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MADRAS

PRELIMINARY OBSERVATIONS ON THE ANIMAL
COMMUNITIES OF THE LEVEL SEA-BOTTOM
OF THE MADRAS COAST

By

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INTRODUCTION.

The study of the animal communities of the sea-bottom has been a subject of great interest for over fifty years. As early as 1883, Petersen carried out extensive work on the animal communities of the Danish Coast, as well as of the adjacent waters down to a depth of about 300 meters and thus laid the foundation for this branch of Biology. No work of this type has been done on the Indian Coast. One of the main objects of this investigation has been to gain a general knowledge of the level bottom fauna of the Madras Coast and to explain, where possible, the difference between the animal communities as met within the different kinds of level sea-bottom. My observations are by no means exhaustive as the facilities for dredging are very limited, though a fairly accurate faunistic picture has been obtained. All that is attempted in this paper is to give a preliminary account of the fauna on the lines adopted by Petersen (1883) and Ford (1922). It is hoped that this contribution will go some way towards creating an interest in the study of the level bottom fauna of the Indian Coast.

PREVIOUS WORK.

The literature dealing with this subject is replete with the work of early Zoologists such as Forbes (1859), Lorenz (1863), Petersen (1915, 1918), Pruvot (1895), Lonnberg (1898) and Appellof (1905) who have shown that there is a great difference in the animal life of the sea-bottom. Allen (1899) has given a detailed account of "the fauna and bottom-deposits near the 30 fathom line from the Eddystone grounds to Start point." The earliest work of this kind by C. G. J. Petersen in the Danish waters, was followed by Allen and Todd (1900 and 1901) who made a detailed study of the fauna and the bottom deposits of

the Plymouth waters, the Exe and Salcombe estuaries. In the course of his work with the bottom-sampler to determine the amount of animal matter available as fish-food at different places in the sea-bottom, Petersen found that certain animal species were distributed over extensive and continuous areas. By a thorough examination of numerous bottom samples, he has been able to distinguish a small number of definite types of animal communities characterised by the occurrence in large numbers of certain forms. By the extensive use of his bottom sampler and by employing other apparatus of his own devising, in the deep waters of the Skagerak upto the Baltic, Petersen has distinguished nine animal communities on the level bottom based on the preponderant occurrence of one or two species in each one of the communities. The index forms may even belong to different groups of animals. Petersen's communities in the Danish waters are:—

1. The Macoma or Baltic community, d, on all our Southern Coast, and in the Baltic.
2. The Abra Community, b±E, especially in the Belt Sea and the Fjords.
3. The Venus Community, v±E, on the open Sandy Coasts of the Kattegat and in the North Sea.
4. The Echinocardium-filiformis Community, E. fil., at intermediate depths in the Kattegat.
5. The Brissopsis-Chiajei Community, B.Ch., in the deepest parts of the Kattegat.
6. The Brissepsis-Sarsii Community, B.S., in the deeper parts of the Skagerak.
7. The Amphilepis—Pecten Community, Al.P., in the deepest water of the Skagerak.
8. The Haploopsis Community, Ha., Locally in the South-eastern Kattegat.
9. The deep Venus Community (v.,) sporadically in the Kattegat."

By further investigations regarding the distribution of animals in other areas, as South Norway and the Christiania Fjord, he came to the conclusion that similar communities as those discovered in Denmark are recognisable there also.

Blegvad, (1914) investigated the "Food and conditions of nourishment among the communities of Invertebrate animals found on or in the sea bottom in Danish waters."

Later on Ford (1922) studied the animal communities of the level sea-bottom in the waters adjacent to Plymouth, following generally the methods adopted by Petersen in Danish waters. The instrument used in the collection of animals with the bottom depo-

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sits was the 1-10 sq. metre Bottom-Sampler. He found that the level bottom in the waters off Plymouth is populated chiefly by Petersen's *Venus* communities with Spatangidae and with some representation of *Echinocardium-filiformis* Community and the shore areas of Plymouth South by Petersen's *Macoma* Community.

Since the present paper was read before the Indian Science Congress in, 1940, two papers on the animal communities of the sea-bottom have appeared, one by Miyadi (1940) entitled "Marine benthic Communities of the Tanabe-wan" and another by Beanland (1940) under the title "The sand and mud communities in the Dovey Estuary." Miyadi describes two *Benthic* Communities. The area investigated can be grouped under sheltered waters. The deeper areas are populated by *Maldane sarsi* with its associated species *Ampelisca* Sp., designated as the *Maldane* Community. The shallow areas are characterised by the dominance of *Cerithium pfefferi* often accompanied by *Terebellides stroemi* and hence named *Cerithium* Community. Both are described as mud communities. The former is confined for the most part to viscid mud containing a small amount of fine shell fragments having a thin brown top layer of fresh silt, while the latter is found in mud "Containing a considerable amount of shell fragments and coral skeletons with a good accumulation of fresh silt upon it." Beanland (1940) deals with the sand and mud communities in the Dovey Estuary. The fauna is analysed with regard to the distance from high-water mark and degree of shelter. The author found that the greater part of the Estuary shows Petersen's *Macoma* Community and the more sheltered part of the Estuary is inhabited by *Corophium* Community characterised by the preponderance of *Corophium volutator*. *Macoma* community according to this author is found in sand, while the substratum for *Corophium* community is mud. A sub-community *Macoma-Bathyporeia* is found in more barren sand.

Material and Methods.

The present investigations on the Madras Coast were started in August, 1938. Dredge collections at a depth of 10 to 15 fathoms were periodically examined and almost every animal captured was preserved in formalin, identified and recorded after noting its relative abundance. The apparatus used for collection of animals along with the bottom deposit in which they occur was the naturalists' dredge. This consists of a rectangular iron frame with a stout gunny bag attached behind which brings up the deposits when

dragged over the sea-bottom together with the animals in them. This method of collection has been found to give fairly good results. Though up-to-date methods could not be employed, since a number of dredge collections each consisting of about half a dozen hauls from the same locality have been made and the fauna tabulated, the general impression one derives from the tables cannot be far from the reality. The collections were made in shallow water within 3—5 miles more or less opposite to the Triplicane beach stretching from the Ice House to the High Court. It is more than probable that a similar fauna occurs all along the coast of the City of Madras extending North to South for about 8 miles.

As already mentioned dredge collections were made at depths from 10 to 15 fathoms during different times of the year. Tabulated statements given at the end show the names of the species, the number of individuals of each species, the nature of the level bottom and their dates of collection. A perusal of the tables gives an idea of the frequency of occurrence of animals in the three main types of level sea-bottom. Needless to say that a number of hauls were made at each locality and the sum total of the collection thus made alone is given. It should not therefore be taken that the tables represent the differences between single hauls on each occasion. The method adopted has a distinct advantage that it will give a fairer idea of the animal composition than could be gained by comparison of single hauls. The areas dredged must be considered as coming under the category of open water as opposed to the sheltered waters and it should also be stated that all the communities described occur at about the same depth.

General.

The importance of the nature of bottom deposits in determining the fauna is one which has received considerable attention. Allen (1899) has made a detailed study of the fauna and bottom deposits near the thirty fathom line from the Eddystone grounds to Start Point. It has been fully recognised by naturalists that variation in the bottom deposit has a great influence on the local distribution of the animal life. Recently Wilson (1937) has shown that the nature of substratum may even influence the ready settling down, or otherwsie, of the free swimming larvae of organisms which lead a sedentary life when adult. Since one of the principal objects of the present investigation was to study the variation in density and composition of the fauna with special reference to the bottom deposit, a quantitative examination of the fauna and

a qualitative examination of the bottom-deposit have been made. The texture of the deposit is of primary importance in considering the relation between the nature of the bottom deposit and the fauna living upon it and attention has been drawn to this factor by many naturalists. Sand with shell gravel, sand-clay, and black clay, form the chief varieties of bottom deposits. Three distinct series of level bottom animals are found to exist almost side by side, on the Madras Coast, the first one restricted to the sandy bottom where the sand is of varying degree of coarseness, mixed with different amounts of shell fragments. The second series is found in sand-clay level bottom consisting of masses formed both of clay and sand (Photograph III). The third one confines itself chiefly to deposits of fine clay.

a. Sandy level Sea-bottom

Sandy level bottom at all points consists of sand of varying degrees of coarseness with varying amounts of silt, shell fragments, and pebbles. The distribution of the fauna seems to be influenced by coarseness of the gravel, the relative abundance of shell fragments, and the amount of silt. There is apparently some variation in the relative abundance of the fauna inhabiting a particular deposit in different parts of the year.

Sandy bottom is characterised by a very great abundance of *Branchiostoma indicum*. The dredge brings up hundreds of them in certain months (April, Table II) though they occur invariably in every haul throughout the year. Mature specimens with well developed gonads have been obtained throughout the year, they being very numerous in the months of March and April. They are very active and have remarkable powers of burrowing in sand. When disturbed they swim swiftly. They vary from white to pink or even light brown in colour, and the gonads of mature male specimens are of milky white colour and of the mature female specimens rose red. The largest examined measures 3.5 cms. in length and 0.5 cm in height.

The sand fauna on the whole, exhibits a variety of colours in sharp contrast to the dull colouration of the animals on the sand-clay and the clay level-bottoms. The eye is captivated by the singularly beautiful hues of varying shades of the echinoderms of the sandy level bottom. The prevailing colours are grey, pink, red and brown. These colours match with the colour of the sandy gravel substratum. Next to *Branchiostoma indicum*, in numbers,

come the Echinoderms, *Temnopleurus toreumaticus* and *Lovenia elongata* which are essentially sand dwelling forms.

Temnopleurus toreumaticus occurs in numbers at certain times of the year ranging from August to January and numbering from seven to fifteen for each dredge collection. It forms an important part of the whole mass of the community, owing to its weight and hence can be considered as one of the characteristic animals in the formation of a community. There is difference of colour in the varieties of *T. toreumaticus*. One is *Temnopleurus toreumaticus* which is olive-greenish grey and its spines are banded reddish-brown. Another is *T. toreumaticus* var. *Perize* in which the test and small spines are merely white and the primary spines pale yellow, banded with reddish white or reddishbrown. This is a very pretty and light coloured form of *Temnopleurus* which at first sight, looks quite different from *Temnopleurus toreumaticus*, but is regarded by H. L. Clark as a colour variety. On a few of them, in amongst their spines, the small gastropod mollusc (*styliifer abyssicola*) has been found along with masses of its eggs.

Amongst the *Lovenia elongata* dredged the youngest ones are only 30 mm. in length while the largest measure about 80 mm. They are extremely pretty with reddish-violet and white markings and the long yellowish spines with light violet bands. They bury themselves in sand with the long spines protruding and in constant movement. *Lovenia elongata* has never been taken from clay. It is capable of burrowing almost vertically into the sand with the aid of its short flat spines. Certain bivalves are occasionally found attached to their long spines. These echinoderms though essentially burrowers can also progress rapidly over the surface of the sand. When placed in a trough of water with sand at the bottom, they quickly disappear into it. In this condition they produce a current of water by constant movement of their spines. This may be a respiratory as well as a nutritive current. The same vibrating movement is observed in the case of the ophiuroids *Ophiocnemis marmorata* and *Ophiothrix longipeda*, also sand dwelling forms, set up by the small spines along their arms which force the water along their surface. The occurrence of *Lovenia elongata* corresponds closely with *Temnopleurus* and both have generally been taken together (Table I). Both the species are usually confined to the sand, though occasionally one or two individuals of *Temnopleurus toreumaticus* have been taken from clay. These appear to be rather of the nature of immigrants from their natural grounds.

As many as 6 species of Ophiuroids have been recorded from the sand. Of these *Ophiocnemis marmorata* was taken in greatest numbers. *Amphiura lobata*, *Amphiura depressa*, *Ophiacantha granulosa*, *Ophiocnida echinata* have been only occasionally captured and that in small numbers. *Ophiocnemis marmorata* is not a burrowing species but is capable of rapid movement over the surface of the sand. When placed in a glass trough, they clump together forming a dense mass of bunched animals with arms interlocking, thus showing a tendency for aggregation. They are somewhat greenish grey in colour, the arms banded with dark grey. Ophiuroids have the practice of fragmenting their arms under certain conditions. It has been observed that there is greater tendency to practise autotomy on the part of the isolated than with bunched *Ophiocnemis marmorata*.

A noteworthy feature characteristic of the ground is the presence in almost every haul of a few representatives of polychaetes. *Euthalenessa djiboutiensis* is the most abundant species on the sandy substratum from where 2 to 17 specimens were obtained in each collection. They are typical of sand and though represented only by a few specimens, occur invariably in all the dredge collections, throughout the year. Owing to their constant occurrence they may be classed among the characteristic animals in the composition of a community. They lie buried in the sand and when disturbed are capable of moving with considerable rapidity. The largest measures about 10 cms. They exhibit a peculiar habit of breaking into bits when handled. The worms, in life, are pale yellowish white with minute pigment spots of a reddish tint.

Another striking feature of the collections from the sandy bottom is the presence of large numbers of membranous tubes coated with sand grains and inhabited by *Owenia fusiformis*. A few of the tubes are empty or contain only fragments of the worm but many of them are inhabited by the slender worms. The tubes measure from 7.5 to 8.5 cms. and are open at both ends. Three species of *Glycera* were obtained from the sandy bottom which can be considered as one of the characteristic polychaete fauna of the region, though few in number. Leathery tubes of eunicid worms with broken bits of shell agglutinated to them are taken at times. Occasional specimens of *Arabella iricolor*, *Drilonereis* sp., *Armandia leptocirrus* and *Lumbriconereis heteropoda* occur on the sandy substratum. The beautifully coloured *Hesione pantherina* has also been found in the sandy level bottom in small numbers.

The crustacean fauna of the sandy bottom is comparatively poor and none of the species occur in large numbers. The well-known habit of the Dromids of seeking protection by covering themselves with a dead lamellibranch shell is often noticed in *Dromia rumphii* which occurs occasionally in the sands. The fifth pair of trunk legs are dorsal in position and the dactyli being prehensile are adapted for carrying the lamellibranch shell over the back. The animal, when deprived of its covering, moves with considerable rapidity and secures another shell. The large chelipeds are massive and setose except the claws which are of a bright red colour. *Pilumnus vespertilio* has been taken, but rarely. They are remarkably slow and sluggish and render themselves inconspicuous by coating themselves with silt and sand particles which are retained by their setose carapace and legs.

Swimming species of Portunidae in the sandy level-bottom are represented in fair numbers by *Thalamita crenata* and *Neptunus pelagicus*. They are extremely active swimmers. Only three Macruran Decapod crustaceans *Penaeus* Sp., *Angasia stimpsonii*, and *Hippolytina vittata* have been recorded. The scarcity of these forms may be due to their free swimming habit. Two specimens of Scyllarids were trawled from the sands, although they are said to frequent generally a rocky substratum.

Broken shells of *Cardium* and other lamellibranchs serve as attachment for the few hydroids present, the only "epifauna" noticed in the collection. The mollusc, *Marginella argustata*, is the best represented species and appears to prefer a sandy bottom although dead shells are taken from the clay with hermit crabs inside. They are very graceful and slow in their movements. They are often brownish yellow matching with the colour of shell gravel effectually to protect them. *Euchelus proximus* is equally frequent. They show a variety of beautiful colours. *Glycimeris trylori* is found in moderate numbers in the collections. The only nudibranch dredged is *Melebe rosea* and it has been recorded twice in the course of the whole year. These are pretty, light greenish white in colour, and graceful in their gliding movements.

b. Sand-clay level sea-bottom.

The sand-clay level sea-bottom consists mainly of masses of sand and clay in almost equal proportion. These masses are full of tubular holes inhabited by sedentary polychaetes (Photograph III). Some of the empty holes afford excellent harbouring

places for anemones and for the hermit crab *Troglopagurus jousseaumii*.

The polychaete fauna is abundant and varied. *Loimia medusa*, *Sabellaria spinulosa* and *Thelepus cinnatus* occur in large numbers and form a characteristic feature of the fauna (Table III). The general colour of *Loimia medusa* when removed from its tube is seen to be greyish red and the buccal tentacles are beautifully coloured uniformly with a reddish purple tinge but in a few the buccal tentacles are banded red. *Thelepus cinnatus* has a more slender build than *Loimia medusa*. These worms creep partly out of their burrows and spread their tentacles to ensnare any small animals near them but at the slightest disturbance they are retracted in and no trace of the worms is visible. *Sabellaria spinulosa* with its operculum of golden setae closing the entrance of their homes as with a lid is a sight of great interest. Varied are the methods by which most of these animals are protected in this level bottom. Grey and dark hues prevail among the species of worms found moving on the agglutinated masses of sand and clay. *Harmothoe dictyophora* has been found creeping very slowly on these substrata, their grey colour matching marvellously with the surroundings rendering them inconspicuous. *Leocrates diplognathus* is very active and moves with considerable rapidity and finds safe retreats inside the cracks and crevices. Occasional specimens of *Tylonereis bogoyawlenskyi*, *Sabella* sp., and *Eunice* sp., were taken along with the more characteristic polychaetes mentioned above.

The only Echinoderm of abundance at certain times of the year is *Ophiactis* sp., which occurs almost to the exclusion of every other species. They are comparatively small in size and can only be made out with difficulty. They are of a greyish brown colour with banded arms matching with that of their surrounding.

The crustacean fauna is represented by *Troglopagurus jousseaumii*, *Polyonyx* sp., and *Porcellana* sp. These curious hermit crabs, *Troglopagurus*, are seen clinging tenaciously to the inside of their tubular homes by means of their dactyli and differ strikingly from *Diogenes* in their habit of inhabiting these holes instead of molluscan shells. Very interesting are the red colour markings on the legs and eye stalks of these hermit crabs which match with the coloured tentacles of *Loimia medusa* with which they are often associated. Most of the specimens are females carrying eggs. *Pilumnus vespertilio* has also been found living concealed in the cre-

vices of the hard masses. Hiding inside the holes are the minute crustaceans *Pisosoma* sp., *Polyonyx* sp., and *Porcellana* sp.

It is interesting to notice that members of the molluscan group are usually absent here.

c. Clay level sea-bottom.

The clay is of a fine texture and contains dead shells of gastropod molluscs which afford safe habitation for numerous hermit crabs (Photograph V). A good number of polychaete worm tubes is also present. As was to be expected from the nature of the clay and the fineness of the particles, the fauna is poor.

During certain months of the year (Table IV) numerous Anomuran crustaceans, belonging to different species of *Diogenes* are found to inhabit the empty shells of a variety of Gastropods such as *Turritella* sp., *Murex tenuispina*, *Oliva gibbosa*, *Marginella argustata* etc. A few of these shells are encrusted with *Hydractinia*. The hermit crabs are so very abundant that it becomes the most striking feature of the ground. Five species of *Diogenes* namely *D. rectimanus*, *D. costatus*, *D. investigatoris*, *D. merguensis*, *D. bicristimanus*, have been recorded from the clay bottom. Of these *Diogenes costatus* is the leading species counting to about 250 in one collection. Next to it comes *Diogenes rectimanus*. A beautiful specimen of *Pagurus hessai* which had established itself in association with a *Turritella* shell was also taken.

Alpheus malabaricus is found in fairly large numbers burrowing into the soft clay. It makes a peculiar clicking noise by rapidly flexing the dactylus of its larger chela against the corresponding immobile finger, when the animal is disturbed. The next most abundant crustaceans met are the minute Porcellanids—*Polyonyx* sp., and *Porcellana* sp. which occur in most of the hauls though only in small numbers. Another clay bottom loving form *Upogebia* sp., also occurs but rarely.

The other decapods dredged from the clay are *Dromia rumphii* and *Schizophrys aspera*. The habit of *Dromia* covering itself with foreign matter has been recorded by many authors. Specimens of *Dromia rumphii* which occur occasionally in the clay are coated with clay, the fine particles of which are held by the short hairs on the upper surface of the carapace and legs. Forms of Malidae such as *Schizophrys aspera* are rendered quite inconspicuous by encrustation of foreign matter entangled by the spines on the back. The more inactive members of the group are just those which resort

to these devices with the object of making themselves inconspicuous. Amphipod crustaceans are quite common in this level bottom. A few stray polychaetes such as *Sabella* sp., *Tylonereis bogoyawenski*, *Diopatra neapolitana*, *Phyllodoce* sp., *Leocrates diplognathus* etc., have been occasionally found.

The chief Echinoderm present here is *Ophiactis* sp. *Amphiura depressa* was also taken. Several gastropods occur in the clay level bottom of which *Oliva gibbosa* is the most common one. *Cantharus tranquebariens* and *Murex tenuispina* are not very uncommon. Other species like *Drupa margariticola*, *Nassa variegata* and *Cancellaria cripsa* are taken occasionally.

By examination of a number of dredge collections, each of about 6 hauls, four animal communities, as characterised by the predominant occurrence of certain forms, have been distinguished.

SPECIAL.

1. Sandy Level Sea-bottom.

a. The Branchiostoma-toreumaticus community

(Photograph I and Table 1)

Branchiostoma indicum is the most characteristic feature of this type of level bottom. In every dredge, there is a very great abundance of *Branchiostoma indicum* numbering from 62 to 296 thus greatly predominating over all other animals, the average number of this characteristic species being about eight times as great as the next best represented form collected. *Temnopleurus toreumaticus* is another characteristic species predominating over others both in weight and number and occurs invariably in association with *Branchiostoma indicum*. Due to the predominance of *Branchiostoma indicum* and *Temnopleurus toreumaticus* over all the other animals, this may be called *Branchiostoma-toreumaticus* Community. It is interesting that *Temnopleurus toreumaticus* and *Lovenia elongata*, another characteristic species, occur regularly together in the same area, though the latter does not occur in equal abundance. Various other species such as *Euthalenessa djiboutiensis*, *Glycera tessellata*, *Owenia fusiformis*, and *Marginella argustata*, occur in association with the *Branchiostoma-toreumaticus* community. They are characteristic of this community as evidenced from their frequent occurrence in the collection but are less numerously represented, *Lumbriconereis impatiens*,

Armandia leptocirrus, *Arabella iricolor*, *Drilonereis* sp. *Ophiethrix longipeda*, *Amphiura lobata* etc., have not been taken in sufficient numbers to be classed as characteristic of this community but when taken always occur in association with *Branchiostoma-toreumaticus* community. It may be noted that the sand is of a fine texture mixed with a small amount of silt.

b. *The Branchiostoma-marmorata Community*

(Photograph II, and Table II).

This community also inhabits the sandy bottom. There is here also the great preponderance of *Branchiostoma indicum*. But instead of the next best represented form being *Temnopleurus toreumaticus*, it is seen to be *Ophiocnemis marmorata*. Hence the association can be called *Branchiostoma-marmorata* community. *Branchiostoma-toreumaticus* and *Branchiostoma-marmorata* are independent communities but with several species common to both. Apart from the species found in both these communities the *Branchiostoma-marmorata* community is characterised by the presence of *Salmacis virgulata*, *Philine aperta*, *Euchelus proximus*, *Glycimeris trylori*, *Clymene* sp. A few species occur in association with both the communities and may be regarded as equivalent to Petersen's "attendant" species capable of associating with different communities. Both these communities are closely related to each other and they occur at similar depths and inhabit almost the same level bottom, although in this case the sand is cleaner and of a coarse texture and contains a large amount of broken pebbles and lamelli branch shells. The difference in the fauna is to be attributed to the coarseness of the gravel, the relative abundance of shell fragments and the amount of silt.

ii. *Sand-clay level sea-bottom.*

a. *The Loimia-spinulosa community*

(Photograph IV and Table III)

Owing to the very special nature of the deposit, the fauna is typical and its most characteristic animals are restricted to this particular substratum. The importance of the nature of the bottom deposit in community formation is well illustrated here. The most abundant species is the polychaete *Loimia medusa* which by its constant occurrence forms a charac-

teristic feature of this type of level bottom. The next important species is *Sabellaria spinulosa*. It is a striking feature that this level bottom consists of masses of agglutinated sand-clay with numerous tubular holes inhabited by dense populations of many tubicolous polychaetes such as *Loimia medusa*, *Sabellaria spinulosa* and *Thelepus cincinnatus*, and the hermit crab *Troglopagurus jousseaumii* (Photograph III). *Harmothoe dictyophora* is another characteristic polychaete worm. The chief and perhaps the only echinoderm which frequents it in numbers at certain times of the year is *Ophiactis* sp. Owing to the preponderance of *Loimia medusa* and *Sabellaria spinulosa* this community is named *Loimia-spinulosa* community. The crustaceans *Troglopagurus jousseaumii*, *Upogebia* sp., *Pisosoma* sp., *Porcellana* sp., *Polyonyx* sp., and the polychaetes *Leocrates diplognathus*, *Eunice* sp., are taken in fair numbers and they occur in association with the *Loimia spinulosa* community.

iii. *Clay level sea-bottom.*

a. *The Diogenes Community*

(Photograph VI and Table IV).

The leading species in the clay level bottom are *Diogenes rectimanus* and *D. costatus*. *Ophiactis* sp. may or may not be present. *Alpheus malabaricus*, *Polyonyx* sp. and *Porcellana* sp. claim some importance as characteristic species more due to the scarcity of other species than to their numerical strength. The very great abundance of dead shells affording habitat for hermit crabs as *Diogenes rectimanus*, *D. costatus*, *D. merguiensis* is a noteworthy feature (Photograph V). The small crustacean *Upogebia* sp., also occurs as in the preceding community. In certain respects there is similarity between the fauna of the clay and sand-clay level bottoms. We can name this *Diogenes* community. *Oliva gibbosa* and *Murex tenuispina* are the two molluscs generally met with. *Dromia rumphii*, *Amphiura depressa*, *Cantharus tranquebariensis*, etc., are occasionally represented but their number is not sufficient to place them in the characteristic species. A careful study of the tabular statements will lead one to the generalisation that all the animals are not of equal importance in the formation of a community. A typical expression of the *Diogenes* community with its leading characteristic species is found in the collection on the 27th April (Table IV).

From a consideration of the fauna of different areas examined it is clear that the controlling factor has been the nature of the bottom and probably also the mutual inter-relationships of the forms constituting the community. Temperature and light cannot have been of any importance as the areas occupied are fairly adjacent.

SUMMARY

1. Dredge collections have been made from the level sea-bottom adjoining Triplicane, Madras. Three main kinds of level sea-bottoms appear to be present. These are i. Sand level sea-bottom, made up mainly of fine or coarse sand, ii. Sand-Clay level sea-bottom consisting mainly of an equal proportion of sand and clay iii. Clay level sea-bottom formed mainly of dark fine clay.

2. According to the nature of substratum three main animal communities have been distinguished:—i. *Branchiostoma-toreumaticus* community characterised by the preponderance of *Branchiostoma indicum* and *Temnopleurus toreumaticus* in fine sand and a sub-community namely *Branchiostoma-marmorata* community, in rather coarse sand. ii. *Loimia-spinulosa* community characterised by *Loimia medusa* and *Sabellaria spinulosa* in sandy-clay bottom. iii. *Diogenes* community in clay-bottom.

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EXPLANATION OF PLATE I.

Photograph I.

'The Branchiostoma-toreumaticus' community showing the characteristic species.

1. Branchiostoma indicum.
2. Temnopleurus toreumaticus.
3. Lovenia elongata.
4. Euthalenessa djiboutiensis.
5. Owenia fusiformis.
6. Glycera tessellata.
7. Marginella argustata.

Photograph II.

'The Branchiostoma-marmorata' community showing the characteristic species.

1. Branchiostoma indicum.
2. Ophiocnemis marmorata.
3. Salmacis virgulata.
4. Philine aperta.
5. Euchelus proximus.
6. Glycimeris trylori.
7. Clymene sp.

Photograph III.

Masses of agglutinated sand-clay inhabited by tubicolous polychaetes and the hermit crab *Troglopagurus* (taken from sand-clay level bottom).

Photograph IV.

'The Loimia-spinulosa' community showing the characteristic species.

1. Loimia medusa.
2. Sabellaria spinulosa.
3. Thelepus cincinnatus.
4. Troglopagurus jousseaumii.
5. Harmothoe dictyphora.

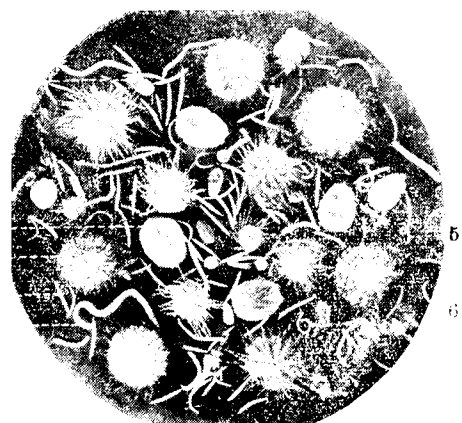
Photograph V.

A collection from the clay level bottom showing the dead shells of gastropods which afford habitation for various species of *Diogenes*.

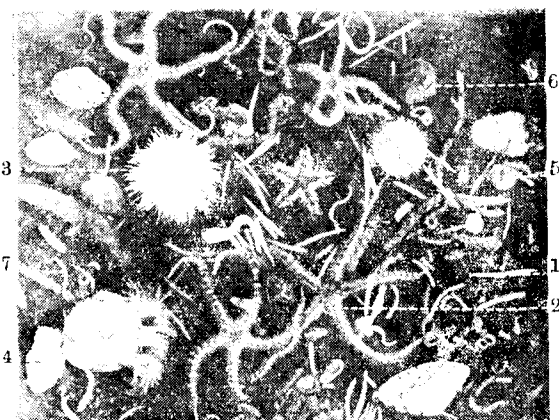
Photograph VI.

'The *Diogenes*' community showing the characteristic animals. The hermit crabs are taken out of the shells and photographed.

1. *Diogenes costatus*.
2. *Diogenes rectimanus*.
3. *Diogenes merguiensis*.
4. *Ophiactis* sp.



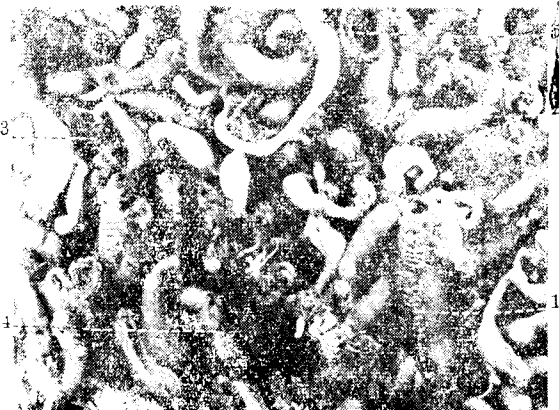
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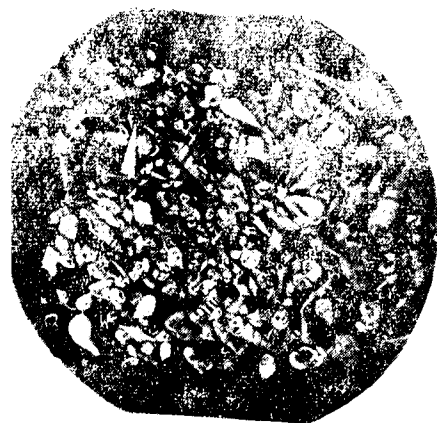
II



III



IV



V



VI

TABLE
"THE BRANCHIOSTOMA-TOREUMATICUS
SANDY LEVEL

Name.	9-8-38	23-8-38
Branchiostoma indicum	.. 125	104
Temnopleurus toreumaticus	.. 12	7
Lovenia elongata	.. 10	3
Ophiocnida sp.	.. 1	
Amphiura lobata?	.. 2	
Ophiothrix longipeda	..	2
Ophiocnemis marmorata	..	
Amphiura depressa	..	
Ophiacantha granulosa?	..	
Salmacis virgulata	..	
Ophiocnida echinata	..	
Synapta sp.	.. 1	1
Euthalenessa djiboutiensis	.. 3	3
Glycera tessellata	.. 1	2
Glycera longipinnis	..	
Glycera cirrata	..	
Eunice tentaculata?	.. 1	
Phyllodoce sp.	.. 1	
Lumbriconereis impatiens	..	
Lumbriconereis heteropoda	..	
Arabella iricolor	..	
Drilonereis filum	..	
Hesione pantherina	..	1
Owenia fusiformis	.. 2	5
Armandia leptocirrus	..	1
Cirratulus sp.	..	
Oliva gibbosa	.. 1	
Marginella argustata	.. 3	1
Conus sp.	..	1
Euchelus proximus	..	
Glycimeris trylori	..	
Turris marmorata	..	
Ancilla ampla	..	
Latrunculus sp.	..	
Nassa gemmulifera	..	
Calliostoma tranquebarica	..	
Dromia rumphii	..	
Pilumnus vespertilio	..	
Albunea thurstonii (n. sp.)	..	
Heteropsammia (sp.)	..	1

L
COMMUNITY"
BOTTOM.

6-9-38	6-10-38	17-10-38	8-11-38	18-11-38	16-1-39
296	108	92	62	110	62
15	15	8	12	8	8
5	4	6	3	4	3
			2	1	1
2					
2	2		1		
				1	
			1		
					1
1	7	9	17	5	2
	4	1	2	1	1
	1				
			1		
			1		1
	1			2	
			2		
		2		1	
1				1	
	2				
3	2	4		2	3
			1	3	
			1		
4	2	3	5	2	5
		3			
			1		
			1		
			1		
			1		
			1		
1			1		
		1	6		1

"THE BRANCHIOSTOMA-MARMORATA

SANDY LEVEL..

Name.	3-3-39	5-4-39	6-4-39
Branchiostoma indicum	89	335	390
Ophiocnemis marmorata	7	30	162
Salmacis virgulata	2	2	3
Synapta sp.			
Euthalenessa djiboutiensis	1	2	
Owenia fusiformis		4	
Clymene sp.	3	3	2
Glycera tessellata			4
Glycera cirrata	1		
Eunice tentaculata?		1	
Eunice sp.	1		
Phyllodoce sp.			
Lumbriconereis heteropoda			
Armandia leptocirrus			
Euchelus proximus	4		6
Glycimeris trylori	4	6	3
Philine aperta	7	3	4
Marginella argustata	2	4	
Oliva gibbosa	1		
Conus sp.	1		
Ancilla ampla	1		
Bursa margaritula		1	
Melibe rosea		1	1
Kalinga ornata			
Nassa gemmulifera			
Nassa variegata			
Calliostoma tranquebarica			
Thalamita crenata			4
Angasia stimpsonii		1	
Penaeus sp.		1	
Hippolysmata vittata		1	
Neptunus pelagicus			
Scyllarid sp.			1
Porcellana sp.			
Squilla sp.			
Cavernularia?			
Callionymus lineolatus			

II

COMMUNITY."

BOTTOM.

27-4-39	17-7-39	9-8-39	9-9-39	19-9-39	9-10-39
255	160	55	98	93	210
8	15	10	5	5	4
5	2	2	6	4	6
		1			
4	5	8	5		3
3	1	2			4
1	3		2	2	3
1	3	1	2		
	4				
	1				
	2	1			
	6				
3	8	4	3	4	4
2	2	7	4	3	
12	6	1	3	2	
			2	7	2
			1		3
1					
			1		
				1	
				1	2
2				3	1
	1				
1					
	1				
	1				
					1

TABLE III

8th Nov. '38 to 6th April '39.

TABLE
"THE DIOGENES
CLAY LEVEL

Name.	23-8-38.	6-9-38.	6-10-38.	17-10-38.	8-11-38.	3-3-39.
<i>Diogenes rectimanus</i>	.. 10	2	18			
<i>Diogenes costatus</i>	..		29			
<i>Diogenes investigatoris</i>	..					
<i>Diogenes merguensis</i>	..			1	3	6
<i>Diogenes bicristimanus</i>	..					
<i>Diogenes diogenes</i>	..					
<i>Pagurus hessii</i>	..					
<i>Pagurus Sp.</i>	..					
<i>Troglopagurus jousseaumii</i>	..				3	
<i>Alpheus malabaricus</i>	.. 3	12		3		
<i>Alpheus crassimanus</i>	..		1			
<i>Dromia rumphii</i>	.. 2	1				
<i>Dorippe dorsipes (?)</i>	..					
<i>Thalamita crenata</i>	..				1	
<i>Neptunus pelagicus</i>	..					
<i>Upogebia Sp.</i>	..	10				
<i>Hippolysmata vittata</i>	..	4				
<i>Porcellana sp.</i>	..	8				
<i>Polyonyx sp.</i>	..	6		3	2	3
<i>Schizophrys aspera</i>	.. 2					
<i>Ophiactis sp.</i>	.. 72	30				
<i>Amphiura depressa</i>	.. 4				2	
<i>Oliva gibbosa</i>	.. 1	2		4	1	
<i>Cantharus tranquebariens</i>	.. 2	8				
<i>Murex tenuispina</i>	..	3				2
<i>Nassa variegata</i>	..					
<i>Cancellaria crispa</i>	.. 1					
<i>Diopatra neapolitana?</i>	..					

IV.
COMMUNITY"
BOTTOM.

5-4-39.	6-4-39.	27-4-39.	17-7-39.	9-8-39.	5-9-39.	19-9-39.	9-10-39.
34		110	3	14	12	15	25
78		250		10			
		8					
		9	3	2		1	
		2					1
						3	
1							2
		1					
		6		1			14
			1				
					1		
1							
		1				1	
2							
		18					
				1	2		
		65	6		5	8	7
							1
4	2	4			3		2
							2
	3	2	1		4	3	4
					1	1	2
							2

A NOTE ON THE DISTRIBUTION OF STUDENT'S RATIO IN SAMPLES FROM NON-NORMAL POPULATIONS*

By

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Introduction.—The variety of uses to which the student's ratio $t = \frac{(x - m)}{s} \sqrt{n}$ where x and m are the sample and population

means and s is such that $s^2(n - 1) = \sum_{i=1}^n (x_i - x)^2$, is put to in statistical analysis has given rise to theoretical and sampling investigations about the distribution of this constant for samples taken from different parent populations. The distribution of 't' is solved completely only when the parent population is normal.² The dependence of mean and variance is noted as the chief difficulty that comes in the way of obtaining the exact distribution for non-normal universes. The error in assuming normality for distributions which are in fact skew or symmetrical in applying Student's Ratio has formed the study of many authors. Writers like Bartlett^a and Gregory carried theoretical investigations and experimental sampling was conducted by many authors.^b The exact distribution of the Student's Ratio was noted to be more accurate in its application to symmetric non-normal than to skew universes.^c

The populations from which samples were drawn by previous authors for experimental investigations varied considerably specified by the values of the first two betas. Samples from parent universes like the rectangular, exponential and other distributions specified by Pearsonal types of curves were utilised. No sampling investigation was carried when the parent population followed the Type I distribution in a limited range. As most distributions in a limited range in practice are never normal, the study of the error involved in assuming normality for the application of student's ratio to samples from a population with a limited

*Taken from a thesis accepted for the M.Sc. degree of the University of Madras.

(a) See references 1, 3, 9 and 10.

(b) See references 4, 7, 10 and 11.

(c) 3, p. 180.

range will be of interest. In this paper a study of samples from type IX distribution which is the extreme case of skewness for type I is undertaken and the extent of error involved in assuming normality to samples in the calculation of Student's ratio is investigated. As any variety of type I distribution is less skew than the type IX, the extent of error in assuming normality for the calculation of Student's ratio will also be less and hence the confidence levels required for Student's 't' calculated for samples from type I can be fixed lower than those necessary for the samples from type IX in this study.

Samples used.—The samples utilised for this study are formed in the following manner. The probability integrals for all the normal deviates given by Mahalanobis⁵ are obtained. These are known⁸ to follow the rectangular law. Retaining the same order the minimum values of samples of size five are taken from these integrals. The 2080 values thus obtained follow the type IX distribution¹² and are specified by the law $Y = \text{const.}(1-z)^4$. These form the parent population. Table I below gives the frequency distribution of these values and the frequency constants with their corresponding expected values are shown in table II.

TABLE I

Class Interval	Frequency
0.000—0.040	424
0.040—0.080	325
0.080—0.120	274
0.120—0.160	211
0.160—0.200	183
0.200—0.240	175
0.240—0.280	118
0.280—0.320	87
0.320—0.360	69
0.360—0.400	63
0.400—0.440	57
0.440—0.480	40
0.480—0.520	22
0.520—0.560	10
0.560—0.600	9
0.600—0.640	5
0.640—0.680	6
0.680—0.720	1
0.720—0.760	2
0.760—0.800	1
0.800—0.840	1
Total	2080

TABLE II

Frequency Constant	Observed.	Expected.
Mean	0.1609	0.1667
Variance.	0.0186	0.0198
μ_3	0.002979	0.003307
μ_4	0.001435	0.001653
β_1	1.378	1.400
β_2	4.145	4.200

The close correspondence between the expected and observed values of the frequency constants shows that the 2080 values obtained follow the type IX distribution. Samples of five each are drawn retaining the order and the values of the mean, standard deviation and Student's 't' are calculated for all the 416 samples thus formed.

Correlation between mean and variance.—As the mean and variance form correlated variables in samples drawn from any parent universe except the normal, the type of relation between these two constants is of importance for the study of the distribution of Student's ratio. It is noted⁶ that if Pearsonian β 's of the universe satisfy the relation $\beta_2 - \beta_1 - 3 = 0$ the regression of variance on $(x - m)$ is linear and in other cases it is well represented by the second order parabola. Table III gives the double entry for the 416 pairs of values of mean and standard deviation of the samples.

TABLE III
Value of Mean

Standard Deviation	Value of Mean																	Total
	.01	.03	.05	.07	.09	.11	.13	.15	.17	.19	.21	.23	.25	.27	.29	.31	.33	
.01	1	1																3
.03			7	1	1	2	2		1							1		15
.05			1	15	9	4	4	4			1							38
.07				8	8	6	5	4	2	1						1		35
.09			1	7	11	16	9	14	2	1	1					1	1	64
.11					2	11	8	6	9	11	4	5	3					59
.13					3	3	8	12	5	6	2	2			1	1		43
.15						3		7	11	10	5	6	5	3			1	51
.17							5	4	6	6	10	2	2	5	1	2		44
.19							1	5	4	4	7	2	2	1	1		1	28
.21							1	2	3	1	1	2	2	1				13
.23								1					3	1	1	1		7
.25									1	1	1	1	1	3			1	8
.27															1	2		3
.29												1						2
.31														1				1
.33																	1	1
.35													1					1
Total			33	34	45	43	59	44	40	32	21	19	17	8	8	2		416

From the table the correlation coefficient $r=0.6900$ and the correlation ratio $\eta=0.6977$.

Applying the significant test^a to r ,

$$z = \frac{1}{2} \log_e \frac{1+r}{1-r} = 0.8479 \text{ and the standard error is}$$

$$\frac{1}{\sqrt{n^1 - 3}} = 0.0492. \text{ So the value of } r \text{ is definitely significant.}$$

For testing the significance of the correlation ratio η , the trans-

$$\text{formation}^b \quad z = \frac{1}{2} \log_e \frac{\eta^2}{1-\eta^2} \cdot \frac{N-p}{p-1}$$

(a) R. A. Fisher.—'Statistical methods for Research Workers', p. 203.

(b) Yule and Kendall.—'An introduction to the theory of Statistics', p. 454.

where N is the total frequency 416, and p is the number of arrays 17 is used. The value of z calculated which is 1.5819 is significant at 1% level obtained with degrees of freedom $n_1=(p-1)=16$, and $n_2=(N-p)=399$.

The closeness of values of the correlation coefficient and the correlation ratio suggests that the departure from linearity is not great for the samples considered.

Distribution of 't'.—As the parent universe is skew and the dependence of the mean on standard deviation is significant for the samples, the distribution of the Student's ratio calculated from these will be of interest. Table IV gives the distribution of 't' for the 416 samples together with the corresponding frequencies calculated from the exact distribution of 't' assuming the parent population to follow the normal law. The histogram of the calculated values of 't' with the expected frequency curve assuming normality is shown in graph I.

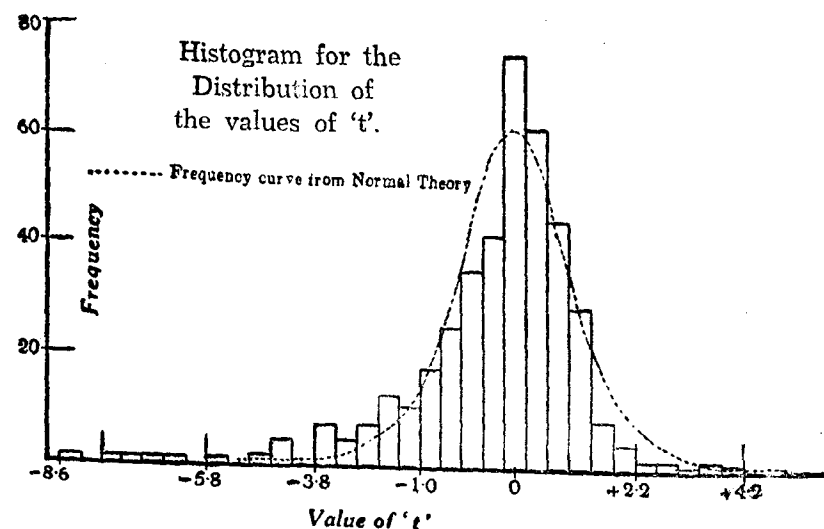
TABLE IV.

Central Values of 't'	Observed Frequency	"Normal Theory"
Below -8.8	5	0.2
-8.4	1	
-8.0	—	
-7.6	1	
-7.2	1	
-6.8	1	0.5
-6.4	1	
-6.0	—	
-5.6	1	
-5.2	—	
-4.8	2	0.4
-4.4	5	0.8
-4.0	1	1.3
-3.6	8	1.7
-3.2	5	2.5
-2.8	8	4.2
-2.4	13	6.7
-2.0	11	11.2
-1.6	18	18.3
-1.2	26	29.2
-0.8	36	42.8

Central Values of 't'	Observed Frequency	"Normal Theory"
-0.4	42	56.6
+0.0	71	61.7
+0.4	62	56.6
+0.8	45	42.8
+1.2	29	29.2
+1.6	9	18.3
+2.0	5	11.2
+2.4	2	6.7
+2.8	2	4.2
+3.2	1	2.5
+3.6	2	1.7
+4.0	1	1.3
+4.4 above	1	2.70
Total	416	

The graph and also the table show a marked difference between the calculated frequencies from the given population and those

GRAPH I



expected from assuming the parent population to be normal. The observed frequency distribution of 't' is unsymmetric with more negative than positive values.

In the absence of symmetry an attempt is made to know the distribution of the observed values of 't' irrespective of the sign. The proportion of the observed frequency falling outside a certain value of $|t|$ is compared with the corresponding proportion of the frequency falling outside the same value when the parent universe is normal.⁷ That is the probability of a given value of the Student's ratio falling outside a particular limit $|t|$ for both the observed and the expected distributions is compared. Table V give the proportions of the total frequency lying outside specified values of $|t|$ for the two distributions. They are also shown in graph II.

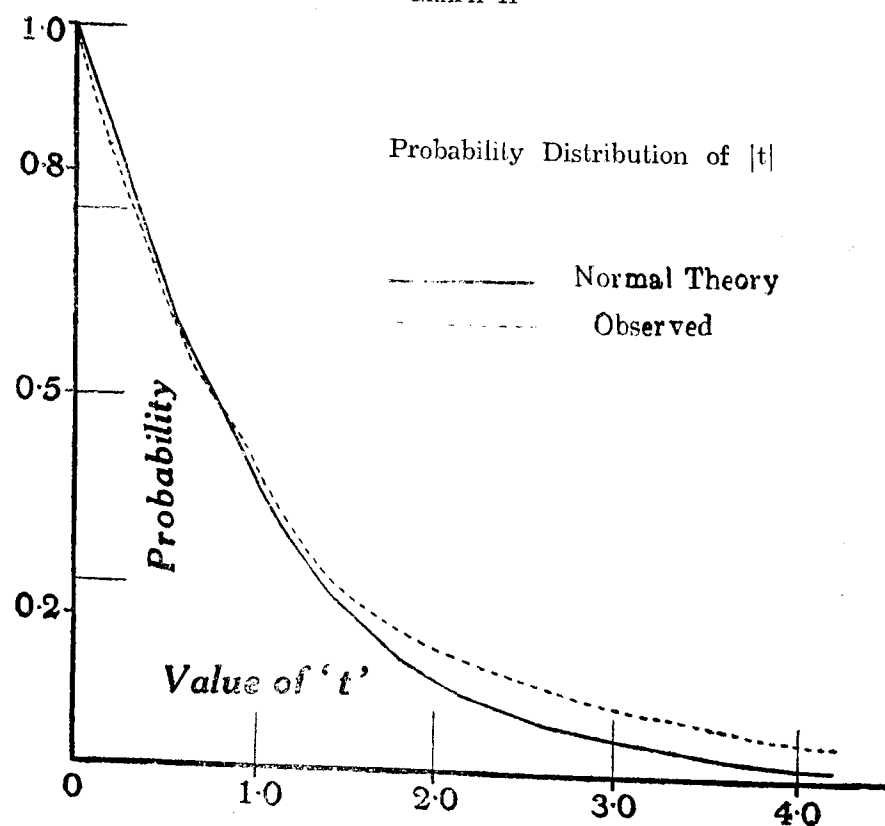
TABLE V

$ t $	Normal Theory	Observed.
0.0	1.000	1.000
0.2	0.851	0.825
0.6	0.581	0.577
1.0	0.374	0.383
1.4	0.234	0.251
1.8	0.146	0.187
2.2	0.093	0.148
2.6	0.060	0.112
3.0	0.040	0.089
3.4	0.027	0.074
3.8	0.019	0.050
4.2	0.014	0.045

From the table after the value 1.0 for $|t|$ the proportions of frequency falling outside the limit are noted to be consistently higher than the corresponding proportions obtained by assuming normality to the parent universe. This fact is clearly seen in graph II. For $|t|=3.0$ only 4% of the values lie outside when normality is assumed whereas 8.9% of the values lie outside for the observed distribution. Now proceeding in the usual manner for testing hypothesis, a hypothesis is rejected when $P \leq 2\alpha$ and it is accepted when $P > 2\alpha$ where the value of α depends on the nature of

the problem. The errors will be in general (i) the hypothesis is rejected when it is in fact true and (ii) it is accepted when it is false.⁷ If the exact distribution of the Student's ratio is used with the assumption that the parent population is normal where as in fact it is skew, we are more likely to reject a hypothesis when it is true rather than accept it when it is false. As the proportions of frequency lying outside a particular value of $|t|$ are noted to be higher for the observed distribution than for those obtained by assuming normality, for any assigned level of significance we are

GRAPH II



more likely to infer that a given value of $|t|$ is significantly different from zero, while the inference is wrong if it is based on the actual distribution of $|t|$. So to judge the significance when normality is assumed for skew populations of the type discussed here a higher level of significance must be fixed than the usually adopted levels of 1% and 5%. The level of significance must be sufficient enough to offset the disparity shown in the distribution

of ' t ' obtained by assuming normality compared with the actual distribution. The exact level α necessary depends on the type of problem present and the extent of the symmetry in the parent population.

Another to note is the lack of symmetry in the observed distribution of ' t '. Beyond the value 3.0 there are only five positive observations where as there are 32 on the negative side. Compared with the distribution of ' t ' for normal samples there is lesser frequency left out on the positive side where as it is more on the negative side. Even though the same level or even a lower level of significance can be utilised for the positive values, a high level of confidence must be set up for negative values. So the significant level must change according as the value of the observed ' t ' is positive or negative.

As type I distribution is more symmetric than the type IX discussed above, the significant level necessary for the testing of hypothesis when normality is assumed will be lower when only $|t|$ is considered than for type IX. When the actual value of t is taken into consideration the significant level necessary depends on whether the value is positive or not. But the difference in the significant levels necessary for the positive and negative values will be much less than those required for the type IX distribution.

Summary.—In this paper the error involved in assuming normality for samples really belonging to a skew population of type IX for the calculation of Student's ratio is investigated. 416 samples of size 5 are taken and the corresponding distribution of the values of ' t ' are compared with the distribution of samples of the same size from a normal universe. It is noted that the observed distribution has no symmetry and if only the absolute values are considered higher level of significance must be used in testing hypothesis if normality is assumed. Taking the actual values different levels of significance are necessary according as the value of t is positive or negative. Here the levels of confidence necessary depend on the asymmetry of the parent population.

I am indebted to Dr. N. S. Sastry, Head of the Department of Statistics, University of Madras, for the suggestions offered during the preparation of this paper.

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NITROGEN METABOLISM AND HUMAN NUTRITION

By

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Pure and applied science are not independent social phenomena. They are inextricably related as shoot and root in the process of healthy growth. Growing science is the unity of theory and practice. Without its roots firmly planted in the moist soil of social practice the green shoot of pure science withers and becomes the dead trunk of metaphysics. Without the aspiring shoot of theory sustaining it with the nutriment of air and sunlight, the root of applied science degenerates into the dry wood of empirical repetition.

Lancelot Hogben.

INTRODUCTION

An attempt is made in these pages to summarize our present knowledge—and ignorance—of the nitrogen metabolism of living organisms and to consider them in relation to human food requirements. The subject matter was first prepared for the Travancore Curzon Lectures in Agriculture delivered under the auspices of the Madras University in 1941 and grateful acknowledgment is made to the University authorities for the opportunity thus provided for discussing the bearing of some aspects of "pure" biochemical research on problems of practical agriculture.

The business of agriculture is to utilize the synthetic powers of the plant to produce those organic substances that are essential for human nutrition. For agriculture to meet successfully the food requirements of populations knowledge is needed (i) of the nutritional requirements of man stated in quantitative terms of the chemical substances necessary for optimal health and (ii) of the most efficient methods by which these substances can be manufactured through the agency of vegetation. The nitrogenous food of man consists of proteins, substances which can best be defined as compounds of high molecular weight built up by the union of amino-acids. The dominant role of protein in the diet arises from

the fact that over 45% of the dry matter of the human body is made up of substances of this class, comprising both structural and protoplasmic material and performing the most diverse functions in the organism. For the protein which has to be supplied in the food for the formation and maintenance of this tissue substance the human being depends upon the plant either directly or through the mediation of his livestock. The ultimate reservoir of the organic compounds that make up living matter is the atmosphere. But while the substances which the plant requires for the synthesis of carbohydrate, viz., carbondioxide and water, exist preformed in the atmosphere, nitrogen as it is present in air cannot be utilized by vegetation. Before elementary nitrogen can be used by plants for protein synthesis it has to be converted into inorganic compounds by the agency of the nitrogen fixing bacteria. Successful satisfaction of the protein requirement of man thus depends upon a knowledge of the transformations which elementary nitrogen of the air undergoes in passing through bacteria to man, in other words, upon a knowledge of the nitrogen metabolism of animals, plants and bacteria. It will be seen from the following pages that our information on these subjects is far from complete and that deficiencies in practice go hand in hand with gaps in fundamental knowledge.

For India the protein problem in nutrition is of paramount importance. Early work on protein deficiency in Indian diets such as those of McCay of the Indian Medical Service has tended to be forgotten mainly because dietetic studies in Western countries have been dominated by the striking discoveries in the field of vitamins. There are several reasons why vitamins should loom large in the popular imagination in considering questions of food. The vitamins exercise their function in quantities so minute and in some cases so dramatically as to become endowed with almost magical properties. Further the results of their absence from the diet are easily recognized in clinical examination. The effects of prolonged protein deficiency on the other hand are likely to manifest themselves in such generalized symptoms of subnormal health as retarded growth and lowered vitality, characteristics which are often apt to be considered "racial". It is often forgotten that the emphasis laid on vitamins and other accessory food factors in dealing with nutrition in Western countries consuming large quantities of highly processed imported food-stuffs is to a large extent misplaced when applied to populations such as those of India whose regimen consists of foods in a more "natural" state. In any population suffering from malnutrition as the result of a low standard of

living dietary deficiencies are likely to be of a multiple nature and it cannot be denied that vitamin and mineral deficiencies are widely prevalent in this country. But the discoveries made in recent years on the chemistry of the vitamins make it increasingly clear that in India satisfaction of vitamin requirements is more a question of proper selection from existing food materials than of increasing food production. Carotene widely distributed in green vegetables is an easily available source of vitamin A while sunlight is the equivalent of vitamin D. The vitamin B problem appears to be the direct outcome of the consumption of highly milled rice and would probably vanish if a return is made to the habit of using hand pounded or parboiled rice. Further, the quantities of vitamins required in the food are extremely small and the supplementing of dietaries by means of concentrates or synthetic products is by no means impracticable. Chemical synthesis has already made available for general use preparations of vitamin D, of two important factors in the vitamin B complex viz., thiamin and nicotinic acid and of vitamin C. The position of protein is however entirely different. The nourishing of human populations on a mixture of synthetic amino-acids provided by chemical industry does not appear to be even a distant possibility and protein of high biological value has to be produced in the only factory capable of such production, viz., in the meat or milk yielding animal. As will be made clear in Part II India produces less than half the quantity of protein that its population requires. To make good this deficit in protein production is from the nutritional point of view the most serious problem that faces Indian agriculture.

PART I

ANIMAL METABOLISM

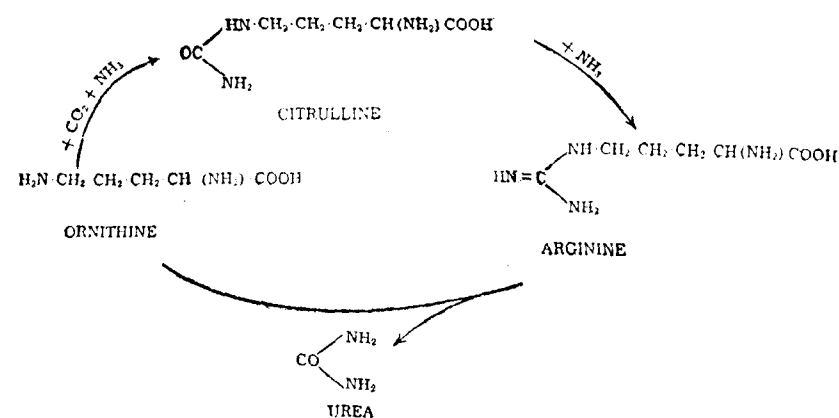
The Urinary Excretion of Nitrogen.—Study of the nitrogen metabolism in the animal has been dominated by the nitrogenous excretion in the urine. The animal organism continually loses nitrogen; the average human adult on a normal diet excretes daily in the urine about 35g. urea, 1.2 to 1.7g. creatinine, 0.6 to 1g. uric acid and 0.5 to 1g. ammonia representing about 16g. of nitrogen or the equivalent of about 100g. of protein. The excretion of nitrogen takes place even on a nitrogen-free diet as well as in complete starvation, the urinary nitrogen in the former case being about 4 to 5g. and in the latter 2 to 3g. This fact has led to the conception of an endogenous and exogenous catabolism; the former results from the break-down of tissue protein, and is considered the inevitable

accompaniment of the life processes of cells and therefore unaffected by the intake in the food, while the latter arises from the decomposition of protein ingested in the food. As far as the adult is concerned the function of food protein is considered to be the replacement of the endogenous nitrogen loss, usually called the "wear and tear quota"; the growing animal requires in addition protein for the building up of new tissue substance also. We have unfortunately little knowledge of the nature of the so called wear and tear, much less of the anabolic processes by which they are repaired or of the reactions by which in the young animal tissue protein is laid down at the expense of food proteins. But a good deal is known about the biochemical processes which result in the excretion in the urine of the nitrogen of the food.

Protein Catabolism—Hydrolysis and Deamination:—Protein ingested in the food is subjected in the alimentary tract to the successive action of a battery of enzymes, of pepsin-hydrochloric acid in the stomach of trypsin, carboxy-polypeptidase and dipeptidase in the small intestine. As a result the protein molecule is almost completely broken down to amino-acids and is carried in that form to the portal circulation leading to the liver. A certain proportion of the amino-acids is carried to the tissues where it is assumed they are utilised for tissue building and the formation of nitrogenous substances required for specific functions. The remainder—the "unwanted" amino-acids—are deaminised and the ammonia excreted mainly in the form of urea. Both the processes of de-amination and the subsequent conversion of the ammonia formed into urea are now fully understood. The process of de-amination consists in the oxidation of the amino-acids to the corresponding keto-acid and ammonia. $R-CH(NH_2) \cdot COOH + O \longrightarrow R \cdot CO \cdot COOH + NH_3$. The first insight into these processes was obtained by Neubauer and Knoop by feeding phenyl-substituted, i.e., non-natural amino-acids. The keto-acids formed in these cases in contrast to those arising from the natural amino-acids were excreted without undergoing further decomposition and could therefore be isolated from the urine. The converse of this process—the change of keto-acid to amino-acid—was accomplished by Embden by perfusion of the isolated liver with the ammonium salts of certain keto-acids. Recently deamination has been elegantly demonstrated by Krebs by the tissue slice method. In these experiments in which practically all the naturally occurring amino-acids were studied the keto-acids formed by de-amination were protected from further change by the addition of chemical inhibitors and finally

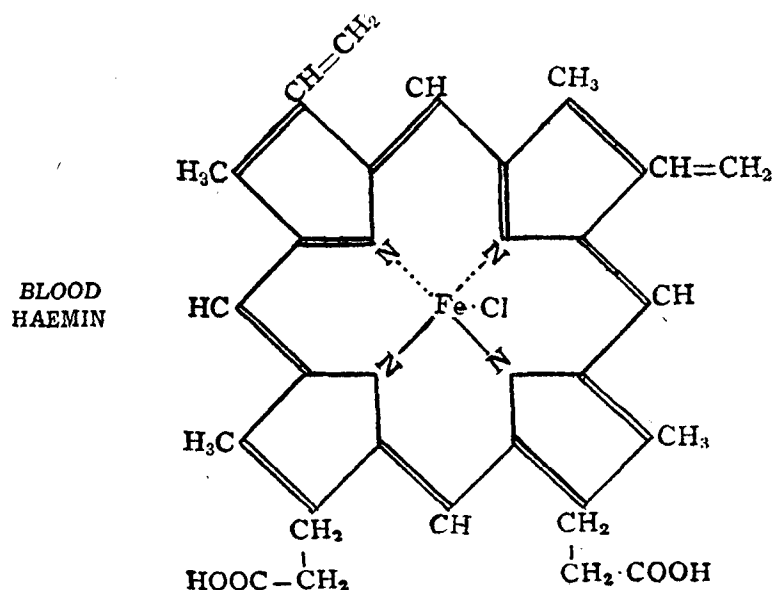
isolated as the 2:4 dinitro-phenyl-hydrazones. Two rather surprising results emerged from these experiments, (i) that the kidney was weight for weight much more active in de-amination than the liver and (ii) the optically non-natural isomers of the amino-acids are more rapidly de-aminised than the natural ones. The kidney thus produces its own ammonia for neutralising the acids it excretes. This organ was further found to have the ability to use ammonia synthetically to convert glutamic acid or the corresponding keto-acid, keto-glutaric acid, into glutamine.

The Formation of Urea.—Krebs has also elucidated the mechanism of urea synthesis about which there had always been much speculation. The reactions by which urea formation takes place in the animal organism provide one of the most beautiful examples of bio-chemical co-ordination. The substances concerned are the three amino-acids arginine, citrulline and ornithine. The existence of an enzyme arginase which hydrolyses arginine into ornithine and urea has long been known. The enzyme has a wide occurrence in animal tissues and is present in particularly high concentration in mammalian liver. This enzyme in itself can however only explain the formation of urea from arginine and not from the ammonia formed from other amino-acids by de-amination. When the effect of amino-acids on urea formation from ammonia by tissue slices was studied, it was found that ornithine had a marked accelerating effect on the process. Krebs further found that another amino-acid, citrulline, discovered not long ago by Wada in water melons, had a similar action. The chemical relationship of these three amino-acids is in fact a biologically important relationship, urea being produced in liver tissue by the following cycle of reactions:

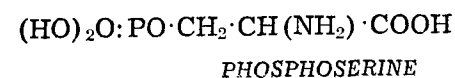


While the nitrogen of amino-acids is in this manner converted first into ammonia and then into urea and excreted via the kidneys the de-aminised residues are assumed to join the pathway of carbohydrate or fat metabolism, although the individual steps by which the different amino-acids are finally oxidised to carbon dioxide and water have not yet been fully traced.

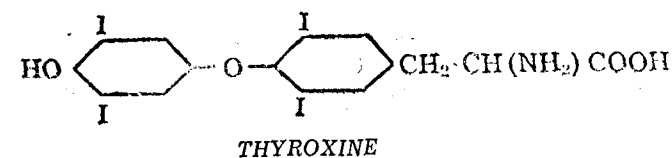
Nitrogen Anabolism.—While thus the fate of the unwanted amino-acids is fairly clear we have very little knowledge of the synthetic processes by which the amino-acids essential to the organism are made use of. We know that the amino-acids must be utilised for the building up of tissues, of blood, of the milk in lactating animals, of specific secretions hormones and enzymes, of the nuclear material of cells and of structural components such as hair and wool. For each of these purposes different amino-acids or a different assortment of them will be required. The different tissues of the body have their own specific amino-acid composition. Specially marked peculiarities occur for example in the case of the scleroproteins of connective tissues, of the keratins of hair, nails and hooves which contain unusually large quantities of glycine and cystine, the protamines and histones of egg cells with their large content of basic amino-acids. Haemoglobin, the respiratory pigment of the blood consists of a protein, globin, of the histone class, rich in basic amino-acids, united to a prosthetic group consisting of an iron porphyrin molecule made up of four pyrrole rings linked by methene bridges.



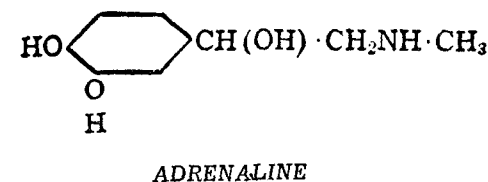
Milk and egg proteins contain a special group, phosphoric acid attached to the amino-acid serine.



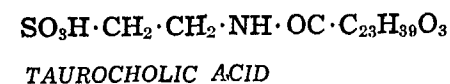
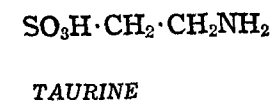
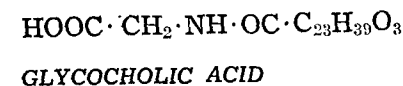
Among the internal secretions we have thyreoglobulin, a protein containing a characteristic amino-acid thyroxine, which is a derivative of tyrosine, and insulin which does not appear to be characterised by the presence of any special amino-acid unit.



Adrenaline, the pressor principle of the adrenal medulla, is also a tyrosine derivative.

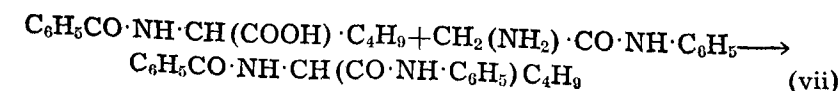
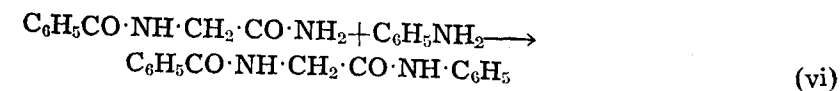
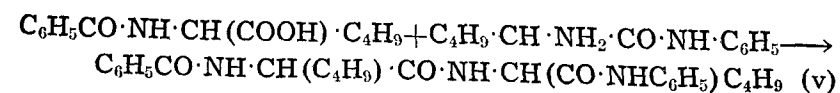
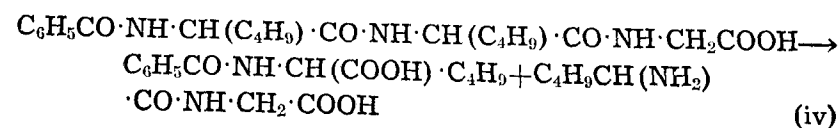
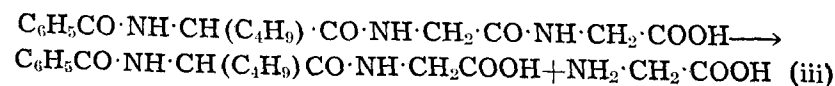
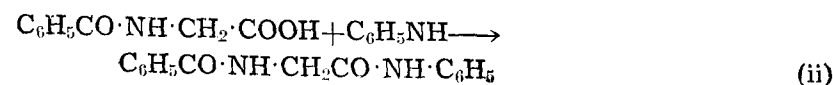
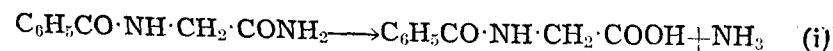


In bile we have the bile acids, derivatives of the sterols, in peptide combination with glycine and with taurine, a derivative of cystine.



Of the biochemical processes leading to the synthesis of these products from the amino-acids arising from food proteins we have at present very little knowledge. The repair of the wear and tear

of tissues in adult animals and the new formation of tissues in growing animals are supposed to take place by a reversal of the enzymic degradation processes which take place in the alimentary tract. Enzymic reactions are essentially reversible reactions and there is an *a priori* possibility that protein synthesis in cells is simply the reversal of hydrolysis. The experimental evidence for such a view is as yet slender. Most claims made about enzymic protein synthesis *in vitro* are disputable. Recently Bergmann has shown that the enzyme papain obtained from the latex of *Carica papaya* and which shares the properties of intracellular proteinases can act synthetically. It was found that while papain hydrolyses hippuryl amide to hippuric acid and ammonia (i), it will under the same conditions form hippuryl anilide from hippuric acid and aniline (ii). Similarly, while benzoylleucyl-glycylglycine (iii) and benzoylleucylleucylglycine (iv) are hydrolysed by this enzyme, it brings about the synthesis of benzoylleucine and leucine anilide to benzoylleucylleucine anilide (v). Papain catalyses replacement reactions also; hippuryl amide and aniline react in its presence to give hippuryl anilide; a mixture of benzoylleucine and glycine anilide gives rise to benzoylleucine anilide (vi).



While these reactions illustrate the versatility of the proteases and their synthetic powers their bearing on the mechanism of protein synthesis *in vitro* is not yet clear. Apart from the obvious objection that the substrates used are not made up entirely of the natural amino-acids there is the more serious difficulty that the peptide models synthesised in these experiments are such as cannot be hydrolysed by papain while the proteins found in tissue cells must be assumed to be hydrolysable during autolysis by the enzymes which brought about their synthesis.

PART II

PROTEIN REQUIREMENTS IN HUMAN DIETARIES

Lack of knowledge of the synthetic processes in which the amino-acids are involved in the body makes it impossible to state nitrogen requirements in quantitative terms of individual amino-acids. We are ignorant even of the amino-acids required for such an important metabolic function as haemoglobin formation. The average life of a red blood cell being about three weeks, continuous regeneration of haemoglobin must be taking place in the body. Study of the active principle in liver which is curative of pernicious anaemia has shown it to be a polypeptide made up of the amino-acids, arginine, leucine, glycine, proline, hydroxyproline, aspartic acid and probably hydroxyglutamic acid, but whether these amino-acids are of significance in normal blood regeneration is not known. There is evidence however to show that tryptophane plays an important part in this process.

The Biological Value of Proteins.—Under the circumstances standards of protein required in the dietary have to be based not upon intimate knowledge of the fate of individual amino-acids in the organism but upon indirect evidence. Three methods of approach have been made to this problem. In the first the biological value of proteins has been determined from their relative efficiency in sparing body protein. As has been mentioned the body loses nitrogen on a nitrogen-free diet and the quantities of different proteins required to offset this loss by "wear and tear" i.e., the quantities required to maintain the body in nitrogen balance vary with the protein. The fact was sought to be utilised as a means of quantitatively evaluating the efficiency of a protein in the diet by Thomas who defined the biological value of a protein as the number of parts of body nitrogen replaceable by hundred parts of the nitrogen of the foodstuff. According to this definition

$$\text{Biological Value} = 100 \times \frac{\text{Body N spared}}{\text{Food N absorbed}}$$

$$= 100 \times \frac{(\text{Urinary N on protein-free diet}) - (\text{Urinary N on protein diet} - \text{N intake})}{\text{N intake}}$$

In using the formula nitrogen excreted in the faeces has to be allowed for to obtain real intake. As the whole of the faecal nitrogen is not of dietary origin various formulae have been suggested to enable faeces nitrogen to be divided into an endogenous or metabolic quota and a portion consisting of un-absorbed food material. But apart from such minor modifications in formulae it is doubtful if the conception of a biological value of protein as defined by Thomas is fundamentally correct. The argument is based upon the assumption that protein is a single entity with a characteristic dietary value. This we know is not the case—a number of different amino-acids are required for specific purposes which are distinct and which have to be separately satisfied. The level of protein intake at which the requirement of each individual amino-acid is satisfied will vary from amino-acid to amino-acid with the result that the biological value of a protein as determined by this method will depend upon the level at which the protein is fed. It is further obvious that Thomas's formula has no meaning when it is applied to levels of protein ingestion higher than is just required to prevent loss of body protein. The practical consequence of this is the necessity for prolonged and tedious experimentation to determine the exact point when nitrogen balance is attained. Another datum required for evaluation of biological value by the Thomas method, the endogenous N output on a protein free diet, also presents considerable practical difficulty. Human subjects of the experiment have to submit to considerable inconvenience and possible impairment of health during a long period of protein-starvation while with animals it is practically impossible to make them take for a sufficiently long period a diet completely devoid of protein. These theoretical and practical difficulties must explain the highly discordant values of the nutritional efficiency of proteins recorded by different observers. A well-known example is that of milk proteins for which Thomas assigned a value of 100 but which in very careful experiments carried out by Martin and Robinson appeared to have a biological value of only 51%.

Amino-acid requirements in growth.—Much more valuable information has been obtained in experiments in which the significance of particular amino-acids has been ascertained by feeding experiments with incomplete mixtures of amino-acids or with proteins known to be deficient in particular amino-acids. Such experiments were commenced by Hopkins and by Osborne. Our present knowledge is based almost entirely on the painstaking and extensive researches of Rose and coworkers in America who fed experimental animals with mixtures of highly purified amino-acids and observed their effects on growth. The result of this work has been to establish that normal growth in animals is impossible unless the following indispensable amino-acids are supplied in the dietary: tryptophane, lysine, histidine, arginine, phenylalanine, leucine, isoleucine, valine, threonine and methionine. The position of arginine is peculiar in that the animal organism is capable of synthesising this amino-acid but not at a sufficiently rapid rate to meet the requirements of normal growth. An extremely interesting aspect of amino-acid metabolism has been revealed by Rose's discovery that in the case of the amino-acids lysine, valine, the leucines and threonine, only the optically natural isomers are effective in nutrition, while with tryptophane, histidine, phenylalanine and methionine it is immaterial which of the optical antipodes are supplied in the diet.

The findings regarding the essential and non-essential amino-acids determined by experiments on rats are considered to apply to all animal species. This is *a priori* probable since animal tissues from different species are similar in amino-acid composition; but it is necessary to keep in mind that animals may differ in their ability to synthesise particular amino-acid units within their own organism.

Dietary Surveys.—With our present ignorance about the utilisation of protein and the unreliability of data obtained from nitrogen-balance studies it is not surprising that prescription of standards of protein in the dietary have to be based, not upon fundamental scientific facts, but upon ordinary experience i.e., upon dietary surveys which ascertain the amount of protein normally consumed in the food by presumably normal persons subsisting on a normal dietary. As the food consumption of populations is usually determined by geographical, climatic, economic and religious factors which have no relevance to metabolic requirements it is only natural that the standards derived from such surveys vary from country to country, from one

economic class to another and curiously enough from time to time. At the beginning of this century it was customary to recommend very high protein levels because surveys carried out at that time showed high protein content in actual foodstuffs consumed. Thus in a survey carried out between 1804-1904 in U.S.A. when the food consumption of 15,000 people was examined, the average protein intake in different classes of the population was found to be between 100 and 175 g. Special categories such as lumbermen and college athletes were found to consume between 160 and 270 g. Similar high levels were found among manual labourers in Russia, Sweden and England and among soldiers in Germany. As an example of a recent survey that of Cathcart and Murray may be mentioned. In a study of the diets of 149 families representing a very varied population these authors found the following values for the protein intake.

TABLE I.

Grouping.	Protein per man per day g.	% of Animal Protein.
I Professional class	100.4	63.7
II Intermediate	90.9	59.4
III Shopkeepers	89.7	34.9
IV Skilled workers	87.5	52.6
V Unskilled workers	72.9	45.9
VI Unemployed	57.9	47.7
VII Mother with young family	79.1	52.1

High Versus Low Protein Diets.—It has been said that if actual foodstuffs consumed are used as the criterion one can with equal justification prescribe a standard of either 300 g. or of 30 g. There are populations that subsist at all levels between these limits, the Eskimo consuming an almost entirely animal diet and the Indian living on a practically complete vegetarian diet representing probably the two extremes. It is therefore not surprising that in contrast to the high protein levels recommended by the older authorities Chittenden and others have suggested that much smaller quantities are sufficient for adequate nutrition. Advocates of low protein diets base their arguments in the first place upon experiments in which individuals have been able to maintain themselves in normal health and activity upon diets

containing the minimum protein required for attaining nitrogen balance, that is on quantities of about 40-50 g. of protein. It is however doubtful if short-term experiments of the type carried out can throw any light on the effect on health and efficiency of prolonged subsistence on a low protein diet. In the feeding of live-stock (q.v.) where long-period trials are possible it has been found that the optimum quantity of dietary protein is twice the amount required for the maintenance of N balance. Statistical studies have shown that under conditions of vigorous muscular exercise there is a greater demand for protein in the food. In the studies made by Cathcart on the food consumption of populations there was a constancy in the proportion of calories derived from protein to the total energy intake in different classes of people. These facts would indicate that there are factors other than the mere maintenance of N equilibrium which determine protein intake.

It has also been suggested vaguely that a high protein diet is undesirable on account of the load it throws on the kidneys. Experiments on animals have shown no evidence of this. Animals on a high protein diet were found to have larger kidneys purely as a natural, physiological response, but manifested no pathological disturbances. As Robertson pointed out the "load" on the kidney is in any case easily lightened by a copious intake of water. Vital statistics pertaining to such countries as Australia would indeed seem to indicate that an unusually high protein intake is positively beneficial to health. Experiments on animals point to the same conclusion. The optimum level of protein for the rat is generally considered to be 14%. But exceptionally fine stocks have been bred on diets containing 30% protein. Further, rats, rabbits and cows allowed a free choice ate a diet containing 30% of protein. There is some reason to believe that the lower basal metabolism recorded for many tropical populations is the result of a low protein diet.

Protein Standards.—It is now generally agreed that the daily requirement of protein for an adult is between 1 and 1.5 g. per Kg. body weight. The Ministry of Health of Great Britain suggested in 1934 a daily intake of 80 to 100 g. per man. 1 g. per Kg. of body weight was laid down as the minimum by the League of Nations Health Committee. Growing children, pregnant women and nursing mothers require a much higher proportion. The allowances of protein recommended by the League of Nations Committee for these categories are as follows:

TABLE II.*

Age (years).	Protein (g. per Kg. body weight).
1-3 ..	3.5
3-5 ..	3.0
5-15 ..	2.5
15-17 ..	2.0
17-21 ..	1.5
21 and upwards ..	1.0
Women :	
Pregnant ..	1-1.5
Nursing ..	2.0

In stating nutritional requirements of protein, quality is perhaps of greater importance than quantity. It is obvious that as the function of food protein is the formation of tissue protein, those proteins will be most useful in nutrition which have an amino-acid composition most closely approximating to human tissues. Proteins of animal origin such as milk, meat, fish and eggs must therefore have a much higher nutritive value than vegetable proteins. Many vegetable proteins are known which are lacking in one or more of the amino-acids. The alcohol soluble proteins of corn (zein), wheat (gliadin), barley (hordein) are well-known examples. Zein is completely lacking in lysine and deficient in tryptophane, while gliadin and hordein are both deficient in lysine. Animal proteins on the other hand have a high content of essential amino-acids. Gelatin lacking in valine, tyrosine, tryptophane and methionine is only an apparent exception since from the method of its preparation it is to be considered a protein degradation product rather than a true protein. Low nutritional efficiency resulting from unsuitability of amino-acid composition is reflected in the determinations of biological value (Table III) carried out by the Thomas method and its modification which, in spite of the discrepant results obtained by different workers, are all in agreement in assigning to vegetable proteins much lower values than to proteins of animal origin.

*From Problem of Nutrition, Vol. II (League of Nations).

TABLE III.†

Food.	Digestibility. %	Biological Value. %
Whole egg ..	100	94
Milk ..	100	85
Egg white ..	100	83
Beef liver ..	90	77
Beef kidney ..	99	77
Beef heart ..	100	74
Beef round ..	96	69
Pork ham ..	100	74
Veal ..	100	62
Rolled oats ..	90	65
Whole wheat ..	91	67
Whole corn ..	95	60
Potato ..	78	67
Navy beans ..	76	38

But apart from amino-acid composition vegetable proteins are at a further disadvantage on account of their low digestibility which makes their nutritional efficiency lower still than is indicated by their biological values. Digestibilities of proteins as determined from the proportion not excreted in faeces in feeding experiments show that while animal proteins such as those of meat and milk are practically completely digested, cereal proteins have a digestibility coefficient of about 90, with the legumes coming still lower in the scale (Table III). The low absorption of vegetable proteins seems to be due, on the one hand, to their inherent resistance to the action of the digestive enzymes as in the case of groundnut globulin and the proteins of many leguminous seeds in the uncooked state and, on the other hand, to the action of indigestible carbohydrate material present in vegetable foods which protects the protein from attack by the enzymes of the alimentary tract.

In the few animal experiments that have been carried out on the utilisation of proteins for specific functions such as haemoglobin formation and reproduction, the decided superiority of animal proteins for these purposes has been clearly demonstrated. Rats suffering from nutritional or phenylhydrazine anaemia recover much more rapidly on diets containing casein or egg albumen than when ingesting wheat gluten. A diet with wheat

†From Mitchell and Hamilton: The Biochemistry of the Amino Acids.

as the exclusive source of protein was found to be inadequate for reproduction in rats. Dogs maintained on a diet free from animal protein have been found to suffer from gastric ulcer and edema and to show a lowering of the blood proteins. In long term experiments carried out in China it was found that animals ingesting a purely vegetarian diet, showed slower growth, a slightly lower basal metabolism and possessed diminished powers of nursing the young than animals on a mixed diet. These effects were found to be accentuated in successive generations.

In formulating dietary standards these facts have to be taken into account and both the British Ministry of Health and the League of Nations Committee stipulate that a certain portion of the protein intake should be first-class protein of animal origin, although the exact proportion has not been specified. In the absence of more precise information a third of the total protein is usually considered adequate for adults. However, in the study made by Cathcart on a group of young women who were allowed a free choice of food, 58.5% of the total protein in the diets eaten was found on the average to be of animal origin. In the survey by Cathcart already referred to (Table I), in most families animal protein constituted nearly half the total consumption. For growing children two-thirds of the total protein is the level of animal protein usually recommended.

The Protein Problem in India.—The issue of the controversy between high and low protein is at present hardly relevant to the protein problem in India as the prevailing protein intake is hopelessly inadequate especially in regard to quality in comparison with any reasonable standard that might be suggested. The general level of protein in our dietary can be gauged from studies on the urinary excretion of nitrogen made in Indian Laboratories. Studies on 30 students including vegetarians and non-vegetarians carried out in Madras showed an average protein intake of about 35 g. the lowest value found being 25 g. McCay has recorded still lower values for Bengalis. This indicates an intake lower than half the League of Nations standard, but in reality the disparity is much greater than these changing standards suggested by various authorities would imply. Dietary surveys have necessarily to be limited to small groups and if experience of populations and not physiological considerations are to be the guide it is well to consider the question broadly. The figures published by the League of Nations for the per caput consumption of animal protein and of milk in various countries (Tables IV &

V) show at a glance the extreme insufficiency of the average Indian diet with respect to protein of high biological value derived from animal sources.

TABLE IV.*

Per Caput Consumption of Animal Protein in Different Countries.

	Meat /day.	Milk Oz/day.	Eggs Annual	Fish Kg Annual.
U. K.	.. 171	39	172	18.1
U. S. A.	.. 170	35	252	6.8
Australia	.. 251	45		
New Zealand	.. 293	56		
Sweden	.. 106	61	110	
Denmark	.. 155	40	86	11.3
Italy	.. 65	10	119	5
Germany	.. 137	34	129	9.5

TABLE V.

Milk Production and Consumption per Head for Twenty Countries.

Country.	Daily Production. per Head Oz.	Daily Consumption per Head Oz.
New Zealand	.. 244	56
Denmark	.. 148	40
Finland	.. 74	63
Sweden	.. 69	61
Australia	.. 69	45
Canada	.. 66	35
Switzerland	.. 65	49
Netherlands	.. 54	35
Norway	.. 45	43
U. S. A.	.. 37	35
Czechoslovakia	.. 36	36
Belgium	.. 35	35
Austria	.. 35	30
Germany	.. 34	35
France	.. 33	30
Poland	.. 27	22
Great Britain	.. 14	39
Italy	.. 11	10
Rumania	.. 9	9
India	.. 8	7

* From Problem of Nutrition, Vols. IV & V (League of Nations).

It has to be noted that the consumption figures in Table IV are incomplete with regard to certain commodities such as fish and that they do not include protein derived from vegetable sources. This latter must amount to considerable quantities in countries where wheat with a protein content of about 11% is the staple cereal in contrast to this country with its food economy based upon rice with a protein content of 6%. For a large proportion of the people of this country the only animal protein that is of any importance is that derived from milk. The present consumption of milk in India has been estimated at 7 Oz. per head, provinces like Madras and Bengal averaging about 2 Oz. (Table VI).

TABLE VI.†

Milk Production and Consumption by Provinces.

Province.	Daily Production. per Head Oz.	Daily Consumption. per Head Oz.
Assam	1.4	2.2
Bengal	3.1	1.9
Madras	3.6	1.6
Bombay	4.7	4.0
United Provinces	4.7	5.0
Central Provinces	6.1	0.8
Bihar & Orissa	6.4	3.2
Punjab	18.3	9.9

To raise it to the levels in European countries (where it must be remembered milk forms only a small fraction of the total intake of animal protein) represents a five-fold increase in milk-production. This is a goal which at present can be looked upon only as an ideal. As an immediate objective we may be content with the standard suggested by Dr. Aykroyd of 65 g. of protein with a fourth of the amount in the form of milk protein. Even this modest requirement fixed admittedly low in view of the economic circumstances and dietary habits of the country implies a doubling of the existing milk production. As the dairy animal can synthesise milk protein only at the expense of suitable nitrogenous material in its food the problem resolves itself into the proper nutrition of live-stock.

(To be continued)

†From Wright: Report on the Development of the Cattle and Dairy Industries of India (Government of India).

THE DEVELOPMENTAL MORPHOLOGY AND CYTOLOGY OF MARCHANTIA PALMATA NEES*

By

K. S. SRINIVASAN, B.Sc. (HONS.), M.Sc.

Three species of *Marchantia* have been so far collected by Prof. M.O.P. Iyengar from South India, viz., *Marchantia polymorpha* from Kodaikanal on the Pulneys and Ootacamund on the Nilgiris, *Marchantia palmata* from Ootacamund and a sterile species, which comes near *Marchantia linearis*, from the sides of the Tiger-hill-shola stream at Kodaikanal. *Marchantia polymorpha*, the classical type which is usually studied in all our University classes, occurs only in a stray manner on the hill tops of South India. But *Marchantia palmata* grows in plenty at Ootacamund. Since this species is so easily available and in such profusion and will serve as an additional type for Indian Universities, a detailed investigation of this liverwort was taken up at the suggestion of Prof. Iyengar who very kindly placed all his valuable material at the writer's disposal.

Material and Methods.

Marchantia palmata grows in plenty almost throughout the year on the sides of the channels and trenches in Ootacamund on the Nilgiris about 7500 ft. above sea-level. In addition to Prof. Iyengar's material, fresh collections of the liverwort were also made by the author from time to time from Ootacamund. Living plants were also brought down to Madras and grown in the laboratory. The material was fixed in Allen Bouin's P.F.A.₃ mixture and Flemming's strong solution in the field and also in the laboratory. It was imbedded in paraffin and sections were cut 2–20 μ thick and stained in Heidenhain's iron-alum haematoxylin, safranin and safranin and gentian-violet.

*From the Department of Botany, University of Madras, Madras.

Description of the thallus.

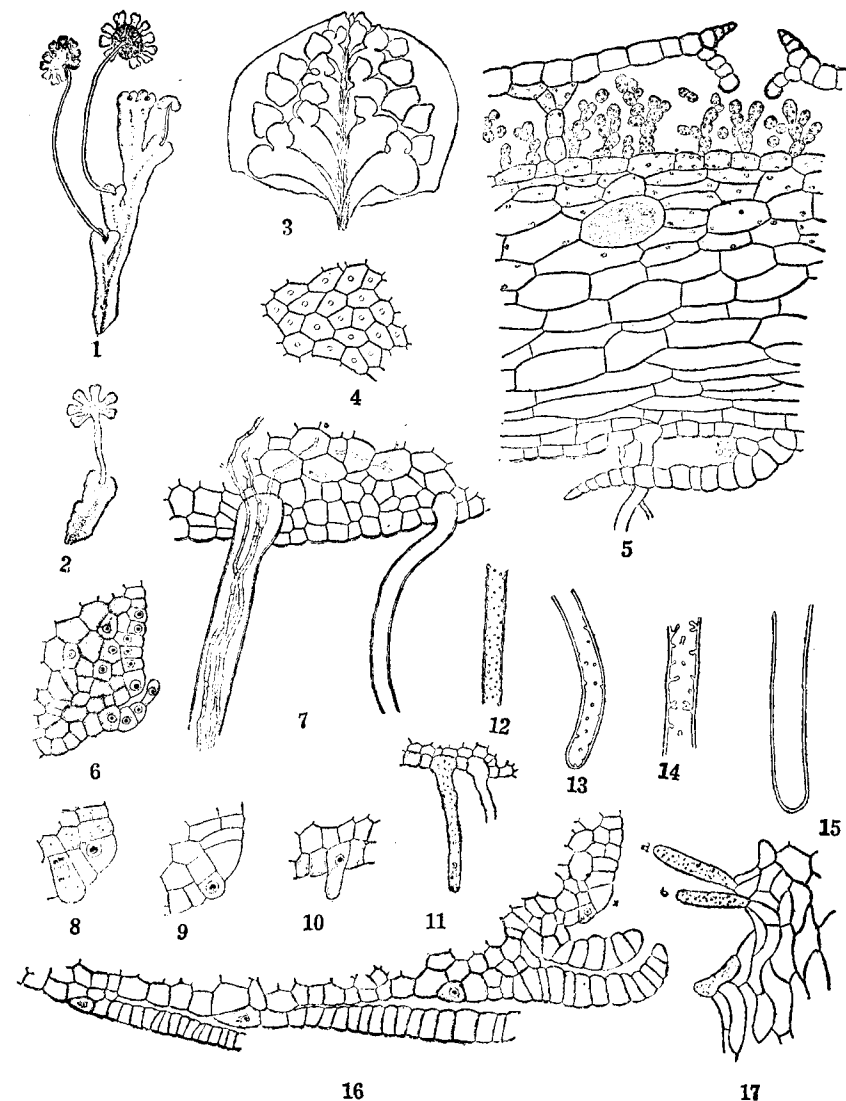
The liverwort grows in dense patches and forms fairly extensive growths on the substratum. The thallus is robust, dichotomously divided and has a more or less entire margin. The lobes do not diverge very much, but run very nearly parallel to each other (Text-fig. 1). The thallus is about 3—12mm. broad and 2.5—7cm. long. The dorsal surface is more or less flat with a dark purple line running along the middle of the thallus. A few gemmae cups are found at long intervals on the middle of its dorsal surface. The ventral surface is brownish with two to three rows of scales on either side of the central region (Text-fig. 3). A dense growth of rhizoids is seen along the central region.

Structure of the thallus.

The dorsal surface of the liverwort, when examined with a hand lens or a dissecting microscope, appears to be divided into a number of polygonal areas, each with a minute pore in the centre (Text-fig. 4). These pores are smaller in the anterior growing region, but become larger in the older posterior region.

The thallus in median longitudinal section shows three regions (Text-fig. 5), (1) the dorsal region, consisting of the upper epidermis and the region of the air-chambers, (2) the middle region, consisting of several layers of thin walled parenchymatous cells, and (3) the ventral region, consisting of the lower epidermis with one or two layers of colourless cells immediately above.

In the dorsal region, the epidermis is made up of a row of regular cells each containing a few small chloroplasts in them. From the floor of the air-chambers arise a number of branched filaments consisting of rows of spherical or ovoid cells containing numerous chloroplasts. The cells lining the floor of the air-chambers are polygonal in section and contain a few chloroplasts. Further down below, the cells have a few chloroplasts, but the cells lower still are devoid of chloroplasts and gradually become more elongated along the longitudinal axis of the thallus and form the central conducting tissue of the thallus (Text-fig. 5). In the middle region are found scattered a number of large mucilage cells (Text-fig. 5). Besides, in this region, several oil-bodies are seen in the older portions of the thallus. From the cells of the lower epidermis are formed the ventral scales and rhizoids which grow closely adpressed to it (Text-figs. 3, 5).



Text-figs. 1-17. Fig. 1. A female plant of *Marchantia palmata* ($\times 5/8$). Fig. 2. A male plant ($\times 5/8$). Fig. 3. Ventral surface of the thallus showing amphigastria and rhizoids ($\times 4 1/2$). Fig. 4. Dorsal surface showing the polygonal areas with pores ($\times 6$). Fig. 5. Section of the thallus ($\times 62$). Fig. 6. Median longitudinal section of the thallus through the apical region ($\times 242$). Fig. 7. Fungal filaments in the rhizoids ($\times 112$). Figs. 8-15. Development of rhizoids. Figs. 8, 9, 10 ($\times 242$). Figs. 11, 12, 13, 14, 15 ($\times 187$). Figs. 16. Development of amphigastria ($\times 242$). Fig. 17. Ventral scale with mucilage hairs ($\times 112$).

The thallus grows by means of an apical cell which is situated in the notch of the thallus. In median longitudinal section, the apical cell is situated more towards its ventral rather than its dorsal portion and is more or less similar to those of the other Marchantiales (Text-fig. 6). It cuts off segments dorsally, ventrally, and also laterally. The dorsal segments form the median dorsal portion of the thallus and the two lateral segments contribute to the formation of the wing portion of the thallus and the ventral segments give rise to the amphigastria and the rhizoids.

Rhizoids.

The rhizoids are of four kinds. Some show in their mature condition a rather thick wall with numerous peg-like thickenings (Text-fig. 12). These rhizoids are about 10–13 μ broad. Others are very similar to the first type, but are 12–15 μ broad and the peg-like thickenings are less crowded (Text-fig. 13). Sometimes the peg-like thickenings in these rhizoids show branching also (Text-fig. 14). The third type, which is still broader and measures about 16–25 μ in thickness, but with the greater part of its wall quite smooth and with only a few pegs here and there. The fourth type, which is the broadest and measures about 25–35 μ in thickness, has quite smooth walls without any pegs whatever (Text-fig. 15). Some of these smooth walled rhizoids occasionally show branching.

Each rhizoid takes its rise from an epidermal cell. The epidermal cell giving rise to the rhizoids becomes enlarged and forms a cylindrical outgrowth downwards (Text-figs. 8, 9, 10). As this projection grows in length, the nucleus passes down into the elongated tubular portion. A certain amount of cytoplasm is seen accumulated at the tip of the cell and also lining the wall of the growing rhizoidal portion (Text-fig. 11). When the rhizoids reach their full length hardly any cytoplasmic contents could be detached in them.

Amphigastria.

One of the superficial cells of the ventral segment very near the growing point divides into an outer and an inner cell by a wall parallel to the outer surface (Text-fig. 16). The outer cell is richer in contents and by further divisions in two directions ultimately forms a flat membranous outgrowth, which in section shows a single layer of cells. These membranous structures (amphigastria) curve outwards and form a sort of a protecting cover over the apical

region (Text-fig. 53). These amphigastria bear long club shaped mucilage cells with rich contents and a large nucleus in each (Text-fig. 17).

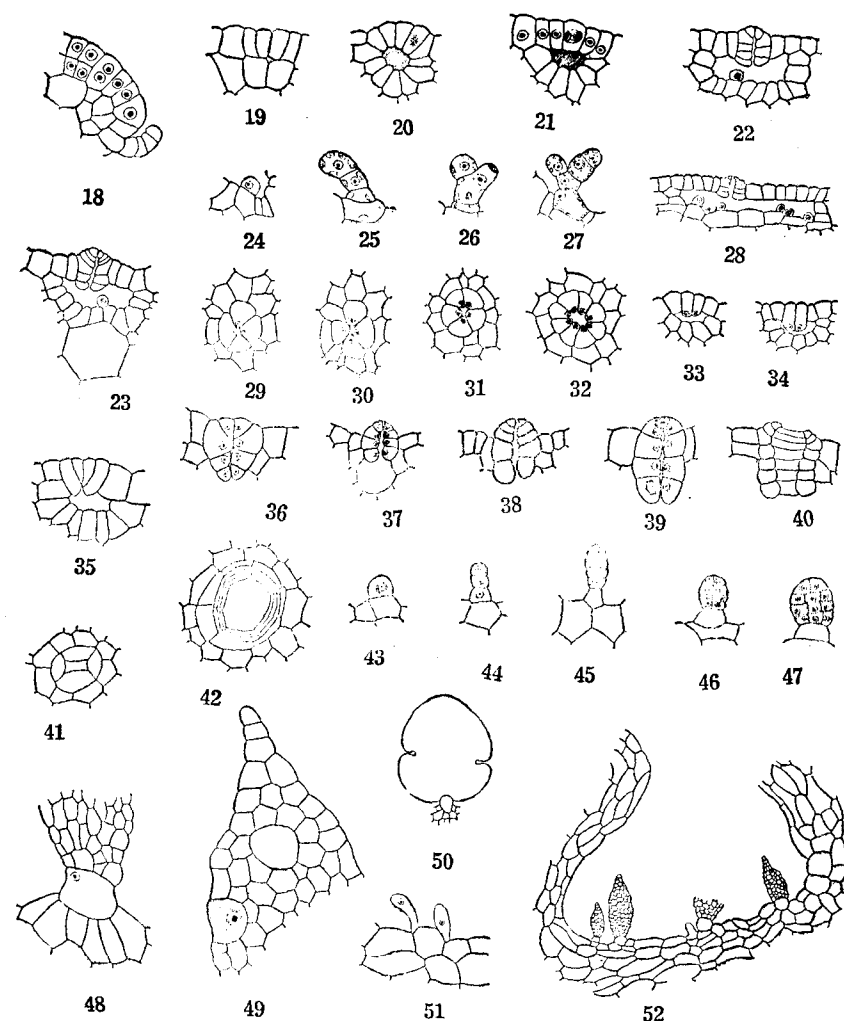
Air-chambers.

The development and the formation of the air-chambers is quite similar to those of *Marchantia polymorpha* (Barnes and Land, 1907). A dorsal epidermal cell, a little behind the apical cell divides first into two by a wall parallel to the outer surface (Text-fig. 18). Very soon another wall at right angles to the first wall is cut off dividing the cell into four cells (Text-fig. 18). Next, by the formation of another wall at right angles to the second wall, two tiers of four cells are formed. A slit appears between these two tiers of four cells and this slit gradually increases in size and separates these two tiers of cells (Text-fig. 19). At the same time the cells surrounding the space divide and increase in number (Text-figs. 20, 21). As the space becomes larger and larger, the upper layer becomes slightly raised by the further division and growth of its cells and soon a small chamber is formed with the upper layer forming the roof and the lower layer the floor as it were of the chamber (Text-figs. 22, 23). Later on, this chamber becomes further elongated (Text-fig. 28). Very soon, small papillate cells grow out from the floor of the much elongated air-chamber (Text-figs. 22, 23, 28). These cells soon grow out into branched filaments and the cells of these filaments contain a single nucleus in the centre and a number of chloroplasts near the periphery (Text-figs. 24, 25, 26, 27).

The development of the chimney-cells and air-pores.

One of the cells about the middle region of the roof portion of the air-chamber divides into two by a vertical wall (Text-fig. 21) and the two resulting cells grow slightly larger than the adjoining cells and become more elongated in a direction perpendicular to the epidermis with their lower portions slightly projecting into the chamber below (Text-figs. 33, 34). Very soon, another vertical wall is formed in each of these two cells at right angles to the first wall. A small space then appears at the crossing of the two primary walls. At this stage, in surface section, this space is seen bounded by four wedge-shaped cells (Text-figs. 29, 30). Next a series of transversely oblique walls, about ten in number, is formed along the whole length of the four primary cells bordering the

central space, thus making up several tiers of cells arranged one above the other (Text-figs. 35, 36, 37, 38, 39, 40). Soon, in each of these tiers, vertical walls are formed which are disposed more or less radially about the central space and ultimately increasing the number of cells bordering the space (Text-figs. 31, 32). This increase in the number of cells bordering the central space is not uniform throughout. The lowermost tier consists of only four or five cells (Text-fig. 41), while the uppermost one consists of five to seven or eight cells (Text-figs. 32, 42).



Text-figs. 18-52. Figs. 18-23. Stages in the development of the air-chamber. Fig. 18 ($\times 242$). Figs. 19-23 ($\times 340$). Figs. 24-27. Development of the assimilatory filaments ($\times 385$). Fig. 28. Elongated air-

The fully developed pore is made up of a number of concentric rings of cells, leading into the air-chamber. About two to three rings of cells of the air-pore stand slightly above the level of the epidermal cells (Text-figs. 5, 22, 23, 37, 39, 40). The pore is circular to elliptic towards the outside (Text-fig. 42), but more or less quadrate towards the inside (Text-fig. 41).

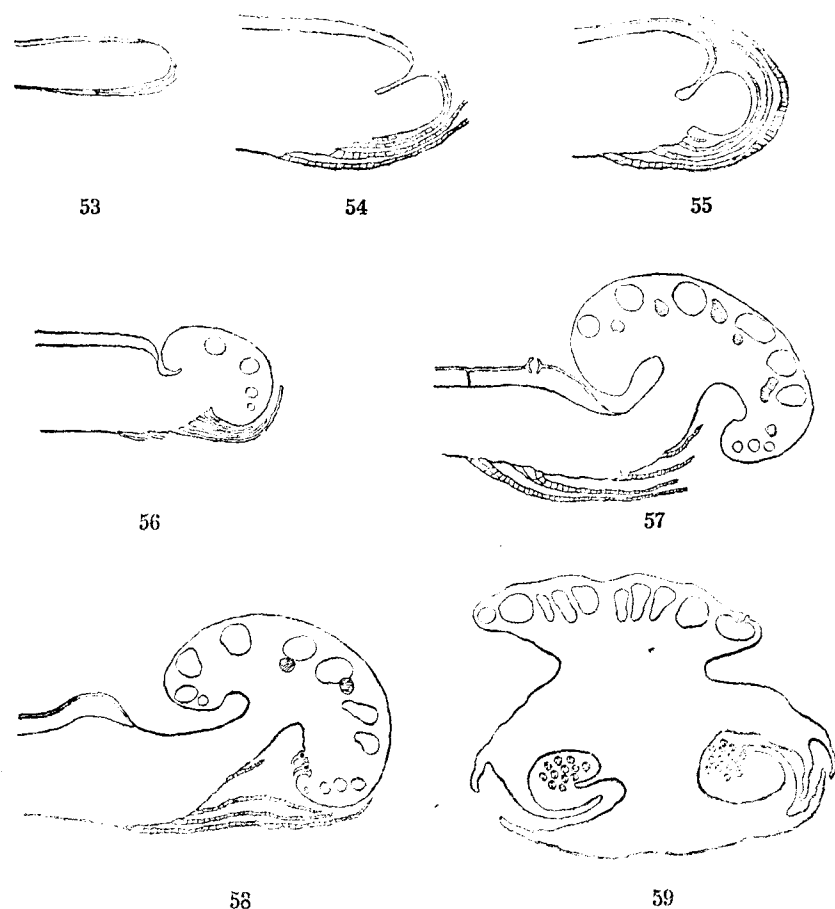
Development of the gemmae.

A large number of gemmae is formed inside each gemmae cup (Text-fig. 52). The development of the gemmae was followed and was found to be similar to what has already been recorded for *Marchantia polymorpha* (Campbell, 1918b; Barnes and Land, 1908). Each gemma arises as a papillate growth of one of the surface cells at the bottom of the cup (Text-fig. 43). In this cell, a series of transverse divisions takes place dividing it into a row of cells (Text-figs. 44, 45). Next, vertical and periclinal walls are formed in all the cells of this row except in the basal cell which remains undivided (Text-figs. 46, 47). Ultimately divisions take place in all directions with the result that a more or less lenticular body with a large basal cell is formed (Text-figs. 48, 52). The fully developed gemma is a fiddle-shaped structure attached to the base of the cup by the single basal cell, which by this time becomes grown into a very large attaching cell (Text-fig. 50). The cells of gemma show a number of chloroplasts and oil bodies. Certain cells, however, attain larger dimensions than the neighbouring cells and become rich in contents (Text-fig. 49). As the gemmae are developing, a number of club-shaped hairs secreting mucilage is also formed from the floor of the cup (Text-figs. 51, 52).

Development of the male and the female receptacles.

Plants bearing the female receptacles are quite common, but plants with male receptacles are comparatively very rare. The

chamber with assimilatory cells developing from the floor ($\times 187$). Figs. 29-32. Surface section of the pore showing development ($\times 270$). Figs. 33-40. Vertical section of the pore showing development. Figs. 33, 34 ($\times 187$), Figs. 35, 36 ($\times 270$), Figs. 37, 38 ($\times 187$); Fig. 39 ($\times 270$), Fig. 40 ($\times 70$). Fig. 41. Inner quadrate pore, ($\times 112$). Fig. 42. Outer circular pore ($\times 112$). Figs. 43-47. Development of gemmae ($\times 270$). Fig. 48. Basal portion of gemma showing the large attaching basal-cell ($\times 187$). Fig. 49. Section of the gemma showing a large mucilage cell and the cell with rich contents ($\times 187$). Fig. 50. Fully developed gemma ($\times 187$). Fig. 51. Mucilage cells starting from the base of the cup ($\times 187$). Fig. 52. Section through a cup showing a few gemmae still attached to the cup, with a few mucilage cells ($\times 47$).



Text-figs. 53-59. Figs. 53-58. Development of the male and the female receptacles. Fig. 53 ($\times 30$). Figs. 54, 55, ($\times 64$). Fig. 56 ($\times 30$). Figs. 57, 58 ($\times 20$). Fig. 59. Transverse section of the stalk of a female receptacle showing the rhizoidal furrows ventrally and air-chambers dorsally ($\times 75$).

male receptacle is a flattened disc with a number of radiating lobes (Text-figs. 2, 61). It starts as a cushion-like out-growth from the apex of the thallus (Text-fig. 54). This cushion-like out-growth, which represents the modified apical portion of the thallus, gradually increases in size and is well protected by a growth of upwardly curved amphigastria. A median longitudinal section of this young receptacle shows a single cell more or less triangular in section, resembling the apical cell of the vegetative thallus. While examining the remaining sections on either side of the median one, a few more such triangular cells could be distinguished showing that there are

several apical cells. These growing points are presumably derived from the original apical cell of the thallus through frequent division. This young receptacle with the several growing points should be considered as a composite structure which has resulted by the repeated dichotomy of the apex of the thallus and may be considered to belong to the "Composite type" of Leitgeb (1881). As the receptacle grows, it forms a disc and the growth of its ventral and dorsal sides is more or less equal as in the case of the vegetative thallus and the several growing points are situated at the marginal portion of the disc. With the growth of the receptacle, the tissue immediately behind it also increases and forms as it were a short stalk (Text-fig. 56). The fully formed male receptacle is carried far above the thallus by the further development of this stalk. The stalk shows two rhizoidal furrows on its ventral side and air chambers on its dorsal side. The fully formed disc is very variable in shape. It may be a mere disc with a number of very short marginal lobes or it may have long, narrow and diverging lobes. The number of lobes in a receptacle varies from one to eight. All the lobes of the receptacle may be equally developed or some of the lobes may attain very large dimensions while the others may remain very small. Their lengths vary from 1-12 mm. The stalk is also likewise variable. Usually it measures from 15-20 mm. in length but occasionally it reaches 25 mm. Sometimes the stalk is very short measuring only 2 mm. in length.

The receptacle develops on its dorsal side the characteristic air-chambers with the assimilating filaments in them. There are also seen large mucilage cells scattered in the tissue of the receptacle. Ventrally it forms amphigastria and a few pegged rhizoids.

The female receptacle, in its earlier stages, does not differ very much from that of the male. It also starts as a cushion-like out-growth in the notch of the thallus (Text-fig. 55, Pl. II, fig. 1.), and as in the male receptacle, this cushion-like outgrowth of the female receptacle represents the modified apical portion of the thallus. It also shows a number of growing points, presumably derived through the division of the original apical cell of the thallus. As the young receptacle grows, the tissue immediately behind it forms a short stalk (Text-figs. 57, 58). In its later stages of development, however, it differs from the male receptacle in that the dorsal surface of the knob-like receptacular portion becomes excessively developed, while the growth of its ventral surface is very limited with the result that the several growing points of the receptacle come to be situated very close to the stalk (Text figs. 58, 63). The short

stalk of the receptacle gradually curves and grows upwards into an erect structure and the young receptacle is seen as a knob-like structure slightly raised on this erect stalk (Text-fig. 1). Along with the further development of the receptacle the stalk also grows in length and ultimately the receptacle is carried far above the thallus on the much elongated stalk (Text-fig. 1).

The margin of the receptacle which is at first more or less entire becomes indented later on and ultimately a number of lobed structures is developed about the margin (Text-figs. 1, 60). The several growing points of the receptacles take a downward and inward direction as in the case of *Marchantia polymorpha* (Text-figs. 63, 65). While in *Marchantia polymorpha* the portions between the growing points grow out into long cylindrical finger-like appendages, in *Marchantia palmata* they form broadly rounded lobes (Text-figs. 1, 60).

The receptacle when young is somewhat hemispherical (Text-figs. 57, 58), but when older, it becomes more flat, and the fully developed receptacle is a more or less flat disc-like structure with seven to eleven lobes (Text-figs. 1, 60). The fully developed female receptacles measures about 12—14 mm. in diameter with a stalk about 30—45 mm. long.

The growing points as in *Marchantia polymorpha* form rows of archegonia on the lower side of the receptacle between the lobes, the youngest archegonium being situated nearest the stalk portion and the oldest furthest from it (Text-figs. 63, 65). Each group of archegonia produced by the several growing points is enveloped in a flap-like membranous structure (velum).

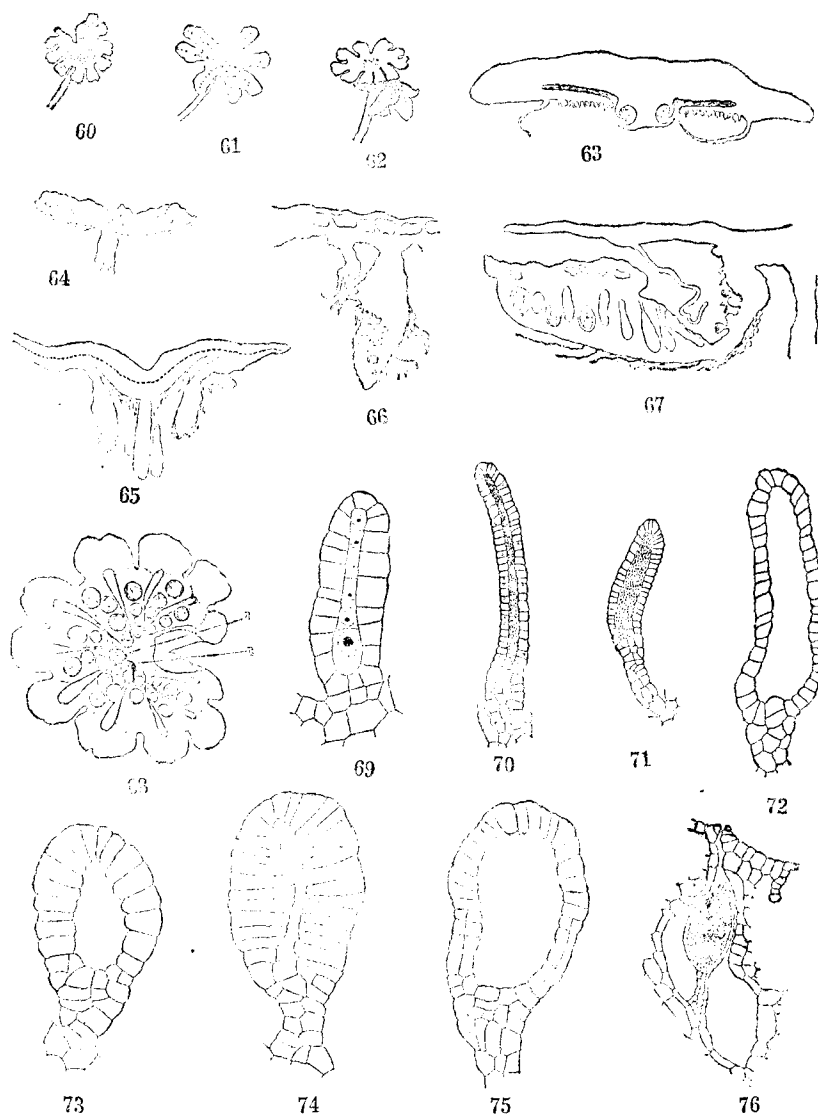
There are two rhizoidal furrows on the ventral side of the stalk clearly indicating that the latter is influenced by the first dichotomy of the shoot (Text-fig. 59).

When the receptacle is very young forming only a small cushion-like outgrowth, its tissue does not show any intercellular spaces (Text-fig. 55). Very soon, however, small spaces are formed on its dorsal surface and these develop into air-chambers with assimilating filaments inside, quite similar to those formed in the ordinary thallus (Text-figs. 57, 58). On the lower side of the receptacle, a number of scales and pegged rhizoids are developed. In many cases several *Nostoc* colonies are seen between the scales and the groups of rhizoids.

As in the case of the thallus, a number of mucilage cells is developed in the tissue of the receptacle (Text-figs. 57, 58). The peculiar oil-bodies which are seen in the older portions of the thallus are seen in several cells of the receptacle also. The mucilage cells are usually single but occasionally a row of them is formed, and ultimately may even become confluent through the absorption of their separating walls.

Usually only one receptacle is formed at the apical notch of the thallus. Very occasionally the stalk of the receptacle may branch very near the base and instead of the usual single receptacle, two receptacles may be formed at the apical notch. Such branching of the stalk of the receptacle in this liverwort is recorded by Kashyap also (1929, p. 35) but he says that the stalk was seen to divide into two near its upper end.

The female receptacle in this liverwort is extremely peculiar. It first forms a number of archegonia, but soon produces proliferations on which are developed only antheridia (Text-fig. 66). In other words the receptacle is female to start with, but ultimately forms male structures and becomes androgynous. The structure and development of these androgynous receptacles are described in full detail elsewhere (Srinivasan, 1939). The several lobes of the archegonia-bearing receptacles first produce a number of archegonia as in the other species of *Marchantia* and even sporogonia are developed from these archegonia (Text-figs. 63, 65). But the growing points of these several lobes become active once again and continue to grow as proliferations outside the umbrella portion of the receptacle (Text-fig. 68). Each of these proliferations, as it grows, turns gradually outwards and slightly upwards and finally brings the morphological dorsal portion to the upper side (Text-figs. 66, 67). The tip portion then continues to grow resembling a green narrow lobe of the thallus. Very frequently this portion may branch dichotomously into two spreading lobes, each of which may occasionally divide dichotomously a second time (Text-fig. 62). On these lobes a number of antheridia is formed (Text-figs. 66, 67). In the portion between the archegonial and the antheridial regions, structures intermediate between archegonia and antheridia are developed (Text-fig. 67). These structures appear to be abortive (Text-figs. 70, 71, 72, 73, 74, 75). Some of these intermediate structures are somewhat similar to those recorded in *Mnium cuspidatum* (Holferty, 1904) and *Mnium horneum* (Wilson, 1915).



Text-figs. 60-76. Fig. 60. A female receptacle ($\times 1$). Fig. 61. A male receptacle ($\times 1$). Fig. 62. An androgynous receptacle ($\times 1$). Fig. 63. Vertical section of a female receptacle ($\times 12$). Fig. 64. Vertical section of a male receptacle ($\times 12$). Fig. 65. Median vertical section of an older female receptacle ($\times 5$). Fig. 66. Section of an androgynous receptacle showing proliferating lobe ($\times 9$). Fig. 67. Section of a proliferation showing intermediate structures at its base and normal antheridia towards its anterior region ($\times 7\frac{1}{2}$). Fig. 68. Ventral view of an androgynous receptacle. Note the apex of two archegonia bearing lobes (P1) and (P2) growing out into a tongue like structure ($\times 2\frac{1}{2}$). Normal archegonium ($\times 385$). Figs. 70-75. Intersexual structures on the androgynous receptacles. Fig. 70 ($\times 72$). Fig. 71 ($\times 65$). Figs. 72, 73

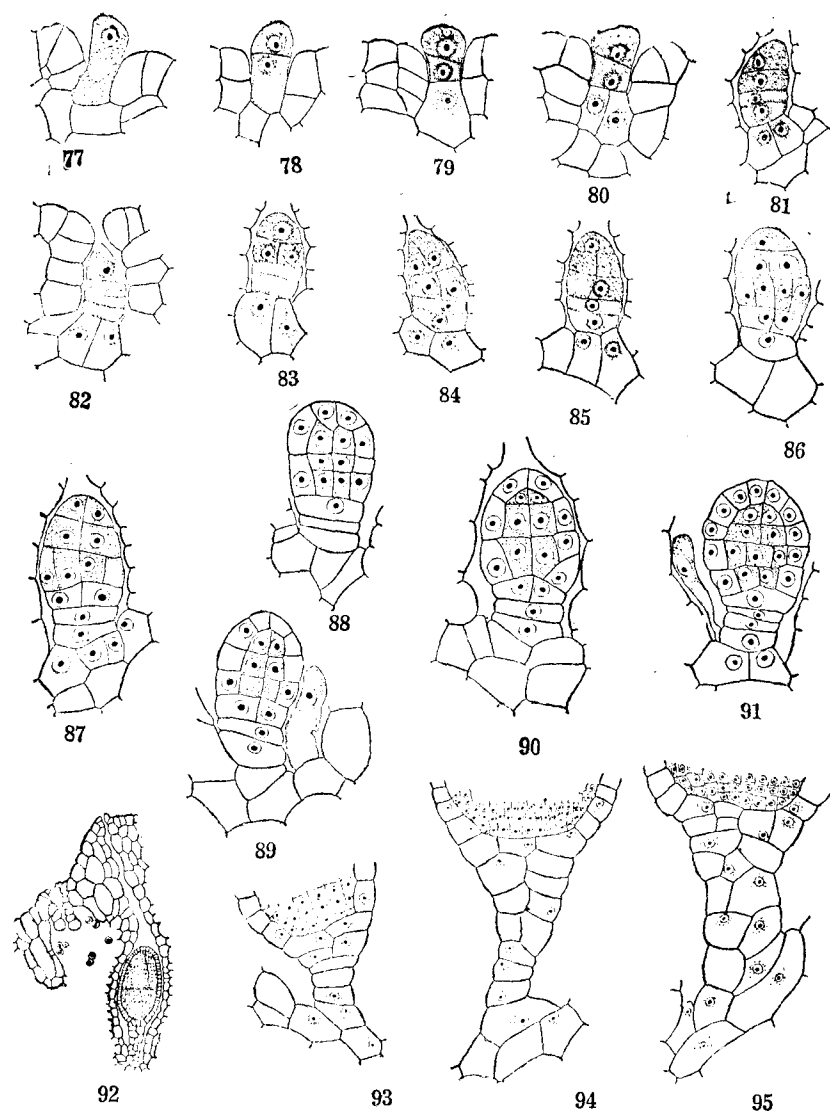
Cutting (1910) has given an account of androgynous receptacles in a species of *Marchantia* which was sent to him from Chelsea Physic Gardens, London, by Williams who referred the liverwort to *Marchantia palmata*. These receptacles resembled ordinary female receptacles, but a number of proliferations were growing from their lower surface. And on these proliferations were formed a number of antheridia sunk in cavities. He states that the male portion of these androgynous receptacles arises from a branch of the receptacle after this branch has been definitely differentiated as a female branch, and says that this is capable of continuing its growth and giving rise to a series of branches, which ultimately bear a large number of antheridia.

Development of the antheridia.

The antheridia are formed dorsally in acropetal succession from the several growing points of the male receptacle. They are arranged more or less regularly in two to four alternating rows in each lobe of the receptacle, but in the central portion they are more or less irregularly arranged.

The development of the antheridium is somewhat similar to that of the other Marchantiaceae. The antheridium-mother-cell is a papillate epidermal cell. This cell by further growth elongates and projects well above the adjacent cells (Text-fig. 77). In this cell, the first division wall is horizontal and nearly on a level with the surface of the thallus (Text-fig. 78). This is soon followed by another similar division in the upper cell with the result that the original cell is divided into three superimposed cells, of which the basal one, which is the largest, is coherent with the adjacent cells of the thallus, while the two upper ones are quite free (Text-fig. 79). Of the two upper ones, the outermost is the antheridial-cell proper and the lower one the stalk-cell. These first divisions exactly resemble those of *Sphaerocarpus* (Campbell, 1918b), *Geothallus* (Campbell, 1896) and *Fossombronia longiseta* (Haupt, 1929). The antheridial-cell then divides transversely once (Text-figs. 81, 82, 83, 84) or twice (Text-fig. 85) and forms two or three cells in a row and the stalk-cell also divides once forming a row of two cells with the result that a row of four (Text-figs. 81, 82, 83, 84) or

($\times 180$), Figs. 74, 75 (192). Fig. 76. A normal antheridium in the androgynous receptacle ($\times 41$).



Text-figs. 77-95. Fig. 77. Antheridium rudiment ($\times 385$). Fig. 78. Two-celled stage of the antheridium ($\times 385$). Fig. 79. Three-celled stage of the antheridium ($\times 385$). Fig. 80. Three-celled stage of the antheridium with the basal cell divided by a vertical wall ($\times 385$). Figs. 81, 82. Division of antheridial and stalk cells ($\times 385$). Figs. 83-85. Formation of vertical walls in the antheridial cells ($\times 385$). Figs. 86, 87. Formation of periclinal wall in the antheridial cells and a median vertical wall in the uppermost stalk cell ($\times 385$). Figs. 88-91. Further development of the antheridium ($\times 385$). Fig. 92. A well developed club-shaped antheridium inside a chamber ($\times 62$). Figs. 93-95. Divisions in the stalk cells ($\times 258$).

five cells (Text-figs. 85, 86), is formed above the large divided basal-cell. Vertical divisions occur in two planes at right angles to each other in the antheridial cells forming two or three tiers of four cells (Text-figs. 83, 84, 85). Soon periclinal divisions occur in these cells, cutting off a wall layer from a central group of spermatogenous cells (Text-figs. 86, 87, 88, 89, 90, 91).

As divisions are taking place in the antheridial-cells, either one or both the stalk-cells may divide transversely once with the result that a row of three (Text-fig. 87) or four (Text-fig. 91) cells is formed. Further divisions follow rapidly in the antheridium and the body of the antheridium grows large in size. The entire product of the antheridium except the wall-layer forms the sperms. As the antheridium enlarges, the stalk-cells also divide both transversely and vertically, the vertical divisions starting from above and progressing downwards (Text-figs. 93, 94, 95). The basal-cell usually divides by two intersecting vertical walls and forms a group of four cells at the base of the antheridium. This is well seen in a cross section at this level.

As these divisions are going on, the divided basal-cells also increase in size and offer a large base for the support of the enlarging antheridium (Text-figs. 93, 94, 95). The mature antheridium is a club-shaped structure with a well developed stalk situated on a group of enlarged basal-cells (Text-fig. 92). As the antheridium is developing, the adjoining cells also divide (Text-figs. 79, 80) and soon grow past the young antheridium with the result that the antheridium is finally left inside a chamber-like cavity communicating with the exterior through a pore (Text-figs. 81, 82, 92).

Usually only one antheridium is found in each chamber. Very occasionally two antheridia are found side by side in a single chamber. A few club-shaped cells grow out from the base of the antheridial-chambers secreting a large quantity of mucilage (Text-figs. 89, 91).

The development of the archegonia.

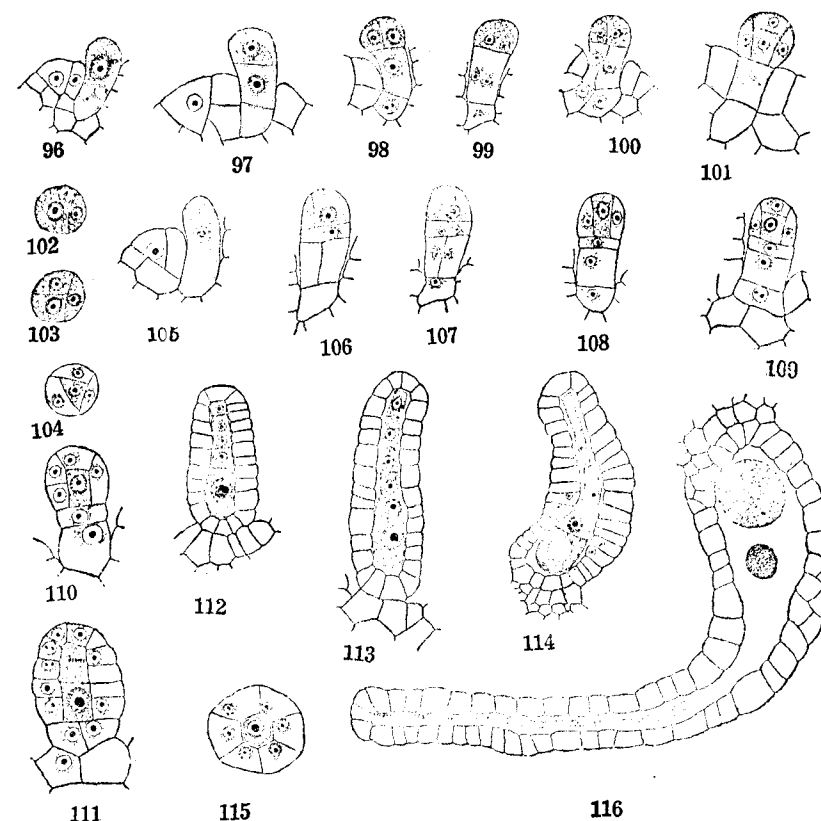
The archegonium starts from a superficial initial cell near the apical portions of the receptacle. This mother-cell of the archegonium is somewhat larger than the neighbouring cells and projects well above the level of the neighbouring cells (Text-figs. 96, 105).

The development of the archegonium from this mother-cell shows two types. In the first type of development, two transverse divisions take place in the archegonium-mother-cell, dividing it into a row of three cells (Text-figs. 97, 98, 99, 100, 101). In this three-celled stage, the lowermost cell is imbedded in the thallus, the middle cell is either completely free (Text-fig. 99) or coherent with the adjoining cells for a short distance near its base, while the uppermost cell is entirely free. The outermost cell gives rise to the archegonium proper, and the lower cells contribute to the stalk portion of the archegonium.

Sooner or later, the middle cell divides into two by a vertical wall (Text-fig. 100). The lowermost cell also may divide likewise vertically (Text-fig. 100). In the outer cell the divisions are those typical of the development of archegonia of liverworts in general. The usual three vertical intersecting walls cut off an axial cell from three peripheral cells (Text-figs. 102, 103, 104). From this axial-cell are formed the cap-cell, canal-cells, the ventral-canal-cell and the egg. And the peripheral cells by further divisions form a jacket for the central row of cells (Text-figs. 110, 111, 112, 113, 114, 116).

In the second type of development, in the mother-cell of the archegonium, transverse walls occur as in the previous case, dividing it into a row of three cells. But in this case, the middle cell cuts off by a transverse wall a very short collar-like cell at its top so that a row of four cells is formed (Text-figs. 106, 107, 108, 109; Cf. Text-figs. 99 and 106). The uppermost cell forms the archegonium proper in the same way as in the previous case, the collar-like cell by further divisions contributes to the floor of the archegonium and the two lower cells contribute to the stalk of the archegonium as in the first type.

The first type of development (Text-figs. 96-101) is more or less similar to that recorded by Humphrey (1906) in *Fossombronia longiseta* and by Campbell (1896) in *Geothallus tuberosus*. The second type of development in which the peculiar collar-like cell is cut off below the archegonial-cell proper (Text-figs. 105-109) shows a certain amount of similarity to that seen in *Pallavicinia Lyellii* (Haupt, 1918) and *Preissia quadrata* as figured by Haupt (1926, figs. 34, 35). In all the species of liverworts previously investigated only one of the above two types of development is recorded. The present liverwort is very interesting in showing both



Text-figs. 96-116. Fig. 96. Archegonium rudiment ($\times 385$). Figs. 97-101. Archegonium of the first type with three cells in a row ($\times 385$). Figs. 102-104. Transverse section of the archegonial cell showing the characteristic wall formations, ($\times 385$). Fig. 105. Archegonium rudiment ($\times 385$). Figs. 106-109. Archegonium of the second type with four cells in a row ($\times 385$). Figs. 110-114. Further development of the archegonium. Figs. 110, 111, 113 ($\times 385$). Figs. 112, 114 ($\times 258$). Fig. 115. Transverse section through the neck region of the archegonium ($\times 385$). Fig. 116. A fully developed archegonium with six neck-canal cells, but with nine nuclei ($\times 258$).

the types of development in the same liverwort, often side by side in the same section.

The mature archegonium usually contains six neck-canal-cells each with one nucleus. Occasionally the nucleus of some of the neck-canal-cells divides without any corresponding wall separating them, with the result that up to nine nuclei could be detected in the neck-canal-region (Text-fig. 116). Haupt (1942) found in *Cryptomitrium* that some or all of the neck-canal-cells may later

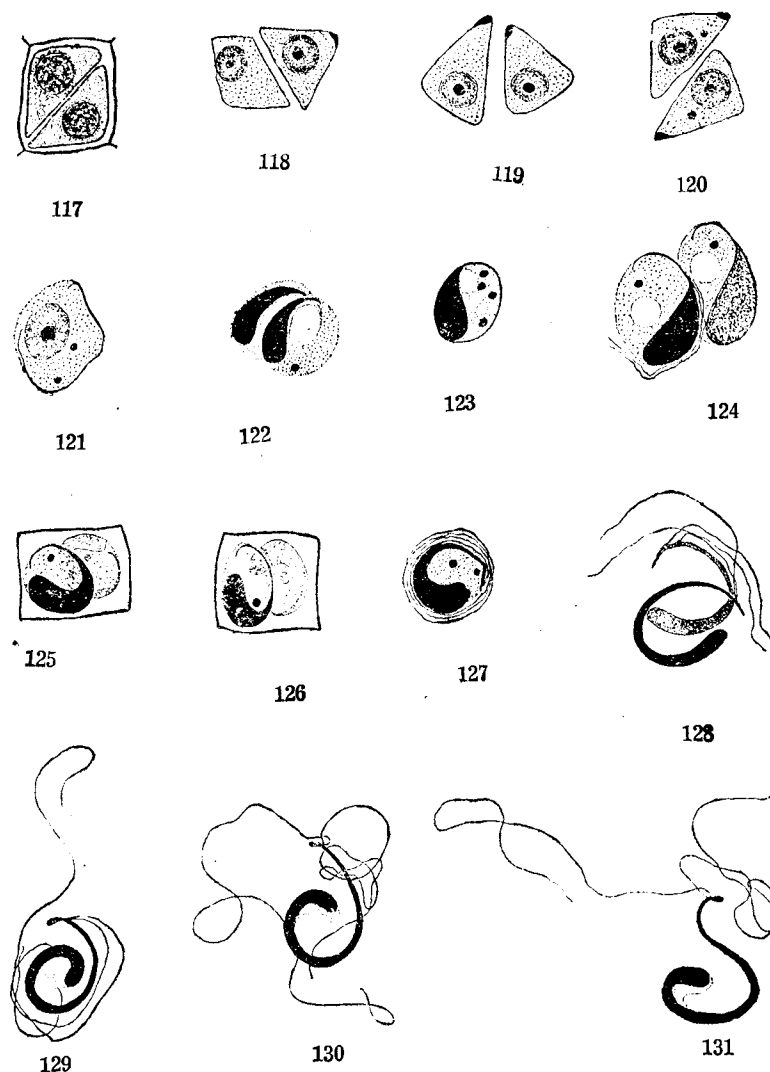
become binucleate. The neck is composed of six rows of cells in the canal region (Text-fig. 115), but in the venter this number is increased by radial division. In the mature archegonium the neck region is usually curved towards the periphery of the disc of the receptacle (Text-figs. 114, 116).

THE SPERMATOZOIDS.

The developmental stages of the spermatozooids were studied from microtome sections of fixed material. Living spermatozooids also were collected and stained preparations made of them. The following method was employed for this. The ripe antheridia are placed in a watch glass of freshwater for some time and examined under a Greenough binocular dissecting microscope. After a few minutes a cloud of spermatozooids is seen escaping out of the antheridium. The spermatozooids then are very carefully pipetted on to a slide and fixed in osmic vapour and stained in iron-alum haematoxylin.

By slightly pressing with a pair of mounted needles, the portion of a receptacle round the antheridial cavity, it is possible to squeeze out the contents of the antheridium in which could be seen the the spermatozooids in various stages of development.

The sperm-mother-cell is cubical in shape and contains a nucleus which very nearly fills the cell. During division the spindle is usually diagonal and after cell division in the sperm-mother-cell two triangular spermatids are formed (Text-fig. 117). Soon after the final division, a small globular or slightly elongated darkly staining body appears in the cytoplasm very near the apex of the triangular spermatids (Text-figs. 118, 119). This body very closely resembles the body that was recorded by Ikeno (1903), Wilson (1911), Woodburn (1911) and Sharp (1934) in the spermatids of various Bryophytes and is evidently the beginning of the blepharoplast. At a slightly advanced stage, besides the blepharoplast already referred to, another body appears close to the nucleus and usually between the nucleus and the blepharoplast (Text-fig. 120). This body more or less resembles the "Nebenkorper" recorded by Ikeno (1903) in *Marchantia polymorpha* and by Humphrey (1906) in *Fossombronia longiseta*. As development proceeds, the spermatids gradually begin to round off and the nucleus then occupies an eccentric position (Text-fig. 121). At a later stage, a small vacuole appears in the cytoplasm (Text-figs. 122, 123, 124). About this stage, sometimes even earlier, some two to six darkly staining small bodies appear in the cytoplasm (Text-fig. 123 Pl. II, fig. 4).



Text-figs. 117-131. Various stages in the development of the spermatozooids. Figs. 117-128 ($\times 2820$). Figs. 128-131 ($\times 2466$).

Similar bodies have been observed in the spermatogenous cells and the spermatids of many Bryophytes by various workers (Wilson, 1911; Woodburn, 1911, 1915; Motte, 1928). As the development proceeds, the vacuole enlarges in the spermatid and the nucleus also begins to elongate (Text-figs. 122, 123, 124). As the nucleus elongates, one end of it becomes very narrow while the other end is broadened. Often the elongated nucleus takes a more or less crescent shape (Text-figs. 125, 126).

The narrow end of the nucleus ultimately becomes the anterior portion and the broadened end the posterior. In a more or less fully developed spermatozoid, the extreme anterior portion is very lightly stained and is clearly marked off from the rest of the spermatozoid body (Text-figs. 128, 129, 130, 131). From the base of this slightly stained portion, two long cilia arise. This region evidently represents the blepharoplast. In the mature spermatozoid the anterior portion of the nuclear material becomes closely applied to the elongated blepharoplast. The darkly staining bodies mentioned above are not to be seen now and what becomes of these bodies could not be definitely stated. The nuclear portion is drawn out into a thread-like structure which stains very deeply with haematoxylin. The fully formed spermatozoid is a coiled structure and in the early stages the two cilia are also coiled close to the body of the spermatozoid (Text-fig. 127). In the later stages, the cilia get gradually loosened (Text-fig. 130), and at the time of escape, the spermatozooids are more or less straight or slightly curved with two long cilia (Text-fig. 131; Pl. II, fig. 5). A small protoplasmic vesicle is seen attached to the posterior end of the spermatozoid (Text-figs. 129, 130, 131).

Fertilization.

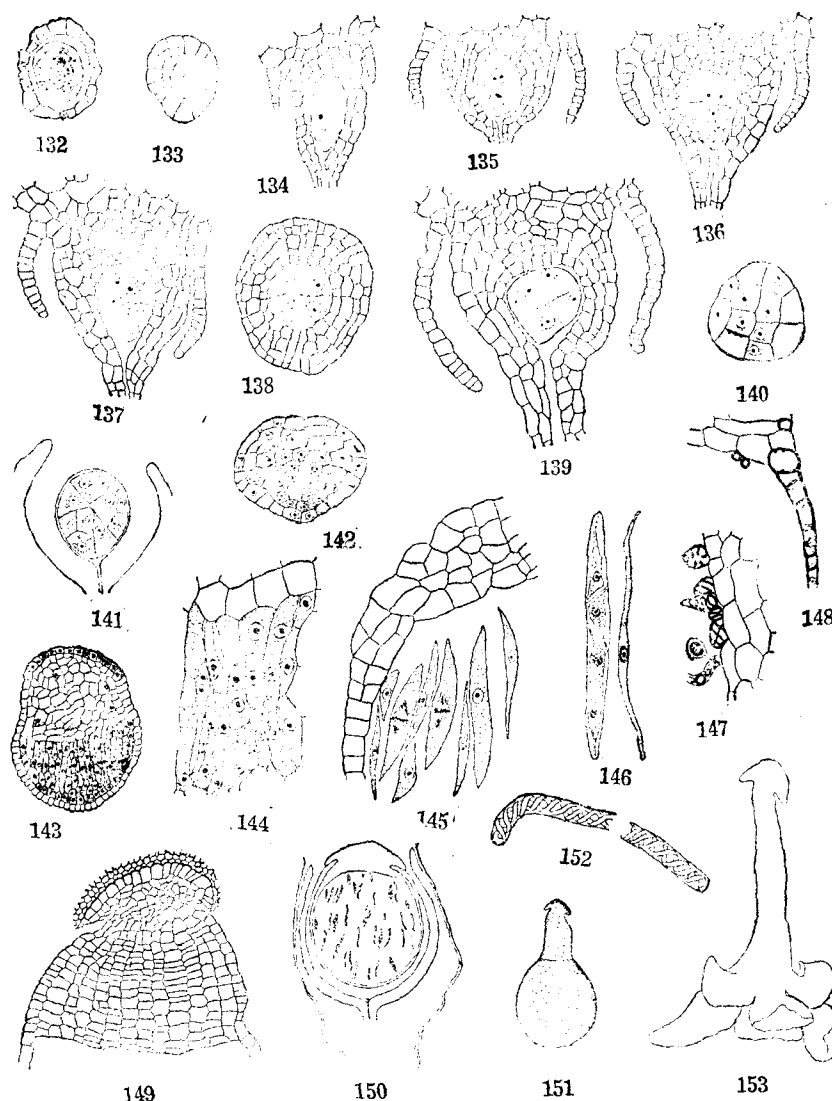
Stages showing the entry of the spermatozoid into the egg-cell were not available in the material. The only stage that was observed in my material was the fertilised egg-cell showing the male and the female nucleus just fusing (Text-fig. 132. Pl. II, fig. 6). The nuclear membranes of the two fusing nuclei at the region of contact have already disappeared though still intact over their remaining portions. When the fusion is complete, the fusion nucleus is more or less spherical with two or sometimes three nucleoli in it (Text-fig. 133).

About this stage, in a few cases, a number of radiating protoplasmic strands were seen connecting the outer surface of the fertilised egg with the inner wall of the venter (Text-fig. 133). A similar condition was seen by the writer in *Riccia himalayensis* also (Srinivasan, 1940). But after the fertilised egg has enlarged somewhat, these strands could not be detected in any of the sections. The fertilised egg is soon surrounded by a membrane of its own. It enlarges and becomes more or less ovoid and highly vacuolated (Text-fig. 134). It divides by an oblique to a transverse wall into an epibasal and a hypobasal cell (Text-figs. 135, 136, 137). A

second division more or less perpendicular to the first follows in each of these two cells resulting in a quadrant stage (Text-figs. 137, 139). In this respect *Marchantia palmata* resembles *Marchantia polymorpha* (Durand, 1908), *Marchantia domingensis* (Andersen, 1929) and *Marchantia* spp. (Heberlein, 1929). McNaught (1929) states that in *Marchantia chenopoda* no quadrant stage is seen but a row of three cells is formed by two primary transverse divisions. No such three-celled stage was found in my material. In the present liverwort, the quadrant stage is followed by an octant stage by the formation of vertical walls in each of the quadrants. In the next stage, anticlinal walls are formed in the cells of the octant and then periclinal walls (Text-figs. 140, 141). The primary transversely-oblique wall could still be distinguished even at this stage. Up to this stage the embryo does not take up much stain. In a slightly advanced stage, the cells increase in number in the developing embryo and their staining capacity increases very much, though the cells of the epi-basal half which are comparatively richer in contents stain more deeply than those of the hypobasal half which are poorer in contents (Text-fig. 142). The cells of the lowermost layer of cells of the hypobasal half, however, are very rich in contents and contain a prominent nucleus and stain very deeply (Text-fig. 143).

The epibasal half forms the capsule and the hypobasal half gives rise to the seta and the foot. In the capsule portion the outermost layer forms the wall and the inner cells form the archesporium (Text-fig. 143; Pl. II, fig. 2). The latter consists of cells with rich contents. The archesporial cells of the capsule soon elongate slightly in the direction of the axis of the archegonium (Text-fig. 144). The uppermost two or three layers of the archesporial cells at the top of the capsule, however, do not elongate but remain more or less isodiametric. These ultimately go to form portions of the cap-cells of the capsule. In the next stage, the elongated cells of the archesporium separate into individual cells (Text-figs. 144, 145; Pl. II, fig. 3), and these by further divisions form several closely placed vertical rows of cells as in *Marchantia polymorpha*. In median longitudinal section of the capsule six to seven vertical rows could be counted (Pl. II, fig. 3). In *Marchantia domingensis* (Andersen, 1929) only two rows of such cells are seen in the median longitudinal section of the capsule. About this stage and even earlier in *Marchantia palmata* several of the elongated cells of the archesporium are binucleate (Text-figs. 144, 145), but in a slightly advanced stage all the cells are found to be uninucleate. At

a later stage, some of the elongated cells in the capsule divide transversely and obliquely into a row of cells while some other cells do not divide (Text-figs. 143, 150). The former ultimately become the spore-mother-cells while the latter develop into elaters. The spore-mother-cells soon become rounded and ultimately get sepa-



Text-figs. 132-153. Fig. 132 Fertilization. Note the male and the female nucleus fusing ($\times 242$). Fig. 133. Fertilised egg with strands of protoplasm radiating from its surface ($\times 125$). Fig. 134. Young embryo ($\times 125$). Figs. 135-142. Various stages in the development of the embryo, Figs. 135-137 ($\times 125$), 138-142 ($\times 180$). Fig. 143. A young

rated from one another (Text-fig. 175). As these changes are going on in the epibasal half, changes take place in the hypobasal portion also. The lowermost layer of cells in the hypobasal half shaped structure (the foot) and is seen imbedded deeply in the gametophyte tissue (Text-figs. 149, 150). The lowermost layer of this foot region may even become two layered by division of its cells. The cells of the upper portion of the hypobasal half divide repeatedly and form the young seta portion (Text-figs. 149, 150).

During the fertilization stages, some of the cells of the wall-layer of the venter divide and become two layered (Text-fig. 133). A few cells at the base of the archegonium also divide and grow out (Text-fig. 134). When fertilization is completed and the embryo enlarges, the wall becomes completely two layered (Text-fig. 134), and the cells around the base of the archegonium grow out into a sort of an envelope (the perianth) consisting of a single layer of cells round the archegonium. When the embryo is in the quadrant stage, the wall of the venter becomes three to four layered and in its early stages takes up more stain than the developing embryo (Text-figs. 137, 139). The same was noticed in *Marchantia polymorpha* by Durand (1908). By the time the embryo differentiates into the capsule, foot and seta, the wall of the venter becomes many layered and forms the young calyptra (Text-fig. 150). The perianth in the meantime grows very much and envelopes the whole developing sporophyte with the calyptra (Text-fig. 150).

As a result of two successive divisions in the spore-mother-cells of which the first division is a reduction division, four nuclei are formed and these are arranged around the periphery of the spore-mother-cell at equal distances in a tetrahedral manner (Text-fig. 174). Cleavages take place in the cytoplasm between the four

sporophyte. Capsule, seta and foot being well differentiated ($\times 112$). Fig. 144. Separation of the archesporial tissue into individual cells. Note some of the cells are binucleated ($\times 385$). Fig. 145. Division in the archesporial cells ($\times 385$). Fig. 146. Differentiation of the archesporial cells into spore-producing cells and elater-producing cells ($\times 270$). Fig. 147. Base of the capsule showing cells with peculiar thickenings on their walls ($\times 112$). Fig. 148. Capsule wall showing annular thickenings ($\times 112$). Fig. 149. Development of the anchor shaped foot and long seta ($\times 62$). Fig. 150. A well developed sporogonium with spore-mother-cells and elaters inside the capsule. ($\times 180$). Fig. 151. Sporogonium showing anchor-shaped foot, elongated seta and a large globose capsule ($\times 7$). Fig. 152. A mature elater showing spiral bands ($\times 270$). Figs. 153. A ripe sporogonium showing the capsule opening by six lobes ($\times 7\frac{1}{2}$).

nuclei dividing the contents into four protoplasts. Walls are formed around the four protoplasts and a spore-tetrad is formed (Text-fig. 176). The tetrad of spores remains intact for a long time. By the successive addition of wall-layers from within, the spore-coat becomes gradually thicker (Text-fig. 177). In the fully developed spore the wall is slightly reticulately thickened on the outside.

As the spores ripen, changes occur in the remaining archesporial-cells which are destined to become elaters. The contents in these elongated cells are at first uniformly granular (Text-figs. 146, 175). but as these cells grow further, the granular contents gradually disappear, and spiral thickenings are formed on their inner wall. In a well developed elater, there are two spirals crossing each other at various points (Text-fig. 152). The elaters are usually broadly rounded at both ends (Text-fig. 152), but occasionally one end is drawn into a very narrow long portion without any thickenings on the wall. Some of the elaters are branched at one end into two lobes. Similar branched elaters have been previously reported in various Bryophytes (Andrews, 1908; Cavers, 1904).

When the sporogonium matures, the membranes of the cells of the wall layer of the capsule become thickened by the formation of annular bands (Text-fig. 148). The few cells next to the wall layer at the apical portion of the capsule also become thick-walled. In *Marchantia domingensis* (Andersen, 1929), there are two layers of cap-cells and these increase in size and resemble the granular basal tissue of the foot. Andersen (1929) suggests that these cells probably function as conducting tissues. In the present liverwort, however, no such granular contents could be seen in the cap-cells.

A number of cells with spiral thickenings are noticed at the base of the capsule also (Text-fig. 147). Up to this stage the seta portion has not grown very much. The cells of the seta portion are arranged in regular longitudinal rows (Text-fig. 149). But soon these cells begin to divide further and the seta elongates and pushes the capsule gradually outside (Text-figs. 151, 153). The foot at this time is very much enlarged and anchor-shaped and is well imbedded in the tissue of the gametophyte (Text-figs. 149, 150, 151, 153). The mature sporogonium finally dehisces by means of five to seven lobes and liberates the spores (Text-fig. 153).

Germination of the gemmae.

The gemmae were germinated by keeping them floating on the surface of some sterilised distilled water in petri-dishes. The

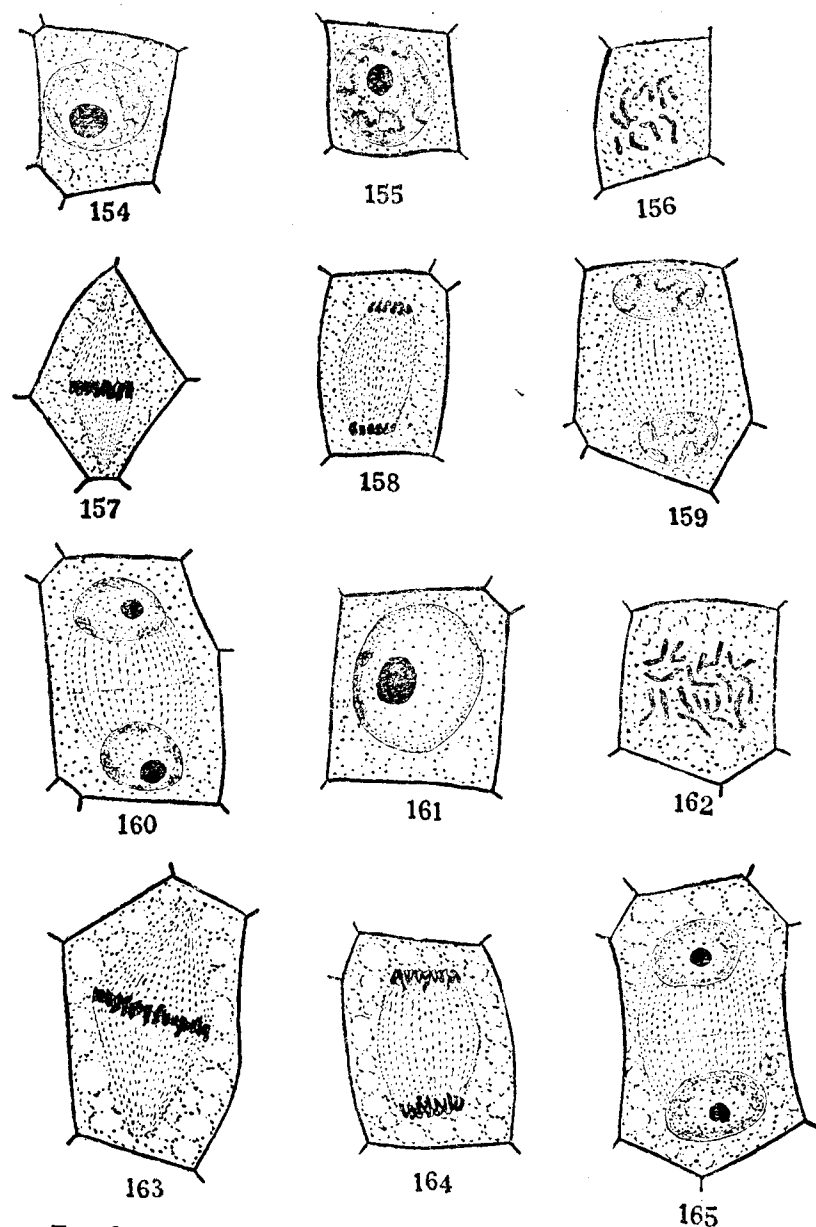
petri-dishes were placed inside a glass moist-chamber. The moist-chamber with the petri-dishes was so placed in the veranda as to receive direct sunlight from 8 a.m. to 11 a.m. only each day, and diffused light for the remaining portion of the day.

The first indication of germination was the production of a few mostly smooth walled rhizoids, on the side which is in contact with the water. Soon the rhizoids grew in length and were seen spreading outwards and the gemmae also grew in length and were assuming various shapes. In about a fortnight, a young shoot is formed at each of the two opposite ends of the elongated gemmae. These shoots are more or less cylindrical at first, but soon become flattened near the distal portion. The flattened part gradually increases in breadth and finally assumes the shape of the thallus of the mature plant. The gemma itself does not show any air-chambers, but in the flattened portions of the shoots which grow out of it, air-chambers and pores are formed on their dorsal surface and a few small scales and rhizoids are formed on their ventral side. These tiny germ-lings in the culture continued to grow for some time, but finally turned pale and died.

A fungus is always seen associated with the liverwort. It is intra-cellular and is found generally in the central portion of the thallus of the liverwort, while the wings of the liverwort are, however, free from it. The fungus very probably enters the tissue of the liverwort from the soil through the rhizoids, where its filaments are found very commonly (Text-fig. 7). Whether the relationship of the fungus with the liverwort is one of parasitism or symbiosis is not clear.

Mitosis

Mitosis was followed in the cells of the vegetative thallus, the female receptacles and the antheridia. The resting nucleus is either round (Text-fig. 154) or ellipsoidal and has a darkly staining nucleolus in the centre and a faintly staining reticulum. During early prophase, the chromatin threads gradually thicken (Text-fig. 155) and by about the late prophase condense still further and are seen as darkly staining long chromosomes distributed more or less towards the periphery of the nucleus. At a later stage, the nuclear membrane gradually disappears. The nucleolus also disappears about the same time. A definite spindle is formed and the chromosomes soon get arranged at the equatorial region of the spindle. Nine chromosomes could be counted in the metaphase plate (Text-fig. 156). The individual chromosomes in the plate



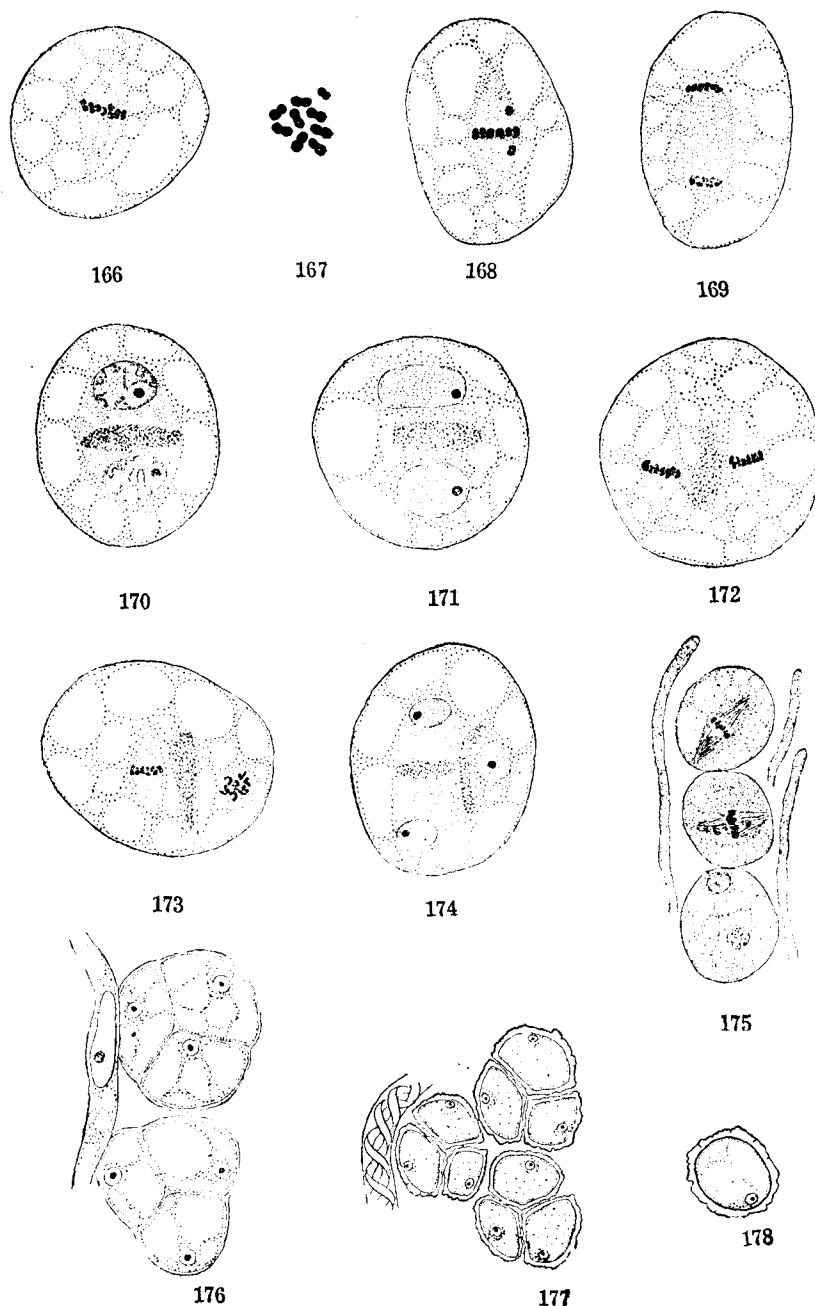
Text-figs. 154-165. Figs. 154-160. Mitosis in the haploid cells ($\times 3180$). Figs. 161-165. Mitosis in the diploid cells ($\times 3180$).

appear as small thick rod-like or hooked structures. During anaphase, the two sets of chromosomes move towards the poles. At telophase, the chromosomes become grouped together at the poles and later become organised as two daughter nuclei (Text-fig. 159).

The mitosis in the sporophyte cells does not show any peculiarities. (Text-figs. 161-165). At metaphase, in polar view about eighteen chromosomes could be counted (Text-fig. 162).

Meiosis.

The spore-mother-cell shows a large nucleus surrounded by a somewhat highly vacuolated cytoplasm, which, however, is quite dense immediately round the nucleus. The resting nucleus shows a reticulum with a prominent nucleolus. The earlier stages of division were not available in my material and hence could not be followed. Division stages from metaphase onwards only of the first division were seen in my material. A number of plates in the polar view at metaphase was examined and in each case nine bivalents were counted (Text-fig. 167; Pl. II, fig. 7). Of these one bivalent is much smaller than the rest. During anaphase, when the individual chromosomes of the bivalents move towards the poles, the two chromosomes of the smallest bivalent are the earliest to separate and move towards the poles of the spindle (Text-fig. 168, Pl. II, figs. 8, 9). Lorbeer (1934) found in *Marchantia polymorpha* nine chromosomes of which one was smaller than the rest. The small chromosome in the female was much larger than that of the male. He considered these two chromosomes as X and Y chromosomes. During anaphase, these X and Y chromosomes were observed by him to precede the other chromosomes. In the present liverwort the two chromosomes of the smallest bivalent are only very slightly different in size. But, since, during anaphase, they separate earlier than the chromosomes of the remaining bivalents, it is very probable that these two chromosomes represent the X and Y chromosomes as recorded in *Marchantia polymorpha* by Lorbeer. The X and Y chromosomes could not, however, be clearly distinguished in the chromosome complements of the vegetative thallus owing to their very small size. During late telophase, the chromosomes become grouped together (Text-fig. 169) and later on they are organised as two daughter nuclei (Text-fig. 170). After the daughter nuclei are organised, the spindle fibres gradually disappear but a mass of cytoplasm is seen at the old equatorial region of the spindle between the two nuclei. Finally the spindle fibres disappear completely, but the aggregation of the cytoplasm mentioned above could still be seen between the two nuclei (Text-fig. 171). A similar condition has been recorded by Meyer (1931, fig. 32) in *Marchantia polymorpha* and by Stevens (1905, figs. 26, 27, 28, 35, 36) in *Botrychium virginianum*.



Text-figs. 166-178. Figs. 166-171. Heterotypic division ($\times 1304$). Fig. 167 ($\times 1480$). Figs. 172-174. Homeotypic division ($\times 1304$). Fig. 175. Spore-mother-cells and elaters ($\times 616$). Fig. 176. Formation of spore tetrads ($\times 616$). Fig. 177. Well developed spore tetrads, and a portion of an elater ($\times 432$). Fig. 178. A mature spore in section ($\times 432$).

Homoeotypic division follows the short interkinesis in the spore-mother-cells. The spindles during the second division may lie either parallel (Text-fig. 172) or at right angles (Text-fig. 173) to each other. Nine chromosomes could be counted at metaphase of the second division (Text-fig. 173). The anaphase stages of the second division are either simultaneous or successive. Ultimately the four nuclei that are formed arrange themselves at equal distance from one another inside the spore-mother-cell, and soon walls are formed between them dividing the spore-mother-cell into four tetrahedral spores (Text-fig. 176).

Summary.

1. The developmental morphology of *Marchantia palmata* Nees, which is commonly growing at Ootacamund on the Nilgiris in South India, is described in detail.

2. The structure and development of the different parts of the thallus including the apical growth are fully described.

3. The development of the male and the female receptacles is followed in detail. The receptacles represent branch systems of a composite nature as in *Marchantia polymorpha*.

4. Each of the fertile lobes of the female receptacles after producing several archegonia, instead of stopping its growth as in the other species of *Marchantia*, continues to grow further as an irregularly strap-shaped proliferation and forms plenty of antheridia on it. Between the archegonial region and the antheridial region structures intermediate between archegonia and antheridia are formed. These female receptacles always end as androgynous receptacles in their final stages.

5. The development of the antheridia and the archegonia are followed in detail. There appears to be two types of development of archegonia in this liverwort.

6. The development of the sporophyte is followed.

7. The haploid number of chromosomes in the species is nine.

8. The chromosome complement in the first heterotypic division includes one small pair. These two small chromosomes, though not showing any marked difference in size, by their behaviour in the anaphase of the first division, show a certain resemblance to the X and Y chromosomes of *Marchantia polymorpha*.

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