A BRIEF RESUME ON THE REGENERATIVE PHENOMENON

Regeneration stands out as a phenomenon known to the scientific world for centuries back. The term regeneration, represents the capacity of animals to generate lost parts; a capacity which has been widely observed in a majority of the lower groups of animals. The marvel of regeneration has attracted the attention of naturalists right from the time of Aristotle. Spallanzani (1768) was one of the earliest experimental zoologists~~,~~ who observed that the tails and limbs of tadpoles of frogs and toads and larval forms of salamanders could regenerate. After this early interest in the phenomenon, there was an unexplainable lag for some time. Renewed interest in regeneration has been marked by a series of monographs. Of the many monographs, those of Morgan (1901), Prizbram (1909), Loeb (1924), Korschelt (1927), Millot (1931) and Abeloos (1932) are the most representative. Another interesting treatise is that of Child (1941) who has reviewed the topic though from a more restricted aspect. Since Morgan, an illustrative general treatment on regeneration which is available, is that of Needham (1952).

Literally, regeneration can be considered to be a rebirth or renewal of lost parts or tissues. All animals have some capacity to restore tissues or body parts which have been lost through normal physiological processes or destroyed accidently. This capacity finds its highest expression in the invertebrates and lower vertebrates, and This fact has led to the suggestion, that, regeneration may be a manifestation of phylogenetic primitiveness. However plausible this proposal appears to be, it is rather difficult to generalize, as animals of very low order incapable of regeneration have been noted to exist (Morgan, 1901). A proposal that the capacity to regenerate is governed largely by its selective value to the animal in reproduction or survival or both, finds favour with Needham (1952), and Vorontsova and Liosner (1960). Regeneration and reproduction appear to be synonymous and both regeneration and asexual reproduction seem to be attuned to the continuity of the species in invertebrates but not in vertebrates. The degree to which the life of the individual organism as opposed to the species is immortal, is reflected in the balance between asexual reproduction and regeneration on the one hand, and sexual reproduction on the other. In many cases, the remarkable regenerative phenomena in the lower animals are closely related to the devices for asexual reproduction that have evolved in the various groups. Regeneration contributes to reproduction only insofar as it helps to maintain the individual; it never produces a new and separate animal. By the observed evolution of a number of asexual modes of reproduction in invertebrates as well as even in some of our close chordate relatives, regenerative capacities are apparently excellent in animals which are capable of reproducing themselves asexually from somatic cells. A complete amoeba may grow from the smallest of a fragment of the original animal, provided, the nucleus is included in the fragment. Only the tiniest part of a hydra or of a planaria is necessary to regenerate a completely new animal. Some of the primitive oligochaete worms can regenerate from a single segment. This potential for extensive regeneration from very small fragments is common in other lower phyla. Sometimes under unfavourable conditions certain sponges are reduced to an amorphous mass which then acquires its original structure when conditions improve. Many protozoans reproduce asexually by binary fission;

hydroids and planarians may also reproduce by fission and regenerate the missing parts. Star fish and brittle stars can regenerate arms from the central disc and can reform a whole animal from an arm if part of the central disc is attached. Sea urchins can repair damage to the skeleton and tube feet. The sea cucumbers respond to certain external stimuli by eviscerating the alimentary canal and other internal organs. The remaining shell of skin and muscle is capable of regenerating the eviscerated (autotomized) organs. Certain sea cucumbers and star fishes, fragment at intervals to produce new individuals by asexual reproduction (Berril, 1961; Hamburger, 1965). Adult echinoderms and annelids live longer than the larvae and in general, have better capacities for regeneration. Needham (1952) suggested that regeneration probably is evoked too rarely in ephemeral forms to have survival value and therefore does not exist. However, Elizabeth.D.Hay, Holt, Rinehart and Winston in ~~their~~ her book on regeneration ha~~ve~~s considered the above view overtly teleological and ~~they~~ opines that regenerative powers which have no survival value are not uncommon among animals, and it would not be surprising to find greater regenerative capacities among the echinoderm larvae, were they studied more thoroughly. In the more advanced phyla, missing appendages are often regenerated and the outer coverings of cuticle, chitin or cornified epidermis are periodically replaced. In man, the germinal layers of the epidermis and the mucous membranes of the digestive tract are continually proliferating to replace cells which are always being rubbed off. Holocrine glands such as the sebacious glands secrete by accumulating a load of secretory materials in the cytoplasm and then disintegrating.

These varied examples are all expressions of the capacities of animal tissues to restore lost parts. Reconstitution was termed as an alternative to regeneration (Child, 1941). This term was considered to be best reserved for regeneration by reaggregation of mere cell masses in sponges and hydroid coelenterates (Beadle and Booth, 1938). Restitution also sometimes used as a general term (Child, 1941) served a more useful purpose in the restricted sense of Korschelt (1927) and Ries (1937) to mean regeneration from dedifferentiated reduction bodies by which some tunicates and polyzoa tide over periods of adverse conditions. In this case too, the development was found to be from an aggregate of either nondifferentiated or of dedifferentiated cells as in typical regeneration. The direct redevelopment in situ of the portion lost, as in the limbs of crustacea and amphibia has been refered to as 'epimorphosis' in contrast to 'morphallaxis' (Morgan, 1901) where the remaining portion of the body is actually remodelled to restore the whole form as is most common in the lower invertebrates, after extensive loss. This distinction may not be held rigid, as the invertebrates are found to use both epimorphic and morphallactic processes. Another type of regeneration observed in the case of liver and other internal organs where, size only and not the specific form is important, is denoted by the term compensatory hypertrophy which is in a way related to morphallaxis in the sense, that the initial form is not restored precisely. Plants are also seen to regenerate in a compensatory way from dormant buds. Physiological regeneration is used to represent the replacement of hair,nails, skin, teeth etc. lost by wear and tear supposed to be a chronic low grade regeneration and extending logically down to the molecular level to the metabolic turnover of the biochemist (Hevesy, 1948). Regeneration capacity could can? be noticed in embryos even. Isolated blastomeres or groups of blastomeres are able to regulate by a process akin to morphallaxis to form complete small scale embryos. Regulation is possible only in the unfertilized egg in mosaic types of eggs (Dalcq, 1938) but in the amphibia, portions of late blastula and even gastrula stages are able to regulate eventually by postgeneration which is again a morphallactic process.

According to Vorontsova and Liosner (1960) the varied forms of regeneration mentioned above are divisible into three general categories: (a) Physiological regeneration, which is a part of the normal and regular functioning of some organs such as sebacious glands, mucous membranes and the outer layers of skin; (b) reparative regeneration, which is provoked by wounding or traumatic destruction, and (c) asexual reproduction, which is a natural process involving the isolation of a part of the animal and its transformation into a daughter organism. It is to be noted that the functional significance of the three processes is quite different. However, since all the three are in reality, "physiological", Leblond and Walker (1956) have opined, that 'cell renewal' is probably a more appropriate term than physiological regeneration and that the word regeneration is usually reserved for reparative and post traumatic processes.

Needham (1952) has ascribed six morphological sequential events during epimorphic regeneration identified closely with the majority of vertebrate regeneration. The first of the events is wound closure, which, in lower forms, involves only a contraction of neighbouring tissues and a stretching of the surrounding cells over the wound; in higher forms, the vascular fluids clot, and thus, form a basis for the latter processes of repair. Wound closure is followed by demolition and defence, which, in the higher vertebrates, begins with a triple response caused by the release of toxic substances from injured tissues and expressed as increased dilation of blood vessels, collection of fluid and eventually the removal of the damaged tissues through autolysis and phagocytosis. The vascular reactions are observed only in the molluscs, arthropods and vertebrates; lower forms probably depend entirely on phagocytosis. Several different processes are associated with the healing which follows demolition and defence. The first of these is often a dedifferentiation of tissues to provide indifferent cells for subsequent regenerative processes. Although dedifferentiation has been described in lower forms, and may occur to some degree in all cases, it is most charecteristic of the vertebrates and has been extensively studied in regenerating amphibian appendages. In lower forms, it is believed that nondifferentiated or pluripotent cells (neoblasts) are always present and that these migrate into the area of injury from nearby tissues. Needham suggests that the development of the highly efficient vertebrate circulatory system reduced the importance of maintaining a stock of undifferentiated migrant cells. The process of dedifferentiation brings to an end the regressive phase of regeneration; this is followed by the progressive phases: formation of the blastema or regeneration bud, its growth and subsequent differentiation into the regenerated structure. The blastema or regeneration bud, is composed of a mass of dedifferentiated or immigrant cells; in the amphibians, the blastema is fully established prior to a sudden initiation of mitotic activity, which heralds the new phase. The intense cellular proliferation which follows, produces a mass of relatively small cells which subsequently increase in size and become somewhat separated as intercellular spaces appear during differentiation.

The mitotic rate declines as the regenerating structure continues to differentiate and become functional.

Regardless of the likely possibility, that the vertebrates are derived from the same primitive stock as did the echinoderms and urochordates, it is quite clear, that, they are a very different kind of creature in the present state of evolution. Asexual reproduction has been completely abandoned by the animals comprising the subphylum vertebrata and terrestrial habitat has been assumed by many. It is not known whether or not primitive vertebrates possessed great regenerative powers. The immediate progeny of the ancestral vertebrates are, of course, animals which have undergone further evolution. The larvae of Petromyzontes the so called ammocoetes, are said to regenerate the tail (Niazi, 1964) and members of the superclass pisces can regenerate the bony fins, optic nerve and taste barbels (Nicholas, 1955; Goss, 1956; Haas, 1962). The fins cannot be cut too close to the body or they fail to regenerate. The anal fin of the male platypoecilus loses the capacity to regenerate in the adult, an irreversible loss which can be induced in the female as well, by early treatment with androgens (Grobstein, 1947). Generally it is realized, that the ability of adult fish to regrow a tail is negative. It is not clear, whether dipnoi and crossopterygii which are closely related to the primitive amphibian ancestor had any greater regenerative capacities than most modern fish. The salamanders seem to have the most remarkable regenerative abilities of all vertebrates. Eventhough the anurans are more closely aligned with the main stem of evolution that gave rise to reptiles than are the urodeles, the regeneration capacities seem quite limited in them with the possible exception of a few species such as <u>Xenopus</u> <u>laevis</u>. The distribution of regenerative capacities among the urodeles support the speculation that the side branch urodeles evolved new regenerative abilities as to make any claim that they retained something the higher vertebrates lost.

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Mechanisms for regenerating the lens are quite different among the salamanders. One family has the capacity to regenerate the whole eye and optic nerve from the pigmented epithelium of the retina. Some adult land salamanders are said to regenerate the appendages well (plethodon), others regenerate poorly (Ambystoma), and there is variability in regenerative capacity among the aquatic forms. Interestingly enough, a genetic mutation in the axolotl, which prevents this neotenic aquatic salamander from regenerating a limb, has been observed (Humphrey, 1966). It is tempting to thnik that a decrease in relative numbers of nerves accompanied by an increased thresh~~h~~old to the trophic action of the nerve is one cause for failure of limb regeneration among the higher vertebrates (Singer, 1952; Zika and Singer, 1965). The frog larva is capable of regenerating the tail and hind limbs. The capacity to regenerate the hind limb is lost at metamorphosis with the proximal parts losing the ability before the distal ones (Schotte and Harland, 1943; Van Stone, 1964). The reptiles derived directly from stem amphibians along the main line of evolution, the most important advance being the acquisition of the terrestrial egg. They have limited regenerative powers as compared with the urodele and frog tadpole, but neverthless have evolved some interesting mechanisms which involve regrowth of body parts. The lizard discards its tail by a process of autotomy not unlike that which has evolved independently among the crustaceans. The regenerated tail, however, is far from perfect. Nerve and muscle are atypical and the cartilaginous axial skeleton does not segment or ossify (Woodland, 1920; Barber, 1944; Kamrin and Singer, 1955; Simpson, 1965). The embryonic lizard does not have the ability to regenerate the tail (Moffat and Bellairs, 1964). In birds and mammals, physiological regeneration is well exemplified. Epidermal appendages, such as feathers, nails, and hairs either grow continually, or, are replaced by periodic molts as was also probably true of the scaly epidermis of our immediate terrestrial ancestors. Regeneration of functional axons in the central nervous system is better developed in birds than in mammals and perhaps is better in embryos than in adults (Windle, 1955). Mammals have a remarkable ability for liver regeneration. In fact, this particular capacity seems better developed in higher vertebrates than in salamanders.

Regeneration being a fascinating physiological phenomenon, the modus operandi and its biological significance, merit the utmost interest and curiosity. As mentioned above, many workers have noted that, the capacity to regenerate is primitive in both the phylogenetic and the ontogenetic sense. It is also noted that, representatives of the lower animal phyla regenerate completely after the loss of large portions of their bodies and younger individuals usually respond much more readily than the older mature ones.

If it is true that the ability to regenerate lost parts is primitive, then it seems curious, that such an important capacity should have been partially lost during the evolution of the higher forms. Schotte (1939) suggested that regeneration is a recapitulation of development process progressively restricted by age and increasing differentiative specialization. Needham (1952) suggested that the advantages of extensive regeneration are less significant in the larger, more complex and more active animals. Whereas a substantial portion of an annelid worm may be sacrificed to a predator while the remainder escapes to regenerate the missing parts, a vertebrate usually escapes with relatively minor injuries or is captured and killed. The more active life of the vertebrates and some of the higher invertebrates reduces their chances of major injury; at the same time, relatively minor damage such as wounds and broken bones are readily repaired. It has also been suggested that the extensive dedifferentiation required for blastema formation in a large vertebrate animal might impose both metabolic and physical disadvantages which would outweigh the advantages. Needham argues in this way that the ability to regenerate lost parts is both pristine and adaptive in the evolutionary sense. This concept has not gone unchallenged (Vorontsova and Liosner, 1960).

In the course of evolution, the capacity to make new cells, heal wounds, renew tissues, indulge in compensatory hypertrophy and reproduce have not declined in the various animal groups. What has declined is the capacity to regenerate substantial parts of the body or appendages thereof. It has been advocated that the fact that higher animals have often not retained the regenerative capacities of their ancestors are indicative that the strategy of evolution has been to select against regeneration, not for it. This has been explained by the suggestion that other advantages of greater importance must have been gained in the bargain in the form of higher specialization and complexity. Why regeneration can take place in some parts of a given organism but not in others ? This has been can be explained on answered by the the basis existance of regeneration territories. With increasing complexity, it is told, that the regenerative ability has become restricted to subfractions of the body. Ultimately, such information as is necessary for regeneration comes to lie within its own structure with the result that a limb could give rise to only a limb and a tail only to a tail. It has also been supposed that carried to the extreme, continued restriction of potentialities might reduce the boundaries of regeneration territory to the vanishing point. It is presumed that this is what might have happened in the metamorphosing tadpoles as it loses its capacity to regenerate legs. Goss (1968) suggests; may be some thing comparable occured during the evolution of reptiles, birds and mammals to account for the extinction of their potential to regenerate limbs. The validity of the proposal that the decline in regeneration capacity during evolution, is compensated by other gains is arguable. Why should the animals during the course of evolution resort to a suppression of regenerative capacities along with the attainment of specialization ? Why can't the two co-exist at the same time when both are advantageous to the animals ? Is it a loss in favour of increasing complexity or is it that the process of specialization created certain factors which might have brought about the inhibition of regeneration capacities ? Even if it be considered, that the loss in regeneration capacity is in favour of other more advantageous processes, the observed capacity of some vertebrates in spite of the evolution of specialization, to regenerate, is unexplainable and merits serious consideration. This capacity of only a few or even of only some parts of an animal to regenerate is said to be due to a restriction of regenerative capacities as mentioned above. The loss of regenerative power in the metamorphosing tadpoles, it is suggested is due to the restriction being carried to the extreme i.e., to the vanishing point. Had it been true, we should have observed a similar fate as in the case of tadpole, in all the regenerating vertebrates. The very fact that other vertebrates do not exhibit a similar fate is in itself contradictory to the above proposal of restriction. Moreover, the observation of a reversed prowess as in the case of reptiles wherein the adult ones retain the regenerative capacities totally unrepresented and unexpressed in the embryos is rather p~~y~~eplexing. Ontogeny recapitulates phylogeny: this clearly states, that all the changes that occur during phylogeny remain more or less represented during the ontogeny or embryogenesis of all higher vertebrates. If as is widely believed that regenerative power is lost during the evolutionary progress, why is it then not recapitulated and ~~be~~ represented in the embryos of all vertebrates ? Another thought thinking which again needs some serious consideration is the one in which regeneration is related to the function to be performed by the structure being replaced. It is said that there is an utilitarian imperative which assures that developmental efforts shall not be expended in the production of useless structures and that in this sense regeneration is to be regarded primarily as a device by which functional competence is recovered with morphological restitution representing only a means to this end. Does this entail that parts capable of undergoing regeneration in the vertebrates are functionally important ? However interesting this possibility might appear, its validity appears to be shallow when one reflects ~~reflected~~ on to the ability of only some of the urodeles and lizards to regenerate their limbs and tails. It becomes imperative in this wake to question whether the limbs and tails have functional significance only in these few Urodeles and lizards ? Does it imply that other amphibians and reptiles have lost the functional imperative for their limbs and tails ? These points of observations then definitely argue in favour of some factor or factors playing an effective role either in the suppression or initiation of the process of regeneration independantly in different vertebrates and/ or even in different parts and organs of the same animal.

These questions and others are as yet unanswered and hence the phenomenon of regeneration phenomenon continues to remain shrouded in mystery, and repeated attempts by scientists all over to unmask this mystery or mysteries have proved unsuccessful. Thus this phenomenon appears to be one of the most challenging encounters ever to be faced by the modern biologists. In the modern era, the phenomenon can be said to have attained more focal attention due to its implications in the neoplasmic development and growth of carcinoma. In this connection, it is again a ~~big~~ largely conjecture as to whether the isolation of factors responsible for the orderly initiation and termination of regenerative process could lead to an understanding of what? and or to the development of curative methods for the much dreaded malady, carcinoma.

Before taking up the general concepts of why, where, when and how, it is imperative to study and understand the process of regeneration itself and the underlying factors in individual cases. Explanations for the causal mechanisms of the various morphological events which characterize regeneration have been persistently sought for more than half a century. Experimental embryologists have been particularly active in the search for factors which induce regeneration, the determination of events within the differentiating blastema, gradients in the regenerative capacity and metabolism in organisms or parts of organisms and the inductive action of different tissue transplants (Needham, 1952; Raven, 1959). Medical physiologists have investigated the post traumatic blood clotting, vascular responses and inflammatory reactions which prece~~e~~de repair. Comparative physiologists have recently demonstrated the prominent ~~a~~ commanding position of the neurosecretory system in the regulation of regeneration among some of the lower forms. In many instances, regeneration is dependant upon physiological factors not of local origin which may be neural or hormonal in nature, and in some cases may even be triggered by environmental conditions. In protozoans, the nucleus is indispensable for regeneration; eye regeneration in flat worms is stimulated by the brain; arthropods fail to grow new appendages unless they can molt; various extremities in the vertebrates require an adequate nerve supply; the newt lens cannot be replaced in the absence of the neural retina; antlers grow in response to changes in daylength. It is thus seen that again and again the initiation of regeneration is linked to physiological conditions which vary from animal to animal.

also in vertebrates

Mabuya carinata