

THE INDIAN HORSESHOE CRAB — A LIVING FOSSIL

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Ocean is a treasure of many living creatures. Surprisingly, quite a few marine organisms, suspected to be extinct, still flourish as living animals. Horseshoe crab, a chelicerate arthropod belonging to the class merostomata is such an amazing creature, considered to be the oldest 'living fossil'. The archeal animal body has not shown any significant phenotypic change (Plate 1) even after a span of millions of years.

The horseshoe crab has descended from mud dwelling primitive arthropods called as trilobite which lived in the precambrian seas, nearly 600 million years ago. After the next 150 million years or so, the horseshoe crab evolved into its present shape, remaining unchanged all these 350 million years. Considering how so many animals including dinosaurs and many other late comers to the mesozoic era have become completely extinct, it is a wonder how the humble horseshoe crab survived for such a long period. The animal can tide over all kinds of situations arising in its estuarine and coastal shallow habitats. It can tolerate a wide range of salinity, temperature, dessication and submergence.

There are only four species of the horseshoe crab reported in the world. The *Limulus polyphemus* occurs along the Atlantic coast of North America from Maine to Yucatan; the *Tachypleus tridentatus* on the Northern shores of Japan up to South Vietnam and along the Western Islands of the Philippines; the *Tachypleus gigas* on the shores of the Bay of Bengal, particularly along the coast of Orissa (India) to Indo-China, North Vietnam, Borneo and Celebes; and the *Carcinoscorpius rotundicauda* on northern shores of the Bay of Bengal to the southern coast of Philippines.

Tachypleus gigas (Muller) and *Carcinoscorpius rotundicauda* (Latreille) are found within Indian limits. The distribution of *T. gigas* and *C. rotundicauda* is restricted to north-east coast of Orissa and

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Plate 1



Sunderbans area of the West Bengal, respectively. The occurrence of both the species at the same place has not been observed. Mature pairs of both the species, in amplexus, migrate towards the shores for breeding purpose, throughout the year. *C. rotundicauda* prefers nesting in mangrove swamps whereas, *T. gigas* breeds on a clean sandy beach.

Observations on the migratory pattern of *T. gigas* for breeding purposes in relation to seasonal and lunar phases along the Indian coast, have been studied in detail. Mature pairs in mating stage are found in most of the months reflecting protracted breeding activity throughout the year. A definite correlation exists in the occurrence of the crab with tidal amplitude and lunar phase. The higher the tidal amplitude the greater is the occurrence of the horseshoe crab. Relatively more numbers of the crabs migrate during full moon tides than the new moon tides. Breeding activity is not found during neap tides. The female crab deposits eggs in comparatively fine and clean sand, in a nest, at the high water mark of spring tides. This protects the eggs from different predators.

Regular shifting of the nesting ground from one place to another

occurs for which sediment characteristics play a significant role. Selection of the nesting ground by *T. gigas* is directly related to the grain size of the sediment. *T. gigas* prefers sediments having mean grain size between 2.18 to 2.46 phi for its nesting. Any change in this range of mean grain size at a particular ground does not seem to favour nesting and the crab shifts its spawning ground to the place where it gets mean grain size between 2.18 to 2.46 phi. The shifting of nesting ground is also indirectly related to the phases of erosion and accretion. The resultant accumulation of particular range of grains at one place provides a suitable environment to the horseshoe crab for laying its eggs for the safer incubation.

The horseshoe crab is a hardy animal and can thrive well in estuarine dilution or saturation of seawater by maintaining osmotic steady state. Salinity changes significantly influence the weight of the horseshoe crab and volume of blood (haemolymph). The body weight displays distinct seasonal fluctuations where maximum weight coincides with low salinity of the environment. At a high salinity, the body weight of the horseshoe crab decreases considerably. Similarly, seasonal variations in the volume of haemolymph also increases at low salinity. The differences in the body weight and volume of haemolymph are more pronounced in females than the males.

T. gigas is basically a benthic feeder and subsists mainly on molluscs, decayed organic matters and polychaetes, in order of prevalence. A strong and positive preference exists for molluscs over other food organisms. The intensity of feeding is maximum during north-east monsoon (November to January) in females and during the intermonsoon season (February to May) in males. The feeding activity reduces during south-west monsoon (June to September).

A mature female carries about 5000 to 6000 eggs under its prosoma but releases only 200 to 300 eggs in a nest in one spawning act. The brooders migrate back to the sea with ebbing tides to their natural habitat after releasing their gametes in sandy nests. After 40 to 42 days, the trilobite larvae are ready to hatch in the nests, depending upon the temperature of the environment. Trilobite larvae swim upside down and migrate to the deeper sea. The larvae go through 15 or more transformations for the next 9-12 years to become an adult. During the first two to four years of the horseshoe crab's life, the shell is moulted

several times.

The horseshoe crab has been useful in different ways to humans. The compound eyes of the horseshoe crab can polarise light and the crystal lining cones concentrate it tenfold — an adaptation to suit their continuous living in translucent and dark muddy waters. The vision is also sensitive to infrared and ultraviolet lights. This helps horseshoe crab to find their way on cloudy days when the Sun can not be seen and a small patch of clean sky is enough for the animal to locate the Sun's position. The principle has been very useful in developing a compass for navigation in polar regions like Antarctica where magnetic compass is found not reliable. The celestial navigation is also difficult in these regions as the Sun and stars are not visible in the long polar twilight. Besides this the design of eye structure has also been successfully adopted in designing solar energy collectors.

The optic nerve cells are cross connected and when one nerve is stimulated, its partner nerve is inhibited. This results in an increase in contrast of the images looked by the horseshoe crab. Prof. Hartline in 1964 shared a Nobel prize for his notable work which shows the functioning of optic nerves of the horseshoe crab. A video system based on the principle to provide sharper TV images was developed by General Electric Company. The principle has also helped in developing an improved radar system.

The blood (haemolymph) of the horseshoe crab is white but it turns blue when it comes in contact with air. The blue colour is attributed to hemocyanin — a copper based molecule that carries oxygen throughout the crab's circulatory system.

In recent years, medical science has found this valuable creature as a potential source of a bioactive substance — a diagnostic reagent - the *Limulus* Amoebocyte Lysate (LAL) from its blue blood (Figure 1). The reagent is highly sensitive and useful for the rapid and accurate assay of gram-negative bacteria even if they are present in a very minute quantity upto the level of 10^{-10} g. Hence the LAL has been proved a valuable diagnostic reagent in the detection of endotoxins in several pharmaceutical products especially injectables. The diagnosis of gram-negative bacteria associated with the cirrhosis, meningitis, endophthalmitis, dental problems, urinary infections, gonorrhoea, typhoid fever and

Figure 1

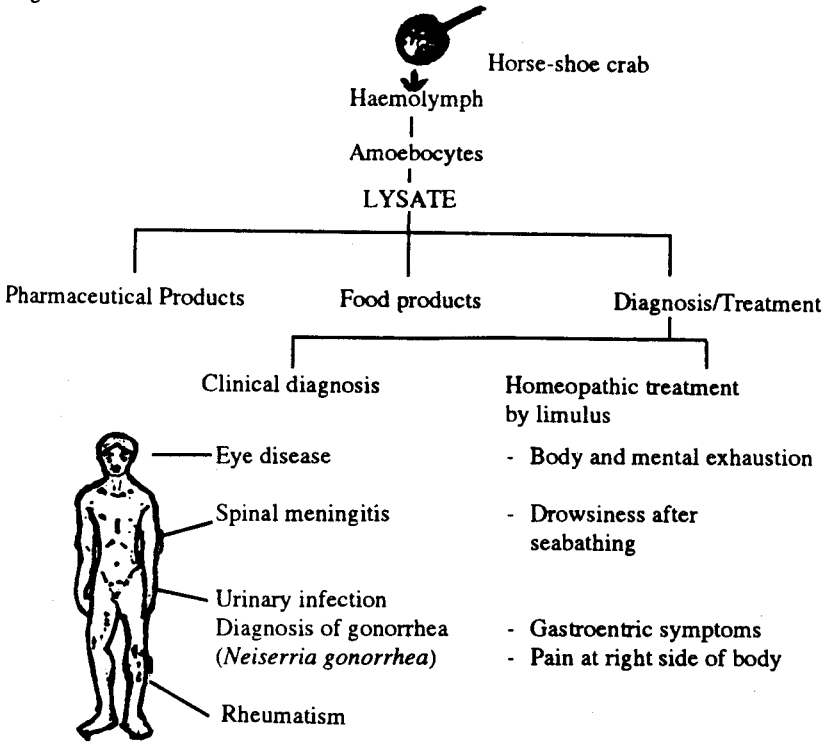
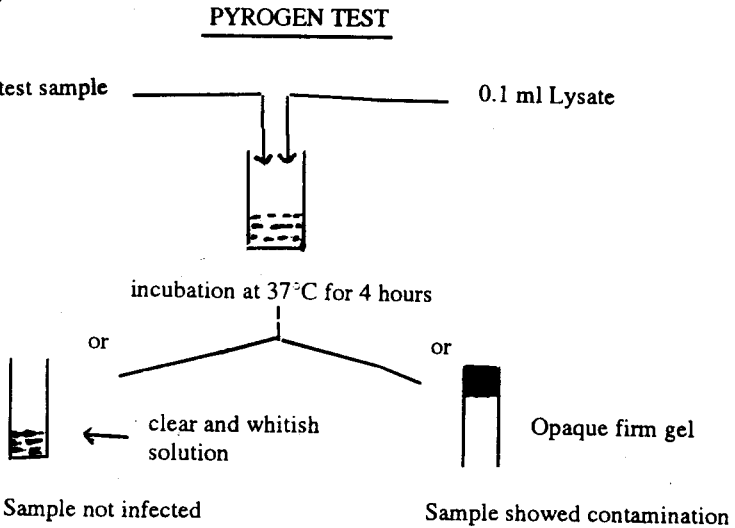


Figure 2



septicemia, is very quick and effective with LAL as compared to the normal traditional Rabbit Test. In addition to this, the LAL is also useful in lipopolysaccharides assay and water quality research. The test takes only 2-3 hours and can be performed *in vitro* (Figure 2).

The amoebocytes in the haemolymph of the horseshoe crab contain a water soluble clotable enzyme which forms a firm opaque gel in no time when it exposes with minute amount of endotoxins or LPS of gram-negative bacteria. Nowadays 98% of the pyrogen tests are performed with LAL in USA. The lysate has also been extensively used for the detection of bacterial contamination of food. The clotting is also useful as an alarm signal while testing substances intended for human use, internally.

The indiscriminate exploitation of the horseshoe crab for medicinal and other purposes, has threatened it with extinction all over the world. In USA, about 50,000 brooder crabs are sacrificed every year for the preparation of LAL. There are about eight pharmaceutical companies holding the approved licences to produce LAL on commercial scale. This resulted in considerable depletion of the population of the horseshoe crab in USA. Japan was the first country to realise the declining trend of *T. tridentatus* population and subsequently took measures of conservation by declaring horseshoe crab as a national monument. The other possible reason for the diminishing population of the horseshoe crab in several places is the degradation and destruction of the habitat specially the breeding ground of the animal.

Considering the economic importance of the horseshoe crab and its declining population due to over exploitation and indiscriminate killing for the preparation of LAL, a technique has been developed successfully by the scientists of the National Institute of Oceanography, Goa, for non-destructive removal of haemolymph from the horseshoe crab. A single crab can be bled several times in a year without disturbing its normal physiology. The technique of the preparation of LAL has also been standardised and patented in the name of Tachypleus Amoetocyte Lysate (TAL). However, the commercial production of the reagent has not been initiated as the Indian Pharmacopoeia has not officially made LAL as an official test or a substitute for Rabbit Vaccine Test. But the time is not very far when the indigenous production of lysate will start which, in turn, will save a huge amount of foreign exchange.