No. 14.—*The Development of Salpa, by* Wm. K. Brooks, Ph. D.

Sketch of Adult Structure.

The accounts of the general anatomy of Salpa, published by Sars,* Krohn,† Huxley,‡ Vogt,§ Müller,‖ and Leuckart,¶ leave little to be done upon this subject; but since these papers cannot be procured easily in this country, and are absolutely inaccessible to the majority of American students, it will not be out of place to give, as briefly as possible, a description of the structure of this aberrant and highly interesting genus. This seems the more important since many parts of the developmental history must be unintelligible to those who are not familiar with the more important peculiarities of the adult structure.

The animals classed together as Tunicata agree very closely, as far as general plan of structure is concerned; and although they vary greatly in form, and in the relative size, position, and degree of development of the various organs, all the important structures found in one may usually be found in the others; and although the genus at present under discussion departs very widely from what may be considered as the typical form, those who are acquainted with the structure of one of the fixed ascidians will find no difficulty in tracing its homology with Salpa.

The chief difficulty in gaining a clear conception of the relations of

* Fauna littoralis Norvegiæ. VII. 63-85.

† Observations sur le génération et le développement des Biphores. Ann. de Sc. Nat., VI. 1846, 110.

‡ Observations upon the Anatomy and Physiology of Salpa and Pyrosoma. Phil. Trans. 1851.

§ Recherches sur les animaux Inférieurs de la Méditerranée. Mém. de l'Institut National Genevois, 1854, II. 1-62.

‖ Verhand. der phys. med. Gesellschaft in Wurzburg. Bd. III. p. 57.

¶ Salpa und Verwandte. Zoologische Untersuchungen, II.

The literature of our subject is so extensive that a complete historical sketch would enlarge our account of the adult structure beyond reasonable limits; accordingly authorities will be referred to, in this chapter, only in those special cases which have a peculiar interest. The chapters which treat of the development will contain references to the various writers, giving a history of the progress of our knowledge of this subject; and very complete historical sketches of our knowledge of Salpa in general may be found in the papers by Huxley and Leuckart, above referred to.

the various parts of a tunicate animal lies in the fact that it is composed of a number of nearly concentric tunics or sacs, which are connected with each other in a rather complicated manner; and the names given to these parts by the various authors are not always used in the same way; the names used by one being applied by another to totally different organs, so that it is difficult to understand what part a name is intended to designate unless the terms used are defined carefully. The precise term used is of minor importance, provided its meaning is clearly stated, and since Huxley, in his paper on Pyrosoma,* has defined, with great clearness and exactness, all the terms which he uses, I shall employ his nomenclature as far as possible.

The Test. — The outer wall of a Tunicate is the "cellulose test" (Figs. 1 and 3, *a*). This is a sac with two openings, — the "branchial aperture" *e*, and the "atrial aperture" *g*.

The Outer Tunic. —Within the cavity of the test, and united to the latter at the two openings, is the "outer tunic" (Figs. 1, 2, and 3, *b*). This also is a sac with two openings, and usually conforms to the shape of the inside of the test, to which it may or may not be united; whenever the two are separated over a considerable area, there is, of course, a chamber between them, but as this is not one of the true cavities of the body and is of no homological importance, the test may be regarded as enclosing no especial cavity. The outer tunic is usually more or less muscular, and is often spoken of as the "muscular tunic"; it is the "second tunic," of most writers. Its cavity — that is, the space between its inner surface and the outer surfaces of the tunics within it — is the true "body cavity," and since all the blood-channels of Salpa and the forms allied to it are parts of this "body cavity," more or less shut off by the union of portions of the outer tunic to those within, it is often convenient to speak of it as the "sinus cavity," or "sinus system." Within the outer tunic are the "branchial sac," with its diverticulum, the digestive organs; and the "atrial tunic."

The Branchial Sac. — During the earlier stages of development the branchial sac is an entirely closed chamber surrounded by a tunic, which is in turn entirely surrounded by the body cavity, by which it

* On the Anatomy and Development of Pyrosoma. Trans. Linn. Soc., 1860, XXIII. pp. 193–250.

is separated from the outer tunic; at an early stage of development, however, the outer and branchial tunics unite at the anterior extremity, and the central portion of the area thus united disappears by absorption, so that an opening is formed through which the cavity of the branchial sac communicates with the outer water; this opening is the branchial aperture. Since the test, the outer tunic, and the branchial sac are all united around its circumference, the free edges are composed of all three of these. Upon the inner surface of the outer tunic around the aperture there is a set of muscles, by the contraction of which the opening may be entirely closed. The interior of the branchial sac bears several structures which are very constant in form and position throughout the group. Upon the hæmal side there are two long parallel folds which project towards the ventral axis of the cavity, and form the boundaries of a deep longitudinal furrow (Figs. 1, 3, and 24, *m*), which projects as a vertical ridge into that portion of the body cavity which forms the hæmal sinus, but remains in free communication with the branchial cavity by a cleft upon its neural side. In consequence of the thickness and opacity of the epithelium which lines the fundus of this fold, it appears (especially in the transparent Tunicata in a fresh state) like a strong hollow rod, mounted upon a thin ridge-like plate, and has been called the "endostyle."

The bottom of the furrow is richly supplied with very long cilia, and its sides are glandular, and secrete an adhesive slime, which serves to entangle the particles of food which are carried with the respired water into the branchial cavity. Upon each side of the endostyle and parallel to it, there is a prominent line of cilia (Fig. 24, *l*, also 1, 3, 32 and 33, *l*) to which the name "epipharyngeal ridge" has been given; these two ridges are continued backward beyond the posterior termination of the endostyle, where they unite to form the "posterior epipharyngeal ridge," which passes backward along the middle line of the posterior wall of the branchial sac to the mouth (Fig. 1, *o*), before reaching which they again separate in Salpa, and pass, one on each side of the tongue-shaped organ shown in Fig. 1, *o*. In front of the anterior end of the endostyle the two epipharyngeal ridges diverge from each other so as to pass around the branchial sac, near its anterior end, and thus form the "peripharyngeal ridges" (Figs. 2, *k*, and 33, *k*). Upon the neural median line of the branchial sac, and therefore opposite the endostyle, there is a row of tongue-shaped organs projecting into the respiratory cavity; these are the "languettes." The number of these is very variable; in Salpa there is only one (Figs. 1 and 3, *w*), in Pyrosoma there are eight, and in most of the fixed ascidians the number is much greater.

At the posterior end or base of the branchial sac, its wall is directly continued into that of the œsophagus (Fig. 24, *o′*,) which is short and opens into a somewhat dilated stomach (Figs. 24, *o″*, and 4 *o″*). The cavities of these organs are continuous with the branchial cavity, and since the branchial sac is shown, by its development, to be nothing more than the anterior end of the digestive tract, Huxley has called it the "pharynx"; but it will be much more convenient, for our purpose, to retain the name which indicates its functional but not its morphological relations. The anterior opening, already described as the "branchial aperture" is undoubtedly the true homological mouth, but it is much more convenient to make use of the name which indicates its function, and to apply the term "mouth" to the aperture of ingestion, at the point where the œsophagus leaves the base of the branchial sac. The intestine bends around the stomach and then runs forward nearly parallel with the œsophagus (Fig. 24), and usually terminates at a point which is anterior to the mouth; the anus opens into the cavity of the "atrium," which is now to be described.

The Atrial Tunic. — Since the form and relations of this structure are very variable in the different Tunicata, a clear conception of it as presented in the typical forms is essential to a correct appreciation of tunicate structure and development in general, and, owing to a lack of such a clear conception, many of the published accounts of these animals are of very little value. In the perfectly transparent genus Perophora, which presents exceptionally favorable conditions for the study of the atrium, the two openings of the external tunic are at the same end of the body; one of these, the branchial aperture, is formed by the union of the branchial sac and outer tunic, as already described, while the other, the atrial aperture, is formed in a similar way by union of the outer and atrial tunics. The latter is a large bag, parallel to the branchial sac, and upon that side of it which bears the languettes; at the bottom of this sac, which Huxley has called the "mid-atrium," are the external openings of the intestine and reproductive organs. On each side of the body the atrium is, as it were, tucked into the space between the branchial and the outer tunic, thus forming two long and broad but very shallow "lateral atria," the cavities of which are continuous with that of the mid-atrium; these lateral atria are extended upon each side of the branchial sac until they almost meet, and thus cover the whole surface of the sac except a narrow line over and parallel with the endostyle. In a transverse section we should have the test and outer tunic as two concentric circles, and, within these, sections of the branchial and mid-atrial chambers side by side and separated from the outer tunic and from each other by the sinus cavity; the cross-sections of the lateral atria would be shown as two long parallel-walled diverticula from the mid-atrium, curving around the branchial sac, and almost meeting upon its opposite side. The outer wall of each lateral atrium is separated from the inner surface of the outer tunic, and the inner wall from the outer surface of the branchial sac by blood sinuses; but the separation from the branchial sac is not complete, for at certain points this is united to the atrial tunic so that the branchial sinus is divided up into a network of longitudinal and transverse vessels or blood-channels, crossing each other at right angles. In each of the parallelograms thus formed the two tunics are absorbed, thus forming a "branchial slit," through which the respired water passes from the branchial chamber into the cavity of the lateral atrium, through which it is driven into the mid-atrium, from whence it escapes through the atrial aperture. It is difficult to give a clear account of the relations of the branchial sac and atrium without the aid of diagrams, but an illustration may help to a conception of this somewhat complicated subject. In the middle of a long glass tube blow a bulb to represent the stomach, and enlarge one end of the tube to represent the branchial sac, and the other to represent the mid-atrium; the small tube uniting the branchial sac to the stomach will represent the œsophagus, and that which connects the stomach to the atrium will represent the intestine. Now bend the intestine around the stomach so that the branchial sac and atrium shall lie side by side, flatten out the latter, and wrap it around the branchial sac, and the whole will form a pretty correct model of these organs in a typical Tunicate. This illustration is open to one objection, inasmuch as it seems to imply that the branchial sac and atrium are serially homologous, while, in fact, the latter is not a part of the digestive tract, but originates independently. Such a model will represent only the condition of the organs in the adult,

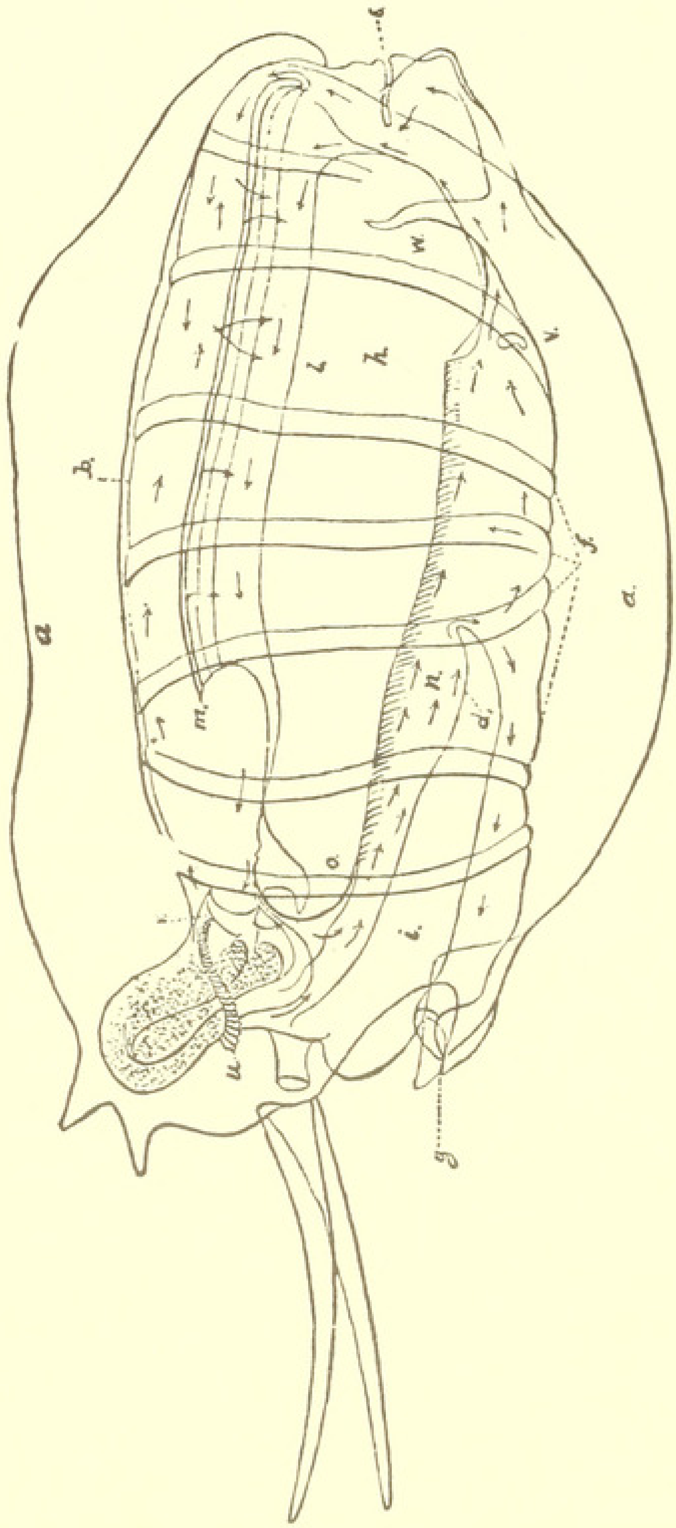


Fig. 1.

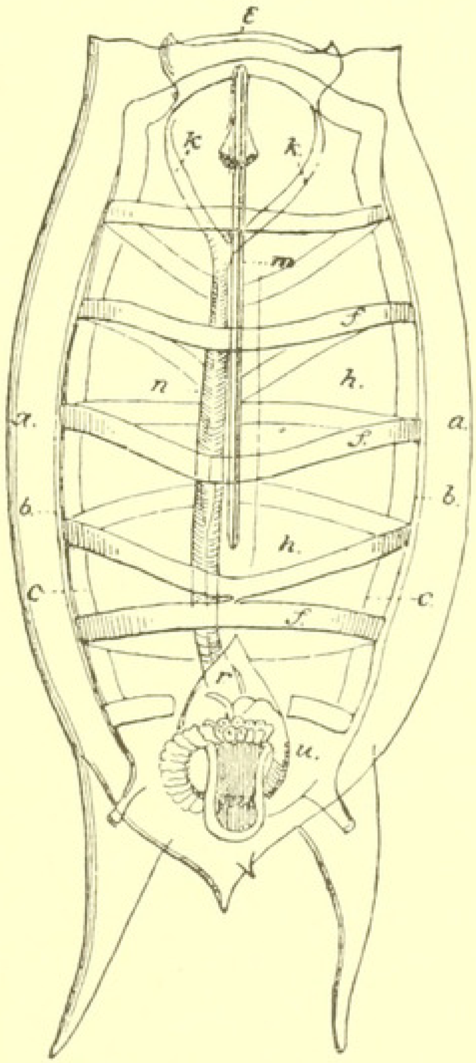
Adult solitary Salpa, viewed from the side: *a*, test; *b*, outer tunic; *d*, wall of atrial chamber; *e*, branchial aperture; *f*, muscular girdles: *g*, atrial aperture; *h*, branchial cavity; *i*, atrial chamber; *l*, epipharyngeal fold; *m*, endostyle; *n*, gill; *o*, mouth; *r*, heart; *u*, chain of males; *v*, ganglion; *w*, languette.

and although the branchial sac may be regarded as a pharynx, tne atrium is in no sense a rectum.

The various tunics above described, with their diverticula and appendages include all the organs of a tunicate animal, with the exception of the nervous system, the heart, and the reproductive organs.

Nervous System.—The nervous system is situated within the body cavity, and is attached to the inside of the outer tunic between the two external openings (Figs. 1, 3, 24, and 33, *v*).

Fig. 2.



Adult solitary Salpa; hæmal view: *a*, test; *b*, outer tunic; *c*, wall of branchial sac; *e*, branchial aperture; *f*, muscular girdles; *h*, branchial cavity; *k*, peripharyngeal ridges; *m*, endostyle; *n*, gill; *nu*, nucleus; *r*, heart; *u*, chain of young males.

The Heart.—The position of the heart varies greatly, but it is usually found near the digestive organs (Figs. 1 and 3, *r*), and in all Tunicates its motion is periodically reversed.

The Reproductive Organs.—These are usually placed upon or near the intestine, and their external openings are near the anus.

We are now prepared to consider the special modifications presented in the organization of Salpa.

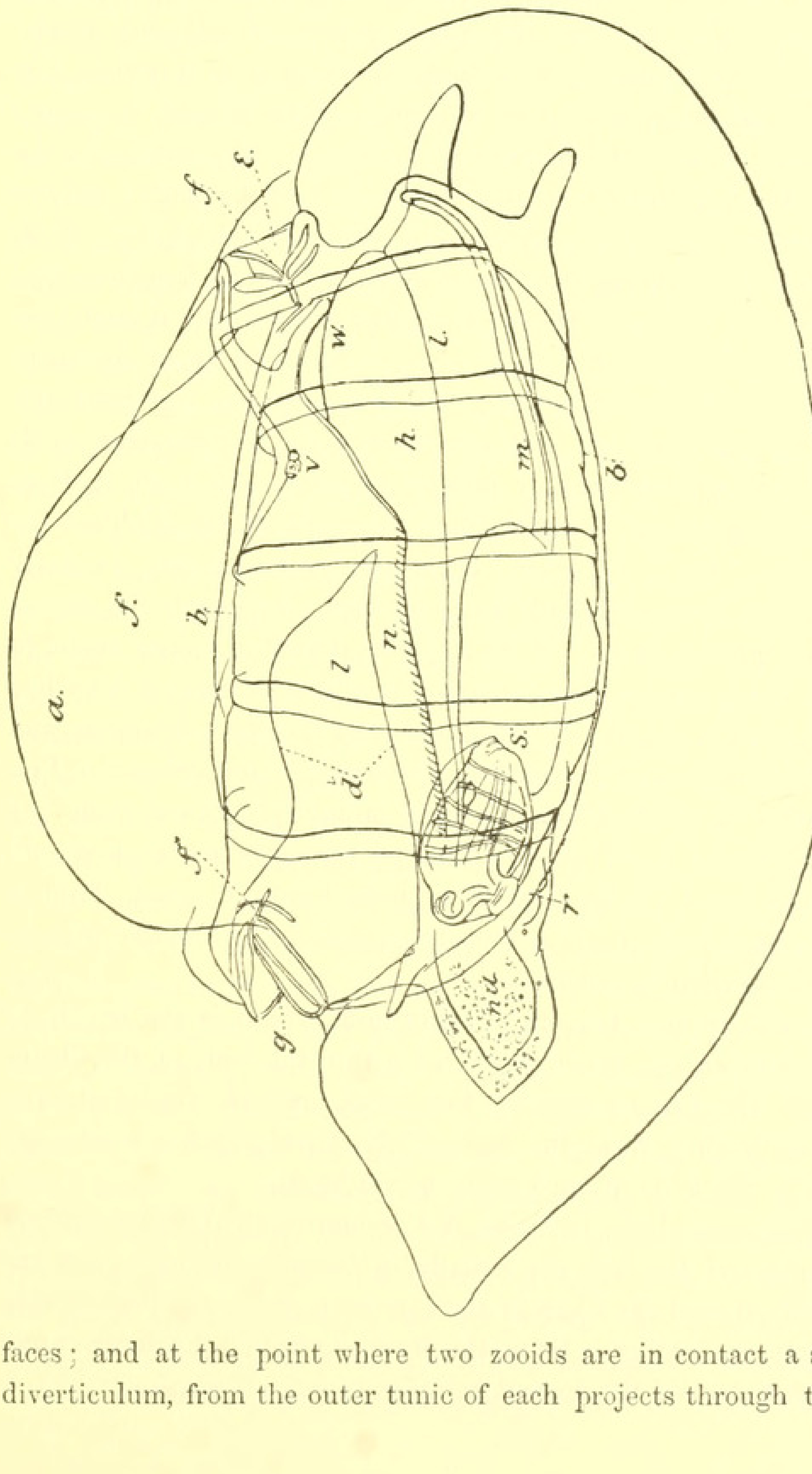
The *test* (Figs. 1, 2, and 3, *a*) is very thick, and, in our species, so perfectly transparent that its outline can be traced only when the animal is seen strongly illuminated upon a dark background. The branchial aperture is at the anterior end of the body of the solitary form (Figs. 1, 2, *e*), although the test of the chain-Salpa (Fig. 3) projects some distance in front of it, upon the hæmal side; in both the test extends posteriorly beyond the atrial aperture *g*. In the young of both forms the latter opening is much nearer the branchial than in the adults, and in this respect the young Salpa resembles the adult of the ordinary fixed ascidians. The shape of the test varies greatly in the different species, as well as in the two forms of the same species. The solitary form of our species,* when seen from above or from below (Fig. 2), is barrel-shaped in outline, with the posterior extremity obtusely pointed and the anterior truncated. At the sides of the posterior extremity the test is prolonged so as to form two long, slightly curved processes, each of which contains a cœcum of the outer tunic, with a cavity which is continuous with the sinus system. When seen in profile (Fig. 1), it is truncated at both ends, from the neural to the hæmal side; the extremities are slightly convex; the posterior truncating plane is more inclined than the anterior. Besides the two large posterior processes there are six much smaller ones, two on the median line and two pairs, all of which are composed of the test only, with no inner chamber. The anterior opening for the admission of water (Figs. 1, 2, 3, *e*) is by far the larger; it occupies the whole width of the body, while the posterior one (*g*) through which the water is expelled is much narrower, and placed at a short distance from the posterior extremity, at the base of the truncating plane, on the neural side; the anterior opening being nearer the hæmal side. The lips which close these openings are quite prominent, and can be thrown considerably beyond the general outline, either when drawing in water or forcing it out.

Since the Chain-salpæ are normally united into a chain composed

* This species is very abundant along the southern shore of New England, and was described by Desor (Proc. Boston Soc. Nat. Hist., III. 1848, p. 75) as Salpa Caboti; and subsequently figured and described by A. Agassiz (op. cit. XI. p. 17) under the same name. It seems to agree, in all respects, with the Salpa spinosa, Otto, figured and described by Sars (Fauna littoralis Norvegiæ, 1846, p. 85, Tab. 10, Figs. 1, 2. and 9). The Salpa spinosa of Otto is stated to be the same as the S. mucronata-democratica of Forskål, but as I have not been able to see Forskål's figures, I am unable to tell from his description whether the American species is the same.

of two rows of alternating zooids, the tests are flattened at the points where they come into contact with each other, and the surface is therefore composed in part of flat facets and in part of curved sur-

Fig. 3.



Adult Chain-salpa; side view: *a*, test; *b*, outer tunic; *d*, atrial tunic; *e*, branchial aperture; *f'*, muscles of branchial aperture; *f''*, muscles of atrial aperture; *g*, atrial aperture; *h*, branchial cavity; *l*, epipharyngeal fold; *m*, endostyle; *n*, gill; *r*, heart; *s*, embryo; *v*, ganglion; *w*, languette.

faces; and at the point where two zooids are in contact a spur, or diverticulum, from the outer tunic of each projects through the test, and meets and is joined to a corresponding spur from the outer tunic of its neighbor. These diverticula are hollow, and the blood passes into and out of them; but the cavity of each is separated by a partition from that of the one to which it is joined, so that there is no communication between the sinus systems of adjacent zooids. Each is furnished with eight of these spurs, by two of which it is attached to the neighbor in front of it on the same side of the chain; two serve to join it to the one directly behind it; two unite it to the neighbor obliquely in front on the opposite side of the chain; and the remaining two connect it with the one obliquely behind it. When the chain is quite young, the test is thin and the surface curved at all points, and the spurs project some distance beyond it, and thus keep the animals apart, and at the same time bind them together; but as they grow larger the tests thicken, embrace, and gradually cover up the spurs, and at last the tests of the two adjacent zooids meet and become flattened by mutual pressure, and tend to force the animals apart, and the chain now falls apart at the slightest disturbance; so that a full-grown chain can be found only in water which has been unusually still for some days. Although the motion of the separated zooids is not as active as that of a united chain, in which all the components act together to effect locomotion, they live and flourish when separated, and all traces of the spurs disappear, and the body again becomes rounded and presents the form shown in Fig. 3, the outline of which differs in several prominent features from that of the solitary form. The long terminal processes are wanting, and the posterior end of the body is prolonged into a broad, bluntly pointed cone, which contains the digestive and reproductive organs, and is the so-called "nucleus."

We may state here that the solitary form, which is the female, is hatched from an egg which is carried within the body of the Chain-salpa; and the Chain-salpæ are the males, and are produced by a process of budding from the body of the female, and a single egg passes into the body of each male before birth.

The *outer tunic* (Figs. 1, 2, and 3, *b*) conforms to the inner surface of the test, and the two are usually in contact, although they are united only around the edges of the apertures, and may easily be separated from each other. Upon the inner surface of the outer tunic, the ganglion (*v*) and the muscular girdles (*f*) are attached.

The *branchial sac* presents all the characteristics already described as peculiar to this structure. It is attached to the outer tunic around the edges of the branchial aperture (*e*), the lips of which are reflected inward and provided with a complicated system of muscles (Figs. 3, 24, and 33, *f'*); the whole forming a valve which usually prevents the water from passing outward, although it may be so arranged, by the contraction of the muscles, that the contents of the branchial sac may be violently expelled through it.

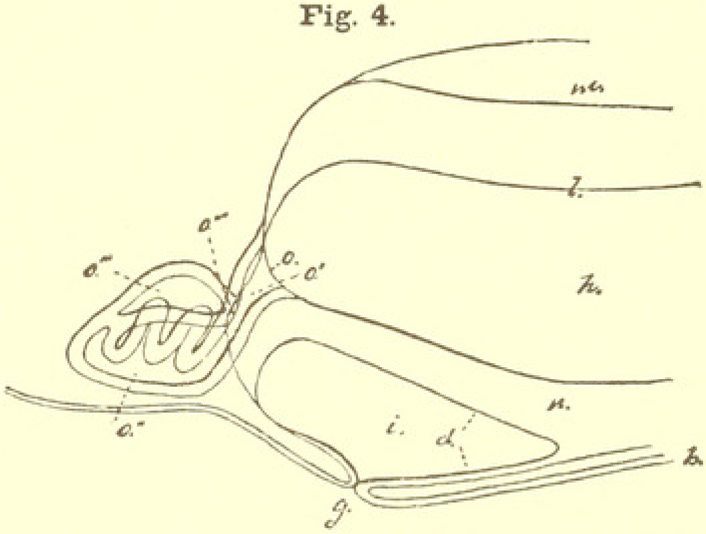
Owing to the transparency of Salpa, the endostyle is very prominent, and has the appearance of a solid rod in the adult, although earlier stages show that it is simply a furrow between two longitudinal folds. There are only two branchial slits, one on each side; but these are very large, and cover almost the whole surface of the branchial sac, except the median dorsal and hæmal lines. On the neural side, the branchial slit opens directly into the atrium, and the line where the two tunics unite is marked by the so-called "gill" (*n*); the posterior border of the slit is seen in Figs. 4, 32, and 33 as a curved line passing by the anus and mouth, and then bending forward to become continuous with the epipharyngeal fold (*l*), which bounds the slit on the hæmal side. Its anterior boundary is the peripharyngeal ridge, and it will be seen that the branchial sac is a complete cylinder only in that short portion which lies anterior to this ridge; while from this backward to the mouth, its sides are entirely wanting, and the branchial cavity opens directly into the atrium.

Although the inside of the branchial sac is supplied with cilia, as in all Tunicates, these do not seem to be of as much functional importance as in the remaining members of the group, since respiration is effected entirely through the action of the muscular girdles, which also assist deglutition and are the organs of locomotion. These contract rythmically, with great regularity, and at each contraction the water is expelled from the branchial sac through the atrial aperture; and when the muscles are relaxed, the elasticity of the test distends the chamber, and a fresh supply is drawn in through the branchial aperture, the lips of which readily admit its passage in this direction, while a similar set of valves allow its passage out of the atrial aperture, but prevent its return. As the result of this rythmical discharge of water, the animal is impelled forward with a motion which is pretty uniform in the case of a perfect chain;

although the solitary individuals, and those which have been set free by the breaking up of a chain, move by jerks. Since the blood circulates in the sinus system which surrounds the branchial sac and atrium, and also penetrates the cavity of the gill, which is simply part of the body cavity shut off by the union of the branchial and atrial tunics, its aeration is amply provided for.

If a little carmine is added to water containing Salpæ, the manner in which the food is conveyed to the mouth can be distinctly seen. The carmine, drawn in with the water, adheres to the inner surface of the branchial sac, anterior to the epipharyngeal ridges, and is then rolled along by the cilia until it reaches these ridges, the cilia of which are so set that they change its direction and convey it to the anterior end of the endostyle, the cilia of which gradually carry it backward toward the mouth. The contraction of the muscles now becomes more vigorous, and at each contraction the body is so compressed that the epipharyngeal ridges come into contact with the hæmal or branchial surface of the gill, and the water is forced along the tube thus formed, driving the food before it to the mouth.

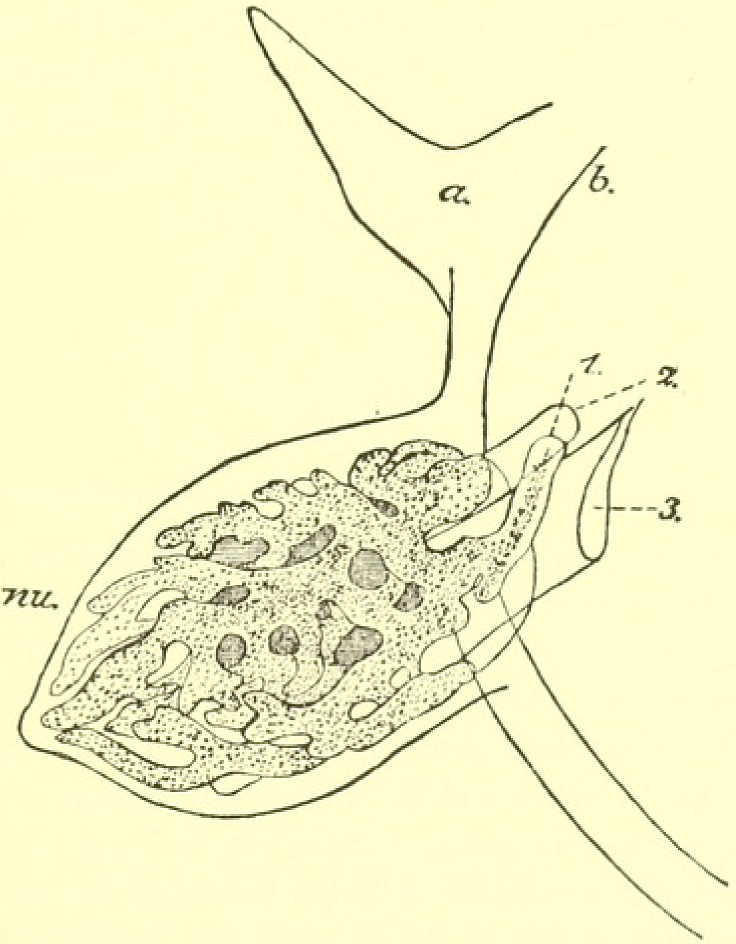
Fig. 4.



Digestive organs of very young solitary Salpa, figured with the neural side below: *b*, outer tunic; *d*, wall of atrium; *g*, atrial aperture; *h*, cavity of branchial sac; *i*, cavity of atrium; *l*, epipharyngeal ridge; *m*, endostyle; *n*, gill; *o*, mouth; *o′*, œsophagus; *o″*, stomach; *o‴*, intestine; *o⁗*, anus.

The Digestive Organs. — In the adult these are so obscured by the organs which overlie them that it is almost impossible to trace their course, which must therefore be studied in the young. Fig. 4 shows the atrial and part of the branchial chamber, and the digestive organs of a very young solitary Salpa, in which the cavity of the intestine is not yet in communication with the stomach, and is still imperforated at the anal end. The mouth (*o*) opens at the base of the branchial sac, on the hæmal side of the posterior end of the gill (*n*), and is joined by a short curved œsophagus (o') to the stomach (o''); the intestine (o''') is parallel to the long axis of the stomach, and the anus (o'''') is close to the mouth, but opens, not into the branchial, but into the atrial chamber, the posterior boundary of which is indicated by the line *l*.

Fig. 5.



Nucleus of adult Chain-salpa, — male; figured with the neural side uppermost: *a*, test; *b*, outer tunic; *1*, external aperture of testis; *2*, anus; *3*, mouth; *nu*, nucleus.

The whole digestive tract is immovable, and without muscles, and the food is driven through the permanently distended cavity by means of the cilia, with which its entire inner surface is lined. The great posterior sinus surrounds the digestive system on all sides, and the nutriment is absorbed directly from its surface by the blood. In the young a layer of large dark-colored cells may be seen, covering the posterior portion of the stomach and intestine (Fig. 33); these seem to be the first traces of the "tubular hepatic system"; a layer of anastimosing tubes which, in the adult, covers the outer surface of the stomach and intestine, and opens into the stomach at its anterior end. The function of this organ is much disputed, but as nothing definite is known upon the subject, a history of the discussion is not necessary here.*

The Testis. — In the adult Chain-salpa, the digestive organs are covered, outside the "hepatic organ," by the glandular organ shown in Fig. 5. This is a layer of arborescent folicles opening into the atrium by two apertures (*1*), one on each side of the anus (*2*); and a microscopic examination shows that it is the testis. It is found only in the Chain-salpa, which is therefore a male; while the ovary is developed within the body of the solitary Salpa, and will be described at length in connection with the development of the chain.

Fig. 6.

Spermatozoa, from the testis of an adult and from the branchial sac of an immature male.

The Ganglion and Sense Organs. — As an adaptation to its locomotive life, the nervous system of Salpa is more highly developed than in most of the adult Tunicata, and is provided with highly specialized sense-organs, which are supposed to be those of sight and hearing. The structure of these organs is described at length in the papers by Vogt, Leuckart, and H. Müller already referred to.

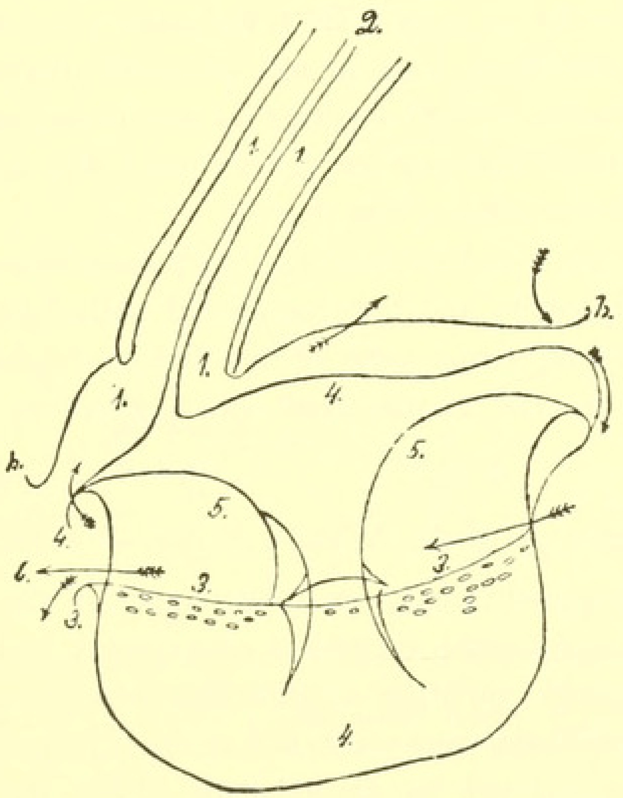
The Heart and the Circulation. — Since the discovery by Van Hasselt in 1824, that the circulation of Salpa is subject to periodical and somewhat regular reversal of its direction, the heart has been made the subject of especial study by numerous observers; but owing to its delicacy and transparency and to its rapid pulsation, its structure and mode of action have never been correctly described, and can be made out only by studying the living animal after it has been treated with ether until the contractions have grown very feeble and slow.

It consists of three concentric saddle-shaped portions, of which the upper (Fig. 7, *4*) and the lower (*3*) are thick and inflexible, while the middle one (*5*) is thin and composed of parallel rows of muscles, which

* Leuckart, pp. 33–38, devotes considerable space to this subject.

extend transversely from one side to the other. The muscular saddle is larger than the upper, and is attached to it around its entire edge, so that there is a closed empty chamber between the two; and the lower edges of all three are united, thus forming a crescent-shaped passage between the lower and muscular saddles. When the muscles of the middle saddle are relaxed, as in death by etherization, this layer is drawn up against the upper one, apparently by atmospheric pressure, and the upper closed chamber is no longer visible, while the

Fig. 7.



Heart and proximal portion of stolon of young solitary Salpa: *b*, outer tunic, continued into the outer wall of the stolon; *1*, sinus chambers of stolon; *2*, prolongation of pericardium; *4*, forming the partition of the stolon; *3*, inner saddle of pericardium; *4*, outer saddle of pericardium; *5*, muscular saddle; *6*, arrow showing the course of the current through the cavity of the heart.

lower crescent-shaped channel is open throughout its entire length; as the heart lies in the sinus system, this channel is of course filled with blood. Now it is plain that if one of the transverse muscular bands which compose the middle layer be contracted, the latter must be drawn down at this point until it comes into contact with the upper surface of the lower saddle, as seen in the figure, in which two such points of contraction are shown. This will of course divide the channel through the heart, so that there will no longer be an unobstructed passage.

The muscles contract successively in order, from one end of the heart to the other, and the middle layer is thus thrown into a wave, which sweeps along the roof of the lower layer and drives the blood before it, and before the first wave has travelled from one end of the heart to the other a second follows it, and so on. After this has gone on for some time the muscles begin to contract in the reverse order, and the waves start from the other end and the direction of the current is reversed. The cause of this change or the meaning of the reversal is as yet by no means clear, although after the pulsations have continued for some time in one direction numbers of blood-globules may be seen crowded together in certain organs, such as the elæoblast; and these block up the sinuses until they are set free by the change in the direction of the current.

In our species the blood-channels are in all cases sinuses, which are parts of the body cavity and have no special walls, although several writers insist that in certain parts of the bodies of other species the blood circulates in true vessels lined with epithelium.

Embryology of the Solitary Salpa,—Female.

We are now prepared to enter upon the history of the development of Salpa. This is rendered somewhat complicated by the fact that the two forms, besides differing considerably in structure, are developed in totally different ways; and before the embryo of the second generation has completed its development within the body of its parent, the formation of the third generation begins within it: accordingly at certain stages we are compelled to study an embryo going through one series of changes, and within this another embryo differing in form, and undergoing an entirely distinct form of development.

On account of the manner in which the two forms of development overlap, it is somewhat difficult to select a point at which to begin our account. The fertilization of the egg seems to be the best point of departure, although some parts of the description cannot be clearly understood until the account of the development of the male has been read.

The Egg: its Fertilization and Segmentation.—At the time when the

Salpa chain is discharged from the body of the solitary form each "zooid" * contains a single egg,† which is situated upon or very near the median plane of the neural side of the animal, within the sinus

Fig. 8.

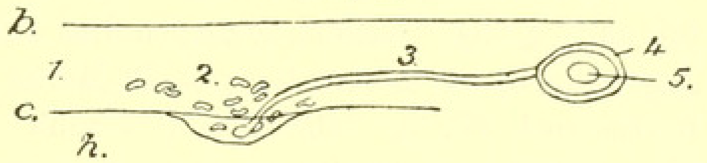


Fig. 9.

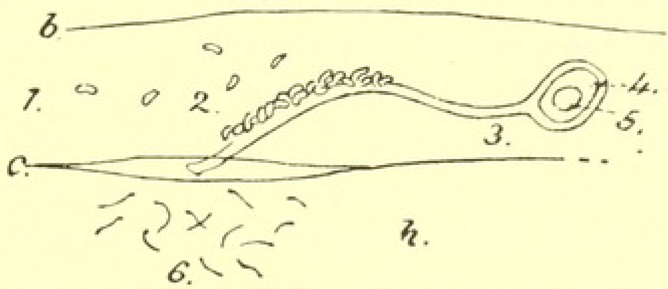


Fig. 10.

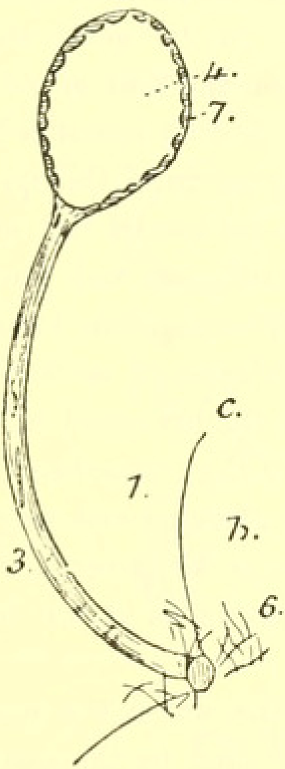


Fig. 11.

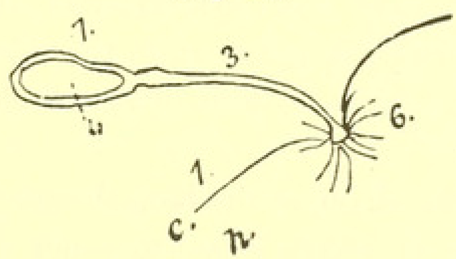


Fig. 12.

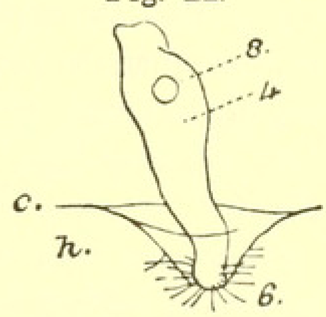


Fig. 8, egg before impregnation. Figs. 9 and 10, egg during impregnation. Figs. 11 and 12, changes following impregnation: *b*, outer tunic of male; *c*, wall of branchial sac of male; *h*, cavity of branchial sac of male; *1*, sinus system of male; *2*, blood corpuscles of male; *3*, gubernaculum; *4*, yolk; *5*, germinative vesicle; *6*, spermatic filaments; *7*, capsule of egg; *8*, nucleus.

system, and midway between the atrial orifice and the stomach (see Figs. 31, 32, 33, and 34, *s*). There is no observable trace of a vitelline membrane; the yolk is transparent without granules, and the germinative vesicle does not contain a germinative dot (see Fig. 8). ‡

* The use of the word "zooid" in this connection must not be understood to imply any opinion as to what is or is not a zoölogical individual; it is used merely as a convenient word to designate one of the males which compose a chain.

† Leuckart says (Ueber Salpen, p. 49) that some species contain more than one egg.

‡ Huxley, who studied what appears, from his description, to be the species here described, says (Salpa and Pyrosoma, p. 577) that there is a germinative dot occasionally. Leuckart (p. 51) gives the germinative dot as one of the characteristics of the egg, but I have not been able to find it in any instance.

The egg is inclosed in a capsule of small epithelial cells (see Fig. 10), which are in direct contact with the surface of the yolk, and upon the lower surface of this capsule there is a long stem or gubernaculum, which passes down the side of the body of the zooid in the sinus between the branchial sac and outer tunic, and is attached to the wall of the branchial sac, upon the right side, near the heart. According to the observations of H. Müller (Ueber Salpen. Zeitsch. f. wiss. zoöl. IV. 3), "the stalked capsule which, in all new-born Chain-salpas, encloses the egg, is an evagination from the wall of the branchial sac, and the epithelium of the latter is directly continued up the stem and over the yolk"; but I was unable to trace the epithelium on to the gubernaculum, which seems to be a solid rod of protoplasm, passing through the wall of the branchial sac and projecting into its cavity. At the point where the gubernaculum joins the wall of the branchial sac, the latter is slightly depressed, so as to form a cup with its convex surface turned towards the branchial cavity, so that the cavity of the cup, which is to form the "brood-sac," is a diverticulum from the sinus system; and blood-corpuscles may usually be found within it, as well as upon the sides of the gubernaculum, adhering together so as to form irregular clusters, as shown in Fig. 9.*

Very soon after the chain is discharged into the water, the egg undergoes impregnation, which, however, is not effected by the spermatic fluid of the zooid which contains the egg, nor by that of any other zooids in the same chain. The testis, at this time, is in a very rudimentary state (see Figs. 33 and 34, *t*), and does not become developed until after the solitary embryo into which the egg is developed has been discharged from the body. Wherever Salpa is found at all, it is very abundant, and individuals at all stages of growth occur together, so that some portion of the fluid discharged into the water from the testis of an adult male readily finds its way into the cavity of the branchial sac of the young male which carries the unimpregnated ovum, and numbers of actively moving spermatic filaments may be found within this cavity at this time. These filaments seem to be drawn to the exposed tip of the gubernaculum by some attrac-

* Leuckart (p. 47) describes this cup as a solid organ developed upon the wall of the branchial sac; although he refers to Vogt's correct description of it (Bilder aus dem Thierleben, 82).

tive force, since they swarm about it; and great numbers become attached to it, as well as to the wall of the branchial sac in its vicinity, as shown in Figs. 9, 10, 11, and 12. A few of them penetrate the gubernaculum, and work their way up towards the egg (Fig. 10); and, although none were actually seen which had travelled over much more than half the distance between the tip of the gubernaculum and the egg, there seems to be no obstacle in their way, and they prob-

Fig. 13.

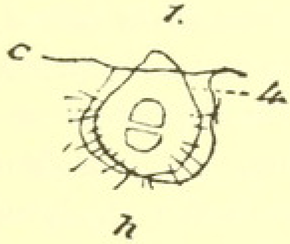


Fig. 14.

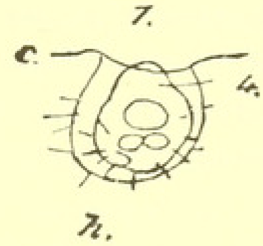


Fig. 15.

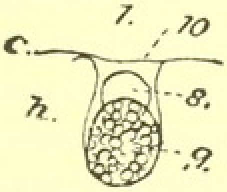


Fig. 16.

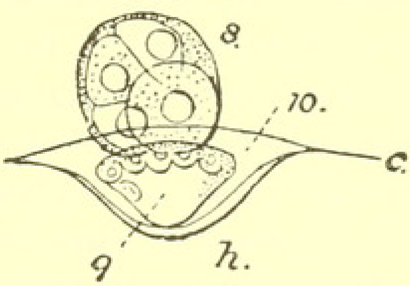


Fig. 17.

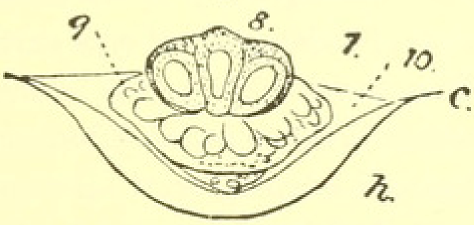
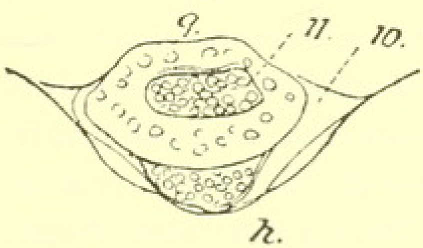


Fig. 18.



Successive stages of segmentation: *c*, branchial tunic of male; *h*, cavity of branchial sac of male; *7*, sinus system of male; *4*, yolk; *8*, food-yolk; *9*, germ-yolk; *10*, orifice of brood-sac; *11*, invagination orifice.

ably reach the yolk; but the subsequent changes follow so rapidly that no egg was found showing them so situated.

Immediately after impregnation the germinative vesicle disappears, but as the actual process was not observed, nothing can be stated as to the manner in which the disappearance takes place. Soon after this is lost sight of, the gubernaculum becomes irregularly swollen and shortened, as shown in Figs. 11 and 12, and the yolk now seems to be prolonged into its cavity, although at an earlier stage the epithelium of the capsule appeared to surround the entire surface of the yolk. The swelling and shortening go on rapidly, and in a short time the egg presents the appearance shown in Fig. 12. A single nucleus can now be seen at the point previously occupied by the germinative vesicle, and the egg, nourished by the blood which bathes it, begins to grow, and is already somewhat larger than before impregnation. The shortening of the gubernaculum continues until the egg is drawn down from the median neural surface of the nurse to the point upon the right side of the lower or hæmal surface of the branchial sac, where the gubernaculum is joined to the latter, as already described. The brood-sac, or cup-like depression of the branchial sac, has meanwhile increased in size, and now forms a nearly hemispherical cup, large enough to contain the egg, which is soon entirely withdrawn into it, as shown in Figs. 13 to 18. Since the cavity of the brood-sac is a diverticulum from the sinus system of the nurse, the blood has free access to it, and bathes the egg on all sides. The latter is perceptibly larger at this time than it was during the stage last described, and this process of growth continues during the whole of the subsequent development; so that the embryo, at the time when the first traces of organs make their appearance, is many times larger than the unimpregnated ovum, and when the solitary embryo escapes from the body of the Chain-salpa it is two or three times as large as the latter itself was at the time when the egg was fertilized. This remarkable growth is mentioned here, in advance, as it will not be referred to in the subsequent description, although it must be understood as going on at all stages.

The egg is now pear-shaped, and is attached by its broad end to the floor of the brood-sac, at the point where the gubernaculum originally joined the latter (see Fig. 13); the nucleus is now divided into two, all traces of the epithelial capsule have disappeared, and nothing more is known about it.

Of the two nuclei now present the lower, with the portion of yolk which surrounds it, is destined to form the "germ-yolk" of the embryo, and soon divides again, as shown in Fig. 14, and at a stage a little later, Fig. 15, it is composed of a mass of minute segments. The upper nucleus of Fig. 13 with its portion of yolk forms the so-called "food-yolk," and segments much more slowly. In Fig. 15 it is not at all divided, and in Fig. 16 it is divided into four large spherules, enclosed in a common membrane. The food-yolk now gradually becomes invaginated into the germ-yolk, as shown in Fig. 17, and is soon entirely surrounded by the latter, forming the symmetrical vase-shaped "gastrula," shown in Fig. 18.* The cavity of the gastrula opens directly into the sinus system of the nurse, and the blood now circulates into and out of the primitive digestive cavity, as well as around the outside of the embryo, which grows rapidly, and soon fills the brood-sac, so that its outer surface comes into contact with the latter, which soon ceases to be visible as a separate covering (Figs. 19 and 20), and of course the blood no longer bathes the outside of the embryo, although it continues to pass into and out of the primitive digestive cavity. The germ-yolk now becomes finely segmented, although the spherules are still somewhat larger than the more transparent ectoderm cells outside them. The invagination cavity or "primitive digestive cavity" becomes separated into two portions; the outer remains in free communication with the sinus system of the nurse and forms the inner chamber (Fig. 19, *10*) of the placenta, and its opening becomes the orifice of the placenta. This persistence and functional importance of the "orifice of Rusconi" are very remarkable, and seem to have no parallel among the other Tunicata, or in any of the various groups of animals with which it has been proposed to associate them. The gastrula of Salpa seems to be a special adaptation to the very anomalous mode of development of the embryo. The lower portion of the primitive digestive cavity now becomes entirely surrounded by the endoderm, and soon becomes obliterated, as shown in Fig. 18. Very soon a cavity reappears in this portion of the embryo, and persists and forms the cavity of the branchial sac (Fig. 20, *13*). A constriction now appears upon the outside of the embryo, separating the placenta from the embryo proper, and soon a body cavity becomes visible, separating the branchial sac from the outer wall, and also extending up around the placenta, to form its outer chamber (Fig. 22, *12*).

The placenta, therefore, consists of an inner chamber communicating with the sinus of the nurse, and having no communication with

* A number of eggs at all stages between the two represented in Figs. 17 and 18 were found, but the two here shown seem to be all that are necessary for clearly representing the process. Huxley (Plate XVI. Fig. 7) gives a figure of a stage in which the invagination is about half completed, but he does not refer to it in the text.

any of the cavities of the embryo; with a cavity which is part of the original "cavity of invagination," and is surrounded by a wall of cells derived from the endoderm: and an outer chamber bounded on the inside by the cells of the endoderm, and on the outside by the ectoderm,

Fig. 19.

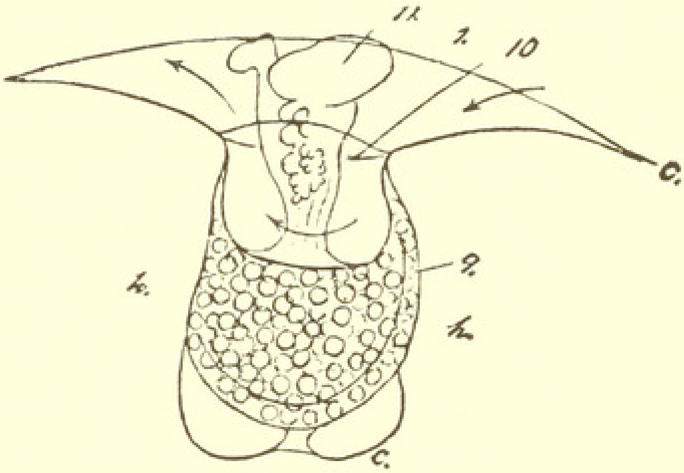


Fig. 20.

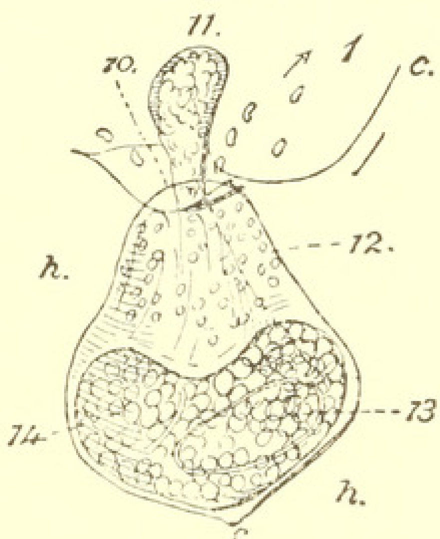
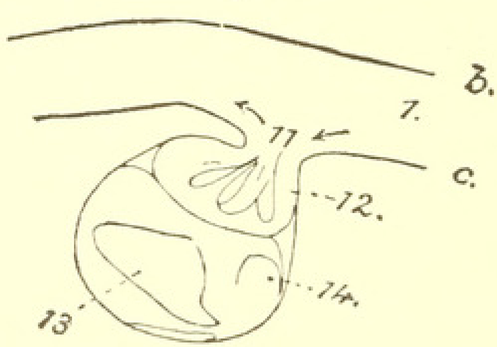


Fig. 21.

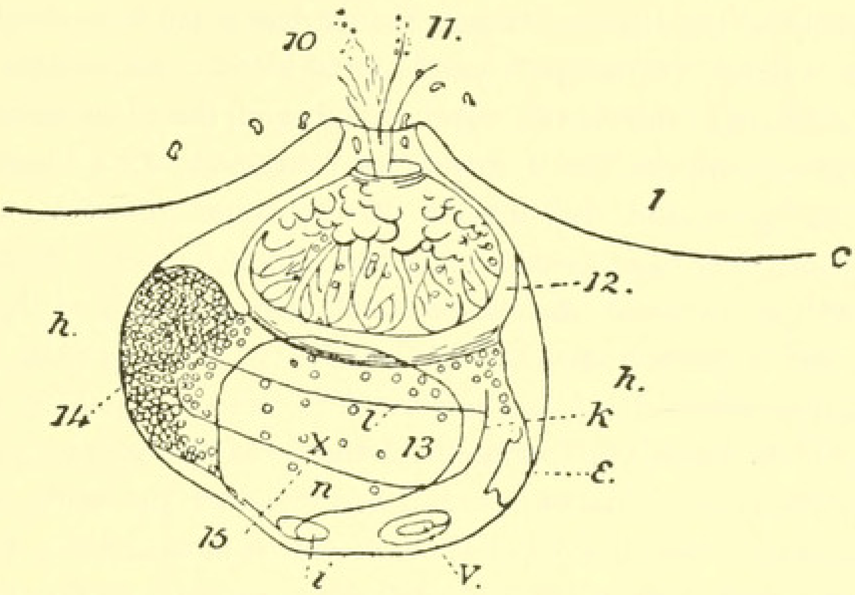


Successive stages in the formation of the embryo: *b*, outer tunic of male; *c*, wall of branchial sac of male; *h*, branchial cavity of male; *1*, sinus system of male; *10*, opening of inner chamber of placenta; *11*, club-shaped organ; *12*, outer chamber of placenta; *13*, branchial cavity of embryo; *14*, nucleus of embryo.

having no communication with the sinus system or other cavities of the nurse, but being directly continued into the body cavity of the embryo.

The Placenta. — For the sake of clearness we will neglect for the present the changes which are now taking place in the body of the

Fig. 22.



Embryo a little more advanced: *15*, lateral atrium; *i*, mid-atrium; *e*, branchial aperture; *k*, peripharyngeal ridge; *l*, epipharyngeal ridge; *v*, ganglion.

embryo, and will describe at once the subsequent development of the placenta. The inner cavity of this is at first one undivided chamber into and out of which the blood of the nurse is constantly passing; but soon a singular club- or stump-shaped structure (Fig. 18, *11*) appears in its opening, dividing this, at first incompletely, into two, and projecting into the sinus system of the nurse as well as into the cavity of the placenta, and serving to more effectually divert the blood into the latter. Appearances seem to indicate that this organ is not derived from any of the parts of the nurse or embryo, but is formed directly from the blood, by the aggregation and fusion of its corpuscles. Observations upon this point were made so frequently and with such uniform results that there seems to be little doubt that it does originate in this way. No traces of a cellular structure were observed in it. It is at first entirely free from all the adjacent parts, but very fine threads are soon visible extending from it, and some of these soon unite to the neck of the placenta and serve to anchor the two together; the terminations of the remaining threads were not traced, but they seemed to float in the blood of the sinus.

The lower end of this organ now extends downward, and joins the bottom of the placenta, and soon becomes divided into a number of root-like portions (Figs. 19, 21, and 22, *11*), which separate the cavity into irregular intercommunicating lacunæ, through which the blood now passes, and among the meshes of which the corpuscles are often entangled, so that the circulation is much less rapid than in the sinus outside. Before this stage is reached the blood of the fœtus also begins to circulate, and as the outer chamber of the placenta is part of its sinus system, the small corpuscles contained in its blood can be seen passing around the outside of the inner chamber, and thus coming into very close proximity to that of the nurses. Huxley's account of the placental circulation at this period is so good that it will not be out of place here, although he adds very little to the accounts previously published by Sars and Krohn. He says (p. 575), that the placenta "contains two perfectly distinct cavities or sacs; of these the outer is concave and cup-shaped and envelops the inner, which is subspherical. Now the outer sac is in free communication by a narrow neck, divided into two channels by a partition, with the dorsal sinus of the fœtus; and the inner sac is in equally free communication by a neck similarly divided, with a short sinus arising immediately behind the heart; and as there is no communication between the two sacs, it follows that the current of blood in each is perfectly distinct from and independent of that in the other. A more beautiful sight, indeed, can hardly be afforded to the eye of the microscopic observer than the circulation in this organ. The blood-corpuscles of the parent may be readily traced entering the inner sac on one side of the partition, coursing round it, and finally re-entering the parental circulation on the other side of the partition; while the fœtal blood-corpuscles, of a different size from those of the parent, enter the outer sac, circulate round it at a different rate, and leave it to enter into the general circulation of the dorsal sinus. More obvious still does the independence of the two circulations become when the circulation of either mother or fœtus is reversed."

The following historical sketch of our knowledge of this structure is also taken from Huxley's paper, page 592: "Cuvier* speaks of finding a fœtus attached to the parent by a pedicle; and, referring to a figure, he says: 'Ce corps rond [evidently the placenta] seroit-il un organe servant uniquement pendant le temps de la gestation pour établir l'union entre la mère et son petit et qui s'effaceroit ensuite?'

Chamisso † calls the pedicle of attachment 'pediculus umbilicalis'; the placenta, 'globulus opacus.'

* Ann. Mus. d'hist. nat. 1804, IV.

† Nova Acta, 1832, XVI. pp. 362-422.

"Meyen was the first to give this structure the name of placenta, and his account of it is so very clear and precise that it is wonderful it should have been subsequently forgotten or overlooked. He says: 'Wir haben bei ganz jungen Individuen den Verlauf der Blut-bewegung selbst by 200-maliger Vergrösserung beobachten können. Der Muttertheil der Placenta hat nur wenig Gefässe, um so mehr aber der Fötus-theil, in dem sich ein ausserordentliches Convolut von Gefässen befindet, das sich in einem Stamme endigt, der sich in das grosse Bauchgefässe ganz in der Nähe des Herzens ergiesst. Ein unmittelbares Uebergehen der Blutgefässe aus dem Muttertheil in den Fötus-theil haben wir nicht sehen können. Hat der Fötus die hinlängliche Ausbildung im Leibe der Mutter erreicht, so verwächst das grosse Blut-gefäss und die Placenta fällt ab.' — Page 440."

Krohn, Huxley, Leuckart, and Vogt subsequently investigated the structure of this organ, and have correctly described its more important features as they exist when fully formed, but its origin and the nature and structure of the club-shaped core are here described for the first time.*

The embryo, at the stage last described (Fig. 20), consists of the outer tunic derived from the outer layer of the gastrula, the body cavity, and the branchial sac, the wall of which is formed from a portion of the cells of the inner layer, while the remainder form a large mass (*14*), at what is to be the posterior end of the body. The subsequent development of these parts, as well as the formation of those which subsequently make their appearance, will now be described.

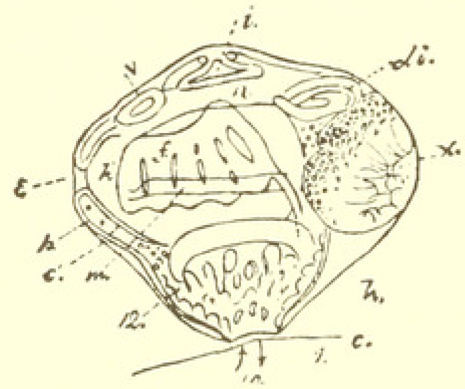
The Outer Tunic. — Little need be said of this; it is not at first covered by the test, which makes its appearance later, apparently by excretion from the surface of the outer tunic; the latter now covers all of the embryo except the part in contact with the placenta, and is reflected on this to form the outer wall of the outer or fœtal chamber, as already described. It is entirely separated, at first, from the branchial sac by the body cavity, but at a later stage an invagination appears at the anterior end (Fig. 22, *e*), which unites with the anterior end of the branchial sac, and a perforation appears which forms the incurrent opening (*e*, Figs. 1, 3, 23, 24, 25, 33, 34). The

* Leuckart says (p. 53), that the residual yolk becomes the placenta, but we have seen that this becomes invaginated to form the digestive layer of the germ, and that the placenta is not formed until the close of the gastrule stage.

atrial opening (*g*) in the same figures is formed in a similar manner, but somewhat later, by coalescence with the walls of the atrial chamber. The muscular bands which adhere to the inside of the outer tunic of the adult are derived from the atrial tunic, and will be described in connection with that organ.

The Body Cavity. — During the early stages the whole surface of the branchial sac, except the region next the placenta, is surrounded by the body cavity, the connection of which with the outer chamber we have already described. As development progresses, the outer tunic and the wall of the branchial sac unite at various points, and the atrial tunic is formed between them and has regions of attachment

Fig. 23.



Embryo more advanced than the one shown in Fig. 22, but less highly magnified: the muscular girdles are partially separated: *1*, sinus system of nurse; *10*, opening of placenta: *12*, outer or fœtal chamber of placenta; *c*, branchial sac of nurse; *h*, branchial cavity of nurse; *b*, outer tunic; *c'*, wall of branchial sac; *e*, branchial aperture; *h'*, branchial cavity; *i*, mid-atrium; *w*, gill; *m*, endostyle; *f*, muscles; *v*, ganglion; *x*, elæoblast; *d i*, digestive organs.

to both, so that we no longer have a single body cavity, but instead of it a sinus system.

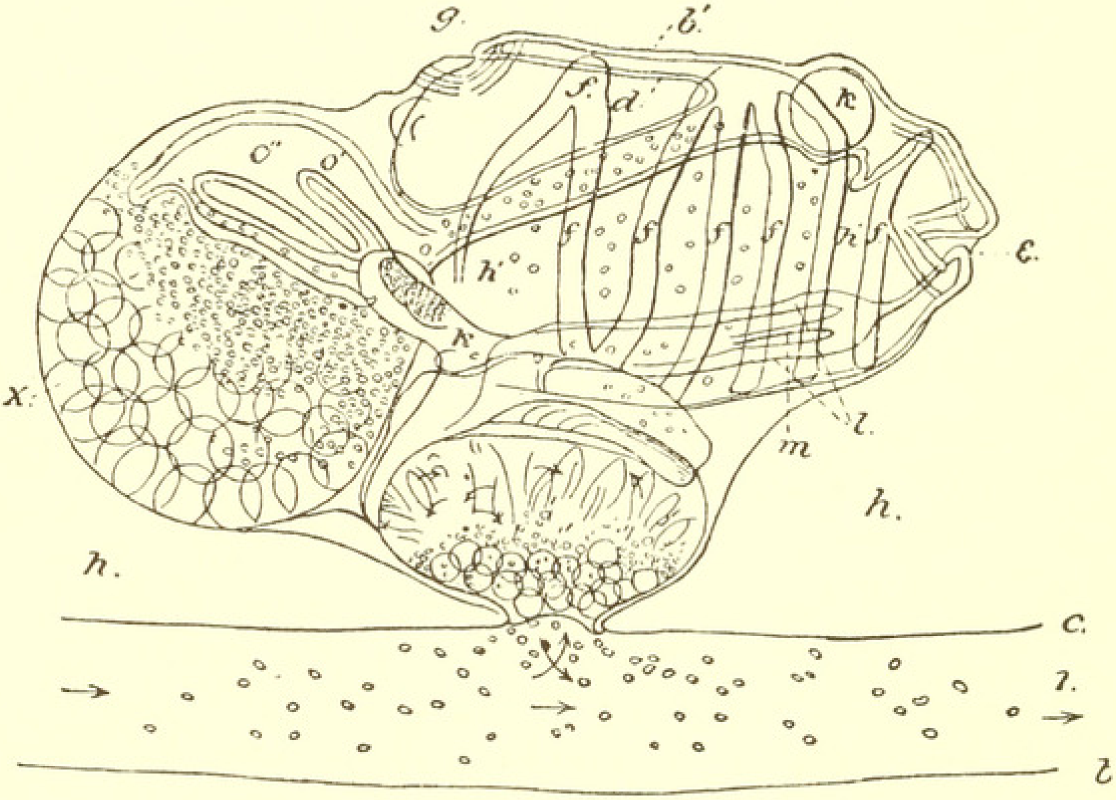
Branchial Sac and Digestive Organs.— The branchial sac is at first a single, nearly oval cavity (*13*, Fig. 20), occupying a little more than the anterior half of the embryo, while the posterior half is filled with a mass of cells (*14*, Fig. 20), which are to give rise to the various organs of the nucleus. The cavity of the branchial sac soon becomes lengthened backward, so as to occupy two thirds or three fourths of the body of the embryo (Figs. 22, *13*; 23 and 24, *h*), and the nucleus is divided, unequally, into two portions, of which the smaller (Fig. 23, *d i*), which is upon the neural side, gives rise to the wall of the digestive organs, while the larger or hæmal portion (Fig. 23, *x*) becomes the elæoblast. The cavity of the œsophagus soon becomes visible as a diverticulum from the posterior end of the cavity of the branchial sac, and is in direct communication with the latter at the earliest stage observed. Two diverticula are now formed upon the lower or hæmal surface of the œsophagus, side by side and parallel with each other (Fig. 4, o''); as these grow they gradually unite and form a single large diverticulum, which is bent forward towards the mouth so that it lies nearly parallel with the œsophagus, as shown in Fig. 24, o''. Since the cavity of the stomach is derived from that of the branchial sac, it must, like the latter, be regarded as a portion of the primitive digestive cavity of the gastrula. The cavity of the intestine, however, first appears as a closed chamber, parallel with and on the hæmal side of the stomach, and having no communication with the cavity of the latter (Fig. 4, o'').

The partition which separates the two soon disappears, and they become continuous, although the anal end of the intestine, which is close to the mouth, is still closed. After the chamber of the mid-atrium or cloaca has been formed the anus unites with this, the partition disappears, and the digestive organs now form an U-shaped tube, connecting the branchial with the atrial cavity. As nothing was learned of the development of the so-called "hepatic organ" of the solitary Salpa, I will now give what little was learned about it in the chain-salpa, in order that this description of the mode of formation of the various organs may be as complete as possible. In the young chain-salpa, at the time it is discharged into the water, the posterior end of the stomach and the side of the intestine are covered with a single layer of large, nucleated, dark-colored cells (Figs. 33 and 34), which present the characteristics of liver cells, and agree in appearance, and approximately in position also, with the liver cells upon the wall of the upper portion of the stomach of a Polyzoön.

At the time that the observations upon Salpa were made a species of Appendicularia was very abundant. The wall of the stomach of this bore, upon the *inside*, two large clusters of cells which were arranged in a single layer, and agreed, in appearance, color, &c., with those found upon the *outside* of the stomach of Salpa, which are undoubtedly liver cells, and the rudimentary "tubular hepatic organ."

The study of this organ was extremely difficult, as the elæoblast of the solitary Salpa obscures this portion of the digestive organs at all stages, while, during all stages but the earliest, it is hidden in the chain-salpa by the testis. The second portion of the nucleus, which is much larger than the portion which forms the digestive organs, is

Fig. 24.



Embryo at the time the first traces of the stolon appear: *1*, sinus system of nurse; *b*, outer tunic of nurse; *c*, branchial tunic of nurse; *h*, branchial cavity of nurse; *b'*, outer tunic; *d*, atrial tunic; *e*, branchial aperture; *f*, muscles; *g*, atrial aperture; *h'*, branchial cavity; *k*, heart; *l*, epipharyngeal ridges; *m*, endostyle; *o*, mouth; *o'*, œsophagus; *o''*, stomach; *x*, elæoblast. Through an error the ganglion, as well as the heart, is marked *k*.

situated upon the hæmal side of the body, as already stated (see Fig. 23, *x*), and soon becomes divided up into large transparent cells, filled with oil globules, or with a body of oil-like appearance and refractive power, but not divided into globules. These cells soon become polygonal by mutual pressure, and form a large spherical mass which increases much more rapidly than the remaining organs of the body (Fig. 24, *x*), and at the time the Salpa chain begins to be formed within the body, the mass of cells is nearly as large as all the other portions of the embryo. The blood circulates freely among the cells and over the outside of the mass, which is not separated from the sinus system by any membrane, and the blood globules, and the oil which escapes from the older cells, often accumulate in large masses near the digestive organs and heart.

This organ, for which Krohn has proposed the name "elæoblast," attains its greatest development in the embryo, and begins to disappear as soon as the chain begins to form within the body, and is therefore provisional, as pointed out by Krohn, and has no permanent place or function in the adult. Meyen regarded it as a yolk (p. 401), and there seems to be little doubt that it is, if not strictly yolk, at least something having a very similar use, and supplying the material which is to be employed in the formation of the zooids of the chain. Leuckart states (p. 57), that it is found in both forms of Salpa, but this is not the case. It is always present in the solitary Salpa during the embryonic development, and the nucleus of the young chain-salpa divides, like that of the solitary form, into an upper smaller portion, which gives rise to the digestive organs, and a lower, larger portion, which thus agrees with the elæoblast in its mode of origin, and, during the earliest stages, in appearance also (Fig. 32, *t*). It never attains a great size, as compared with the other organs, and soon forms a compact globular mass of cells (Fig. 34, *t*), which in the adult chain-salpa forms the testis (Fig. 5). Since the testis of the chain-salpa agrees so closely with the elæoblast of the solitary one, it seems very probable that this is also a reproductive organ, and that Meyen was right in calling it a yolk-deposit. It is not a true ovary, for this originates in a different way, as will be shown further on.*

The Atrium. — The adult Salpa is composed of three principal tunics, each enclosing a special cavity. The formation of the outer one of these, the outer tunic, has already been described, and we have seen that it is derived from the outer layer of the gastrula; its cavity has

* Few organs have had their functions more disputed than this elæoblast. Many of the earlier observers considered it a liver, and Sars repeats this error, although Meyen had previously determined its connection, in some way, with development. Krohn discovered that it was transitory and provisional, but does not commit himself in regard to its function. Huxley described the true liver, and, although acquainted with all that had been written upon the elæoblast, says (p. 571), "There would seem to be no clew either to the homology or to the function of the elæoblast. Without hazarding a conjecture, it may be remarked, as a curious fact, that these animals, so remarkable for possessing in the fœtal state a true though rudimentary placental circulation, possess an organ which in structure and duration somewhat calls to mind the thymus gland." Leuckart (p. 57) concludes that it is a depot of nutriment stored up for future use, but he does not state whether it is to be used in the formation of the embryo or in the development of the chain. We will return to the subject of its homology with the testicle further on.

also been described as the body cavity or sinus system. The inner tunic, or branchial sac, has been shown to be part of the inner or digestive layer of the gastrula. The third tunic, now to be described, is the atrial tunic, and its cavity the atrial chamber or cloaca. The earliest stages in the formation of this tunic were not traced in either form of Salpa, but as it presented, when first observed, an appearance very similar to that described by Kowalevsky,* as presented during the early stages in the development of Pyrosoma, it is possible that it originates in the same way; although in the absence of any observations upon its origin, the fact that Kowalevsky's observations upon Pyrosoma are here referred to must not be regarded as implying anything more than a belief that the similarity which is known to subsist between the two genera, in many other respects, may extend to the way in which the atrium is formed. In the egg-embryo of Pyrosoma two circular depressions appear upon the neural side of the outer tunic, near the anterior end, and these deepen so as to form tubes derived from the outer layer, with cavities which open externally; these tubes lengthen and penetrate the body cavity upon each side of the branchial sac; their external openings close, and they become separated from the outer tunic, and form the "lateral atria" of Huxley.† According to Huxley (pp. 215, 216), — and Kowalevsky's account corroborates that of Huxley in every particular, — these at first "are very small and thick-walled; they soon become larger and the walls proportionally thinner, and the sacs themselves are both absolutely and relatively larger.

"In Fig. 29 they are very much larger and thinner, and their relations to other organs are especially worthy of attention. The outer layer of each is applied to the outer tunic of its side, leaving a small interspace, which communicates freely with the great posterior sinus, in which the intestine and genatilia are disposed, and with the anterior sinus which lies between the pharyngeal wall [branchial sac], and the external tunic. This interspace is, in fact, the parietal sinus. The internal layer, continuous with the outer anteriorly and posteriorly, but separated from it by a wide chamber for the rest of its length, is applied against the wall of the pharynx [branchial sac], for

* Ueber die Entwickelungsgeschichte der Pyrosoma. Von A. Kowalevsky. Archiv. für Mikr. Anat., XII., 1875, p. 597.

† Huxley, Anatomy and Development of Pyrosoma, p. 205. Trans. Linn. Soc., XXIII., 1860, p. 193.

four fifths of the extent of the latter, and then coats the lateral portions of the gastro-intestinal tract, forming the antero-lateral boundary of the great posterior sinus. The space between the wall of the pharynx and the inner layer of the sac communicates anteriorly with the anterior sinus, posteriorly with the posterior sinus, and it is interrupted at several points by the union of the pharynx and inner layer with one another. It represents the system of branchial sinus." In Salpa this union of the inner wall of the lateral atrium with the branchial sac does not take place. "In side views it is not easy to make out the boundaries of the lateral sacs; but it is most important to observe that, as has been already mentioned, in the middle of the lateral face of the pharynx, and therefore also in the middle of the lateral face of the inner wall of the sac, a series of opake rings with clear centres, the rudiments of the branchial stigmata, make their appearance." In this respect also Salpa differs from Pyrosoma; no branchial clefts are ever formed in connection with the lateral atria. "These correspond with the points of union of the pharynx and the inner wall of the sac. They are at first small, round, and very indistinct, but by degrees they elongate in a direction perpendicular to the long axis of the pharynx, and their real nature becomes apparent. Hence it is clear that these stigmata must eventually open into the lateral sacs, as indeed they may be seen to do in such buds as that represented in Fig. 30; and hence also it follows that the lateral sacs are the rudiments of the lateral atria.

"At first the lateral atria appear to be perfectly distinct from one another, and no atrial aperture is discernible. In buds, such as that represented in Fig. 29, again, they do not extend, posteriorly, further than the sides of the alimentary canal; but in more advanced buds they are produced backward on each side until they pass beyond the level of the posterior margin of the stomach, so that they now constitute the entire lateral boundaries of the great posterior sinus. The longitudinal section of a somewhat smaller bud shows that in this condition the atria are no longer distinct, but are united together below [on the neural side of] the stomach, by a comparatively narrow and short canal, which is the mid-atrium.

"I have not traced out all the details of the process of coalescence of the lateral atria; but I suppose that each branchio-parietal portion of the atrium, at first a distinct sac, is prolonged downwards and inwards, under the stomach, and that the opposed walls of the prolongation become applied to one another, coalesce, and then become perforated.* At any rate, the mid-atrium is now surrounded by a membranous wall, continuous on all sides with the lining of the lateral atria, and applied superiorly and anteriorly against the stomach and œsophagus, posteriorly and inferiorly against the external tunic, but not touching either of these parts, except for a small space on the floor of its chamber, where it becomes united with the external tunic to allow of the formation of the atrial aperture. This aperture is situated on the neural side of the body, in front of the posterior end, which is chiefly occupied by the genitalia; but as development goes on the mid-atrium increases, disproportionately, and encroaches upon the other organs, upwards and forwards, in such a manner that its anterior wall invests the whole posterior and lateral faces of the gastro-intestinal division of the alimentary canal. . . . . The facts which I have detailed are exceedingly important for the comprehension of ascidian structure in general." This account of the form and connections of the atrial chamber applies to those zooids of Pyrosoma which are produced by budding, as well as to the egg-embryo, but according to the observations of Kowalevsky the diverticula which give rise to the lateral atria of the bud-zooid are derived, not as in the egg-embryo, from the outer tunic, but from the branchial sac, or inner tunic, and the cavities are therefore diverticula from the cavity of the latter.†

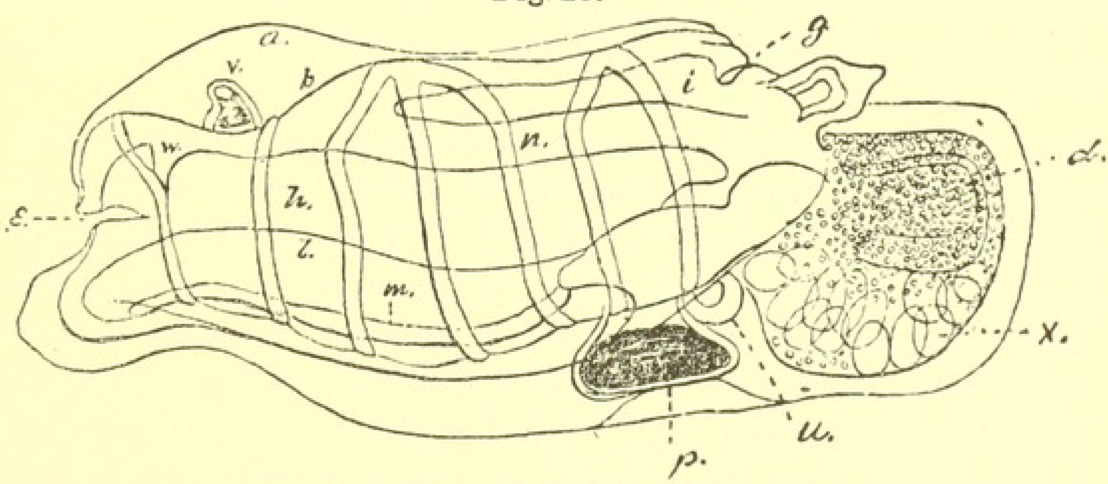
The atrium of Salpa, when first observed (Fig. 22, *15*), was composed of two broad lateral atria within the body cavity, one on each side of the branchial sac, and a very small mid-atrium (*i*); so that we have all the essential parts of this structure, occupying similar positions to those of Pyrosoma, and bearing almost identical relations to the surrounding parts. The lateral atria do not, however, as in most Tunicata, remain connected with the mid-atrium, and unite with the wall of the branchial sac to form the branchial slits, but soon become entirely separated (Fig. 23, *f*), and the two walls of each unite so as to form a broad solid sheet of tissue, which soon splits up to form the muscular bands of the branchial sac, of which there are six in the solitary form, and five in the chain-salpa.

* This conjecture is fully proved by the observations of Kowalevsky.

† Compare also the manner in which the atrium is formed in the bud-zooids of Amauricium and Dideminum. Ueber die Knospung der Ascidien. von A. Kowalevsky. Archiv. für Mik. Anat. X, 1874, p. 441.

The Muscles. — The bands of the two sides of the body are at first entirely distinct from each other, as noticed by Krohn, and all of those upon one side are, at first, united above and below, as in Fig. 23; the hæmal ends of the first, second, third, and sixth, in the solitary Salpa, soon become free, as shown in Fig. 24, and the neural ends separate into two bundles, composed of the first, second, third, fourth, fifth, and sixth, respectively (Fig. 24). The first now separates at both ends (Fig. 25), and after a time the hæmal ends of the fourth and fifth also become free, but the amount of separation is not uniform in specimens of the same age, and the union between the fourth and fifth may persist until the animal is nearly full grown (Figs. 1, 2, and 23); and to this the discrepancy in the various descriptions of the species is due. The neural ends of all the corresponding bands upon the two sides of both forms of Salpa soon unite, as well as the hæmal ends of the first, second, third, fourth, and fifth of the solitary Salpa, which thus form closed muscular belts or girdles encircling the body; while the hæmal ends of the sixth pair of the solitary Salpa, and of all in the chain-salpa, are permanently free. Soon after the muscular layer begins this process of division, it ad-

Fig. 25.



Solitary Salpa, — female, at the time when it escapes from the body of the male, viewed from the side, with the neural surface uppermost: *a*, test; *b*, outer tunic; *d*, digestive organs; *e*, branchial aperture; *g*, atrial aperture; *h*, branchial cavity; *i*, atrial cavity; *l*, epipharyngeal fold; *m*, endostyle; *n*, gill; *p*, placenta; *u*, stolon; *v*, ganglion; *w*, languette; *x*, elæoblast.

heres to the inside of the outer tunic, in which situation the muscles of the adult are always found; and this tunic has accordingly been called the muscular tunic, although, as we have seen, the muscles really belong to the atrial portion of the body. The muscles of the branchial and cloacal apertures do not appear to be derived from the atrial tunic, as they make their appearance in the positions which they subsequently occupy. See Figs. 24 and 33.

The Branchial Slits. — During the changes which have been described as taking place in the lateral atria, the mid-atrium has increased in size (Figs. 23, 24, and 25, *i*), and at last extends almost from the stomach to the ganglion (*v*). When seen in section (Figs. 24 and 33, *i*), it presents an irregularly triangular outline, with its base

Fig. 26.

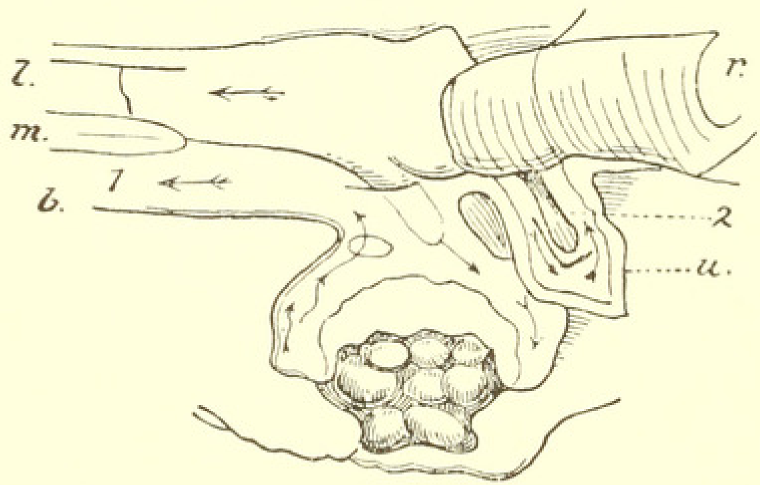


Fig. 26, from Huxley: *b*, outer tunic; *l*, epipharyngeal ridge; *m*, endostyle; *r*, heart; *u*, stolon; 2, central tube of stolon, which in Huxley's figure stops short, and does not reach the outer wall of the heart; but as its connection with the latter is unmistakable, the figure has been altered accordingly.

parallel to the posterior neural surface of the branchial sac, which surface, like the corresponding one of the atrium, is flattened so that they would be shown as parallel by a section at right angles to the one represented in Fig. 33. The branchial and atrial tunics now unite upon each side, so that the sinus (*u*, Fig 4) is converted into a tube which communicates, at its posterior end, with the heart and perivisceral sinus (Fig. 24), and at the anterior end with the neural sinus. This tube is the gill or "hypopharyngeal band," and it is evident that its cavity is part of the primitive body cavity, and its walls are derived from or rather are parts of the branchial and atrial tunics. The centres of the two regions upon the sides of the gill, where these two tunics have become united, are now absorbed, so that a single long and narrow branchial slit is produced upon each side of the gill. The branchial cavity is thus thrown into communication with that of the atrium, and the upper surface of the latter now unites with the outer tunic, and the external atrial opening is formed by absorption. See Figs 1, 24, 25; 3, 33, and 34, *g*.*

The Heart. — I was prevented, by lack of time, from making a series of observations upon the manner in which the heart originates, and accordingly quote what Leuckart (p. 55) gives upon this subject: "The heart presents, at the earliest stages, an oval form, and lies in the space between the nucleus and the ventral surface above the placenta." See Fig. 24, *k*, of the present paper. "One end is directed obliquely upward and backward, and the other forward and downward. At first, as already stated, it is a solid mass of cells, within which a cavity gradually appears [according to Vogt, p. 84, the heart is hollow from the first] and thus becomes transformed into a pouch, whose walls quickly become thin, and also, very early exhibits a pericardium. The first faint pulsations are separated from each other by long intervals, but are to be seen at a period when the ends of the heart seem to be closed. A circulation becomes visible only after most of the other organs are formed; but as the blood contains no globules during the earliest stages, the absence of a circulation cannot be absolutely affirmed."

The Nervous System. — The ganglion, when first observed (Fig. 22, *v*), was a hollow oval chamber, with indications of an opening at the anterior end. As it was impossible to devote much time to the

* Most of the writers upon Salpa have entirely mistaken the nature of the branchial sac, and its relation to the typical form among the Tunicata, and Leuckart seems to be the only one who has recognized the fact that the so-called "gill" is simply the sinus between two large branchial slits. His statement is clear, and, as far as it goes, correct. He says (p. 56): "Kurz nach der Aushöhlung der Ganglionkapsel beobachtet man in der Rückenwand des Embryos eine neue Bildung. (Tab. 11, Fig. 6.) Es entsteht hier in der Mitte, zwichen der Ganglionkapsel und der Wurzel des Nucleus wie früher im Innern des Embryonalkörpers, eine lichte Stelle, die sich allmählig in einen länglichen Hohlraum verwandelt, und jederseits durch die Wand der Athemhöhle hindurchbricht. Die Innenlage der Rückenwand, die Anfangs beide Höhlen von einander trennte, wird durch diesen Durchbruch in einen cylindrischen Strang verwandelt, der von der Wurzel des Nucleus nach dem spätern Nervenknoten hinzieht, und naturlicher Weise nichts Anderes, als die erste Anlage der Kieme sein kann. Die Höhle durch welche die Kieme von der Körperwand abgetrennt wird, ist die Kloakhöhle, die also auch bei den Salpen, als ein eigner, von der Athemhöhle (im engeren Sinne) verschiedener Hohlraum ihren Ursprung nimmt."

As far as this goes it is correct, but he makes no mention of a special tunic around the cloacal chamber, and says nothing of the lateral atria, or of the origin of the muscular bands.

study of this organ, Leuckart's account is again referred to, in order to furnish as complete an account as possible of all that is known of the evolution of all the organs of the fœtus.

According to Leuckart (page 56), "The mass of cells, which is the first indication of the ganglion, and which lies upon the anterior end of the branchial sac, diagonally opposite the heart, to which it is not inferior in size, soon shows traces of a cavity, surrounded by very thick walls, and it continues in this condition for some time. After the remaining organs have gradually been developed, and histological differentiation begins, the cavity of this organ becomes filled up, thus transforming it into a solid mass of cells, which is easily recognized by its great size, and is surrounded by a capsule. The ganglion proper does not seem to exist before this stage is reached, for the primitive mass of cells with the cavity which appears within it are to be regarded, not as the ganglion, but as the capsule within which the ganglion is to be formed."

I have now described the way in which all the organs of the solitary Salpa are formed, with the exception of the ovary, and the stolon which gives rise to the chain; and these can be best treated in connection with the history of the development of the chain-salpa.

Development of the Salpa-Chain.

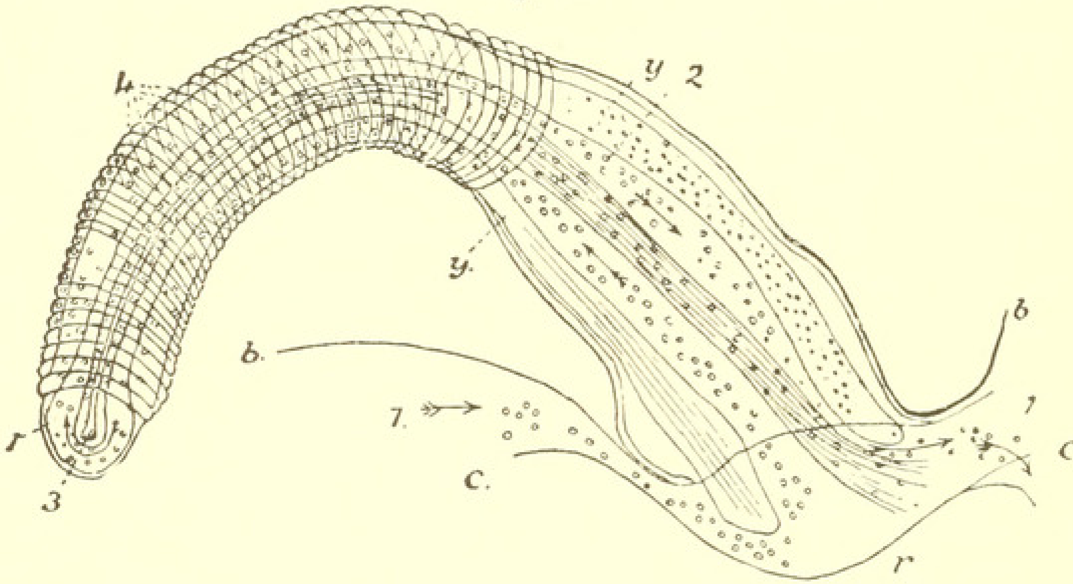
When the solitary embryo has reached the stage of development shown in Figs. 24, 25, 33, *s*, at which time it is about one thirtieth of an inch long, the tube which is to give rise to the chain appears within its body, and is at first simply a cup-like protrusion of the outer tunic into the cellulose test which now surrounds the embryo. This cup (Fig. 25, *u*) is situated midway between the nucleus and the placenta, upon the hæmal side of the body and directly opposite the heart, and its cavity is a diverticulum from the sinus system, into and out of which the blood passes. Since the wall of the cup is derived from the outer wall of the body cavity, while the heart is upon the inner or branchial tunic, it is plain that the cavity of the cup is separated from the pericardium by the width of the sinus system. A small bud-like protrusion now appears upon the surface of the pericardium, and lengthens so as to form a long rod, which extends across the sinus and projects into the cavity of the cup, as shown in Huxley's Fig. 4, Plate XVI., which is copied as Fig.

26 of the present paper. This rod, crossing the blood current at right angles, more effectually diverts this into the cup, so that a steady stream now passes into and out of the latter, which rapidly lengthens so as to form a tube projecting from the outer tunic into the cellulose test (Fig. 27).

The prolongation from the pericardium (Fig. 27, *2*, and Fig. 7, *2*) also lengthens, and reaches almost to the tip or blind end of the tube, and soon shows traces of a central longitudinal cavity (Fig. 27, *3*), which appears to be entirely closed at both ends. In a cross-section at this stage we should have: first, the outer tube, thin-walled and derived from the outer tunic; within this a chamber continuous with the sinus system, and within this a second tube, derived from the pericardium, with very thick walls and a cavity without connection with any of the pre-existing cavities of the embryo. This inner tube now becomes flattened until its edges unite with the inner wall of the outer tube, the cavity of which thus becomes "divided by a partition into two canals, which are distinct for the whole length of the tube, except at its very extremity, where they communicate just as the two scalæ of the cochlea do; and it thence happens that, in the living animal, a constant current passes up on one side of the partition and down on the other, the direction of the two currents being generally, but not always, reversed with the reversal of the general circulation" (Huxley, p. 573).*

* Huxley's figures of the early stages in the formation of the chain are very correct, and exhibit the relations of the various parts correctly; but the passage above quoted includes nearly all of his description, which is correct as far as it goes, but very brief. Vogt also (Sur les Tuniciers nageants de la Mer de Nice, p. 36) gives a correct account of the tube, but Leuckart, although he was acquainted with and refers to Huxley's description, disputes its correctness. It is so easy to make observations at this period, and their result is so satisfactory, that it is hard to understand how such a difference of opinion could arise; but as Leuckart's statement is made with the greatest confidence, it cannot be passed without notice, and is accordingly quoted here: "Man hat behauptet, dass der röhrenförmige Keimstock der Salpen aus mehreren übereinander gelegenen Häuten bestehe. Ich habe indessen — abgesehen natürlich von der äussern Cellulosescheide, die sich bei der Entwickelung der Knospen in keinerlei Weise betheiligt — vergeblich versucht, diese beiden Häute darzustellen. Das Keimrohr der Salpen zeigt nur eine einzige Substanzlage, und hat eine einfach-zellige Beschaffenheit. . . . . Der Hohlraum, den die Keimrohre einschliest, communicirt, wie wir schon früher beschrieben haben, mit dem Lacunen-system des mütterlichen Leibes. Man sieht auf das Deutlichste, wie die Blutkörperchen an der einen Seitenwand der Keimröhre emporsteigen und später an der entgegengesetzten Wand wiederum in den Kreislauf des mütterlichen Körpers zurückkehren" (page 69).

Fig. 27.

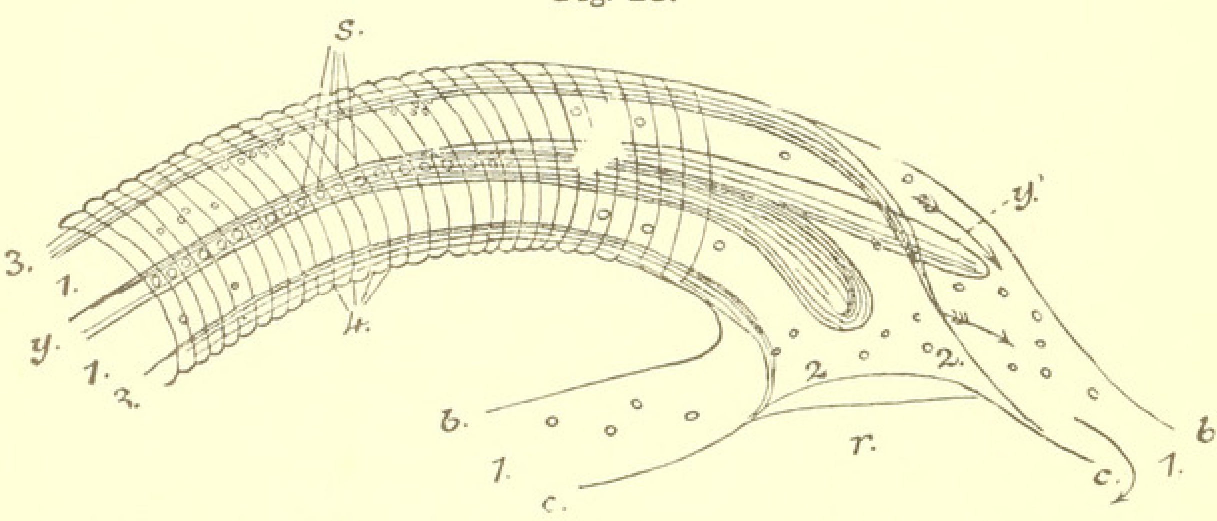


Very young stolon : *b*, outer tunic ; *c*, wall of pericardium ; *r*, heart ; *1*, sinus cavity ; *2*, central tube of stolon ; *3*, cavity of central tube ; *4*, constrictions in outer wall of stolon ; *y*, *y*, ovaries.

At about this period a long club-shaped mass of protoplasm (Figs. 27 and 28, *y*) appears within each of the sinus chambers of the tube; and soon after the constrictions (*4*) make their appearance upon the outer wall. We now have all the parts which are to take part in the development of the chain, and it will be best to state here the future history of each, and to give an outline of the development, before entering into the details.

The wall of the outer tube, which, as we have seen, is part of the outer tunic of the solitary form, is destined to form the outer tunics

Fig. 28.



Proximal portion of stolon a little more advanced, and rotated 90° from the position represented in Fig. 27 : *b*, *c*, *r*, *y*, *1*, *2*, and *4* as in Fig. 27 ; *3*, thickened edges of inner tube ; *s*, germinative vesicles.

of the zooids, and the constrictions upon it indicate the bodies of the latter. By the deepening of these constrictions, each of the sinus chambers, which are diverticula from the body cavity of the solitary form, becomes divided up to form the body cavities of the zooids upon one side of the chain.

The central partition gives rise, near one edge, to a row of bud-like protrusions upon each side, which become the branchial and digestive organs of the zooids of each side of the chain; while a similar double row, upon the other edge, give rise to the ganglia. It is probable that the cavities of the branchial sacs and ganglia originate as lateral diverticula from the tubular chamber of the partition into these buds; but this, for reasons which will be stated presently, could not be determined with certainty. The club-shaped organs within the sinus chambers become divided up into single rows of eggs, one of which passes into the body cavity of each zooid at a very early period of development.

It will be seen from this account that, in Salpa, as well as in Pyrosoma, "gemmation takes place, not, as in so many of the lower animals [e. g. the Hydrozoa and Polyzoa], by the outgrowth of a process of the body wall whose primarily wholly indifferent parietes become differentiated into the organs of the bud; but, from the first, several components, derived from as many distinct parts of the parental organism, are distinguishable in it, and each component is the source of certain parts of the new being, and of these only." * While these changes are in progress the tube lengthens, so that it at length encircles the nucleus, as shown in Figs. 1 and 2, *u*. The constrictions upon its surface deepen, and the wall protrudes between them, and each is soon seen to mark off, on each side of the stolon, the body of a young Salpa, and these soon become large enough to be visible to the unaided eye.

They do not increase in size gradually, from one end of the tube to the other, but develop in sets of from thirty to fifty each, and the development of all which are embraced within a set progresses uniformly. There are usually three of these sets upon the tube of an adult solitary Salpa, but sometimes there are four, two, or only one.

* Huxley, on the Anatomy and Development of Pyrosoma (p. 211).

I have taken the liberty of slightly changing this quotation, since the portion in brackets reads, in the original, "the Hydrozoa and Polyzoa, or *Salpa* and Clavelina among the Ascidians."

The zooids which compose the set nearest the proximal end of the tube are very much smaller than those of the second, which again are sharply distinguished from those of the third, and so on; and at a time when the first are very rudimentary indeed the last present all the organs of the adult, with the exception of the testis, and are ready to be discharged into the water. The carefully drawn, but somewhat rudely engraved figure of the fully developed chain, given by Eschricht,* gives an excellent idea of the position and relations of the various parts at this stage, and is, by far, the best figure which has ever been published. The position of the zooids is uniform throughout all parts of the stolon. The incurrent openings of all are upon the convex, and the excurrent upon the concave or inner side of the tube. The zooids of the opposite sides alternate with each other, and their hæmal surfaces face inwards. They are not perfectly parallel, for the visceral ends of their bodies are nearer each other than the neural ends, which incline outward. Fig. 34 shows a few zooids from a set which is nearly large enough to be discharged. The young chain, when first set free, is about half an inch long, and the single zooids, of which there are from twenty to thirty on each side, measure about one tenth of an inch in length. They grow very rapidly and soon reach their full size, when the chains "are often a foot or even a foot and a half long, and contain two rows of individuals, which are united together in such a way that they stand obliquely to the axis of the chain; the branchial openings are all on the upper side of the chain as it floats in the water, while the posterior openings are all on the lower side of the chain, close to the edge. Each individual is connected both with its mate on the right or left side, and to those immediately in front and behind on the same side. The succeeding individuals in the chain overlap considerably. The chains do not appear to break up spontaneously, but when broken apart by accident, the individuals are capable of living separately for several days. . . . . The individuals composing the chain, when full grown, are about three quarters of an inch long."† I do not think that the separation of the zooids makes the least difference in the length of their life, and the full-grown chains fall apart at the slightest touch, and, unless the water has been perfectly still for several days, chains more than four

* Undersögelser over Salperne, Tab. IV.

† Verrill, Invertebrate Animals of Vineyard Sound (p. 447).

or five inches long are not met with, and those a foot or more in length are very rare indeed, even when the water has been still for some time, and are only found in sheltered places, although full-grown chain-zooids are very abundant.

Huxley states of the species studied by him, which seems to be the same as that found on our coast, that it " but rarely happened that even two or three adhered together, and they never formed the remarkable free-swimming chain of other species. Generally they were found solitary, presenting only on their lateral faces traces of their former adhesion" (p. 574).

The chain-salpa, like the solitary form, moves by means of the stream of water which is continually discharged from the atrial aperture, and since the apertures of all the zooids of a chain are turned the same way, the current produced is quite powerful, and a chain, two inches long, placed in a glass bowl, with a pint of water, will soon set the whole of it in rotation. As we should expect, the motion of a chain is much more uniform and rapid than that of a single Salpa, and they usually move in nearly straight lines, although Mr. Agassiz states (Proc. Boston Soc. Nat. Hist.) that they sometimes change their course to escape capture.

Having now given a sketch of the formation of the chain, we will go back and follow the development of the various parts more minutely.

The Outer Tunic. — Little need be said of the formation of this portion, — the body wall of the zooid. It is derived directly from the outer wall of the tube, and its growth keeps pace with that of the contained organs, which are entirely separated from it, at all the earlier stages, by a distinctly visible body cavity (Fig. 30). The branchial

Fig. 29.

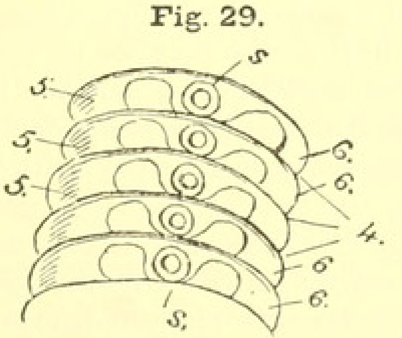
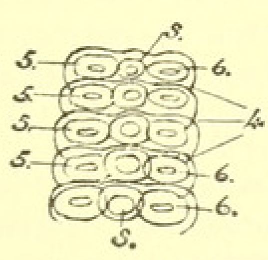


Fig. 30.



Two stages in the development of the zooids upon the stolon : *4*, constrictions which extend into the sinus chambers, and mark off the separate zooids ; *5*, the ganglia of the zooids ; *6*, primitive digestive organs ; *s*, a single ovum within the body cavity of each zooid.

and atrial apertures are formed after the embryo is considerably advanced (Figs. 31 and 32), precisely as in the solitary form. According to Vogt,* this tunic splits into two layers, the outer becoming the cellulose test, and the inner the outer tunic; nothing of this kind was observed, and appearances seemed to indicate that the test is an excretion from the surface of the body, as stated by Huxley (p. 585), and Leuckart; but this must still be regarded as one of the unsettled points in the history of the genus.

The Body Cavity. — The constrictions (Figs. 27, 28, 29, and 30, *4*) which mark out the zooids upon the surface of the tube gradually extend inward, and enclose part of the sinus cavity, which thus becomes converted into a body cavity, into and out of which it is quite easy to watch the blood corpuscles of the solitary form make their way. This direct connection of the sinus systems of the young zooids with that of the solitary Salpa persists long after all the principal organs of the former have attained their essential characteristics; and Huxley states (p. 574) that he has seen "one of the large blood corpuscles of the parent entangled in the heart (which was then not more than one five-hundreth of an inch long) of a very young fœtus." This connection of the two circulations may be seen with such perfect distinctness in our species that the only way in which we can reconcile Vogt's observations upon the circulation of the chain-zooid of S. pinnata with those here detailed is by assuming that there is a very decided and remarkable difference between the species in this respect.†

Digestive and Nervous Organs, and the Egg. — In Fig. 28 part of the proximal portion of a stolon a little more advanced than the one

* Sur les Tuniciers nageants de la Mer de Nice (p. 42).

† According to Vogt (p. 47), the chain-salpa of S. pinnata is provided with an organ, the "stoloblast," homologous in structure and function with the placenta of the solitary embryo. He says that this is situated near the nucleus and heart, and is composed of two chambers, into one of which the blood of the embryo makes its way, and thus comes into close contact with the blood circulating in the sinus system of the parent, which has access to the second chamber. He says that there is no communication between the two, and that none of the blood of the parent gains access to the body cavity of the zooid. Unfortunately his figures, and the letters of reference especially, are so indefinite that very little can be made out by the study of them; but the explanation has suggested itself that the "stoloblast" may be nothing more than the rudimentary testicle, as Vogt says that it begins to disappear at the same time that the development of the latter begins, and disappears entirely after this is formed.

represented in Fig. 27 is shown, and, in order to exhibit the relations of the various parts more clearly, it is figured in the position which the latter would occupy if it were rotated 90° upon its axis. The side, instead of the edge, of the inner partition is therefore seen, and one of the sinus tubes (*1*), with its enclosed ovary (*y*), is above, and the other below it. The edges of the partition soon begin to thicken and spread out, so that a section would present nearly the shape of a letter H, with a cavity in the cross-bar. The four flaring edges now divide up into four rows of bud-like prominences, and two of these extend into the space between the constrictions which mark out the body cavity of each zooid, and lengthen until they meet upon the median line and surround the egg, which is now found in this position, as shown in Fig. 29. In this figure the prominences as well as the egg seem to be in contact with the outer tunic; but as it was necessary to use pressure in order to obtain a side view, this appearance is to be explained as caused in this way, for at a stage only a little later, but sufficiently advanced to be studied without pressure (Fig. 30), a very distinct body cavity is seen separating them from the body wall. In this figure the portion which surrounds the egg (*s*) has now separated from the portions upon each side of, or more strictly before and behind it, and forms the egg capsule. The two remaining portions (*5* and *6*) have also separated from the partition, and form oval masses, which are free within the body cavity of the zooid. One of these (*5*) is destined to form the ganglion, and the other (*6*) the branchial sac and digestive organs. They are very similar in shape and size, and each contains a cavity entirely surrounded by a thick wall. These cavities are, without doubt, at first diverticula from the cavity of the inner tube or partition, and are invisible in Fig. 29, on account of the pressure to which the specimen there figured was subjected; but the early changes in these parts take place so rapidly that no specimen was found at a stage which admitted of the determination of this point, although hundreds were examined with this end in view. The portion which is to form the digestive organs increases in size much more rapidly than the ganglionic portion, and extends forward under the egg and ganglion, to the anterior end of the body, and thus gives rise to the branchial sac. The subsequent development of these parts does not differ essentially from that of the same parts in the solitary embryo.

The Heart. — This, when first seen, was a granular body beneath and close to the stomach; and its position seems to confirm Vogt's conjecture (p. 45) that it is derived from the digestive tract.

The Testis. — This is at first (Figs. 33 and 34, *t*) a mass of cells, under the digestive organs and behind the heart, and therefore, as has been already pointed out, in the position occupied in the solitary embryo by the elæoblast, with which it agrees in appearance at first, but it does not become excessively developed, as this does; and in Fig. 34, *t*, it is shown as a compact globular mass of cells. As development advances, it spreads out over the surface of the digestive organs, and in the adult it presents the irregularly branched glandular appearance shown in Fig. 5.

The Ovary and Eggs. — Soon after the partition grows out from the pericardium and divides the tube into two chambers, and before the constrictions make their appearance upon the outer wall of the tube, a long club-shaped organ (Figs. 27 and 28, *y*) is seen within each chamber. These seem to lie free within the sinus cavity, and they could not be seen to be derived from any of the pre-existing parts of the embryo or of the tube; and the way in which they make their appearance seems to indicate that they are formed directly from the blood. The elæoblast begins to disappear at about the same time, and as the blood which passes through the latter goes directly to the tube, it is possible that some of its nuclei or smaller cells are thus transported to the tube, and form the basis of the new organs. This, however, is purely conjectural, as nothing was seen which indicated that the club-shaped masses were thus formed.

Fig. 31.

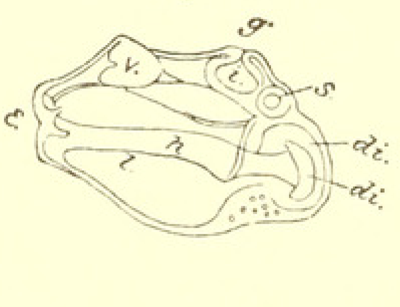


Fig. 32.

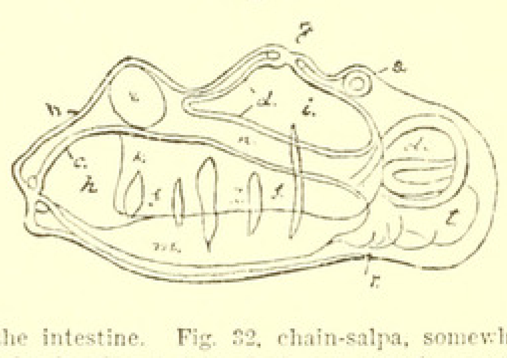
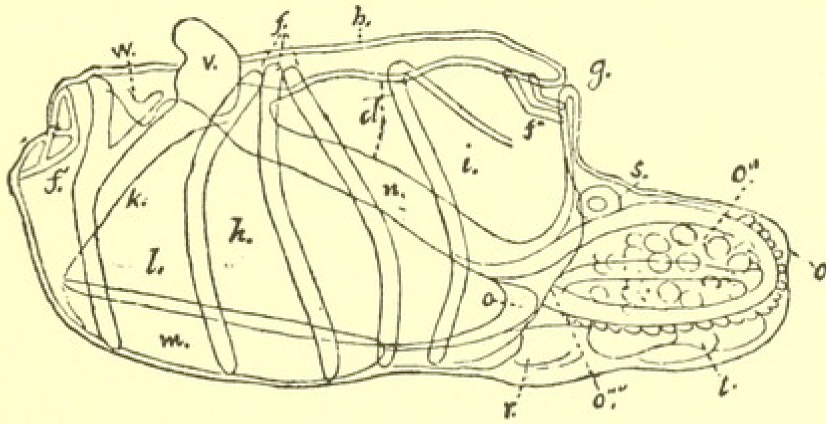


Fig. 31, chain-salpa, before the formation of the intestine. Fig. 32, chain-salpa, somewhat older, showing the manner in which the muscular bands are formed; *b*, outer tunic; *c*, wall of branchial sac; *d*, atrial tunic; *e*, branchial aperture; *f*, muscular girdles; *g*, atrial aperture; *h*, branchial cavity; *i*, atrial cavity; *d i*, digestive cavity; *d i'*, wall of digestive cavity; *k*, peripharyngeal ridges; *l*, epipharyngeal folds; *m*, endostyle; *n*, gill; *r*, heart; *s*, egg; *t*, testicle; *v*, ganglion.

As the tube grows, these organs lengthen also, and soon a single row of germinative vesicles is seen extending along each of them (Fig. 28); they are therefore the ovaries. At the time that the constrictions, which are the first indications of the zooids, appear in the outer wall of the tube, each ovary is seen to be made up of a row of eggs, equal in number to the constrictions; and as the zooids are developed, and their body cavities are separated from the sinus chambers of the tube, the chain of ova also divides, so that a single egg passes into the body cavity of each zooid (Figs. 29 to 34, *s*) and becomes suspended there by a gubernaculum, by means of which it is attached to the wall of the branchial sac, as already described.

Since the chain-salpa, at birth, always contains a single unimpregnated egg, organically connected with its body, and since this egg and the resulting embryo are nourished by the blood of the chain-salpa, by means of a true placenta, and since no reproductive organs have hitherto been described in the solitary Salpa,* it seems most natural

Fig. 33.



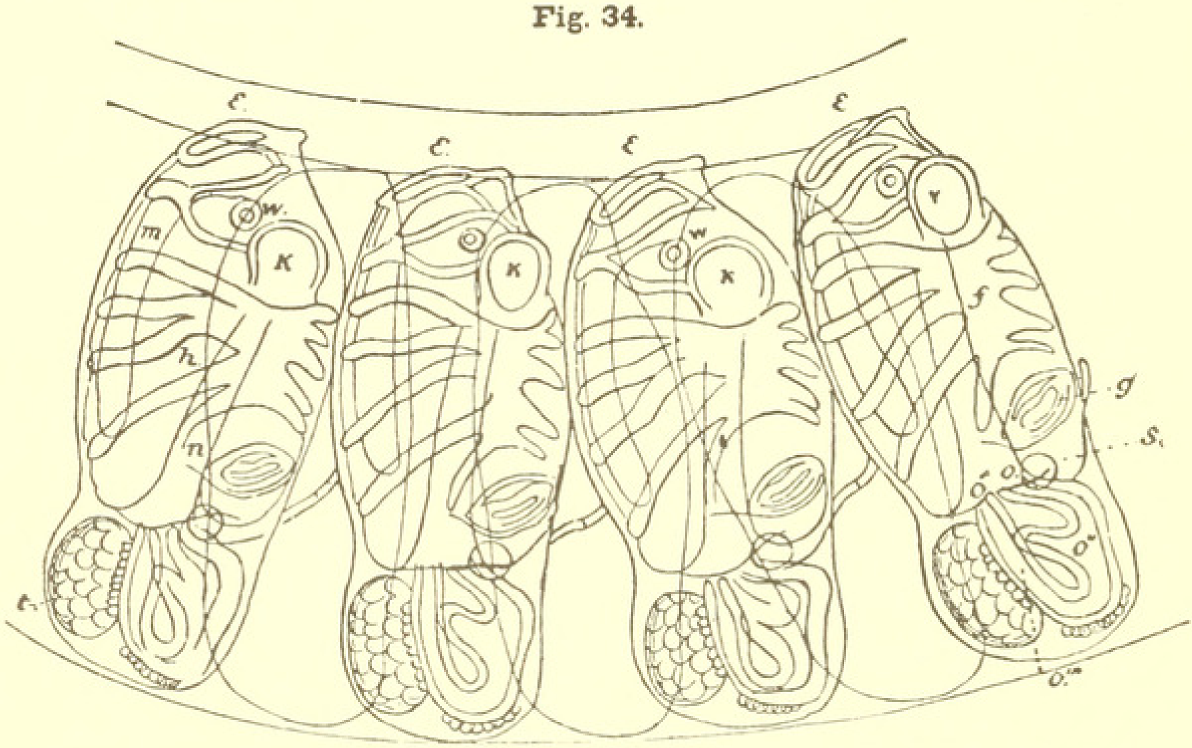
Side view of a single zooid from a chain at the stage shown in Fig. 34; the neural side is uppermost: *b*, outer tunic; *d*, wall of atrial chamber; *f*, respiratory muscles; *f'*, muscles of branchial aperture; *f''*, muscles of atrial aperture; *g*, atrial aperture; *h*, cavity of branchial sac; *i*, cavity of atrium; *k*, peripharyngeal ridge; *l*, epipharyngeal folds; *m*, endostyle; *n*, gill; *o*, mouth; *o''*, stomach; *o'''*, intestine; *o''''*, anus; *r*, heart; *s*, egg; *t*, testis; *v*, ganglion; *w*, languette.

* Although I made my observations without any knowledge that the origin of the eggs within the solitary Salpa had ever been traced, I cannot claim originality for the observation; since Kowalevsky states incidentally, in his paper upon the "Development of Pyrosoma," that this is the case in Salpa. He gives no description or figures, and confines himself to a bare statement of the fact, which occupies not quite one line. The passage containing the reference will be quoted farther on.

My observations were made in August and September, 1875, and Kowalevsky's paper on Pyrosoma was printed in August, 1875, and reached the library of the Boston Society, where I first saw it, on October 11.

to accept the view which has been generally held since the time of Chamisso's famous paper; that is, that Salpa presents an instance of "alternation of generations." This view, in its most modern form, may be stated as follows: "It is now a settled fact that the reproductive organs are found only in the aggregated individuals of Salpa, while the solitary individuals, which are produced from the fertilized eggs have, in place of sexual organs, a bud-stolon, and reproduce in the asexual manner exclusively, by the formation of buds. Male and female organs are, so far as we yet know, united in the Salpæ in one individual. *The Salpæ are hermaphrodite.*" (Leuckart. Salpa und Verwandten, pp. 46 and 47.) When, however, we trace backward the history of one of the individuals which compose a chain, and find that the egg is present at all stages of growth, and has exactly the same size and appearance as at the time when it is impregnated; when we find one organ after another disappearing until at last we have nothing but a faint constriction in the wall of the tube, indicating what is to become the animal, the conclusion seems irresistible, that the animal, which as yet has no existence, cannot be the parent of the egg which is already fully formed.*

Fig. 34.



Seven zooids from a fully developed chain, immediately before its discharge from the body of the female; the references are the same as in Fig. 33.

* The development of the eggs in the body of one zooid, and their passage into the body of another produced by budding from the first is not unusual among the

Summary and General Conclusions.

The life history of Salpa may be stated briefly, as follows :

The solitary Salpa — female — produces a chain of males by budding, and discharges an egg into the body of each of these before birth.

These eggs are impregnated while the zooids of the chain are very small, and sexually immature, and develop into females, which give rise to other males in the same way.

Since both forms are the offspring of the female, the one by budding, and the other by true sexual reproduction, we have not an instance of " alternation of generations," but a very remarkable difference in the form and mode of origin of the two sexes.

After the fœtus has been discharged from the body of the male the latter grows up, becomes sexually mature, and discharges its spermatic fluid into the water, to fertilize the eggs carried by other immature chains.

The fact that impregnation takes place, not, as we should expect, within the body of the solitary, but within that of the chain-salpa is no valid objection to the view that the latter is simply a male, for the number of animals whose eggs undergo impregnation within the body of the female is quite small, and in at least one genus, " Hippocampus," the unfertilized ova are received into a specialized brood-sac upon the body of the male, and there impregnated.

We can also find an analogy for the singular fact that the eggs of Salpa always develop females, while the males originate by budding. The fertilized eggs of the bee always develop female embryos, while the virgin bee, or one from the body of which the spermathecæ have been removed, produces only males, and Professor McCrady has pointed out that the production of the male bees in this manner can be best interpreted as a process of ovarian gemmation. The reproductive process of the bee accordingly presents a very striking parallel to that of Salpa. We cannot fail to associate the fact that in these two ani-

Tunicata. Huxley pointed out, in 1860 (Anatomy and Development of Pyrosoma, p. 212), that each bud carries away part of the ovary of its parent, and one fully developed ovum ; and he says : " It is not a little remarkable that the first recognizable part of the new organism should be the foundation of that structure which will eventually develop into a creature distinct from it." Other observers have described a precisely similar occurrence in other Tunicata.

mals the males are developed through a process which falls short of perfect sexual reproduction, and are therefore lower than the female, as far as their origin is concerned, with the well-known fact that throughout the animal kingdom cases occur in which the male is to a greater or less degree supplemental to, and more or less degraded in accordance with the degree to which it is subsidiary to the female.

The supplemental males of certain cirrhipeds, the male Argonaut, with its parasitic hectocotylus arm, and the small males carried upon the backs of certain female spiders are well-known instances of the existence of such a relationship between the sexes. I am not prepared at present to connect these two sets of facts in any way, but we can hardly avoid the conclusion that there is a real connection between them, and that one may furnish the means for explaining the other.

The fertilization of the eggs within the bodies of zooids, produced by budding from the body of that whose ovary gave rise to the eggs, is not unusual among the Tunicata.

As already mentioned, this was first observed in Pyrosoma by Huxley, and has since been seen in Didemnium, Perophora, Amauricium, Botryllus, and Salpa, and there is good reason for believing that it will be found to occur in most tunicates. The zooids of these tunicates are hermaphrodite, and develop eggs of their own, which, however, must pass into the bodies of the zooids of the next generation before they can be impregnated, and the ova which are formed in the ovaries of this generation must pass into the bodies of the third, and so on.

The essential difference between this process and that in Salpa, a difference which is here pointed out for the first time, is, that since the sexes are here distinct, the chain-salpa contains no ovary, and the process therefore comes to an end, while the zooids of the other tunicates are hermaphrodite, and the process may therefore go on indefinitely.

The way in which close interbreeding is prevented in these Tunicata is worthy of notice, and shows that in cases where this would seem likely to occur special arrangements may exist to render it impossible. In most hermaphrodite animals self-fertilization is prevented by the existence of a difference in the periods at which the two reproductive elements ripen. In the hermaphrodite Tunicata just mentioned, self-impregnation is impossible, since the egg must pass into the bud before it can reach a position to which the spermatic fluid can gain access; but the zooid which contains the egg, and the embryo into which the latter is to develop are the children of the same parent, and very close interbreeding would be apt to occur if the testis became mature before the egg had been impregnated. In Salpa, which is not hermaphrodite, the same provision against incest exists, and seems to have been inherited from a hermaphrodite ancestral form, which in turn may have inherited it from a still more remote ancestor before the peculiar method of throwing off the eggs had been acquired, in which its object was, as in most invertebrates, simply the prevention of self-impregnation.

As the remainder of this paper will be mainly theoretical and speculative, I must state here that while I fully realize the difference between observations as to the way in which a phenomenon has been, and speculations as to the way in which it may have been brought about, it does not seem best to omit all theoretical discussion, although the views here advanced may be very much modified or entirely replaced by subsequent discoveries.

Exact observations are permanent additions to our stock of knowledge, and although they may be supplemented they cannot be superseded; while any theoretical views which are reached in the present imperfect state of our knowledge of zoölogy are liable to be entirely set aside by the discovery of new facts, the history of our knowledge of Salpa shows that a theoretical interpretation may be of the greatest utility, and yet be entirely false. Chamisso's theory of the "alternation of generations" in Salpa has resulted in the discovery of the numerous and instructive instances of true "alternation," which now form so large a chapter of zoölogical science, although the facts detailed in this paper show that, as applied to Salpa, this theory is absolutely without basis. I am encouraged by this to give my views of the relationship of Salpa, and of the origin of the separation of the sexes, although I have kept this discussion as distinct as possible from my record of observations.

The free-swimming Tunicata have generally been regarded as the lowest representatives of the group, and Huxley (Salpa and Pyrosoma), gives a series of diagrammatic sections, to show a gradual transition from Appendicularia through Salpa and Doliolum to Pyrosoma and the ordinary Ascidians. Bronn (Klassen und Ordnungen) gives a somewhat similar series, arranged in the same order, but beginning with a Polyzoon and ending with a Lamellibranch and a Brachiopod. It is, of course, unnecessary to enlarge, in this place, upon the fact that the features which have been supposed to unite the Tunicata with the Polyzoa, Brachiopods, and Lamellibranchs are superficial and absolutely without scientific value, as this is now recognized by all who are familiar with what is known of the embryology of these various animals.

That the position assigned to Salpa, in the series above referred to, rests upon a false conception of the nature of the "gill" and its relations to the branchial sac in the ordinary Tunicates, has never been pointed out, but the history of the formation of this structure, as it has been described in this paper, shows that it is not a rudimentary but a specialized form of the branchial sac; and this, as well as all the other peculiarities of Salpa, seem to be special adaptations to its mode of life, and seem to show that it occupies a very high position among the Tunicata.

So little is known of the life history of Appendicularia and Doliolum that their relation to the remaining members of the group cannot be stated with any certainty; but as far as we now know, Appendicularia seems to be an adult representative of the "tadpole larva" stage, and must be regarded, for the present at least, as a very low and embryonic form, while Doliolum appears to stand between Salpa and the ordinary Tunicata as a transitional form.*

Salpa lacks the "tadpole larva" stage, and this is what we should expect in a Tunicate which had become adapted to a locomotive life. The power of locomotion during the earlier stages is very necessary to those animals which subsequently become fixed, and we should expect, according to the theory of evolution, that, wherever these fixed animals are descended from a free ancestor, they should retain, during the early stages, the free locomotive form of their remote predecessors.

* During my work on Salpa specimens of Appendicularia were frequently met with, but no thorough study of them was made, owing to lack of time. Although a constant search for Doliolum was kept up, only two dead specimens were met with during the summer, so that no opportunity for filling the gaps in our knowledge of this form was afforded.

The Cirrhipeds, for instance, pass through a "nauplius" stage, which has been retained because it is necessary, but if a Cirrhiped should now become adapted to a locomotive life, the nauplius stage would lose its importance, and might, in time, disappear by the process of acceleration of development. This seems to have taken place in Salpa. The "tadpole larva" appears to represent a form similar to Appendicularia, from which the Tunicata are descended; and this tailed stage has been retained by most of the fixed Ascidians, but in Pyrosoma, where the embryo does not need special locomotive power, the tail is not formed, although the embryo in its development passes through this stage, which is represented by the so-called Cyathozooid.

In Salpa, not only the tail but all traces of the larval stage have disappeared, and the egg shapes itself at once and directly into the perfect animal. As Herbert Spencer would state it, the indirect method of development has given place to the direct,* as is so often the case in the higher forms of a group; the Cephalopods, among the Mollusca, for instance.

The relations of the branchial sac and atrium may be explained in a similar way. Leuckart has pointed out (loc. cit.) that the "gill" of Salpa is simply a sinus between two large branchial slits, and we have seen that the atrium of the embryo has the two lateral chambers upon the sides of the branchial sac, which in ordinary tunicates communicate with the branchial chamber by the formation of the branchial slits. In Salpa it has been shown that no branchial slits are ever formed upon the sides of the sac, and that the lateral atria become converted into the respiratory muscular girdles. If we attempt to follow out in imagination, the manner in which a tunicate might become adapted to a free locomotive life, we can see that if an ordinary ascidian were to be loosened from its attachment the branchial current would give it more or less motion in the water, and if this free life were advantageous natural selection might in time lead to the separation of the branchial and atrial openings until they reached opposite poles of the body, in which position the current of water would be most efficient as a motive force. (Sars calls attention to the fact that the two openings are nearer each other in the embryonic

* According to Krohn (Wiegem. Arch. 1852, XVIII., I., 53, Taf. 2), Doliolum passes through a larval stage, and at this time is provided with a tail.

than in the adult Salpa, and shows that in this respect the embryo resembles the adults of other tunicates.) As soon as this locomotive life had been established it would be of advantage to have as much water as possible pass through those branchial slits which lie in the straight line connecting the two external apertures, and accordingly those at the base of the branchial sac would become excessively developed at the expense of those upon the sides, and these latter, being no longer of functional importance, would tend to disappear, and if the walls of the lateral atria were at all muscular, as they undoubtedly are in all Tunicata, we can easily understand how they might be so modified as to violently expel the water from the body, and thus still further assist in locomotion. If Salpa is descended from a form having the ordinary branchial sac the presence of the lateral atria in the embryo can be understood, but if we deny all evolution or consider Salpa a low form, they are as meaningless as the aortic arches of an embryonic mammal would be if we did not refer them to a gill-bearing ancestor.

In one respect Salpa is more embryonic than the fixed Ascidians. The structure of its ganglion and sense organs is much more specialized than in them, and resembles that of their larvæ in this respect; but we can see that while a fixed animal, having little need of a specialized nervous system would be likely to possess one much more rudimentary than that of its locomotive larva, a form which remained free throughout life would be likely to retain it in its highly developed form.

We come now to the question: Will the theory that Salpa has been adapted to a locomotive life throw any light upon the separation of the sexes?

"It must have struck most naturalists as a strange anomaly, that both with animals and plants, some species of the same family and even of the same genus, though agreeing closely with each other in their whole organization are hermaphrodites, and some unisexual." In Salpa we have such a case, which a comparison with the other Tunicata shows to have been originally composed of two hermaphrodites; the male organ of the solitary form having been converted into the elæoblast, which becomes excessively developed, and supplies the material for the formation of the chain, while in the chain-salpa the ovary has entirely disappeared.

The life-history of a typical tunicate seems to be about as follows: The tailed larva becomes converted into an animal, which may become sexually mature, as in Ascidia, or may remain rudimentary, as in Pyrosoma, but which, in all cases, develop zooids by budding, and, in most cases at least, discharges eggs into the bodies of these zooids, which are hermaphrodite, and discharge their eggs into other buds in the same way. The process of vegetative reproduction by budding is well known to be antagonistic to true sexual reproduction, and although it is very common among the lower representatives of most of the larger groups of animals, it is not usually found to occur among the higher forms, and is replaced by sexual reproduction, unless there is some special necessity for its retention, as there is in those forms which are fixed, as the Cirrhipeds and Ascidians. Wherever these fixed animals are united into a colony, the power to multiply by budding is of course essential, and however disadvantageous it may be in other respects, it must be preserved, and we see that in the Tunicata it has been so modified that instead of being antagonistic to, it has become to a certain degree accessory to reproduction by eggs. Wherever a colony is arranged in a definite form, as in Pyrosoma, there must be a point at which any further increase in size would be of no advantage; budding will therefore continue until this point is reached, but we should expect that the last series of zooids in each colony would gradually lose the tendency to multiply in this way. After this change had taken place, no more eggs could be discharged from the body, and the ovary, being now of no functional importance, would also tend to disappear. We should therefore have, first, the tailed larval stage, then a variable but limited number of hermaphrodite zooids, and finally, a zooid with the male organs only developed, but containing an egg derived from the ovary of the one next before it in the series.

Suppose, now, that this series of zooids should become adapted to a free locomotive life. The larval stage would disappear, as already shown, and we should now have a series of hermaphrodite zooids, ending with an egg-bearing male. The necessity for budding would cease to exist as soon as the solitary locomotive life was entered upon, and as this process is known to be antagonistic to high evolution and great specialization, it would gradually disappear until the series had been reduced to two, an hermaphrodite zooid hatched from the egg, and an egg-bearing male produced by budding. The series could not be still further reduced to one, for at the period when it had been useful it had been made subservient to reproduction by eggs, and this necessity for throwing off the eggs into the bodies off new zooids still existing, one generation of buds must be formed to contain them.

This "syncopation of development" leads to a diminution in the number of egg-producing zooids, and however advantageous it might be in other respects, it would lead to the diminution and final extinction of the species, unless the number of eggs discharged from each ovary could be in some way increased. If the solitary salpa produced but one bud at a time, only one egg at a time could be placed under the conditions necessary for development, and of course, the number of new embryos hatched during a given period would be only a little more than one tenth as great as at that time when there were ten egg-producing zooids in the series; and in order to allow the syncopation to take place, the number of egg-bearing buds produced by each egg-producing zooid must increase inversely as the number of the latter in the series is diminished. Although most Tunicata produce only a single bud at a time, some, as Amauricium, form a long tube which divides up into a series of five or six buds, which develop simultaneously;* and in Pyrosoma, the Cyathozooid forms a stolon almost exactly like that of Salpa, and this becomes constricted so as to form a chain of four Ascidiozooids.† If, as there seems to be so much reason to suppose, Salpa has been derived through a form like Doliolum, from one similar to Pyrosoma, it must have begun its solitary life with a tendency to produce several buds and thus set free several eggs at a time; and we can readily understand that, as the series was shortened, and it became necessary for the eggs to be discharged more rapidly, this stolon might lengthen, and thus give rise to a greater number of eggs at once. This would demand that, in some way, a supply of nutriment should be provided to supply the material used in their formation, and since the number of males would now greatly exceed that of the hermaphrodites the fertilization of the eggs would now be amply provided for, and the development of the testis of the egg-producing form would no longer be necessary. As this does not develop until late in life, it would exist, during the

* Kowalevsky. Knospung der Ascidian.

† Kowalevsky. Entwickelung der Pyrosoma.

formation of the chain, only as a rudimentary mass of formative cells, which might easily be turned to another use, and some portion of it would accordingly be devoted to the formation of the chain; as no injurious effect would follow, and as more buds could now be produced, the process would go until the testis had been converted into the elæoblast, and we should now have a solitary female hatched from an egg, and giving rise by budding to a chain of egg-bearing males.*

The number of theoretical questions which the development of Salpa suggests is very great, and only a few of those which are the most easily discussed have been noticed here. There are many others upon which the development of Doliolum, when it is better known, may be expected to shed light, and for this reason they are omitted here.

The vertebrate affinity of the Tunicate is now a subject of great interest, but if Salpa is a highly specialized form, departing widely from

* Kowalevsky appears to entertain a somewhat similar opinion of the relation of Salpa to Pyrosoma, as he concludes his paper upon the development of the latter as follows: "Wir finden hier, in geschlechtlicher Beziehung, die beiden Salpen vereinigt.

"Bei den Salpen giebt es bekanntlich zwei Generationen, in der einen entwickelt sich der aus vielen Eikeimen bestehende Eierstock, welcher in den Stolo hineingeht, und sich hier zu je einem einzigen Eie vertheilt, sodann die einzelnen Knospen-resp. Kettensalpen, in welchen weiter aus diesem Eie ein Embryo entsteht, wieder mit einem aus mehreren Eikeimen bestehenden Eierstock.

"Bei Pyrosoma, enthält jede Knospe auch wie die Kettensalpa das einzige grosse Ei zur unmittelbaren geschlechtlichen Vermehrung, und wie die Salpen-Amme, den Eierstock mit vielen Eikeimen zur Bildung der Geschlechtsorgane der künftigen Knospen. . . . . Möchten wir diese Bildung der vier Ascidiozooide mit ähnlichen Vorgängen bei anderen Tunicaten vergleichen, so fällt uns besonders in die Augen die Aehnlichkeit mit den Salpen, bei denen die aus dem Eie sich entwickelnde Salpe noch während der embryonalen Stadien schon den Stolo bildet auf dem auch die einznenlen Knospen angedeutet sind. Bei den Salpen geht aber die Bildung des Stolo langsamer vor sich als die der Amme selbst, und deshalb entwickelt sich die erste früher, wird zu einen freilebenden Thier und um während der letzten Periode ihres Lebens entfaltet sich die Kette.

"Bei der Pyrosoma ist der ganze Vorgang ganz entgegengesetzt und namentlich die Kettenindividuen resp. die Ascidiozooiden entwickeln sich schneller, dagegen wird der Cyathozooid (resp. Amme) nie zu einem freilebenden Geschöpfe, sondern bildet sich nur so weit aus, um in Stande zu sein, den schon angehauften Nahrungsdotter aufzulösen und die ernährende Flussigkeit den wachsenden Ascidiozooid zuzuführen. Ist diese Aufgabe erfüllt, so geht der Cyathozooid almählig zu Grunde und bei dem Freiwerden der aus vier individuen bestehenden jungen Colonie der Pyrosoma ist er ganz verschwunden."— Pp. 604 and 621. It will be noticed that Kowalevsky fails to see that the formation of the eggs within the body of the solitary Salpa proves this to be the female, and the chain-salpa a male.

the normal course of development and omitting the larval stage entirely, we cannot expect it to throw any additional light upon this question.

The presence of a placenta is of course only an analogy with the Mammalia, since the resemblance is simply functional and not in any sense morphological.*

* A memoir by Todarro (Sopra lo Soiluppo, e l' Anatomia delle Salpe, del Dott. Francesco Todarro, Roma, 1875), on the development of Salpa, reached me after I had finished writing my own account. In this he refers to an abstract published by Kowalevsky in 1868 (Nachrichten von der K. Gesellschaft der Wissenschaften. Göttingen, 1868, p. 407-415); as this is not referred to by Kowalevsky in any of his later papers on the development of the Tunicata, and is omitted from the index of the Nachrichten for that year, it had escaped my notice until my attention was called to it by the reference in Todarro's paper. It is simply a very brief and condensed preliminary abstract, without figures, and although it agrees in general with my own observations, it seems to conflict with them in several important particulars. The developmental history of Salpa is so very complicated that it is hardly possible to decide, in the absence of figures, exactly how much weight to attach to this apparent lack of agreement; and although anything upon the embryology of the Tunicata by this distinguished embryologist, to whom we owe so large a part of our knowledge of this subject, must be regarded as having the highest authority, it does not seem advisable to rewrite my paper in order to refer to views which, from the condensed way in which they are stated, and from the lack of illustrations, it is very possible that I may have misunderstood. In a few cases, however, the want of agreement is too evident to be explained in this way. He says (pp. 109, 110) "that no direct communication, such as has been stated by some to exist, is to be found between the cavity of the placenta and the body cavity of the mother."

The animal which contains and gives birth to the embryo is, according to our view, not the mother, but the male nurse. Regarding the existence of a communication between the body cavity of the nurse and the inner chamber of the placenta, I can only reiterate my statement that, at all stages, from the first appearance of the cavity of the gastrula until the embryo is fully formed, the blood of the nurse can be seen passing into and out of the cavity of the placenta. It is possible that there is a difference between the various species in this respect, for Vogt and Todarro agree with Kowalevsky in stating that at one period at least there is no such communication, while most of the other writers are equally confident that the facts are as I have stated them.

His account of the formation of the stolon also differs somewhat from mine. He says (pp. 412, 413) that this is made up of the following parts: (1) the outer wall, derived from the outer tunic of the parent; (2) the digestive tube, derived from the intestine of the parent; (3) two cloacal tubes, continuations of the posterior ends of the cloaca of the parent; (4) a bunch of cells, which gradually lengthens, becomes tubular, and gives rise to the ovaries of the chain-salpæ; (5) a tube which becomes converted into the nervous systems of the chain-salpæ. According to my observations, the digestive tube is not derived directly from the digestive organs of the parent, but from the pericardium. The two cloacal tubes mentioned are without doubt the same as those which I have called the sinus-chambers of the stolon; and the fact that the blood circulates within, not around them, proves that their cavities are continuations, not of the cloacal, or, as I have termed it after Huxley, the atrial cavity, but of the sinus system or body cavity of the parent. Instead of one ovarian rod, I found two, and failed to discover that they are hollow. In this paper Kowalevsky says that this tube gives rise to the ovaries of the chain-salpæ, although in his paper on Pyrosoma, published in 1875, and already quoted, he seems to agree with me in holding that they develop eggs before the chain-salpæ are formed, and discharge a single egg into each one of these. He fails to see, however, that this proves the solitary salpa to be the true female. His fifth or nerve tube I did not find.

I was unable to homologize the placenta of Salpa with any organ of any other Ascidian. Leuckart compares the foot of a Lamellibranch, the tail of Appendicularia, and the placenta; but this comparison seems to be rather fanciful. Kowalevsky suggests that the placenta may be the homologue of the cyathozooid of Pyrosoma, and the first generation of Doliolum. He says (p. 414): "At the close of this communication I may be permitted to call attention to the general analogy which is to be remarked between the development of Salpa, Pyrosoma, and Doliolum. In Salpa the egg forms an embryo which divides into two parts; one forms the placenta, and the other the embryo proper, which bears a dorsal [hæmal] stolon from which the sexual individuals bud. The egg of Pyrosoma forms a very rudimentary embryo, which produces, through budding, four embryos, which now, in turn, develop four sexual individuals from a dorsal stolon. The egg of Doliolum forms a perfect but sexless individual, which puts forth a ventral stolon, from which are formed individuals with a dorsal stolon, out of which the sexual individuals bud. From this comparison it is manifest that, between the placenta of Salpa, the rudimentary embryo of Pyrosoma, and the free-swimming stolon-bearing Doliolum an analogy exists; that, in a word, we here have before our eyes the different steps in the same developmental process."

I think there can be no doubt that Salpa has originated in a manner somewhat similar to that here pointed out; although the fact that the solitary form or egg-embryo is not sexless, but a female, would seem to indicate that the relation between the three genera cannot be exactly as it is here described. In my account of the manner in which the evolution of Salpa may be supposed to have taken place, I purposely refrained from referring to Doliolum, as we know so little of its development. A complete history, by one observer, of all the stages of one species of this genus, from the egg through all the alternations around to the egg, would be of the greatest interest. At present our knowledge is made up of fragments by various observers of isolated stages in the development of various species.

The Memoir by Todarro now remains to be noticed. This is an elaborately illustrated quarto of 150 pages; and the observations recorded, as well as the conclusions reached, are so utterly at variance with all that has been done by previous observers that it seems impossible to reconcile them. According to this writer, Salpa is the synthetic type of all the Vertebrata, and presents, during its development, peculiarities which are characteristic of each of the classes of this group, including the Mammalia. It is an allantoïdian vertebrate, developed in a true uterus, which is composed of a muscular, a vascular, and a mucous layer; and after impregnation the neck of the uterus becomes closed by a plug of mucus, and the embryo forms an allantois, exactly as in the higher Vertebrata. The sections which are represented in the figures are so strikingly like those of the earlier stages of the higher vertebrates that I am unable to make any comparison between them and my own observations. The work seems to have been done almost entirely upon sections of specimens hardened and treated with reagents, and every embryologist is well aware how untrustworthy results reached in this way often are, unless they are carefully compared with those reached by a thorough study of the living embryo, and also verified in every possible way. As I made no sections, but confined my attention to the living animal, I am unable to offer any suggestion as to the way in which what I believe to be the errors of interpretation in this paper are to be explained, and am therefore compelled to confine myself to this very short and unsatisfactory notice.