintained. Thus, cancer cells avoid aging, mitosis does not slow down or cease, thereby immortalising the cancer cells.

5. Continued perfusion of cancer: Tumour angiogenesis

Cancer can only survive and thrive if the cancer cells are adequately nourished and perfused, as otherwise they cannot grow further. Neovascularisation in the cancers not only supplies the tumour with oxygen and nutrients, but the newly formed endothelial cells also elaborate a few growth factors for progression of primary as well as metastatic cancer. The stimulus for angiogenesis is provided by the release of various factors:

(i) Promoters of tumour angiogenesis- include the most important vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF).
(ii) Anti-angiogenesis factors- inhibiting angiogenesis include thrombospondin-1, angiostatin, endostatin and vasculostatin.

6. Invasion and distant metastasis: cancer dissemination

One of the most important characteristics of cancers is invasiveness and metastasis. The mechanisms involved in the biology of invasion and metastasis have already been discussed.

7. DNA damage and repair system: Mutator gene and cancer

Normal cell, during complex mitosis suffer from minor damage to the DNA which is detected and repaired before mitosis is completed so that integrity of the genome is maintained. Similarly, small mutational damage to the dividing cell by exogenous factors (e.g. by radiation, chemical carcinogens etc) is also repaired. TP53 gene is held responsible for detection and repair of DNA damage. However, if this system of DNA repair is defective as happens in some inherited mutations (mutator genes), the defects in DNA is passed to the next progeny of cells and cancer results.

The examples of mutator genes exist in the following inherited disorders associated with increased propensity to cancer: (i) Hereditary non-polyposis colon cancer (Lynch Sundrom) is characterized by hereditary predisposition to develop colorectal cancer. (ii) Ataxia telangiectasis (AT) has ATM (M for mutated) gene. These patients have multiple cancers. (iii) Xeroderma pigmentosum is an inherited disorder in which there is defect in DNA repair mechanism. UV radiation damage to DNA cannot be repaired. Thus, such patients are more prone to various forms of skin cancers. (iv) Bloom syndrome is an example of damage by ionizing radiation which cannot be repaired due to inherited defect and the patients have
increased risk to develop cancers, particularly leukaemia. (v) *Hereditary breast cancer* patients having mutated BRCA1 and BRCA2 genes carrying inherited defect in DNA repair mechanism.

### 8. Cancer progression and heterogeneity: Clonal aggressiveness

Another feature of note in biology of cancers is that with passage of time they become more aggressive; this property is termed tumour progression. Clinical parameters of cancer progression are: increasing size of tumour, higher histologic grade, areas of tumour necrosis, invasiveness and distant metastasis.

In terms of molecular biology, this attribute of cancer is due to the fact that with passage of time cancer cell acquire more and more heterogeneity. This means that though cancer cells remain monoclonal in origin, they acquire more and mutations which in turn produce multiple-mutated subpopulations of more aggressive clones of cancer cells (i.e. heterogeneous cells) in the growth which have tendency to invade, metastasis and be refractory to hormonal influences.

### CLINICAL ASPECTS OF NEOPLASIA

Two major aspects of clinical significance in assessing the course and management of neoplasia are: tumour-host inter-relationship (i.e. the effect of tumour on host and vice versa) and laboratory diagnosis of cancer (Mohan S, 2005).

1. **Effect of tumour on host**: Malignant tumours produce more ill-effects than the benign tumours.
(a) **Local effects-** Both benign and malignant tumours cause local effects on the host due to their size or location. Some of the local effects of tumours are: i) Compression, ii) mechanical obstruction, iii) tissue destruction, and iv) infarction, ulceration, haemorrhage.

(b) **Cancer cachexia-** Patients with advanced and disseminated cancers terminally have asthenia (emaciation), and anorexia, together referred to as cancer cachexia (meaning wasting). Possibly, cachectin or tumour necrosis factor α (TNF-α) and interleukin-1 derived from macrophages play a contributory role in cachexia.

(c) **Fever-** Fever of unexplained origin may be presenting feature in some malignancies such as in Hodgkin’s disease, adenocarcinoma kidney, osteogenic sarcoma and many other tumours.

(d) **Paraneoplastic syndromes-** Paraneoplastic syndromes (PNS) are a group of conditions developing in patients with advanced cancer which are neither explained by direct and distant spread of the tumour, nor by the usual hormone elaboration by the tissue of origin of the tumour. About 10 to 15% of the patients with advanced cancer develop one or more of the syndromes included in the PNS. Rarely, PNS may be the earliest manifestation of a latent cancer.

2. **Host response against tumour (Immune surveillance of cancer):** It has long been thought that host defence mechanism in the form of immunological response exists so as to counter the growth and spread of cancer. The following observations provide basis for this.

   (a) Certain cancers evoke significant lymphocytic infiltrates composed of immunocompetent cells and such tumours have somewhat better prognosis e.g. medullary carcinoma breast, seminoma testis.
(b) Rarely, a cancer may spontaneously regress partially or completely e.g. malignant melanoma.

(c) It is highly unusual to have primary and secondary tumours in the spleen due to its ability to destroy the growth and proliferation of tumour cells.

(d) Immune surveillance exists is substantiated by increased frequency of cancers in immunodeficient host e.g. in AIDS patients, or in organ transplant recipients.
SECTION 6

CANCER & APOPTOSIS
Apoptosis is one of the most potent defense against cancer, since this process eliminates potentially deleterious mutated cells. The pathogenesis of many diseases, including cancer, is closely connected with aberrantly regulated apoptotic cell death (Reed, 1999). It is estimated that excessive or inadequate cell death contributes to approximately half of all medical illness. It is intriguing that key components in cellular regulation of apoptosis have been identified and thus may be targeted by therapeutic strategies. These targets include death receptors that trigger apoptosis from the cell surface, Bcl-2 proteins as integral regulators of the mitochondrial apoptotic pathway, caspases as the executioner enzymes, and endogenous caspase inhibitors (Fischer & Schulze-Osthoff, 2005).

Programmed cell death (PCD) is a distinct genetic and biochemical pathway of cell death essential to metazoans in maintaining tissue homeostasis without any specification of the mode. Apoptosis, however, is one specific mechanism of cell death with a distinctive phenotype, which regulates tissues homeostasis. The term apoptosis in Greek meaning “Falling of the leaves”; the term describes the distinctive phenotypic phenomenon related to cellular shrinkage. Depending on the trigger and the mode of cell death, PCD and apoptosis can occur simultaneously or independently as elements of physiologic cell death (Holdenrieder & Stieber, 2004). Apoptosis has further been defined as “a sequence of events based on cellular metabolism that leads to cell destruction with a specific morphology”; this definition distinguishes this process from other forms of cell death, such as autophagy, oncrosis, and necrosis (Guimaraes & Linden, 2004; Lockshin & Zakeri, 2004; Nicotera & Melino, 2004). Typically, apoptosis, an active energy-requiring process, that is activated in single cell that are aged, dysfunctional, or damaged by external stimuli.
Phenotypically and morphologically apoptosis is characterized by chromatin condensation, nuclear fragmentation into mono- and oligonucleosomal units, cell shrinkage, and plasma membrane blebbing (Rodriguez & Schaper). Ultimately, cells break into small membrane-surrounded fragments (apoptotic bodies) that are phagocytosed without inducing inflammation (Lauber et al., 2004). Additional early features of apoptosis include marginalization in the nucleus, karyorrhexis, packaging of organelles, and dilation of the endoplasmic reticulum (Holdenrieder & Stieber, 2004). The early events occur within minutes, while final stages involving lysosomal degradation of cellular components typically are complete in hours. The entire apoptosis process is regulated very precisely by several intrinsic and extrinsic factors (discussed below).

**Regulation of Apoptosis**

The inducers of apoptosis include both intra- and extracellular stimuli, such as DNA damage, disruption of the cell cycle, hypoxia, detachment of cells from their surrounding tissue, and loss of tropic signaling (Sun et al., 2004). Apoptosis occurs primarily through two well-recognized pathway in cells. Both effector mechanism of apoptosis are associated with caspase activation and include the intrinsic, or mitochondrial-mediated, effector mechanism and the extrinsic, or death receptor-mediated, effector mechanism (Reed, 2004).

In addition to mitochondria, other organelles, including the endoplasmic reticulum, golgi apparatus, and lysosomes, may also contribute to damage sensing, pro-apoptotic signaling, and caspase activation (Sun et al., 2004; Jaattela, 2004). The endoplasmic reticulum, as an important apoptotic control point, displays anti-apoptotic Bcl-2 and proapoptotic Bax and Bak proteins (Danial & Korsmeyer, 2004).
The intrinsic pathway of apoptosis relies primarily on the permeabilization of mitochondrial membranes, with associated release of apoptogenic mitochondrial proteins, leading to activation of caspase 9 and downstream cleavage of caspases 3, 6 or 7 (Guimaraes & Linden, 2004). A third pathway involving granzyme has been identified, one that directly activates caspase 3. A critical element and commonality among these pathways is the involvement of caspases and specifically and activation of caspase 3, the pivotal committed executioner caspase of apoptosis.

**Intrinsic Mitochondrial Pathway** – Although apoptosis may be mediated by the interaction between death receptors and their ligands, many dietary bioactive agents induce apoptosis through the intrinsic mitochondria-mediated pathway (Chen & King, 2005). This pathway is characterized by alternations in mitochondrial polarization and release of mitochondrial proteins, including cytochrome c, endonuclease G, secondary mitochondria-driven activator of caspase (Sma)/direct inhibitor of apoptosis protein (IAP) binding protein with low pi (DIABLO), Omi/HtrA2 apoptosis-inducing factor (AIF), and its homolog AIF-homologous mitochondrial-associated inducer of death (Sun et al., 2004). The release of cytochrome c, trigger caspase activation and ultimately execution of apoptosis.

**Mitochondrial Polarization/Depolarization** – Mitochondria are intracellular organelles that generate energy for the cell and are thus known as the powerhouse of the cell. Normally the mitochondrion possesses an electrochemical gradient across the inner membrane, which is critical for proper function of the energy-yielding electron-transport chain. The mitochondrion is also a pivotal organelle for the induction of apoptosis via the intrinsic pathway. Increased mitochondrial permeability and dissipation of the electrochemical gradient or membrane potential (MMP) via opening of the mitochondrial
permeability transition pore (MTP) triggers cell death by release apoptogenic factors form within the mitochondria, with subsequent cytochrome c release, apoptosome formation and ultimately apoptosis induction.

**Alteration of Bcl-2/Bax Ratios** – Failure to active apoptosis is one of the major impediments to the successful treatment of cancer. Novel compounds that target apoptosis regulatory pathway and specifically, the proteins involved are potentially chemopreventive. Such targets include the Bcl-2 family of proteins, since these proteins are molecular integrators of both simultaneous cellular pro-death and pro-survival signals (Heil, 2003). This balance dictates the decision to die or not, based on the release of apoptogens from the mitochondria to the cytosol. As a result, Bcl-2 and Bcl-xL have emerged as major new chemopreventive targets.

*In the intrinsic mitochondrial pathway,* the Bcl-2 family of at least 18 pro- and anti-apoptotic proteins are pivotal regulators of apoptosis, all of which may be targets. Moreover, these proteins may directly or indirectly antagonize each other’s functions and are important in connecting signals from the extrinsic death receptor pathway to the mitochondrial pathway. Bax, a Bcl-2 family protein, potentially regulates the pro- and anti-apoptotic balance within a cell by regulating mitochondrial function (Cory & Adams, 2005).

**Mitochondrial Cytochrome c Release** – Cytochrome c is found in cells attached to the outer surface of the inner mitochondrial membrane and is largely localized in the cristae, where the protein functions in the electron-transport system. During apoptosis, cytochrome c is released from the cristae into the cytosol, a pivotal step in apoptosis initiation. This can be induced by various stimuli, including elevations in pore-forming pro-apoptotic Bcl-2 family proteins such as Bax. Once released to the cytoplasm, cytochrome c binds and activates
apoptotic protease activating factors (Apaf-1), enabling binding and activation of procaspase 9, an initiator caspase. This process is suppressed by molecules that prevent cytochrome c release, including the anti-apoptic Bcl-2 proteins.

**Activation of Caspases** — Caspases, comprised of 12 proteins, are a family of cysteine asparatate-specific protease involved in apoptosis and are subdivided into initiator (caspases 8, 9, 10) and executioner (caspases 3, 6, 7) caspases (Fischer & Schulze-Osthoff, 2005). Modulating the mechanisms of caspase activation and suppression is a critical molecular target in chemoprevention, since these processes lead to apoptosis (Philchenkov, 2004).

The intrinsic and extrinsic pathways converge at caspase 3. Active caspase 9 and caspase 8 of the intrinsic and extrinsic pathways, respectively, have been shown to directly cleave and activate the effector protease caspase 3. Caspase 3 cleaves and activates directly or indirectly other effector caspases, such as caspases 6 or 7. Active caspases, including caspases 3, 7, and 9, can be directly inhibited by some IAP family proteins, such as X chromosome-linked IAP (XIAP). Inhibitor of apoptosis proteins are suppressed by Smac/DIABLO, which is released from mitochondria. The transcription factor Nf-kB induces expression or apoptosis suppressors, including certain IAP family genes and some anti-apoptotic Bcl-2 family genes. The kinase Akt can phosphorylate and inactivate Bad as well as caspase 9 (Reed, 2001). Thus, there is considerable overlap between the intrinsic and extrinsic pathways.

**Extrinsic Death Receptor Pathway** — The extrinsic pathway is triggered by members of the tumor necrosis factor (TNF) receptor superfamily, which comprises almost 20 members of cytokine receptors, such as TNFR 1. Fas, and TNF-related apoptosis inducing ligand (TRAIL) receptors (Bouralexis et al., 2005 & Reed, 1999). These proteins recruit adapter
proteins, including FADD, to their cytosolic death domains, with subsequent binding to pro-
caspases, particularly caspase 8, which contains a protein interaction motif (the death effector
domain, or DED) that binds a complementary domain in FADD. Next, intracellular
recruitment of the death-inducing signaling complex (DISC) occurs by means of
protein/protein interactions involving death domains (2). The DISC plays a central role in the
extrinsic pathway by activating the initiator caspases 8 and 10 (Perik et al., 2005).

Inhibitor of apoptosis proteins are a family of evolutionarily conserved anti-apoptotic
proteins that bind caspases 3, 7, and 9 and modulate cell division, cell cycle progression, and
signal transduction (Reed, 2001). They are potentially clinically useful in the diagnosis and
treatment of occult malignancy and, thus, are considered valid therapeutic targets (Schimmer,
2004).

Gene Expression and Apoptosis

In the past decade, the identification of genes and gene products that regulate
apoptosis coupled with increasing elucidation of mechanisms targets and mode of action have
been critical in the search for compounds targeting remains a considerable focus of attention
as a means of chemotherapy (Lotan, 1995; Holzman, 1996; Fesus, 1995).

Cellular Signaling – The reduction of growth factor-induced proliferative signaling in
many cases permits the initiation of apoptotic cascades. The signals exerted through
interaction of growth factor receptors and their ligands, including insulin-like growth factor
(IGF), EGF, and vascular epithelial growth factor (VEGF), strongly drive cells to proliferate
in carcinogenesis. As a result, those dietary agents that can interrupt this signaling would be
beneficial via decreased proliferative and proapoptotic signaling. In fact, that TNF family
member Apo2L TRAIL receptor has received considerable recent attention as a therapeutic target, since many cancer cells appear sensitive to Apo2L/TRAIL-induced apoptosis (Bouralexis et al., 2005). The MAPK kinase signaling cascades include extracellular signal-related proteins kinases (ERKs), JNKs/stress-activated protein kinases (SAPKs), and p38 kinases. The ERKs transmit signals, initiated by growth promoter, including EGF, PDGF, and fibroblast growth factor (FGF) and may ultimately foster cell growth and survival (Bode & Dong, 2004). The polyphenols curcumin, EGCG, and resverator downregulate phosphorylation and ligand binding of growth factor receptor including EGF, FGF, and PDGF (Manson et al., 2005). Consequently, this quenches MAPK signaling, transcription factor activation (i.e., AP-1), and ultimately gene expression. It is noteworthy that many cells require such signals to avoid apoptosis, and, as a result, interruption of this signaling encourages induction of apoptosis in many cell types. For example, the indirect inhibition of PI3-Akt anti-apoptotic signals might contribute to cell death through modulation by diet (Chen & King, 2005). The MAPKs are activated by translocation to the nucleus, where they phosphorylate numerous substrates, including the transcription factors AP-1 and NF-kB. Activation of both are linked to carcinogenesis and tumor promotion (Bode & Dong, 2004).

Indeed numerous mutations can occur in tumor suppressor genes involved in induction of apoptosis, and these include p53, p19ARF, Rb, PTEN, TRAIL, and CD95/Fas. Numerous oncogenes may also be activated through mutation to inhibit or circumvent the inherent controls of apoptosis, and these include Bcl-2, MDM2, IAPs, NF-kB, Akt, P13K, Ras, Myc, and FLIP (Johnstone et al., 2002). Blocking the expression of genes, and in particular oncogenic ras, is currently an active pharmacological approach for cancer therapy (Adjei, 2001). Clearly mutations in genes that regulate apoptosis pathways are common in most
cancers, emphasizing the importance of apoptosis in carcinogenesis and protection of these genes against DNA damage (Sun et al., 2004).

**Transcription Factors NF-κB** - Activation of NF-κB promotes survival and cellular proliferation, and down regulation sensitizes cells to apoptosis. NF-κB is a key transcription factor involved in integration of multiple survival signaling pathways, including upregulation of Bcl-xl, IAPs (XIAP and cIAP-2), and the antagonist of death receptor signaling Flip (Cumming et al., 2004).

It is widely accepted that NF-κB, as well as its regulators IKK and IκB, are associated with survival from apoptosis and other physiologic processes (Courtois, 2005).

**Transcription Factor p53** - p53 is a sequence-specific transcription factor and critical tumor suppressor gene that is the most frequently mutated in human cancer. P53 transactivates genes that mediated apoptosis and has roles in DNA repair, senescence, and cell cycle arrest (Levin, 1997). There is broad consensus that the primary physiologic role of p53 in DNA damage-induced apoptosis is to function as a transcriptional activator of genes encoding apoptosis effectors. P53 directly activates transcription of several genes encoding members of the Bcl-2 family, but it also mediates cell death through a variety of mechanisms, including downregulation of anti-apoptotic genes such as Map4 and surviving and upregulation of pro-apoptotic genes such as Bax, IGF-BP3, DR5, Fas and Apaf-1, as well as various other apoptosome components representing potentially key therapeutic targets (Hajra & Liu, 2004; Slee et al., 2004; Woods & Vousden, 2001). P53 has also been demonstrated to exhibit a direct apoptogenic role in the mitochondria, where it translocates and interacts with Bcl-xL and Bcl-2 proteins to induced mitochondrial permeability zation (Mihara et al, 2003). Given the central nature of p53 in the apoptotic response, it is not surprising that...
perturbations of proteins known to regulate p53 also affect the apoptotic program. Moreover, p53 deficiently leads to inappropriate survival of cells with DNA damage and therefore predisposes one to develop neoplasia.

Recently, p63 and p73 proteins have also been identified that bind p53 response elements and transactivate p53-associated genes and, as a result, induce apoptosis. Furthermore, there is extrinsic overlap of p53 and multiple transcriptional targets, in which p53 can activate at least two proteins in the intrinsic pathway, including Bax and p53-apoptosis including factor (Harms et al., 2004). Reactive oxygen species have been strongly correlated with p53-mediated apoptosis. Upon overexpression of p53, ROS levels rise, and mitochondrial apoptosis is induced as described above (Johnson et al., 1996).

**AP-1 Transcription Factor** – In addition to intrinsic genetic variability, inhibition of growth factor pathway and pathways associated with antagonism of apoptosis has been shown to be beneficial in numerous cell models. Specifically, the AP-1 activation pathway is oncogenic and antagonizes apoptosis in neoplasia. Numerous reports indicate NF-kB activation fosters cell survival through maintenance of cellular proliferation and decreased sensitization to apoptotic signaling. This occurs through alteration of gene expression with upregulation of NF-kB, Bcl-2, Bcl-XL, cIAP, surviving, cyclin D1, TRAF-1, and TRAF-2 (Garg & Aggarwal, 2002).

Some of the factors and signaling pathways, that involved in the induction of apoptosis have been presented in Fig. 2.6.1.
Fig. 2.6.1. The factors and pathways involved in the induction of apoptosis
SECTION 7

CANCER & CELL CYCLE
The cell cycle is a term used to describe an orderly series of events that ensure the duplication of all the cellular components, such as organelles and DNA, in their correct sequence and the faithful partitioning of those components. Traditionally the cell cycle has been divided into four parts based upon measurable or visible events in the cycle. The S phase (synthetic phase) is the period for the complete duplication of nuclear DNA. Mitosis, or the M phase, results in the condensation of chromosomes, attachment to the spindle, and segregation of the duplicated chromosome into two equal parts. The M and the S phases are separated in time from other by two gaps termed G1, between M and S, and G2, between S and M.

Two different categories of genes and their protein products participate in the cell cycle: (1) functions that are obligatory for progress through the cell cycle from G1 to S to G2 to M and (2) checkpoints that monitor the efficacy and completion of these obligatory events and stop the progress of the cycle if conditions are not perfect (Hartwell et al., 1989). An example of an essential function in the cell cycle is the synthesis and/or activation in the late G1 phase of enzymes that are required to produce the deoxyribonucleotide triphosphate precursors for DNA synthesis. Without such precursors, DNA could not be replicated, and so the regulation of these enzymes and their activities is essential for progression through the cycle. Similarly, the synthesis of DNA polymerase, ligase, etc., is required in S phase to progress in the cycle. Histone synthesis and DNA packaging are required in S phase in an obligatory fashion to progress to G2. Chromosome condensation begins in G2, and condensation, spindle attachment, and segregation are all examples of obligatory functions in
the cell cycle. Checkpoint controls monitor and regulate these events. The cycle progresses unless it is stopped and restarted by a checkpoint control (Fig. 2.7.1.).

**Checkpoint control of the cell cycle**

There are several examples of checkpoint controls acting in the G1, S or G2 phase of the cell cycle (Fig. 2.7.2.). One of these measures the nutrient levels in the environment and determines whether they are sufficient to duplicate the cell. If they are not, the cycle is blocked at a specific phase. Replication of damaged DNA results in mutations, cell death, or the origin of cancerous clones of cells. Mammalian cells frequently receive signals from their neighbors that are commonly mediated by hormones (both growth stimulation and inhibition), adhesion molecules, integrins, or external events. Many of these signals act as checkpoints for progression through the cell cycle.

How do checkpoint controls act in the cell cycle to stop progression of cells into the next phase of the cycle? While the entire pathway that regulates these processes is not clear, some answers are now available. Some checkpoints act upon a group of proteins called cyclins that in turn activate the catalytic subunits of protein kinases called p34 cdc-2 kinases. The cyclin activated cdc-2 kinase plays a critical role in providing an essential function for progression of the cell cycle at several different stages. Cyclins are proteins that were first described (in sea urchins) and were so named because they increase in amount as the cell cycle progresses from G1 to M and are then abruptly degraded at a specific stage in the cell cycle (Evans et al., 1983).
The different cyclins accumulate with variable kinetics, magnitude and timing across the cell cycle. The timing of the cyclin protein appearance gives the first clue about the stage of the cell cycle that is regulated by the particular cyclin. Since most decisions about the cell cycle progression in mammalian cells are made in G1, so the G1 cyclins are the best candidates for alternations giving rise to uncontrolled cell growth. These includes cyclins A, E and D type. The overexpression of certain types of cyclins appears to be a common event in tumors.

There is a small but increasing number of CDKs substrates that have known roles in proliferation. These include tumor suppressor proteins p107 and p130, oncogenes c-src, c-mos, cable, c-myc and myb, transcription factors E2F and RNA polymerase II and replication factor RPA. In addition to receiving correct environmental cues for cell division, a successful completion of the cell cycle also depends on the efficient completion of the previous processes in the cycle. These feedback controls have come to be known as cell cycle checkpoints and they represent intracellular signaling pathways that allow cells to monitor the correct completion of the cell cycle events. The following checkpoints that have been identified:

- One that monitors for DNA damage and arrests cells in G1.
- One that monitors DNA damage and arrests cells in G2.
- One that signals that DNA synthesis has been completed before mitosis begins.
- One that monitors completion of mitosis before allowing entry into S phase.
- One that ensues that the chromosomes are correctly aligned at the metaphase plate prior to the initiation of anaphase.
Identification of cell cycle components as potential tumorigenic agents have implications in cancer therapy. Disruption of such pathway must give the cell a proliferation advantage and allow it to grow unchecked by normal monitoring systems. Most chemotherapeutic agents act by blocking DNA replication, inducing DNA damage or by interfering with chromosome segregation. However, the interplay of these interlocking regulatory system is lost in tumorigenesis.

An understanding of cell-cycle kinetics is essential for the proper use of antineoplastic agents. Many of the most effective cytotoxic agents act by damaging DNA. Their toxicity is greater during the S, or DNA synthetic, phase of the cell cycle, while others, such as the vinca alkaloids and taxanes, block the formation of a functional mitotic spindle in M phase. These agents have activity against cells that pass through the most vulnerable phase of the cell cycle. Accordingly, human neoplasms that currently are most susceptible to chemotherapeutic measures are those with a high percentage of cells undergoing division. Similarly, normal tissues that proliferate rapidly (bone marrow, hair follicles, and intestinal epithelium) are subject to damage by most cytotoxic drugs, which often limits their usefulness. Conversely, slowly growing tumors with a small growth fraction (for example, carcinomas of the colon or non-small cell lung cancer) often are less responsive to cycle-specific drugs. Although differences in the duration of the cell cycle occur between cells of various types, all cells display a similar pattern during the division process: (1) a phase that precedes DNA synthesis (G1); (2) a DNA synthesis phase (S); (3) an interval following the termination of DNA synthesis (G2); and (4) the mitotic phase (M) in which the cell, containing a double complement of DNA, divides into two daughter G1 cells. Each of these daughter cells may immediately re-enter the cell cycle or pass into a nonproliferative stage, referred to as G0. The
Go cells of certain specialized tissues may differentiate into functional cells that no longer are capable of division. On the other hand, many cells, especially those in slow-growing tumors, may remain in the G0 state for prolonged periods, only to re-enter the division cycle at a later time. Each transition in the cell cycle is controlled by the activity of specific cyclin-dependent kinases (CDKs), which are activated by their corresponding small regulatory proteins called cyclins, and inhibited by proteins such as p16. Mutations or loss of p16 or other components of the so-called retinoblastoma pathway such as retinoblastoma protein itself, or enhanced cyclin or CDK activity, will lead to relentless proliferation in tumor cells. Consequently, CDKs and their effector proteins have become attractive molecular targets for new antineoplastic agents. Because of the central importance of DNA to the identity and functionality of a cell, elaborate mechanisms have evolved to monitor DNA integrity. If a cell expresses normal p53 protein, DNA damage activates a normal checkpoint function and damaged cells undergo apoptosis, or programmed cell death, when they reach the G1/S boundary. If the p53 gene product is mutated or absent and the checkpoint function fails, damaged cells will not be diverted to the apoptotic pathway but will proceed through S phase. At the G2-M interface, other checkpoint proteins monitor DNA integrity and may delay progression into M phase. Absence or mutation of these checkpoints allows cells to pass through mitosis and survive DNA damage (Lane and Fischer, 2004). These cells can proceed through S phase and some will emerge as a mutated and potentially drug-resistant population.

Normally, cells in a differentiated state are stimulated to enter the cell cycle from a quiescent state, or G0, or continue after completion of a prior cell division cycle in response to environmental cues including growth factor and hormonal signals. Cells progress through G1
and enter S-phase after passing through “checkpoints,” which are biochemically regulated transition points, to assure that the genome is “ready” for replication. The cyclin-dependent kinases (CDKs) are enzymes that critically regulate cell cycle progression from one phase to the next. One important checkpoint is mediated by the p53 tumor-suppressor gene product, acting through its upregulation of the p21^WAF1 inhibitor of CDK function, acting on CDKs 4 or 6. These kinase molecules can also be inhibited by the p16^{INK4A} and p27^{KIP1} CDK inhibitors, but in turn are activated by cyclins of the D family (which appear during G₁) and the proper sequence of regulatory phosphorylations. Activated CDKs 4 or 6 phosphorylate, and thus inactivate, the product of the retinoblastoma susceptibility gene, pRb, which in its nonphosphorylated state complexes with transcription factors of the E2F family. Phosphorylated pRb releases E2Fs, which activate genes important in completing DNA replication during S-phase, progression through which is promoted by CDK2 acting in concert with cyclins A and E. During G₂, another checkpoint occurs, in which the cell assures the completion of correct DNA synthesis. Cells then progress into M-phase under the influence of CDK1 and cyclin B. Cells may then go on to a subsequent division cycle or enter into a quiescent, differentiated state. Thus, an understanding of cell-cycle kinetics and the controls of normal and malignant cell growth is crucial for the design of current therapeutic regimens and the search for new drugs (Fig. 2.7.3.).
THE CELL CYCLE

Fig. 2.7.1. The cell cycle check points

Fig. 2.7.2. The cell cycle and its controlling factors
Fig. 2.7.3. The cell cycle proteins and cellular homeostasis
SECTION 8

ANTINEOPLASTIC AGENTS
AND LIMITATIONS
Cancer is a complex multifactorial disease of the cell. Only single drug or more than one drug can not control all the different types of cancer. Complexity of cancer differ according to type of cancer, site of origin, place of tumour in the body and the genes involved. Different therapeutic approaches have been developed to counteract different types of cancers. Surgical therapy, radiation therapy and chemotherapy are the main therapies till now available throughout the world. However, each therapy has its own limitations. Leukaemia is usually always treated with chemotherapy. The drugs kill the leukaemia cells but also damage the normal cells and follows several complications. Commonly used antineoplastic agents and their limitations have been discussed (Fauci A S, 2006; Brunton L L, 2006) and shown in Table 2.8.1.

ALKYLATING AGENTS

Alkylating agents are so named because of their ability to add alkyl groups to many electronegative groups under conditions present in cells. They stop tumour growth by cross-linking guanine nucleobases in DNA double-helix strands - directly attacking DNA. This makes the strands unable to uncoil and separate. As this is necessary in DNA replication, the cells can no longer divide. It is a potent activator of cell cycle checkpoints and signaling pathways that can activate apoptosis (Fig. 2.8.1.).

*Nitrogen mustard* (mechlorethamine) - is the prototypic agent of this class, decomposing rapidly in aqueous solution to yield potentially a bifunctional carbonium ion. It is a powerful vesicant, and infiltration may be symptomatically ameliorated by infiltration of the affected site with 1/6 M thiosulfate. Even without infiltration, aseptic thrombophlebitis is frequent. It causes moderate nausea after intravenous administration.
Cyclophosphamide - is inactive unless metabolized by the liver to 4-hydroxy-cyclophosphamide, which decomposes into alkylating species, as well as to chloroacetaldehyde and acrolein. The latter causes chemical cystitis; therefore excellent hydration must be maintained while using cyclophosphamide. Liver disease impairs drug activation. Sporadic interstitial pneumonitis leading to pulmonary fibrosis can accompany the use of cyclophosphamide, and high doses used in conditioning regimens for bone marrow transplant can cause cardiac dysfunction.

Several alkylating agents are less commonly used. Chlorambucil causes predictable myelosuppression, azoospermia, nausea, and pulmonary side effects. Busulfan can cause profound myelosuppression, alopecia, and pulmonary toxicity but is relatively “lymphocyte sparing.” Its routine use in treatment of chronic myeloid leukemia (CML) has been curtailed in favor of imatinib (Gleevec), hydroxyurea, and interferon (IFN), but it still is employed in transplant preparation regimens.

Nitrosoureas - break down to carbamoylating species that not only cause a distinct pattern of DNA base pair-directed toxicity but also can covalently modify proteins. They share the feature of causing relatively delayed bone marrow toxicity, which can be cumulative and long-lasting. Streptozotocin is unique in that its glucose-like structure conveys specific toxicity to the islet cells of the pancreas (for whose derivative tumor types it is prominently indicated) as well as causing renal toxicity in the form of Fanconi’s syndrome, including amino aciduria, glycosuria, and renal tubular acidosis. Methyl CCNU (lomustine) causes direct glomerular as well as tubular damage, cumulatively related to dose and time of exposure.
Procarbazine - is metabolized in the liver and possibly in tumor cells to yield a variety of free radical and alkylating species. In addition to myelosuppression, it causes hypnotic and other CNS effects, including vivid nightmares. Altretamine (formerly hexamethylmelamine) and thiotepa can chemically give rise to alkylating species, although the nature of the DNA damage has not been well characterized in either case. Thiotepa can be used for intrathecal treatment of neoplastic meningitis. Dacarbazine (DTIC) is activated in the liver to yield the highly reactive methyl diazonium cation. It causes only modest myelosuppression 21 to 25 days after a dose but causes prominent nausea on day 1.

Cisplatin - was discovered fortuitously by observing that bacteria present in electrolysis solutions could not divide. Only the cis diamine configuration is active as an antitumor agent. It is hypothesized that in the intracellular environment, a chloride is lost from each position, being replaced by a water molecule. The resulting positively charged species is an efficient bifunctional interactor with DNA, forming Pt-based cross-links. Cisplatin requires administration with adequate hydration, including forced diuresis with mannitol to prevent kidney damage; even with the use of hydration, gradual decrease in kidney function is common, along with noteworthy anemia. Hypomagnesemia frequently attends cisplatin use and can lead to hypocalcemia and tetany. Other common toxicities include neurotoxicity with stocking and glove sensorimotor neuropathy. Hearing loss occurs in 50% of patients treated with conventional doses. Cisplatin is intensely emetogenic, requiring prophylactic antiemetics. Oxaliplatin is used in colon cancers refractory to other treatments. Its place in the primary and adjuvant treatment of colon tumors is being defined, along with tests of activity in other tumors. It is prominently neurotoxic.
ANTITUMOR ANTIBIOTICS AND TOPOISOMERASE INHIBITORS

Antitumor antibiotics are substances produced by bacteria that in nature appear to provide chemical defense against other hostile microorganisms. As a class they bind to DNA directly and can frequently undergo electron transfer reactions to generate free radicals in close proximity to DNA, leading to DNA damage in the form of single strand breaks or cross-links. Topoisomerase poisons include natural products or semi-synthetic species derived ultimately from plants, and they modify enzymes that regulate the capacity of DNA to unwind to allow normal replication or transcription. DNA damage from these agents can occur in any cell cycle phase, but cells tend to arrest in S-phase or G$_2$ of the cell cycle in cells with p53 and Rb pathway lesions as the result of defective checkpoint mechanisms in cancer cells.

*Doxorubicin* is the most widely active and frequently used antineoplastic agent. It can intercalate into DNA, thereby altering DNA structure, replication, and topoisomerase function. It can also undergo redox cycling by accepting electrons into its quinone ring system. It causes predictable myelosuppression, alopecia, nausea, and mucositis. In addition, it causes acute cardiotoxicity in the form of atrial and ventricular dysrhythmias, but these are rarely of clinical significance. The incidence of cardiomyopathy appears to be related to schedule (peak serum concentration), with low dose, frequent treatment, or continuous infusions better tolerated than intermittent higher dose exposures. Its cardiotoxicity is increased when given together with trastuzumab (*Herceptin*), the anti-HER2/neu antibody. The drug is a powerful vesicant, with necrosis of tissue apparent 4 to 7 days after an extravasation; therefore it should be administered into a rapidly flowing intravenous line. The drug is metabolized by the liver, so doses must be reduced by 50 to 75% in the presence of liver dysfunction. *Daunorubicin* is closely related to doxorubicin and was actually introduced
first into leukemia treatment, where it remains part of curative regimens and has been shown preferable to doxorubicin owing to less mucositis and colonic damage.

**Bleomycin** - refers to a mixture of glycopeptides that have the unique feature of forming complexes with Fe$^{2+}$ while also bound to DNA. Oxidation of Fe$^{2+}$ gives rise to superoxide and hydroxyl radicals. The drug causes little, if any, myelosuppression. Bleomycin is not a vesicant and can be administered intravenously, intramuscularly, or subcutaneously. Common side effects include fever and chills, facial flush, and Raynaud's syndrome. The most feared complication of bleomycin treatment is pulmonary fibrosis, which increases in incidence at >300 cumulative units administered and is minimally responsive to treatment (e.g., glucocorticoids). Bleomycin is inactivated by a bleomycin hydrolase, whose concentration is diminished in skin and lung.

**Dactinomycin** - intercalates into DNA and appears to have less, but not absent, capacity to undergo electron transfer reactions. It causes severe myelosuppression, nausea, alopecia, and mucositis. Mithramycin historically was used against testicular and other neoplasms; however, in addition to causing nausea, myelosuppression, and vesicant properties, it causes an acute hemorrhagic syndrome consisting of platelet function defects in association with indicators of disseminated intravascular coagulation.

**Mitomycin C** - undergoes reduction of its quinone function to generate a bifunctional DNA alkylating agent. It is a broadly active antineoplastic agent with a number of unpredictable toxicities, including delayed bronchospasm and chronic pulmonary fibrosis syndrome more frequent. Cardiomyopathy has been described, particularly in a setting of prior radiation therapy. Mitomycin is a notable vesicant and causes substantial nausea and vomiting.
Mitoxantrone - is a synthetic compound that was designed to recapitulate features of doxorubicin but with less cardiotoxicity but may cause alopecia.

Etoposide - was synthetically derived from the plant product podophyllotoxin; it binds directly to topoisomerase II and DNA in a reversible ternary complex. It stabilizes the covalent intermediate in the enzyme's action where the enzyme is covalently linked to DNA. This "alkali-labile" DNA bond was historically a first hint that an enzyme such as a topoisomerase might exist. The drug therefore causes a prominent G$_2$ arrest, reflecting the action of a DNA damage checkpoint. Prominent clinical effects include myelosuppression, nausea, and transient hypotension related to the speed of administration of the agent. Teniposide is a structural relative with unique activity in childhood acute lymphoid leukemia. When given at high doses or very frequently, topoisomerase inhibitors may cause acute leukemia associated with chromosome 11q23 abnormalities in up to 1% of exposed patients.

Camptothecin- was isolated from extracts of a Chinese tree and had notable antileukemia activity. Early clinical studies with the sodium salt of the hydrolyzed camptothecin lactone showed evidence of toxicity with little antitumor activity. Identification of topoisomerase I as the target of camptothecins and the need to preserve lactone structure allowed additional efforts to identify active members of this series. Topoisomerase I is responsible for unwinding the DNA strand by introducing single strand breaks and allowing rotation of one strand about the other. In S-phase, topoisomerase I–induced breaks that are not promptly resealed lead to progress of the replication fork off the end of a DNA strand. The DNA damage is a potent signal for induction of apoptosis. Camptothecins promote the stabilization of the DNA linked to the enzyme in a so-called cleavable complex, analogous to
the action of etoposide with topoisomerase II. *Topotecan* is a camptothecin derivative approved for use in ovarian tumors. Toxicity is limited to myelosuppression and mucositis.

**ANTIMETABOLITES**

A broad definition of antimetabolites would include compounds with structural similarity to precursors of purines or pyrimidines or that interfere with purine or pyrimidine synthesis. Antimetabolites can cause DNA damage indirectly, through misincorporation into DNA, abnormal timing or progression through DNA synthesis, or altered function of pyrimidine and purine biosynthetic enzymes. They tend to convey greatest toxicity to cells in S-phase, and the degree of toxicity increases with duration of exposure. Common toxic manifestations include stomatitis, diarrhea, and myelosuppression. Second malignancies are not associated with their use.

*Methotrexate* - inhibits dihydrofolate reductase, which regenerates reduced folates from the oxidized folates produced when thymidine monophosphate is formed from deoxyuridine monophosphate. Without reduced folates, cells die a “thymineless” death. N-5 tetrahydrofolate or N-5 formyltetrahydrofolate (leucovorin) can bypass this block and rescue cells from methotrexate, which is maintained in cells by polyglutamylation. The drug and other reduced folates are transported into cells by the folate carrier, and high concentrations of drug can bypass this carrier and allow diffusion of drug directly into cells. Methotrexate is cleared by the kidney by both glomerular filtration and tubular secretion, and toxicity is augmented by renal dysfunction and drugs such as salicylates, probenecid, and nonsteroidal anti-inflammatory agents that undergo tubular secretion. In addition to bone marrow suppression and mucosal irritation, methotrexate can cause renal failure itself at high doses.
Methotrexate can cause prolonged myelosuppression. Chronic low-dose methotrexate can cause hepatic fibrosis.

5-Fluorouracil (5FU) - represents an early example of “rational” drug design in that it originated from the observation that tumor cells incorporate radiolabeled uracil more efficiently into DNA than normal cells, especially gut. 5FU is metabolized in cells to 5’FdUMP, which inhibits thymidylate synthetase (TS). In addition, misincorporation can lead to single strand breaks, and RNA can aberrantly incorporate FUMP. 5FU is metabolized by dihydropyrimidine dehydrogenase, and deficiency of this enzyme can lead to excessive toxicity from 5FU. Oral bioavailability varies unreliably, but orally administered analogues of 5FU such as capecitabine have been developed that allow at least equivalent activity to many parenteral 5FU-based approaches to refractory cancers. Intravenous administration of 5FU leads to bone marrow suppression after short infusions but to stomatitis after prolonged infusions. Less frequent toxicities include CNS dysfunction, with prominent cerebellar signs, and endothelial toxicity manifested by thrombosis, including pulmonary embolus and myocardial infarction.

Cytosine arabinoside (ara-C) - is incorporated into DNA after formation of ara-CTP, resulting in S-phase-related toxicity. Continuous infusion schedules allow maximal efficiency, with uptake maximal at 5 to 7 μM. Ara-C can be administered intrathecally. Adverse effects include nausea, diarrhea, stomatitis, chemical conjunctivitis, and cerebellar ataxia. Gemcitabine is a cytosine derivative that is similar to ara-C in that it is incorporated into DNA after anabolism to the triphosphate, rendering DNA susceptible to breakage and repair synthesis, which differs from that in ara-C in that gemcitabine-induced lesions are very inefficiently removed. In contrast to ara-C, gemcitabine appears to have useful activity in a
variety of solid tumors, with limited nonmyelosuppressive toxicities. 6-Thioguanine and 6-
mercaptopurine (6MP) are used in the treatment of acute lymphoid leukemia.

*Fludarabine phosphate* is a prodrug of F-adenine arabinoside (F-ara-A), which in turn
was designed to diminish the susceptibility of ara-A to adenosine deaminase. F-ara-A is
incorporated into DNA and can cause delayed cytotoxicity even in cells with low growth
fraction, including chronic lymphocytic leukemia and follicular B cell lymphoma. CNS
dysfunction and T cell depletion leading to opportunistic infections can occur in addition to
myelosuppression. *2-Chlorodeoxyadenosine* is a similar compound with activity in hairy cell
leukemia. *2-Deoxycoformycin* inhibits adenosine deaminase, with resulting increase in dATP
levels. This causes inhibition of ribonucleotide reductase as well as augmented susceptibility
to apoptosis, particularly in T cells. Renal failure and CNS dysfunction are notable toxicities
in addition to immunosuppression.

*Hydroxyurea* inhibits ribonucleotide reductase, resulting in S-phase block. It is orally
bioavailable and the drug of choice for the acute management of myeloproliferative states.
Asparaginase is not classically considered an antimetabolite as it causes breakdown of
extracellular asparagine required for protein synthesis in certain leukemic cells. However, it
effectively stops DNA synthesis by preventing the requisite concurrent protein synthesis, and
therefore it has a similar functional outcome as the classic antimetabolites. As asparaginase is
a foreign protein, hypersensitivity reactions are common, as are effects on organs such as
pancreas and liver that require continuing protein synthesis. This results in decreased insulin
secretion with hyperglycemia, with or without hyperamylasemia and clotting function
abnormalities.
ANTIMICROTUBULE AGENTS

Microtubules are cellular structures that form the mitotic spindle and in interphase cells are responsible for the cellular "scaffolding" along which various motile and secretory processes occur. Microtubules are composed of repeating noncovalent multimers of a heterodimer of α and β subunits of the protein tubulin. Vincristine binds to the tubulin dimer with the result that microtubules are disaggregated. This results in the block of growing cells in M-phase; however, toxic effects in G₁ and S-phase are also evident (Fig. 2.8.1.). The drug is metabolized by the liver. It is a powerful vesicant, and infiltration can be treated by local heat and infiltration of hyaluronidase. At clinically used intravenous doses, neurotoxicity in the form of glove-and-stocking neuropathy is frequent. Acute neuropathic effects include jaw pain, paralytic ileus, urinary retention, and the syndrome of inappropriate antidiuretic hormone secretion. Vinblastine is similar to vincristine, except that it tends to be more myelotoxic, with more frequent thrombocytopenia and also mucositis and stomatitis.

The taxanes include paclitaxel and docetaxel. These agents differ from the vinca alkaloids in that the taxanes stabilize microtubules against depolymerization. The "stabilized" microtubules function abnormally and are not able to undergo the normal dynamic changes of microtubule function necessary for cell cycle completion. Taxanes are among the most broadly active antineoplastic agents for use in solid tumors, with evidence of activity in ovarian cancer, breast cancer, Kaposi's sarcoma, and lung tumors. They are administered intravenously, and paclitaxel requires use of a cremophore-containing vehicle that can cause hypersensitivity reactions. Docetaxel uses a polysorbate 80 formulation, which can cause fluid retention in addition to hypersensitivity reactions, and dexamethasone premedication with or without antihistamines is frequently used. Paclitaxel causes hypersensitivity reactions,
myelosuppression, neurotoxicity and paresthesia. Cardiac rhythm disturbances were observed in phase I and II trials, most commonly asymptomatic bradycardia but also, much more rarely, varying degrees of heart block. Docetaxel causes comparable degrees of myelosuppression and neuropathy. Hypersensitivity reactions, including bronchospasm, dyspnea, and hypotension, are less frequent but occur to some degree in up to 25% of patients.

Estramustine was originally synthesized as a mustard derivative that might be useful in neoplasms that possessed estrogen receptor sites. However, no evidence of interaction with DNA was observed. Surprisingly, the drug caused metaphase arrest, and subsequent study revealed that it binds to microtubule-associated proteins, resulting in abnormal microtubule function. Estramustine binds to estramustine-binding proteins (EMBP), which are notably present in prostate tumor tissue. The drug is used as an oral formulation in patients with prostate cancer. Gastrointestinal and cardiovascular adverse effects related to the estrogen moiety occur in up to 10% of patients, including worsened heart failure and thromboembolic phenomena. Gynecomastia and nipple tenderness can also occur.

HORMONAL AGENTS

The family of steroid hormone receptor–related molecules have emerged as prominent targets for small molecules useful in cancer treatment. When bound to their cognate ligands, these receptors can alter gene transcription and, in certain tissues, induce apoptosis. The pharmacologic effect is a mirror or parody of the normal effects of the agent acting in nontransformed tissue, although the effects on tumors are mediated by indirect effects in some cases.
Glucocorticoids are generally given in “pulsed” high-dose exposure in leukemias and lymphomas, where they induce apoptosis in tumor cells. Cushing's syndrome or inadvertent adrenal suppression on withdrawal from high-dose glucocorticoids can be significant complications, along with infections common in immunosuppressed patients, in particular Pneumocystis pneumonia, which classically appears a few days after completing a course of high-dose steroids.

Tamoxifen is a partial estrogen receptor antagonist; it has a tenfold greater degree of antitumor activity in breast cancer patients whose tumors express estrogen receptors than in those who have low or no levels of expression. Side effects include a somewhat increased risk of estrogen-related cardiovascular complications, such as thromboembolic phenomena, and a small increased incidence of endometrial carcinoma, which appears after chronic use.

Progestational agents including medroxyprogesterone acetate, androgens including fluoxymesterone (Halotestin), and, paradoxically, estrogens have approximately the same degree of activity in primary hormonal treatment of breast cancers that have elevated expression of estrogen receptor protein. Estrogen is not used often owing to prominent cardiovascular and uterotrophic activity.

Prostate cancer is classically treated by diethylstilbesterol (DES) acting as an estrogen at the level of the hypothalamus to downregulate hypothalamic luteinizing hormone (LH) production, resulting in decreased elaboration of testosterone by the testicle. For this reason, orchietomy is equally as effective as moderate-dose DES, inducing responses in 80% of previously untreated patients with prostate cancer but without the prominent cardiovascular side effects of DES, including thrombosis and exacerbation of coronary artery disease. In the event that orchietomy is not accepted by the patient, testicular androgen suppression can also
be effected by luteinizing hormone–releasing hormone (LHRH) agonists such as leuprolide and goserelin. These agents cause tonic stimulation of the LHRH receptor, with the loss of its normal pulsatile activation resulting in its desensitization and decreased output of LH by the anterior pituitary.

**IMMUNE MEDIATORS**

Tumors have a variety of means of avoiding the immune system: (1) they are often only subtly different from their normal counterparts; (2) they are capable of downregulating their major histocompatibility complex antigens, effectively masking them from recognition by T cells; (3) they are inefficient at presenting antigens to the immune system; (4) they can cloak themselves in a protective shell of fibrin to minimize contact with surveillance mechanisms; and (5) they can produce a range of soluble molecules, including potential immune targets, that can distract the immune system from recognizing the tumor cell. Some of the cell products initially polarize the immune response away from cellular immunity (shifting from $T_{H1}$ to $T_{H2}$ responses; and ultimately lead to defects in T cells that prevent their activation and cytotoxic activity. Cancer treatment further suppresses host immunity. A variety of strategies are being tested to overcome these barriers.

**Cell-Mediated Immunity**: The strongest evidence that the immune system can exert clinically meaningful antitumor effects comes from allogeneic bone marrow transplantation. Adoptively transferred T cells from the donor expand in the tumor-bearing host, recognize the tumor as being foreign, and can mediate impressive antitumor effects (graft-versus-tumor
effects). Three types of experimental interventions are being developed to take advantage of the ability of T cells to kill tumor cells.

- Allogeneic T cells are being transferred to cancer-bearing hosts in the form of allogeneic bone marrow transplantation or pure lymphocyte transfusions. The donor T cells recognizes the tumor as being foreign, probably through minor histocompatibility differences. The main risk of such therapy is the development of graft-versus-host disease because of the minimal difference between the cancer and the normal host cells. This approach has been highly effective in certain hematologic cancers.

- Autologous T cells are being removed from the tumor-bearing host, manipulated in several ways in vitro, and given back to the patient. The two major classes of autologous T cell manipulation are (1) to develop tumor antigen–specific T cells and expand them to large numbers over many weeks ex vivo before administration, and (2) to activate the cells with polyclonal stimulators such as anti-CD3 and anti-CD28 after a short period ex vivo and try to expand them in the host after adoptive transfer with stimulation by IL-2.

- Tumor vaccines are aimed at boosting T cell immunity. The finding that mutant oncogenes that are expressed only intracellularly can be recognized as targets of T cell killing greatly expanded the possibilities for tumor vaccine development. However, major difficulties remain in getting the tumor-specific peptides presented in a fashion to prime the T cells. Tumors themselves are very poor at presenting their own antigens to T cells at the first antigen exposure (priming). Vaccine adjuvants such as GM-CSF appear capable of attracting antigen-presenting cells to a skin site containing a tumor antigen. Purified antigen-presenting cells can be pulsed with tumor, its membranes, or particular tumor
antigens and delivered as a vaccine. Tumor cells can be transfected with genes that attract antigen-presenting cells. Vaccines against viral cancers (papilloma virus in cervical cancer), lymphomas, and melanomas have had modest clinical success.

**Antibodies**: In general, antibodies are not very effective at killing cancer cells. Because the tumor seems to influence the host toward making antibodies rather than generating cellular immunity. Many patients can be shown to have serum antibodies directed at their tumors, but these do not appear to influence disease progression. However, the ability to grow very large quantities of high-affinity antibody directed at a tumor by the hybridoma technique has led to the application of antibodies to the treatment of cancer.

The humanized antibodies against the CD20 molecule expressed on B cell lymphomas (rituximab) and against the HER-2/neu receptor overexpressed on epithelial cancers, especially breast cancer (trastuzumab), have become reliable tools. Antibodies to CD52 and vascular endothelial growth factor are active in chronic lymphoid leukemia and colon cancer, respectively. Conjugation of antibodies to drugs and toxins, and conjugates of antibodies with isotopes, photodynamic agents, and other killing moieties may also be effective.

**Cytokines**: There are >70 separate proteins and glycoproteins with biologic effects in humans: IFN-α, -β, -γ; IL-1 through -29 (so far); the TFN family [including lymphotoxin, TFN-related apoptosis-inducing ligand (TRAIL), CD40 ligand, and others]; and the chemokine family. Only a fraction of these has been tested against cancer; only IFN-α and IL-2 are in routine clinical use.

About 20 different genes encode IFN-α, and their biologic effects are indistinguishable. Interferon induces the expression of many genes, inhibits protein synthesis,
and exerts a number of different effects on diverse cellular processes. Its antitumor effects appear to be antagonized in vitro by thymidine, suggesting that de novo thymidylate synthesis is also affected. The two recombinant forms that are commercially available are IFN-α2a and -α2b. In general, interferon antitumor effects are dose-related, and IFN is most effective at its MTD. Interferon is not curative for any tumor but can induce partial responses in follicular lymphoma, hairy cell leukemia, CML, melanoma, and Kaposi’s sarcoma. It produces fever, fatigue, a flulike syndrome, malaise, myelosuppression, and depression and can induce clinically significant autoimmune disease.

IL-2 may exert its antitumor effects indirectly through augmentation of immune function. Its biologic activity is to promote the growth and activity of T cells and natural killer (NK) cells. High doses of IL-2 can produce tumor regressions in certain patients with metastatic melanoma and renal cell cancer. IL-2 is associated with myriad clinical side effects: intravascular volume depletion, capillary leak syndrome, adult respiratory distress syndrome, hypotension, fever, chills, skin rash, and impaired renal and liver function.

GENE THERAPIES

No gene therapy has been approved for routine clinical use. Several strategies are under evaluation, including the use of viruses that cannot replicate to express genes that can allow the action of drugs or directly inhibit cancer cell growth; viruses that can actually replicate but only in the context of the tumor cell; or viruses that can express antigens in the context of the tumor and therefore provoke a host-mediated immune response. Key issues in the success of these approaches will be in defining safe viral vector systems that escape host immune function and effectively target the tumor or tumor cell milieu. Other gene therapy
strategies would utilize therapeutic oligonucleotides to target the expression of genes important in the maintenance of tumor cell viability.

The basic strategies for gene therapy that have been explored include immunogene therapy such as cytokine gene transfer, selective prodrug activation, so-called suicide genes, transfer of a tumor suppressor gene, and inhibition of activated oncogenes by antisense mechanisms (Gunji Y et al., 2000).
Fig. 2.8.1. Cancer preventing agents acting through the cell cycle
### Table 2.8.1. Commonly used antineoplastic agents

<table>
<thead>
<tr>
<th>Category</th>
<th>Agents</th>
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<tbody>
<tr>
<td><strong>Alkylating agents</strong></td>
<td>Cyclophosphamide, Ifosphamide, Melphalan, Carboquone, Nimustine, Ranimustine, Cisplatin, Carboplatin</td>
</tr>
<tr>
<td><strong>Antitumor antibiotics &amp; - topoisomerase inhibitor</strong></td>
<td>Mitomycin C, Bleomycin, Peplomycin, Chlomomycin A3, Neocarzinostatin, Actinomycin D, Adriamycin, Daunomycin, Pirarubicine, Epirubicin, Aclarubicin, Mitoxantrone, Etoposide, Camptothecin</td>
</tr>
<tr>
<td><strong>Antimetabolites</strong></td>
<td>Methotrexate, 5-Fluorouracil, Thioinosine, Cytosine arabinoside, Vincristine, Vinblastine, Videsine, Paclitaxel</td>
</tr>
<tr>
<td><strong>Antimicrtubules agents</strong></td>
<td>Glucocorticoids, Estrogen, Progestational agents, Diethylstilbesterol</td>
</tr>
<tr>
<td><strong>Hormonal agents</strong></td>
<td>T-Cells, GM-CSF, Antibodies, Cytokines (IFN-α, β, γ, IL-1, IL-2 etc.), Cytokine gene, suicide gene, Oncogene antisense</td>
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