IX. DISCUSSION

The present studies have brought forth some interesting results regarding the nature and origin of the air-breathing habit among fishes. The origin of the air-breathing habit among the fishes has been an important event in the evolution of terrestrial life and several workers have attempted to explain this phase of evolution from different aspects and the results obtained in the present studies make it necessary to reexamine the whole problem from a completely objective point of view. It was therefore, thought proper to discuss the problem under three sections. The first deals with a discussion on the light thrown by the results obtained in the present investigations with regard to the nature of the internal environmental conditions and that of the aquatic respiratory organs (viz. the gills) and the associated structures (viz. the muscles) in both water-breathing as well as air-breathing fishes. In the second, the discussion is centred on the questions as to how far the study relating to the oxygen content of the different habitats and a survey of their teleostean fauna supports the well known contention that the deficiency of oxygen in the waters in which the ancestors of the air-breathing fishes lived has been a prime factor which has induced the development of the air-breathing habit in these forms. The third and final part has been devoted to exploring the possibility of suggesting a comprehensive and tangible view on the problem of the origin of the air-breathing habit in fishes based on the various investigations made so far including the present one.

1. THE ADAPTATIONAL FEATURES OF THE AIR-BREATHING TELEOSTS

A. THE OXYGEN-LOADING TENSION OF THE BLOOD

It is seen as a rule that the haemoglobin concentration of the blood of the air-breathing teleosts based on the estimation of iron, is higher than that of the normal non-migratory water-breathers. In water-breathing teleosts investigated here, the iron content per 100 cc. of the blood varied from a minimum of 16.13 mg. in *Labeo rohita*, to a maximum of 27.52 mg. in *Catla catla*. In air-breathers, the corresponding figure varied from a minimum of 29.41 mg. in *Heteropneusteus fossilis* to a maximum of 40.0 mg. in *Ophiocephalus striatus*. In other words it means that the oxygen-loading capacity of the blood of the air-breathers is markedly higher.

The investigations of Hall & Gray (1929), Redfield (1933), Willmer (1934) and Black (1940) show that besides air-breathing fishes, the oxygen-loading tension is higher in certain other fishes also. These fishes include the well known migratory marine fishes Bonito and Mackrel, Myleus setiger, an active fish of the open river, a swampy acid-water inhabiting cat-fish, Ameiurus and a sucker fish, Catostomus. A higher oxygen-carrying capacity of the blood of Bonito and Mackrel is perhaps an index of their physiological activity as stated by Hall & Gray. The same seems to be the case with Myleus setiger. As regards Ameiurus, it seems to be a safeguard against the difficulties encountered by the fish during respiration, on account of the peculiar nature of its habitat known to contain acidic water as well as a higher amount of carbon dioxide in its waters. Such conditions are known to affect the utilization of oxygen in a number of fishes (Fry-1957). Regarding Catostomus which is a sucker fish, a higher loading-tension of oxygen is also an advantage since its respiratory movements are limited to a certain extent by its sucking habits.

The blood of a non-migratory water-breathing fish, Cyprinus carpio (Redfield-1933 & Black-1940) has also been mentioned of having a higher oxygen-tension. Not much is known about this fish except that it is sluggish in habit. It is not possible therefore to state the exact cause of the presence of the higher oxygen-carrying capacity of this fish. It is likely that this feature is an adaptation to the peculiar conditions of the environment.

The air-breathing fishes studied during the present investigations include Ophiocephalus striatus and Heteropneusteus fossilis which normally lead an inactive life. The water-breathing fishes such as Cirrhina mrigala, Labeo rohita and Catla catla inhabiting similar waters are comparatively more active. So, a higher concentration of iron in the blood of the air-breathers cannot be explained on the basis of their activity since they are inactive nor could it be explained on their adaptations to the peculiar conditions of their environments because normal water-breathers also inhabit the same environment. An explanation other than one based on activity or the nature of the environment, must therefore be found for the higher percentage of iron present in the blood of the air-breathers. The only reasonable explanation seems to be that this feature is in some way or the other intimately related with the air-breathing habit.

B. LINING OF THE RESPIRATORY EPITHELIUM BY A MUCOUS AQUEOUS LAYER

As stated earlier, respiration is essentially aquatic in the sense that any respiratory surface is always covered by a stationary aqueous layer. It is also known that in land vertebrates this layer is in the form of a thick mucous aqueous layer to prevent desiccation.

A fish always lives in water and ordinarily there is no possibility of the aqueous lining getting dried up. The air-breathing fishes however are met with at situations adjoining lands and a mucous covering is an advantage because of the possibility of their respiratory epithelium being exposed to air. Moreover, if a part of a pond dries up, there is a possibility that the fishes inhabiting it, are forced to struggle their way through, to reach other parts. Fishes such as Ophiocephalus, Mastecembalus and certain others thus often migrate from the drying portion of the ponds and reach safe situations during droughts. The presence of a mucous aqueous layer which is found to be present in the air-breathing forms protects the gills from drying up quickly. It is noteworthy that the gill-filaments of a water-breathing fish Wallago attu are also lined by a mucous covering. This fish is known to survive out of water for quite a long period.

The presence of a mucous aqueous layer which is found to be present in the air-breathing forms helps them in preventing asphyxiation at least for some time when the fish is forced out of water, though the advantage may be limited by the coherence of the gill-filaments as stated by Schöotle (1931) and Gray (1954). The development of this layer seems to be one of the earlier achievements of the air-breathing fishes living in aquatic habitats adjoining land and can be looked upon as a preadaptation enabling the animal to switch over to land life during the course of evolution.

C. THE EXTENT OF GILL-SURFACE

From the study made, it has been found that the extent of gill-surface in water-breathers per unit volume, varies from 132.7 sq.mm. to 165.9 sq.mm. The corresponding figures for occasional air-breathers vary from 148.68 sq.mm. to 163.9 sq.mm. For habitual air-breathers the figures vary from 56.8 sq.mm. to 98.1 sq.mm. The extent of such a wide difference has not been pointed out before.

Carter (1931) has estimated the area of the gill--surface in sq.cm. per gm. body weight in some fishes. In 6 fishes examined by him the area varied from 0.5 sq.cm. to 2.9 sq.cm. Possibly most of these fishes are water-breathers. However, he has neither mentioned the names of the fishes nor has he given the figures for individual fishes. The average figure for these fishes as given by him is 1.7 sq.cm. For *Hoplosternum littorale*, an air-breathing fish for which he has given separate figures, it is 0.7 sq.cm. For another air-breathing teleost, *Erythrinus*, this figure is as high as 3.56 sq.cm. It means that, though *Erythrinus* is an air-breather, the gills are well developed. His observations (1931 & 1957) about this fish are that it can maintain an active life with the help of gills alone in well oxygenated waters. It can equally do so in completely deoxygenated waters as a portion of the air-bladder subserves the function of a lung. Willmer (1934), who has studied the respiratory habits of Erythrinus in details, observed that this is a fish which uses either its gills or air-bladder as a means of respiration, depending upon the pH value, oxygen and carbon dioxide contents of the water.

Schöotle's measurement of the gill-area, reveals that this area is less for gobiids including Periopthalmus and others which can lead a more or less terrestrial life for some time. Gray, who has estimated the gill-area, in some marine fishes, has concluded that a definite correlation exists between the size of the gill-area, the degree of activity and the habits of the fishes. In a very recent paper (1957) on crabs, he has found that the above mentioned statement holds good for crabs too. It is also interesting to note another observation of his, which he has recorded in the same paper, that, in crabs there is a tendency towards reduction in gill-area per unit weight going from wholly aquatic to intertidal species. Earlier Pearse (1929) had shown that there is a tendency towards reduction in the gill volume as crabs emigrate from over the beach to land.

From the evolutionary stand point, the reduction of the gill-surface met with in air-breathers with accessory respiratory organs is very significant. It means that as air-breathing organs developed, the gills got reduced in size. Their total respiration is thus through the gills plus the air-breathing organs. In *Erythrinus*, it is through the gills and/or air-breathing organs.

D. THE DISPOSITION OF MUSCLES ASSOCIATED WITH RESPIRATION

Whether the gills of the air-breathing fishes are capable of absorbing the full complement of oxygen required for the metabolic activities or not, still in majority of the air-breathing teleosts the gills play a predominant role in obtaining the oxygen from the environment. Some air-breathers are found to utilise the gills for breathing atmospheric air also. Moreover, the aerial accessory respiratory organs are associated mostly with the passage of aquatic respiration viz. the buccopharyngeal and branchial chambers. In short, the aerial respiratory organs are so situated that the very muscles which take part in aquatic respiration can also be used for aerial respiration. Under the circumstances, therefore, it should not be surprising that no modifications as such have taken place in the disposition of the muscles associated with aquatic respiration.

E. OXYGEN-CONSUMING CAPACITY OF THE GILL-TISSUE

From the investigations conducted, it is seen that the oxygen-consuming capacity of the gill-filaments estimated in cc. per gm. weight of the tissue per hour varies from 2.56 to 6.31 in water-breathers. In air-breathers, the corresponding figures vary from 2.48 to 6.48. It means that no detectable difference exists in the capacity of cellular respiration of water-breathers and air-breathing ones living in similar habitats.

The fact that no change as such has taken place in the disposition of the muscles associated with respiration or in the oxygen-consuming capacity of the gills shows that the air-breathing habit has been brought about without affecting the aquatic respiratory organs as far as possible. The only major change is the degeneration of the gills which is correlated with the development of the accessory respiratory mechanism and an increase in the oxygen-carrying capacity of the blood; another change being the formation of an aqueous mucous layer which has conferred an advantage on the air-breathing fishes which are often exposed to terrestrial conditions.

2. DEFICIENCY OF OXYGEN IN THE MEDIUM AS A CAUSATIVE STIMULUS FOR THE ORIGIN OF THE AIR-BREATHING ORGANS

Granted for a moment, that the air-breathing habit in fishes has been evolved to survive in a medium deficient in oxygen, a dictum so commonly expressed in many original publications dealing with air-breathing fishes: It is seen from the analysis of the mode of life of the air-breathing fishes during several investigations including the present one, that these fishes live in a variety of habitats, some of them rich in oxygen. As regards the water-breathing fishes, the literature surveyed by Rounsefell and Everhart (1953) reveals that the minimum oxygen-tension value for the fishes investigated by several authors is less than 0.3 cc. per litre (i.e. 0.5 parts per million by weight) and that the fish is found to live in a healthy condition even at extremely low concentrations. Even in a high concentration, both the water-breathers and air-breathers live equally at ease. For instance, a fish is found to live when the oxygen content is as high as 9.0 cc. per litre (i.e. 12.9 parts per million by weight). Under controlled conditions the amount of oxygen can be increased to 9.03 cc. per litre (i.e. 12.95 parts per million by weight). This has been demonstrated from the analysis of water in the Laboratory aquarium planted with *Vallisneria*, conditions almost similar to those which occur in most of the fresh-water tanks during the monsoon period from July to September.

The habitats of the air-breathing teleosts as stated previously, can be divided into two broad categories. In one category come the marshy places, swamps, puddles, ponds, pools etc. These contain a lower amount of oxygen than in the other, which comprise the rivers including the hill-streams, estuaries, shore waters etc. Assuming that the fishes living in waters having a less percentage of oxygen became adapted to live there, by evolving organs to supplement the gills for the intake of oxygen, can it also be said that the same has been the case with all the fishes of the water-holds having sufficient oxygen in their waters?

It is true that the living fauna of to-day does not necessarily occupy the same type of habitat as the progenitors and it can be argued that the fishes of to-day living in waters rich in oxygen have migrated to their present habitats from those having a lower content of this gas. It is highly doubtful whether this holds good in all the cases, because the air-breathing fishes are found not only in fresh-water habitats but also marine ones. It is hard to presume that all the marine air-breathing fishes evolved first in waters deficient in oxygen and later migrated to their present habitats. Sea-shores, estuaries etc., which are subject to tide action can hardly be said to have a deficiency of oxygen in their waters. Moreover marine water is hardly at any time in a stagnated condition. Temporary shallow pits formed if any, during the low tide on or near the sea-shores are subject to breeze action and there is no likelihood of deficiency of oxygen in them. Therefore, even presuming that the fresh--water air-breathing fauna originated in waters deficient in oxygen, some alternative explanation has to be sought for the origin of air-breathing fishes of marine fauna which cannot be said to have suffered for such a long time so as to leave any permanent effect on their ancestors. Hora (1933) who conducted a detailed survey of the fauna of the sea-shores etc., made the following suggestion regarding the origin of the air-breathing habit in them. He stated "During the ebb-tide, shore animals and burrowing forms are stranded on the banks or pools etc., which are liable to stagnate or dry up altogether. In such situations, the animals are forced to make use of the atmospheric air." Das (1940), who has also made a reference to the air-breathing fishes inhabiting marine waters and others inhabiting waters rich in oxygen, while generally agreeing with Carter's statement that deficiency of oxygen has been the causative stimulus which has induced the development of air-breathing habit, treats the development of the air-breathing habit in fishes inhabiting waters rich in oxygen as an exception to the rule.

Majority of the fishes living in swamps, pools, puddles etc., having less quantity of oxygen in their waters are water-breathers and only a small number are air-breathers. The swamps of Paraguayan Chaco for instance which hardly contained 0.5 cc. of oxygen per litre in its waters, nevertheless was inhabited by atleast 20 species of fishes of which only 8 were air-breathers. All these fishes according to the statement made by these authors, are permanent members of the fauna. The presence of fishes with normal sized gills in waters deficient in oxygen cuts at the root of the suggestion of the paucity of oxygen in the medium as a factor in the evolution of the accessory respiratory organs. If the amount of oxygen is insufficient to meet the requirements of the water-breathing fishes, how do these fishes manage to live there? The only reasonable conclusion we can arrive at is that, though this theory is interesting and a highly suggestive one, it does not stand close scrutiny, since the facts of fish-life are overwhelmingly against this theory at least in the majority of cases.

It may be noted here that the fossil deposits of Ophiocephalus, have been discovered in the lower Siwaliks (Lydekker-1886 & Wadia-1950). Besides Ophiocephalids, Clariids have also been traced in the pliocene and pleistocene periods. Menon (1951), who has made an extensive study of the Clariid fishes has concluded that these fishes originated during the early pliocene period, somewhere in South China, when the climatological conditions of the Siwaliks extended as far east as China. Clariids as is well known, form a group of highly specialised air-breathing fishes. This Siwalik system according to Krishnan (1949), "has the characters of the fluviatile deposits of torrential streams and floods in shallow water basins. The earlier Siwalik period was apparently a wet period, or alternately the sediments were deposited in the shallow waters." So during this period which is known to be one of mountain formation, it is quite unlikely that the water remained in stagnated condition for long. On the other hand, the conditions suggest that, the water-hold must have been periodically disturbed by heavy rains and water flowing into them from rivers. It is quite unlikely therefore that water under such circumstances will be deficient in oxygen.

3. THE ORIGIN AND THE EVOLUTION OF THE AIR-BREATHING HABIT AND THE EVOLUTIONARY TRENDS IN FISHES VIEWED AGAINST THE LARGER SETTING OF VERTEBRATE EVOLUTION

A change over from aquatic to aerial respiration has taken place in the evolution of a number of groups of animals. Among the invertebrates this habit is observed in many groups of animals such as annelids (e.g. the earthworm), molluscs (e.g. slugs and snails) and arthropods. Perhaps the arthropods were among the earliest of the air-breathers (Swinnerton-1947). These animals are better suited for a terrestrial mode of life, on account of the presence of chitin, which checks the evaporation of the tissue fluid. Even a predominantly aquatic group among the arthropods viz. Crustacea has certain animals such as terrestrial and intertidal crabs which are adapted to air-breathing.

Among the vertebrates - as has already been stated in the introduction - the aerial respiratory organs are believed to have been present in the early bony fishes of the Devonian period. It is also interesting to note in this connection that, atleast three elasmobranchs viz. *Chilloscyllium indicum*, *C. griseum* and *Aetomyleus maculatus* which are found in abundance along the Bombay sea coast are found occasionally to come to the surface to breathe atmospheric air.

A student of vertebrate evolution is immensely interested in the transition of the water-breathing habit to the air-breathing one, since the acquisition of the latter habit had been an epoch making event in the history of vertebrates. Apart from the ancestors of amphibia, a large number of other fishes including some actinopterygians, dipnoans and some others had acquired the air-breathing habit, demonstrating the multiferous paths tread in evolution.

Water adjoining the land always forms a transitional habitat. It is in this laboratory that the first experiments of the air-breathing habit were conducted. Their inhabitants form naturally a link between the aquatic and terrestrial fauna in several cases. The piscine inhabitants of such waters, before the advent of Amphibia, represented the transitional vertebrates on their onward journey towards higher evolutionary levels. There is no doubt however that, they were well adapted to live in their habitats; yet they were in a state of disequillibrium in their adaptation to the environment as a result of the diverse factors which influenced their life on account of the proximity of the habitat to land. A biotic population of this kind is full of potentialities due to the preadaptive tendencies which are likely to appear in it.

The fact that both water-breathers as well as air-breathers live in waters deficient in oxygen side by side with equal ease suggests that this aquatic habitat adjoining the land, has offered an opportunity to these organisms in taking to different ways of living with equal ease. To state in Dobzansky's terms (1951), it means that both the categories of fishes viz. water- and air-breathing forms are sympatric and live in a heterogenous territory. This heterogeneity is such that it permits the development of sympatric diversity. In this context, it can be said that both these categories of fishes are equally well adapted to live in their peculiar habitat viz. the water adjoining the land. While the water-breathers are better adapted for drawing oxygen through their gills, the air--breathers are well adapted for drawing atmospheric air under the normal conditions. It is reasonable to assume that the adaptive value is the same in either case. Otherwise according to Gause's principle, the significance of which is emphasized by several authors including Lack (1947), Mayr (1947) and Dobzansky (1951), the less adapted forms will be eliminated in due course of time. If the adaptive value of one is unity and that of the other is 1-S no matter how small this 'S' is, the less well adapted will, given enough time, be eliminated. If therefore, deficiency of oxygen has compelled the fishes to take to air-breathing habit, one should expect the extinction of water-breathers in these habitats. The facts of fish-life are quite contrary to these expectations.

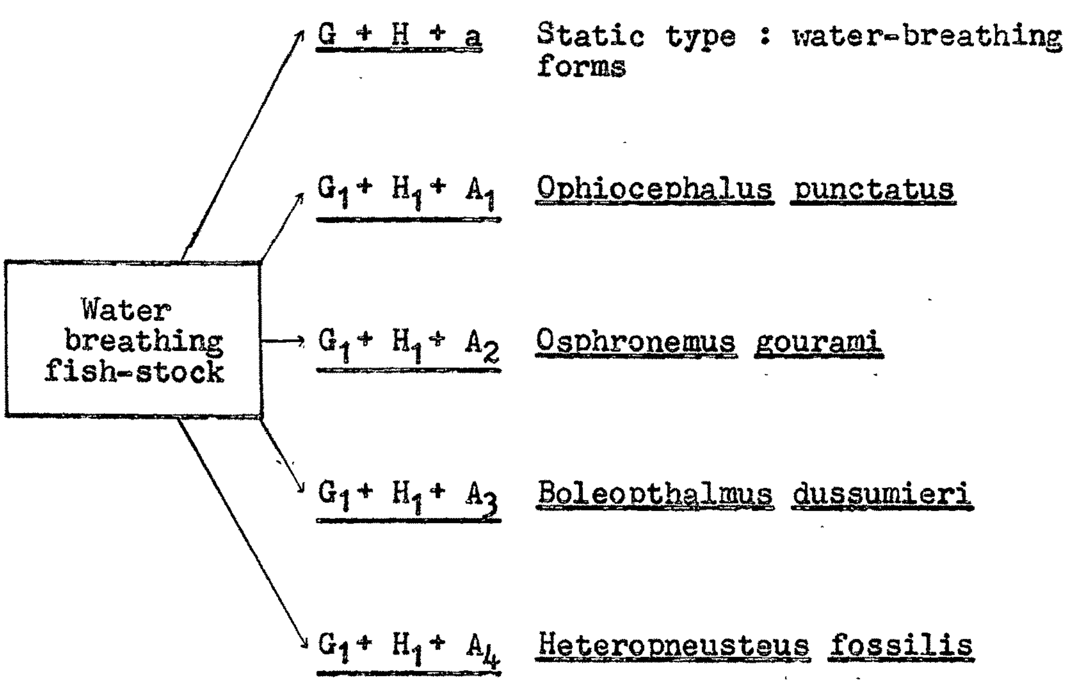
Whereas one litre of water hardly contains a maximum of 8 to 10 cc. of oxygen per litre, an equal quantity of air contains as much as 209.0 cc. of this gas. The importance of this gas to an organism need not be emphasized here as the role played by this gas in the release of chemical energy is too well known. Further it is also a well known fact that the evolutionary trend is often directed towards exploiting the available resources of nature with ease. It is quite likely therefore that the evolutionary trend relating to the development of the air-breathing organs among fishes was one which directed them towards a final acquisition of the organs through which the oxygen available in abundance in the atmosphere could be absorbed with ease. It was not the paucity of oxygen then, which compelled the ancestors of the air-breathing teleosts to breathe atmospheric air: rather fishes living in a habitat - which is in the state of disequillibrium - found a new and an easy way of obtaining oxygen from the environment. The development of the air-breathing habit among a number of invertebrates including a predominantly aquatic group viz. crustaceans, also suggests the same thing. A highly suggestive statement made by Professor J.Z.Young (1950) while referring to the evolution of fishes may be quoted here : "It is true that the sea- and fresh-water have been in existence relatively unchanged throughout the period that we are considering. In a sense, the fishes have not found a new environment. But they have found endless ways of living in the water."

One cannot lose sight of the fact however, that during the Devonian period, which is characterised by the appearance and the predominance of the lung-fishes, drought conditions were prevalent (Gregory & Barret-1936, Romer -1953 etc.). Similarly it is also quite likely that the fluviatile deposits of the torrential streams in shallow waters of the Siwaliks, reduced the volume of the water-holds and created conditions similar to droughts on account of the withdrawal of water from such habitats. To such fishes air-breathing has a survival value. It is quite likely therefore, that mutations involving the development of the air-breathing habit occurred in the piscine fauna during the postulated period in those waters and were later fixed by selection. In either case, linking the present day air-breathers to the ancestral types, we can say that they are evolutionary descendants of the types which had preadaptive tendencies towards air-breathing, amidst a multitude of ordinary forms. The present day water-breathers inhabiting the water adjoining land are on the other hand descendants of the static types that existed then.

Having stated elsewhere that the air-breathing habit represents a forward step, one can as well speculate on the evolutionary path traced by these air-breathers. One of the preliminary steps towards the acquisition of the air-breathing habit seems to be the development of a thick mucous aqueous layer to protect the delicate gill-filaments and the other respiratory organs against desiccation. In this connection, the occurrence of this feature in *Wallago attu*, a water--breather capable of surviving for some time in air comes to mind.

It is observed from the investigations that the majority of the air-breathing teleosts show a reduction of gill-surface and an increase in the capacity of the blood to fix oxygen. The mechanism of respiration and the oxygen--consuming capacity of the gill-tissue in both the water and air-breathers, however, is the same. It is legitimate then to connect these phenomena with the development of the air-breathing habit and treat them as intimately related. In essence, it means that the mutations which induced the growth of the aerial respiratory organs are correlated with those inducing a reduction in the gill-surface and an increase in the oxygen-fixing capacity of the blood. This combination of features is to be regarded as an example of the principle of functional correlation in evolution.

Since the evolution of the air-breathing organs should have occured through successive mutations, it is reasonable to assume that the mutations involving those three modifications viz. (i) the appearance of the air--breathing organs, (ii) a reduction in gill-surface and (iii) a change in the nature of the blood, must have occured in all air-breathing types. However, since the air-breathing organs are of diverse types, the mutations which produced them could not have been of a uniform nature. The following representation will elucidate the common factors and the variations in the evolution of the air-breathing habit :



Where,

G - represents a set of genes inducing the development of the normal sized gills.

G_1 - represents a set of mutations which culminated in under-sized gills.

H - represents a set of genes responsible for the normal oxygen-fixing capacity of the blood.

H_1 - represents a set of mutations responsible for an increase in the oxygen-fixing capacity of the blood.

a - denotes the absence of mutations inducing the development of the air-breathing organs.

A_1, A_2, A_3, A_4 etc. - represent different sets of mutations which culminated in different air-breathing organs in different species.

The diversity in the organs concerned emphasizes the degree of instability which was peculiar to the fishes which inhabited the waters during the postulated transitional period.