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MEMOIRS:

On the Relation of Pathogenic to Septic Bacteria, as illustrated by Anthrax Cultivations.

By

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The research, of which in the following report I propose to give the first instalment, had for its object, first, to investigate whether and how far the Bacillus anthracis undergoes any change, morphologically and physiologically, when cultivated artificially; and secondly, whether ordinary bacteria of putrefaction and septic fermentations can by artificial cultivations be so modified as when introduced into the body of an animal to be productive of disease, that is to say, whether it is possible for an innocuous saprophyte to assume the properties of an obnoxious pathogenic organism.

It is well known that bacteria of ordinary putrefaction may be introduced, either by ingestion with the food into the alimentary canal, or by inoculation into the skin, the mucous membranes, the subcutaneous or submucous tissue, or by direct injection into the vascular system, without there being produced in the animal experimented upon any appreciable dis-

[1] Reprinted from the 'Reports of the Medical Officer of the Local Government Board for 1881.'

order that could be directly brought into connection with the bacteria, provided that these latter be introduced in small quantities only. Large quantities, on the other hand, are productive of putrid intoxication, not so much on account of the presence of bacteria as on account of the now well known and generally accepted chemical putrid poison of Panum, Bergmann, and others.

It is equally well known that pathogenic bacteria prove their efficacy when introduced in minimal quantities, for it is essential to their character to find in the animal organism a suitable soil for multiplication and by their increase of numbers to produce directly or indirectly a definite disorder in the animal economy.

In order, then, to investigate whether saprophytic bacteria have assumed the properties of pathogenic organisms it is necessary to bear in mind that they must be expected to show these properties after their introduction in minimal quantities into the animal organism ; that is to say, they must be capable to resist and overcome the effects of the healthy tissues—effects proving invariably deleterious to ordinary saprophytic bacteria—and, having done so, of starting a definite disorder in the tissue.

It will be admitted, I presume, that it is not necessary that the disorder be of a general nature ; in some well-established instances, such as anthrax, febris recurrens, pneumo-enteritis of the pig, the malignant œdema (Koch), a general disorder ensues on the introduction of the pathogenic organism, but in other instances, such as the actinomycosis in cattle and man, known through the researches of Bollinger, Jahn, Israel, Ponfick, the effect of the invasion by the actinomyces is at first, at any rate, of a purely local nature, being generally limited to the lungs ; similarly the introduction of tubercular virus into the anterior chamber of the eye is followed by an eruption of tubercles in the iris (Cohnheim and Salomonsen), and of the same character is the pulmonary tuberculosis occurring in dogs after inhalation (Tappeiner) of the tubercular virus. The malignant ulceration in mice (Koch) is in the same way at first a

purely local effect of the introduction of a pathogenic organism. And it will be necessary to postulate at least this of a would-be pathogenic organism, viz. that its effect after its introduction into an animal should be local, but need not be so if general.

As is well known, Professor v. Nägeli is a firm advocate of the "sporting" of harmless saprophytes, they becoming converted under certain conditions into pathogenic organisms. But, on the other hand, Professor v. Nägeli also maintains that pathogenic bacteria may become converted into harmless saprophytes. His views are stated with great clearness in his work, 'Die niederen Pilze,' &c., Munich, 1877. From many years' studies, carried on with patience, and after a strictly experimental method, he arrived at these important conclusions.

Professor v. Nägeli makes the very widest allowance for bacteria, inasmuch as a harmless form, when brought under suitable conditions, may become the origin of an infectious disease, may through generations retain this power, and when again placed under different conditions may change into an inactive form.

Dr. Hans Buchner, a pupil of v. Nägeli, and working under this latter's directions, put these general statements to a special test, and succeeded, or maintains to have succeeded, in confirming them. He claims to have succeeded in changing, by successive artificial cultivations under constantly varying conditions, the Bacillus anthracis, of previously deadly power, into a perfectly inactive and harmless bacillus, which in morphological respects appeared then identical with the motile Bacillus subtilis (Cohn) of hay infusion. But he also thinks he has succeeded, what is of even greater and more fearful consequence, in transforming, through artificial cultivations under ever varying conditions, the notoriously harmless Bacillus subtilis of hay infusion into deadly Bacillus anthracis.

Dr. Buchner's paper, "Ueber d. exper. Erzeugung d. Milzbrandcontag. aus d. Heupilzen . . . .," which gives the results of a very large number of observations, is published in the

'Sitzungsb. d. Math.-Physikal. Classe d. k. b. Akad. d. Wiss. zu München,' 1880. Heft iii, p. 368 et passim. I shall have opportunity to return below to Buchner's assertions in detail, as I shall have to criticise some of his facts and deductions, but at present I wish to point out that one of Buchner's fundamental propositions, viz. that the Bacillus anthracis and the bacillus of hay are morphologically (with the exception of the motility of the latter) identical, is altogether erroneous. The two kinds of bacilli are not identical, and never become identical, however long they may be cultivated in artificial cultivations; and on this point I must with Koch (Cohn's 'Beiträge,' ii, Bnd. iii, and 'Aetiol. d. Milzbr.,' p. 21), most decidedly oppose Buchner. It is true that Buchner admits some very essential differences between the two, but these differences refer to chemical and functional relations. I shall point out below in detail these differences.

Buchner cultivated, at a temperature of 35°—37° C., the Bacillus anthracis, originally derived from the spleen of a white mouse dead of anthrax, in 0·5 per cent. solution of Liebig's meat extract, with or without the addition of peptone or sugar. As a first result of his observations on white mice Buchner found (p. 383) "that the infectious activity of the fungus becomes the more diminished the more generations it had passed in the artificial cultivations." But on looking carefully into his facts we notice that the above result does not come out in so simple and regular a manner as is represented in the above sentence; for in one series of cultivations of Bacillus anthracis, carried on in a nourishing fluid of 10 parts of Liebig's meat extract, 8 parts of peptone, and 1000 parts of water, Buchner found (p. 383 et passim) that the inoculations with the first, second, third, and fourth remove or generation produced always anthrax, whereas those with the fifth, sixth, seventh, and eighth did not yield any positive results "if the same quantity of infective material was used," but "if larger quantities are used positive results were obtained." In like manner in other series of cultivations he finds great differences as regards the activity of the bacillus of the

various "generations." Thus he found (p. 384) that minute quantities of the first cultivation proved effective, whereas the second, third, and fourth, in small quantities, proved ineffectual; but the fifth cultivation proved effective in large quantities; the sixth was ineffective. In a series of cultivations in meat extract, peptone, and sugar, the second cultivation proved active; the third and fourth inactive; the fifth active; the seventh, eighteenth, and even the thirty-sixth generation proved active. From all these and similar observations Buchner deduces that the Bacillus anthracis undergoes a gradual change, by which sooner or later it is rendered altogether harmless and inactive. This change is, however, not one of morphological character. I shall, further below, when describing my own observations on these subjects, have to return to Buchner's observations, and I shall then show that all his facts are easily explained by the aid of my own observations, but not by the theory of a gradual diminution of activity of the Bacillus anthracis. There is such a thing as a real diminution of activity of the Bacillus anthracis in artificial cultivations; the inactivity on white mice of some cultivations of the Bacillus anthracis and not of others is due to a variety of circumstances, one of which, at any rate, is this, the absence of spores. If Buchner had tested his cultivations on guinea-pigs or rabbits he would have obtained altogether a different result, always supposing that he worked with pure cultivations of Bacillus anthracis.

Koch ('Zur Aetiologie d. Milzbr.,' p. 22 et passim) considers it probable that Buchner's uncertain and unequal results are explained by the fact that his (Buchner's) cultivations not being absolutely guarded from contamination with other non-pathogenic bacilli, he may have had, and probably did have, in some of his cultivations the Bacillus anthracis, originally sown, diluted, or altogether suppressed by the overgrowth of the non-pathogenic bacillus. With Koch I fully think this objection well justified, especially since Buchner does not admit a difference between non-moving non-pathogenic bacilli and the non-moving Bacillus anthracis.

[I am acquainted with Dr. Greenfield's paper on cultivations of the Bacillus anthracis communicated to the Royal Society on June 17th, 1880; but I am unable to find in it anything to which I can attach importance, with the exception of some observations that repeat earlier experiments of Dr. Burdon-Sanderson and of Dr. Buchner. In my opinion some of Dr. Greenfield's observations contain internal evidence that he was occasionally operating with some harmless bacillus and not with anthrax bacillus at all; and I cannot admit his claim to speak with authority on the etiology of splenic fever. It may be noticed in this connection that Koch, in his last elaborate paper on the subject, has not made a single mention of Dr. Greenfield's assertions.]

Buchner noticed, as his cultivations rose in degree of generation, that the bacilli showed a change in their general mode of growth, inasmuch as, unlike their previous behaviour so often described by Pasteur, Koch, and others, they ceased to form the beautiful cloudy and flaky felt-work (Pasteur's "en filaments tout enchêvetrés, cotonneux") rising from the bottom of the culture-vessel into the otherwise perfectly clear nourishing fluid, but they gradually assumed the tendency to adhere to the walls of the vessel. After a duration of cultivation of ninety days (Buchner calls this the 900th generation), this condition became very pronounced, and in later generations the bacilli formed the same pellicle on the surface as do the harmless hay bacilli. On changing the mode of growth he ultimately succeeded in obtaining bacilli that in this respect did not differ from the typical hay bacilli. With reference to this point I am inclined to think that Koch ('Aetiologie d. Milzbr.,' p. 22) is right in refusing to accept this as proven; he says that Buchner had not sufficiently guarded himself against outside contamination, and therefore it is quite possible that he introduced into his cultivations at one or another step a common bacillus which after several cultivations became so numerous as to replace altogether the original Bacillus anthracis.

With reference to Buchner's cultivations, by which (using blood for his nourishing fluid) he gradually changed the

common hay bacillus into the Bacillus anthracis, Koch (l. c., p. 26) maintains that what Buchner really had before him in those cases in which he had animals die after the inoculation with the hay bacillus cultivated in blood for many generations, was not anthrax bacillus, but the baccillus of malignant œdema (Koch). I think Koch's criticism is very thorough and has every probability for itself. I certainly should be surprised to find that a cultivation in blood treated in Buchner's manner (l. c., p. 405), viz. without any precaution against accidental contamination, and which naturally would undergo putrid changes, did not yield the œdema bacillus; this, when cultivated for several successive generations, as was the case in Buchner's experiments, would at last yield a cultivation in which the œdema bacillus is the only organism present.

I now come to the most important research of this cycle, viz. that of M. Pasteur. A succinct summary of his results he himself has given us in a remarkable address given during the last International Medical Congress in London in August, 1881. This address has been reprinted, with a translation, as a Parliamentary paper, under the title "Animal Inoculation." It refers to the micrococcus of fowl cholera and to the Bacillus anthracis. It is only the latter that interests us here. By numerous previous observations M. Pasteur has found that cultivating the Bacillus anthracis in chicken broth at a temperature of 42° and 43° C., the bacillus, although vigorously growing in the shape of the characteristic convolutions of threads, nevertheless does not form spores (p. 10).

"In a month or six weeks the culture dies; that is to say, if one impregnates with it fresh decoction, the latter is completely sterile. Up to that time life exists in the vessel exposed to air and heat. If we examine the virulence of the culture at the end of two days, four days, six days, eight days, &c., it will be found that long before the death of the culture the microbe has lost all virulence, although still cultivable. Before this period it is found that the culture presents a series

of attenuated virulences; everything is similar to what happens in respect to the microbe of chicken cholera. Further, each of these conditions of attenuated virulence may be reproduced by culture. Lastly, since splenic fever does not recur (ne récidive pas), each of our attenuated anthracoid microbes constitutes for the superior microbe a vaccine, that is to say, a virus capable of producing a milder disease. Here, then, we have a method of preparing the vaccine of splenic fever."

[These are stated as general propositions, and M. Pasteur makes them without mention of any particular kind of animal. He cultivates the anthrax bacillus at 42 C°. in fowl-broth, and treats as indifferent the class of animal into which he inoculates the cultivation.]

"I was asked to give a public demonstration at Pouilly-le-Fort, near Melun, of the results already mentioned. This experiment I may relate in a few words. Fifty sheep were placed at my disposition, of which 25 were vaccinated, and the remaining 25 underwent no treatment. A fortnight afterwards the 50 sheep were inoculated with the most virulent anthracoid microbe. The 25 vaccinated sheep resisted the infection; the 25 unvaccinated died of splenic fever within 50 hours. Since that time the capabilities of my laboratory have been inadequate to meet the demands of farmers for supplies of this vaccine.[1] In the space of fifteen days we have vaccinated in the departments surrounding Paris more than 20,000 sheep, and a large number of cattle and horses. This experiment was repeated last month at the Ferme de Lambert, near Chartres. It deserves special mention. The very virulent inoculation practised at Pouilly-le-Fort, in order to prove the immunity produced by vaccination, had been effected by the

[1] It is matter of regret that the exact methods of preparation used by M. Pasteur in his laboratory are not made public; so that the present research has had to be conducted in ignorance of his details. The fact of his success in producing what he calls a "vaccine"—a something which when inoculated into sheep produces some modified splenic fever that protects the sheep against the after-production of fatal splenic fever when the virulent material is inoculated into the sheep—may be taken as established.

aid of anthracoid germs deposited in a culture which had been preserved in my laboratory more than four years, that is to say, from the 21st March, 1877. There was assuredly no doubt about its virulence, since in 50 hours it killed 25 sheep out of 25. Nevertheless, a commission of doctors, surgeons, and veterinary surgeons of Chartres, prejudiced with the idea that virus, obtained from infectious blood, must have a virulence capable of defying the action of what I call cultures of virus, instituted a comparison of the effects upon vaccinated sheep and upon unvaccinated sheep of inoculation with the blood of an animal which had died of splenic fever. The result was identical with that obtained at Pouilly-le-Fort; absolute resistance of the vaccinated, and death of the unvaccinated."

I have hitherto had no experience with the inoculation of sheep with cultivated Bacillus anthracis (but hope soon to be able to gain some); and I cannot therefore say anything about it, nor do I for one moment question the absolute reliability of M. Pasteur's successful vaccination of sheep with Bacillus anthracis, and the immunity thus conferred upon them, altho gh no such uniform results were obtained by his assistant when repeating M. Pasteur's experiments in Buda Pesth, but what I will take the liberty of questioning is the general application of these results by M. Pasteur and his followers to anthrax in animals other than sheep, or to the other infectious maladies. For I am able to show, that not only does no such mitigation of activity on rodent animals take place in the Bacillus anthracis when artificially cultivated and precluded from forming spores, but that no immunity is conferred on rodent animals, if not succumbing to the effect of such cultivated Bacillus anthracis. There are a number of statements by M. Pasteur, such as the oxygen of the air being the cause of the attenuation of the virulence; further, the inability of the cultivated Bacillus anthracis to form spores at a temperature of 42° and 43° C.; then the assertion that the attenuated virulence once obtained is transmitted to the next cultivation, the accuracy of some of which my experience obliges me to question, of others directly to contradict.

Koch ('Zur Aetiologie des Milzbrandes, in Mittheil. de Kais. Gesundheits-Amtes.,' Bnd. i, Berlin, 1881) made some interesting contributions to the etiology of anthrax. The most important points in his publication are the criticisms of Buchner's and Pasteur's work on the subject. Although a great deal of what Koch says when speaking in a perfectly objective manner of Buchner's observations is justified, I do not think that there exists the same justification for all he says of Pasteur's work. That in the present extended knowledge of anthrax, both in its etiology and pathology, but especially the former, we owe more to Koch's brilliant researches than to the researches of all other observers taken together, including Pollender, Brauel, and Davaine, the discoverers of the Bacillus anthracis, will, I think, be readily conceded by all who have had the opportunity of repeating some of Koch's experiments and observations, and have read his several communications on this subject, and it is not want of respect to the other workers in this field to concede this much. It will likewise be conceded, I think, by all who read Pasteur's various contributions on the subject of the etiology of anthrax, that Pasteur would have profited by a more careful study of Koch's observations and writings, particularly that the error of Pasteur's earth-worm theory [1] might have been avoided if he had felt the significance of Koch's previous observations on the incapability of the Bacillus anthracis to form spores within the body of an animal owing to the want of sufficient amount of oxygen, and especially of Koch's valuable observations on the inability of the Bacillus anthracis to form spores in the depth of the soil. And, again, objection may perhaps be taken

[1] According to this theory spores having been formed in the bacilli within the organs of a buried animal that has died of anthrax, such spores are taken up by earth-worms, carried up to the surface, and then deposited with their castings. From the surface of the soil they find easy access into the mouth or nostrils of animals grazing on that soil.

I shall show in a future report that the bacillus threads, as such, do not survive even the initial stages of decomposition of the buried body. And it cannot be supposed that earth-worms can feed on buried bodies in the few days that may elapse before decomposition has set in.

with reason to Pasteur's dogmatic way of dealing with pathological facts, such as his arbitrary definitions and descriptions of what constitutes anthrax and what constitutes malignant œdema.

But while allowing all this, and perhaps even more, I think all must extremely regret the tone in which Koch's criticism is made; all must think that his criticism would have been much more valuable had it kept within strict bounds of an objective statement. Koch shows by means of most valuable observations, some of earlier date, published in his previous writings (Cohn's 'Beiträge zur Biologie d. Pflanzen,' 2 Heft), some of recent date, that no spore formation is possible in Bacillus anthracis at a temperature below 15° C. or 59° Fahrenheit. At a depth of 1 meter the temperature of the soil in middle Europe is so low that the formation of spores in Bacillus anthracis is practically impossible in an animal buried unopened at that depth. Koch also proves (l. c., p. 20) by very instructive and direct experiments with earthworms and spores of Bacillus anthracis mixed with earth, that Pasteur's earth-worm theory cannot be correct.

Various considerations of the distribution of anthrax in Germany and of the manner of the outbreaks of its epidemics lead Koch to the assumption (p. 29, et passim) that the natural habitat of the Bacillus anthracis is really in the soil, and that its casual introduction into the body of an animal and the production of anthrax in it, is only "a casual excursion of a micro-organism not generally limited to such a parasitism."

As mentioned just now, there is a great deal of evidence for such an assumption, especially the way in which in Germany the disease makes its appearance in animals grazing in fields and meadows which occasionally become flooded. In these instances it is assumed that the Bacillus anthracis growing in vegetable infusions (that it does readily do so in some of them is shown by Koch) of a distant locality or in the depth, is carried by means of water to the surface, and is left here when the water is receding or drying up, to find

ultimately entrance into the body of an animal grazing on that field. Hay infusions and other vegetable matter, it is true, owing to their acid reaction, do not, according to Koch, form a suitable nourishing fluid for the Bacillus anthracis; but Koch draws attention to its being a known fact that the dangerous anthrax localities have a chalky or loamy soil, and it is therefore possible that even where the vegetable infusions in the soil would be acid (hay, some kinds of straw, barley, grass, &c.), the carbonate of lime of the chalky soil would suffice to neutralise the infusions.

Koch (l. c., p. 25) failed to find any diminution in virulence of the Bacillus anthracis cultivated artificially up to the fiftieth successive cultivation, an experience which I am able, as I shall presently show, to confirm.

Before I give a detailed description of my own observations on the cultivation of Bacillus anthracis, I have to state the method of cultivation which was employed in this inquiry. As nourishing fluid I have employed broth prepared from fresh pork. About a pound and a half to two pounds of pork are boiled in water for an hour or so down to about two pints of fluid. The fat scum is removed, and the fluid, provided the pork employed has been lean, filters tolerably clear through filter paper. To obtain it, however, perfectly limpid, the broth is cleared à la cuisine with egg-albumen and then filtered. The filtrate, which will be spoken of as "the pork broth," is of a neutral or faintly acid reaction; in this latter case a sufficient amount of carbonate of sodium is added in order to make it neutral; it is then received in long-necked flasks, which are then plugged with cotton wool.

In all cases, and I wish to state this once for all, where a cotton-wool plug is spoken of, whether in connection with a flask or a test-tube, it will be understood that a cotton-wool plug of about two inches length is meant, in some instances two plugs one above the other, each about an inch long, being used. The cotton wool, the flasks, beakers, filters, filter paper, test-tubes, and all vessels used, are invariably disinfected by

exposing them for several hours, generally repeated several times, to a temperature varying between 140° to 150° C. To use cotton wool disinfected by prolonged (for several days or weeks) steeping in absolute alcohol, or concentrated carbolic acid solution, is not absolutely reliable. Overheating the cotton wool in an air chamber to the above temperature till singed has proved invariably and absolutely safe for all cultivations; I have had to my regret failures in my cultivations which could be referred to cotton wool soaked in concentrated carbolic acid solution even for months. The same is to be said of the flasks and test-tubes used. No amount of cleaning, even with strong acid, is to be relied on; nothing but overheating gives reliance. I at first always used to heat the vessel well all round over the open flame of a Fletcher's burner almost till the glass becomes glowing, and soon after when the glass is still hot, but not as to do more than just singe the cotton wool, I place in its neck the cotton-wool plug, this having previously been overheated in the air chamber. But lately I have overheated the vessels in the air chamber to about 140°—150° C. for several hours, several times repeated, having previously cleaned them with distilled water, and dried them as far as possible; and I have found this perfectly sufficient to disinfect them thoroughly. It cannot be too strongly insisted on with Koch that the flasks and test-tubes, and especially the cotton wool used as plugs for the vessels, should be thoroughly sterilised by overheating, for it is as much and as often that cultivations become thereby contaminated as by the non-sterility of the nourishing fluids or the accidental entrance of organisms from the air.

The filtered nourishing fluid (pork broth) having been received in a clean flask plugged, as above stated, with long and clean cotton wool, is boiled for about ten to fifteen minutes. I never fill the flask to more than half its volume with the broth, in order to avoid the fluid rising too high during boiling, and thus wetting the cotton-wool plug. This, although not necessarily fatal, owing to the sterillity of the cotton wool, nevertheless I always avoid in this and other cases, for the sake of cleanli-

ness, and to avoid all possible contamination. Immediately before turning off the flame of the burner, and while the fluid is still boiling, I place over the mouth of the flask a cotton-wool cap, and keep this pressed over the mouth and upper part of the neck of the flask by an inverted beaker pushed firmly over it. The flask is then placed into an incubator, and kept here at a temperature of about 32°—35° C. After two or three days the flask, plugged but without the cotton-wool cap, is again placed over the gas-flame, and the broth subjected to boiling for five to ten minutes. While still boiling the cotton-wool cap and beaker are placed over the mouth and neck, and the flame is turned off. Such a flask with broth may now be considered absolutely sterile; it may be kept in the incubator for weeks and months—it will always remain absolutely limpid and free of any organisms. Such broth will in the following be always spoken of as "sterile pork broth."

This broth I use either as such, i. e. as pure broth, or in combination with gelatine, as "gelatine pork," in order to have, as recommended by Koch (l. c.), a nourishing material, not of fluid but of solid consistency. I consider, with many others, this method of Koch's, viz. of using gelatine as an admixture to a nourishing fluid, and thus converting it into a solid state, a very great advance indeed in the methods of cultivating bacteria, especially in securing pure cultivations not contaminated accidently, for then the sowing of a particular species of bacterium is possible in a particular spot or spots, the growth and progress can be easily watched and controlled, and accidental contaminations can be readily recognised; but I shall show below that it is quite possible also without the gelatine admixture after the method I use to be almost absolutely guarded from accidental contamination, i.e. to have pure cultivations. Koch has very minutely described the advantages of the gelatine method and his modus procedendi, and he has given numerous photographic illustrations of various species of bacterium in pure cultivations effected by his gelatine method.

Koch recommends, in order to solidify the nourishing

material (in his case it was a solution of Liebig's meat extract), to mix with it purified and well sterilised and neutralised gelatine solution in such a proportion that the gelatine would form 2—3 per cent. Such a nourishing mixture is solid at ordinary temperatures, and represents an excellent soil for sowing on or in it the desired species of bacterium in dots or lines; kept in flat glass dishes or slides the examination with the microscope can be easily carried out from time to time, and it can easily be ascertained how and whether the sown species is making progress, and accidental contaminations can thus be easily detected and removed, all growths, owing to the solid state of the nourishing material, being naturally limited to the spot or line on which the bacterium has been sown. It is necessary to keep the dish or glass in a chamber (under a bell-jar) saturated with moisture. This is, in short, the essence of Koch's method. He maintains that such a gelatine material remains solid at a temperature of 20°—25° C., sufficiently high for the growth of all species of bacteria.

All this sounds very excellent, but when one comes to work with it practically one finds that everything is not as perfect and excellent as one imagines at first.

As is well known from the researches of Brefeld, Grawitz, Wernich, and others, nourishing material in a solid state, such as gelatine, boiled potato, bread, paste, &c., has been used for the sake of obtaining pure cultivations, and for the sake of easily watching and keeping under control the progress and growth of particular organisms, e. g. Penicillium, Aspergillus, Micrococcus prodigiosus, &c.; but most of these observations were carried on at ordinary temperatures. Koch, however, recommends it, after many observations, in the above form for pure cultivations, even in the incubator, at 20°—25° C. for all species of bacteria (Micrococcus, Bacterium termo, and various species of baccilli, &c.).

The first difficulty one has to overcome is to obtain a sterile and neutral clear and limpid gelatine solution. I have tried every obtainable kind of gelatine, in which I was much assisted by Dr. George Maddox, to whom I am under great obligations,

such as ordinary French gelatine, best Swiss gelatine, much recommended to photographers by Dr. Eder of Vienna, best French gelatine, called gold-label gelatine, isinglass, a peculiar lichen-gelatine used by Chinese cooks to get a very firm jelly, and various other kinds of gelatines; and after a great many experiments, both time-consuming and patience-trying, to enumerate which would be a very unnecessary infliction on the reader, I have found best answering our purpose a gelatine solution prepared in the following manner: one part of "gold-label gelatine" (the tablets in which it is sold being cut up into small strips) is soaked overnight in six parts of cold water, it is then dissolved on the water bath; this solution has a slightly acid reaction; to it is added carbonate of sodium just sufficient to give it a neutral reaction. While quite hot it is filtered through filter paper once or twice. (It must be borne in mind that the filter paper, the vessels receiving the solid gelatine or the filtrate, and all other vessels subsequently used for its reception, are perfectly disinfected by overheating them.) The process of filtering is carried out by using hot filters and filter paper, keeping up the warmth by placing at two opposite sides, as close to the filter as practicable, Bunsen burners. The filtrate is tolerably clear, but can be obtained perfectly clear by adding to it after neutralisation egg albumen, and then boiling it for several minutes. In this latter case it may be filtered through calico or flannel previously disinfected. To the filtered gelatine are then added three parts (not three times its volume) of the above pork broth, so that we have now altogether one part of solid gelatine, six parts of water, and three parts of pork broth, which would be equal to one part of solid gelatine in nine parts of fluid, or 11$\frac{1}{9}$ per centum. This mixture is received in several sterilised flasks, closed well with long and thoroughly sterilised cotton-wool plugs, and is subjected to boiling for 5—10 minutes. While still boiling, and just before removing from the flame, the mouth of the flask is covered with a cotton-wool cap, and a beaker is inverted over it. The flasks are then placed in the incubator and kept there at 32—35° C. for twenty-four hours, after which they are again

subjected to boiling for about five minutes. This I have found to be sufficient to keep them sterile for ever after. This mixture, which I will speak of as "sterile gelatine pork," remains, even in the smallest quantity, solid up to a temperature of 25° C., a temperature generally sufficiently high for the growth of bacteria.

Koch ('Zur Unters. d. Pathog. Organ.,' p. 24) recommends a mixture of gelatine and nourishing fluid in such proportions that the gelatine amounts to about 2½ to 3 per cent., and he states this to have served in a solid state for the cultivation of bacteria, not only at the ordinary temperature of the room, but at temperatures varying between 20° and 25° C.

Now, no kind of gelatine which I have been able to lay hold of has kept solid at a temperature of 20°—25° C. in such percentage, nor as 5 per cent. mixture, not even as 7·5 per cent. Ten per cent. mixture is the lowest that I have been able to keep solid at such a temperature. It is true a good many bacterial organisms grow tolerably well in a temperature about 15°—18° C., at which the 2½—3 per cent. gelatine mixture is solid, but their growth is very slow. In some instances, e. g. Bacillus anthracis, the growth progresses tolerably well, but in others it is extremely slow. To make spores of hay bacillus sprout at such a temperature is exceedingly difficult, and so it is also with the spores of some other kind of bacilli. I have seen bacilli which absolutely refuse to grow at such a temperature. Of micrococci some grow well, others do not.

It is clear, then, that if, as is the case in laboratory experiments, one requires growth of a particular organism to take place within a reasonable time, not to mention those cases in which organisms do not grow at all at so low a temperature, the above temperature, viz. 15°—18° C., is not sufficiently high, and it is necessary to use gelatine mixtures stronger than 2½—3 per cent. The above 11 per cent. mixture of gelatine and pork keeps well and solid at 25° C., and at this temperature all bacteria that I have tried grow well and abundantly.

Thus far I have been describing the manner in which I prepared the nourishing material, viz. sterile pork broth and sterile

gelatine pork, which is to serve as stock for the cultivations. I have used also other nourishing material, such as beef broth, rabbit broth, &c., for the cultivation of various organisms; but the subject of the present report is the observations made with Bacillus anthracis, and for this I have used, hitherto with satisfactory results, the pork broth and gelatine pork only, and I shall not enter on the present occasion into a consideration of the other nourishing materials.

I now come to the description of the method of using the above stock of nourishing material for the special cultivations of the Bacillus anthracis.

(A.) A number of disinfected test-tubes and small flasks are used, the latter of the capacity of an ounce or so, plugged with disinfected cotton wool, the plug lifted, and each charged as rapidly as possible for a fraction of their volume with the nourishing material from the stock flask, and then plugged with cotton wool. In the case of the gelatine pork, this is of course first liquefied over the flame. The stock flask, if not emptied by this process of charging, is subjected to boiling from five to ten minutes. When charged and plugged each test-tube and small flask is subjected to boiling for a few minutes; the boiling is effected over a small flame in order to prevent the over-boiling; this is not so much to be feared in the case of the flasks as in that of the test tubes. Thorough boiling for once is generally sufficient to destroy every organism that may have accidentally entered during the process of charging. Kept for an indefinite time in the incubator at 32°—35° C. the fluid in them remains bright and clear.

(B.) Glass cells of exactly the same nature as those that were of so great use to me in my research on the pneumo-enteritis of the pig (see these Reports for 1877, p. 210), in the majority of instances without any addition, in some with the addition of a thin glass tube cemented to the glass slide and leading into the cell; the outer opening of this glass tube is plugged with cotton wool. This tube was chiefly added with the view of facilitating the formation of spores, but as a rule I found, cæteris paribus, if the other conditions for the spore

formation are present, the amount of air present in the glass cell sufficiently large to enable the spore formation.

As in my former work so also now, I use olive oil to fix the cover glass over the glass ring forming the sides of the cells. The cover glass before being used is well heated over the flame. A small quantity of the nourishing fluid (pure pork broth or liquefied gelatine pork) is withdrawn from the stock flask by a freshly drawn-out small capillary pipette; this is effected in this manner: the cotton-wool plug of the stock flask is drawn up for about half its length, and the one end of the pipette being drawn out into a long capillary tube is gradually pierced through the remaining half length of the plug and pushed down till it reaches the fluid; the pipette is filled and withdrawn, and the plug is again pushed down into its previous position. By this means absolutely no access is allowed to particles from the air into the stock flask, and at the same time the capillary tube, while being pushed through the cotton-wool plug, is cleaned from accidentally adhering particles. It must be borne in mind that for the above purpose the cotton wool must have been well sterilised by heat, because if not so, the nourishing material in the stock flask is sure to become contaminated by impurities adhering to the cotton-wool fibres, some of these being pushed down as well as carried down into the fluid by the capillary tube. From this pipette a drop is quickly deposited in the centre of the cover glass, and this is inverted and fixed on the ring of the glass cell, a drop of distilled water having been previously placed at the bottom of the cell at a peripheral place. The cell is now "charged" and ready to receive the organism that is to be cultivated in the drop of nourishing material attached to the centre of the lower surface of the cover glass. The process of charging the test-tubes and flasks, as well as the glass cells, being carried out in the air, is of course subjected to the complication of a contamination with air organisms. In the case of the test tubes and flasks this is remedied by subsequent boiling of the charged and plugged vessels; but in the case of the glass cells a sterilisation after charging is for obvious reasons impossible,

and it is therefore necessary to take one's chance, so to speak, of having a number of failures owing to accidental contamination. And it is this very point, viz. the chance of contamination with air organisms, which makes the Koch's method, as recommended by him, impracticable in the case of many cultivations, as I shall have to point out below in detail.

It depends very much on the place and season where and when the charging is carried out, as regards the accidental contamination with air organisms. I have made some comparative studies on these points, and I think it worth while to enter here more fully into them.

At first, when working at the laboratory of St. Bartholomew's Hospital Medical School, I charged my test-tubes from my stock flask under carbolic-acid spray, the carbolic acid being of the strength of about 5—6 per cent. From my note-book I gather that in one series I charged sixteen test-tubes carefully under the carbolic-acid spray, and placed them into the incubator at about 35° C. Of these test-tubes one went bad in the course of twenty-four hours, at which time it became turbid owing to the presence of actively moving bacilli. In another series of fourteen test-tubes two went bad. In a third series of twenty-two test-tubes every one went bad, although the method of charging under the carbolic-acid spray was the same as in the other cases; but the conditions of the atmosphere were not the same. While I had tolerably good results in July and August I had very bad results in October, and my failures, both in preserving sterile my stock fluids and my test-tubes charged with them, became during this month so numerous and persistent that I had to give up work altogether for this period. To have cultivations exposed to the air and not afterwards sterilised, as is almost the general rule in Koch's method of gelatine cultures, and to keep them pure was altogether out of the question. The cause of these universal and unconditional failures was not far to seek. During October we had a good deal of dry weather with strong winds, and the laboratory in which I worked faces Smithfield hay market,

whence a good deal of dust was blown into the laboratory. The dust contained an enormous number of spores, especially of bacilli, as was proved not only by the direct observation, but also by the fact that every kind of nourishing fluid, Cohn's nourishing fluid, hay infusion, beef broth, mutton broth, pork broth, &c., previously sterile, when exposed to the air on such windy days for a second became very difficult of sterilisation; boiling for ten minutes and sometimes fifteen minutes, or even more, did not produce sterilisation. After forty-eight hours' incubation the fluid was invariably swarming with bacilli.

During July, August, and September, when days were tolerably still, especially on rainy days, and there were no high winds, test-tubes containing sterile nourishing fluid could be kept open, i. e. the cotton-wool plug could be altogether removed under carbolic-acid spray for several seconds, and without being subjected to boiling after this remained sterile at a temperature of 35° C., only a relatively small percentage, varying from five to seven, being lost by air organisms. This is not at all an unsatisfactory result, considering that the laboratory faced the hay market, and considering how easily a contamination could occur under these conditions. But at a time with high winds the contamination was so serious that even prolonged boiling after exposure did not sterilise. This is not to be wondered at if we remember that fluids containing hay bacillus spores and some other bacillus spores require for absolute sterilisation boiling extending up to and even over half an hour (see Cohn's 'Beiträge,' ii Bnd., ii Heft). The results obtained subsequently, when resuming my work, not in the previous locality, but in the laboratory of the Brown Institution, near Vauxhall, situated in a less contaminated atmosphere, were very much more satisfactory. Comparative experiments which I here made showed that exposing to the air for half a minute sterile nourishing fluids contained in test-tubes during windy weather yielded about 50 per cent. failures, while exposing them to air under the carbolic-acid spray yielded no failures in one series, it yielded 5 per cent. failures in another series.

As I mentioned above, I charge my test-tubes rapidly either with pure pork broth or with liquefied gelatine pork without spray, and then boil them well for a few minutes, and doing this in the laboratory of the Brown Institution I find it sufficient to thoroughly sterilise the fluids.

In connection with this subject I would draw the attention of the reader to the very important investigation made by Mr. Watson Cheyne ('Transact. of the Pathol. Society of London,' 1879, p. 577) on the value of the carbolic-acid spray in the inoculation of artificial cultures.

The plan of Koch of spreading out a large drop or small quantity of liquefied gelatine nourishing fluid on a glass slide, or on a flat glass dish, and having inoculated this on its surface with the desired organism to place it in a chamber closed by a bell-jar or the like, which is kept moist by putting into it (the chamber) moist filter paper, has not been found practicable, owing to the fact that the gelatine nourishing fluid having solidified again dries up too soon, before the sown organisms have had time to make a start, the moisture all settling on the inner surface of the bell-jar. This condition I have invariably found to obtain, even when the chamber was closed air-tight, the bell-jar with ground margin being fixed by lard or oil on to a ground-glass plate. Not only in ordinary temperature, but still more so in the incubator, was this drying up of the gelatine nourishing fluid found to happen, and I have therefore modified Koch's plan by using the arrangement mentioned before, viz. the closed glass cells, and the test-tubes plugged with cotton wool.

The next important step in the cultivation of bacteria in the nourishing material hitherto described, as contained in the test-tubes or glass cells, is the inoculation of these materials with the organisms it is desired to grow, i. e. the process of sowing. It is, of course, obvious that if it is desired to cultivate a single species of organism, it is necessary to sow a single species, and to prevent contamination with air organisms, the nourishing fluid itself being sterile. With reference to the first, it is necessary to be certain that the material containing the seed

and to be transferred into the nourishing material contains no other but the desired species. This is, however, not always a simple matter. It is simple enough in the following cases:— If I transfer to my nourishing material a droplet of blood taken from the heart or the spleen of an animal just dead or dying of anthrax, I am certain to have no other organism in the blood except the Bacillus anthracis; or if I have an artificial cultivation of Bacillus anthracis which from certain definite naked-eye appearances (see below), and still more from microscopic examination of anilin-stained specimens, I can pronounce with certainty to be a pure cultivation of Bacillus anthracis, I shall be certain that I shall again, cæteris paribus, obtain a pure cultivation, if sowing out from this cultivation. Again, if I take an infusion of hay in which fermentation produced by the hay bacillus has been completed— that is to say, in which the bacillus has passed its whole cycle and has yielded an abundant crop of spores forming a fine brown precipitate at the bottom of the infusion—and if I boil this infusion for several minutes, I shall be sure to destroy everything living except the spores of the hay bacillus, and if I sow out from this so-boiled infusion I shall have the satisfaction to find that the new growth contains only hay bacillus.

The above modified use of Koch's method, viz. charging the covering glass of the glass cell with a drop of liquefied gelatine nourishing fluid, and when this has become solidified again to inoculate it in one or two straight lines with matter containing the bacteria to be sown, i. e. to dip a needle previously heated, or the end of a freshly drawn-out capillary tube into the fluid containing the seeds, and then to draw this needle or the capillary tube quickly across the surface of the drop of gelatine nourishing material once or twice; this method, I say, is invaluable for the study of the gradual changes those bacteria undergo when subjected to incubation, the manner in which they multiply; further, to ascertain whether the desired organism has been sown, and whether only one kind of organism or several are growing in the nourishing material; for the glass-cell specimen can be easily examined, even with high

powers of the microscope from time to time, without in the least disturbing the growth. Koch, in his paper above quoted, has minutely described all these advantages, and therefore I need not further enter into this part of the subject, as I have no doubt it must be obvious to every one who has the slightest acquaintance with artificial cultivations of bacteria.

If you have sown in this manner a particular organism well known to you, it is of course easily ascertained on microscopic examination immediately after, whether the same is present in any part of the line you have drawn over the gelatine drop in the above glass-cell specimen with your needle or capillary tube. Thus, inoculating the gelatine drop with the Bacillus anthracis or with its spores, or the spores of hay bacillus, with sarcina, with torulæ, with Micrococcus prodigiosus, &c., you can at once find these seeds in the streak you have drawn on the gelatine drop; according to the number of seeds present in the material to be sown there will be more or less numerous seed in that streak. If in addition to this you have sown only one species of those named any accidental contamination will soon be detected under the microscope in the gelatine drop, say after a day or two or longer.

But supposing you are sowing a material of which you do not know whether it contains any organism, or, if so, what kind of organism, the case is altogether different, and the value of this method is not obvious; on the contrary, may lead to serious errors; in this way: the inoculation of the solidified gelatine nourishing material, whether in my glass-cell specimens or after Koch's plan, on glass slides or flat dishes, must take place in the air, and there is no means to prevent contamination with air organisms. Under ordinary circumstances and working quickly the chances of such contamination are not very great, but are, nevertheless, objectionable. Now, supposing that you inoculate your gelatine in several specimens with the material to be tested for organisms, you may find after a day or two or more of incubation that in one or more of the specimens in the streak you have drawn there is no growth whatever of any organism, but outside it at other points an organism or

several organisms begin to grow, you will justly say that all these organisms are accidental contaminations, air organisms; but if you found in the streak one species or more growing you cannot conclude from this that you have transferred this or these species from your original material, because your moist needle-point or capillary glass tube may have caught these seeds while passing through the air; and this has actually happened to me, not once, but repeatedly. I have several instances in which I have sown or meant to have sown in one streak over the gelatine drop of my cell specimen a particular species of bacillus, and to my great annoyance I found, after several days' incubation, in that very streak growing three different species of organisms, viz. one kind of micrococcus and two different species of bacilli. In another instance I wished to test a fluid for the presence of an organism or organisms, consequently I sowed it out on the gelatine in several of my glass cells, and I obtained in the streak drawn over the gelatine drop two species of organisms, a micrococcus and a bacillus; but as I ascertained with a more precise method, the fluid contained no organism whatever. These facts, it must be conceded, prove that the method of Koch, although of great value in certain cases, is less to be recommended in others, and therefore does not deserve that unqualified praise which its author accords to it (l. c.), saying as much as that this is the only method after which cultivations of micro-organisms are to be carried on. I shall presently show that there is a more reliable method (provided the question is one of transferring one definite organism from one fluid into a vessel containing the nourishing material), a method in which the chances of contamination are less and the method also, for other reasons more practicable.

The method of inoculation of the nourishing material which I at first used was under the protection of the carbolic-acid spray: a freshly drawn out capillary pipette is dipped into the material to be sown, the cotton-wool plug of the test-tube or flask containing the nourishing material is lifted under carbolic-acid spray on one side just sufficient to admit the end of the capillary pipette; this being done the plug is again closed

over the mouth, the capillary tube is pushed down into the nourishing fluid and then quickly withdrawn, and the plug completely replaced. In this manner I have been very successful in inoculating, without contamination with air organisms, nourishing fluids with the special organism desired to be sown.

But this method is in so far unpleasant as the spray prevents one from seeing easily the capillary tube while being pushed down into the test-tube. Although I used this method a good deal, I have nevertheless recently employed a much simpler method, which yields as good if not better results. In the carbolic-acid spray the chances of contamination with air organisms are small, as I have above stated, and when a contamination with them occurs it is probably through the spray catching them and carrying them into the test-tubes; but it must be obvious that this is really only a remote chance, considering that in my cases I only momentarily lift one side of the plug sufficient to admit the end of a capillary tube.

The best and most practicable method which I am now in the habit of using, and which is almost absolutely safe against accidental contamination, is this: the cotton-wool plug of the test-tube or flask containing the nourishing material is pulled out for about half its length; a capillary pipette having been charged with the fluid to be sown is then gradually and carefully pierced through the remaining part of the cotton wool (thereby clearing itself of adhering particles), introducing it between it and the sides of the vessel; it is then pushed down into the nourishing material and a trace of the seed fluid emptied into the former. The capillary pipette is quickly withdrawn, and the cotton-wool plug pushed down into its old position. If the nourishing material is gelatine pork, it is of course easily possible at will to deposit the seed from the capillary tube either on to the free surface or into the depth. If the seed fluid is to be obtained from an artificial cultivation contained in a test-tube or a flask, it is withdrawn with a freshly made capillary pipette in exactly the same manner as the seed material is introduced into the new

cultivation just described. It must be borne in mind that for the success of this method it is imperative, in a greater degree than in the other previously mentioned methods, that the cotton-wool plug is thoroughly sterilised. For it is obvious that if this is not the case, by the piercing of the cotton-wool plug with the capillary pipette wool fibres are always carried down into the nourishing material, and if these are not thoroughly sterilised a contamination of the latter must inevitably follow.

I had charged twelve test-tubes with pork broth, and had them well plugged with cotton wool, well boiled on two successive days, and placed into the incubator at 32°—35° C.; they were kept here for two weeks, and remained perfectly limpid and sterile. I then inoculated six of them in the above manner with Bacillus anthracis of an artificial cultivation, viz. introducing the bacilli by piercing the capillary tube containing them through the cotton-wool plug. After twenty-four hours all showed signs of accidental contamination. I remembered that I had kept the test-tubes for several hours, and at two successive days, at 140°—150° C.; but the cotton wool had been tightly compressed in a beaker, and exposed only for about an hour to a temperature of about 120° C. From the remaining six test-tubes I removed the plugs of this cotton wool, and closed them with fresh plugs of thoroughly sterilised cotton wool. They were well boiled and kept in the incubator for several days; as they remained quite limpid they were inoculated after the same maner and with the Bacillus anthracis of the same cultivation as in the case of the first six test-tubes; the result was completely satisfactory; no accidental contamination occurred. From this it is clear that the test-tubes and the nourishing material were sterile in both instances, and also the bacillus to be sown was the same, and in a pure state in both cases, but in the first the cotton wool was at fault, hence the accidental contamination introduced into the nourishing material.

As a rule, in cases where the naked-eye appearances do not and cannot give indications of the actual state of the cultiva-

tions, i.e. whether pure or not, as is the case in most cultivations of bacteria, except, perhaps, of Bacillus anthracis, I have employed both methods, i.e. I cultivated it in the test-tube or flask, and at the same time controlled it under the microscope, by cultivating in the above glass cell a specimen in a drop of solid gelatine nourishing material.

In the cultivations of Bacillus anthracis in the above-named neutral pork broth in test-tubes or small or large flasks with which I worked, after three or four or more days' incubation, even at a temperature so low as 20° to 25° C., a beautiful whitish crop of the bacilli is visible at the bottom of the vessel in the shape of a fluffy, nebulous, more or less filamentous mass as incubation proceeds, gradually extending into the further layers of the fluid, this latter being tolerably bright and limpid. These appearances have been well described by Pasteur, and have been also noticed by Buchner (l. c. p. 376). The cultivations which I carried on in the pork broth, from one transfer to the other, all showed these characteristic appearances, except in those few instances in which, as mentioned above, an accidental contamination occurred. These appearances are so striking and peculiar that it can with certainty, from the naked-eye inspection alone, be recognised whether a given cultivation is one of pure anthrax.[1] If the growth after the first few days does not present the peculiar nebulous and filamentous masses at the bottom of the fluid, if the supernatant fluid remains clouded and turbid, and especially if a scum appears on the surface, either only where the surface of the fluid adheres to the glass or over the whole surface, it can be concluded with probability that the cultivation is impure, there being generally present a micrococcus or a scum-forming bacterium or a bacillus, and this can be easily verified by microscopic examination. During the first two or three days of incubation, however, the fluid is not limpid, but more or less

[1] These appearances are much more striking in neutral cultivations than in those of acid or alkaline reaction. In the latter instances there is never the same copious growth as in the first.

uniformly turbid, the growth not being limited to the bottom layers; but soon this changes, and the characteristic nebulous masses are visible at the bottom, while the rest of the fluid is perfectly clear. The first turbidity is due to the uniform distribution in the fluid of the bacilli, isolated and in chains.

If the vessel in which the cultivation is carried on is kept quiet (I generally keep the test-tubes in a beaker with a layer of cotton wool at its bottom), the peculiar anthrax bacillus growth retains its original naked-eye appearances for a considerable time; shaking the test-tube up after several days' incubation destroys the coherence of the bacillus mass, and this latter breaks up into small flaky particles, which, however, readily sink to the bottom, so that the supernatant fluid again becomes limpid. As long as sufficient nourishing material is present in the fluid the bacillus mass will of course continue to grow in amount, and when this does not any longer take place the fluid is "exhausted." Now, watching the behaviour of the bacillus mass afterwards, i. e. after the mass has ceased increasing, these important facts become obvious: that the bacillus mass becomes gradually smaller; this diminution is in some instances so rapid and conspicuous that at a first inspection it seems that the cultivation is not the same, but that it might have been changed for another; but there can be no doubt about it if the inspection is made a few days later. This diminution goes on till only a few flaky transparent masses are left in the fluid. Below I shall show what the reason of the diminution is and what the microscopical appearances are. During this process of diminution and disappearance of the bacillus mass the fluid remains always perfectly limpid. But I may at once state that this disappearance of the bacillus mass has nothing whatever to do with spore formation, as might be at first supposed; for we know from the researches of Cohn that in a bacillus mass composed of long and convoluted threads, such as the Bacillus anthracis forms in these artificial cultivations, the formation of bright oval spores soon sets in. The newly-formed spores become liberated, the bacillus threads become trans-

parent and disintegrate, while the spores sink to the bottom to form a minute precipitate. There is nothing of the sort in our cultivations. As a general rule in the flasks and test-tubes with fluid neutral pork broth no spore formation takes place. Whether the cultivation is carried on at an ordinary temperature or at a temperature of 20°—25° or 32°—35° C. here is no spore formation. I have test-tubes with neutral, pork broth in which exceptional spores have been formed in the threads. These test-tubes were of the following nature: in one test-tube an enormous mass of bacillus threads had made its appearance while the cultivation was kept in the incubator at 22° C. for about three weeks, the masses of threads were very loose and consequently occupied a large volume, so large that the growth extended almost to the surface of the fluid; here numerous spores were found in the superficial threads. Another test-tube of exactly the same material inoculated with exactly the same generation of Bacillus anthracis, and kept under precisely the same conditions, did not develop any trace of spores; here the mass of bacillus threads formed a more dense growth, and kept its place at the bottom of the test-tube far away from the surface of the fluid. In another test-tube I found that the mass of bacillus threads at first growing at the bottom of the fluid after some time sent out some bundles of threads which grew along the glass wall to the surface of the fluid. Here also numerous spores were found in the threads. Buchner (l. c., p. 370) says: "The physiological cause of the spore formation lies in the commencing deficiency of nourishing material." This is proved to be incorrect by our cultivation. The exhaustion of the nourishing fluid is long apparent and no spore formation occurs, and vice versâ, spore formation may be observed long before there is any sign of exhaustion of the nourishing matter, so much so that spores appear early in the culture and again become converted into bacilli, but no spores may be formed in this new generation. Pasteur says that he prevents the formation of spores in the Bacillus anthracis cultivated in chicken broth, by keeping the cultivation exposed

to a temperature of 42°—43° C., which is still suitable for the growth of the bacilli, but not for the spore formation; in many instances this is unnecessary, because even at ordinary temperatures no spores are formed. Cohn and Koch maintain that for the formation of spores in the bacillus of anthrax as in other bacilli, a certain degree of warmth, a certain degree of moisture, and a sufficient supply of air are indispensable for the formation of spores. There can be no doubt that this is so; Bacillus anthracis does not form spores in the body of an animal, as I can fully confirm Koch against Pasteur, who makes this assumption, and as I have repeatedly convinced myself by direct and systematic observations, to be mentioned in my next Report; but I cannot admit, if it is said that given those three conditions, viz. a certain degree of warmth and moisture, and a sufficient supply of air, supposing other things unchanged, the Bacillus anthracis must of necessity form spores. This is by no means the case, for I have seen numerous cultivations in which these conditions were present, but no spore formation ever occurred, although the bacillus went on increasing in numbers in a most satisfactory way. In the cultivations of Bacillus anthracis in neutral pork broth in test-tubes or flasks, if they are kept quiet, no formation of spores ever occurs. In these instances the absence of a sufficient amount of air is no doubt the cause, as I shall show below, the growth taking place at the bottom of a fluid which had been well boiled for the sake of sterilisation. Dr. Löffler ("aus dem Kaiserl. Gesundheitsamte," 1881, p. 134) also suspects that in Pasteur's cultivations it is perhaps not the temperature of 42° or 43° C. which prevents the spore formation, but the immersion of the bacilli in the fluid, for the bacilli form spores at this temperature when cultivated in flat dishes (see below).

Apart from spore formation, inoculation with Bacillus anthracis of gelatine pork in test-tubes or flasks (and kept solid) yielded slightly different results, according as the inoculation was established on the surface of the gelatine or in the depth. In the first case the growth proceeds with rapidity,

the bacillus forming fluffy masses of convoluted filaments; where these appear the gelatine becomes liquid; and as the growth gradually extends downwards, deeper and deeper layers of the gelatine material become liquefied, the bacillus growth of course occupying the deepest layer of the liquefied material; the liquefied layers above remaining perfectly limpid. In this way the growth gradually comes to lie deeper and deeper, and when the deepest layer of the gelatine has become liquefied, the bacillus mass is at the bottom of the test-tube. When this stage is reached the growth does not differ in any respect from one in a test-tube of pure pork broth. The same changes, described above, of diminution and gradual dwindling away of the bacillus mass is also here noticed.

Differing, however, from the bacillus growth in pure pork broth, the bacillus growth on the surface of the gelatine pork is capable of spore formation, as long as the growth is still close to the surface; but as the superficial layer of the gelatine becomes liquefied by the bacillus growth, this latter gradually takes a deeper position, and since the spores previously formed again germinate into bacilli, a time arrives when no more spores are formed in the bacilli. When a sufficient amount of the gelatine pork has become liquefied so that the bacillus mass being placed at the lowest part of this liquefied portion is away from the surface, no more spore formation is observed; the bacillus threads, however, continue to increase in length and numbers till all the nourishing material is exhausted. But I have some instances where no spore formation took place even at the commencement; that is the case when the inoculation of the gelatine pork in test-tubes takes place (though it be at the surface) at a point between the gelatine and the glass. Here the growth increasing burrows itself at once into the depth and liquefies the gelatine on one side in the shape of a pit or channel passing downwards, and as the bacillus mass occupies the deepest position in this pit of liquefied gelatine it becomes removed from the surface, while yet only composed of bacillus threads. And the growth proceeding into the depth and from here into the sides it may happen that the whole

gelatine pork becomes liquefied without there having occurred a trace of spore formation in any part of the gelatine material. But I have seen a cultivation in which spore formation nevertheless appeared subsequently, although the bacillus mass has become deeply placed in the fluid gelatine. In this case the same process occurred as is mentioned above to have happened in a test-tube of pure pork broth, viz. bundles of threads grew along the glass wall of the test-tube towards the surface of the liquid, and having reached this spores made their appearance.

Spore formation can be, however, easily procured and kept up in gelatine pork in this manner: A flask containing gelatine pork, for about a quarter or a half of its volume, is inoculated with the Bacillus anthracis in the middle of the surface of the solid gelatine material. The growth naturally spreads from here to all parts of the surface, since growth in this direction is much easier than into the depth owing to the resistance offered by the solid material. The layer of gelatine on which the growth takes place becomes liquefied, and thus the growth passes downwards. Owing, however, to the large surface presented by the gelatine material, the bacillus threads show very copious spore formation, and this spore formation is kept up by the bacillus threads for a long time, since even when the superficial layer of the gelatine has become liquefied the bacillus mass is still near a very large surface of air. This superficial fluid layer can be easily drawn off with a glass pipette drawn out into a thin tube at one end, which is introduced into the flask through the cotton-wool plug, in the same manner as for the purpose of withdrawing a single drop, or of inoculating it in the first instance with some seeds, a method that has been minutely described above. This liquefied mass thus drawn off teems with bacillus threads and spores, multitude of them being quite isolated. It can be easily discharged into a sterilised test-tube plugged with cotton-wool, without contamination with air organisms, and kept here ad infinitum. The fluid mass being nearly or quite exhausted of the nourishing parts, at any rate for the Bacillus anthracis and its

spores, is naturally unable to supply the spores with the pabulum necessary for germination, and hence these spores remain as such in the fluid. These relations are perfectly in harmony with all that Cohn has taught ('Beiträge zur Biol. d. Pfl. II.,' Band ii.), about the behaviour of the spores of other bacilli, notably the spores of the hay bacillus. Such spores I have kept in the above test-tube as a sort of stock, both for the production of fatal anthrax in animals as well as for the establishment of new artificial cultivations.

A new layer of liquefied gelatine teeming with spores is gradually formed in the above flask, owing of course to a continuation of the growth of the bacillus threads and spores left behind, and this layer can be drawn off in the same manner as the former; thus liquefied masses teeming with spores can be obtained and drawn off in succession, until a thin layer of the gelatine pork is left in the flask, in which, owing to the enormous surface, abundant spore formation takes place in the bacillus threads, and for the reasons above stated, many of these spores are retained as such. If we start at the outset with only a thin layer of gelatine pork or pure pork broth at the bottom of a flask, and if we inoculate this with Bacillus anthracis, we also obtain here, after a certain progress of the growth of the bacillus threads, a copious crop of spores. This has also been observed by Dr. Löffler, as mentioned above. Many of these spores remain naturally as such in the fluid ad infinitum. We have, then, several methods by which we can with certainty obtain a crop of spores and preserve them ad infinitum. All these observations prove in a most definite manner that for the formation of spores in the Bacillus anthracis a rich supply of air is required, and unless the bacillus threads are well exposed to the air, no spore formation takes place in them. Thus the statement of Cohn and Koch are fully borne out by my observations.

If on the other hand the inoculation of the gelatine pork in test-tubes or in flasks takes place in the depth, that is to say, if the seed is deposited at the outset at the bottom of the solid gelatine mass, the growth proceeds slowly owing to the re-

sistance of the solid material, but in the same manner as is the case in the pure pork broth. Like a beautiful bed of more or less distinctly dendritically branched weeds the masses of bacillus threads springing up at the bottom of the vessel rise into the superimposed layers. The appearance produced hereby is very fine after the growth has made some progress; we perceive the growth to rise perpendicularly from a common bed at the bottom of the vessel like a forest of dendritically branched plants. The gelatine of the deepest layer becomes of course here first liquid. This growth does not yield spores at any time owing to being far away from the surface, and it always remains in the state of masses and convolutions of bacillus threads.

The following confirms in a marked manner what has just been said about the spore formation. In a flask filled to a third or fourth of its volume with solid gelatine pork, Bacillus anthracis is introduced on to the middle of the surface, as in the case above described. Masses of bacilli soon spread over the surface; the superficial layer of the gelatine becomes liquefied by the growth, and this liquid layer teems with spores. Now, I decant this liquid layer, and having plugged the flask again, subject it to boiling. What will happen? By the process of decanting a quantity of the growth is removed, but a great deal (spores and threads) is still left behind on the surface ready for fresh growth. Next, heating the gelatine mass, and thereby making it liquid, of course all growth (spores and threads) sinks to the bottom of the flask. But boiling the mass for about a minute or so does not kill all living matter. The threads of bacillus are indeed necessarily killed by the boiling, but not the spores. This is proved by the fact that on allowing the gelatine to cool again it becomes solid, and now all particulate matter is kept enclosed at the bottom of the flask; and a new and beautiful growth of typical Bacillus anthracis growing from the spores soon makes its appearance at the bottom of the flask, while the surface of course remains free. One of the finest growths of Bacillus anthracis threads in the shape of a forest-like mass of per-

pendicularly ascending branched minute plants at the bottom was obtained in this very manner; but under these conditions no new spore was formed—the growth of the threads did not reach the surface.

In cell specimens of the kind mentioned above the Bacillus anthracis grows very well both in the neutral pork broth as well as in the gelatine pork. In the latter, when kept solid, i. e. at a temperature of about 20—25° C., the progress of the bacillus can be readily watched. The change of the bacilli into the very characteristic homogeneous-looking long threads forming bundles twisted round one another in a spiral manner so as to resemble a cable; the extension of the threads in all directions; the appearance of spores and their full development, come out here with the same beauty as in the previous cultivations. One drawback to the cultivation in cell specimens is the possibility of contamination with air organisms, as has been mentioned before. In many instances the growth of Bacillus anthracis proceeds all right for two or three days, even to the formation of spores, but then an unpleasant crowd of moving bacilli, or what is equally common, zoogloœa masses of innumerable micrococci cover everything in the field, including the Bacilli anthracis. But I have had a good many specimens in which the growth of Bacillus anthracis remained free of contamination. In such cases it is noticed that after some days the gelatine becomes also here liquefied. While in some specimens active spore formation is observed, in others, kept and established under apparently the same conditions, there never is a trace of real spores, or at the utmost there is a sort of abortive or imperfect spore formation. In the latter cases the whole growth of bacilli in the preparation gradually disappears, and dwindles down to an insignificant number of hyaline threads, just as was the case with the cultivations in the test-tubes and flasks. But whether the growth leads to the formation of spores or not, there is always, already in the early stages when the bundles of bacillus threads are yet few and not very long, this fact to be noticed, viz. that in many threads there are a good many shorter or longer spaces

in which nothing but a hyaline sheath or tube is noticeable, the highly refractive contents or the protoplasm within the tube being wanting; as the growth proceeds the number of such threads with empty spaces in their sheath increases, and whole threads of immense length may be found in this condition, i. e. in the state of hyaline tubes or sheaths from whose interior the protoplasm has altogether disappeared. This is always noticed in the growth of the Anthrax bacillus threads in cell specimens; samples taken out at any stage from the cultivation in test-tubes or flasks show the same condition, viz. there are always present longer or shorter threads, which either entirely or partially have become barren of the protoplasm inside the sheath. In cell specimens, it is possible to ascertain that the growing ends of the threads which may be found in the peripheral part of the drop as straight filaments with a rounded end, are always full of protoplasm, and that the deficiency in protoplasm commences at some distance from the end. By-and-by the greater number of threads may thus lose altogether their protoplasm, and hereby become quite transparent and almost lost to sight, but a careful inspection can still detect their presence. Some appear ultimately to break up altogether. This change, viz. the disappearance of the protoplasm in the threads from place to place, is associated, generally but not always and in all places, with the appearance of irregularly sized granules in the tubes, these disappear gradually, becoming evidently dissolved and absorbed, and the then sheath appears at such a place or places quite empty, i. e. without containing any solid protoplasm. These granules are not spores, as I shall show below. I consider it merely a form of degeneration or death of the protoplasm. Another change in the cell specimens is the appearance of spherical corpuscles, either isolated or in close rows or chains; in this latter case we have a thread of regular varicose appearance, not unlike a chain of torulæ. The size of these spherical corpuscles is in their best development that of a human red blood-corpuscle, and in aspect are identical with the gonidia of an oïdium or the cells of torula, i. e. within a cell membrane they contain

clear contents and in this a minute nucleus. They become elongated and by fission divide into, or by gemmation produce, two new spherical corpuscles. The growing ends of the threads seen at the marginal part of the specimen sometimes are connected with such a chain of spherical torula-like corpuscles, and in this respect the appearances bear a striking resemblance to the formation of gonidia by mycelium threads. This change also has nothing whatever to do with the formation of spores. It can be ascertained to exist always also in the cultivations in pure pork broth and gelatine pork in the test-tubes and flasks. In some cultivations in the neutral pork broth I have met with it very extensively already after a few days' incubation. With great distinctness and profusion I have seen it in cultivations in gelatine pork carried on at the temperature of the room.

The observations which I have made on the life-history of the Bacillus anthracis differ in some respects from those of previous writers. Starting with the Bacillus anthracis of the blood, introduced into the cultivations of neutral pork broth or of the mixture of this broth with gelatine, it is invariably the rule that, as noticed by other observers (Koch, Pasteur, Buchner, and others), the bacilli grow out sooner or later into long homogeneous-looking threads which form bundles, the individual threads coiling round one another in the manner of the wires of a cable. But there are always some short bacilli, or chains of them and short threads; especially in the former it is noticed in the fresh state and after staining, that their ends are not so blunt as is usually represented, but that they are slightly rounded; and the same rounded appearance is also noticed on the ends of the threads, which are undoubted anthrax bacillus threads. In all specimens of gelatine pork above described the rounded conditions of the ends of the threads is easily perceived.

In the first few days the cultivations of neutral pork broth invariably show, as mentioned above, a uniform distribution of shorter or longer bacilli, isolated and in chains. These gradu-

ally lengthen and then of course by their weight settle down at the bottom of the fluid whence they grow upwards into the characteristic long convolutions. I presume the uniform distribution of the bacilli and the general turbidity of the fluid caused hereby in the early days is due to the bacilli following Brownian molecular movement, as well as to their being able to float in the fluid, but when they grow out into long threads their weight and the cessation of molecular movement draws them down to the bottom of the fluid, which for this reason then becomes clear. That the isolated and short chains of bacilli causing the general turbidity of the cultivation in the first few days are really anthrax bacilli as much as the typical long threads afterwards formed, is shown by the fact that if with these latter a cultivation is started it presents the turbid appearance in the first few days, and secondly, at any stage the smallest quantity of the cultivation kills with typical anthrax guinea-pigs and rabbits.[1]

With Abbe's condensor and Zeiss' oil immersion objectives it is possible to discover in these threads, already in the fresh and living state from place to place, a differentiation of a thin sheath forming the tube, as it were, and a protoplasmic contents, and this protoplasmic contents appears subdivided into a single row of short, almost cubical blocks or cells. In many places this subdivision of the protoplasm into cells or even the differentiation into sheath and protoplasm is not distinct in the fresh state, but comes out with greater or lesser distinctness after staining or after certain reagents. Thus, for instance, careful staining them fresh with anilin dyes (gentian violet, methyl violet, methyl blue, Spillers' purple, &c.) brings out in many places this differentiation into sheath and protoplasm,

[1] These peculiarities of the early bacillus growth may or may not be connected with the ability of that growth, which is not possessed by later stages of the same growth, to kill mice that are inoculated with it. However that may be, these early peculiarities have no relation to spore formation. Spores have, indeed, nothing in common with these rounded ends and cubical cells, which stain in a way that spores do not stain, and have a quite different shape and refractive power.

and the subdivision of the protoplasm into square or rather cubical individuals or cells. Adding a nearly concentrated solution of acetate of potash to the fresh preparation brings out these appearances also very well. Still more, and in fact with marvellous distinctness, does it come out in dried and stained specimens (after Weigert and Koch). Watching the bacilli of the spleen or any other organ while drying under the microscope, the gradual differentiation into sheath and cubical cells can be followed very readily. I have made an endless number of stained specimens of bacillus threads of my cultivations in pork broth and gelatine pork, and have invariably found the same appearances, provided the specimens be not overstained, or if so, well washed with alcohol, viz. the whole protoplasm of each thread is subdivided into a single row of cubical cells, stains well with the anilin dye, and distinct from the general sheath of the thread. Koch has pointed out that the Bacillus anthracis shows in dried and stained specimens a very characteristic subdivision into shorter or longer rod-like structures, and by this alone Bacillus anthracis can be distinguished from other bacilli. He gives in his work ('Cohn's Beiträge II,' Bnd. iii) a photograph to illustrate this point. In this illustration the subdivision of the protoplasm is not by any means numerous, far less than in my case, for I find the individual members not rod-shaped but cubical. It is true here and there it is seen that instead of a cubical we have an elongated or rod-shaped cell, but in some of these we can clearly discover a slight constriction in the middle, a sign of commencing division into two. The independence of the common sheath of the thread and these cells Koch has not noticed.

But also in the bacilli of the blood and spleen of mice, guinea-pigs, and rabbits, dead of anthrax, I have noticed precisely the same distinction into common sheath and the subdivision of the protoplasm into cubical cells, or when the cells are elongated a middle constriction was noticeable. Accordingly the length of a bacillus, viz. whether a longer or shorter rod or a longer or shorter thread, depends entirely on the

number of cubical protoplasmic cells contained within the common sheath. The cells may be seen aggregated into twos or into fours, that is, forming longer or shorter rod aggregations.

Bacilli obtained fresh of the spleen of an animal dead of anthrax show in some instances an absence of the protoplasmic contents within the sheath; this may be only limited to a small spot or may involve the greater part of the length of the bacillus. In sections made of hardened or fresh organs of an anthrax animal, after staining with logwood, or, still better, with anilin dyes, the same appearance may be met with, viz. limited deficiency of protoplasm in some bacilli. Koch (l. c., p. 40, and Plate v, figs. 29 and 30) mentions this appearance of stained bacilli from the spleen of an anthrax rat.

On a former page I have described a similar local deficiency of the protoplasm in bacilli and bacillus threads in the artificial cultivations at all periods of growth, even at the earlier stages, and in these circumstances the deficiency extends in some threads over long distances, and in consequence only the hyaline transparent sheath of the original thread is left, and this ultimately may also become broken up.

It appears to me very probable from numerous observations that in every bacillus at some period of its growth, one, two, or more consecutive cells may cease to grow and to multiply. These cells die if spore formation does not occur in them, and their death is indicated by a granular disintegration of their protoplasm and a final solution and absorption of it. In this case the sheath of the bacillus thread remains empty at this place. Such bacilli and bacillus threads are thicker than the unaltered ones.

The division and gemmation of the above-mentioned torula-like corpuscles or gonidia leads to the formation of chains, at first entirely composed of torula-like gonidia; by active division of these gonidia the chains rapidly elongate; in a further stage the gonidia are transformed into oval elongate cells, which are thinner than the original gonidia, and ultimately

they change into rod-like cells, again thinner than the oval cells. When the latter stage is reached we have already to do with the typical thread of an Anthrax bacillus. In some such threads there are seen numerous places in which the preceding stages of oval cells and of spherical gonidia can be easily recognised. We have, then, here before us a new form of growth of the Bacillus anthracis very similar to that of an oïdium growing in a fluid.

The diminution of the bacillus mass in the artificial cultivations in test-tubes and flasks described on a previous page is due to the degeneration and disappearance of the protoplasmic cells in the threads, so that at first the transparent sheaths are left, and they also break up ultimately. This degeneration takes place chiefly on the plan of a gradual granular disintegration of the cells within the sheath. In the first stage of this process, and especially if the preparation has been stained, it is noticed that instead of the cubical mass of protoplasm representing one cell, we find either one large granule or a delicate dumb-bell joined by a shorter or longer thin pale bridge. These appearances I have seen in many places in undoubted Anthrax bacillus threads; of an accidental admixture there can be no manner of talk, since the general sheath passes uninterruptedly over all unaltered and altered cells. In a further stage of disintegration the granules dwindle down, and are ultimately altogether lost. Koch figures ('Unter u. path. Organismen,' Plate vii, fig. 39) thin bacilli showing a similar appearance to that just described; In Koch's case they were not Anthrax bacilli, and Koch does not decide whether this appearance means spore formation or not. I am quite confident it has nothing whatever to do with spore formation, although I at first thought this to be the initial stage of it (see Cohn and Koch); but in my case there is at no time to be seen in them a trace of a bright oval spore. The whole protoplasm of a thread may give origin to these granules; they become smaller and smaller and more numerous, and irregularly distributed in the sheath, and ultimately altogether disappear.

In some instances I can see something like a thin septum stretching between some of the cells and fixed to the membrane of the common sheath, but I cannot be quite certain about a septum being present between each two cells. I think that the sheath of many a bacillus, be it short or long, be it a straight thread or a curved one, is traversed by such septa at relatively few places; in many places it is a continuous membranous tube in which the protoplasmic cells lie in a single row. In threads in which the above-mentioned granular degeneration occurs the presence of septa can be easier ascertained than in perfect bacilli, but one must not confuse them with transverse discoid débris within the tube. In some such tubes it is seen that many compartments contain one cell; others contain two cubical cells or one oblong, and still others there are that contain three cells, one oblong and two cubical ones. In threads in which the above-mentioned nodose swelling up of the cells has taken place there are distinct signs of septa between the individual cells, especially where the degeneration comprises a whole row of cells.

Comparing the Bacillus anthracis of heart's blood or spleen of a mouse, guinea-pig, or rabbit, dead of the disease, with the bacilli and bacillus threads grown in the cultivations of neutral pork broth, or in a mixture of this and gelatine, it is found that the organisms are almost twice the thickness of those taken from the animal. I do not refer to the bacilli and threads in which either granular degeneration or the torula-like swelling of its cells or spore formation is going on, for these are naturally thicker, but I refer to bacilli in which unaltered protoplasm is contained within the sheath.

Ewart ('Quarterly Journ. of Micr. Scien.,' April, 1877) maintains to have observed a transition of the ordinary non-moving Bacillus anthracis into flagellate-moving bacillus. I can only say with reference to this, that in all my observations, whether conducted in test-tubes or in cell specimens, there was never anything of the sort observable. Ewart took no precaution whatever against contamination with other

bacillus, and therefore his observations lose all value, since it is probable that he had before him an ordinary flagellate bacillus.

Buchner (l. c., p. 394) also claims to have seen a transition of a non-moving typical Bacillus anthracis into a flagellate bacillus, but in Buchner's case this is supposed to have come about in a gradual manner after more than 1100 generations. Notwithstanding the imposing number of generations, I nevertheless doubt the reality of this transformation, since Buchner's cultivations are open to the objection that they were contaminated with an air bacillus. Besides, Buchner, in connection with this very transformation, makes certain statements as regards the influence on this transformation of the acid reaction of the hay infusion in which the bacilli were cultivated and transformed into flagellate innocuous hay bacilli, statements, I say, which I know to be incorrect, as I shall show later on. He says, for instance (l. c., p. 392), "that the slight acidity of hay infusion prevents altogether the growth of the true Bacillus anthracis." This statement is to me unintelligble, since I have seen Bacillus anthracis starting off into a very good growth in acid hay infusion, as well as in other acid nourishing fluids. This statement of Buchner's, if it is to be accepted at all, must be accepted to mean something else than what Buchner infers, viz. that the true Bacillus anthracis in the acid hay infusion used by Buchner had no chance against the hay bacillus growing in it, and of which Buchner had not quite got rid previous to the inoculation with Bacillus anthracis.

I have mentioned above some of the conditions under which spores were formed in my cultivations, and I wish now to state the manner in which this takes place. Examining in the first state the bacillus of such cultivations in which spore formation is just commencing, it will be found that the protoplasm of the bacilli and bacillus threads appears slightly granular. Where no spores appear it is uniform in aspect. In the granular ones are seen here and there bright, glistening,

spherical, or rather cubical and elliptical, or rather rod-shaped spores. The spores are slightly truncated at their ends, and slightly convex where in contact with the sheath. Staining such specimens with Spiller's purple it will be found that the granular as well as the homogeneous protoplasm stains readily and deeply, whereas the spores, both the spherical as well as the oval ones, remain unstained, and therefore contrast well with the rest of the bacilli. This same relation is exhibited by specimens stained with other anilin dyes, but best with gentian violet and Spiller's purple. The different parts of the bacillus threads show a great difference with respect to the number of spores. In some places there is for long distances a single cubical or oblong spore contained in the thread; in others they are more numerous; and still in others they follow one another as numerously as the elementary cells. It is found that wherever a spore appears it is at once either cubical or elongated, and conspicuous by its glistening appearance and remaining unstained. The cubical ones when ripening become elongated, but always remain of the bright appearance; it is further true that each cubical spore belongs to an elementary cell, but where this latter has become elongated and slightly constricted, preceding division, as mentioned on a former page, we may find two such pores contained in it. In some places the cubical spore remains single in the oblong cell. The elongated spores are as a rule placed parallel to the long axis of the bacillus; but in some places I have seen one or the other spore placed in a diagonal direction. The elementary cell containing a spore still possesses a trace of protoplasm around the spore; but this remnant of protoplasm sooner or later breaks altogether away, and the spore is free of it. I have, however, seen spores which after having left the sheath of the thread, still showed at one end a trace of the protoplasm.

According to the facility with which spores are formed in a cultivation we find the number of spores formed in a bacillus thread varying. In some threads every cell for some distance may develop a spore, in others numerous cells remain always

without spores, and their protoplasm crumbles down into a granular débris. Under all conditions, however, the thread becomes much thicker, the sheath swells up, and gradually is lost as such. In some cell specimens I noticed an abortive formation of spores; these appeared as irregularly distributed spherical small spores, which never grew into the typical fully-formed large elongated spores.

The conclusion we then arrive at from all these observations is this:—Under most favorable conditions every elementary cell is capable of forming a spore; these spores are bright and glistening, and do not stain (see Koch). At first they are spherical, afterwards larger and oblong. If the cell is an elementary or cubical one it forms one spore; if it is elongated and constricted, i. e. before dividing, it may form two spores not all the protoplasm of the cell is involved in the formation of the spore, a trace of it is left around the spore, but sooner or later crumbles away as a granular débris. If the conditions are not so favorable only a limited number of cells form spores, in the rest the protoplasm degenerates into granular débris, and under unfavorable conditions, especially in the absence of a sufficient supply of air no spore is formed in any of the cells. When spores are formed they escape after the sheath breaks down.

Ewart (l. c.) maintains to have observed a division of the spores after they had become freed of the bacillus sheath. This statement also requires confirmation. The above-mentioned couples of spherical spores Ewart also noticed, but they are not due to a division of spores as Ewart maintains, but are developed as such in an oblong dividing cell.

I now enter one of the most important parts of this research, viz. the results of the inoculation of rodent animals with the Bacillus anthracis of the artificial cultivations described above.

At the outset I wish to state the manner in which the inoculations were carried out. The animals used were white and tame brown mice and offsprings of both; further, guinea-pigs

and rabbits. As a rule the mice were inoculated into the subcutaneous tissue of the tail, the guinea-pigs and rabbits into the inguinal skin or subcutaneous tissue, or into the skin of the ear-lobe. The infective material—blood of an anthrax animal or bacillus of a cultivation—is collected in a capillary tube freshly drawn out, and is then blown out into a small incision made into the true skin or subcutaneous tissue, according as desired, with a sharp blade that has been before perfectly disinfected in the gas blowpipe. By this method of inoculation I always made certain of not getting any contamination by the instruments—syringe and canula—that may have been used in previous inoculations. In some cases I used also Pravaz syringes, viz. when I inoculated with blood of anthrax, and expected fatal anthrax; for in this case, even if the syringe should not have been cleaned of anthrax particles of former inoculations it did not matter. Knowing the great difficulty of thoroughly disinfecting Pravaz syringes, heating not being available, I did not practise as a rule inoculation by means of the syringe. In the case of guinea-pigs and rabbits a syringe can be always dispensed with, since a capillary pipette drawn out to a fine point and charged with the infective material is as easily inserted to any distance into the inguinal subcutaneous tissue as the canula of a hypodermic syringe. A minute incision having previously been made, the capillary pipette is emptied of its contents as usual i. e. by blowing into the near end of it. But even into the subcutaneous tissue of the tail of a mouse a capillary pipette drawn out into a fine point can be easily advanced for a distance sufficiently long for safe inoculation. In all my inoculations with blood and with fresh cultures I have used only very minute quantities of the infective material, a portion of a droplet to a drop, and I found that, as a rule, the quantity of the material introduced was, if other conditions were equal, seldom a matter of any importance. Buchner and Greenfield, speaking of the early stages of successive cultivations, maintain to have had to introduce in some cases larger quantities of the same material than in others, in order to produce an effect, owing to the activity of the material

having become diminished by cultivation in the former and not in the latter. These statements may be, and probably are, best explained after Koch, by the assumption that the original cultivation had become contaminated by another bacillus, the Bacillus anthracis remaining in the minority is gradually overgrown after a certain period or after a certain number of cultivations by the contamination of bacillus, and hence larger quantities of the fluid are to be used to get hold of one or the other stray Bacillus anthracis left therein, and after some more transfers the original number of Bacillus anthracis had become so much diminished that perhaps even a larger quantity contains no other than the contamination bacillus. In my cultivations I never noticed such a condition, i. e. I never found reason for supposing the anthrax bacillus to undergo change in its virulence, otherwise than as there might be question of spore formation on the one hand, or of degeneration (that rendered the bacillus completely inert) on the other hand. I have, indeed, on occasion found an exceptionally large quantity of inoculating material to be required; but this circumstance has always appeared to me perfectly well explained through the small number of really active bacilli existing in the particular material. This has taken place, e. g. in cultures of pure Bacillus anthracis without spores, that had been kept for some weeks (see below), and where there had been a gradual diminution of the number of active anthrax bacilli.

A point of importance which I wish to mention here refers to the time of death of rodents after inoculation with anthrax. As a rule they die within forty-eight hours from the time of inoculation; some within twenty hours, others in thirty or thirty-six hours, and a few others between this and forty-eight hours. Few animals survive the third day, although I have seen mice and guinea-pigs die after five days of typical anthrax. A given cultivation used after the same method and in the same quantity for the inoculation of several mice will kill some of them more rapidly than it will kill others, and the same is true if guinea-pigs are the animals under experiment. I have seen animals (mice and guinea-pigs) die within twenty hours after

inoculation with infinitesimal doses of artificially cultivated Bacillus anthracis. The presence of spores makes no difference to the rapidity of death. A guinea-pig inoculated with spores that had been first of all frozen with ether spray and then allowed to thaw died within twenty-four hours.

Observations on Inoculation.

It is not necessary for me to enter here into a description of the symptoms of anthrax in rodents, since these are well known through the various descriptions already existing, and I may refer the reader especially to those given by Koch in his several writings on anthrax.

But one fact I must mention here, viz. the great irregularity presented by the spleen in the animals (mice, guinea-pigs, and rabbits). In some instances the spleen is very much enlarged, in others it is not enlarged at all, but in all instances it contains bacilli, though the number of these varies considerably without, however, standing in any relation to the duration of the illness.

1. Inoculation with blood of an animal dead of anthrax:

The blood was derived from the heart of mouse, guinea-pig, or rabbit, either quite fresh or after one, two, three, to eight days. In all instances death followed after the introduction of infinitesimal doses. Before inoculation I ascertained that the blood contained the bacilli in sufficient numbers to make it pretty certain that some bacilli will be present even in so minute a quantity. When using infinitesimal doses it is necessary to bear this in mind for the following reasons:—Supposing the point of a needle is well steeped into the blood of a guinea-pig or rabbit dead of anthrax, and then with this needle the skin of a mouse, or guinea-pig, or rabbit is pricked down to the subcutaneous tissue, out of ten such inoculations the chances are that all ten will be successful; but supposing the point of the needle be well dipped into the blood of a mouse dead of anthrax for some hours, and the inoculation be performed as above, out of ten such inoculations the chances are that there will be

several failures. The reason lies in the peculiar way the bacilli are distributed in the blood fluid, being now most of them collected in masses, owing to being held together by a granular, imperfectly-coagulated fibrine. Dipping the point of a needle into the blood, it may chance that the point of the needle does not take up one of these coagula, they being sometimes large and far between, and in this case the inoculation will be unsuccessful. A similar connection, i. e. the aggregation of the bacilli by granular coagula, may be also observed in guinea-pigs and rabbits some time after death, but not to such an extent as in mice. I well remember to have been rather puzzled one day about the inexplicable cause of death of four of my mice that had been inoculated with anthrax, but apparently did not show any bacilli in the blood. The animals had been dead for some hours (less than twenty), and specimens of blood of the jugular vein and heart withdrawn with a capillary pipette, and used for microscopic preparations, did not reveal the presence of the bacilli. I then made specimens of the tissue of the spleen, which organ was only slightly enlarged, and found it teeming with the characteristic anthrax bacilli. I examined the blood again, and especially collected from the cavity of the heart blood with the blade of the knife, so as to get out not only fluid blood, but coagula as well, and then I met a number of large coagula crowded with the bacilli. These peculiarities, in fact, account for several cases in which I thought at first to have to deal with animals refractory against anthrax; but on inoculating them again with guinea-pig's blood containing uniformly distributed bacilli they all succumbed. The older the blood, and the longer it has remained within the body of the dead animal, the less chance there is of its retaining the bacilli in a living condition, and the greater also the chances of these bacilli having disappeared and other saprogenic bacilli having made their appearance. These points have been well ascertained by Koch, and I can fully confirm them. I shall have to return to this point in a later report, in which I shall give the results of a systematical inquiry into this death of the bacilli in the organs of an animal dead of anthrax. In some instances

I have had opportunity of using grey mice for my inoculations, and also bastards of white and grey mice, but did not notice any refractory power possessed by the former against anthrax blood. Some observers have noticed a certain amount of resistance offered to the anthrax virus by the grey mouse, but in these instances they were wild mice, whereas in my cases they were born in captivity and tame.

At the seat of the inoculation with blood in mice the place is always more or less marked as slightly discoloured and a little tumid, but since death as a rule occurs within thirty-six hours there is not much chance of inflammation. After the inoculation of mice with blood, and the same applies to other anthrax material, as well as to artificially-cultivated anthrax, the animals appear in perfect health until a short time, sometimes half an hour or an hour before death; they are very lively and feed well; nothing in their condition reveals the presence in them of the seed of death, when suddenly they become quiet, their movements become impeded, their breathing rapid, and their temperature begins to sink; and after half an hour, or even less, sometimes more, up to two hours, they are dead.

So remarkably sudden is this change from apparent health to a sickness that is rapidly and surely fatal, that this form of the disease has in Germany received the name of fulminirender Milzbrand, Teufelsschuss (Röll, 'Lehrbuch d. Path. und Ther.,' &c., Wien, 1876, i, p. 493).

In guinea-pigs I noticed on inoculation of blood or any other anthrax material and artificial cultivations into the subcutaneous tissue of the inguinal region, as a rule before the day is over, a distinct œdematous swelling; this increases gradually till the animal dies, which in the case of blood happens generally within thirty-six or forty-eight hours. Death is also here rapid, but not so rapid as in mice, since the animal becomes quiet and weak several hours before death takes place. On post-mortem examination the subcutaneous tissue of the inguinal region and abdomen, especially on the side of inoculation, shows much œdematous swelling. Near the seat of the

inoculation there are a few hæmorrhagic spots, but the œdema is generally free of blood; cutting into the tissue a quantity of clear serum flows out that contains only very few bacilli, if examined soon after death. Later on their number is much increased. In some cases, especially of prolonged illness, this œdema is so extensive that the subcutaneous tissue of the inguinal region, abdomen, and chest is uniformly infiltrated with the serum. This œdema I have not missed, with very few exceptions, in the cases of inoculation of Bacillus anthracis and their spores, no matter whence derived, if the inoculation is made into the subcutaneous tissue of the inguinal or abdominal region, but it was absent if that of the ear-lobe was the seat of inoculation. That in our case we have to do with veritable anthrax there can be no manner of doubt from the symptoms, the nature of the bacilli, and their distribution in the organs. Pasteur mentions a similar appearance of œdema in some of his sheep.

In rabbits the œdema is not so frequent as in guinea-pigs, nor is it so pronounced, but nevertheless I have met with it in several instances of inoculation into the subcutaneous tissue of the inguinal region.[1]

It must be, however, understood that this symptom of subcutaneous œdema in guinea-pigs after inoculation into the subcutaneous tissue of the inguinal region occurs not only after the inoculation with blood or with tissues, but equally distinct with artificially cultivated bacillus and its spores.

Inoculation into the corium itself of the inguinal region of guinea-pigs produced only very slight œdematous swelling about the point of inoculation; in rabbits such an inoculation is not associated with œdema.

2. Inoculations with artificially cultivated Bacillus anthracis.

[1] What the meaning of the statement of Wernich's ('Central f. med. Wiss.,' No. 12, 1882, p. 217) is, that rabbits, "although not absolutely refractory, nevertheless are very little susceptible" to anthrax, I cannot comprehend, since I have never found a rabbit escape death after inoculation with anthrax blood or artificially cultivated active Bacillus anthracis.

The experiments which I wish to mention here were made with Bacillus anthracis derived from the mouse, guinea-pig, or rabbit, killed by inoculated anthrax, after this bacillus had been cultivated in the neutral pork broth or the neutral gelatine pork above described. In the following I shall, of course, only take into account the cultivations which from the unaided-eye aspect, the microscopic examination, and the experimental results of inoculation with them, are to be considered as undoubtedly pure cultivations of Bacillus anthracis. The first remove of Bacillus anthracis from the anthrax animal will be considered as the first cultivation; the remove from this into a new cultivation, the second cultivation; from this, again, a third cultivation; from this, again, a fourth, and so on. As a rule, as soon as a cultivation showed a good crop of the bacillus, a next cultivation was established from it by transferring into the sterile nourishing material an infinitesimal part of a drop of the parent cultivation. In some instances, especially when the cultivations were kept at a temperature of 32—35° C., there was a copious growth of bacillus obtained already after two or three days; in other instances, if the incubation was carried on at 20—25° C., I generally waited about six or seven days before utilising the cultivation for the establishment of a new cultivation.[1] To term the cultivations "generations," as Buchner does, seems to me altogether arbitrary; his "1500 generations" are no more in reality 1500 generations than they are "150 generations," as Koch is only too leniently inclined to admit (l. c., p. 24). A "generation" could really only be called a new crop of bacilli produced from spores of bacilli. If a bacillus grows out into a long convoluted thread or threads, i. e. if one or several elementary cells continue to divide till they have formed a chain of enormous length, then we have no more right to call this chain a full generation than we have to consider the initial cell of the bacillus thread as the parent, the second cell derived from this as the first generation, the third cell as the second generation,

[1] For convenience's sake, I shall speak of the days of exposure of a cultivation to a constant temperature in the incubator, as being days of "incubation."

and so on, for then we could no doubt get millions of generations in the very first cultivation that we established with the bacillus taken from the blood. But even if in a cultivation spores are formed this cultivation need not represent one generation only, because the spores first formed may, and as a rule do, germinate into new bacilli; these or their offsprings again form spores, then germinate into bacilli, and so on within the same cultivation as long as nourishment is present. But in Buchner's case there must have been cultivations in which spores were never formed. In whatever way we look at it we cannot fix the meaning of the term "generation," and therefore we cannot speak of a cultivation as a "generation."

At the commencement of my experiments I used white mice for testing the activity of fluids and cultivations containing Bacillus anthracis, since, as is well known from Koch and others, these animals are very susceptible to anthrax. Inoculation with a given cultivation of blood bacillus in neutral pork broth kills mice, guinea-pigs, and rabbits, when used during the first few days of incubation; but soon a difference sets in, for after the first few days inoculation of mice with the same cultivation proved fatal only in a certain percentage of cases, and after several days more a good many mice remained perfectly unaffected by the cultivation. My cultivations were typical and perfectly pure, as I ascertained by microscopic examination and further experiments; there were the typical convoluted cable-like bundles of the threads; on staining they were beautifully "cellular" in structure; inoculations of new cultivations which I made came up very finely; and inoculations with infinitesimal doses in guinea-pigs and rabbits produced typical anthrax. The above mice were inoculated, some by Pravaz syringe, others by deep incision, and placing into this a drop of the cultivation. The inoculation was repeated in some instances, but without effect; the mice remained perfectly free of illness. This result was obtained with the cultivation, in some cases within a week, in others after a longer period. How could this be explained? Were all these mice refractory to anthrax. Were they refractory only to the artificially-cultivated

Bacillus anthracis? Were they attacked by it, but did not die, as was the case in Pasteur's experiments with the "vaccine?" Had the cultivated bacillus lost its virulence or altogether its specific effect after the first remove from the blood, and thus putting the cultivations of Buchner and Greenfield altogether in the shade? These were questions that presented themselves for solution.

As I mentioned above, of the purity of the cultivation I had no manner of doubt; for this I had the most cogent reasons. I must state in connection with this, that I refer not only to the use for inoculation of the cultivations in fluid pork broth, but also to cultivations carried on in gelatine pork in a microscopic cell specimen above described, where from day to day I could follow the increase in number and length of the bacillus threads. Such cultivations were also used for the inoculation of mice, and if containing no spores, proved without effect. Another point I must not omit to mention, the method or rather methods of inoculation used with mice were perfectly reliable, since really active material introduced in the same manner proved efficacious.

That the mice were not refractory to anthrax—[it would have been a most extraordinary thing if I should have happened to get hold of one refractory mouse after another; I have not found a mouse that was really refractory[1] to anthrax, if the

[1] In several instances of mice I have noticed what seems to denote a certain resistance offered to the anthrax virus on the part of white mice, viz. that some of my animals did not become affected by the virus the first or even the second time they were inoculated with it. Thus I noticed some that resisted the action of the cultivated Bacillus anthracis of a first cultivation; then they remained also unaffected by the introduction of typical anthrax bacillus threads of a second cultivation; and on a third time being inoculated with blood bacillus remained nevertheless alive. They succumbed, however, on a fourth inoculation with bacillus spores of an artificial cultivation. Another mouse remained unaffected after the introduction of anthrax blood filled with bacilli, but succumbed to the influence of an artificial cultivation of anthrax bacilli filled with spores. The inoculation in these cases was carried out in the way described above, and I have no doubt that the material was properly introduced into the subcutaneous tissue of the tail. Not seeing any reason to accept in the first instances a refractivity of these particular animals to the anthrax virus,—for

proper material is used; every one of them inoculated once or twice with it died]—was proved by the fact that they one and all succumbed to anthrax afterwards when inoculated with a different but active material. And this latter circumstance proves at once that they had not been "vaccinated," in the sense of Pasteur, or any other sense, by the first inoculation, that in fact the first inoculation produced absolutely no disease. But, secondly, had the bacillus as such lost its virulence by being taken from the blood and cultivated in an artificial medium? Not in the least, because the very same bacillus killed mice in the first few days of the cultivation, and later on it killed guinea-pigs and rabbits within forty-eight hours by typical anthrax, and blood of these animals killed without fail within thirty-six hours. What is more than this, the very same cultivation which failed to kill a mouse or mice at one time, killed them without fail at another, provided the bacillus had in the meantime had the opportunity to form spores. And this fact, viz. the presence of spores in the cultivation, is of the utmost importance in respect of the fatal efficacy of the artificially cultivated bacillus on the mice.

Pasteur, as mentioned above, produced a certain incapability of the bacillus to kill sheep by growing it at 42°—43° C. By these means he maintains that he can prevent the bacilli from forming spores which prove fatal to sheep when inoculated. I have mentioned above that in my cultivations in neutral pork

they succumbed to it ultimately, thus proving that they were susceptible to the virus,—it remains as the most probable explanation to assume that the virus, although locally introduced, was for some unknown reasons not carried into the general circulation. That in our instances it was the resistance offered by the tissue of the tail to the life of the Bacillus anthracis, which prevented the development of the disease, is not a probable reason, since there exists no real resistance to the anthrax bacillus, of either mice, rabbits, or guinea-pigs to prevent the fatal result generally produced after such incubations.

A similar negative result after first inoculation I have noticed also in a few of my guinea-pigs, where the fluid had been introduced into the subcutaneous tissue, and also in a sheep. But in both cases a second inoculation with the same virus produced positive results. The virus was introduced during the first inoculation in sufficient quantity very safely into the subcutaneous tissue.

broth, in which the bacillus mass remains quiet at the bottom of the vessel, no spores are formed, and it is such a cultivation which proves inactive on mice only. In Pasteur's case the sheep inoculated with such bacilli (prevented from forming spores) are not killed by anthrax, but "vaccinated," and protected against the most virulent anthrax material. I have not yet succeeded in discovering the method employed by M. Pasteur (and the details of which he has not published) for the production of "vaccine" protective against anthrax; and I can only say that in the case of mice there is no such diminution of virulence as Pasteur has obtained in the cultivation with which he inoculates his sheep. The mice not killed or even injured by the pure bacillus threads of our cultivations succumb without fail to an inoculation with spores or blood bacillus, or to an inoculation with the early stage of a new cultivation of bacillus derived from the former cultivation. This inefficacy of the bacillus of the cultivation on the mice, after several days' cultivation, must be borne in mind when judging of Buchner's results above quoted. Buchner (l.c., p. 384) finds the greatest irregularity in respect of the supposed deterioration in virulence of the cultivations, for while in one series, the third and fourth cultivation is inactive; the fifth active if used in large quantity; in other series other results are observed. Granted that Buchner had pure cultivations, of which, however, there is no sufficient evidence—see Koch (l.c., p. 25)—these irregular results, I think, might be explained by the assumption that the active cultivations were fresh or contained spores, the inactive ones were of some age and had no spores, Buchner's cultivations being carried on in a fluid medium, and being used solely on white mice. More difficult is it to explain Greenfield's statements. He speaks of mice, guinea-pigs, and rabbits as all giving identical results under all circumstances, and this as if the identity of result were matter of course and of necessity. I do not propose to comment on his statements.

As has been already indicated, a given cultivation of Bacillus anthracis, although speedily becoming inactive on

some mice, proved under all conditions and for a considerable length of time, fatal to guinea-pigs and rabbits, no matter whether spores had developed in it or not. This different behaviour of mice on the one hand and guinea-pigs and rabbits on the other, towards an artificial cultivation of Bacillus anthracis without spores, came indeed after a while to be a useful means to decide whether a given cultivation of Bacillus anthracis, after several days' incubation, contained spores or not. I have so often repeated the following experiment that I am confident it can serve as a typical one. A sample of a cultivation of Bacillus anthracis in neutral pork broth, which appears to the unaided eye a typical growth, and in which cultivation the bacillus mass is left quiet at the bottom of the test tube or flask for a week or two, when examined under the microscope does not contain any spores. Inoculate with it half-a-dozen mice and half-a-dozen guinea-pigs or rabbits. All or most of the mice will probably remain well, all the guinea-pigs and rabbits die within forty-eight hours. Allow the bacillus of the above cultivation to form spores, by sowing them on to gelatine pork, and keeping them well exposed to the surface, or establish a new cultivation in neutral pork broth, and now inoculate the above six mice, or as many other mice as you like, with this new cultivation in its early stage or with the above spores, every one of them will be probably dead within thirty-six or forty-eight hours.

The conclusions to be drawn from this seems to me obvious. Mice, unlike guinea-pigs and rabbits, are insusceptible to the Bacillus anthracis when cultivated artificially in neutral pork, after this cultivation has been kept for some time, provided no spores are formed in the bacilli. But no immunity of any kind is by such inoculation conferred on the mice. Since mice are very susceptible to the Bacillus anthracis of the blood and tissues of an anthrax animal in which notoriously no spores occur, and since they are (equally with guinea-pigs and rabbits) susceptible to the spores of the artificially cultivated Bacillus anthracis and to the bacillus of a fresh cultivation, it seems to me it follows from the facts, as

a necessary conclusion, that this insusceptibility must depend both on the mice as well as on a change in the bacillus.

I have made several series of observations, to be detailed at a future period, by cultivating Bacillus anthracis in acid pork broth, and to my great surprise the first cultivation, and also sometimes the second cultivation, of blood Bacillus anthracis in this acid pork broth formed spores, and consequently killed all mice when inoculated into them in infinitesimal doses; but as cultivation was carried on into a third and fourth, the bacillus, although still copiously and typically growing during the first four or five days, nevertheless did not form spores at any time. As a consequence it did not prove effective on many mice; but it proved fatal on guinea-pigs and rabbits when inoculated into them in minimal doses.

What has been said in the foregoing paragraphs, respecting the effects of inoculating with a given cultivation of Bacillus anthracis in neutral pork broth and in gelatine pork, applies not only to the first or the second cultivation, but also applies, in exactly the same manner and to exactly the same degree, to the third, fourth, fifth, sixth cultivation, and even (as I have proved by my own observation) to the twentieth or thirtieth cultivation of the bacillus in like nourishing material. As each new sample of sterile material is inoculated from a former sample, a typical and copious growth of bacillus threads takes place in it. If the material be pork broth, spores will not be formed in it as long as the growth takes place undisturbed below the surface of the liquid. And each successive sporeless cultivation will after a few days (and always within a week or two) lose its power to kill mice, though it will retain for about two months the same power that preceding cultivations possessed of killing guinea-pigs and rabbits when inoculated into them.

If any of these cultivations of Bacillus anthracis in neutral pork broth or gelatine pork are kept for several weeks, it will be noticed, as described above, that the mass of bacilli gradually diminishes in a conspicuous manner. On a former

page I have pointed out that already while active growth is going on in the cultivation, some threads undergo degeneration, and when the pabulum in the cultivation is exhausted, this degeneration gradually extends over the whole growth. As a rule, as pointed out before, if during active growth the bacillus mass has been kept at the bottom of the fluid, no spores are formed, and therefore degeneration after the exhaustion of the pabulum gradually destroys every active particle of the growth. Thus it happens that the cultivation, taken as a whole, gradually loses its virulence, inasmuch as with the progress of the degeneration extending over greater numbers of bacilli, larger doses must be injected into guinea-pigs and rabbits to produce fatal result. But this must not be taken as identical in meaning with what Buchner calls a diminution in virulence of a cultivation. According to Buchner larger quantities have to be used of a later generation (cæteris paribus) to produce the same result as with a former generation, because, he tells us, the Bacillus anthracis is gradually changing its nature, becoming gradually converted into an innocuous hay bacillus.

In our case the diminution in virulence of a cultivation is entirely due to a diminution in the number of active bacilli, and not to any progressive weakening of the potency of each several bacillus. Wherefore the greater the number of bacilli destroyed, the fewer undestroyed or active bacilli will be found in a given quantity, or what comes to the same thing, a larger quantity of material must be used in order to meet with an active bacillus.

While there exist any living bacilli in the cultivation it is possible to start new cultivations, which when used in the early stage of the new cultivation or when allowed to form spores kill without fail all rodents. I have made several experiments in this respect, and I have invariably obtained the same results.

A first cultivation, which promptly killed guinea-pigs during the first fortnight when used in infinitesimal doses, failed to kill a guinea-pig when injected into the subcutaneous tissue

after one month; a sample examined under the microscope showed hardly a trace of a well-preserved bacillus and no spores. I inoculated a test-tube of pork broth with it and produced a beautiful growth of typical Bacillus anthracis; some of these extended up to the surface and formed a copious crop of spores. This killed a guinea-pig three weeks after it was established. In the above case it was evidently only a chance of our missing to have an active bacillus in the samples which we used for the inoculation of the guinea-pig; but more luckily we got one in the sample used for the inoculation of the pork broth in the test-tube.

The above guinea-pig which escaped anthrax was not immune against the introduction of the active virus, since it succumbed to an inoculation afterwards with bacillus and spores of a cultivation in gelatine pork.

All these observations seem to point out that there are two conditions to be borne in mind: (a) a peculiarity possessed by mice and not possessed by guinea-pigs and rabbits; and (b) a peculiar change that the bacilli of the artificial cultivation undergo as the duration of incubation advances. As regards the first of these conditions it is known through Chauveau that Algerian sheep are altogether refractory against anthrax, and consequently these Algerian sheep possess a peculiarity not owned by the French sheep.

According to Pasteur the influence of the air (oxygen) on the artificial cultivations of the micrococcus of fowl cholera, and the artificial cultivations of the Bacillus anthracis has a deleterious effect, inasmuch as it gradually weakens and ultimately altogether destroys the activity of the respective organisms. As I have no experience of the micrococcus of fowl cholera, I cannot say anything about it; but of the Bacillus anthracis I can say something from my own observations, and I will undertake to offer to this theory of Pasteur, viz. of the deleterious influnace of the oxygen of the air on the Bacillus anthracis, an unconditional opposition. If in a cultivation we meet with a copious production of typical

and beautiful anthrax bacilli and threads thereof; and if we find that owing to the absence of sufficient oxygen these bacilli fail to produce spores; and if we further find that after a certain time the bacilli undergo degeneration, and not being able to form spores owing to the absence of sufficient oxygen, they generally all disappear from the cultivation, I think we are justified in concluding that the conditions are exactly the reverse of what is postulated by the theory of Pasteur; in concluding, viz. that it is the want of sufficient oxygen which destroys the bacilli. If oxygen had been present in sufficient quantities, the bacilli would have formed spores and the cultivation would have preserved its full virulence for an indefinite period.

Pasteur, as mentioned above, maintains that his cultivations, kept without spores, gradually lost all activity. "If we examine the virulence of the culture at the end of two days, four days, six days, eight days, &c., it will be found that long before the death of the culture the microbe has lost all virulence, although still cultivable." My observations bear out to a certain extent this statement of Pasteur, inasmuch as the cultivation lost its power to kill mice before it lost its power to kill guinea-pigs and rabbits. But as regards guinea-pigs and rabbits it does not hold good; for in their case complete want of power to kill has appeared in my experience to be the same thing as want of power to grow in a cultivation. Pasteur further states that the animals inoculated with the mitigated virus remain immune against further attacks of anthrax. It is evident that Pasteur's process of cultivation must in some way have differed from my own, or that his assertion for "animals" generally is too broad, for as regards the mice of my experiments there is no immunity of any kind conferred on them.

Pasteur in his cultivations, found, that owing to the diminution of virulence, as time went on, he could at will choose for inoculation a fluid of less and less virulent effect, from one that would produce a fatal effect to one that would have only a slight or local effect. But, says he, sheep inoculated with such

a cultivation, which, owing to having been kept for a certain length of time, produced no fatal effect, are "vaccinated" and protected from anthrax in a virulent form.

As regards my mice, guinea-pigs, and rabbits, I have not found anything of the sort. Either the inoculation with my cultivations is fatal or it is not; in the latter case it has no effect whatever, and does not at all protect against active virus; in the former case it is always fatal. The inactivity on mice of a cultivation may be due to the absence of spores, or to the age of a cultivation—Pasteur's statement of a diminution in virulence in two days and four days, does not quite cover my facts—or the bacillus mass in a cultivation, not being able to form spores and gradually degenerating and dwindling away and becoming macerated into a granular débris, loses after a time altogether its power to infect mice, guinea-pigs, or rabbits, or to start new cultivations.[1]

These latter conditions come out especially strikingly in

[1] As regards the slight effect (constitutional disturbance and rise of temperature) produced in cattle after inoculation with anthrax blood of rodents (Sanderson and Duguid), or with artificially cultivated Bacillus anthracis(?) of a rodent (Greenfield), as well as the non-fatal effect produced on sheep by Pasteur with his vaccine, we have to deal with peculiar conditions, not solely due to a diminution of virulence of the bacillus, but chiefly to some peculiarity (breed appears to be one of such peculiarities) of the animal inoculated. These cases are comparable in a certain sense to those mild cases of other infectious maladies, which not occurring more than once during the lifetime of an individual, would naturally confer immunity on this individual against a second attack. Thus, a person once having had a mild attack of scarlatina, measles, &c., very likely remains free from a second attack. In cases of scarlatina the differences in the severity of the attacks are due to differences of the source of the virus [i. e. differences of the nature of the virus], as well as to differences of the individuals attacked,—cases of varying severity being derived from the same source, i. e. the same virus. The same is also noticed in the cases of anthrax produced by the Bacillus anthracis; the bacillus of some cultivations is altogether ineffectual on mice, deadly on guinea-pigs and rabbits, while it appears to produce, according to Pasteur, only a slight effect on sheep. Now, no one could say this difference is due entirely to a change of the bacillus, since it is equally due to the difference of the individual. Again, the non-fatal result with the blood bacillus of a guinea-pig, dead of anthrax, produced in a cow contrasts strongly with the

cultivations of the Bacillus anthracis carried on in acid pork broth. I have made a sufficient number of observations to state this positively, and I have seen such a cultivation losing its infective power both for animals and for new cultivations after five days, no other organism making its appearance in it, and the original mass of Bacillus anthracis having altogether broken up.

The important statement by Pasteur that "each of these conditions of attenuated virulence may be reproduced by culture," is not borne out by my observations, since every one of the cultivations containing only anthrax bacilli but no spores, and incapable of producing any effect on mice, is invariably capable of starting a new cultivation proving fatal to all rodents when used fresh.

I have before me a fourth cultivation of Bacillus anthracis in neutral pork, which had proved fatal to guinea-pigs and rabbits. It had never any spores, and the days for its activity on mice had passed. After the lapse of two months it was again examined, and there were found in it bundles of degenerated bacilli, as well as a few good bacilli. Inoculated into a guinea-pig in minute doses it proved without result, but it started a good and copious new cultivation of typical anthrax bacillus threads, which killed a guinea-pig with typical anthrax in twenty hours. Pasteur maintains that if a cultivation is weakened in activity by keeping it for some days, it is capable

invariably fatal result produced by the same bacillus on mice, guinea-pigs, and rabbits.

It is very curious to find that Greenfield talks ('Veterinarian,' 1881) of a certain immunity against fatal anthrax conferred on cattle by his artificial cultivations, although these animals showed considerable illness after a further inoculation with blood of man or guinea-pig dead of anthrax. He had already learned that cattle do not die after blood inoculation, even when not inoculated previously with any artificial cultivation. If he had inoculated his cattle with the blood of a guinea-pig or man (woolsorters' disease), without previously inoculating them directly with artificial cultivations, the result would have been precisely the same This appears to me to furnish decisive evidence of Greenfield having had to deal, not with cultivations of Bacillus anthracis, but with some other harmless bacillus.

of starting a new cultivation, whose activity is also weakened, that is to say, the bacillus having become modified by time, transmits to its offspring this acquired mitigation. In the case of the cultivations of Bacillus anthracis in neutral pork or gelatine pork there was nothing of the sort. As long as a cultivation, no matter which, contains living Bacillus anthracis, it is capable of starting a new cultivation, and this as well as its parent is capable of killing guinea-pigs and rabbits.

All that has been said of the first, second, third, fourth, fifth, and sixth cultivations of Bacillus anthracis in neutral pork broth holds good for the tenth, eleventh, twelfth, thirteenth, and so on cultivations. In no instance have I seen, with reference to its infective power on mice, guinea-pigs, and rabbits, any difference of behaviour from that mentioned of the previous cultivations.

It is altogether impossible for me to understand how Greenfield ('Veterinarian,' 1881) could have come to the conclusion, that once arrived at the eighth cultivation, he already knew that no fatal effect could be produced with it. He has tried the effect of his cultivations on mice, guinea-pigs, and rabbits; but with pure cultivations of anthrax bacillus, the result is to some extent the reverse, since guinea-pigs and rabbits are killed by any cultivation, provided there are living anthrax bacilli in it.

The conclusion, it seems to me, forces itself on us, that Greenfield's like Buchner's cultivations were impure, and the further away from the earlier cultivations the smaller the number of the anthrax bacilli, until the contaminating innocuous bacillus gets altogether the mastery in the cultivations, and then the anthrax bacilli gradually disappear altogether.

Of the power of resistance spores are capable of, an idea may be gained from the following facts:

I have tried to ascertain whether the spores of Bacillus anthracis in my cultivations become killed, like the bacilli themselves, through boiling or freezing. As regards the first

process, boiling a minute or two does not destroy the life of the spores. I have thus treated, as mentioned on a former page, spores contained in a flask of gelatine pork, and have obtained afterwards from them a copious crop of bacilli proving fatal to guinea-pigs and rabbits. I have similarly exposed in a capillary pipette fluid full of spores to the influence of ether spray, and having thus kept the fluid well frozen for several minutes, have injected it into the guinea-pig and rabbit with fatal result. I then subjected spores in the same manner to repeated freezing, each time for several minutes, the freezing being also carried out by the ether spray; but these spores nevertheless retained their full virulence. Before forty-eight hours were over the inoculated animals were dead of anthrax. I then placed a capillary tube filled with spores in a mixture of ice and salt, and kept it here for one hour exposed to a temperature of 12° to 15° C. below freezing point; after thawing the material was injected into the subcutaneous tissue of a guinea-pig. This animal died of typical anthrax during the third day. There was, however, no œdema about the seat of inoculation.

Such a low temperature, viz. 12° to 15° C. below freezing point (or 21°—27° Fahr. below freezing point), does not occur in the soil of middle Europe or of these kingdoms, even in the depth of the coldest winter, and therefore spores of Bacillus anthracis formed in the soil from the Bacillus anthracis that happens to be growing there in a suitable nourishing material (vegetable infusion, &c.), are practically indestructible.

As an addition to our knowledge of the mode of propagation of anthrax in animals the following facts may not be valueless. In several instances I found that of a mouse that had died of anthrax a great portion had been eaten by its fellow-companion not inoculated with anthrax; its neck, heart, lungs, and liver had all been swallowed up, but, nevertheless, the mouse that had thus feasted on anthrax remained perfectly well. Koch ('Die Aetiologie d. Milzbrand,' p. 13) is very strong on the communicability of anthrax through simple in-

gestion from the alimentary canal in animals and man. The above facts do not support this theory as regards mice, for no mouse would escape inoculation with an infinitesimal dose of blood of anthrax. They are, however, well in harmony with those of Pasteur and Toussaint, who maintain that in the case of sheep the production of anthrax in these animals by ingestion is in reality due to an inoculation with anthrax into the mucous membrane of the mouth, owing to small wounds and abrasions produced there by the material mixed with the food.

The important question as to the preservation of the activity of the Bacillus anthracis within the body of the dead animal, although commenced, is not advanced enough to be here reported, and therefore must be reserved for a further report. Also the observations on the changes the Bacillus anthracis undergoes when cultivated in acid and alkaline fluids. Some of these observations have been already touched upon, but others of equal importance cannot find room here to be discussed. But this much I will state already now, that I have ascertained that, contrary to what Koch and Buchner have found, Bacillus anthracis is capable of growing in acid fluids, and that it most undoubtedly behaves in a different manner, both as regards size, mode, and time of degeneration and formation of spores, in alkaline and acid cultures, from what it does in neutral nourishing fluids.

In conclusion, and as concerns the more general question which has been held in view throughout the present report, it is to be observed that the theory of the transformation of a pathogenic organism into a non-pathogenic septic organism, as expressed by v. Nägeli and regarded by Dr. Buchner as being capable of direct proof, is not supported by my own investigations into the characters of the Bacillus anthracis, which has appeared to retain its full power to produce specific disease as long as it has retained any power at all. And in regard of that particular bacillus, it is further to be noted

that its behaviour towards sheep in Pasteur's hands is not the same as its behaviour towards rodents in my own experience; so that we must remain unable to accept, as a general proposition, the general view of attenuation that Pasteur would propose.

The Tongue of Perameles nasuta, with some Suggestions as to the Origin of Taste Bulbs.

By

Edward B. Poulton, M.A.

With Plate I.

I am indebted to the kindness of Professor Moseley for the opportunity of working upon the tongue of this little-known animal. I had expected to meet with interesting details in this investigation, but I hardly ventured to hope for characters with so important a bearing on general development as, it appears to me, are to be found in this organ, especially when such suggestive structures are combined with so much highly specialised peculiarity.

The animal to which this organ belonged was caught in the summer of 1874, and its capture is described in the "Notes by a Naturalist on the 'Challenger,'" page 269. Professor Moseley had hardened the back part of the tongue in chromic acid, and since that time it had been kept in strong alcohol. This treatment was so successful that the cells came out in my sections fully as well as in recently hardened structures. The following is a description of the obvious characters of the piece of tongue when it came into my possession. The length was 18 millimeters, including the limits of the papillate surface behind, but cut transversely across this surface in front. The width was 12 mm. and the thickness 9 mm. There is a description of the whole tongue in vol. vi of the 'Memoirs of the Wernerian Natural History Society,' by Dr. R. E. Grant (in a paper dated January 26th, 1828, on the "Anatomy of Perameles nasuta"). He states that the tongue is very long, flat, narrow, and rather thin; of equal breadth from the root

to near the extremity, which has an elliptical form. Its length is 3 inches from root to apex, and it is quite free for nearly 2 inches from the frænum, and thus capable of great freedom of motion.

I thus had rather less than a quarter of the length of the tongue, but probably including all the most interesting details. At the posterior part of the papillate surface are three large circumvallate papillæ, each situated at the angle of an isoceles triangle with the base directed forwards, and 4·75 mm. in length (measured from the centres of the papillæ), the sides being 2·5 mm. in length. The papillæ themselves are about 1 mm. in diameter, and are encircled by a peculiarly deep and narrow trench.

The general surface in front of these three papillæ is densely covered by very small papillæ, of a type which I believe to be entirely peculiar. Each papilla is crowned by a circlet of 8—10 fine, long, bristle-like filiform papillæ, whose points are directed backwards, thus causing a slight roughness to the finger when drawn from behind forwards. Thinly scattered among these excessively numerous papillæ are others of the "fungiform" type, of which about twenty were present on this piece of the tongue, but as they were more thickly placed in the front part they are no doubt commoner on the organ in front of this piece. They are chiefly arranged as an irregular single line on each side, beginning about 7·5 mm. in front of the circumvallate papilla on the same side; but they also occur on the upper surface, about 11·5 mm., in front of two anterior circumvallate papillæ. Their appearance is quite normal. Beneath the lateral row of fungiform papillæ (of which the papillæ are separated by the small peculiar papillæ) is a row, from two to three deep, of large filiform papillæ of ordinary appearance, and beneath these the papillate surface ends abruptly in a perfectly smooth epithelium. These rows of filiform papillæ are continued backwards and upwards until they meet at the circumvallate papillæ, between and around which they are thinly scattered, and are also longer than elsewhere.

There is a slight trace of a median raphe, in the form of a shallow groove, in the anterior part. Dr. Grant also mentions a close covering of minute papillæ, no doubt referring to those of peculiar type to be described further on; and he speaks of others of larger size more thinly scattered (probably the fungiform papillæ), and describes the arrangement of the three circumvallate papillæ. At this latter part of the tongue he mentions the orifices of minute ducts, which I was unable to detect (except deep in the trenches round the papillæ), although I made horizontal sections in this part. He states that the circumvallate papillæ of the opossum are similar in form and arrangement. The slight groove which appeared in the anterior part of the upper surface of my specimen he describes as running the whole length; and he also mentions that there is a median ridge on the lower surface from the apex of the tongue to the frænum, bordered by a fold on each side, and a corresponding groove on the floor of the mouth beneath, with a ridge on each side. The roof of the palate is covered by a black cuticle, and is traversed by about fourteen transverse elevated ridges slightly curving forwards; and as Dr. Grant suggests, of importance in grinding down the hard coverings of insects when the sharp points of the papillæ are rubbed against them.

I have now mentioned the details of this organ given by Dr. Grant, and I have been careful to include all points, since this animal seems to be very slightly known in Europe. The structure of the abundant peculiar papillæ renders it almost certain that the animal is insectivorous. Yet Gould, in the 'Mammals of Australia,' says that its "food consists of bulbous and other roots, obtained by its powerful fore feet and claws," but he adds that there is very little information known respecting it. Waterhouse, however, quotes Dr. Grant as the authority for the insectivorous habits of P. nasuta.

In the paper above-mentioned Dr. Grant says that the fæces were composed of insects, together with some tufts of woolly hair and some vegetable matter, probably taken in accidentally. The stomach and small intestines also contained a little hair,

sand, and vegetable matter, with no regular food. The simple character of the digestive tract and short cæcum is also evidence that the animal does not subsist on vegetable food. E. Geoffroy first named and described the genus Perameles from this species and another in the 'Annales du Muséum d'Histoire Naturelle' (1804); and in describing this species he infers that it is insectivorous, from the characters of the teeth, and suggests that this food is obtained by digging. This latter habit probably explains Gould's mistake and that of the colonists, for I believe that in Australia the bandicoots are generally believed to be plant-eating.

I have now mentioned all the points bearing upon my subject that I can find in any former writings upon this animal. The structures I am about to describe admit of a simple classification under two heads. First, those structures which are probably concerned with taste—the circumvallate and fungiform papillæ, together with some suggestions as to the origin of taste bulbs. Secondly, those structures of mechanical or tactile use—the papillæ of peculiar type and the filiform papillæ.

I. Gustatory Structures.

The Circumvallate papilla.—The surfaces of these papillæ are circular, and a little more than 1 mm. in diameter. The sides (protected by the trench) are vertical for ·33 of a mm., and then incline inwards for about the same distance, making an angle of about 36°, with the vertical side above. Below this the side of the papilla turns inwards for a short distance almost horizontally, and by this and the inward slope above, the diameter of the papilla is only about ·5 of a mm. at its base. The taste bulbs are arranged only upon the side inclining inwards and downwards, and are thus peculiarly protected. The depression of the epithelial surface forming the outer wall of the trench follows the sides of the papilla with a curved and smooth outline in vertical section, but seen to be vertically ridged by horizontal sections. The papilla itself is not similarly ridged. The outer wall of the trench

is prolonged upwards very nearly to the level of the surface of the papilla, and as this is rather above the level of the tongue the papilla is surrounded by a slight ridge. The trench is very narrow, and its depth and relation to the shape of the papilla is shown in fig. 1, which is magnified 24·5 diameters. Thus, in shape this papilla is peculiarly specialised in the way of protection; its minute structure will also be found to be highly specialised in many points. Glands are very abundant within the bodies of the papillæ, between the three papillæ, and for a considerable distance around them. They are almost entirely of the granular "serous" type, which Klein points out as always accompanying taste bulbs. Their structure is exactly as described by Klein. Their ducts open into the trench, especially in its deeper part, and are very numerous. In one horizontal section I counted twenty-six ducts, probably all separate, and at a lower level I calculated that there must have been at least forty at one horizon, while the bottom of the trench is completely surrounded by thickly crowded gland-ducts radiating inwards (see figs. 1 and 2 for vertical sections). The body of the papilla, as usual, bears secondary papillæ on its upper part, the depressions between which are filled up to one level by the epithelium.

The most remarkable structure, and as far as I am aware one hitherto undescribed, is a large and distinct ganglion in the form of a thick axial column making up a great part of the bulk of the papillary body. It is surrounded by a clearly defined connective-tissue capsule, which enters and supports the nervous elements. Above, the ganglion breaks up into branches, which stream outwards towards the sloping side of the papilla containing the taste-bulbs. The gradual collection of the scattered branches above and to the sides into the dense and compact ganglion centrally and below is especially well seen in successive horizontal sections. The nerve-fibres appear to be almost entirely non-medullated, but they possess a distinct sheath of Schwann. Among the nerve-fibres occur primitive nerve-fibrils. The nerve-cells are few in number, very large and distinct (they are indicated even in the lowly magnified

fig. 1), and always situated at the base of the ganglion close to the fibrous capsule. In vertical sections two to six cells appear in one section. In a horizontal section across the base of the ganglion about twelve cells are seen, and many others also present appear to be belong to small ganglia in the course of nerves entering the larger ganglion. The nerve-cells are very large and fusiform, with branched ends (the branches losing themselves among the nerve-fibres of the ganglion). The large oval nucleus, containing a distinct nucleolus, is coarsely granular in appearance, and does not stain in hæmatoxylin, although the finely granular cell-body stains deeply. Large nerves with non-medullated fibres are distinctly seen entering the base of the ganglion, and in a deep horizontal section a ground-plan, as it were, of the ganglionic nerves is seen, as they radiate outwards from the base of the ganglion. These nerves contain isolated nerve-cells, and also small groups, in their course, the cells exactly resembling those of the ganglion, except that they become polyhedral when crowded together

It thus appears almost certain that nerve-cells are intercalated in the course of sensory impulses from the peripheral organs to the nervous centres. This is of interest in bringing these terminations into closer connection with the related terminal organs of sight and hearing, where ganglion cells similarly intervene. If this be a true correlation, it seems likely that ganglion cells will be found generally in the nervous masses of the large gustatory papillæ, now that attention is directed to their existence. Microscopic ganglia on the nerve-branches have been described, and a nerve is generally figured in the axis of the papilla, but a true, large, compact ganglion making up most of the papillary body is, I think, as yet unmentioned.

Beneath the epithelium containing the taste bulbs the nerve-fibres (in the course of or between which there appear to be many nuclei) are very numerous, and are cut in all directions owing to their irregular and sinuous course. There is no doubt of their connection with the bulbs, but it would

probably need the fresh tissue to trace the actual union. The connective-tissue matrix, in which these fibres ramify, is less dense than the mucous membrane of the organ generally, although derived from the latter. This mucous membrane is peculiarly firm and tendinous in appearance, with its fibres arranged transversely to the long axis of the organ and containing many interfascular spaces. Striated muscle fibres appear to terminate very abruptly in it. The ganglion and nervous elements are shown in fig, 2, which is taken vertically through the base of the papilla.

The taste bulbs of the circumvallate papillæ are fairly numerous. They are arranged in a zone of seven or eight tiers, exactly filling up the overhanging side of the papilla. The calculation of the number of bulbs in a tier from horizontal sections cannot be very exact. In one semicircle towards the upper part I counted fifty bulbs; in the lowest there seem to be not less than forty, although here they are somewhat larger. Thus, allowing the mean, ninety in each tier, and allowing for eight tiers, the number of bulbs in each of the three papillæ becomes 720. The length of the bulbs appears to be about ·07 mm., but the lower are always larger, and the size is somewhat irregular throughout. Their shape is often a perfect oval, but sometimes rather like that of a peg-top without the spike, for there is hardly any neck, but in longitudinal section the sides seem to meet almost at the surface of the epithelium in a blunt point. The only representative of a neck is the gustatory pore itself, which perforates a very thin apparently homegeneous superficial layer, probably formed by coalesced epithelial cells of the surface (see fig. 2). This layer is also seen covering other parts of the tongue, and is cornified, as it stains yellow in picrocarmine. The perforation of this thin layer is very often seen both in horizontal and vertical sections. In some cases there was a distinct protrusion through the gustatory pore, but anything like a circlet of hair-like processes projecting from it could not be identified. The cells do not appear to be collected together into a distinct basal pole, although they most distinctly converge at the apex.

This contact of the cells of the bulb with a tolerably extended surface of mucous membrane at the base is still more apparent in a simpler form of bulb to be described as occurring on the fungiform papillæ. Another indication of simplicity is the persistence of papillary elevations of the mucous membrane between adjacent bulbs. This was most distinct in horizontal and vertical sections of the circumvallate papillæ, and was especially marked at the demarcation between the lowest bulb and the ordinary epithelium (of course in vertical sections).

The great irregularity of size and shape also appears to favour the view that these bulbs are of a peculiarly undeveloped and ancient type. This will be further considered in the discussion upon the origin of taste bulbs. No very distinct separation of the cells of the bulbs into central and peripheral could be made out, and it is possible that this structural difference is not yet established, but this suggestion needs confirmation by work upon a fresher tissue. The cells of the bulbs stain very slightly in carmine or hæmatoxylin. Fig. 2 shows the structure and arrangement of the bulbs.

Thus these circumvallate papillæ in their shape and structure are peculiarly highly developed, notably in the abundant glandular and nervous elements and the presence of nerve-cells in the ganglion of the papilla. The overhanging sides of the papillary body must also be regarded as marks of great specialisation, carrying still further the protective function of the trench. Yet combined with these extremely developed structures are terminal organs of a lower type than have yet been described.

The inference is that the former structures have reached their high specialisation by assisting another form of terminal organ, which has been comparatively recently replaced by the bulbs. This probability will be further discussed after the fungiform papillæ have been considered, for these latter possess structures with an important bearing on the argument.

The fungiform papillæ.—These papillæ are entirely of normal shape, appearance, and distribution. The only noteworthy fact about their distribution is the collection into a line

on each side of the tongue. The peculiar papillæ which everywhere surround them always leave a little space immediately round the fungiform papilla, in the centre of which it stands. The shape is shown in fig. 3, which is a vertical section through a papilla. The mucous membrane in the centre is of the ordinary type, but less dense than that below, from which it is prolonged.

A large non-medullated nerve occupies the axis, and is well seen in vertical and horizontal sections. The epithelium resembles that of the general surface of the organ, and, like it, is penetrated by papillary upgrowths from the mucous membrane below.

Taste bulbs are not very common on the fungiform papillæ of the higher animals, and seem to be always isolated when they are present.

It was therefore unlikely that they would be common here, and I examined very many sections without meeting any traces of them. At length I found some indications, and finally the specimen shown in fig. 3. In this section (at the top of the papilla amongst the diagrammatically-shaded epithelium) there are two distinct bulbs of a very low order. They have not yet anything of the bulbous shape, and the basal ends of their cells are spread out over a wide extent of mucous membrane. In this section they do not reach the surface of the epithelium, but it is probable that in a section through the true longitudinal axis the surface may be reached or even perforated. They are seen to be merely the lowest columnar cells of an interpapillary process, greatly prolonged towards the surface, and it is interesting to see one or two columnar cells outside the chief mass, in both cases, also elongating and applying themselves to the others. It is also noteworthy that the apex is not produced by any curve of the cells, as in true bulbs, but merely by the cells being prolonged from a curved surface, and so, like radii, meeting at a common centre. Three papillary upgrowths distinctly separate the two developing bulbs from each other and from the surrounding epithelium.

There were no glands of the "serous type," as far as I

observed, near the fungiform papillæ, or indeed anywhere in my sections, except in or around the area of the circumvallate papillæ.

Mucous glands were, however, common in other parts and of the type described (Klein, in the 'Atlas of Histology.')

The origin of taste bulbs.—This low form of bulb, found upon the fungiform papillæ, suggested to me a possible explanation of the manner in which taste bulbs have arisen in Mammalia. At the outset it seems probable that in Marsupials or Monotremes we have the best chance of finding the course followed in the development of these and other structures, as we know them in the higher mammals.

For in these extremely ancient types, owing their existence to isolation, with little rivalry to render structural advance and complexity necessary in such forms, it is certain that long halts will be made at stages long since left behind in the development of other animals living on more warmly-contested areas.

There is little doubt that the gustatory terminal organs have more in common with those of the general surface of the body than any other special sense. There is great structural resemblance, actual structural continuity, between skin and the oral mucous membrane with its epithelium. Certain sensory terminal organs are found in both, although it is probable that they subserve the tactile rather than the gustatory sense in the mouth.

Before the appearance of taste bulbs actually opening on the surface of the epithelium, when the gustatory surface was less specialised, the terminal organs (similar to those of skin or modified to receive at first feeble gustatory stimuli) would be placed as in skin, namely, in the papillary upgrowths from the mucous membrane. In this position they would be nearer to the gustatory stimuli than any other, without actual perforation of the epithelium by the terminal organ, for, of course, the layers of cells over a papillary process are far thinner than elsewhere. It appears to me that then the serous glands were modified from those of the general mucous type in the parts where the terminal organs had been chiefly specialised. Simul-

taneously the nerve-supply would be increasing in amount and advancing in complexity.

The chief taste areas will have also been sheltered by becoming enclosed in folds, either of the circumvallate or foliate type. Of course the exact order of these events cannot be made out, nor is it of great importance. The important point is that a time must have come when a more specialised form of terminal organ, coming into closer relations with the stimulus, was substituted for one of a more general type. It is not necessary or possible to exactly define this point of time relatively to that of the advancing accessory structures, but, as before mentioned, the high place reached by these in the circumvallate papillæ, accompanied by a low type of bulb, renders it probable that the latter was subsequently developed. It is also noteworthy that these accessory advantages may have been more necessary with a less advanced type of terminal organ.

This substitution of a higher form of terminal organ seems to have taken place by the growth of the columnar cells forming the lowest layer of an interpapillary process. In this way the cells would approach the surface, converging as they elongated. There would also be a gradual concentration of the nervous elements upon these new end organs and a corresponding withdrawal from the papillary structures.

It seems to me that this stage is reached in the fungiform papilla (fig. 3) above described. The deeply placed columnar cells of the interpapillary process have elongated, and so come into closer relation with the surface than the less deeply placed cells of the papillary process; that is to say, the former would be more advantageous as terminal organs. This account of the origin of taste bulbs explains one important difference between them and the other structurally related end organs, as those of the olfactory region, or sacculi and ampullæ, i.e. in the fact that the gustatory cells are massed together in little groups surrounded by protective cells, while the auditory cells in the positions above mentioned and the olfactory cells are isolated, each being separately protected by columnar cells.

This difference, it appears, is simply due to the latter elongating from a tolerably plane surface, while the gustatory cells have elongated from the curved surface of an interpapillary process—approximately the segment of a sphere—and therefore have met and penetrated the surface in a group. In the further development of the bulbs the external columnar cells would become protective, the axial cells alone acting as end organs; the columnar cells would converge to form a basal pole, as the nerve supply was limited to a small area in this region (being connected only with the axial cells). In fig. 3 the basal convergence has not commenced, and in the circumvallate bulbs (fig. 2) it is not nearly as complete as in higher animals. In the former case I do not think that there is yet any trace of a division into protective gustatory cells in the bulb, and in the circumvallate papillæ I am sure that the difference is not well marked, even if begun. Finally, the papillary elevations would disappear between the bulbs, and the latter would rest in the cavities of an epithelium with a nearly plane surface below. This, which is reached in the higher animals, is apparently never attained in the tongue of Perameles nasuta. The papillary upgrowths separating the bulbs give to them the appearance of interpapillary processes to a marked degree. In fact, there is the greatest resemblance between the bulbs and the interpapillary processes of the epithelium on the outer wall of the trench—a resemblance so great as to suggest this explanation of the origin of bulbs.

And yet indications of the ultimate disappearance of the papillæ between the bulbs are seen in the fact that the papillæ between the lowest tier of bulbs and the ordinary epithelium below, are always far more marked than those between the bulbs themselves (see fig. 2). I think that a comparison of figs. 2 and 3 with the figures of taste-bulbs in 'Stricker's Handbook,' by Engelmann, or in Klein's 'Atlas,' will at once suggest an explanation of their origin similar to that which I have given above. I must express my thanks to Mr. W. H. Jackson, M.A., for kind help and suggestions in working out the above theory.

II. Structures with Mechanical or Tactile Functions.

The papillæ of peculiar type.—These papillæ are very numerous, thickly covering the upper surface of the tongue in front of the circumvallate papillæ, and certainly continuous over the organ in front of the anterior limits of the piece in my possession.

They are smaller and more thickly placed anteriorly, and here I counted thirty-four on a square mm. of surface. Posteriorly there were only sixteen on the same area. These papillæ appear to be closely related to the compound filiform type of other animals, differing in the regular ring-like arrangement and the number of the secondary papillæ, and also in certain points of minute structure.

Their appearance when examined as opaque objects is given in fig. 10 (× 55·25), A and B; A representing an anterior, B a posterior papilla. The summit of each papilla is surrounded by a ring of fine hair-like papillæ, generally ten in number, which sweep backwards, and must act very effectively in retaining small insects.

The hair-like papillæ are longer and finer anteriorly, and form a more complete ring; but even here the ring is most developed posteriorly in each papilla, and tends to become incomplete anteriorly with feebler secondary papillæ. This arrangement becomes gradually more marked posteriorly, until around the circumvallate papillæ the anterior part of each ring finally disappears, while the posterior part becomes immensely developed as a very thick, blunt, secondary papilla, with one or two small ones on each side of it. (One of these depressed forms of papilla is seen in vertical section in fig. 1, *p*.) Part of this transition is seen in fig. 4. I believe that in front of this piece of tongue the papillæ are in a short distance surmounted by a symmetrical ring of hair-like processes. The same transition occurs from the centre to the side of the tongue, but is far less marked. Here the fine processes bend upwards

and backwards instead of backwards merely, and the superior and posterior side of the ring is chiefly developed.

Within the ring the summit of the main papilla is concave, the greatest depth being attained near the anterior part of the ring (or inferior and anterior side in the case of the lateral papillæ). This is because a far longer incline of central cells leads up to the posterior hair-like processes than to the anterior (see fig. 11, &c.), and this difference is especially marked in the posterior part of the tongue, while it almost disappears in front (fig. 4). Transverse vertical sections show that the rings are developed equally on both sides of the antero-posterior diameter, but as the hair-like papillæ bend sharply backwards they are only cut through near their bases (fig. 5). The same bilateral structure is seen in horizontal sections (figs. 6 and 12). Each papilla is seated upon a single main papillary upgrowth from the mucous membrane.

The relation of this to the secondary papillæ is best studied in horizontal sections at various depths (figs. 6, 7, and 12). In such sections the papillary process is almost circular in section at the lowest level, but a very little higher there is an interruption at one point of the margin by a small ingrowth of cells, which a little higher becomes so large as to convert the original simple involution into a ring, incomplete only where the central mass of cells is continuous with the epithelium outside the ring. These appearances are due to the primary papillary involution growing upwards as a ring everywhere except anteriorly, and therefore the first appearance of the ingrowth of cells indicates that this is the anterior part of the margin. The mass of cells within the ring is convex below, and at the same time slopes slightly downwards anteriorly, and is continuous with the wall of the ingrowth (see fig. 4, *A* end). Therefore, this point is first reached in horizontal sections from below upwards. The extension of the primary process into the ring is well seen in transverse vertical sections (fig. 5), and less well in longitudinal vertical sections, for here the section passes through the incomplete point of the ring or near it, where the ring is less developed.

In the anterior papillæ the ring is more perfect and the incomplete point is much narrower. Hence longitudinal vertical sections, which do not strike this point, show a well-developed upward growth anteriorly (compare the ends of fig. 4 in this respect, and the ring p'' of fig. 7 with p' of fig. 6). This explains why the vertical longitudinal sections (figs. 8 and 11, and the anterior [B] end of fig. 4) do not often show the convex surface within the ring sloping down to be continuous with the anterior wall. Horizontal sections of course do indicate this.

At higher levels the ring gives off small secondary papillary processes for the hair-like papillæ. These are first met with in the feebly developed anterior horns of the ring, and gradually extend backwards as the ring (becoming semicircular, finally a semilunar remnant) rises higher. Finally, the last semilunar trace of the ring gives origin to the largest and most posterior papillary upgrowths (fig. 6 shows all these changes very distinctly in passing from A to B). Although, strictly speaking, no papillary upgrowth can take place in the exact anterior margin of the ring (incomplete), it is common to meet a papillary process almost at this point at a rather higher level (fig. 6, &c.). This is due to a papillary process rising a little obliquely from one side to the anterior point, and explains why two hair-like papillæ are almost invariably cut through in longitudinal vertical sections (figs. 4 and 11), although the corresponding papillary upgrowth for the anterior papilla is often wanting (see posterior end of fig. 4). The upper surface of the papilla within the ring of hair-like papillæ corresponds to the under surface within the ring-like extension of the papillary process from the mucous membrane. The concave upper surface corresponds to the convex lower surface, and the downward anterior slope of the latter to the upward posterior slope of the former. From side to side there is a regular concavity above corresponding to a regular convexity below (fig. 5). The curves are, however, always more marked below than above, as the great thickness of intervening cells tends to partially fill up the hollows (figs. 5, 11, &c.). The inferior convexity is

obviously an interpapillary process between the small secondary papilla. It now remains to describe the minute characters of the cells of the papillæ and epithelium around, and the relation of both to the hair-like papillæ.

When the mass of cells within the ring is cut vertically it is at once seen to be divided into two chief layers, sharply marked from each other. The upper stains deeply and the cells appear homogeneous, the lower does not stain (or very slightly), and the cells are extremely granular (figs. 4, 5, 11, and also seen in horizontal sections, figs. 6 and 12).

The transition of characters met with in passing upwards through the central mass of cells is very remarkable. The mass may be divided into two chief parts (already indicated), each of which may be further subdivided (see fig. 8 and description). From below these are (*A*) granular cells hardly staining in picrocarmine or logwood.

(1) The lowest columnar and succeeding small polyhedral cells. The columnar cells are shorter than in the rest of the epithelium, and both kinds of cell more granular with less distinct outlines. Indications of karyokinesis are not uncommon.

(2) The cells are fusiform and granular with indistinct outlines; the nuclei frequently have a vacuolated appearance. This is commoner over the secondary papillary processes beneath the hair-like papillæ than over the convex interpapillary part.

(3) A very thick layer of cells with distinct outlines, fusiform in shape, attenuated below but much swollen above. The contents are very large granules, arranged in groups or rows in a fine granular matrix, staining slightly, and especially distinct at the margin, where a thin layer is generally free from the larger granules. The nucleus is often indistinct, shrunken, and sometimes absent, filled with large and small granules. The large granules are rounded or angular, and do not stain at all. (See fig. 9, *a* and *b*, for the outline of a swollen cell from the upper part of this layer.) Attenuated cells often have a single row of granules from end to end.

(4) A narrow layer of very attenuated cells, still granular, and usually having no trace of a nucleus. This layer appears to be more constant in the posterior papillæ.

B. Deeply staining cells :

(5) A narrow layer of attenuated, deeply staining, finely granular cells with no nuclei.

(6) Homogeneous, swollen, fusiform cells, very deeply staining, and rarely containing faint traces of a nucleus, as a stellate mass, commoner in the anterior papillæ.

The demarcation between A and B is extremely sharp, and the cells which are at length formed at the surface, after such varied changes, are wonderfully like those formed by the simpler transitions of the ordinary epithelium outside the papillæ. This epithelium is of the normal stratified type, and its cells have very distinct nuclei. Traces of karyokinesis can be distinguished in the lowest layer. The superficial cells only differ from the highest cells within the papillæ in possessing nuclei and in staining rather less deeply.

The hair-like papillæ are formed of cornified fibre-like cells derived from three sources. First, from the small secondary papillary processes on which each is situated. The rapid transition from columnar and polyhedral forms to cells with apparently vacuolated nucleus, and finally to vertical fibres in the axis of the hair-like papillæ, is well seen in a vertical section through the side of a papilla. Secondly, the cells within the ring of hair-like papillæ apply themselves to the latter on all sides. The cells at all depths and of all the different kinds of structure mentioned, seem to apply themselves to the hair-like papillæ, either before or after the emergence of the latter at the surface. Thirdly, the external cells of the regular stratified epithelium of the tongue apply themselves to the outside of the hair-like papillæ. (These three sources are best seen in fig. 8.) Thus the cells are received from different sources, and one of these (the second) is extremely complex.

As to the explanation of the granular cells, it seems most probable that the appearance is due to the development of the corneous material. It appears that this material (or some

necessary precursor of it), at first formed as large granules, becomes gradually more finely granular, undergoing suddenly a change which enables it to become coloured deeply by staining fluids, and shortly after becoming entirely homogeneous. If this be correct the granular cells must be looked upon as representing on a very large scale the stratum granulosum and lucidum of skin, which are generally considered to be stages in the development of the corneous condition reached at a higher level.

The difficulty remains, that outside the papilla no trace of the granular cells is seen, although very similar cells are met with at the surface, both within and without the papillæ. It also appears remarkable that the granular cells should be applied at all stages of structure to the hair-like papillæ, and that the latter should receive accessions from the outside where there are no granular cells, and from its own secondary papilla, where the granular cells are not much developed.

I have described the structure of these papillæ minutely, as they seem to me to be an entirely new and hitherto undescribed type, modified in a remarkable manner for the capture of small insects.

Filiform papillæ.—These are very large and long (1·5 mm. in length), and entirely normal in appearance. Their direction is backwards in the row along the sides (limiting the papillate surface), upwards and backwards among the circumvallate papillæ. They have no corneous investment, and a large non-medullated nerve may often be seen in the axis. It is therefore probable that they are tactile rather than mechanical in function, a conclusion which is also confirmed by the greater specialisation of the peculiar papillæ for these very mechanical purposes. The distribution of the filiform papillæ is also strangely antagonistic to the view that they are mechanical, and certainly not opposed to the supposition that they are tactile.

Plant Cells and Living Matter.

By

Louis Elsberg, M.D.,
of New York.

To botanists biology owed its first knowledge of ultimate structure and of living matter. The names "cell" and "protoplasm" testify to the epoch-making researches of Schleiden and Von Mohl. And in accumulation and classification of further biological knowledge botanists have taken so prominent a part that even those of us who are interested only in animal morphology have had to keep some track of the labours of Nägeli, Pringsheim, De Bary, Hofmeister, Sachs, Prantl, Strasburger, and many others. It is all the more remarkable, therefore, that the investigations carried on during the past decade, which have resulted in proving that all the so-called "cells" constituting animal tissues are interconnected by filaments of living matter emanating from these "cells," seem to have borne no fruit for the study of plants. It was in the hope of being able to repay histological botany for some of the light it has thrown on animal histology that I engaged in the researches, the account of a few of which I am about to detail.

A small portion of a delicate blade of grass, cut off with a pair of scissors, transferred to a slide together with a drop of dilute glycerine (two parts of pure glycerine and one part of distilled water), was examined with a power of 1200 diam. I had at my disposal for these examinations two excellent immersion lenses, made respectively by Tolles, of Boston, and Vérick, of Paris. In some parts, in trichomes, stomata, air-vessels &c., nothing more could be seen with such amplification than with comparatively low powers of the microscope; the epi-

dermal fields as well as the surrounding frames of cellulose appeared structureless, or at most only very indistinctly granular. The main mass of tissue enclosed by the epidermal system, the parenchyma, presented blunt polygons separated from each other by a shining narrow rim of cellulose, and containing numbers of chlorophyll-granules. Some contained only very few and very small such granules, surrounded by an extremely delicate uncoloured reticulum, of which the filaments were of about the same breadth as the points of their intersection. In some polygonal fields there were a number of coarse chlorophyll-granules interspersed in a network, the threads of which had points of intersection that were thickened so as to constitute distinct though not green minute granules, while in other fields there were so many coarse and smaller green granules that they nearly completely filled up the polygon. Under all circumstances, however, the granules, closely focussed, appeared stellate, and were interconnected by means of delicate filaments running in large numbers from each granule to all its neighbours. If of small size a chlorophyll-granule appeared homogeneous, of a comparatively higher lustre, and of less intense green colour; larger granules exhibited an indistinct reticular structure in their interior; the largest showed the reticular structure very plainly, and not infrequently in the centre a small shining body was observed sending radiating spokes toward the periphery, inosculating with a thin wall that enclosed the granule in toto. Toward the apex of the blade the granules became fewer in number and smaller in size; at the apex there were no chlorophyll-granules.

In fig. 1 are represented chlorophyll-granules (CHL.) interspersed in the reticulum (R), surrounded by the cellulose frame (C).

These observations show that the vegetable living matter enclosed by the wall of cellulose is arranged in the form of a network, and that a similar reticular arrangement exists in the chlorophyll-granules. It is well known that chlorophyll-granules are themselves minute masses of the living matter of plants, coloured green by a colouring matter, to which the

name chlorophyll is given. Living matter has been called by Hugo von Mohl "protoplasm," by Lionel Beale "bioplasm,"

Fig. 1.

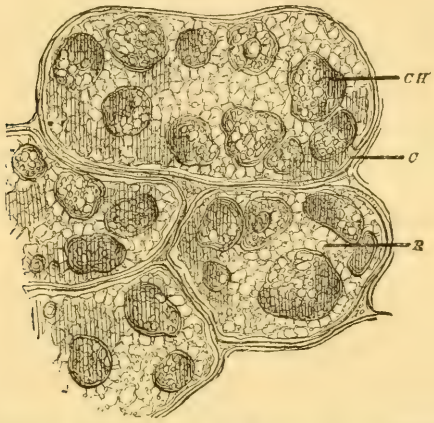

FIG. 1.—Cells from blade of grass, showing—*CH.* Chlorophyll granules. *R.* Reticulum of protoplasm, and *C.* Cell-wall.

and by me, because etymologically more correct, "bioplasson." I am no stickler for new names, but in scientific discussions we should use, if possible, correct names; and of the four synonymous designations, viz. living matter, protoplasm, bioplasm, and bioplasson, I therefore confine myself generally to the first and last, although the term protoplasm is best known and by others most used.

In the year 1873, in a communication to the Vienna Academy of Sciences, entitled "Phases of Living Matter," Carl Heitzmann first described, in Amœba, the youthful condition of masses of living matter as being constituted by homogeneous granules, and advanced stages as being characterised by vacuolation followed by reticulation. These statements were confirmed as regards vegetable organisms in a paper on "The Structure and Growth of some Forms of Mildew," in the 'New York Medical Journal,' November, 1878, by William Hassloch, who says that the first visible form elements of the plant are homogeneous granules, and the first appearing buds

compact projections, either globular or elongated, the first differentiation consisting in the occurrence of a central vacuole, while after a certain development has been attained the plant protoplasm appears in the form of a network.

Many botanists have observed and described reticulated living matter, not only when in its naked condition, as plasmodium, as it is called, but also when enclosed in a cellulose wall. Allow me to cite a few examples: Sachs has figured "a cell of Zygnema cruciatum, with two stellate chlorophyll-bodies which are suspended in the interior of the cell; they are united by a colourless bridge of protoplasm in which lies a nucleus; the rays which form the union with the parietal sac are already nearly colourless in the middle. In each of the two chlorophyll-bodies lies a large grain of starch (amplification 550)," also " forms of the protoplasm contained in cells of Indian corn (Zea mais); A, cells from the first leaf-sheath of a germinating plant, showing the frothy condition of the protoplasm, i.e. the many vacuoles separated by thin plates; B, cells from the first internode of the germinating plant; the protoplasm is broken up into many rounded masses in each of which there is a vacuole (*b*); these are the so-called 'sap-vesicles.'" Sachs has also figured "parenchyma cells from the central cortical layer of the root of Fritillaria imperialis, longitudinal sections, A, very young cells, lying close above the apex of the root, still without cell sap or vacuoles. B, cells of the same description about 2 millimètres above the apex of the root; by the entrance of cell sap the vacuoles s, s, s, have been formed. C, cells of the same description about 7 to 8 millimètres above the apex of the root," in one of which the reticulum is very plainly seen. Bessey says "in the stamen-hairs of Tradescantia Virginica the protoplasm forms a rather thick layer over the inner surface of the cell wall, and in some part of this layer the nucleus lies embedded. From the nucleus, and from various parts of the protoplasmic layer, there pass to the opposite side of the cell thicker or thinner bands and strings, and gives a figure of the same after Hofmeister. Prantl has figured Meristem cells of the stem of

Vicia faba in which filaments of living matter emanating from the nucleus go to the peripheric layer of living matter, and also hairs from the epidermis of ovary of Cucurbita, in some of the compartments of which the reticulum is very distinctly shown with quite low power (× 100).

Heitzmann, the discoverer of the reticulum of living matter and of its continuity throughout the entire animal organism, states in his magnificent work just published, entitled 'Microscopical Morphology of the Animal Body in Health and Disease,' p. 57, "My own limited researches enable me to assert that the granules of living matter in vegetable protoplasm are, as a rule, united in the shape of a reticulum, in the same manner as in animal protoplasm. Besides, the researches of W. Hassloch elucidate the identity of both animal and vegetable living matter in a satisfactory manner. I may add that all cells of the vegetable organisms are uninterruptedly connected by means of delicate offshoots piercing the walls of the cellulose. The granules of amylum are transformed living vegetable matter. The plant in toto is an individual and not composed of individual cells." But demonstration of this statement is wanting. Low powers of the microscope, and even high powers, show that a less or more thick cloak of cellulose surrounds each plant "cell," and separates it from its neighbours. The observations of the chlorophyll-granules and of the interior of the polygonal cellulose frames of blades of grass herein detailed, while they fully bear out the assertions of Heitzmann and Hassloch as to the reticular structure, and perhaps even as to the growth phases, at least so far as dimension is concerned, of masses of living matter of plants, do not advance our knowledge much further. All my endeavours definitely to determine whether the plant "cells" are interconnected or not were unsuccessful with the means I employed in both transparent specimens and in sections. The inspection, under all sorts of circumstances, of the wall of cellulose, although it frequently gave me the impression that it was faintly granulated, and although delicate filaments emanating from the most peripheral chlorophyll-granules were often seen tending towards the wall,

did not enable me to arrive at a conclusion concerning its intimate structure.

Francis Darwin has discovered protoplasmic filaments protruding from the cellulose investment of the glandular hairs on the leaves of Dipsacus sylvestris ('Quarterly Journal of Microscopical Science,' 1877, p. 245). Previously, Hoffman ("Ueber contractile Gefilde bei Blatterschwämmen," 'Botan. Zeitung,' 1853, p. 857, and 1859, p. 214) had described contractile filaments projecting from cell walls in Amanita (Agaricus) muscaria, and although De Bary has expressed the opinion that these are not protoplasmic, Darwin believes them to be so ('Quart. Journ. Mic. Sc.,' Jan., 1878, p. 74). Later, W. J. Beal ('American Naturalist,' October, 1878, p. 643) described threads, but does not say that they are protoplasmic, projecting from the end of hairs of several plants. Darwin has observed filaments of living matter, emanating from the interior of plant cells, pierce the cellulose frame. They protruded from terminal cells only, and of course showed no interconnection between neighbouring cells. Such interconnection I can now demonstrate.

My first successful observations were made in specimens of the flowers of flowering flax (Norimbergia gracilis), and of the leaf and stem of the common india-rubber plant (Ficus elastica), and were obtained as follows. The analogy between epidermal layers, as well as other parts of a plant, and animal epithelia, led me to the inference that reagents successfully applied for elucidating the structure of animal epithelia might serve for the same purpose in plants. Now, each epithelial body is a nucleated, reticulated bioplasson mass, enclosed by a continuous layer of bioplasson and separated from all its neighbours by a cloak of cement-substance. The cement-substance answers to the cellulose wall of plant cells, and as a memento of Schleiden and his cell doctrine, I would advocate not only the retention of the term cellulose, but its extension to animal tissues, i.e. to take the place of the term cement-substance. It is known to histologists that the cement-substance is traversed by numerous conical filaments which by

their discoverer, Max Schultze, were termed "thorns or prickles." It is also known that upon applying a 2 per cent. solution of silver nitrate to fresh epithelia, the cement-substance assumes a dark brown hue, and appears perforated by numerous light transverse lines; while if, on the contrary, a one half per cent. solution of gold chloride be applied to epithelium, the bioplasson reticulum in its interior assumes a dark violet tint, the cement substance remains unstained, and in it Max Schultze's thorns, also coloured deep violet, appear very plainly. Thus it has been proved that the wall of cement-substance does not completely isolate the single epithelia, but is pierced by bridges of living matter which interconnect all epithelia into one continuous bioplasson mass.

I placed pieces of the flower of "Norimbergia" into a 2 per cent. solution of silver nitrate for about half an hour, then washed the specimens with distilled water and exposed them to daylight. I found that nitrate of silver does not invariably affect the cellulose alone, but sometimes stains also the "cell"-contents; a corresponding general tinction occasionally happens in the case of animal epithelia. Frequently, while the cellulose wall on the inner surface of the flower was comparatively little coloured by the silver salt and dark granular precipitates filled the spaces between the radiating cellulose offshoots, the polygonal frames on the outer surface of the flower were beautifully stained dark brown by the silver salt; and examined with Tolle's immersion lens, showed numerous interruptions in their continuity, as represented in fig. 2, exactly like the light-coloured transverse markings seen in cement-substance of animal epithelia under similar circumstances. Usually the hairs were stained deeply brown; in many compartments one or several light fields were seen, of irregular shapes, freely branching; the periphery of such a light-coloured field often looked serrated, and a reticulum proceeding from it pervaded the whole compartment. This appearance is shown in fig. 3. In a number of instances I observed that the septum separating two neighbouring compartments was marked by light-coloured lines, as represented in

fig. 3. The branching light fields were the smaller the nearer

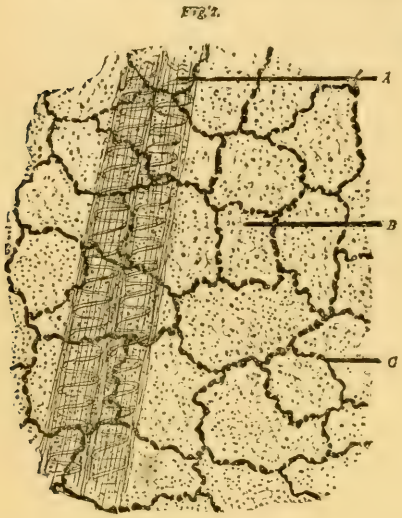

Fig. 2.—Cells from the flower of Norimbergia, stained with nitrate of silver.

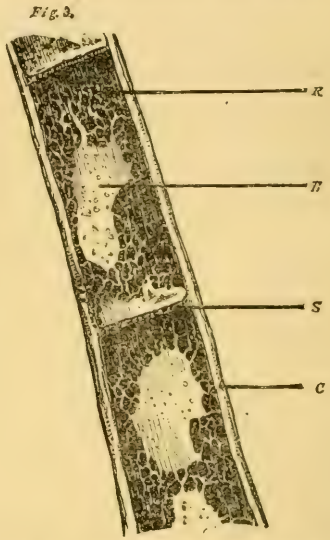

Fig. 3.—Hair of flower of Norimbergia, stained with nitrate of silver.

the compartment was to the apex of the hair; at the end, the whole hair, as a rule, appeared uniformly dark brown, or contained in its interior an extremely delicate, light-coloured reticulum only.

After a one half per cent. solution of gold chloride had been brought to bear upon pieces of the flower for about forty minutes, the wall of cellulose became more distinct although not coloured by the gold salt. In the interior of the polygonal fields, on the inner surface, a scalloped body had made its appearance; it was slightly retracted from the cellulose frame and offshoots, bordered by a continuous delicate layer, and filled with a very distinct reticulum in connection with a central coarsely granular and also reticulated nucleus. The bordering layer and the reticulum around the nucleus, as well as the nuclear wall and the intranuclear granules and reticulum, were of a dark violet colour, just as in animal epithelia

(see fig. 4). On the outer surface the epidermal bodies

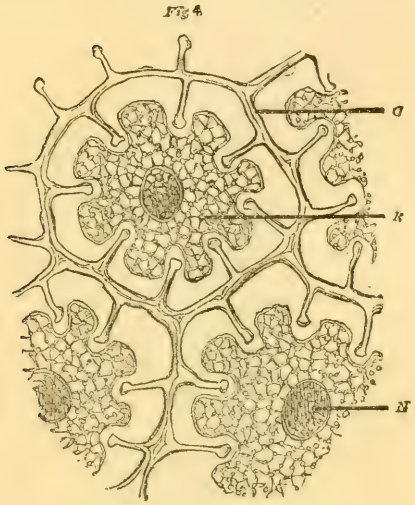

Fig. 4.—Cells from flower of Norimbergia, stained with gold chloride.

exhibited a distinctly reticular structure. The hairs showed dark violet granules and clusters of granules in the interior of the compartments; these granules had radiating offshoots which formed a network, with frequently distinctly granular thickened points of intersection, as represented in fig. 5. There could be no doubt that this was the positive image of the structure that was demonstrated by the silver staining in a negative manner as depicted in fig. 3. In some, especially in small hairs, the dark violet reticulum in the compartment was very dense. Frequently, delicate violet filaments pierced the transverse septa of neighbouring compartments and interconnected the reticula and bioplasson formations in their interiors, as seen in fig. 5.

But the most complete proof of the existence of living matter within the cellulose walls of plant "cells" I obtained in sections of the stems of leaves of the common india-rubber plant (Ficus elastica), a silver-stained specimen of which

is represented in fig. 6. The latex oozing out of the stem proved to be composed of a viscid, as if mucous, colour-

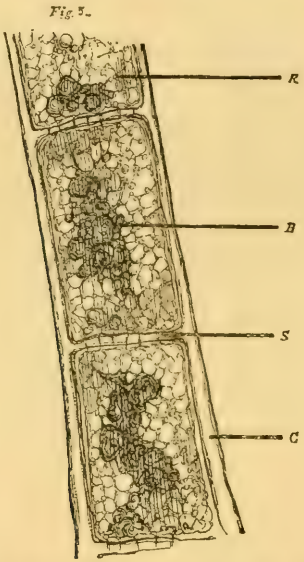

Fig. 5.—Hair of flower of Norimbergia, stained with gold chloride.

less liquid, in which were suspended innumerable isolated granules of a high lustre, somewhat similar to that of fat; gold chloride staining made the smallest granules appear dark violet, while the larger were only indistinctly coloured, retaining their high lustre. Transverse sections of the stem, examined in dilute glycerine, showed chlorophyll-granules and the reticular structure. The parenchyma of some specimens, especially those treated with strong alcohol, plainly exhibited the layer of living matter in the interior of the "cell," which Von Mohl called "Primordial utricle," and sacs, more correctly "protoplasmic sac;" and in many cases the bioplasson mass showed the reticular structure. Treatment of gold chloride not only rendered the network of many bioplasson bodies distinctly visible, but in some cases offshoots emanating from such bodies were seen to penetrate more or less far into the cellulose investment; what has been sometimes de-

scribed by authors, especially in growing tissues, as "intercellular spores" and "middle lamellæ," in the cellulose were

FIG. 6.—Cells from petiole of Ficus elastica, treated with silver nitrate.

revealed to be in a number of instances accumulations and filaments of living matter wedged in between the "plant cells," very much like the wedges of bioplasson and the medullary elements which I have found to grow between animal epithelia in cases of new growths ("Microscopical Study of Papilloma of the Larynx," 'Archives of Laryngology,' March, 1880). Treatment with the solution of silver nitrate revealed in the darkened substance of the cellulose light spaces occupying the position of such wedges. These light spaces sent off comparatively broad offshoots parallel to the inner surfaces of the cellulose frame, and innumerable delicate light offshoots from both the central space and the broad offshoots traversed the brown cellulose in uninterrupted connection with the delicate light reticulum seen here and there within the so-called "plant cell." The appearance of the silver-stained cellulose frame in a portion of such a specimen is accurately reproduced in fig. 6, and the results obtained in

these specimens I have verified by very numerous other examinations.

My researches demonstrate, and so far as I know, demonstrate for the first time, that the frame of cellulose, analogously to the cement substance of animal epithelia and the basis substance of other animal tissues, is pierced by either single filaments of living matter or a reticulum with more or less large accumulations of living matter, interconnecting all neighbouring tissue elements, and that the plant, therefore, like the animal, is one continuous mass of living matter, with interspaces which contain some non-living material.

The structure of plant tissue may be illustrated by the structure of hyaline cartilage of animals. For many years it was believed that cartilage consists of a homogeneous non-living basis substance in which are embedded, at various distances apart, isolated living cartilage corpuscles—cartilage-"cells" as they were called. The more or less convincing observations made by Heitzmann, and after him by Hertwig, Thin, Prudden, Spina, and Flesch, have shown this to be a mistake; and the results which I obtained in the histological examination of the cartilages of the larynx (published in the 'Archives of Laryngology,' October, 1881, and January, 1882), have proved beyond question that hyaline cartilage is a filigree of living matter, in the meshes of which lumps of basis substance are embedded. According to the former view cartilage could be compared to a pudding, in the dough of which a certain number of raisins are embedded; in truth, it is like a framework composed of larger and smaller raisins and bands and strings of raisin substance, in the meshes or interspaces of which blocks of dough are embedded.

Just so in the tissue of plants, the so-called plant "cells" are connected one with the other, and blocks of cellulose fill up the interstices in the network of living matter.

Not to trespass too much upon the patience of the reader, I must leave undetailed here the far-reaching consequences of the "bioplasson doctrine" for the better understanding of the relations and phenomena of plant life.

The Life History of the Liver-Fluke (Fasciola hepatica).

By

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With Plates II and III.

It has been known from very early times that the liver-rot of various herbivorous mammals is a parasitic disease due to the presence of very numerous flukes in the liver of the affected animals. Amongst our domesticated animals the sheep is by far the most frequent victim. The fluke disease is always common in certain districts in England and in many parts of the world; but in consequence of a succession of wet seasons there was a serious outbreak of it in the winter 1879-80, and it is estimated that in the United Kingdom 3,000,000 sheep were then destroyed by it. Hence special attention was called to the subject, and the research summarised in the following paper was undertaken on behalf of the Royal Agricultural Society of England.

For the sake of convenience the subject is divided into the following sections:—I. Historical. II. Methods of Investigation. III. Life-history.

I. Historical.

From what was known of the course of development amongst the digenetic Trematodes, the nearest allies of Fasciola he-

patica, there was reason to believe that here also an alternation of generations existed, and that one or more molluscs served as intermediate host for the asexual forms. Many attempts had been made to discover the intermediate host by various eminent biologists, including Professor Leuckart, von Linstow, Ercolani, &c., but all had proved fruitless, and notwithstanding its important practical bearing the problem remained unsolved.[1]

Very many suggestions had been made as to the nature of the intermediate host. Moulinié[2] had found in Limax cinereus and Arion rufus (ater) sporocysts containing cercariæ with a rudimentary tail, and suggested that these might have some connection with the liver-fluke. I met with this species in Arion ater early in the course of my investigations, and was able to disprove experimentally the conjecture that this was the cercaria of Fasciola hepatica. Willemoes-Suhm[3] had drawn attention to the fact that liver-rot was very prevalent in the Faroe Islands, the molluscan fauna of which was restricted to eight species, viz. Arion ater, A. cinctus, Limax agrestis, L. marginatus, Vitrina pellucida, Hyalina alliaria, Limnæus pereger, and L. truncatulus. Of these Limax agrestis, our common grey slug, was by far the commonest and most injurious, and he suggested that this slug might act as intermediate host. Von Linstow[4] had mentioned Planorbis vortex as being possibly the host. Weinland[5] had found the liver of L. truncatulus infested with nurse forms. The

[1] A statement has been published in several text-books, English and American, to the effect that Cercaria cystophora inhabiting Planorbis marginatus is the larva of Fasciola hepatica. This, of course, is erroneous, and the mistake appears to have been copied from an abstract in the 'Zoological Record' for 1872 of a paper by Willemoes-Suhm. The suggestion really made in the original paper was that C. cystophora is the larval form of Distoma lanceolatum. This species is known on the Continent as the small liver-fluke, and is far less formidable than the larger, F. hepatica, the true liver-fluke. It appears not to exist in England.

[2] 'Mémoires de l'Institut Genevois,' vol. iii, p. 267.

[3] 'Zeitschrift für wissentschaftliche Zoologie,' 1873, vol. xxiii, p. 339.

[4] 'Arch. für Naturgeschichte,' 1875, p 194.

[5] Abstract in 'Archiv für Naturgeschichte,' 1874, vol. ii, p. 423.

cercariæ had the habit of throwing off their tails and crawling about by the aid of their suckers, and he thought that the larvæ of the liver-fluke might encyst upon plants. Küchenmeister had suggested certain slugs as possible hosts of Fasciola hepatica. On April 7th, 1880, the 'Times' published a letter written by Dr. Cobbold to contradict the statements made by Dr. J. Harley, who denied the existence of any intermediate host. The letter contained the following sentence:—"The investigations of the lamented Willemoes-Suhm render it almost certain that Cercaria cystophora infesting Planorbis marginata is the higher larval state of the small fluke (Distoma lanceolatum), and the still later researches of Leuckart point to the mollusc called Lymnæa truncatula as the bearer of the cercarian stage of Fasciola hepatica." Seven days later, on April 14th, 1880, the late Dr. Rolleston gave, in a letter to the 'Times,' reasons for regarding the black slug, Arion ater, as the intermediate host of Fasciola. Since that time Ercolani[1] has made a similar suggestion for certain terrestrial molluscs. He found that larval trematodes were of far more frequent occurrence in land-molluscs than had hitherto been supposed, and this circumstance, together with the failure of the most illustrious helminthologists to discover the genesis of F. hepatica, led him to think that it was in this direction that success would eventually be obtained.

On the 2nd of the following June the Royal Agricultural Society of England offered Dr. Rolleston a grant for an investigation into the life-history of the liver-fluke. Dr. Rolleston being unable himself to undertake the work, mentioned my name to the Society, and the research was begun by me on June 7th.

On Dec. 22nd, 1880, I found in a Limnæus truncatulus, captured on an infected field at Wytham, near Oxford, on the 24th Sept., and since that time kept in an aquarium in the

[1] "Dell' Adattamento delle specie all' ambiente," 'Memorie dell' Accademia delle Scienze dell' Istituto di Bologna,' serie iv, tomo ii, 1881, pp. 241, 327.

laboratory, a cercaria, which has since been proved to be the larva of the liver fluke.

The reasons which led me to regard this as the cercaria of Fasciola hepatica need not be explained here, as they are given in another part of this paper. In a report in the Royal Agricultural Society's 'Journal' for April, 1881, I described this cercaria as a new species, and at the end of the description wrote as follows:—"The structure and habits of this cercaria render it possible that it may prove to be the larva of Fasciola hepatica, but want of material has prevented my testing the question by giving the cysts to rabbits. I intend, however, to pursue this case further."

On Dec. 12th, 1881, a paper appeared in the 'Zoologischer Anzeiger,' in which Professor Leuckart announced that he had succeeded in infecting young specimens of L. pereger, but had been unable to obtain the development of the expected cercaria. He also made it known, for the first time, that the statement made by Dr. Cobbold in the 'Times' for April 7th, 1880, was founded on a private letter from himself, and that the announcement that his researches pointed to L. truncatulus had proved to be premature and incorrect, for on further examination of his snails he had found them to be not L. truncatulus but L. pereger juv.

In the first number of the 'Archiv für Naturgeschichte' for 1882, which, however, bears no further date, the same results were given at greater length. Professor Leuckart stated that in a number of specimens of L. truncatulus sent him by a friend he had found three kinds of rediæ. One of these contained tailless distomes, which, he held, probably belonged to the developmental cycle of the liver-fluke. He considered this supposition to be entirely justified until further results were obtained. A second form was not absolutely excluded from all connection with the liver-fluke, but no such statement could be made with respect to the third form. But in this third form I at once recognised the cercaria found by me at Wytham, of which a description had been published some eight months earlier.

During the summer of 1882 I at length obtained L. truncatulus for my experiments, and succeeded in infecting them with the embryos of F. hepatica. Before the end of August the development of the species was obtained up to the time when the tailed cercariæ were nearly mature, and, as I had by me well-preserved specimens of the rediæ and cercariæ found at Wytham, I was able to compare the two forms step by step, and see that they were identical. A paper was drawn up for the October number of the 'Journal of the Royal Agricultural Society,' giving these results, and was sent to the printer on Sept. 1st. A fortnight later it received a revision, which was merely verbal, and the whole of the copies were printed off by Oct. 2nd.

Separate copies of the paper were received for distribution on the 24th Oct., but the journal was not published until nearly the end of the month.

In the mean time a paper by Professor Leuckart appeared in the 'Zoologischer Anzeiger' for the 9th October. In this he stated that he too had succeeded in rearing the cercaria of F. hepatica in L. truncatulus, "the only true intermediate host," and that it had proved to be not the tailless form, but, on the contrary, the third form mentioned above, which he had supposed early in 1882 could have no connection with the liver-fluke.

On the 19th Oct. a *résumé* of my completed researches was published in 'Nature,' attention being called to the fact that the cercaria of the liver-fluke was the one already discovered by me in Dec., 1880, and described in April, 1881, as probably belonging to F. hepatica, and that the necessary proof had been furnished by myself, and also independently by Professor Leuckart.

II. Methods of Investigation.

On commencing the investigation into the life-history of the liver-fluke it was felt that where so many different molluscs had been suggested as possible intermediate hosts, it was necessary to examine the question anew, and not to be guided by

the numerous conjectures already expressed, some of which had very scanty evidence to support them. Work was begun in two directions, first, by endeavouring to infect many of our commoner molluscs, both terrestrial and fresh-water. In the second place, numerous localities in the country around Oxford, in which sheep were known to have incurred liver-rot, were visited, the whole of the invertebrate fauna was carefully studied, and many specimens brought home to be dissected and searched for larval trematodes. Very numerous places were examined in this way, but mention will only be made here of the one which proves finally to have given the correct solution of the problem. At Wytham, near Oxford, was a clearly circumscribed area of infection. The fields, five in number, were situated on the side of a hill far above the reach of river floods, lying upon the Oxford clay. They were searched thoroughly by day and by night, and the various invertebrate animals found, including snails, slugs, aquatic insect larvæ, crustacea, worms, &c., brought home and examined. Fresh-water snails were very scarce on the ground; all that were brought to light were two small specimens of Physa fontinalis, a small Cyclas, and Limnæus truncatulus in moderate numbers. The last-named species was found in a boggy spot in one of the fields. In one of them was discovered, on the 22nd December, 1880, the peculiar and interesting form of cercaria to which allusion has already been made. Its most striking character was due to the presence of very coarsely granular cells arranged in lobed masses along each side of the body. It was very active, but soon came to rest, encysting itself upon surrounding bodies. The cyst was snowy white, from the presence in its substance of the highly refractive granules already seen in the granular cells forming the lateral masses, which were thus shown to be cystogenous organs.

The other points in the structure of the cercaria were all favorable to the supposition that I had here discovered the long-sought cercaria of the liver-fluke. I had already found in a lamb's liver exceedingly minute flukes, smaller than any yet recorded, one of them being only 1·1 mm. in length, i.e.

only 1-30th part as long as the adult, and these immature forms gave me valuable information as to the structures to be expected in the cercaria, the relative size of its suckers, &c. It was usually supposed that the sheep when grazing picked up the parasites whilst they were still within the snail. This view was upheld by so eminent an authority as Professor Leuckart in a paper published in the beginning of 1882.[1] But I had already collected evidence from independent sources, which inclined me to the belief that the larvæ were picked up in the encysted condition attached to the grass. Hence the presence of a special cystogenous organ in the cercaria, and its habit of encysting on grass were highly suspicious. Further, the suspicions raised by the structure and habits of the cercaria were increased by the fact that its nurse-form was the only one found upon the ground, although there was every reason for expecting to find the larva of the liver-fluke, as an infected sheep had been seen a few months earlier wandering over the boggy spot from which the L. truncatulus was obtained. I can testify that the sheep harboured numerous flukes, for its liver was sent me for examination; and there could be no doubt that large quantities of fluke eggs had been scattered all over the fields. My suspicions were accordingly expressed in a report to the 'Royal Agricultural Society's Journal' for April, 1881.

During the summer of 1881 I was anxious to try infective experiments with Limnæus truncatulus, but was unfortunately unable to obtain any specimens; the localities near Oxford, where I had formerly found it, were searched in vain. I went out repeatedly in quest of this snail, having on several occasions the skilled assistance of my friend and colleague, Mr. W. Hatchett Jackson, but we never found any other trace of this species than the empty shells. It could not be discovered in the localities given for it by Whiteaves in his paper on the "Mollusca inhabiting the Neighbourhood of Oxford."[2] My friends at a distance were appealed to, but were

[1] 'Archiv für Naturgeschichte,' 1882, p. 80.

[2] 'Proceedings of the Ashmolean Society,' 1857.

unable to assist me. The comparative freedom from rot of sheep in the neighbourhood of Oxford last year was probably due to the real scarcity of this snail.

In 1882, however, there were floods in July, and the waters of the Isis brought down vast multitudes, probably from its breeding haunts in marshy places up the river. So numerous was it, that a single sweep of a small hand-net repeatedly gave me more than 500 examples, and this was in a ditch where last year not a single L. truncatulus could be found.

On obtaining the snails I had so long been searching for, I exposed a number to infection by placing fluke embryos in the vessel with them. The snails were speedily found to have afforded a suitable place for the further development of the embryos; indeed, infection was too successful, for very large numbers of them died simply from exhaustion owing to the excessive number of parasites they contained.

It may be well to mention here that infection experiments have been tried upon the following species of molluscs, as well as upon L. truncatulus, viz., Limnæus pereger, L. palustris, L. auricularius, L. stagnalis, Physa fontinalis, Planorbis marginatus, P. carinatus, P. vortex, P. spirorbis, Bythinia tentaculata, Paludina vivipara, Succinea amphibia, Limax agrestis, L. cinereus, Arion ater, A. hortensis. None of these could be infected, with the partial exception of Limnæus pereger. With regard to this last species I can corroborate Professor Leuckart's statement that the youngest specimens only of this species can be infected, and that even here development does not proceed beyond an early stage.

Although it appears that L. truncatulus is the only English mollusc which can serve as intermediate host to the liver-fluke, it is quite possible that elsewhere some other mollusc of similar habits may be victimised. L. truncatulus has a very wide geographical distribution, but so, too, has the liver-fluke; and if the latter has the wider distribution, there must of course be some other intermediate host. Leuckart[1] states that

[1] 'Die menschlichen Parasiten,' p. 531.

Fasciola hepatica is reported from Australia, and a similar assertion has been published in the 'Veterinarian.' According to Hutton[1] and Wallace,[2] the genus Limnæus does not exist in Australia. Hence if both these statements are correct there must be another intermediate host. The liver-fluke is also found in North America, where the genus Limnæus occurs indeed, but not the species L. truncatulus. Sheep-rot is also found in the Shetland Islands, where, according to Forbes,[3] the genus Limnæus is represented by the species L. pereger alone. It is, however, quite possible that in the last case L. truncatulus has been overlooked on account of its minute size.

III. Life-History.

A. First generation—1. Egg.—The eggs of the liver-fluke occur in very large numbers in the contents of the bile ducts and gall-bladder of the infected animal. They give a dark brown colour and sandy appearance to the bile, and in some of the smaller terminal ducts often form a stiff brown mass, completely plugging up the lumen. They pass with the bile into the intestines, and may be found abundantly in the droppings of animals suffering from liver-rot.

The egg is an oval body, with a smooth, transparent, yellowish-brown chitinous shell. The average size may be said to be 0·13 mm. in length by 0·08 mm. in breadth, but the dimensions vary greatly, the length from 0·105 to 0·145 mm., and the breadth from 0·066 to 0·09 mm. The anterior end is a little more rounded than the posterior, and a slightly serrated line running around it marks off a circular segment, forming an operculum 0·028 mm. in diameter. The opposite end is frequently a little thicker, and slightly roughened.

The number of eggs produced by a single fluke is

[1] 'Transactions' of the New Zealand Institute, vol. v, p. 18.

[2] 'Geographical Distribution of Animals,' vol. i.

[3] 'Brit. Ass. Report,' 1859, p. 127.

exceedingly large, and its fecundity has been underrated. In one case I obtained 7,400,000 from the gall-bladder of a sheep suffering from the rot, and, as the liver contained about 200 flukes, this gives an average of 37,000 eggs to each fluke. And these eggs were found in the gall-bladder alone; the liver must have contained at least as many more, and eggs had been passed copiously by the sheep for several months. The number of eggs produced by a single fluke may be safely estimated at several hundred thousands.

When first formed, the egg includes a single germinal cell, supplied by the germarium, and fertilised by a spermatozoon, and a considerable number of secondary yolk-cells supplied by the vitellaria, which serve as food to the growing embryo. Segmentation of the ovum takes place during the descent of the egg through the oviduct, but no further development takes place so long as the egg remains with the body of the host. Fig. 1 on Plate II represents a fluke egg in the condition in which it is found within the bile ducts of the sheep; the embryo is represented by a pale spherical mass of delicate nucleated cells, and is situated near the opercular end of the shell. It is surrounded by the secondary yolk-cells, which are filled with refractive spherules, both large and small, so that the examination of the embryo is rendered very difficult.

The further development of the embryo can only take place out of the body of the bearer of the adult fluke and at a lower temperature. Eggs kept in an incubator, at the temperature of the mammalian body, do not make any progress, whilst the eggs kept at a lower temperature complete their development in a few weeks. The conditions necessary for development are moisture and a certain moderate degree of warmth. Light I have found to exert no influence; eggs taken from the gall-bladder and placed directly with water into an opaque vessel, develop as soon as similar eggs exposed to light but otherwise kept under the same conditions. A temperature of about 23° C. to 26° C. is most favorable, and with this degree of warmth the embryo is formed in about two or three weeks. At a lower temperature development takes place much more

slowly, and with an average warmth of 16° C. occupies two or three months. During the winter no progress is made unless artificial heat is supplied.

All the eggs under the same conditions, however, do not produce embryos in the same time, a certain number are hatched out on every successive day for some weeks or even months, and at the end of this time some of the eggs may remain in the same condition as when just taken from the liver. No explanation can be discovered in the eggs themselves of the very variable time required for the development of the embryo, but the fact is of much practical importance, for eggs scattered over any damp ground may render it dangerous for a long period.

The granular character of the secondary yolk-cells render it very difficult to follow in detail the growth of the embryo whilst still within the egg, and as the matter is one of theoretical importance only, the examination of the development by more elaborate methods has been postponed in favour of matter of more practical interest. I hope, however, to have the opportunity before long of observing the formation of the layers in the embryo. All that can be seen in the egg by direct examination is as follows. The embryo increases in size, being nourished by the absorption of the secondary yolk. The outlines of the yolk-spheres become more distinct and the granules less numerous, whilst some of them appear to coalesce or disintegrate (fig. 2, Pl. II). Within them the outline of the embryo is visible, often showing one or more annular constrictions. As the yolk-cells are gradually used up the body of the embryo becomes larger and more plainly visible, and comes to occupy the whole length of the egg (v. fig. 3). Its surface is somewhat bossy owing to the projection of the cells forming the outermost layer of the body. A papilla appears at the anterior end, which is always directed towards the opercular pole of the shell, and a little way behind a quantity of dark brown pigment is produced, giving rise to a double eye-spot, while the surface loses its bossy appearance. Wave-like peristaltic contractions pass along the body from the anterior towards the opposite end.

In the last stage, when the embryo is ready to emerge from the shell, it lies slightly curved upon itself at one side of the egg (v. fig. 4), the remainder of the space being occupied by the fluid remains and refuse of the yolk-spheres. At the anterior end, just beneath the operculum, is a quantity of viscid mucus, which forms a sort of lining or cushion against which the head-end of the embryo is pressed. Around the body of the embryo may be distinguished a bright border, which is formed by the cilia covering its surface; these cilia, however, can only in exceptional cases be seen in motion before the animal quits the egg.

2. Free Embryo.—The embryo is now ready to come forth; its movements become more marked, and at length a vigorous extension of the body causes the operculum to fly open, as if moved by a spring. The cushion of mucus pours out, the embryo thrusts the fore part of its body out of the shell, the cilia begin to move instantly the water touches them, and the animal, after a short struggle, succeeds in drawing the whole of its body through the narrow opening of the shell, and glides away with ease and rapidity through the water. Although light has no influence in accelerating development, the embryo itself is very sensitive to it. Thus they congregate especially on the light side of a vessel containing them; and I have repeatedly observed that, although on removing a vessel of eggs from the darkened incubator in which they were being hatched, not more than two or three embryos could be seen, yet after it had stood in a window for twenty minutes the water was quite nebulous from their presence.

The form of the free-swimming embryo is an elongated cone, with rounded apex (fig. 5, Plate II), its average length 0·13 mm., its breadth at the anterior end 0·027. The broader end or base of the cone is directed forwards, and in its centre is a short retractile head-papilla. The whole of the surface, with the exception of the head-papilla, is covered with long cilia, which are borne by an outer layer of flattened ectoderm cells. These cells are arranged around the body in transverse rings, usually five in number, though occasionally six may be

counted; they vary in length from ·025—0·35 mm.; each has a very small nucleus, 0·003 mm. in diameter. The cilia are of the same length (·012 mm.) over the whole of the surface, but on the cells of the anterior ring they are more numerous, and hence more conspicuous. This first row is composed of four or sometimes five cells, arranged round the papilla, and these are thicker than the other cells belonging to the same layer, often forming ear-like projections at the side of the embryo, and resembling epaulets. The second ring contains five or six cells, the next two rows, each four, as a rule, whilst the last ring is formed by two cells only. In the last two rings the cells are of greater length than in the others. Seen in a surface view the cells of this outer layer are polygonal and sometimes hexagonal. They overlap one another at their edges, and it is probably owing to this fact that the outlines appear double in silver nitrate preparations. In the small number of examples possessing six rows of cells, the second and third rows are formed by smaller cells.

Beneath the ciliated cells the body wall is formed by a granular layer, the cellular nature of which is not easily made out. In favorable preparations, however, nucleated cells can be seen slightly projecting on the inner surface. In the outer parts of the layer are situated both transverse and longitudinal muscle-fibres. The longitudinal are more feebly developed than the transverse, and are only seen with difficulty. The double eye-spot belongs to this deeper layer; it has been figured as having a form of the sign of multiplication. This, however, is not the case, for it is really double, and has commonly the form of two crescentic masses of dark pigment, placed with their convex sides turned towards each other, and in contact near the anterior horns. On closer examination it is seen that each eye-spot is composed of a cell in which the pigment is arranged at one side in a crescent, the hollow of which is filled up by refractive material which will act as a rudimentary lens. The body wall also contains numerous yellowish refractive granules, especially just behind the eye-spots, and to it belong the two ciliated funnel-shaped spaces of the excretory system. These

are situated one on either side of the middle of the body, in each is a large cilium carried by a nucleated cell, and usually directed forwards. The cilium is connected with the cell by a disc at its base, it is tongue- or flame-shaped, and is constantly in motion, waves passing along the cilium from the base to the tip, and hence towards the apex of the rather narrow infundibulum. Just behind the head-papilla is a granular mass, which reacts with staining fluids differently from the adjacent tissues. This, from comparison with other trematode embryos, would seem to be a rudimentary digestive tract. Behind, the rest of the body cavity is occupied by delicate round nucleated cells—the germinal cells.

The embryo is exceedingly active, and with head-papilla retracted swims swiftly and restlessly through the water, not unlike some of the larger infusoria, though more rapidly. Sometimes it goes directly forwards, and then rotates on its longitudinal axis, just turning a little from side to side, as if searching for something. At other times, by curving its body, it sweeps round in circles, or, curving itself still more strongly, spins round and round without moving from the spot. When the embryo, in moving through the water, comes in contact with any object, it pauses for a moment, and feels about as if trying to test its nature, and, if not satisfied, darts off hastily again. But if the object be a Limnæus truncatulus it at once begins to bore. Prof. Leuckart has said of the head-papilla of the embryo, that "it seems to have the function of a tactile organ." But I have no doubt that it has the function assigned to it in my former papers, viz. that it is a boring-organ.[1] The papilla is ordinarily short (about ·006 m. in length), and the end is quite blunt, or may have a slight depression in the middle. A differentiation in the tissue of the head-papilla is visible in the form of a delicate rod-shaped structure, occuping the axis, certainly not distinct enough to be called a spine, though the papilla seems to possess considerable rigidity. It is particularly evident in preparations of embryos killed with osmic acid and stained with picro-carmine.

[1] 'Roy. Agric. Soc. Journ.,' 1881, p. 7.

As soon as the embryo begins to bore the head-papilla becomes longer, conical, and pointed. The embryo spins round on its axis, the cilia working vigorously and pressing the embryo against the surface of the snail. This pressure is increased by the body of the embryo being alternately drawn up and then suddenly extended. As the papilla sinks further into the tissues of the snail, it becomes longer and longer until it may reach five times its original length (Plate II, fig. 6), and the tissues of the snail are forced apart, as if by a wedge, leaving a gap through which the embryo squeezes its way into the snail.

The embryo appears to exert an instinctive choice in selecting the host into which it enters. It is conceivable that the tissues of Limnæus truncatulus are softer than those of other molluscs, and that the embryo is able to bore its way into the former whilst it cannot do so into the latter. But from the greater eagerness which the embryo exhibits when placed on a slide with Limnæus truncatulus I do not think that this is the correct explanation. Moreover, the tissues of such snails as young specimens of Physa fontinalis or Limnaeus palustris appear to be quite as soft as those of L. truncatulus, and yet if a quantity of embryos are placed in a vessel containing equal sized examples of the three species just named, it is found, on subsequent examination, that whereas each L. truncatulus may contain fifty or more intruders, the other snails are quite free from them. The most probable explanation seems to be that there is some difference in the nature of the secretion of the surface of the body in these snails, which is sufficient to serve as a guide to the instinct of the embryos.

But, although the embryo instinctively chooses the snail in which its further development is possible, it does not always make an equally happy selection of the part of the snail into which it enters. I have found as many as a dozen embryos embedded in the substance of the foot of a L. truncatulus, such a place of course is unfavorable to further development, but they may remain alive there for two or three days. Once

only I found a full-grown sporocyst in the foot of a snail; the survival of this one in so unsuitable a place was probably owing to its having accidently forced its way into a connective tissue space or into a venous sinus. The most natural situation for the development of the embryo seems to be the pulmonary chamber, and this organ is, of course, from its position and the thinness of its walls, most easily accessible to the embryo. Other embryos, however, may be found in the body cavity of the snail.

The average maximum duration of the embryo's free and active life in water is only about eight hours, though occasionally one may live over night. During the last portion of the time its movements become slower, and it will then in desperation often endeavour to bore into any object which presents itself, even into its own empty egg-shell. If an embryo has not succeeded in finding a host, its motion becomes gradually feebler, and at length ceasing the body assumes an oval or elliptical shape; the outer ciliated cells absorb water and swell up into round vesicles, and the whole body disintegrates. In a feebly alkaline solution of peptone I have kept them alive for three days. The cilia were not lost until the third day, though their motion became very sluggish; the embryos increased a little in size and remained alive even after a number of the ciliated cells were detached.

3. Sporocyst.—Arrived within the suitable snail the embryo undergoes a metamorphosis, loses its organs of locomotion, and degenerates into an inactive sporocyst. The outer layer of ciliated cells is lost, whilst the embryo changes in form. The ciliated cells absorb water and appear as round or hemispherical vesicles with the cilia standing out perpendicularly from their surface (fig. 7). During the metamorphosis embryos may have various irregular shapes, but sometimes retain a less elongated conical form, even after they have lost the ciliated cells. The conical form is, however, soon lost, and the embryos take an elliptical shape such as is shown in fig. 8. The eye-spots of the embryo become detached from one another and lose their crescentic form; but they, as well as the head-

papilla, persist, showing the identity of the young sporocyst with the embryo of the liver-fluke. After the change in form has taken place the length is only about ·07 mm. The rudimentary digestive tract remains for a time, but later on is no longer distinguishable. The growth of the various larval forms of trematodes depends very much on the temperature to which they are exposed. During warm summer weather it is very rapid, and in the case of Fasciola hepatica, the sporocyst may reach its full size before the end of a fortnight; in autumn development to the same stage takes a period of double the length. The sporocyst commonly preserves the elliptical shape until it reaches the length of ·15 mm., after this time the growth is most rapid in the longitudinal direction and the form becomes sac-shaped. The contents of the sporocyst are formed by a number of very clear rounded cells, some of which are the germinal cells of the embryo or cells derived from them by division, others are formed by a proliferation of the epithelium lining the cavity of the sporocyst. If the sporocyst be contracted, these cells seem to fill up the whole of the space, and the cells which are still attached to the body wall, and form part of its inner surface, cannot be properly distinguished from those which are lying free. But if a sporocyst be chosen for examination which is not in a state of contraction, cells of various sizes, with very large nuclei, may be seen projecting here and there from the inner surface; sometimes in a single layer, at other times in rounded heaps, two or three cells deep. It is very difficult to follow the earliest stages in the formation of the spores within the sporocyst, but by the time the sporocyst has reached the size of ·2 mm., there are always indications that the contents are becoming arranged in separate balls of cells—the germs of the next generation.

The sporocyst continues to increase in size, and ultimately reaches the length of ·5—·7 mm. (Plate III, fig. 10). On the outer surface is a structureless cuticle, and beneath this is the thin layer in which the external circular and internal longitudinal muscle-fibres are often the only structural elements which can be distinctly observed; but in some cases, though not in

all, there is visible beneath the cuticle a finely granular layer in which the muscle-fibres appear to be embedded.

It appears probable that some of the most superficial cells of the body, or at least portions of them, are converted into muscle-fibres, whilst others undergo more or less degeneration. These muscle-fibres are more feebly developed in the sporocysts than in the rediæ which form the next asexual generation, and in accordance with this feeble development the sporocysts are exceedingly inert, and rarely show any movements. In the redia active movements are necessary in order that the digestive tract, which is present, may be filled with food, and the muscular system accordingly reaches a greater development. In the sporocyst the digestive tract is altogether rudimentary, and as no exertion is required to procure the nourishment, the degeneration of the muscle-fibres has to some extent followed that of the enteron. In those sporocysts, however, in which the power of performing active movements is useful for some other reason, the muscles may retain a high degree of development; as is, for instance, the case in the sporocysts of Cercaria limacis, which, as soon as their included cercariæ are sufficiently matured for transference to the ultimate host, bore their way out, through the thick integument of the slugs (Arion ater and Limax cinereus), which serve as intermediate hosts, and are then left behind in the mucous track ofthe slug.

Immediately following the layer of muscle-fibres is an epithelium, which lines the cavity of the sporocyst, and forms the greater part of the thickness of the body wall. It is composed of cells of very various sizes, round or polygonal in form, and containing large nuclei (fig. 11). The layer in most places is only one cell deep, though adjacent cells may overlap one another; but in places, and especially in the less mature sporocysts, it is two or three cells deep. The excretory system is lodged in the body wall of the sporocyst; on each side may be distinguished an irregular group of about half-a-dozen ciliated infundibula. They have the same structure as the two described above as being present in the embryo, and are always found in the middle third of the length of the body. No clearly defined

or regular canals can be distinguished, but the ciliated infundibula appear to communicate with an extensive system of irregular lacunæ between the cells of the body wall. Numerous yellowish refractive granules occur in the tissues of the sporocyst, or in the body cavity; they are found within the cells, but are especially numerous between them, or on their surface. They are also present in large numbers in the lacunæ, and there frequently exhibit molecular motion, thus showing that the lacunæ contain a fluid of some tenuity; occasionally a whole group of them may be seen to move en masse, or by careful pressure on the cover-glass may be made to travel for a short distance along the lacunar passages. No external opening of this system of passages can be seen, nor any communication with the body cavity be clearly proved. In the ciliated infundibula, however, there is present in the lower wall of the space and close to the base of the flame-shaped cilium, an elliptical structure, closely resembling that which Fraipont has described in the ciliated infundibula of certain other trematodes, as an opening into the body cavity. This interpretation has more recently been called in question, but whatever the real nature of this structure may be, and it is difficult to see what else it can be, there can be no doubt of its presence in the sporocysts. The yellowish granules described appear to be excretory products formed within the cells of the sporocyst and then ejected. They are partially soluble in acids, leaving an organic basis.

Wagener[1] found in the sporocysts of Cercaria macrocerca (the larva of Distoma cygnoides) vibratile lobules (Flimmerläppchen) or ciliated spots, which he did not describe in detail. Thiry[2] described in the same sporocysts a system of vessels, the branches of which had ciliated terminations opening into the body-cavity. The vessels, however, were very pale and difficult to follow, and only in one instance, where the animal was exceptionally transparent, did he plainly see the whole system with its branches. The ciliated ends, on the

[1] 'Beiträge z. Entw. d. Eingeweidewürmer,' p. 65.
[2] 'Zeitschrift für wissentschaftliche Zoologie,' x, p. 272.

other hand, were present in almost all fairly developed examples; in form they most closely resembled the ciliated openings in Clepsine complanata, as figured by Leydig. The vessel was opened on one side and expanded into a two-horned lobe, covered on its inner surface with cilia. In short, the ciliated opening was described as similar to the inner ends of the segmental organs of various annelids. I have never found the sporocysts which Thiry studied, but it seems improbable that there should be any great difference in the structure of the ciliated ends in question in the various species of sporocysts. The isolated cilia really present are very large and their motion peculiar, so that it is not difficult to understand that any one who had before his mind the segmental organ of the earthworm, and was not prepared to see a large isolated cilium within an infundibulum, might take the waves passing along the cilium for waves travelling over a series of small cilia. There can be no doubt, therefore, that the ciliated infundibula have essentially the same structure in the asexual generations (sporocysts and rediæ) as in the adult sexual trematodes.

Amongst the digenetic trematodes the reproduction of sporocysts by sporocysts takes place, either by transverse fission, which may be continued through several generations, as in the case of Cercaria limacis, or by the formation of sporocysts within the parent, or both methods may occur in the same species (e. g. Cercaria chlorotica, &c.). But the only way in which the sporocysts are multiplied in the case of Fasciola hepatica is by transverse division, and this is of far less frequent occurrence than in some other species of trematodes. There appears to be a great and invariable increase of the nurse-forms amongst the Distomidæ, and in Fasciola hepatica the multiplication is effected by the production of numerous broods of the more highly organised rediæ. Fission does, however, sometimes occur, and usually at an early stage in the growth of the original sporocyst. A constriction appears about the middle of the body, and becomes deeper and deeper (fig. 9), and finally the two halves are completely severed. One of these contains the remains of the head-

papilla and the two separated eye-spots, whilst the other is, of course, without the signs of any such structures. Hence sporocysts can be found, which even at an early stage show no trace of head-papilla or eye-spots; and in the majority of adult sporocysts these organs have degenerated entirely.

B. 1. Development of Redia within Sporocyst.—It has been mentioned above that the germinal cells which give rise to the rediæ are in part already present in the embryo, but that they gain an increase in their numbers by the proliferation of cells lining the body cavity. The earliest stages in the development of the spores cannot be so well distinguished in the sporocyst as in the redia. The cells within a sporocyst having the length of about ·2 mm., begin to show an arrangement into rounded masses or solid morulæ. One side of the morula then becomes flattened, and the cells here then appear to be invaginated, producing a gastrula, whilst the surface becomes smooth, and its outline first round and then oval. The cells forming the opposite sides of the archenteron are in contact, so that there is no archenteric cavity. Each spore may now be seen to be surrounded by a delicate membrane, and as it increases in size its form becomes more nearly quadrate. At one end a number of cells are separated to form a spherical pharynx leading into the blind digestive tract, which now extends a little beyond the middle of the body. A little behind the pharynx, the body shows a slightly raised annular ridge, whilst more posteriorly two short blunt processes are formed. Germs, as described above, and in various stages of development, are found in each mature sporocyst; there is usually one redia (or less frequently two), nearly ready to leave the sporocyst, with two or three germs of medium size, and several small ones. Owing to the varying size and shape of the included germs, the sporocysts have frequently a very irregular outline.

As soon as the redia is ready to issue from the sporocyst, which is usually the case by the time it has reached the length of ·26 mm., it shows active movements, which increase in strength until at length it succeeds in rupturing the wall of the sporocyst, and as this state of contraction is continued, the

wound produced by the forcible exit of the redia is kept closed until it has healed up. Meanwhile the development of the remaining germs proceeds. Many of the nurse-forms of trematodes are known to possess a special birth opening for the escape of the brood, and even amongst the sporocysts such an opening is present in those possessing a filiform shape; and I have myself observed this structure in the sporocysts of Cercaria gracilis. But in F. hepatica no such definite opening can be detected in the sporocyst.

2. The Free Redia.—The sporocysts of the liver-fluke are found in the pulmonary chamber of the snail, or less abundantly in the body cavity. But the free rediæ force their way through the tissues of the host, and wander into the other organs, and especially into the liver. They are usually found, with the digestive tract quite yellow from the remains of the snail's liver-cells, with which it is filled. In thus forcing their way through the tissues they necessarily inflict much injury on their host, so much, in fact, that comparatively few snails survive three weeks from artificial infection, and the majority, even of these, die before the time when the cercariæ are completely mature. Thus, in the laboratory at any rate, the fluke-disease is more fatal to the snail than it is subsequently to the sheep.

The redia increases in size until it may reach the length of 1·3 mm. to 1·6 mm. It has an elongated cylindrical form (Pl. III, figs. 12, 13), and at a little distance behind the pharynx there is present an annular ridge or collar projecting from the surface, the use of which will be explained below. From this ring or collar the body tapers gently towards the anterior end, which is abruptly truncated, and includes in its centre the mouth. Behind the collar the body becomes a little narrower, but then swells out gently again until it reaches the middle of its length, from which point it tapers, at first almost insensibly, and then more rapidly, the extremity being conical with rounded apex. At a distance from the posterior end, equal to about one fourth of the total length of the body, are situated two short and bluntly conical processes, which serve as rudimentary feet, and are no doubt of much service in steadying the redia and preventing it from slipping backwards

whilst wandering through the tissues of the host. They are not situated on opposite sides of the body, but are close together on the same surface, and their bases may even be connected by a low transverse ridge. They are directed outwards and somewhat backwards, their axes being usually inclined at an angle equal to or rather less than a right angle.

The body-wall has a similar structure in both redia and sporocyst, so that it will only be necessary to describe the points in which a difference exists. The muscle-fibres are far more strongly developed, especially in the anterior part of the body, so that the rediæ show considerable activity as compared with the sporocyst. When the body is fully extended it may have a length twice as great as when in a state of contraction. If an example of the host be chosen, which has a clear and transparent shell, and has had the greater part of its liver consumed by the parasites, the rediæ may be observed performing movements of elongation and contraction whilst still within the living snail.

In the collar or ring mentioned above the muscle-fibres are strongly marked, and have a peculiar arrangement. The transverse muscle-fibres appear to lie directly under the cuticle, and are closer together at the sides of the ridge then near its most convex part. The longitudinal muscle-fibres, however, do not follow the curve of the surface, but stretch across from one to the other side of the base of the ridge. Sometimes, when the ring is strongly marked, the longitudinal muscle-fibres as they pass forwards may spread out in a fan-shaped fashion before they are finally inserted in the cuticle (fig. 16). The extent to which the ring projects above the rest of the surface of the body varies very greatly according to the size and condition of the redia, and may be altered from time to time by the contraction of the muscle-fibres. It is greatest in those which show the most active movements, least in those which are the most passive. The smaller or half-grown rediæ commonly show the greatest activity, and in one of these I have observed the ring so enormously developed that the diameter of the body was almost doubled at this point. Those rediæ in which fully-

developed cercariæ are present are frequently very sluggish in their behaviour, and the ring may then be relatively inconspicuous. In very young rediæ the outline of the body appears to present a slight process on each side anteriorly, and without the most careful focussing it is often impossible to see that these are simply the optical expression of the collar, the tissues of which are still so delicate that the ridge is flattened above, and therefore, owing to the transparence of its substance, not readily recognised.[1] The function of the collar is to maintain the shape of the body and to produce a firm basis upon which the neck of the redia can be moved. I have observed a redia, whilst the whole of its body behind the ring was at rest, stretch forth its neck in such a way as to sweep a considerable area in front, and thus be enabled to reach conveniently the tissues of the snail upon which it was browsing. When disturbed the neck was retracted and the pharynx drawn back close to the collar. But although the collar has thus a supporting function, there is no thickening of the structureless cuticle in it, such as could be termed a definite skeletal structure.

The excretory system is better marked in the redia than in the sporocyst, and definite canals can be distinguished in the body-wall. Sinuous longitudinal vessels, one on each side, have been described in the rediæ of several other Trematodes, and

[1] Diesing ('Wien. Sitzungsberichte,' vol. xxxi, p. 248) has described the redia of Cercaria fallax as having two short processes situated anteriorly, and two, of thrice the length, posteriorly. De Filippi ('Memorie della Reale Accademia delle Scienze di Torino,' Ser. ii, tomo xviii, p. 207) has described the redia of Cercaria tuberculata as having four lateral processes, two anteriorly and two posteriorly. I have met with a species which appears to be identical with Cercaria tuberculata, and in the redia recognised a collar. The same writer has figured (ibid., vol. xvi, pl. i, fig. 13) in the young redia of Cercaria coronata four processes; the two placed in front are slightly smaller than the two posterior, but otherwise they are drawn as if exactly alike. There can be no doubt that in all these cases the structures described as anterior lateral processes are simply the projecting borders of the transparent collar, seen perhaps in the flattened redia. From comparison with the descriptions given by these distinguished observers I was led in my first paper ('Roy. Agricult. Soc. Journ.,' 1881, p. 19) to similarly misinterpret the corresponding projections in the young rediæ of Fasciola hepatica.

may also be distinguished here, though the main trunks are less distinctly visible than their ramifications, and can rarely be followed for any great distance. Hence it is impossible to discover whether the system of vessels opens externally. The branches begin with a long narrow infundibulum, in which a flame-shaped cilium is constantly working, as described in the sporocyst. The ciliated infundibula are arranged in two groups on each side of the body; the anterior group on each side lies a short distance behind the collar, the posterior close to the processes which serve as feet (fig. 15). The ciliated cells do not all lie at the same level beneath the surface, so that occasionally two of the infundibula may be seen lying across one another, and sometimes the cells may lie free within the body-cavity, the end of the cell opposite the cilium being connected with the wall by one or more processes (fig. 14).

The digestive tract is the characteristic structure of the redia, and at once differentiates it from the simple sporocyst. Quite at the anterior end of the body is the mouth surrounded by projecting folds, which may be termed the lips. The transverse muscle-fibres are especially well developed in the lips, and assisted by the transverse muscles of the following part of the body-wall, serve as a sphincter muscle in closing the orifice of the mouth. The space within the lips is very small, and leads almost directly into the pharynx, an elliptical muscular organ by means of which the animal draws in and crushes the tissues which serve as food. Its outer surface is formed by a clearly marked limiting membrane, so that it is everywhere distinguished with readiness from the mass of ill-defined cells in which it is embedded, and its cavity is lined by a thickened cuticle. To the pharynx immediately succeeds the digestive sac, a blind tube of very simple structure. Its wall is composed of a single layer of clear nucleated cells (fig. 12), supported by a basement membrane, and when it is distended the cells are flattened out till they are little more than discs, in which the nucleus causes a distinct swelling. The digestive sac is seldom more than ·3—·4 mm. long, and may be less than this, but its length differs a good deal, not only in different indivi-

duals, but also according to the amount of food contained in it. It reaches its full size early; indeed, in a redia not half grown it may be as long as in a full-grown example.

The body-cavity is traversed in different directions by bridges or trabeculæ of tissue, in which cells of various shapes, some of them with long processes, can be distinguished. This tissue is most abundant in the anterior part of the redia around the pharynx and digestive tract, and here often contains fibres, probably contractile. Its amount varies very greatly in different specimens, and behind the digestive tract is sometimes altogether absent. At other times it is so extensively developed that the cavity of the redia appears to be divided up into a number of imperfect compartments, in which the germs lie loosely. De Filippi appears to have observed similar trabeculæ in the redia of Cercaria coronata, of which he says,[1] "La cavité du corps est traversée sans ordre par des brides."

There is always a good deal of tissue around the pharynx and beginning of the digestive tract, and embedded in it may be seen, in favorable specimens, a few large round cells with clear protoplasm and large nucleus; each has a process or duct (?) passing towards the angle formed by the junction of the digestive sac with the pharynx. They are probably glandular in function. At the side of the redia, a little behind the collar, there is present a birth-opening (Pl. III, fig. 13, *v*), which permits the exit of the brood when ready to leave the parent. Such an opening has been seen in a number of rediæ of different types, and probably exists in all.

The germs produced within the redia develop either into daughter-rediæ or into cercariæ, and it appears to me that slight differences exist between the individuals giving rise to one or the other of these generations. A redia producing rediæ is usually smaller, but its pharynx and digestive sac are larger; for example, two rediæ were taken from the same snail, one producing rediæ measured less than 1 mm. long, with a pharynx ·117 mm. and an intestine ·41 mm. in length, whereas in the slightly larger redia containing cercariæ the pharynx measured ·078 mm. and

[1] Ibid., vol. xvi, p. 426.

the digestive sac ·24 mm. A further distinction lies in the number of the progeny; a mother-redia may contain from one to three well-formed daughter rediæ with a few germs in various stages of growth; the highest total observed was ten. On the other hand, in a well-grown redia producing cercariæ, I have counted a total of twenty-three.

The early stages in the development of the spores is the same, whether they are destined to become rediæ or cercariæ. Some of them may be formed from the cells which fill the body-cavity in the very young rediæ, but the majority seem to be formed in the following way:—Some of the cells lining the body-cavity of the parent, especially those at the posterior end, are greatly enlarged, and each of these germinal cells undergoes segmentation, giving rise to a morula. Fig. 18 represents a large number of germinal cells in the hind end of a young redia, in which no morulæ were yet present. Similar cells may be found in the mature rediæ (fig. 13 *k'*), for they retain the power of producing more spores as the older ones reach their full development and quit the parent. Hence we find in the adult redia germs in all the successive stages of growth. Each morula or germ is enclosed by a delicate membrane forming a loose envelope. The germs are usually detached from the body-wall whilst still small and lie free in the cavity of the parent, but occasionally they may remain in sitû in the body-wall until they have attained a considerable size (fig. 13 *w'*). The morula soon becomes flattened on one side (fig. 12 *s*), and the cells of this area are then invaginated, giving rise to a gastrula (fig. 12 *m*), whilst the germ again becomes round. The opposite sides of the archenteron are in contact, so that there is rarely any archenteric cavity, and as growth proceeds and the cells become more numerous it is no longer possible to distinguish the cells of the endoderm, for the cells have the same size and appearance. Nevertheless it appears to me probable that the cells invaginated form the digestive tract, which becomes visible at a later period in the development, rather than any other cells in the germ. As the germ continues to increase in size the surface becomes smooth and the outline oval.

Further growth in size is accompanied by a change in shape, and it then becomes possible to distinguish between the germs destined to become rediæ or cercariæ. The growth of the young redia within the redia agrees in every respect with the development of the mother-redia within the sporocyst. The growth of the cercaria follows a different line.

It may be asked what determines the character of the progeny, whether the germ shall become redia or cercaria. My observations are not sufficiently extensive to definitively decide the question, but it appears to me that the season of the year is one of the principal determining causes. Rediæ producing rediæ were only found during warm weather, in the cold months cercariæ were always produced directly. Further, it is a noteworthy fact that I found at the beginning of the autumn a redia, containing a single daughter-redia in addition to numerous cercariæ and their germs (fig. 13). I am inclined to think that the redia was producing rediæ but that a fall of temperature induced the formation of cercariæ instead. The explanation suggested is the more likely to be correct, since such an arrangement would be highly advantageous to the species.

C. 1. The development of the cercaria within the redia. —The earliest stages of the development have already been described up to the time when the germ is an oval mass of cells. As this continues to increase in size it assumes a more elongated shape, whilst one end becomes rather more attenuated than the other. The more slender end becomes slightly constricted off to form the rudiment of the tail, which as yet is very stumpy. The remainder of the germ forms the body of the cercaria, it becomes more depressed in shape, whilst cells are separated at the anterior end to form an oral sucker, in the midst of which opens the mouth, and in the centre of the inferior surface to form a ventral sucker equal in size to the oral. The digestive tract is now visible as a solid mass of cells. Immediately following the oral sucker is the rounded pharyngeal bulb. Then comes a narrow œsophagus ascending slightly towards the dorsal surface, and at a short distance in front of

the ventral sucker it bifurcates to form the two limbs of the intestine, which reach, one on each side of the ventral sucker, to nearly the end of the body. The limbs of the digestive tract are solid, being formed for the most part by single rows of thick disc-shaped cells (fig. 13). The cells are finely granular at this stage, and show out distinctly against the clear spheroidal cells which surround the limbs and produce concave impressions on the surface. At the sides of the body refractive granules begin to collect in certain of the cells, which are destined to assist in the formation of the cyst of the cercaria, and may conveniently be termed cystogenous. At first the granules are few and inconspicuous, but gradually become more and more numerous until at length they may obscure the nuclei, and render the cells opaque. Many of the cells in the body of the cercaria are crowded with most remarkable rod-shaped bodies closely resembling bacteria in size and shape (fig. 20). They reach the length of ·006 mm., and are often arranged in rows side by side, whilst the long axes of nearly all the rods in each cell have approximately the same direction. Both Wagener and De Filippi appear to have observed similar structures in the cercaria of Amphistoma subclavatum. The former speaks of them as "rod-shaped corpuscles," and the latter says that "their form may not inaptly be compared to that of a shuttle or spindle, with thick walls, and truncated at both ends. They are destined to disappear later." These bodies are not precisely like the narrower ones found in the cercaria of the liver-fluke, but they are probably corresponding structures.[1]

An adult redia generally contains a brood numbering about a score; amongst these there will be one, two, or three cercariæ

[1] Prof. Leuckart ('Zool. Anz.,' Oct. 9th, 1882) has also found these curious bodies (which had already been described by me in the 'Journ. Roy. Agric. Soc.,' for April, 1881), and as he was unable to find any spines on the cuticle of his cercariæ, he suggests that the rod-like bodies are subsequently arranged in bundles to form the spines of the adult fluke. But I have found the spines in the most mature cercariæ, and can say that these rod-shaped bodies have no connection with them, though I am unable to suggest any probable explanation as to their nature.

approaching complete development. On one occasion I counted as many as six.

2. Free Cercaria.—As soon as the cercaria has reached the limit of development within the redia, it escapes from the parent by the birth-opening (fig. 13, *v*) and then by the aid of suckers and tail, crawls or wriggles its way out of the host. The free cercaria is very active, and its tissues so contractile that the form and dimensions of the body are constantly changing. When in a relatively quiescent condition, the body has a depressed oval form (Plate III, fig. 19), its average size is ·28 mm. long and ·23 mm. broad, though the largest may measure over ·3 mm. in length. The tail is more than double the length of the body, and is exceedingly contractile. The oral sucker is subterminal, the opening of the mouth being directed downwards and forwards, and has a diameter of ·06 mm.; the pharynx is ·034 mm. in diameter. The ventral sucker is situated slightly behind the centre of the ventral surface, and is equal in size to the oral, or is sometimes a little larger. As is the case with all the cercariæ produced in rediæ (with the partial exception of Distoma Paludinæ impuræ armatum) the cercaria has no head spine. In the most mature specimens, and especially in such as have left the redia in the natural course, and have not been disturbed by the dissection of their host, the surface of the body is beset anteriorly with exceedingly minute spines. But the most striking character is due to the presence of the cystogenous cells, large nucleated cells so crowded with coarse, highly refractive granules as to be rendered quite opaque. They are arranged in two lobed masses extending along each side of the body (Plate III, figs. 19 and 21), from the level of the pharynx to the posterior end of the body. Just in front of the ventral sucker is another group of these cells, which is often large enough to connect the two lateral masses, and behind the ventral sucker others are scattered. Cells of the same kind, and showing a similar arrangement, are found in Cercaria tuberculata (inhabiting Bythinia tentaculata), a species which shows at first sight a remarkable resemblance to the cercaria of Fasciola hepatica. I have,

however, myself met with C. tuberculata, and from comparative measurements, as well as the difference in the host, can state with confidence that the species are quite distinct. And even in an armed cercaria, recently found in Limnæus pereger, I found cells, showing a similar arrangement, distinguished from the remaining cells of the body, not, indeed, by coarsely granular contents, but by the possession of a protoplasm of a finely granular nature. In this case also the more granular cells are probably cystogenous.

The other organs of the body are much obscured by the presence of the opaque cystogenous cells, but the contractile vesicle of the excretory system, together with the principal lateral vessels, one on each side, which contain small highly refractive concretions, can be made out.

3. The Cyst.—When the snails infested with the larval forms of Fasciola hepatica are kept in an aquarium, the cercariæ may occasionally be found swimming about in the water, for the granular cells which render the body nearly opaque when viewed under the microscope by transmitted light, give it a snow-white appearance by reflected light, and it is thus rendered conspicuous for its size. The life as a free-swimming animal, however, never seems to last long, for, on coming in contact with the side of the aquarium or the water-plants contained in it, the cercaria proceeds to encyst itself. Numbers of minute snow-white cysts may thus be seen adhering to the walls of the aquarium or to the dark-green leaves of the water-plants. The way in which the cyst is formed can be readily observed under the microscope, for when examined on the glass slide the cercaria soon comes to rest, and assumes a rounded form, whilst a mucous substance is poured forth all over the body, together with the granules forming the contents of the cystogenous cells already mentioned. The tail is sometimes shaken off before the encystation begins, but, as a rule, the tail remains in connection with the body during the process, and continues to be energetically lashed from side to side, until finally a more vigorous movement detaches it. The whole process of forming the cyst is very rapid, and in a few

minutes a layer of considerable thickness is formed, whilst its substance begins to harden. The cysts, as already remarked, are snowy-white, but the body of the included Fasciola is quite transparent.

The habits of the intermediate host (Limnæus truncatulus) are of much importance, as showing the manner in which the cysts are distributed in places where they are likely to be picked up by some herbivorous mammal, within which they can attain the adult state. Limnæus truncatulus belongs to the group of fresh-water Pulmonata; it is a common snail, but one which is often very difficult to find on account of its small size and peculiar habits. It has a very wide geographical distribution, being found, according to Dr. Gwyn Jeffreys, throughout Europe, in North Asia, Morocco, Algeria, Madeira, and (doubtfully) in Guatemala. Several species belonging to the genus Limnæus occasionally crawl for short distances out of the water, but in L. truncatulus this habit is so much more strongly developed that the snail should be termed amphibious. Indeed, it is oftener found out of the water than in it. When kept in an aquarium it quits the water, and as often as it is put back crawls forth again so long as the necessary strength remains. It is said to breed on the mud at the sides of ditches. To show how much it lives out of the water I may briefly relate my own experience. There were floods on the Isis in July last, and the waters brought it down in vast multitudes, probably from its breeding haunts in marshy places far up the river. It was extremely abundant, and a single sweep of a small hand-net repeatedly gave me more than 500 examples, and this was in a ditch where previously I could not obtain a single L. truncatulus.

All along the margins of the ditches the ground was covered by them, and they were found in numbers on the flooded ground when the flood waters had retired. On returning a month later to the same ditches I was unable to find a single example alive in the water. There had been dry weather since the flood, but early that morning heavy rain had fallen, and I found numbers of specimens of L. truncatulus out on the gravel of

a path near the ditch, and these seemed to have crawled out of the grass when revived by the rain. At the roots of the grass, along the margin of the ditch, others were found in abundance. Some few shells were quite empty, but the majority contained the dried remains of the snail, which had shrunk far back into the spire of the shell. Most of these appeared to be quite dead, but were, however, merely dormant, for on placing them in water the tissues imbibed moisture and assumed their normal bulk, and after a few hours the snails had regained their full activity, and were seemingly none the worse for their prolonged desiccation. To test their power of resisting drought I collected specimens of L. truncatulus and placed them in an open vessel on a shelf in a dry laboratory, in a position where the sunshine fell on them for an hour or so daily. I found that rather more than 50 per cent. withstood twenty-six days of this treatment, and some few revived after more than six weeks. That the snails can live on moist ground quite away from any quantity of water for considerable periods, is sufficiently proved by the fact that I have kept them alive for eleven weeks on moist grass and moss, even when infested with Fasciola hepatica.[1]

It is clear, therefore, that the species of snail under consideration, when left on the fields by the passing away of a flood, continues to wander and feed so long as the bottom of the grass remains moist. It is equally clear that the numbers so left are recruited from surrounding ditches and streams. A drought may render the snail dormant, but, unless too long continued, it revives at the first shower of rain. If there are fluke-eggs on the ground and water in puddles or ditches for them to develop in, the L. truncatulus will most certainly

[1] Sir Charles Lyell ('Life,' vol. ii, p. 212), in speaking of Madeira, says that Limnaeus truncatulus was unintentionally introduced by the Portuguese thirty years before, and has spread so widely that it is now found even in the pools and ruts in the roads, so that it must have a mode of distribution which needs investigation. It will be seen from the above account that the terrestrial habits of this snail, and its power of withstanding drought, are amply sufficient to explain its spread in Madeira.

be infected with the larval forms of the liver-fluke; and owing to the habit this particular snail has of living so much out of water, either on the banks of ditches or further away towards the centre of the fields, if they are damp enough, the cercariæ will, on leaving their host, encyst on the grass in the places where they have the best chance of being transferred to the herbivorous mammals grazing on the ground. Having thus gained a suitable home they will attain the mature sexual condition, and reproduce their species by means of ova, thus completing the developmental cycle.

Man himself sometimes serves as host to the liver-fluke, and in this case the cysts are probably eaten with water-cress.

4. Growth of Sexual Fluke.—From observations,[1] which I need not describe here, it appears probable that six weeks elapse from the time of the entrance into the ultimate host before the fluke begins to produce eggs. During growth the body undergoes a very great change in form; the posterior part, which contains the reproductive organs, far outstrips the anterior part (figs. 24—26). The ventral sucker shares in some degree the greater growth of the hinder portion of the body; in the cercaria the suckers are of nearly equal size, and the same was the case in a young fluke 1·1 mm. in length. But in specimens 2—3 mm. long, the diameters of the oral and ventral suckers have usually the ratio of 1 : 1·1, and in still larger examples 6—8 mm. long, the ratio is 1 : 1·2, whilst in the adult the ratio is 1 : 1·35, though there is much individual variety.

The smallest fluke I have yet found in the liver of a sheep is represented in fig. 23; the digestive tract, which in the cercaria was simply forked, has already acquired a large number of branches, though they are comparatively simple as yet. They subsequently attain a much more complex form, owing to the number of secondary branches. This branched intestine is highly characteristic, and affords the principal reason for separating the three species which constitute the genus Fasciola from the species forming the distinct genus Distoma, none of

[1] 'Journ. R. A. S.,' 1881, p. 25.

which have a branched digestive tract. It is usually supposed that the liver-fluke passes out of the sheep at the beginning of the summer, i.e., life lasts only about three quarters of a year. But I have shown elsewhere[1] that the life of the liver-fluke may extend beyond one year, and have found both digestive and reproductive organ in full functional vigour in flukes at least thirteen months old; the oviduct was filled with eggs, and there was no indication of any exhaustion of the supply.

For an account of the economic aspects of the subject, including the discussion of preventive measures, I may refer to a paper in the forthcoming number of the 'Journal of the Royal Agricultural Society.'

It gives me much pleasure to take this opportunity of thanking Dr. Acland for kindly permitting me to use the Sanitary Laboratory of the Oxford Museum for my experiments, and Professor Moseley for kindly placing apparatus, &c., in the Anatomical Department at my disposal.

[1] Ibid., p. 26.

Note on the Early Development of Lacerta Muralis.

By

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With Plates IV, V, VI.

The following paper contains an account of some observations on the early stages in the development of Lacerta muralis, begun during the summer of this year at the zoological station at Naples and completed in the morphological laboratory at Cambridge. It relates chiefly to the mode of formation of the germinal layers and to the early development of the kidney.

On my return from Naples I found that in June last Professor C. K. Hoffmann[1] had published an account of the mode of formation of the germinal layers, and the results obtained by him agree generally with my own. As, however, Professor Hoffmann has published very few figures of the stages observed by him, and as my observations lead me to differ from him in one or two points of detail, it has seemed to me that it would not be useless to give a short account of my own results.

The segmentation, which conforms to the ordinary meroblastic type, has already been fully described and figured by

[1] C. K. Hoffmann, "Contribution à l'Histoire du Développement des Reptiles," 'Arch. Néerlandaises des Sciences exactes et Naturelles,' t. xvii.

Kupffer and Benecke[1] and by Balfour.[2] Neither of these observers describes a segmentation cavity; but Hoffmann[3] states that during the later stages of segmentation a cavity is present, the floor of which is formed by the yolk, the roof by the lower layer cells. Towards the close of segmentation it disappears.

This cavity Hoffmann considers equivalent to the segmentation cavity of the Ichthyopsida.

I have observed cavities similar to that described by Hoffmann, but I have been unable to satisfy myself that they were not due to the action of the hardening reagents employed. The cavity described by Professor Hoffmann differs strikingly, as he himself points out, from the segmentation cavity of other vertebrates, in the fact that its floor is never formed of lower layer cells.

At the close of segmentation the blastoderm consists of a superficial layer of epiblast cells, which is generally stated to be a single cell thick; in my sections, however, the arrangement is very irregular, the epiblast being in some places two cells deep, in others more.

Beneath the epiblast is an irregular sheet of lower layer cells; this layer is in many places two or three cells deep, and the cells of which it is composed are large, irregular, loaded with yolk-granules, many having two or even more deeply-staining nuclei.

In the centre of the blastoderm the epiblast cells become more columnar than in the peripheral parts, and the lower layer cells become slightly more regular in their arrangement. An oval area pellucida is thus formed.

Hoffmann finds at this stage a marked thickening of the lower layer cells at the posterior extremity of the blastoderm.

The posterior region of the area pellucida now becomes dis-

[1] Kupffer u. Benecke, 'Die erste Entwicklung am Ei der Reptilien.' Königsberg, 1878.

[2] Balfour, "On the Early Development of the Lacertilia," this Journal, vol. xix.

[3] Loc. cit.

tinguished from the anterior by the presence of the primitive streak.

A median longitudinal section through an embryo with a commencing primitive streak is shown in fig. 1. Anteriorly the area pellucida is seen to be formed by an epiblastic layer of irregular columnar cells and a sheet of lower layer cells, the two layers being quite distinct. At a point (*bp*), however, the position of the future blastopore, these layers are replaced by a mass of closely-packed cells (*pr*), exhibiting no division into layers, and forming the primitive streak, which may in some cases at least extend backwards as far as the commencement of the area opaca.

The blastopore commences at the anterior end of this streak as a pit, open above, and closed below. This is shown in fig. 2.

The floor of this pit presently breaks up, and the blastopore assumes its normal condition, forming a communication between the archenteron and the exterior, its anterior wall forming a communication between the epiblast and the lower layer cells (see fig. 3).

From this time a change in the character of the lower layer cells takes place, beginning from the anterior wall of the blastopore, where they pass into the epiblast, and proceeding forwards. Instead of being large, irregular, full of yolk, as in the previous stages, they become columnar, lose their yolk, arrange themselves in a definite layer several cells deep, and take on the characters of normal hypoblast. A median longitudinal section through an embryo, in which about half the lower layer cells are thus converted, is seen in fig. 4.

This process is evidently an invagination comparable to that which takes place in an Elasmobranch. It especially resembles the process described by Scott and Osborne[1] in the newt.

The first traces of mesoblast appear at a stage slightly earlier than that represented in fig. 4. Fig. 5, which shows a portion of a lateral section from the same series as that to which

[1] Scott and Osborne, "On the Early Development of the Common Newt,' this Journal, vol. xix.

fig. 4 belongs, shows the condition of the mesoblast shortly after its origin.

The blastopore being funnel shaped, with its narrow opening directed downwards, it appears in a lateral longitudinal section as a pit, closed below, and from its closed extremity the mesoblast grows forwards as a solid cap, separate from epiblast and hypoblast.

Transverse sections show that the mesoblast is in connection not only with the walls of the blastopore, but also with the axial strip of invaginated hypoblast. Figs. 6—13 are selected from a series of transverse sections of an embryo slightly older than that represented in fig. 4, and show the relations of the mesoblast. The figures are arranged in order from behind forwards, fig. 6 being posterior. Figs. 6—9 pass through the blastopore, and a sheet of mesoblast, continuous with its walls, is seen growing out on each side. In figs. 10 and 11, which pass through the posterior embryonic region in front of the blastopore, each sheet of mesoblast is seen to be free laterally, but to be continuous near the middle line with the axial strip of hypoblast, the cells of which will give rise to the notochord, and are easily distinguishable from the more peripheral hypoblast cells by their more elongated forms and by being more than one layer deep.

This mode of origin of the mesoblast, however, only holds good for the posterior part of the embryo. Anteriorly (fig. 11) the mesoblastic sheet loses its connection with the axial hypoblast and finally disappears (fig. 12), being replaced by branched cells, which are budded off, partly from the axial, partly from the lateral hypoblast. This mode of origin of the anterior mesoblast has been overlooked by Hoffmann.

The account above given is obviously in complete accord with the observations of Balfour,[1] who described a stage a little later than that represented in figs. 6—13, with a widely-open, neuro-enteric canal, and a sheet of mesoblast on each

[1] Balfour, "On the Early Development of the Lacertilia," &c., this Journal, xix.

side, which had separated from the axial hypoblast—all the layers being, however, still fused in front of the blastopore.

The statement of Kupffer,[1] that the blastoporic invagination gives rise to a closed sac, the walls of which become the allantois, is of course inconsistent with the truth of the above observations; but it has been already so abundantly disproved, first by Balfour and afterwards by Stahl and Hoffmann, that it is not necessary here to do more than refer to it in passing.

The actual mode of development of the allantois was first figured by Balfour,[2] a copy of whose drawing is reproduced in the woodcut. The details of the process were worked out by Strahl.[3]

I have nothing to add to the account given by these authors, but I would call attention to a consequence of it which neither observer has, to my knowledge, remarked.

It is obvious from the woodcut that, as has been shown in detail by Strahl,[4] the allantois arises as a process of the primitive streak, which projects at first backwards into the body cavity.

Now, if this be the case, when the primitive streak is bent ventralwards during the establishment of the tail fold, the primitive streak must extend in the middle line from the posterior extremity of the medullary canal, round the end of the embryo, as far forwards as the point of connection of the allantoid stalk, with head cut; and therefore the proctodœum, when it arises, must not pass through the primitive streak.

Therefore, if we adopt the view of Balfour, that the primitive streak represents the position of the blastopore of other gastrulæ, we shall be forced to conclude that, at any rate in this group of Craniata, the anus is in the position of a part of

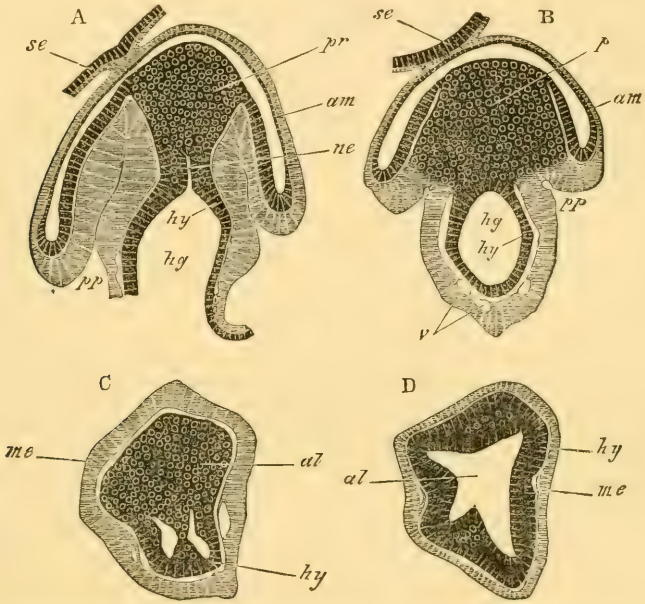
[1] Kupffer, "Die Gastrulation an den Meroblastischen Eiern der Wirbelthiere und die Bedeutung des Primitiv Streif," 'Arch. f. Anat. u. Phys.,' 1882.

[2] "On the Early Development of the Lacertilia," &c., this Journal xix.

[3] Strahl, "Ueber die Entwicklung des Canalis Myelocnteriens und der Allantois der Eidechse," 'Archiv. f. Anat. u. Phys.,' 1882.

[4] Loc. cit.

the blastopore—a supposition which simplifies our ideas as to the origin of the vertebrate anus in general.



FOUR TRANSVERSE SECTIONS THROUGH THE HINDER END OF A YOUNG EMBRYO OF LACERTA MURALIS (Balfour).

Sections A and B pass through the whole embryo, while C and D only pass through the allantois, which at this stage projects backwards into the section of the body cavity behind the primitive streak. *ne.* Neurenteric canal. *pr.* Primitive streak. *hg.* Hind gut. *hy.* Hypoblast. *pp.* Body cavity. *am.* Amnion. *se.* Serous envelope (outer limb of amnion fold not yet separated from the inner limb or true amnion). *al.* Allantois. *me.* Mesoblastic wall of allantois.

The development of the kidney has been described by Braun.[1] My observations lead me, however, to believe that his account of the mode of origin of the segmental tubules and of the Wolffian duct is erroneous.

[2] Braun, "Das Urogenitalsystem der einheimischen Reptilien," 'Arb. aus d. Zoolog. Inst. z. Würzburg,' Bd. iv, 1878.

According to him, the first part of the urino-genital system which appears is the Wolffian duct. He says: "In an embryo of Lacerta agilis, barely 5 mm. long, in sections just below the heart, I find the Wolffian duct lying close to the lateral mesoblast plates, in a region belonging neither to these nor to the protovertebræ, but lying between the two as a semicircular mass of cells, sharply defined towards the ectoderm, but passing gradually into the lateral mesoblast; in the middle of this cell mass is a lumen . . . . " which he considers to be the lumen of the Wolffian duct.

In the next stage described by Braun, a number of segmentally arranged vesicles are present, which are for a short time attached to the peritoneal epithelium, their cavities also opening for a short time into the body cavity, but which afterwards break away, form the well-known S-shaped tubes, and communicate with the Wolffian duct.

From this account it is evident that Braun has not investigated embryos less than 5 mm. long. I have been fortunate enough to obtain younger embryos, and have been led to somewhat different conclusions.

On the formation of the protovertebræ, each protovertebra does not at once become completely separated from the lateral mesoblast, but remains connected at a certain point with a continuous solid ridge of tissue, generally in early stages about two cells thick, which projects inwards from the peritoneal epithelium, thus forming an "intermediate cell mass" comparable with the structure so called in birds.

Figs. 15 and 16 show the characters of this ridge in an embryo of about seven protovertebræ; fig. 15 is taken from a vertebral region, and shows the ridge (*i. c. m.*) connecting the protovertebra with the peritoneal epithelium; fig. 16 is from the next intervertebral region, showing the ridge projecting freely inwards from the peritoneum. In fig. 16 traces of a prolongation of the body cavity into the intermediate cell mass may be observed. In an embryo with ten protovertebræ this cell mass, without losing its connection with the protovertebræ, swells up and becomes semicircular in

section, the convexity being directed outwards; this condition is shown for vertebral regions in fig. 17, for intervertebral in fig. 18.

At a stage with eleven protovertebræ, the vertebral portions of the intermediate cell mass, behind the fourth protovertebra, acquire a circular lumen, which is bounded by a single layer of columnar cells; this condition is seen in fig. 19. In fig. 20, which represents a section passing through the end of the same protovertebra as that from which fig. 19 is taken, the lumen is smaller; in the intervertebral region behind the lumen altogether vanishes, and the solid, swollen cell mass presents an appearance exactly like that seen in the preceding stage (fig. 18).

There is thus formed a series of cavities in the continuous intermediate cell mass, each situated opposite a protovertebra, and having its walls continuous both with the protovertebra and with the peritoneal epithelium. These cavities are separated from one another by the solid intervertebral parts of the intermediate cell mass.

In embryos with eleven protovertebræ there are five of these vesicles, opposite the fifth to the tenth protovertebra, the last two somites being as yet without them. In these last somites the intermediate cell mass is swollen and solid, as in the anterior region of an earlier embryo.

These cavities are, as will be seen from their subsequent history, the segmental vesicles described by Rathke and subsequent writers.

They have hitherto been described entirely separate from one another, and have been supposed (Braun., loc. cit) to arise as invaginations of the peritoneal epithelium.

When twelve protovertebræ are present the Wolffian duct begins to appear as a solid cord of cells, splitting off in the intervertebral region only from the intermediate cell mass, and passing, in the region of each protovertebra, into the wall of a segmental vesicle.

Figs. 21—23 represent three sections through about the sixth and seventh somites of an embryo with twelve proto-

vertebræ. Fig. 21, the most anterior, passes through a vertebral region, and shows the segmental vesicle, with its lumen; the section passes through the attachment to the peritoneum (which in the vertebral regions is becoming smaller), but not through the connection with the protovertebra. The next section (fig. 22), through the commencement of the intervertebral region, shows the solid cell mass, with a few cells (*w. d.*) split off from its outer portion. These cells are the rudiment of the Wolffian duct. In the next protovertebral region this cord ceases to be visible. Fig. 23 shows a section through the commencement of the next protovertebra, passing through the solid wall of the corresponding vesicle, which has no trace of the duct.

These cords of cells are present at this stage in four intervertebral areas, behind protovertebræ five to eight inclusive.

With the formation of the thirteenth protovertebra the solid rudiment of the Wolffian duct becomes more distinctly split off in the intervertebral regions, while opposite the protovertebræ it appears as a solid appendage of the wall of the segmental vesicles, with which it is perfectly continuous.

At the same time it extends backwards into the ninth intervertebral region.

In an embryo with fourteen protovertebræ there are eight segmental vesicles with a lumen opposite the protovertebræ five to twelve inclusive. All these have the Wolffian duct as a solid knob on their outer wall, while in the corresponding intervertebral regions there appears a distinct lumen in the duct, which is more or less completely split off from the rest of the intermediate cell mass.

The relations of the duct and vesicle in an embryo with fourteen somites are shown in fig. 24, from the second segmental vesicle of such an embryo. In this figure the segmental vesicle (*s. v.*) is seen to have a large lumen, and the solid Wolffian duct (*w. d.*) appears attached to its outer wall.

In fig. 25, from the next intervertebral region behind fig. 24, the Wolffian duct has a large lumen, and is attached to the solid intervertebral cell mass.

A section through the next protovertebra would repeat the features shown in fig. 24.

On the appearance of the fifteenth protovertebra the lumen of the Wolffian duct becomes continuous throughout the region of the first eight segments, and at the same time it acquires a communication with the cavity of each segmental vesicle in its course.

The first eight segmental tubules are therefore differentiated, continuously with the Wolffian duct, from a ridge of cells, continuous at first along its entire length with the peritoneal epithelium, and at certain points with the adjacent protovertebræ.

With regard to the tubules behind the eighth, they are developed from the intermediate cell mass in exactly the same way as those in front; but the Wolffian duct, instead of arising continuously with them, grows backwards as a free projection of the above-described portion, without coming into relation with adjacent structures. It is at first solid, but afterwards acquires a lumen, and becomes connected with the segmental vesicles in order from before backwards.

On the subsequent behaviour of the tubules and on the development of the metanephros I have no observations.

The most interesting feature in the preceding account of the early development of the lacertilian kidney is the close resemblance which it shows to exist between the process of development in that group and the process which has been shown by Sedgwick[1] to exist in birds and Elasmobranchs. In both these groups Sedgwick has shown that the segmental tubules arise from a continuous cell mass connected with the peritoneal epithelium and with the mesoblastic somites, which cell mass is present from the very beginning of the process of mesoblastic segmentation.

In the anterior part of the Wolffian body of the chick

[1] Sedgwick, "Development of the Kidney in its relation to the Wolffian Body in the Chick," this Journal, vol. xx; and "Early Development of the Wolffian Duct and anterior Wolffian Tubules in the Chick," &c., this Journal, vol. xxi.

Sedgwick has shown that the Wolffian duct and segmental tubules arise continuously by differentiation of the cell mass. In the chick, as in the lizard, the independent origin of duct and tubules in the posterior region is probably a secondary character.

In conclusion, I wish to express my gratitude to the authorities of the Zoological Station at Naples for their kindness to me during my visit, and to Mr. Sedgwick for the advice and assistance which he has given me since my return to Cambridge.

On a Crustacean Larva at one time supposed to be the Larva of Limulus.

By the late

R. von Willemoes-Suhm, Ph.D.,
Naturalist on board H.M.S. "Challenger."

With Plate VII.

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[The manuscript and figures of Dr. v. Willemoes-Suhm relative to a Crustacean larva, which he at one time was led to regard as the larval form of Limulus molluccanus, have been placed in my hands for publication. I am informed by Professor Moseley that before his death Suhm was definitely of opinion that the larva in question belonged to a Cirrhipede. In the mean time a reference to its supposed connection with Limulus had been published in Suhm's 'Challenger Briefe,' In consequence of this, as well as in view of the intrinsic interest of the form, I think it well to publish Suhm's drawings and description in full. In the description I have in brackets made such additions as are consistent with Suhm's later interpretation of the larvæ.—E. Ray Lankester.]

On her way from the Moluccas to Hongkong, and when sailing from this port to New Guinea, H.M.S. "Challenger" went twice through the Philippines, and both times touched at Zamboanga. This is a port in the south-western part of Mindanao, on the Straits of Basilan, so called from the large island which, with numerous small ones, forms the opposite side of the straits. There a very intelligent native brought a

living Limulus rotundicauda, Latr, which, according to Milne-Edwards, is also found in the Moluccas. The man was, of course, asked to bring more of them, but declared he could not do so unless we went over to the other side of the straits to Basilan or Malamani, where his specimen had been caught. This was done afterwards, when the ship had to go there in order to take in coals; but during the short time of our stay no king-crabs could be procured, though I offered high rewards, and some men, who said they knew where to find them, were sent out, but never came back to bring anything. It appears that this Limulus inhabits the shallow water round the small islands, a great many of which are to be found in these straits. The native who had brought me the first specimen was further asked whether he knew what they did with their eggs, and he at once pointed to the swimmerettes as the place where these were attached. It appears also that L. molluccanus carries its eggs about, for it is stated that the animal with its eggs is frequently brought to the market of Batavia, where they are both eaten. If this is the case the American king-crabs differ in habits very much from their eastern cousins, for, according to Lockwood, they deposit their eggs in a sand-hole, where these are fecundated by the male, and then left to themselves. Unfortunately the shortness of our stay in Zamboanga did not allow me to investigate this question more thoroughly, nor did I attach at first so much importance to the matter, thinking the development of the eastern king-crab would be very much the same as that of the American one. But to my greatest astonishment I found one day among the surface animals brought up by the towing-net from behind the ship, Nauplii and larvæ in different stages, which [at the time appeared to me to] clearly belong to Limulus. The next days my attention was, of course, entirely directed to them, and I succeeded in getting the whole series of stages from the newly-hatched Nauplias to the larva, which shows already under its skin the abdominal and the first traces of the thoracic appendages. In the whole these larvæ were rare, at night commoner than in daytime. Altogether I

think they had been seized by the current and carried away from their breeding places, otherwise they would have been more common. I found these larvæ not only in Zamboanga, but also during the night which we spent in the narrow channel between Basilan and Malamani. At our return to the former place another current had set in, bringing in pelagic animals and sweeping away all the small larvæ.

After this we took to determining the surface animals, which had been kept in spirit during the time when the [supposed] young Limuli were found, and among them we got some more larvæ, so that satisfactory drawings, and also some preparations showing the Nauplius with only one eye and the larvæ with the additional large lateral eyes, could be made. (See Plate VII.)

The Nauplii [in question] are easily recognisable from the position in which they hold their antennæ, which are never carried in an upward position, but always either at a right angle to the body, in a horizontal position or directed backwards. As soon as the animal rests or is touched by the covering glass all the antennæ take the latter position, which makes it rather difficult to the observer to make them out. Another very characteristic point in all the Nauplii, except in the very earliest stage, is the pointed caudal portion, [which erroneously suggested] the future spine [of a Limulus]. Even before the large lateral eyes have come out, and before the division of the body into three regions is complete, i.e. after the first moult, this [larva supposed to be a] young Limulus is very easily to be distinguished by the cordiform shape of its body, its peculiar antennæ, and the pointed abdomen. This guided me with great certainty, for it is at first by no means easy to find out the [supposed] small king-crabs among the enormous number of larvæ which swarm on the surface near these tropical shores.

In the first stage, the one in which I imagine the (supposed) young Limulus to leave the egg, we see a somewhat oval-shaped embryo filled with yolk and granulations, with one central eye and three pairs of appendages (Pl. VII, fig. 1). I am,

however, not quite sure whether this first stage really belongs to the larvæ, which I am now going to describe, as I did not see it leaving the egg, but consider this to be the case from the form and position of its antennæ. In the second stage, which undoubtedly belongs to this series, the body is divided into a thoracico-abdominal portion [the shield being not yet formed] and the jointed spine, the whole Nauplius being of an elongate heart shape (fig. 2 *a*, 2 *b*). The inferior part of the body, the future abdomen, is divided into nine segments, separated by a ridge, which gives their edges a spiny appearance. The last of these ends in two large lateral spines. [What seemed to correspond to], the spine of Limulus in this stage consists of seven segments, the last of which ends in a point. Of appendages there are three pairs, the first being just opposite the Nauplius eye, and showing no joints, with three hairs at the top. The second consists of five joints and has a two-jointed flagellum. The third pair finally has about the same number of joints and very nearly the same length, also showing a small flagellum. There are as yet no parts of the mouth, nor does the interior, which is filled with large globules of yolk, show as yet any differentiation. Above the Nauplius eye, which is very conspicuous, we remark a small lens. In this stage the embryo has a length of about 0·14 mm., and it is the one in which it was most commonly met with at Zamboanga.

In the next stage, the third, it has grown a little in size, but shows as yet very few differences from the former stage, except that the walls of the intestine begin to form and show contractions, without as yet communicating with the anal opening (fig. 3).

After this, one of the most important changes goes on, for with the fourth moulting the shield appears, and the body up to this period, consisting of two divisions only, shows three parts simulating those of a full-grown Limulus, the head and thorax with its shield, the abdomen, and the jointed spine, The Nauplius has now a length of 0·36 mm. (figs, 4 *a*, 4 *b*).

The shield is rounded at the edges, just as Milne-Edwards

describes it in the young Limulus which he figured in ' Cuvier's Règne animal.' Only once I saw the edges ending in a rounded point, a case which I think was due to a folding in of the skin, and therefore could not be taken as evidence. In all the subsequent free swimming stages the edges of the shield (the diameter of which is 0·20 mm.) were round. The abdomen shows its nine larval segments, and under the thin chitinous covering you distinguish already clearly six newly formed segments, with lamellar appendages on all of them. The latter can be best observed in a side view, when also the hairs or their ends are already to be remarked. They consist of two joints, the terminal being the shortest.

The [region which simulated the] spine [of an adult Limulus] being 0·056 mm. long [about one sixth of the whole length] now shows eleven larval segments, with slightly serrated edges. The terminal point has about the length of three of the preceding segments.

The appendages are the same as in the former stages—the three nauplial antennæ. The eye also is still the simple Nauplius eye with a lens, but in one case we saw two lenses above the black spot, perhaps the earliest trace of the later subdivision of the central eye into two. To the right and the left of the eye there are still large globules of yolk, filling the carapace. The organs of digestion show some changes, for the upper part of the intestine is widened (the future muscular stomach), and the position of the mouth, with which there is as yet, however, no communication, is indicated by a chitinous under lip. The anus is formed and communicates with the intestine by a short rectum. It opens between two large spines at the base of the last abdominal segment. Some yolk globules are as yet left in the thorax, and some others are included in the intestine.

The fifth stage is signalised by the appearance of the two large lateral eyes, which are situated a little below the base of the first antennæ. They consist of intensely black pigment, and a circle round them indicates already the growing connection between these large globular spots and the surface of

the carapace. In this stage the six segments of the thorax shine still more clearly through the chitinous covering, and in a side view one gets a most perfect idea of the swimmerettes. The animal remains, however, as far as its appendages are concerned, in the Nauplius stage, and in this it remains as long as it swims on the surface, for in the last stage, in which three pairs of thoracic legs were visible on both sides of the mouth under the larval skin, no abdominal feet had as yet disengaged themselves. The animal has grown a little, its spine is somewhat longer and more pointed, but it still must be considered as a larva.

[It will be interesting to know precisely to what animal Suhm's Philippine larva above described belongs. It differs from all known Cirrhipede larvæ in the structure of the tail. There is no ground whatever for reviving the view, discarded by Suhm himself, that this larva belongs to a Limulus.—E. R. L.]

On Plasmolysis and its bearing upon the Relations between Cell Wall and Protoplasm.

By

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(From the Jodrell Laboratory, Royal Gardens, Kew.)

With Plate VIII.

It is not surprising that, after devoting their efforts for so long to the study of the nucleus, botanists should again turn their attention to the cell wall and its relation to the protoplasm. It was only to be expected that by the application of those accurate methods of study, elaborated during investigations of the nucleus, to the formation and origin of the cell wall, new results would be obtained. Such expectation has been amply justified by the works of Dippel, Schmitz, and Strasburger. The mode of increase of substance of cell walls by apposition, and more especially the mode of formation of walls in the first instance in cell division by the lateral coalescence of "microsomata,"[1] leads naturally to the supposition that if there be such a genetic connection between the cell wall and the protoplasmic body, it would also be possible to demonstrate that the physical connection between them is very close. Further, the idea that cells may be connected with one another by delicate threads of protoplasm, which keep up a protoplasmic continuity through their cell walls, also presents itself as a natural corollary on these observations.[2] Such con-

[1] Strasburger, 'Ueber den Bau und das Wachsthum der Zellhaüte,' p. 174.

[2] Cf., Strasburger, l. c., p. 246.

tinuity has actually been observed by Tangl[1] in the endosperm of certain seeds, and by Gardiner[2] in the pitted cells of the pulvinus of Mimosa, Robinia, and Amicia. Again, Fromman[3] states that he has been able to observe, in various cases, a continuous network extending from the protoplasm into the cell wall.

Such observations as these do not harmonise with the view at present held of plasmolysis, which is derived mainly from the writings of H. de Vries.[4] According to his descriptions of the process of plasmolysis (l. c., pp. 37—39, and esp. pp. 47, 48), the protoplasmic body would appear to separate with a "smooth surface" from the cell wall on treatment with the plasmolysing solution, and, when the solution is strong enough, to be completely isolated from the cell wall. Hence is derived the idea, which is, it is true, more often tacitly understood than directly expressed in words, that the smooth surface of the protoplasmic body is merely in apposition with the cell wall, and not more closely connected.

The observations detailed below will tend to show that results obtained by plasmolysis do not disagree with those obtained by the direct observations of the above-named authors, i. e. that the connection of the protoplasmic body with the cell wall is very close.

Before entering upon the description of my own observations it would be well shortly to review the chief sources from which our present information on the subject is derived.

V. Mohl, in his treatise on the vegetable cell, speaking of the "primordial utricle," remarks that "it usually adheres firmly to the cell wall."[5] His results were, however, obtained for the most part by treatment with acids, &c.

[1] Pringsh., 'Jahrb.,' vol. xii, p. 170.

[2] 'Quart. Journ. Micr. Sci.,' Oct., 1882; 'Roy. Soc. Proc.,' Nov. 11, 1882.

[3] 'Beob. über Structur und Beweg. d. Protoplasma der Pflanzenzellen.' Jena, 1880.

[4] 'Unters. über die Mechanischen Ursachen der Zellstreckung.' Leipzig, 1877.

[5] V. Mohl, 'Vegetable Cell,' English translation, p. 37.

In an early work by Pringsheim[1] a description is given of the process of plasmolysis, in which (pp. 12, 13) he compares the separation of the protoplasm from the cell wall with the separation of a sticky substance from a membrane to which it had hitherto adhered. He further notices the way in which the protoplasm remains here and there adherent to the cell wall, while sometimes, though separated almost entirely from the wall, it remains connected with it by isolated threads of protoplasm. He goes on to describe how these strings, after undergoing various changes of form, finally break off (cf. his Taf. iii, figs. 16—21).[2] Naegeli ('Pflanzenphysiologische Untersuchungen,' 1855, Heft 1) also observed and described strings of protoplasm which connect the contracted protoplasmic body with the cell wall in plasmolysed cells. He observed them in various instances (epidermis of petals, Spirogyra, &c.), but did not recognise their appearance as of general occurrence (cf. his Taf. i, 23; Taf. ii, 2—6; Taf. iii, 4, 5, 12). He also notes in Spirogyra that strings are often attached at corresponding points on opposite sides of the wall, but leaves it an open question whether this is significant or not.

Hofmeister[3] describes the appearance of the contracted protoplasm of cells with large vacuoles (p. 8, &c.) as lying free in the cell cavity, but makes no mention of any connecting protoplasmic strings as of general occurrence, though (p. 15) he notices the occurrence of such strings connecting the contracted protoplasm of cells of certain Algæ with the terminal walls.

It is to H. de Vries[4] that we owe the most extended treatment of the subject of the action of dehydrating reagents upon the

[1] 'Bau und Bildung der Pflanzenzelle,' 1854.

[2] From his description and figures, I conclude that Pringsheim has only seen the coarser strings to be described below. As I there point out, however, the difference between these and the finer strings, which appear to have escaped his observation, is only one of degree.

[3] 'Die Pflanzenzelle,' 1867.

[4] 'Unters. über die Mechanischen Ursachen der Zellstreckung.' Leipzig, 1877.

living cell. We may leave on one side the very valuable conclusions as to the connection between turgescence and growth, which he obtained by the use of plasmolysis, since these fall outside my present subject. It is unfortunate that the importance of these conclusions made him lose sight of the structural details, which had already been partially observed by Naegeli and Pringsheim. He even quotes (p. 38) the description of the latter word for word, though in the text he repeatedly ignores his results, speaking of the contracted protoplasm as free on all sides ("allseitig frei," pp. 9, 38, &c.). His figures (p. 35) also represent the contracted protoplasm as completely disconnected from the wall, with which it was originally in contact.

The results of these earlier observations being thus but little taken into account in what is certainly the most important of the more recent works on this subject, it was only natural that for a time no further advance should be made. The description of de Vries and his figures were adopted in text-books subsequently written, and, as far as I know, there has been no further statement on this subject[1] till Gardiner, in a notice communicated to the Royal Society (Nov. 11, 1882), described observations on plasmolysis of cells of the pulvinus of Robinia pseudacacia, which were made in connection with his work "On the Continuity of Protoplasm in the Motile Organs of Leaves."[2] He also extended his observations to pulvini of a number of other plants, and also to stems and roots. He found that in a very great number of cases strings of protoplasm connect the contracted protoplasmic body with the cell wall. His attention was naturally attracted to the relation of these strings to the pits, and he found that "in several well-defined instances many threads do go to pits, and also that in two adjoining cells many threads on different sides of the common cell wall are exactly opposite one another."

Before these observations of Gardiner were published, and

[1] The matter seems to have been entirely overlooked by Pfeffer in his 'Osmotische Untersuchungen,' and in his 'Pflanzenphysiologie.'

[2] 'Quart. Journ. Micr. Sci.,' 1882.

quite independently of them, I had already arrived at conclusions in the main similar to them, as the result of observations on the plasmolysis of the prothalli of ferns, which were instituted with a very different object, viz. that of finding whether plasmolytic contraction of the protoplasmic body would be a good method for preparing the apical region of the prothallus, so as to show the form and arrangement of the individual cells. It was impossible to overlook the fact that strings of protoplasm are very universally to be seen connecting the contracted protoplasm with the cell wall in cells of prothalli thus prepared.

For the reasons which determined the choice of De Vries (l. c., pp. 7—13) I have adopted as the dehydrating agent solutions of common salt of varying strength, from 1 per cent. to 10 per cent., according to the requirements of the object under treatment. It has been my practice to use as weak a solution as will suffice to bring about the desired result, and it will be seen that in the majority of cases solutions varying from 2 per cent. to 5 per cent. have proved strong enough. As changes in the appearance of the protoplasm follow slowly upon its contraction, the time at which certain appearances are presented is usually given. The following are the details of the experiments:

Prothallus of Nephrodium villosum and Aspidium Filix-mas.

On treating a prothallus of either of the above species (others have not been examined) with a 2 per-cent. solution of common salt, the protoplasmic body in each cell is seen to separate itself gradually from the cell wall, the process beginning as a rule at the corners of the cells. The contraction goes on slowly for a considerable time, and usually results in the protoplasmic body assuming a more or less regular spherical form, as has been frequently described by former writers. When stronger solutions are used the contraction is more rapid but usually less regular.

When the protoplasm first contracts in this way there is

often little or no visible connection remaining between it and the cell wall. Frequently, however, there is to be seen from the first a faint silky striation in the space between the protoplasmic body and the cell wall, running in a radiating manner between them. This is in most cases extremely delicate, and even with Zeiss, obj. F, it is sometimes impossible to define the appearance as any distinct system of lines. Again, in other instances coarser threads, the outlines of which can readily be made out with high powers, are seen from the first to maintain a connection between the cell wall and the contracted protoplasm. On these coarser threads are often to be seen nodal thickenings, similar to those described by Gardiner. Though the above difference is easily recognised under the microscope, there can be little doubt that the appearances are merely phases of one and the same phenomenon.

In those cases where there is at first no visible trace of a connection between the protoplasm and the cell-wall, there usually appears, after the lapse of a short time, a striation of the intervening space similar to that which may often be observed from the first; while in the latter case the striation becomes more obvious, and after a short time (e. g. quarter or half an hour) it may be seen that it is due to the existence of numerous very delicate threads, which extend from the protoplasmic body to the cell wall. Some idea of the fineness of these threads in the first instance may be gained from the fact that they cannot be individually defined even with high power (F., Zeiss). Fig. 1 represents cells as they appear about a quarter of an hour after plasmolysis;[1] the threads, being tense at first, appear quite straight. Some time after plasmolysis has taken place, and the strings have become more obvious, they may be seen to be executing rapid and more or less irregular vibratory movements; these show that they are not then very tightly stretched. The strings run not only to those walls which separate contiguous cells, but also to the free marginal walls as represented in the figure, and further, as may be ascertained by careful focussing, to the walls which

[1] Compare Pringsheim's fig. 16, Taf. iii, l. c.

form the upper and lower surfaces of the prothallus. As far as I was able to judge, they run as a rule in just as large numbers to the free walls as to the walls separating contiguous cells.

It may often be seen that strings appear to cross one another, as in the lower cell in fig. 1. This appearance may be explained by reference to the protoplasmic body, which will in such cases be found to have contracted irregularly. It may also be seen that strings, which thus cross one another, are not in the same plane, a conclusion which might easily be drawn from fig. 1. Where, as in other cells of fig. 1, the contraction goes on more regularly, such crossing of the strings is not seen.

Remembering Strasburger's observations on the formation of the walls in cell division, as well as the results obtained by Tangl and Gardiner, it was of course a matter of interest to observe whether these strings in two contiguous cells are opposite to one another, and thus point to a direct continuity of protoplasm through the walls, or whether this is not the case. In many instances it does appear that the strings on opposite sides of a wall are attached at corresponding opposite points; in a much greater proportion of cases, however, they appear to have no relation to one another, but to be distributed quite independently over the walls. It should be remembered in connection with this that the strings run with equal frequency to the free walls, and to those separating contiguous cells.

Such connection of the contracted protoplasm with the cell wall, as that above described, is found to exist in the cells throughout the prothallus. It has been observed in the cells at the extreme growing point in young prothalli, and also in the root hairs at points close to their apex. In such cells, however, the phenomenon is not so well marked as in cells of medium age, the threads being of finer texture.

That these connecting strings consist of protoplasmic substance can hardly be doubted from their mode of origin and their properties to be detailed below; the application of re-

agents to them is, however, a matter of difficulty, as, under the action of reagents which injure living protoplasm, they assume a ropy appearance, and often break away, while they refuse to take up neutral colouring matters. It has been ascertained that they stain slightly brown with iodine solution, while they give a characteristic reaction with gold chloride.[1]

It has been stated above that the strings, which are at first as a rule extremely thin, become more obvious a short time after plasmolysis, there being usually a marked change in the first quarter of an hour (fig. 11). This is due to an increase in thickness of the strings, which might be produced by either of two processes, or by both simultaneously—(1) by the supply of fresh substance from the main protoplasmic body; (2) by the lateral coalescence of two or more originally separate strings.

Exact observation shows that the first process does take part in the change. It has been noted above that nodal swellings are sometimes to be found on the threads. By fixing the pointer of an indicating eyepiece upon one of these swellings, on a thread of a recently plasmolysed cell, and watching it for a period of a quarter of an hour or more, it has been seen and verified in a number of instances that the nodal swelling moves slowly from the main mass of protoplasm. Since the motion is, as far as my observations go, always from the main mass of protoplasm, we have thus an indication of the supply of fresh substance from it to the threads, which may account for the increasing prominence of the latter. The lateral vibratory motion, which is seen in the strings some time after plasmolysis, but is not so marked or is absent immediately after the contraction, has been alluded to above. From these movements it is inferred that the strings, though apparently tightly stretched at first, become gradually slacker as time goes on, a conclusion which harmonises with the observations

[1] The method adopted was as follows: after plasmolysis with 3 per-cent salt solution treat with a solution containing 3 per cent. salt and 1 per-cent. gold chloride, then wash with water and expose to the light in very dilute acetic acid.

on the movement of the nodal swellings from the main mass of protoplasm. It may then be inferred that fresh substance is derived from the main mass of protoplasm after the original plasmolytic contraction.

The question still remains, whether the increase in prominence of the strings may not in part be due to lateral coalescence of originally separate strings. I have no direct evidence that such coalescence does occur. Branching strings, such as those represented in figs. 2, B, and 3, A, B, are often to be found, which might appear to give colour to the idea that the branches had originally been separate, and had subsequently coalesced. It is, however, as far as my observations go, a universal rule that the branching is in the direction of the cell wall. This being the case, and taking into account the process of drawing out of fresh material from the main mass of protoplasm as above described, the following is a more probable explanation of such branchings. That two strings (or more), originally separate but attached to the main body of protoplasm at points very close to one another, had drawn out from that body a common string on which they appear as branches. Direct evidence that such a process does take place is afforded by such objects as are represented in fig. 3, A and B. In A are seen numerous strings, branched and unbranched, as they appeared twenty minutes after plasmolysis. B represents the same cell half an hour later; only one of the most prominent branched strings is drawn; on comparing it with the corresponding string in A it will readily be seen that the change of appearance points to a process such as that above suggested. The instances of branching, which are represented in the figures, are only the last and roughest examples of the process above described. On examining cells of prothalli soon after plasmolysis with a high power (Hartnack, 13), it was seen that not uncommonly strings, which appeared single throughout the greater part of their length, branched close to the cell wall, and were thus attached at a number of points.

It would appear, then, that the change, which gradually

comes over the strings after plasmolysis, is due, at least in a great measure, to a drawing out of fresh substance from the main protoplastic body, and a consequent thickening of the individual strings, which at the same time become less tightly stretched. It is not, however, asserted that lateral coalescence of strings never occurs; it is only to be expected that in their rapid vibratory movements strings should come into contact with one another and remain coherent, but this has not been directly observed.

It has been stated that before reagents, which are liable to injure living protoplasm, the strings alter their appearance, become ropy and slack, and often break away. Similar changes occur after plasmolysis has been continued for a long time, and death supervenes in the plasmolysed cells (cf. de Vries, l.c., p. 66, &c.). In dead cells the contracted protoplasm is completely isolated, or only connected with the walls by a few ropy strings, which differ in general appearance from those of living cells, and do not show the vibratory movements.

When the strings break away their free ends often execute irregular movements, while they contract gradually, as described by Pringsheim and Gardiner, on the one hand to the protoplasm, on the other to the cell wall.

It has already been noted by several observers in various plants that the protoplasm does not always contract as a single mass. This is sometimes the case in cells of the prothallus, the protoplasm dividing into two (or more?) rounded portions; when this occurs the masses are usually seen to be connected by strings of protoplasm of rather coarse texture; these are, doubtless, of a similar nature to those which connect the contracted protoplasm with the cell wall.

In conclusion, it may be noted that the walls separating contiguous cells of the prothallus of the above species have not a perfectly smooth surface, but show, after the protoplasm has receded, slight inequalities in thickness when observed with a high power.

Such being the results obtained by the study of plasmolysis of cells of prothalli of ferns, the next step was to see whether

these phenomena are of general occurrence in vegetable cells, and more especially whether they are to be observed in those of which the plasmolysed condition has already been described by other writers.

It being already late in the year, young flower stalks of Cephalaria leucantha (the plant used by de Vries) were not to be had: experiments were, however, made with a 5 per-cent. salt solution upon sections of young leafy stems of this plant, with the result that, though the material was not very favorable, strings of protoplasm, similar to those seen in the prothallus, were found connecting the contracted protoplasm with the cell wall in a large number of cells of the cortical parenchyma.

Young flower stalks of an allied species (Cephalaria rigida) were also used: sections were cut through the cortical parenchyma and treated with 5 per-cent. salt solution. The same phenomena, as seen in the prothallus, were again reproduced here in their chief features: the strings of protoplasm, at first not well seen, were quite obvious in the cells after the lapse of one hour (fig. 4).

The observations of Gardiner on the beet were also verified, it being found that here, on plasmolysis with 10 per-cent. salt solution, strings of protoplasm remain connecting the contracted protoplasm with the cell wall. They have frequent nodal swellings, but the strings are not so numerous nor so regular as in the prothallus.

Sections of the flesh of a ripe apple were also subjected to the same treatment with results similar to those obtained in the beet.

In leaves of Vallisneria spiralis strings, forming a fine radiating system, are seen some time after plasmolysis with a 5 per-cent. salt solution.

The diaphragms of the intercellular spaces of water plants supply very good material for the study of the phenomena of plasmolysis in parenchymatous cells. Those of the petioles of Limnocharis, sp. Aponogeton distachyon, Alisma Plantago, and Pontederia (Eichornia) cœrulea,

were used; in all of these the process of plasmolysis was observed, its main features being the same here as above described for the prothallus.

Special attention was given to the diaphragms of the petiole of Pontederia (Eichornia) cœrulea, which consist of flattened, polygonal, thin-walled cells, in close contact with one another, except at the angles where three or more cells meet; at these points are intercellular spaces, which act as channels of communication between the cavities above and below the diaphragm (cf. figs. 5, 6). On treating a transverse section, including a diaphragm, with 1 per-cent. salt solution, a slight contraction of the protoplasm takes place. In a very large number of cases it is found that the protoplasm first leaves the wall at those points where two cells are separated from one another by a thin septum, while it still remains in contact with the parts of the wall adjoining the intercellular spaces. This would not be the case, if the connection of the protoplasm with the septa were more close than with the walls adjoining intercellular spaces; hence it may be inferred that it is not so. In fig. 5, which illustrates this, and which was drawn immediately after plasmolysis, there are no strings to be seen running to the cell walls; but when plasmolysis is more complete, and, after the lapse of a short time, numerous strings may here be seen, as in other cases (fig. 6). It is found that strings run both to the septa and to the walls adjoining intercellular spaces, and no distinction in the numbers which run to these different parts of the wall has been observed. Comparing this observation with the fact that the strings run in as large numbers to the free walls as to the septa in the prothallus, it is seen that the same inference may be drawn from both cases, viz. that as far as evidence from plasmolysis goes, the connection of the protoplasm is just as close with the free walls as with walls separating contiguous cells.

It should be noted that also in Pontederia the septa dividing contiguous cells have not a perfectly smooth surface, though there are no obvious pits in the usual sense of the term.

Observations were also made on the cells of the amphigastria of Lunularia and Marchantia, a 2 per-cent. solution of salt being found strong enough to induce plasmolysis. The protoplasm in these cells is very meagre; when contracted it was seen to be connected with the cell wall by a few long, fine strings of protoplasm.

Filaments of Spirogyra were also treated with salt solutions of various strengths (2, 5, and 10 per cent). The protoplasm of each cell contracts into a rounded mass, usually leaving the septa entirely, but often remaining in contact with the lateral walls. Here also fine strings of protoplasm run from the contracted mass to the walls, more especially to the septa. They often have nodal thickenings, and execute obvious vibratory movements. The phenomenon is better seen on plasmolysis with 10 per cent. than with weaker solutions, and even then it is seen only with difficulty.

The above observations having been made upon cells with approximately smooth walls, the question suggests itself, what will be the relation of these strings of protoplasm to the pits in walls where these are present? Peculiar interest is attached to this question since the publication of the observations of Gardiner on plasmolysis of pitted parenchymatous cells of the pulvinus of Robinia pseudacacia, and other plants, in which he had previously demonstrated the continuity of the protoplasm through the pits.

The leaves of species of Trichomanes serve as excellent material for the study of this point, since the lateral portions of the lamina consist of a single layer of cells, of which the walls separating contiguous cells are thick and have numerous pits (fig. 7, A, B); the walls in these figures are represented as rather thicker in proportion than they appear in nature. A 10 per-cent. solution of salt was found to give good results. Here, as in other cases described, there is usually no very obvious system of strings to be seen immediately after the contraction of the protoplasm connecting it with the cell wall; but, as before, the intervening space soon assumes the silky

striated appearance noted in other objects (fig. 7, A). As time goes on the striæ become more plain, and resolve themselves into protoplasmic strings. These were observed to run not only to the lateral walls separating contiguous cells, but also, and apparently in equal numbers, to the free walls of the cells, which are not pitted. As in the prothallus, so here fresh substance is drawn out from the main mass of protoplasm, in this case as thick conical processes (fig. 7, B), which give a very striking appearance to the whole protoplasmic body about two hours after plasmolysis. The observations made in the prothallus as to apparent branching of strings were confirmed in the behaviour of the strings of protoplasm in these cells.

It being possible after their thickening to trace the individual strings, it could be seen whether they run as a rule or chiefly to the pits, or whether there is any constant relation between them and the pits. On examining a large number of cases I have found that strings of protoplasm often do run to pits, and that strings from the contracted protoplasm of contiguous cells are often opposite to one another; but that a much larger proportion of the strings are not opposite to one another, and run to points on the cell wall where there are no pits. In other words, I conclude that in Trichomanes pyxidiferum my observations on plasmolysis give no clue to there being any special relation of the protoplasm to the pits. This is, however, no proof that some special relation does not exist.

Concluding Remarks.

From the above observations it is seen that the connection between the protoplasm and the cell wall, as shown by plasmolysis in those cases which have been observed, is closer than is usually described, or at least implied in current botanical writings. The objects were selected from very different systematic groups; it is true their number is small, and it must be admitted that the effect is not visibly produced in every cell; nevertheless, though it cannot be asserted that the

phenomenon described is universal, it must at least be admitted that it is very general. I may here suggest that some difference may be found between the relation of the protoplasm to the cell wall in young and in old cells; no such difference has been uniformly observed by me, though it has been alluded to by Naegeli.

It has been repeatedly observed in various instances that according to the evidence of plasmolysis the connection between the protoplasm and free cell walls is as close as between the protoplasm and walls separating contiguous cells; also it has been seen, in the one example investigated in connection with that point, that there was no evidence to show any special relation between the protoplasm and pits of an ordinary parenchymatous tissue [this will of course require confirmation in other cases]. From these results it may be inferred that the phenomena observed are due to a close mode of connection between protoplasm and cell wall, which is uniform wherever they are in contact with one another in the living cell. In the light of recent observations on the mode of formation and growth of cell walls by apposition and coalescence of microsomata, the connection thus demonstrated by plasmolysis acquires a special interest. Unfortunately, the kernel of the whole matter, viz. the ultimate mode of application of the protoplasm to the cell wall, cannot be arrived at with certainty by plasmolysis, owing to the obvious difficulties of observation of minute details with high powers in uninjured cells. Still, collateral evidence may be gained, and as such I regard the observations above described.

I would suggest two possible explanations of the phenomena observed in plasmolysis, and their bearing upon the ultimate mode of application of protoplasm to cell wall—(1) that the main mass of protoplasm on retreating may leave the cell wall still completely lined with a thin film of protoplasm; (2) that the peripheral part of the protoplasm being entangled, as a network, among the deposited microsomata may, on the contraction of the main mass, be drawn out at the points of entanglement, into fine strings like those observed, while the

surface of the wall is for the most part left free, and not covered by a film of protoplasm.

In the former case the phenomena observed would be entirely intra-protoplasmic. The process might in fact be compared with what is seen when two surfaces, having a layer of a semi-fluid plastic substance, such as canada balsam, between them, are suddenly separated. Both surfaces remain covered with a film of the balsam, while between them run strings of balsam of varying thickness, which are occasionally branched, and sometimes have nodal thickenings. If the formation of strings in plasmolysis be thus intra-protoplasmic, their position would, as in the case of the balsam, be mainly determined by the conformation of the surface of the wall, and by internal determining causes in the plastic substance itself, and would not throw light on the present question of the mode of connection between cell wall and protoplasm. I have repeatedly examined the cell walls of plasmolysed cells, both in surface views and when seen edgeways, and have not been able to observe any continuous film of protoplasm covering their surface. Having, however, learned from the experiments above detailed that protoplasm may be drawn out into strings so thin as to remain undefined with very high powers, the failure to observe such films does not prove their absence, as they might also be exceedingly thin.

Taking the second possible explanation of the phenomena into consideration, we have a strong presumption in its favour from recent observations. In the first place, those of Strasburger on the deposition of microsomata on the cell wall, would suggest that the protoplasm might be, so to speak, entangled between these microsomata, and thus be continuous into and held fast by the cell walls. Thus the attachment would not be equally close over the whole surface of the wall, but would be most strong at a number of points where the processes of protoplasm are continued into the body of the wall. Secondly, Fromman asserts that he has seen a continuous network extending from the protoplasm into the cell wall. Further, we have evidence that where the protoplasm certainly does pene-

trate the cell walls (i. e. in sieve plates) it still may retain its connection with the cell wall after contraction by means of a number of strings, which run severally to the pores of the sieve (De Bary, 'Vergl. Anat.,' figs. 72, 75). These, if the second explanation of the above phenomena were true, would differ in degree but not in kind from the strings of protoplasm observed on plasmolysis. It is probable, from Gardiner's account (l. c.), that the same may be the case in the perforated pits in the cells of the pulvini on which he has worked.

I have already stated that on careful observation of the terminal parts of the strings soon after plasmolysis in the prothallus they are often seen to split up close to the cell wall into fine branches, and that they are thus attached to the cell wall at a number of points. This observation gives still further support to the second mode of explanation of these phenomena of plasmolysis.

Though it is impossible at present to decide with certainty which of these interpretations of the phenomena is nearer the truth, the latter seems to me to coincide best with the facts.

It is unfortunately hardly to be anticipated that the phenomena of plasmolysis will yield us any very certain conclusions as to the ultimate structural relations between cell wall and protoplasm, since the difficulties are so great in using high powers on objects at least as thick as one whole cell; and it is only by the use of high powers that this point can be decided. We must, therefore, look to the study of fine sections for further and secure information on this most important question.

On Haplobranchus, a New Genus of Capitobranchiate Annelids.

By

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With Plate IX.

Occurrence.—This very interesting worm was kindly placed in my hands for description by Professor Lankester, who received it last November in quantity from Mr. Thomas Bolton, F.R.M.S., of Birmingham, accompanied by a sketch of the animal in its tube, which I have reproduced in a modified form in fig. 1. Respecting its habitat, I may quote the words of Mr. W. H. Shrubsoll, who writes as follows:—

"The worms have been familiar to me for a long time, and occasionally I have met with them in great abundance on the coast of Sheppey. Not having books of reference at command I had no means of knowing whether they were described or not, and I assumed that they were.

"As far as my experience goes, they are always found associated with diatoms on the surface of soft mud at the bottom of gullies, and the presence of the diatoms insures to the animal a plentiful supply of oxygen.

"There is generally an inch or so of water overlying the mud, and the diatomaceous film at the bottom is ornamented with silvery-looking globules of oxygen.

"As it is impossible to collect either the diatoms or the

worms without getting well besmeared, and sometimes walking knee-deep in the tenaceous mire, it is hardly to be wondered at that they have hitherto escaped attention."

Associated with the worm was a species of Nais, which Professor Lankester has identified as the Nais littoralis of O. F. Müller, which has for many years been unrecorded, no naturalist, in fact, having seen it since Oersted's description and figure in 1842. There were also numerous free-living nematoids and rhabdocœl planarians.

Mr. Bolton tells me that he has seen the worm once before in a gathering from the mouth of the Liffey.

Anatomy.—The animal is minute, adult specimens not exceeding 6 mm. in length.

The tube is about twice the length of the animal, and is composed of particles of mud, with here and there a diatom (Pleurosigma, sp.).

Segments and appendages.—The "head" consists of a prostomium and a peristomium.[1]

The prostomium is much reduced and hidden by the peristomium, which rises to form a "collar" around it; this collar is higher upon the ventral than upon the dorsal surface. There are two prostomial tentacles, which are short, have pigment in the walls, and are not ciliated; they are much obscured by the palps and peristomial tentacles (figs. 1, 2, 3, and 5, prost.

[1] In the description, I make use of the following nomenclature:—The Prostomium (Præstomium, Huxley) is the lobe in front of the mouth; it may bear two kinds of appendages, (1) prostomial tentacles (antennes, Milne-Edwards and De Quatrefages; cirri, Kinberg), which spring from its dorsal surface, and generally resemble in character the appendages of the peristomial somite; and (2) palps (palpi, Kinberg; infero-lateral præstomialcirri, Huxley; antennes externes, Milne-Edwards and De Quatrefages), which spring from the lower surface of the prostomium and differ very considerably in character in different families of annelids. The Peristomium (Peristomium, Huxley; Mund-segment, Grube), which is the first somite of the body, and, it may be, the second and third fused with it; and although retaining ordinary characters in a few families of annelids, *e.g.* Syllidæ, very generally becomes much modified. Its appendages are peristomial tentacles (tentacules, De Quatrefages).

tents.) They are united at their base with the palps and more posteriorly with the two most dorsal of the peristomial tentacles.

The palps are very long, and, springing ventrally, bend over at their free ends towards the dorsal region. They are richly ciliated upon their dorsal surface, and each contains a large blood-vessel, with the green blood nearly filling up its lumen; they can thus be instantly recognised, as the peristomial tentacles have no such blood-vessel, but merely prolongations of the general body cavity.

The prostomium bears at its sides a pair of black pigment spots (fig. 5, *oc.*), which can be seen through from the ventral surface, and appear at first sight to lie upon the collar, but transverse sections have demonstrated their true position (fig. 5).

The peristomium, which forms the collar, as stated above, bears two pairs of appendages, each consisting of a very short basal piece and two long rami (noto- and neuropodial), of these the ventral ramus is the longer in each case, but is not so long as a palp. They are all richly ciliated upon their inner faces, and contain prolongations of the general body cavity, but no special blood-vessel.

The mouth, which is hidden by the collar, lies between the palps and the bases of the prostomial tentacles.

The somites of the body (counting the peristomial somites as the first) are twelve in number, of these somites 1—9 form the "thorax," and differ from somites 10—12 which form the "abdomen."

The parapodia are very slightly raised from the surface of the body, and slightly more so in the posterior than in the anterior somites.

As the peristomum bears no setæ the second somite is the first setigerous somite and bears dorsal capillary setæ only they are of two varieties placed in two bundles with usually three in each bundle, and resembling respectively figs. 8 and 9, which represent setæ from the following somite. The one variety has a very long and delicate blade, while in the other

the blade is shorter and wider; in both cases the blade only occurs on one side of the axis.

The third somite has similar setæ but slightly more numerous (the usual number of the various kinds of setæ is accurately shown in fig. 2), and bears in addition ventral "crochet" setæ (fig. 10). These are not actually forked but evidently correspond to the "crochet" setæ of allied worms.

The remaining somites in the "thorax" bear similar setæ, the crochet setæ present slight variations. Figs. 10 and 11 represent the extreme conditions, the capillary setæ in the posterior segments of the thorax gradually approach the condition shown in fig. 12, intermediate in character between figs. 8 and 9.

In the "abdomen," an inversion in the position of the setæ occurs, the capillary setæ becoming ventral, and the crochet setæ dorsal, at the same time their character is changed (fig. 14); the capillary setæ become longer, more flexible, are often bayonet-shaped (not well shown in fig. 13), and the blade occurs on both sides of the axis; the "crochet" setæ become very numerous and are closely placed in a transverse row, they are finely serrated on one side at the free extremity.

Alimentary canal.—The alimentary canal is simple, there is a constriction between the fifth and sixth somites, and between the sixth and seventh; in the sixth somite it is dilated, from the seventh somite to the anus, in the terminal somite, it gradually narrows, immediately in front of the anus it is slightly dilated (fig. 4). The canal is ciliated at any rate in its posterior half, the anterior portion presents a brown pigment in the walls.

Blood-vessels.—The complete distribution of the circulatory system is not easily determined.

The blood is green.

There is a dorsal vessel which bifurcates in the peristomial and terminal segments, the two branches in each case turning round and uniting to form a ventral vessel. The dorsal and ventral vessels are connected in the tenth and eleventh somites

by a pair of lateral commissures. Lateral commissures were also observed in the somites which contained the ova in the female (somites 4 and 5).

In the central region, the dorsal and ventral vessels pass towards the sides and appear to form a sinus around the intestine, Claparède thought to have observed this condition in Fabricia armandi, and De Quatrefages in Amphicorina.

Vessels pass to the head, but it was not possible to make out their exact origin; these dilate into a sinus at the base of the peristomial tentacles (Claparède, 'Rech. Anat. sur les Annélides, &c., dans les Hebrides,' 1861, describes similar sinuses at the bases of the branchiæ in Fabricia quadripunctata and calls them "branchial hearts"), but vessels pass from them into the palps only, a single trunk to each, which alternately fills and empties, as is the case in Fabricia and in Polydora and Spio.

Nephridia.—It was impossible to determine definitely the Nephridia; in somites 10 to 12 (fig. 4, Neph.) paired bodies are seen at the base of the parapodia, which I take to be Nephridia.

In the third somite there are two bodies, the structure of which could not be ascertained, owing to the amount of pigment in the wall, but they are doubtless modified Nephridia and function as tubiparous glands; they open at the bases of the parapodia on each side in the same somite.

Gonads.—The sexes are distinct. The spermatozoa are seen floating in various stages of development in the body cavity in the thoracic somites 7, 8, and 9, but not in the abdominal somites (fig. 4).

They are confined to the central region of the somite around the alimentary canal by a membrane. The spermatospheres are not spherical, but much elongated rope-like bodies.

They cannot be passed from segment to segment, and the manner in which the spermatozoa are shed is uncertain.

In the females the ova are found in somites 4 and 5 (fig. 2), and attain a very large size in the body cavity; their shape is continually being altered by the movement of the wall of the

intestine. Their large size probably necessitates their passing to the exterior by rupture of the body wall.

Nervous system.—I have been unable to determine the structure of the nervous system. The supra-œsophageal ganglion nearly fills the prostomium.

There are no caudal eyes.

There are no auditory capsules.

Affinities.—Haplobranchus comes into the family Serpulidæ on account of its capitobranchiate nature, but differs from all hitherto known genera of the family in that the tentacles, while they remain free, are devoid of any secondary filaments and of any trace of cartilaginous support. It agrees with the sub-family Sabellidæ in the absence of any thoracic membrane and operculum.

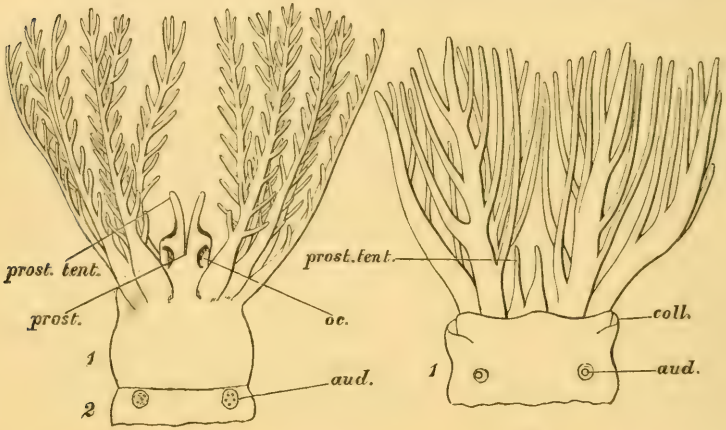
There are certain genera of the Sabellidæ which present some approach to its simplicity of structure. These are: Amphiglena, Clap.; Fabricia, Blainville; and Amphicorina, De Quatr. These forms present certain characters in common which are absent in Haplobranchus. The first pair of Nephridia, belonging to the second somite, which are modified as tubiparous glands, and which in all true Sabellids open on each side at the base of the parapodium, are united in these three genera in the dorsal region, and open by a single median dorsal pore at the base of the branchiæ. In Haplobranchus, although I have not been able to make out their exact relations, there is no doubt they are not thus specially modified. These three genera present auditory capsules in the peristomial segment and caudal eyes, of neither of which is there any trace in Haplobranchus.

On the other hand, these three genera agree with one another and with Haplobranchus in the comparatively simple structure of the head; the prostomium not being completely fused with the peristomium is still recognisable, and presents prostomial tentacles and palps. The peristomial collar, completely absent in Amphiglena, is only slightly developed in the other forms. There is little differentiation of the regions of the body

—thorax and abdomen; the setæ are simpler than in other sabellids, and the copragogal groove is absent.

A comparison of the heads in these genera seems to throw considerable light upon the nature of the processes of the head in the Serpulidæ.

In Amphiglena[1] the prostomium remains well developed, bear-



A. Diagram of head of Amphiglena, dorsal view.
B. Diagram of head of Fabricia,, dorsal view. *prost.* Prostomium. *prost. tent.* Prostomial tentacles. *l.* Peristomial somite. *coll.* Peristomial collar. *oc.* Eye-spots. *aud.* Otocyst. After Claparède.

ing lateral pigment spots and a pair of tentacles. It is difficult to make out the nature of the palps from Claparède's figures; there are two ventral processes which may represent them. The branchiæ which, according to Claparède, vary in number, from eight to twelve, have taken up such a position as to form a circular crown, and bear along their whole length a series of short, opposite, secondary filaments.

The branchiæ appear to spring from the peristomium.

In Fabricia,[2] also, prostomial tentacles may be definitely determined.

[1] Claparède, 'Glanures Zootomiques parmis les Annélides de Port Vendres,' p. 32, pl. 3.

[2] Claparède, loc. cit., p. 36, pl. 3.

The branchiæ have become modified owing to the great development of the secondary filaments, which arise alternately and attain the same length as the main stems.

In Haplobranchus, prostomium and tentacles closely resemble those of Amphiglena, but the palps are well developed. There is some little uncertainty about the determination of the organs I have so marked as palps, but their slightly greater muscular development, their blood supply, and their close connection with the ventral region of the prostomium, point to their being different in nature to the other tentacles.

The branchial tentacles it is which are so especially interesting in Haplobranchus, their definite arrangement, united in pairs at the base, the absence of any secondary filaments, the rich ciliation upon their inner faces,[1] and the absence of any branch of the closed vascular system in this lumen are all interesting characters, and point to their being similar to the peristomial tentacles of other annelids which are just taking on that branchial function which is the most marked feature of the whole family. These tentacles on account of their united bases may really be said to represent two parapodia on each side, each possessing a notopodial and a neuropodial ramus, indeed, they remind one very forcibly of the peristomial tentacles of such an annelid as Nereis. Their condition seems to me very strong evidence in favour of their peristomial nature, and consequently of the peristomial nature of the branchiæ of the Serpulidæ. This view was entertained by Milne Edwards,[2] but De Quatrefages [3] states that the branchiæ of the Serpulidæ receive their nerve supply from the supra-œsophageal nerve ganglion, and consequently he considers them to be prostomial.

Claparède and Mecnikow [4] have shown that in Dasychone

[1] Since sending my drawings to the press, I have observed a distinct tendency to a grouping in the arrangement of the cilia; upon the surface of the branchiæ, groups of cilia springing from a very slightly raised serpentine ridge.

[2] 'Règne An. ill.,' pl. 1 E, explanation of fig. 2.

[3] De Quatrefages, 'Hist. Nat. des Ann.,' tome ii, p. 401.

[4] 'Zeit. für wiss. Zoologie,' Bd. xix, 1869, Taf. xvi.

lucullana, the first rudiments of the branchiæ arise as two processes which soon bifurcate, and are clearly placed below the very large prostomium. This is at a stage when three setæ bundles are visible.

The prostomial lobe, which in Dasychone never bears any tentacles or palps, gradually aborts, and the secondary branchial filaments appear to have a terminal origin. Thus developmental history, so far as we know it, favours the view of the peristomial nature of the branchiæ.

Systematic Description.

Family—Serpulidæ. Tribe—Sabellidæ.

Haplobranchus, g. n.

Head distinct.

Pro- and peristomium almost fused, two prostomial tentacles, two palps.

Collar slightly developed.

The paired branchiæ consist each of four free tentacles united at the base in pairs, and entirely devoid of secondary filaments; they are richly ciliated. No blood-vessel in the branchiæ, a single blood vessel in each palp.

Tubiparous glands not united.

Caudal eyes absent.

Auditory capsules absent.

Sexes distinct.

H. aestuarinus, sp. n. Isle of Sheppey, England, W. H. Shrubsoll; Mouth of the Liffey, Ireland, Tho. Bolton.

Specific characters where a single species only is known must always be guardedly put forward, but judging from allied forms, the following would seem to have such weight:

Length 4—6 mm.

Nine somites (8 setigerous) in the thorax.

Three somites in the abdomen.

The shape of the setæ, figs. 8—14.

Blood green.

The Minute Structure of the Lateral and the Central Eyes of Scorpio and of Limulus.

By

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and

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With Plates X, XI and XII.

Scope.—In the essay entitled "Limulus an Arachnid," published in this Journal in 1881, it has been pointed out by one of us (Prof. Lankester) that, amongst other very numerous agreements of structure exhibited by the Scorpions on the one hand and the King Crab on the other, there is a close superficial coincidence in the disposition and the character of the eyes; in both we find a single pair of simple central eyes, and a lateral or marginal pair of "grouped" or aggregated eyes—the multicorneal lens of the King Crab's lateral eye corresponding to the numerous (two to seven) small lenses placed in groups laterally on the Scorpion's head.

The object of the investigation, of which the present memoir records the results, was to ascertain how far there is a real identity in the minuter structure of the eyes thus compared, and what precisely is the morphological relationship between the multicorneal lateral eye of Limulus and the groups of unicorneal eyes occupying a corresponding position in the Scorpions.

The evidence adduced in the essay above cited in favour of a close genetic relationship between the King Crabs and the Scorpions was so overwhelming and convincing to our minds, that we entered upon this inquiry with the strong anticipation

that most important points of agreement would be revealed in the comparison of the minute structure of the soft parts of the eyes in question. And we consider that the results we have obtained confirm the opinion expressed by Prof. Lankester, to the effect that the minute structure of the eye, when thoroughly studied, will furnish even more valuable evidence than that given by any other structural features, in the attempt to trace out the genetic relationships inter se of the great groups of Arthropoda.

Previous observations.—The classical researches of Grenacher[1] have laid the foundations of a new and rational study of the minute structure of the Arthropod eye. Our observations have led us to accept, as thoroughly justified, the main conclusions of that anatomist in reference to the nature and structure of the morphological factors of the simple and compound eyes of Arthropoda. To these we shall have to allude in the course of our descriptions. Here, we have to point out, that in his large work Grenacher has not given any account of the eyes of the Scorpions, and only a fragmentary account of the lateral eye of Limulus.

Von Graber,[2] writing subsequently to the publication of Grenacher's large work, has endeavoured to "correct" the conclusions arrived at by Grenacher, and has offered some original observations on the structure of the lateral and central eyes of Scorpions. So far as Graber's "corrections" relate to the fundamental points, such as the ultimate structure of a retinal or optic cell, and the relation of optic cells to the cells of the vitreous body, we have no hesitation in stating that he is totally wrong and that Grenacher is right. With especial reference to the Scorpion's eye, Graber's observations and drawings are very defective and, indeed, altogether misleading in regard to simple and fundamental features of structure. Apparently the method of manipulation, the great thickness of the sections, and similar circumstances, are the cause of Graber's errors.

[1] 'Sehorganen der Arthropoden,' Gottingen, 1879.

[2] 'Archiv. f. Mikrosk. Anatomie,' vol. xvii, 1880.

Nevertheless, Graber has the merit of having, as we shall mention below, correctly observed some important facts as to the Scorpion's retina—for the first time. In reply to Graber's article, Grenacher has published a memoir in which, whilst he very justly rejects the "corrections" attempted by Graber, he gives an account of certain observations on the structure of the Scorpion's eye—made in order to control the statements of Graber. Unfortunately these observations of Grenacher were confined to the central eye, and did not extend to the very differently-built lateral eye; and moreover, the observations are not illustrated by any figures. We shall not have occasion to refer to them again, since they merely furnish the observational basis which enabled Grenacher categorically to deny some of Graber's assertions.

Both this paper of Grenacher's and that of Graber, to which it is a reply, deal very largely with the eyes of Myriapods; and the structure of these, though not of those of the Scorpion's, is beautifully illustrated in Grenacher's plates.

Hence it is actually the case that no figures, except the very erroneous ones of Graber, have been published of the eyes of Scorpions; whilst the structure of the lateral eyes of those animals has not been looked at by the most capable student of the Arthropod eye.

With regard to Limulus, there is practically nothing else published relating to the structure of the eyes than the results given by Grenacher in his large book, of the examination of the lateral eye of a not too well preserved specimen. No one has given any account of the minute structure of the central eye of Limulus.

It is true that Dr. Packard has alluded to this matter in his memoir on the "Anatomy, Histology, and Embryology of Limulus," but it is so abundantly evident that Dr. Packard has not made use of the ordinary methods of histological inquiry in dealing with this and other parts of the King-crab, that it seems to be the proper course to omit any further reference to the drawings and descriptions in his memoir which are supposed to have reference to the histology of the central and

the lateral eyes of Limulus. It is to be regretted that the sections used by Dr. Packard were not made transparent before they were put under the microscope and drawn.

Material.—We have studied the central and lateral eyes of two species of Scorpion, viz. Androctonus funestus, var. citrinus, Ehr., obtained in the living state by Prof. Lankester from North Africa, through the kindness of Prof. Carl Vogt, and of Euscorpius Italicus, Roess. (also of the allied E. Carpathicus), kindly forwarded to us in the living state by Mr. Carmichael Gibson, and by Mr. Neville Reid, from the South of Italy.

We have been able from time to time to purchase living specimens of the American King-crab, Limulus polyphemus, Latr., in London, though we have felt here very much the want of small specimens not exceeding one or two inches in the diameter of the prosomatic shield, which would have been easier to cut and in other respects advantageous.

Methods.—We have been able to ensure, as above shown, the perfect freshness of the material used.

The soft tissues of the eye were placed, with the piece of chitinous cuticle adjacent, in absolute alcohol. In the case of the central eye of Limulus it was found necessary to separate the soft tissues from the chitin before immersion in alcohol.

Sections were prepared by the method of long soaking, first in turpentine then in paraffin, and slicing with the improved Rivière's microtome. Most of the sections were then, after removal of the paraffin, carefully depigmented whilst under observation by the use of dilute nitric acid (about 5 to 10 per cent.). The process of the destruction of pigment was arrested at various stages. Some of the depigmented sections, and some of those not treated to remove pigment, were stained with borax carmine and mounted in Canada balsam. Others were preserved in glycerine unstained.

We may point out that the excellent method of thorough impregnation with paraffin enabled us to obtain exceedingly thin sections of the Scorpion's eyes, and to preserve complete series for study.

The lateral eyes of Limulus required special treatment, on account of the density and extent of the chitinous lens-area. The vertical sections in this case were cut with the hand, and were more difficult to obtain in satisfactory condition than any of the others.

Drawings.—The figures which illustrate this memoir are not drawn to a constant scale. With a few exceptions, they are not representations of actual sections, but combination-drawings, intended to place before the reader results, and not the crude material from which the results are derived. The colouring of the drawings is purely conventional. In most cases the eye-pigment forms, on solution by the nitric acid, a fine madder-brown tint, which stains the nuclei, and often the protoplasm, of the cells of an entire section. The pigment thus becomes diffused as soon as the attempt is made to remove it. Pigment granules in process of solution appear of a deep red-brown colour; when not acted on at all they are absolutely black, or, in some cases, greenish grey.

The Lateral Eyes of Scorpions.

Of Androctonus funestus, Ehr.—The lateral eyes of Scorpions are placed on the margin of the prosomatic shield, in a group on each side anteriorly. The number of separate lenses developed differs in various sub-genera, each lens indicating a separate eye. In Androctonus the eyes are more numerous than in other Scorpions, each lateral group showing in A. funestus as many as five lenses, three larger and two smaller. The smaller lenses are equally entitled to count as eyes with the larger. It is, however, difficult without great care and minute examination to distinguish mere tubercles of the chitinous integument from eye-lenses. [This is, of course, readily done either when sections are cut, or when the subjacent tissues are cleaned away from the ocular area of the chitinous shield, and the tubercles and lenses are examined by transmitted light.

It is convenient to speak of the region in which the lateral eyes of the Scorpion develop as the "ocular area."

A section vertical to the chitinous surface of the shield, and parallel with the optical axis of the lens through one of the lateral eyes of A. funestus, presents before the pigment is removed the appearance shown in Plate X, fig. 1, after the removal of pigment, the appearance shown in fig. 2.

In the first place we notice in this, as in other Arthropods' eyes, that the lens is simply a local enlargement of the cuticle, and that the layer of epidermic cells usually called "hypodermis," which produces the general cuticle, and is observable at the sides of the section (Pl. X, fig. 2 *c*), is continued beneath the enlarged boss of cuticle, which acts as lens, and is correspondingly increased in dimensions. This enlarged portion of the hypodermis is, in fact, the soft or living tissue of the eye, and may be distinguished from the lens in front of it by a special name. We propose to call it the "ommatēum." The ommateum and the lens together form the eye.

When series of sections of the ommateum of a lateral eye of Androct. funestus are carefully studied it is found that the ommateum is a simple enlargement of the single layer of cells forming the hypodermis. It consists of a single row or stratum of cells, which present a distinction among themselves into two kinds. The two kinds of cells in the ommateum of the Scorpion's lateral eye are—firstly, the retinal or NERVE-END CELLS (fig. 2, *h*), in which the nerve filaments of the optic nerve terminate (fig. 2, *m*); and, secondly, INDIFFERENT CELLS (fig. 2, *f* and *g*), which are narrow and columnar in form, similar in every respect to the ordinary hypodermis cells in the neighbourhood of the eye, and like these latter pigmentiferous.

Both nerve-end cells and indifferent cells of the lateral ommateum apparently belong to the epiblastic layer, and are shut off together with the layer of hypodermis cells from the subjacent connective tissue by a well-marked "basement membrane," which in the region of the ommateum may be called the eye-capsule, or, better, the "ommateal capsule."

The nerve-end cells of the ommateum of a lateral eye of Androctonus funestus are much larger than the neighbouring indifferent cells. They are elongated, and are disposed somewhat radially, reaching from the lower surface of the cuticular lens to close upon the ommateal capsule. The nucleus is placed near the capsular or filamentary extremity (that which is connected with the nerve filament) of the nerve-end cell, and is of large relative size, spherical, and with well-marked nucleolus. The nerve-end cells appear to possess over their whole surface a well-marked cuticular substance which encloses the soft protoplasm. The minuter structure of these cells we are not prepared to discuss on the present occasion, our object being morphological rather than histological. A very important feature in the structure of the nerve-end cells is the existence of a special rod-like cuticular thickening on the side of each cell. This thickening is highly refringent, and very possibly is of a chitinous nature, though we are unable to offer any evidence as to its chemical nature. In the section drawn in fig. 2 fragments of these lateral hard-pieces are seen of a yellow colour (*i*). It appears, from further examination of sections, that the lateral thickening in each nerve-end cell is so placed as to adjoin and even fuse with the similar lateral piece of a neighbouring nerve-end cell. The resulting hard-piece has been called by Grenacher, when observed in the compound eyes of Insects and Crustaceans, a "rhabdom." We may make use of the same term for the composite body formed by the union of the lateral hard-pieces of the nerve-end cells of the Scorpion's eye. At the same time, each hard-piece in a nerve-end cell may be called a "rhabdomere."

The rhabdoms of the lateral eye are not so well developed as those of the central eye. They appear to be irregular in shape, and inconstant in the number of cells and rhabdomeres which take part in their formation. They will be best understood when the structure of the central eye has been described.

The indifferent cells of the ommateum of a lateral eye of Androctonus funestus are to be distinguished into

two kinds according to their position in the ommateum. There are, firstly, those which form the periphery of the ommateum, and are contiguous with the extra-ocular hypodermis cells. These are very long columnar cells, which fill in, as it were, the optically valueless circumference of the ommateal capsule. They may be called perineural cells (fig. 2, *f*).

The second kind of indifferent cells are placed between the diverging filamentary extremities of the nerve-end cells. They are very small columnar, closely-fitting cells, quite similar in character to the general hypodermis cells. They may be called "interneural cells." They are by no means easy to observe, being liable to be destroyed by the action of the acid which is necessary to remove the abundant pigment with which they are charged (see fig. 1).

Pigment granules appear to be developed in all the cells of the ommateum as well as in the neighbouring hypodermis cells. But it is difficult to make out in this and in all Arthropod eyes what precisely is the situation and the limit of the pigment. Before the pigment is removed observation is impossible; when it is dissolved by acid it diffuses and stains structures previously devoid of pigment. A partial removal of the pigment by means of solvents seems to be the only method which can give any indications on this matter, and even that is unsatisfactory. Pigment granules appear to be very freely developed in the protoplasm of the ordinary hypodermis cells and of the indifferent cells (both perineural and interneural) of the ommateum. But in the nerve-end cells the pigment granules are confined to the surface of the cell, leaving the axis transparent. It will be seen subsequently that in the central eyes the nerve-end cells are very nearly if not quite devoid of intrinsic pigment granules, and one is led to question whether the pigment which clothes the nerve-end cells may not in all cases be of external origin. This, however, cannot, it seems, be maintained. We have to admit that the nerve-end cells sometimes produce peripheral pigment granules, and sometimes are devoid of pigment.

The relation of pigment to the optical apparatus cannot be

said to be at present properly understood. It is perfectly certain that in some eyes, and possibly in all, pigment does not play a primary part in the physiological process set going by light. Light acts with full effect upon transparent protoplasm, and no pigment is necessary, converting the energy of light into the energy of heat, in order that the protoplasm of cells may constitute an apparatus sensitive to light. The function of pigment in an eye is a secondary one, as we learn from the sight of albino varieties. What precisely the significance of pigment may be in relation to the cells in which the optic nerve ends, is not yet agreed upon by physiologists.

Of Euscorpius Italicus, Kös.—In figs. 3 and 4 two sections are drawn of the lateral eye of the little Italian scorpion, as seen after removal of pigment. They are more highly magnified than the eyes drawn in figs. 1 and 2, being in actual size considerably smaller than the corresponding eye of Androctonus funestus, as shown by a comparison of the measurements given in the explanation of the plate. The hypodermis cells are relatively to the nerve-end cells coarser than in Androctonus funestus. In all essential respects the eyes of the two species agree. The marginal indifferent cells or perineural cells of the ommateum are larger relatively than in A. funestus, and so are the interneural cells, which are correspondingly less numerous than in A. funestus. The rhabdom is larger and thicker in E. italicus, whilst further the nerve-end cells present a special structure which is not in any way indicated in the nerve-end cells of A. funestus. Each nerve-end cell contains, besides its nucleus, a globular, highly refringent body (figs. 2, 3, *k*), quite unconnected with the rhabdom, though of a substance similar to that of the rhabdom. These bodies, which it is convenient to term "phaospheres," are usually to be found below the nucleus of the nerve-end cells, that is to say, near the filamentary extremity of those cells. But there are some nerve-end cells in every lateral eye of E. italicus which have the phaosphere placed in front of the nucleus (fig. 4, *l*). This

irregularity in the position of the phaosphere is very remarkable. It is to some extent paralleled by Grenacher's observation of the fact that in Epeira diadema all the nerve-end cells in one eye (posterior dorsal) present a rod-like body in front of the nucleus, whilst in a neighbouring eye (anterior dorsal) all the nerve-end cells present a rod-like body posterior to the nucleus. At the same time it is to be observed that the axial rod of the Spider's nerve-end cell must be considered as representing not only the phaosphere but also the laterally-placed rhabdomere of the nerve-end cell of the Scorpion.

The rhabdoms of the lateral eye of E. italicus are very nearly as indefinite in their development as in the corresponding eye of A. funestus.

It will be seen below that in the central eye groups of five nerve-end cells unite by means of their rhabdomeres to form what Grenacher has called, in the multicorneal eye of Insects and Crustaceans, a "retinula," and in the centre of this retinula is a five-ridged rhabdom (see Pl. XI, fig. 14).

In the central eye of Scorpions this grouping or segregation can be made out, though it is by no means so fully expressed as in the multicorneal (polymeniscous) eye of Insects. In the lateral eyes of the Scorpions, on the other hand, the grouping into retinulæ of the nerve-end cells and the formation of rhabdoms from rhabdomeres is merely foreshadowed and not completely carried out.

In fig. 6 a portion of a section transverse to the long axis of the nerve-end cells of the lateral eye of E. italicus is shown. Rhabdomeres coloured yellow are seen in section, and it appears that they have a tendency to unite with one another, though such a five-sided figure as that to be observed in the corresponding region of the central eye (Pl. XI, fig. 15) is not yet attained.

In fig. 5 the appearance of the ends of the nerve-end cells, as seen when the cuticular lens is removed, is shown. A tendency of the cells to group in fives can be traced. The pavement-like appearance of the ends of the long nerve-end cells when thus viewed has given rise to the erroneous statement on the part of

Von Graber, that a pavement epithelium is disposed on the deep face of the cuticular lens.

Von Graber's statements.—Of the numerous points concerning which Von Graber has made erroneous statements in his writings on Scorpion's eyes,[1] the most important is that which relates to the fundamental structure of the lateral eye. Von Graber states (and emphasises his statement by a drawing professing to be an accurate copy of a preparation of the lateral eye of Scorpio Europæus, Schr.) that the lateral eyes of the Scorpions are provided with two rows of cells—a vitreous body and a retinal body—just as are the central eyes, the two rows of cells being separated by a membrane. This statement is altogether erroneous. The description and figures which we here publish show that the ommateum of the Scorpion's lateral eye has no "vitreous body," and consists of a single layer of cells, some larger (nerve-end cells), some smaller (interneural and perineural cells).

Von Graber's error in this matter has apparently arisen, like most of the errors to which he commits himself in the same memoir, from the defective character of his methods of investigation. His sections were too thick and ill directed, and his macerating and decolorising fluids were allowed to act too rapidly or for too long a period.

A further error of Von Graber in regard to the lateral eye is his description of the ommateum as composed of three layers (besides his non-existent vitreous body)—a layer of nerve-fibres, a layer of ganglion cells, and a layer of "rod cells" (nerve-end cells). There are no "ganglion cells" within the eye-capsule distinct from the nerve-end cells. Von Graber holds an altogether erroneous view as to the structure of the nerve-end cells, which, in opposition to Grenacher (who has studied and described these structures in other Arthropoda), he declares to possess three nuclei—an anterior, a middle (that of the rod cell or rod region), and a posterior (that of the ganglion cell). The nerve-end cell is thus, according to Von Graber, a compound body, consisting of three fused cells. He terms it a "retinal

[1] 'Archiv f. Mikrosk. Anat.' vol. xvii, 1880, p. 58.

sac" (Schlauch). Grenacher, on the other hand, denies the existence of Von Graber's anterior and middle nuclei; for him the nerve-end cell is a single cell of elongated form, with one large nucleus—that which Von Graber calls nucleus of the ganglion cell. Our conclusion as to the nerve-end cells of the lateral eyes of Euscorpius and Androctonus entirely agree with those of Grenacher as to these structures in general. Grenacher has not examined, it must be remembered, the lateral eyes of Scorpions. In describing the nerve-end cells of the central eyes of Scorpions we shall have occasion to point out the existence of structures adjacent to the nerve-end cells, which have probably led Von Graber to hold the erroneous views as to the nerve-end cell which he has advanced. These structures have escaped the notice of Grenacher.

Central Eye of Androctonus funestus.

The central eyes of the Scorpions are considerably larger (from twice to three times linear) than the lateral eyes. As in the lateral eye, we distinguish lens and ommateum. The lens is a simple laminated mass of cuticle, which we must dismiss on the present occasion without attempting any examination of its optical properties. It would, no doubt, be important to compare these with those of the lateral eye, &c.

The ommateum of the central eyes differs essentially from that of the lateral eyes, in the fact that it is not composed of one layer of cells, but consists of two layers of cells, one superimposed upon the other, and separated from it by a strong laminated membrane (Pl. X, fig. 8 *n*; Pl. XI, fig. 11 *n*).

The anterior layer (*o* in the figures) is known in other similarly constructed eyes as the "Glaskörper," or "vitreous body," whilst the hinder layer may be called the "retina," or "retinal body." As will be seen when we examine the retinal body more fully, it does not consist of a simple layer of nerve-end cells, but is complicated by the presence of a large bulk of nerve-fibres within the eye-capsule, and by the presence of what is of more importance morphologically, viz. intrusive connective tissue.

The EYE-CAPSULE or OMMATEAL-CAPSULE (fig. 8 *d'*) is, as in the case of the lateral eye, a continuation of the well-developed basement membrane (fig. 8 *d*), which marks off the hypodermis of the prosomatic shield from the subjacent connective tissue. It is finely laminated, and devoid of nuclei. The septum (fig. 8 *n*), which divides the vitreous body from the rest of the ommateum, is continuous with and part of the capsule. Von Graber has the merit of having discovered this septal membrane.

The CELLS OF THE VITREOUS BODY (figs. 8. 9, 11 *o*) are closely similar to those of the general hypodermis, with which they are in direct continuity ; but they are devoid of pigment.

The long axes of these cells are curiously bent in one portion of the vitreous body (see fig. 8). Instead of radiating in lines, which would meet if continued at some geometrical centre related to the curved surfaces of the cuticular lens, the vitreous cells exhibit a bending towards the side marked B in the figure, which possibly has some relation to the optical axis of the eye. This seems to lead to the inference that the optical axis differs considerably from the geometrical axis of the lens; and this inference is confirmed by the one-sided development of the hinder part of the ommateum (see figs. 7 and 9).

There is no concretion or formation of refringent substance in any of the cells of the vitreous body.

The RETINAL BODY may be divided into the layer of nerve-end cells and the layer of nerve-fibres—interspaces, and the whole inner surface of the ommateal capsule being filled in by intrusive pigmentary connective tissue.

The NERVE-END CELLS abut upon the septal membrane, which divides the vitreous body from the retinal body. The opposite extremity or filamentary extremity of the elongated nerve-end cells does not come into such close proximity with the ommateal capsule as in the lateral eyes; a large mass of intracapsular nerve filaments (fig. 8 *q*) separates the nerve-end cells from the capsule. In the lateral eye this mass of intracapsular nerve filaments does not exist, the nerve filaments perforating the capsule more immediately than they do in the central eye.

The nerve-end cells are arranged in the central eye in definite groups of five, more clearly marked than in the lateral eyes, though not so obviously segregated as in the multicorneal (polymeniscous) eyes called "compound eyes" in Insects and Crustacea.

Each group of five cells is entitled, as in the latter case, to the name proposed by Grenacher of "Retinula." Each retinula is provided with a five-fluted rhabdom, formed by the union of the five rhabdomeres, which are produced laterally each by one nerve-end cell. The nature of these dispositions is exhibited in the diagrams drawn in Pl. XI, figs. 14, 16, 17. A single nerve-end cell with its rhabdomere is shown in fig. 12, where, however, it is drawn of insufficient proportionate length.

In a view of a horizontal plane (at right angles to the long axes of the retinulæ) it is possible to observe the five-fluted rhabdoms in optical section when they have the appearance of five-rayed stars (Pl. XI, fig. 15). This appearance was observed, and described and figured by Von Graber, who appreciated its significance. We thus owe to him, in spite of his other interpretations which are erroneous, the important discovery that the nerve-end cells of the central eye of Scorpions are grouped in retinulæ and possess a compound rhabdom. This discovery is of great importance, since in the unicorneal (monomeniscous) eyes described by Grenacher, whether of Arachnida or of Insecta Hexapoda, no such segregation of the nerve-end cells was detected, although the existence of such an arrangement serves more directly than anything else to connect the structure of so-called simple (monomeniscous) eyes with that of so-called compound (polymeniscous) eyes.

The INTRA-CAPSULAR NERVE FILAMENTS which are given off from the filamentary extremities of the nerve-end cells are in fig. 8 seen to run parallel with the plane of section and issue from the capsule in groups (nerves) which are placed to the outer side (B) of the eye. In fig. 9 the section is taken in a plane which cuts these nerve filaments at right angles to their long axes, and accordingly they are seen as irregular masses.

The INTRUSIVE PIGMENTARY CONNECTIVE TISSUE is a very important element in the building up of the retinal body, which has been on the one hand misinterpreted by Von Graber, and on the other hand overlooked by Grenacher, whose observations upon the central eye of Scorpions were undertaken with a view to the controlling of Von Graber's results.

No intrusive connective tissue (except in the form of blood-vessels) is described by Grenacher in other monomeniscous eyes (such as those of Spiders) similar to the central eyes of Scorpions. It would, perhaps, be worth while searching for it in those eyes. The structures which we consider as intrusive connective tissue in the central eyes of the Scorpion may be compared to the interneural cells of the lateral eyes. Like these they are pigmentiferous and serve to fill up the spaces between the several nerve-end cells, and between these and the ommateal capsule. But whilst we regard the interneural cells as ectodermal in origin, that is, as belonging to the same germinal layer as the cells of the hypodermis and the great nerve-end cells, we find reasons for considering the intracapsular pigmentary tissue of the central eyes of Scorpions as derived from mesoblast and of the nature of connective tissue.

We have not embryological evidence for this conclusion, and depend entirely upon the branching, inosculating character of the pigmentary cells, and upon the analogy of the pigment-cells surrounding the retinulæ of the polymeniscous eyes of Insects and Crustacea, which are very generally held to be of the nature of connective tissue, as also upon that of the "packing-tissue" to be described below in the central eye of Limulus.

We are by no means anxious to maintain that the more epithelium-like cells amongst what we are about to describe as "intrusive intracapsular connective tissue" may not be of distinct origin from other portions of this pigmentiferous framework, and referable to interneural cells of ectodermal nature, but any such distinctions must be based upon embryological facts, which we do not possess. In the present state of knowledge it seems most convenient and justifiable to hold that in the

central eyes of the Scorpions there are no interneural cells of ectodermal origin, as there are in the lateral eyes, and that their place is taken by intrusive connective tissue. In any case it is by this name that we shall designate a largely developed pigmentiferous framework, which pervades the hinder chamber of the ommateal capsule, and has not hitherto been described in any similar eye.

In fig. 8 (as also in fig. 7), for the sake of clearness in other details, a large part of the pigmentiferous intracapsular connective tissue has been omitted. But a series of epithelium-like cells (*p*) and a group of cells resembling adenoid tissue (*r*) has been retained in the drawing. All these cells possess before the de-pigmenting process abundant black pigment granules in their protoplasm.

The layer of cells (*p*) closely adheres to the inner wall of the ommateal capsule, and when the pigment is present gives a deep black limiting border to the capsule, as shown in fig. 9. The cells are again seen in the partially de-pigmented preparation drawn in fig. 10. They may be called the "intracapsular pavement." At the periphery of the capsule these cells become continuous with a series of very delicate pigmentiferous cells, which lie close beneath the capsular septum (*n*), between the anterior extremities of the nerve-end cells. These are seen as flakes of pigment in fig. 9 *s*, more clearly in fig. 10 *s*, and diagrammatically in fig. 11 *s* and fig. 14 *s*. They may be known as the "anterior intra-retinular pigment cells." These cells are exceedingly thin and delicate, and readily destroyed by the acid which is used to remove the pigment. It is on this account that they have escaped the observation of Grenacher, whilst on the other hand Von Graber has seen their nuclei, and attributed them not to interstitial cells, but to the nerve-end cells themselves. These nuclei are undoubtedly the so-called "anterior nuclei" of Von Graber, which he has seen and figured with especial clearness in the central eye of Buthus.

A very thin section of the ommateum of the central eye of Androctonus, which has been but little or not at all acted upon by acid, shows a second series of pigmentiferous cells similar

to the anterior intra-retinular cells. These lie about the middle of the length of the retinulæ, and are somewhat less compressed than the anterior cells. They are seen as fusiform patches of pigment in the section drawn in fig. 9 *t*. In fig. 10 *t* they are seen more clearly, and in fig. 11 *t* and fig. 14 they are represented diagrammatically. These we call the "median inter-retinular pigment cells." They appear to have given origin to Von Graber's "middle nuclei of the retinal sacs."

Again, at the base of the retinulæ, fitting to the rounded ends of the nerve-end cells is a third series of pigmentiferous cells, indicated by the letter *v* in the figures, whilst closely connected with these is a wide-meshed reticulum of pigmentiferous branching cells (*w* in the figures), which embraces the bundles of intra-capsular nerve-fibres, and becomes continuous with such masses of connective-tissue cells as those marked *r* in figs. 8 and 10, and also forms junctions at intervals with the pigmentiferous pavement cells which line the back of the ommateal capsule (as shown in fig. 9).

The three series of inter-retinular pigment cells and their relation to the five nerve-end cells which build up a retinula is shown diagrammatically in the drawing (fig. 14).

Representations of actual preparations showing these constituents of the retinal body are given in figs. 9 and 10.

Central eye of Euscorpius Italicus, Rös.

The central eye of Euscorpius, the structure of which is illustrated by the section drawn in fig. 7, requires no special description. The same elements are present as in Androctonus, but the cells are relatively of larger size, and consequently the retinulæ and rhabdoms are fewer in number.

We have not been able to study the distribution of the pigmentiferous tissue of the retinal body so fully in Euscorpius Italicus as in Androctonus, owing to the treatment to which the sections were subjected.

It is noteworthy that post-nuclear phaospheres (fig. 7, *k*) occur in the nerve-end cells of the central eye of Euscorpius just

as they do in the lateral eye; and occasionally we find prœ-nuclear in place of post-nuclear phaospheres. No phaospheres occur in the nerve-end cells of Androctonus.

The peculiar shape of the ommateum (as shown in a right and left vertical section at right angles to the animal's long axis) is exhibited in the figure and also the peculiar bending of the rhabdoms. The drawing is diagrammatic in so far as that only one plane of nerve-end cells is drawn, whilst the rhabdoms are represented as uncut. They, of course, really lie each in the axis of a group of five cells, of which some are omitted from the drawing for the sake of clearness. Very perfectly preserved and well-stained sections, similar to the drawing, were obtained by the use of nitric acid, followed by borax carmine (after washing). The inter-retinular pigment cells were, however, not preserved.

Pigment in the Optic Cells of the Central Eyes.

We find it difficult to decide as to whether pigment granules are ever to be found actually within the nerve-end cells of the central eyes of Scorpions. Analogy with the nerve-end cells of the lateral eyes would render it highly probable that the nerve-end cells in both cases are pigmentiferous, the pigment being limited to a superficial layer of the cell. At the same time very thin sections, such as that drawn in fig. 9, seem to show that in the special instance of the central eye the pigment granules are not really in the protoplasm of the nerve-end cells, but always in fine branches and processes of interstitial cells. The question is one which must be left undecided for the present. It is, however, important to notice (what will be further described below) that in Limulus the nerve-end cells of the central eye certainly contain pigment granules within their own proper substance.

Comparison of the Lateral and Central Eyes of Scorpions with those of other Arthropods hitherto described.

In his great work Grenacher has described the so-called "unicorneal" eyes of several Spiders, the "unicorneal" eyes of Insect larvæ and adult Insects, and the "multicorneal" eyes

adult Insects and of Crustacea ; also the " multicorneal " lateral eye of Limulus. In a more recent work Grenacher has described ('Archiv für Mikr. Anat.,' Bd. 18) the eyes of Myriapods.

One of the chief conclusions which appears to us to follow from Grenacher's work, when combined with our own observations on the central eyes of Scorpions, is that the primary distinction which has to be made amongst the various forms of Arthropod eyes is not, as has hitherto been maintained, a distinction into (A) simple or unicorneal eyes, and (B) compound or multicorneal eyes, but a distinction into (A) eyes with a one-cell-layered ommateum (i. e. with no vitreous body separated from the retinal body), and (B) eyes with a two-cell-layered ommateum (i. e. with a vitreous body, or layer of cells placed in front of the retinal body, and usually separated from it by membrane). These may be called respectively Monostichous and Diplostichous eyes. In both these primary groups it appears to be possible for the nerve-end cells (see woodcut, fig. 1) to remain ungrouped—each equal and similar to its neighbour, as is usual with cells building up cell layers—or, on the other hand, the nerve-end cells of the ommateum may segregate and group themselves as Retinulæ (see fig. 14, Pl. X). These two conditions we propose to speak of as (1) eyes non-retinulate, i. e. with the nerve-end cells autonomous, and (2) eyes retinulate, i.e. with the nerve-end cells segregated.

We have monostichous eyes which are non-retinulate in the larvæ of Insects, according to Grenacher's descriptions. We have a monostichous eye which is feebly retinulate in the lateral eye of the Scorpion, and a highly developed retinulate monostichous eye in the lateral eye of Limulus. In the dorsal eyes of Spiders and simple eyes of adult Insects we have examples of (according to Grenacher's description) a diplostichous non-retinulate eye. In the central eyes of Scorpions, on the other hand, we find for example of the strongly retinulate diplostichous class.

Further, the so-called compound eyes of Insects and Crustacea are examples of diplostichous retinulate eyes, with certain additional peculiarities now to be noticed.

Subsequently, as it would seem, to the segregation of the layer of nerve-end cells into retinulæ, optical advantage may be found in the segregation of the cuticular lens often called cornea. Thus, from the so-called unicorneal the multicorneal condition of eye is developed. It seems to be undesirable to speak of the cuticular lens as a "cornea," with which it has little analogy, if by cornea we understand in the first instance the vertebrate cornea. It will be therefore best to distinguish the simple one-lensed eyes as "monomeniscous," that with a segregated lens as "polymeniscous." This third alternative of structure is presented by Arthropod eyes, which differ in other respects inter se, but it seems that a non-retinulate eye cannot be polymeniscous, since the segregation of retinulæ is the developmental antecedent of the segregation of the lens. Hence we may have and actually can point to monostichous polymeniscous eyes (lateral eyes of Limulus) as well as diplostichous polymeniscous eyes, but all non-retinulate eyes are monomeniscous.

In this way we are led to correct the conception of the so-called compound or polymeniscous eye which is current, and and is thus enunciated by Gegenbaur ('Comp. Anatomy,' English translation, p. 266): "A reduction of the retinal elements of the simple eye gives rise to the retinula, and a compound eye is formed by the gradual concrescence of a number of simple eyes." On the contrary, it seems that the compound eye is formed, not by the gradual concrescence of a number of simple eyes, but by the segregation of the elements of a simple eye, which affects first the retina and then the lens. It appears from a consideration of the structure of the polymeniscous lateral eyes of Limulus that even the groups of monomeniscous lateral eyes found in Scorpions (and similarly, also, the closely-set groups of monomeniscous eyes of Myriapods, which can in some cases be regarded as one large polymeniscous eye) must be looked upon as resulting from a process of segregation carried further than is necessary for the production of a compound eye—carried so far, in fact, as to produce from one original large simple (monomeniscous) eye, not a continuous

polymeniscous eye, but a number of contiguous secondary simple (monomeniscous) eyes.

The polymeniscous eye presents various elaborations of structure. In the lateral eye of Limulus we have a monostichous polymeniscous eye of relatively simple character. On the other hand, polymeniscous eyes which have developed by the differentiation of diplostichous monomeniscous eyes exhibit the highest elaboration of structure known in the Arthropod series. In the first place the retinulæ become exceedingly well defined, and separated from one another by intrusive connective tissue; that is to say, by connective tissue which, not belonging originally to the hypodermis layer from which the nerve-end cells and vitreous body-cells are developed, yet pushes its way in amongst these elements. This intrusive connective tissue is pigmentiferous.

We have seen it already making its appearance (figs. 9, 10, 11, 14) in the monomeniscous retinulate central eye of Scorpions. It is more strongly developed in the typical "compound eye," and serves to isolate very completely each retinula from its neighbours.

A further speciality of that higher form of polymeniscous eye known as the compound eye (of Insects and Crustacea) is the segregation of a third element of the eye in addition to the segregation of retinulæ and lens facets; this third element is the vitreous body. Whilst in the monomeniscous diplostichous eye, even when retinulate (central eye of Scorpion) the vitreous body remains homogeneous, consisting of uniformly distributed columnar cells, in the higher form of polymeniscous diplostichous eye (compound eye of Insects and Crustacea), the vitreous body, as might be expected, joins in the segregation which characterises the cuticular lens formed upon it. Not only do we find the cells of the vitreous body arranging themselves in isolated groups similar to, and super-imposed each upon a retinula, but the intrusive connective tissue advances into the vitreous layer and cuts off with its pigment cells each group of vitreous cells from its neighbour, in the same way as it separates neighbouring retinulæ.

Each group of vitreous cells corresponding to a retinula

should be called a "vitrella." Just as the retinulæ develop axial hard structures called "rhabdoms" by Grenacher, so do the vitrellæ in varying degree develop dense hyaline bodies within them known as "crystal-cones."

The preceding sketch of the degrees of complication of structure presented by Arthropod eyes which the reader will find more readily intelligible if reference be made to the plates of Grenacher's large work, serves to enable us to estimate correctly the morphological significance of the two kinds of eye present in the Scorpions.

In the lateral eyes we have an example of the simplest kind of Arthropod eye, the monostichous monomeniscous eye. It may be compared with the simplest eye of this kind studied by Grenacher, namely, that of the larva of an Insect (Dytiscus). The woodcut reproduces diagrammatically one of Grenacher's

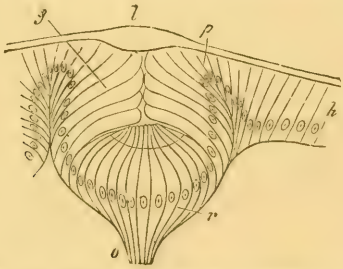

Fig. 1.—Eye of larva of Dytiscus, with monostichous, non-retinulate apostatic ommateum. *l*. Lens. *g*. Perineural cells (rudimentary vitreous body). *p*. Pigment cells. *r*. Nerve-end cells. *o*. Filaments of the optic nerve.

figures of this eye. As in the Scorpion's lateral eye, we find a single row of cell elements continuous with the hypodermis, of which the lateral members (*p. g.*) are narrow and columnar, whilst the more median are connected with nerve filaments and differentiated as nerve-end cells (*r*).

As compared with the eye of the larval Dytiscus, the lateral eye of the Scorpion is in one respect more primitive, in other respects more elaborate. It is more primitive in this, viz. that the row of cells forming the ommateum is in continuous contact with the cuticular lens, whereas in the larval Dytiscus eye

the ommateum is deeply cupped leaving a tubular cavity immediately below the cuticular lens. This condition is a step towards the complete pinching in of the perineural cells of the ommateum, and their separation as an anterior "vitreous layer" from the deeper lying nerve-end cells.

No Arthropod eye has as yet been described which is so strictly "monostichous" as the lateral eye of the Scorpion, that is to say, which presents so little evidence of any tendency of the perineural cells to take up a position in front of the nerve-end cells. The position of the ommateum in relation to the lens in the lateral eye of the Scorpion is more nearly like that of the ordinary hypodermis cells in relation to their cuticle, and may be called "epistatic," whilst the monostichous eyes described by Grenacher (in Myriapods as well as in Insect arvæ), are all characterised by a tubular cupping of the ommateum, which may be called "apostatic."

The relation of the long axes of the cells of the ommateum to the geometrical (and optical) axis of the lens is widely different in the two cases. Similar facts as to the direction of the axes of the cells of the ommateum, which are not unfrequently to be observed in the eyes of Arthropods, must be taken into account in any attempt at an explanation of the Arthropod eye as an optical apparatus; but we are not at present in a position to make such attempt.

The features in which the lateral eye of Scorpions is more elaborate than that of the larval Dytiscus are (1) the existence of interneural cells in the former, and (2) the tendency in the former of the rhabdomeres of neighbouring nerve-end cells to unite as rhabdoms.

The two agree in the possession of a well-marked ommateal capsule continuous with the basement membrane of the hypodermis, and in the "purity" of the ommateum, that is to say, its freedom from intrusive connective tissue. Since such intrusive connective tissue, when it does enter into the ommateum, appears to enter there with the function of a pigmentary investment to the optical elements, we may call an ommateum which is devoid of such adventitious pigmentiferous tissue

"autochromic," whilst one which is penetrated by pigmentiferous connective tissue is "exochromic."

The central eye of the Scorpion is seen from what has been said above to take a position among Arthropod eyes very distinct from that occupied by the lateral eyes. It is diplostichous, it is definitely retinulate, it is exochromic, and only agrees in fact with the lateral eye in being monomeniscous. As compared with all other Arthropod eyes hitherto described, the central eyes of the Scorpion stand alone in being definitely retinulate, whilst retaining the primitive monomeniscous character of lens.

It is highly noteworthy that the central eyes of Scorpions, which in position and naked-eye appearance agree with the centrally grouped eyes of the Scorpion's congeners, the Spiders, should nevertheless present such marked differences from those eyes as appear from a comparison of the description given in this memoir of the one and by Grenacher of the other. The Spiders have apparently simple axial rhabdomeres in their nerve-end cells in place of laterally developed rhabdomeres, uniting to form rhabdoms, as in Scorpions. Further, it would appear from Grenacher's description that the Spider's eyes are autochromic, whilst the Scorpion's central eyes are exochromic—i.e. the ommateum is penetrated by intrusive connective tissue.

Having thus noted the peculiarities of the Scorpion's lateral and central eyes, we are in a position to compare them with the lateral and central eyes of Limulus, of which we shall now give a description. It will be seen that though differing in some important details, there are, taking all things into consideration, no Arthropod eyes which so closely agree in their plan of structure with those of the Scorpions as do those of Limulus. Whilst this conclusion might be impugned were we to separate the consideration of the two kinds of eyes, the lateral and the central, it is indisputable if we compare the whole set of optical organs in the one animal with the whole set in the other.

The Lateral Eyes of the American King Crab, Limulus polyphemus, Latr.

The two lateral eyes of the King Crab exist on either side of the prosomatic shield, each as a reniform, smoothly polished protuberance. The protuberance is the cuticular lens; it is of great thickness, smooth on its surface, and produced on its inner surface into a number of conical processes, each of which is to be regarded as a secondary or segregate lens. The general features of the structure of this eye have been made known by Grenacher; we have only to add certain details to the description and figures given by him.

In Pl. XI, fig. 18, a diagram is given representing a portion of the polymeniscous lens with its subjacent ommateum.

Making use of the terminology which has been explained in the course of this paper, we can briefly describe the structure of the ommateum. Corresponding to each conical facet of the polymeniscous lens is a retinula (*Rn*). The ommateum is (as observed by Grenacher) essentially monostichous. The retinulæ correspond in position to the apices of the conical secondary lenses.

That part of the ommateum which clothes the sides of the lens-cones consists of simple cylindrical cells corresponding to the perineural cells of the lateral eye of Scorpions, and in the valleys between neighbouring lens-cones, the ommateal cells are not to be distinguished from ordinary pigmentiferous hypodermis cells.

The nerve-end cells, which are combined to form retinulæ, are of very large size, as much as $\frac{1}{800}$th long. Transverse sections of the ommateum show that ten cells are united in each retinula. The difficulties of observation are here even greater than in the eyes of Scorpions (owing to the thickness and density of the cuticular lens, which prevents the preparation of satisfactory sections); but we are inclined to think that ten nerve-end cells, being double the number present in the Scorpion's retinula, is the rule, although Grenacher has figured a retinula

of Limulus with as many as fifteen nerve-end cells. Possibly there is a superior and inferior series of retinula cells interlocking with one another, and amounting to fifteen in number, which might in certain sections show ten in others, fifteen areas in section; but we have not obtained definite evidence that such is the case, and are disposed to consider the retinula as having the arrangement of cells shown in fig. 19 and in fig. 20. The nuclei of some of the retinula cells lie nearer to the lens extremity, in others nearer to the filamentary extremity. Each retinula cell gives off a coarse nerve filament from its filamentary extremity.

The rhabdom of the retinulæ of the lateral eyes of Limulus is formed by the union of ten rhabdomeres, as shown diagrammatically in fig. 20. That end of the retinula nearest the lens touches the conical extremity of the secondary lens, but leaves an axial space, which is filled neither by the lens nor by the rhabdom (*xy*). The rhabdom itself is hollow in its more anterior portion, the constituent rhabdomeres only thoroughly uniting along the common axis in the deeper region of the retinula. This is seen in the transverse sections of three retinulæ at different horizons drawn in fig. 26, where the section with hollow rhabdom is more anterior (that is, nearer the lens) than is that to the left with solid rhabdom.

The perineural cells (fig. 19 *f*) are delicate columnar cells, much elongated where they adjoin the retinula, and deeply charged with pigment. They pass over in the lateral regions of the lens-cones into ordinary pigmentiferous hypodermis cells (*c*).

Vertical sections through a lens-cone and subjacent ommateum (de-pigmented as a matter of course) show, besides the retinula cells and the perineural cells, small cells, which are disposed upon and between the adjacent large nerve-end cells of the retinula. At first sight these might be interpreted as perineural cells, but their position and distribution do not seem to admit of this view of their nature. They are apparently intrinsive connective tissue, which enters the ommateal capsule with the large group of nerve-fibres attached to the retinula cells.

These cells are seen in fig. 19 and fig. 22, where they are

coloured pink to distinguish them from the perineural cells of hypodermic origin, and are marked with the letter *r*. In transverse sections, owing to the delicacy of the structures and the destructive action of the acid used to remove the obscuring pigment, great difficulty is found in tracing the perineural cells and these intrusive connective-tissue cells. In fig. 26 the letters *f* and *r* indicate what appear to belong to these two sets of cells respectively.

The whole question of the distribution of pigment granules in the three sets of cells, viz. nerve-end (retinular), perineural, and intrusive, is bound up with the proper distinction of the distribution of the last-named cells.

The nerve-end cells undoubtedly contain some pigment granules both in the lateral and central eyes of Limulus. The perineural cells are intensely pigmentiferous. But it is probable that the chief clothing of pigment to each nerve-end cell—for example, that which is seen in the right-hand retinula of the section fig. 26—is furnished by the intrusive connective-tissue cells disposed between neighbouring retinula-cells.

Connected with the fact that the ommateum of the lateral eye of Limulus is invaded by intrusive connective tissue, is the incomplete character of the ommateal capsule. Whilst well marked in every other region (figs. 19, 25, 26 *d*), the capsule is deficient immediately below the retinula where the group of optic nerve filaments passes out of or into the capsule, and it is here that the intrusive connective tissue (*r*) is seen to be continuous with the extra-capsular connective tissue (*e*), as shown in fig. 19.

Comparison of Lateral Eye of Limulus with the Lateral Eyes of Scorpion.

The lateral eye of Limulus is shown above to be monostichous, polymeniscous, exochromic (i.e. not purely autochromic), and seems, therefore, at first sight, to differ largely from a lateral eye of a Scorpion.

But, as was stated at the commencement of this memoir,

it has been suggested by Prof. Lankester that the comparison to be made is not between a single lateral eye of the Scorpion and a whole lateral eye of Limulus, but between the latter and the complete group of lateral eyes occurring in Scorpions.

When the comparison is thus made, we see that if we supposed a common ancestor of the Scorpion and King Crab to have exhibited a lateral "ocular area," which possessed a single feebly developed cuticular lens, then by two slightly divergent lines of differentiation we can obtain the grouped eyes of Scorpio on the one hand, and the polymeniscous eye of Limulus on the other hand.

The ancestral eye was undoubtedly monostichous, an archaic character which is retained by both descendants. The archaic eye was at first non-retinulate, and commenced to exhibit a tendency to retinulation before the actual divergence of the Scorpionids and the Limuloids. In the Limuloids the differentiation of retinulæ and the corresponding differentiation of lens-cones (facets) became definite and characteristic. It is probable, if we may judge from the condition of the extinct Eurypterina, that the lens-cones were at first relatively larger and shallower and the retinulæ less concentrated (composed of more numerous cells) in the Limuloids than they subsequently became.

In the Scorpionids the segregation of ommateum and lens took different proportions. The original lens segregrated, not into a number of contiguous lens-cones, but broke up into a number of quite separate lenticules, to each of which a portion of ommateum corresponded. This process was no doubt a very gradual one, and was essentially determined by the fact that well-marked retinulæ did not develop themselves as optical units in the ommateum of the lateral eye of these ancestral Scorpions. Probably the secondary eyes of these Scorpionids were at first much more numerous and more closely set than in living Scorpions. Gradually they have become reduced in number and more widely separated from one another. At the present day various genera of Scorpions differ in the number of eyelets present on the lateral ocular area (from two to seven). It is also

important to notice that the number varies even in individuals, and that supernumerary eyelets of small size are distinguished as of irregular occurrence by the side of the larger eyelets, thus seeming to indicate that we have in this region of the Scorpion's prosomatic shield an "ocular area," which by reversion may occaionally reproduce the more numerous and closely set eyelets into which the single ancestral eye of the common parent of Scorpio and Limulus was divided in the Scorpionid line of descent.[1]

The Central Eyes of the American King Crab, Limulus polyphemus, Latr.

The central eyes of Limulus have not hitherto been examined. Grenacher appears not to have had an opportunity of studying them, although he has figured sections of the lateral eyes. Packard has published some figures relating to them, which are valueless, because he did not attempt to remove the pigment which necessarily obscures their structure.

Hence we have here a perfectly novel subject to deal with.

These eyes are very difficult to study on account of the great strength and thickness of the lenses and adjacent cuticle. The ommateum must necessarily be drawn away from the lens in the fresh state, and then hardened and cut. Our conclusions are founded on the examination of the central eyes of four fresh specimens of Limulus.

The anticipation which immediately forces itself on the mind is, that the central eye of Limulus will prove—if the assimilation of Limulus and Scorpio be justified—to be, like that of Scorpio, diplostichous, monomeniscous, and retinulate, with a more or less abundant intrusive connective tissue in the

[1] It is important to note the following difference between the lateral eyelet of a Scorpion and a single element of the King Crab's lateral eye—in the former the ommateum contains more than one retinula, it is retinulate, in the latter it contains but one group of nerve-end cells, truly a retinula when the whole eye-group is considered but in itself non-retinulate. Thus the eyelet of the Scorpion is morphologically more (a larger segment of the original ocular area) than the lens-cone element or eyelet of Limulus.

ommateum (exochromic). This is precisely the character which is revealed by our sections. In fig. 27 an approximately median vertical section of one of the two central eyes of Limulus is represented. The lens is coloured yellow, and the intrusive connective tissue pink. It is at once clear from the figure that we have an anterior layer of cells (vitreous body) (*o*), separated by firm membrane from a posterior retinal body. The vitreous body is peculiar in the fact that its cells are not specially elongated, but are small like those of the adjacent hypodermis. The retinal body is highly remarkable. Though identical in plan of structure with that of Scorpions, it differs in the exaggerated development of the intrusive connective tissue, which forms an abundant growth both in front of (*s s*) and around (*s x*) the retinulæ (*h*). The individual nerve-end cells are very large, exactly corresponding to those which form the well-defined retinulæ of the lateral eyes. Large groups of nerve-fibres (*m*), surrounded by connective tissue, are seen at the base of the section passing away from the retinulæ to form the optic nerve. We could not define an ommateal capsule, though there is a perceptible difference between the tissue of the ommateum and the loose reticulate connective tissue (*e*), which is abundantly developed around the eye and in all other parts of the King Crab's body.

The retinulæ are much less definitely constituted in the central eyes of Limulus than are those of the lateral eyes. The nerve-end cells are sometimes grouped quite irregularly, but here and there a definite arrangement of five around a common axis can be observed (fig. 32, Ret. 2). Those towards the periphery are less defined, those nearer the centre of the ommateum more clearly differentiated. In teasing a fresh ommateum of the central eye a retinula was isolated, which is diagrammatically represented in fig. 28. It appeared to be built up of seven nerve-end cells, and was only partially disengaged from the adherent connective tissue. In fig. 33 a drawing is given showing nerve-end cells, and surrounding pigmentiferous connective tissue. The action of nitric acid has dissolved some of the pigment contained in the nerve-end cells, and stained the proto-

plasm of these cells with the madder-brown colour formed by the dissolved pigment.

The rhabdoms of the retinulæ are very irregularly developed. Those nearer the centre show, however, under favorable circumstances in transverse section, a five-fluted or seven-fluted rhabdom, as represented in figs. 29, 30.

It is difficult to estimate the number of the retinulæ present in a central eye of Limulus, on account of the want of definite segregation of the nerve-end cells at the periphery of the ommateum. But indications of about twenty are seen when a complete horizontal section is studied.

The most remarkable feature in the central eye of Limulus is the great development of intrusive connective tissue in front of and around the retinulæ. Much of this connective tissue is pigmentiferous, but we must suppose that certain tracts of it in front of the retinulæ are free from pigment, and leave a path for the rays of light. Nevertheless we are not able to say with any certainty which cells are pigmentiferous and which cells are transparent. The large vesicular cells in front of the retinulæ, marked *s s*, are probably free from pigment.

The branched and fusiform cells, which are seen everywhere in sections creeping over and clothing the retinulæ (*s x*), are undoubtedly pigmentiferous.

Comparison of Central Eye of Limulus and Scorpions.

The great mass of connective tissue present in the ommateum of the central eye of Limulus is to be regarded as a development of the intrusive connective tissue which we have already seen in the central eye of Scorpions. It is so largely developed in Limulus as to lead one to regard the retinulæ as sunk and buried in it, and suggests the possibility that, at any rate in the adult, the central eye of the King Crab may have partially lost its function. At any rate, the irregularly constituted retinulæ and the abundant connective tissue of the central eye contrast markedly with the cleanly cut retinulæ and simple perineural cells of the lateral eyes.

Although there is this strange exaggeration in parts, the essential agreement of the central eyes of Limulus with those of Scorpions is obvious. No other known eye approaches it in constitution. This agreement is more marked than that of the lateral grouped eyelets of Scorpions with the lateral polymeniscous eye of Limulus, though there is no parallel to the monostichous eyelet of the Scorpion with its "epistatic" ommateum to be found so close as that presented by a single element of the King Crab's lateral eye, also monostichous and epistatic.

When the two kinds of eyes are compared in the two animals, each to each, we are abundantly justified in saying not only that there is no other animal which presents so close an approach to either of these animals in respect of its eyes as they do to one another, but even more emphatically that the agreement is one comprising such a large number of important details that we are compelled to conclude from it that the Scorpions and the King Crabs are closely allied representatives of one class, the Arachnida.

Summary and Tabular Statement.

The facts set forth in the preceding memoir are to a large extent summarised by the plates which accompany it, and the explanatory description of those plates.

The more general conclusions to which our observations tend may be gathered from the following tabular arrangement of some of the chief varieties of Arthropod eyes which are at present known. The technical terms which have been introduced in the present memoir and recur in the tabular statement are explained in a list appended.

Without assuming to assign their due significance to all varieties of the Arthropod eye, some of which may very possibly (e. g. those of certain Crustacea) find no proper place in the scheme here submitted, we yet think that it is useful to tabulate the principal facts of structure known as to a large number of Arthropod eyes in the following way.

We take as primary divisions those eyes with monostichous

Group I.—Monostichous Eyes.

	Dytiscus and other Insect Larvæ (Single Stemma).	Scorpion, Lateral Eyes (the whole group of eyelets considered as a unit).	Scorpion, Lateral Eyes (a single eyelet considered as a unit).	Limulus, Lateral Eyes (a single lens cone and ommateum considered as a unit).	Limulus, Lateral Eyes (the whole ocular area considered as a unit).	Scolopendra and Julus (the whole ocular area or group of eyelets considered as a unit).	Scolopendra and Julus (a single lens and ommateum considered as a unit).
Non-retinulate	*	...	...	*	...	...	*
Retinulate	...	*	*	...	*	*	...
Monomeniscous	*	...	*	*	...	...	*
Polymeniscous	...	*	...	...	*	*	...
Ommateum epistatic	...	*	*	*	*	...	...
Ommateum apostatic	*	...	...	...	...	*	*
Intrusive connective tissue absent (autochromic)	*	*	*	...	...	*	*
Intrusive connective tissue present (exochromic)	...	...	...	*	*	...	...
Ectodermal interneural cells absent	*	...	...	*	*	*	*
Ectodermal interneural cells present	...	*	*	...	...	...	...

Group II.—Diplostichous Eyes.

	Spiders. Stemma.	Adult Hexapod Insect. Stemma.	Scorpion, Central Eyes.	Limulus, Central Eyes.	Facetted or Compound Eye of Hexapod Insect and Crustacea (whole ocular area considered as a unit).	Facetted Eye of Hexapods and Crustacea (a single facet and corresponding ommateum considered as a unit).
Non-retinulate	*	*	...	..	...	*
Retinulate	...	...	*	*	*	...
Monomeniscous	*	*	*	*	...	*
Polymeniscous	...	...	...	...	*	...
Intrinsic connective tissue . absent (autochromic)	*	*	...	...	...	...
Intrinsic connective tissue . present (exochromic)	...	...	*	*	*	*
Vitreous body, simple	*	*	*	*	...	*
Vitreous body, segregated to form "vitrellæ"	...	...	...	...	*	...

N.B.—The deposition of refringent substance in the vitreous body (anterior layer of ommateum), apart from its segregation of the cells to form vitrellæ, is not mentioned in the table. This physical metamorphosis of substance occurs in the vitreous body of the stemmata of some Insects, as well as in the vitrellæ of the compound eyes of both Insects and Crustacea (crystal-cones).

ommateum, the more archaic, and those eyes with diplostichous ommateum derived from the monostichous condition. A few examples clearly transitional between the monostichous and the diplostichous condition have been described by Grenacher (among Myriapods).

It is especially to be noted in reference to the comparison of monomeniscous and polymeniscous eyes, that the comparison yields totally different results accordingly as we may choose to compare with the non-facetted eye of a Spider or of an Insect larva, on the one hand, a single eyelet of a "grouped" eye or of a "compound" eye, or on the other hand, the whole group or the whole "compound" eye.

Explanatory List of Terms introduced in this Memoir.

Ommatēum.—All the soft tissues of an Arthropod eye, as distinguished from the cuticular lens.

Nerve-end cells.—The cells of the ommateum, in which the filaments of the optic nerve terminate.

Perineural cells.—Cells having the same ectodermal (hypodermis) origin as the nerve-end cells, and surrounding a group of the latter; the rudimentary vitreous body and marginal cells of monostichous eyes.

Interneural cells.—Cells having the same ectodermal (hypodermis) origin as the nerve-end cells, but remaining small and unrelated to nerve filaments whilst wedged in between the bases of adjacent nerve-end cells. Only known as yet in the lateral eyes of Scorpions.

Monostichous.—Of an ommateum which consists of a single layer of cells.

Diplostichous.—Of an ommateum which consists of two layers of cells, one superimposed on the other.

Vitreous body.—The anterior cell-layer of a diplostichous ommateum.

Retinal body.—The posterior, or deep-layer, of a diplostichous ommateum, often, but not always, separated from the anterior by a septal membrane.

Retinulate.—Of an ommateum in which the nerve-end cells are segregated to form definite groups, or "retinulæ."

Rhabdomere.—The axial or lateral hard-piece which is frequently formed by a nerve-end cell in front of its nucleus.

Rhabdom.—The compound hard-piece formed in the axis of a retinula by the union of the laterally formed rhabdomeres of its constituent nerve-end cells.

Phaosphere.—A brilliantly refringent spherical body found in the nerve-end cells of Euscorpius italicus, Rös, usually behind, but sometimes in front of the nucleus.

Monomeniscous.—Of the chitinous cuticle in front of an ommateum when it has the form of a single lens.

Polymeniscous.—Of that condition of the chitinous cuticle in front of an ommateum when it is segregated or broken up into many lenses, only found, and then not always, when there is a retinular segregation of the ommateum itself.

Epistatic.—Of a monostichous ommateum when the anterior ends of its nerve-end cells abut upon the cuticular lens. Only observed in the lateral eyes of Scorpio and Limulus.

Apostatic.—Of a monostichous ommateum when it forms a cup or tube-like depression below the cuticular lens, the lens not entering the lumen of the cup or tube.

Autochromic.—Of an ommateum in which all the pigment is developed in cells of ectodermal (hypodermis) origin, and into which no connective tissue penetrates.

Exochromic.—Of an ommateum in which some, if not all, the pigment is developed in intrusive connective-tissue cells, which penetrate deeply between the proper ectodermal elements of the ommateum.

Vitrella.—A group of cells of a vitreous body which has become segregated in correspondence with the segregation of the retinal body and of the lens. A lens-facet, a vitrella and a retinula surrounded by pigmentiferous cells constitute a single "element" of the compound eye of Insecta hexapoda and of Crustacea.

The Anatomy and Development of Peripatus capensis.

By the late

Francis Maitland Balfour, LL.D., F.R.S.,

Fellow of Trinity College, Professor of Animal Morphology in the University of Cambridge.

With Plates XIII—XX.

Introduction.

The late Professor Balfour was engaged just before his death in investigating the structure and embryology of Peripatus capensis, with the view of publishing a complete monograph of the genus. He left numerous drawings intended to serve as illustrations to the monograph, together with a series of notes and descriptions of a large part of the anatomy of Peripatus capensis. Of this manuscript some portions were ready for publication, others were more or less imperfect; while of the figures many were without references, and others were provided with only a few words of explanation.

It was obviously necessary that Professor Balfour's work—embodying as it did much important discovery—should be published without delay; and the task of preparing his material for the press was confided to us. We have printed all his notes and descriptions without alteration.[1] Explanations which appeared to be necessary, and additions to the text in cases in which he had prepared figures without writing descriptions, together with full descriptions of all the plates, have been added by us, and are distinguished by enclosure in square brackets.[2]

[1] Excepting in an unimportant matter of change of nomenclature used with regard to the buccal cavity.

[2] The account of the external characters, generative organs, and development, has been written by the editors.

We have to thank Miss Balfour, Professor Balfour's sister, for the important service which she has rendered by preparing a large part of the beautiful drawings with which the monograph is illustrated. Many of these had been executed by her under Professor Balfour's personal supervision; and the knowledge of his work which she then acquired has been of the greatest assistance to us in preparing the MSS. and drawings for publication.

Since his death she has spared no pains in studying the structure of Peripatus, so as to enable us to bring out the first part of the monograph in as complete a state as possible. It is due to her skill that the first really serviceable and accurate representation of the legs of any species of Peripatus available for scientific purposes are issued with the present memoir.[1]

We have purposely refrained from introducing comments on the general bearing of the new and important results set forth in this memoir, and have confined ourselves to what was strictly necessary for the presentation of Mr. Balfour's discoveries in a form in which they could be fully comprehended.

Mr. Balfour had at his disposal numerous specimens of Peripatus novæ zealandiæ, collected for him by Professor Jeffrey Parker, of Christchurch, New Zealand; also specimens from the Cape of Good Hope collected by Mr. Lloyd Morgan, and brought to England by Mr. Roland Trimen in 1881; and others given to him by Mr. Wood Mason, together with all the material collected by Mr. Moseley during the Challenger voyage.

A preliminary account of the discoveries as to the embryology of Peripatus has already been communicated to the Royal Society.[2] It is intended that the present memoir shall be followed by others, comprising a complete account of all the species of the genus Peripatus.

H. M. Moseley.
A. Sedgwick.

[1] The drawings on Pl. XIV, figs. 9 and 10 on Pl. XV, and the drawings of the embryos (except fig. 37), have been made by Miss Balfour since Professor Balfour's death.

[2] 'Proc. Royal Soc.,' 1883.

PART I.

Description of the Species.

Peripatus capensis (fig. 1).

[The body is elongated, and slightly flattened dorso-ventrally. The dorsal surface is arched, and darkly pigmented; while the ventral surface is nearly flat, and of a lighter colour.

The mouth is placed at the anterior end of the body, on the ventral surface.

The anus is posterior and terminal.

The generative opening is single and median, and placed in both sexes on the ventral surface, immediately in front of the anus.

There are a pair of ringed antennæ projecting from the anterior end of the head, and a pair of simple eyes, placed on the dorsal surface at the roots of the antennæ.

The appendages of the body behind the antennæ are disposed in twenty pairs.

1. The single pair of jaws placed within the buccal cavity in front of the true mouth opening, and consisting each of a papilla, armed at its termination with two cutting blades.

2. The oral papillæ placed on each side of the mouth. At their apices the ducts of the slime glands open.

3. The seventeen pairs of ambulatory appendages, each provided with a pair of chitinous claws at its extremity.

4. The anal papillæ placed on each side of the generative opening.

Colour.—The following statements on this head are derived from observations of spirit specimens. The colour varies in different individuals. It always consists of a groundwork of green and bluish grey, with a greater or less admixture of brown. The chief variations in the appearance of the animal, so far as colour is concerned, depend on the shade of the green.

In some it is dark, as in the specimen figured (fig. 1); in others it is of a lighter shade.

There is present in most specimens a fairly broad light band on each side of the body, immediately dorsal to the attachment of the legs. This band is more prominent in the lighter coloured varieties than in the dark, and is especially conspicuous in large individuals. It is due to a diminution in the green pigment, and an increase in the brown.

There is a dark line running down the middle of the dorsal surface, in the middle of which is a fine whitish line.

The ventral surface is almost entirely free from the green pigment, but possesses a certain amount of light brown. This brown pigment is more conspicuous and of a darker shade on the spinous pads of the foot.

In parts of the body where the pigment is scarce, it is seen to be confined to the papillæ. This is especially evident round the mouth, where the sparse green pigment is entirely confined to the papillæ.

In some specimens a number of white papillæ, or perhaps light brown, are scattered over the dorsal surface; and sometimes there is a scattering of green papillæ all over the ventral surface. These two peculiarities are more especially noticeable in small specimens.

Ridges and Papillæ of the Skin.—The skin is thrown into a number of transverse ridges, along which the primary wart-like papillæ are placed.

The papillæ, which are found everywhere, are specially developed on the dorsal surface, less so on the ventral. The papillæ round the lips differ from the remaining papillæ of the ventral surface in containing a green pigment. Each papilla bears at its extremity a well-marked spine.

The ridges of the skin are not continued across the dorsal middle line, being interrupted by the whitish line already mentioned. Those which lie in the same transverse line as the legs are not continued on to the latter, but stop at the junction of the latter with the body. All the others pass round to the ventral surface and are continued across the middle line;

they do not, however, become continuous with the ridges of the other side, but passing between them gradually thin off and vanish.

The ridges on the legs are directed transversely to their long axes, i.e. are at right angles to the ridges of the rest of the body.

The Antennæ are ringed and taper slightly till near their termination, where they present a slight enlargement in spirit specimens, which in its turn tapers to its termination.

The rings consist essentially of a number of coalesced primary papillæ, and are, therefore, beset by a number of spines like those of the primary papillæ (described below). They are more deeply pigmented than the rest of the antenna.

The free end of the antenna is covered by a cap of tissue like that of the rings. It is followed by four or more rings placed close together on the terminal enlargement. There appears to be about thirty rings on the antennæ of all adults of this species. But they are difficult to count, and a number of small rings occur between them, which are not included in the thirty.

The antennæ are prolongations of the dorso-lateral parts of the anterior end of the body.

The Eyes are paired and are situated at the roots of the antennæ on the dorso-lateral parts of the head. Each is placed on the side of a protuberance which is continued as the antenna, and presents the appearance of a small circular crystalline ball inserted on the skin in this region.

The rings of papillæ on that part of the head from which the antennæ arise lose their transverse arrangement. They are arranged concentrically to the antennal rings, and have a straight course forwards between the antennæ.

The Oral Papillæ are placed at the side of the head. They are attached ventro-laterally on each side of the lips. The duct of the slime gland opens through their free end. They possess two main rings of projecting tissue, which are especially pigmented on the dorsal side; and their extremities are covered by papillæ irregularly arranged.

The Buccal Cavity, Jaws, and Lips are described below.

The Ambulatory Appendages.—The claw-bearing legs are usually seventeen in number; but in two cases of small females we have observed that the anal papillæ bear claws, and present all the essential features of the ambulatory appendages. In one small female specimen there were twenty pairs of claw-bearing appendages, the last being like the claw-bearing anal papillæ last mentioned, and the generative opening being placed between them.

The ambulatory appendages, with the exception of the fourth and fifth pairs in both sexes, and the last pair (seventeenth) in the male, all resemble each other fairly closely. A typical appendage (figs. 2 and 3) will first be described, and the small variations found in the appendages just mentioned will then be pointed out. Each consists of two main divisions, a larger proximal portion, the leg, and a narrow distal claw-bearing portion, the foot.

The leg has the form of a truncated cone, the broad end of which is attached to the ventro-lateral body-wall, of which it appears to be, and is, a prolongation. It is marked by a number of rings of primary papillæ, placed transversely to the long axis of the leg, the dorsal of which contain a green and the ventral a brown pigment. These rings of papillæ, at the attachment of the leg, gradually change their direction and merge into the body rings. At the narrow end of the cone there are three ventrally placed pads, in which the brown pigment is dark, and which are covered by a number of spines precisely resembling the spines of the primary papillæ. These spinous pads are continued dorsally, each into a ring of papillæ.

The papillæ of the ventral row next the proximal of these spinous pads are intermediate in character between the primary papillæ and the spinous pads. Each of these papillæ is larger than a normal papilla, and bears several spines (fig, 2). This character of the papilla of this row is even more marked in some of the anterior legs than in the one figured; it seems probable that the pads have been formed by the coalescence of

several rows of papillæ on the ventral surface of the legs. On the outer and inner sides of these pads the spines are absent, and secondary papillæ only are present.

In the centre of the basal part of the ventral surface of the foot there are present a group of larger papillæ, which are of a slightly paler colour than the others. They are arranged so as to form a groove, directed transversely to the long axis of the body, and separated at its internal extremity by a median papilla from a deep pit which is placed at the point of junction of the body and leg. The whole structure has the appearance, when viewed with the naked eye, of a transverse slit placed at the base of the leg. The segmental organs open by the deep pit placed at the internal end of this structure. The exact arrangement of the papillæ round the outer part of the slit does not appear to be constant.

The foot is attached to the distal end of the leg. It is slightly narrower at its attached extremity than at its free end, which bears the two claws. The integument of the foot is covered with secondary papillæ, but spines and primary papillæ are absent, except at the points now to be described.

On each side of the middle ventral line of the proximal end of the foot is placed an elliptical elevation of the integument covered with spines. Attached to the proximal and lateral end of this is a primary papillæ. At the distal end of the ventral side of the foot on each side of the middle line is a group of inconspicuous pale elevations, bearing spines.

On the front side of the distal end of the foot, close to the socket in which the claws are placed, are two primary papillæ, one dorsal and the other ventral.

On the posterior side of the foot the dorsal of these only is present. The claws are sickle-shaped, and placed on papillæ on the terminal portion of the foot. The part of the foot on which they are placed is especially retractile, and is generally found more or less telescoped into the proximal part (as in the figure).

The fourth and fifth pairs of legs exactly resemble

the others, except in the fact that the proximal pad is broken up into three, a small central and two larger lateral. The enlarged segmental organs of these legs open on the small central division.

The last (17) leg of the male (Pl. XIV, fig. 4) is characterised by possessing a well-marked white papilla on the ventral surface. This papilla, which presents a slit-like opening at its apex, is placed on the second row of papillæ counting from the innermost pad, and slightly posterior to the axial line of the leg.

The Anal Papillæ, or as they should be called, generative papillæ, are placed one on each side of the generative aperture. They are most marked in small and least so in large specimens. That they are rudimentary ambulatory appendages is shown by the fact that they are sometimes provided with claws, and resemble closely the anterior appendages.

PART II.

Alimentary Canal.

The alimentary canal of Peripatus capensis forms, in the extended condition of the animal, a nearly straight tube, slightly longer than the body, the general characters of which are shown in figs. 6 and 7.

For the purposes of description, it may conveniently be divided into five regions, viz. (1) the buccal cavity with the tongue, jaws, and salivary glands, (2) pharynx, (3) the œsophagus, (4) the stomach, (5) the rectum.

The Buccal Cavity.—The buccal cavity has the form of a fairly deep pit, of a longitudinal oval form, placed on the ventral surface of the head, and surrounded by a tumid lip.

[The buccal cavity has been shown by Moseley to be formed in the embryo by the fusion of a series of processes surround-

ing the true mouth-opening, and enclosing in their fusion the jaws.

The lip is covered by a soft skin, in which are numerous organs of touch, similar to those in other parts of the skin having their projecting portions enclosed in delicate spines formed by the cuticle. The skin of the lips differs, however, from the remainder of the skin, in the absence of tubercles, and in the great reduction of the thickness of the dermis. It is raised into a series of papilliform ridges, whose general form is shown in fig. 5; of these there is one unpaired and median behind, and a pair, differing somewhat in character from the remainder, in front, and there are, in addition, seven on each side.

The structures within the buccal cavity are shown as they appear in surface views in figs. 5 and 7, but their real nature is best seen in sections, and is illustrated by Pl. XVI, figs. 11 and 12, representing the oral cavity in transverse section, and by Pl. XVI, figs. 17 and 18, representing it in horizontal longitudinal sections. In the median line of the buccal cavity in front is placed a thick muscular protuberance, which may perhaps conveniently be called the tongue, though attached to the dorsal instead of the ventral wall of the mouth. It has the form of an elongated ridge, which ends rather abruptly behind, becoming continuous with the dorsal wall of the pharynx. Its projecting edge is armed by a series of small teeth, which are thickenings of the chitinous covering, prolonged from the surface of the body over the buccal cavity. Where the ridge becomes flatter behind, the row of teeth divides into two, with a shallow groove between them (Pl. XV, fig. 7).

The surface of the tongue is covered by the oral epithelium, in parts of which are organs of special sense, similar to those in the skin; but its interior is wholly formed of powerful muscles. The muscles form two groups, intermingled amongst each other. There are a series of fibres inserted in the free edge of the tongue, which diverge, more or less obliquely, towards the skin at the front of the head anteriorly, and towards the pharynx behind. The latter set of fibres are directly continuous with

the radial fibres of the pharynx. The muscular fibres just described are clearly adapted to give a sawing motion to the tongue, whose movements may thus, to a certain extent, be compared to those of the odontophor of a mollusc.

In addition to the above set of muscles, there are also transverse muscles, forming laminæ between the fibres just described. They pass from side to side across the tongue, and their action is clearly to narrow it, and so cause it to project outwards from the buccal cavity.

On each side of the tongue are placed the jaws, which are, no doubt, a pair of appendages, modified in the characteristic arthropodan manner, to subserve mastication. Their structure has never been satisfactorily described, and is very complicated. They are essentially short papillæ, moved by an elaborate and powerful system of muscles, which are armed at their free extremities by a pair of cutting blades or claws. The latter structures are, in all essential points, similar to the claws borne by the feet, and, like these, are formed as thickenings of the cuticle. They have therefore essentially the characters of the claws and jaws of the Arthropoda, and are wholly dissimilar to the setæ of Chætopoda. The claws are sickle-shaped and, as shown in Pl. XIV, fig. 5, have their convex edge directed nearly straight forwards, and their concave or cutting edge pointed backwards. Their form differs somewhat in the different species, and, as will be shown in the systematic part of this memoir,[1] forms a good specific character. In Peripatus capensis (Pl. XV, fig. 10) the cutting surface of the outer blade is smooth and without teeth, while that of the inner blade (fig. 9), which is the larger of the two, is provided with five or six small teeth, in addition to the main point. A more important difference between the two blades than that in the character of the cutting edge just spoken of, is to be found in their relation to the muscles which move them. The anterior parts of both blades are placed on two epithelial ridges, which are moved by muscles common to both blades (Pl. XVI, fig. 11). Posteriorly,

[1] Some material for this memoir was left by Prof. Balfour, which will be published separately.

however, the behaviour of the two blades is very different. The epithelial ridge bearing the outer blade is continued back for a short distance behind the blade, but the cuticle covering it becomes very thin, and it forms a simple epithelial ridge placed parallel to the inner blade. The cuticle covering the epithelial ridge of the inner blade is, on the contrary, prolonged behind the blade itself as a thick rod, which, penetrating backwards along a deep pocket of the buccal epithelium, behind the main part of the buccal cavity for the whole length of the pharynx, forms a very powerful lever, on which a great part of the muscles connected with the jaws find their insertion. The relations of the epithelial pocket bearing this lever are somewhat peculiar.

The part of the epithelial ridge bearing the proximal part of this lever is bounded on both its outer and inner aspect by a deep groove. The wall of the outer groove is formed by the epithelial ridge of the outer blade, and that of the inner by a special epithelial ridge at the side of the tongue. Close to the hinder border of the buccal cavity (as shown in Pl. XVI, fig. 12, on the right hind side), the outer walls of these two grooves meet over the lever, so as completely to enclose it in an epithelial tube, and almost immediately behind this point the epithelial tube is detached from the oral epithelium, and appears in section as a tube with a chitinous rod in its interior, lying freely in the body cavity (shown in Pl. XVI, figs. 13—16 *le*). This apparent tube is the section of the deep pit already spoken of. It may be traced back even beyond the end of the pharynx, and serves along its whole length for the attachment of muscles.

The greater part of the buccal cavity is filled with the tongue and jaws just described. It opens dorsally and behind by the mouth into the pharynx, there being no sharp line of demarcation between the buccal cavity and the pharynx. Behind the opening into the pharynx there is a continuation of the buccal cavity shown in transverse section in fig. 13, and in longitudinal and horizontal section in fig. 17, into which there opens the common junction of the two salivary glands. This diver-

ticulum is wide at first and opens by a somewhat constricted mouth into the pharynx above (Pl. XVI, fig. 13, also shown in longitudinal and horizontal section in fig. 17). Behind it narrows, passing insensibly into what may most conveniently be regarded as a common duct for the two salivary glands (Pl. XVI, fig. 17).

The Salivary Glands.—These two bodies were originally described by Grube, by whom their nature was not made out, and subsequently by Moseley, who regarded them as fat bodies. They are placed in the lateral compartments of the body cavity immediately dorsal to the ventral nerve cords, and extend for a very variable distance, sometimes not more than half the length of the body, and in other instances extending for nearly its whole length. Their average length is perhaps about two thirds that of the body. Their middle portion is thickest, and they thin off very much behind and to a slight extent in front. Immediately behind the mouth and in front of the first pair of legs, they bend inwards and downwards, and fall (fig. 7) one on each side into the hind end of the narrow section of the oral diverticulum just spoken of as the common duct for the two salivary glands. The glandular part of these organs is that extending back from the point where they bend inwards. This part (fig. 16) is formed of very elongated cells supported by a delicate membrana propria. The section of this part is somewhat triangular, and the cells are so long as to leave a comparatively small lumen. The nuclei of the cells are placed close to the supporting membrane, and the remainder of the cells are filled with very closely packed secretory globules, which have a high index of refraction. It was the presence of these globules which probably led Moseley to regard the salivary glands as fat bodies. The part of each gland which bends inwards must be regarded as the duct.

The cells lining the ducts are considerably less columnar than those of the gland proper. Their nuclei (fig. 14) are situated at the free extremities instead of at the base of the cells, and they are without secretory globules. The

cells lining the ducts of the salivary glands pass, without any sharp line of demarcation, into those of the oral epithelium, which are flatter and have their nuclei placed in the middle.

The Pharynx.—The pharynx is a highly muscular tube (fig. 7) with a triangular lumen (figs. 14, 15), which extends from the mouth to about half way between the first and second pair of legs. It is lined by a flattish epithelium bounded by a cuticle continuous with that of the mouth. On the dorsal side is a ridge projecting into the lumen of the pharynx. This ridge may be traced forwards (Pl. XVI, figs. 11—14) into the tongue, and the two grooves at the side of this ridge, forming the two upper angles of the triangular lumen, may be followed into those at the sides of the tongue. The muscles of the pharynx are very highly developed, consisting of an intrinsic and an extrinsic set. The former consists, as is best seen in longitudinal sections, of (Pl. XVIII, fig. 23) radial fibres, arranged in somewhat wedge-shaped laminæ, between which are rings of circular fibres. The latter are thicker externally than internally, and so also appear wedge-shaped in longitudinal sections. Very characteristic of the pharynx are the two sympathetic nerves placed close to the two dorsal angles of the triangular lumen (fig. 14, *sy*).

The pharynx of Peripatus is interesting in that it is unlike, so far as I know, the pharynx of any true Arthropod, in all of which the region corresponding with the pharynx of Peripatus is provided with relatively very thin walls.

The pharynx of Peripatus has, on the other hand, a very close and obvious resemblance to that of many of the Chætopoda, a resemblance which is greatly increased by the characteristic course of the sympathetic nerves.

The form of the lumen, as already pointed out by Grube, resembles that of the Nematoda.

The Œsophagus.—Behind the pharynx there follows a narrow œsophagus (fig. 7, *o e*) shown in section in fig. 16. It has somewhat folded and fairly thick walls, and lies freely in the central division of the body cavity without any mesenteric

support. Its walls are formed of five layers, viz. from without inwards.

(1) A peritoneal investment.

(2) A layer of longitudinal fibres.

(3) A layer of circular fibres, amongst which are numerous nuclei.

(4) A connective-tissue layer supporting (5) a layer of fairly columnar hyaline epithelium, bounded on its inner aspect by a cuticle continued from that of the pharynx. In front it passes insensibly into the pharynx, and beyond the region where the dorsal walls of the pharynx have clearly commenced, the ventral walls still retain the characters of the œsophageal walls. The œsophagus is vertically oval in front, but more nearly circular behind. Characteristic of the œsophagus is the junction of the two sympathetic nerves on its dorsal wall (fig. 16). These nerves cannot be traced far beyond their point of junction.

The Stomach.—The next section of the alimentary tract is the stomach or mesenteron (fig. 6). It is by far the largest part of the alimentary tract, commencing at about the second pair of legs and extending nearly to the hind end of the body. It tapers both in front and behind, and is narrowest in the middle, and is marked off sharply both from the œsophagus in front and the rectum behind, and is distinguished from both of these by its somewhat pinker hue. In the retracted condition of the animal it is, as pointed out by Moseley, folded in a single short dorsal loop, at about the junction of its first with its second third, and also, according to my observations, at its junction with the rectum; but in the extended condition it is nearly straight, though usually the posterior fold at the junction of the rectum is not completely removed. Its walls are always marked by plications which, as both Moseley and Grube have stated, do not in any way correspond with the segmentation of the body. In its interior I have frequently found the chitinous remains of the skins of insects, so that we are not justified in considering that the diet is purely vegetable. It lies free, and is, like the remainder of the alimentary

tract, without a mesentery. The structure of the walls of the stomach has not hitherto been very satisfactorily described.

The connective tissue and muscular coats are extremely thin. There is present everywhere a peritoneal covering, and in front a fairly well-marked though very thin layer of muscles formed of an external circular and an internal longitudinal layer. In the middle and posterior parts, however, I was unable to recognise these two layers in section; although in surface view Grube found an inner layer of circular fibres and an outer layer formed of bands of longitudinal fibres, which he regards as muscular.

The layer supporting the epithelium is reduced to a basement membrane. The epithelial part of the wall of the stomach is by far the thickest (fig. 20), and is mainly composed of enormously elongated, fibre-like cells, which in the middle part of the stomach, where they are longest, are nearly half a millimètre in length, and only about ·006 mm. in breadth. Their nuclei, as seen in fig. 20, are very elongated, and are placed about a quarter of the length from the base.

The cells are mainly filled with an immense number of highly refracting spherules, probably secretory globules, but held by Grube, from the fact of their dissolving in ether, to be fat. The epithelial cells are raised into numerous blunt processes projecting into the lumen of the stomach.

In addition to the cells just described there are present in the anterior part of the stomach a fair sprinkling of mucous cells. There are also everywhere present around the bases of the columnar cells short cells with spherical nuclei, which are somewhat irregularly scattered in the middle and posterior parts of the stomach, but form in the front part a definite layer. I have not been able to isolate these cells, and can give no account of their function.

The rectum extends from the end of the stomach to the anus. The region of junction between the stomach and the

rectum is somewhat folded. The usual arrangement of the parts is that shown in fig. 6, where the hind end of the stomach is seen to be bent upon itself in a U-shaped fashion, and the rectum extending forwards under this bent portion and joining the front end of the dorsal limb of the U. The structure of the walls of the rectum is entirely different to that of the stomach, and the transition between the two is perfectly sudden. Within the peritoneal investment comes a well-developed muscular layer with a somewhat unusual arrangement of its layers, there being an external circular layer and an internal layer formed of isolated longitudinal bands. The epithelium is fairly columnar, formed of granular cells with large nuclei, and is lined by a prolongation of the external cuticle. It is raised into numerous longitudinal folds, which are visible from the surface, and give a very characteristic appearance to this part of the alimentary tract. The muscular layers do not penetrate into the epithelial folds, which are supported by a connective tissue layer.

Nervous System.

The central nervous system consists of a pair of supra-œsophageal ganglia united in the middle line, and of a pair of widely divaricated ventral cords, continuous in front with the supra-œsophageal ganglia.

It will be convenient in the first instance to deal with the general anatomy of the nervous system and then with the histology.

Ventral Cords.—The ventral cords at first sight appear to be without ganglionic thickenings, but on more careful examination they are found to be enlarged at each pair of legs (Pl. XV, fig. 8). These enlargements may be regarded as imperfect ganglia. There are, therefore, seventeen such pairs of ganglia corresponding to the seventeen pairs of legs. There is in addition a ganglionic enlargement at the commencement of the œsophageal commissures, where the nerves to the oral papillæ are given off (Pl. XVIII, fig. 22 *or. g.*), and the region of junction between the œsophageal commissures with the supra-

œsophageal ganglia, where another pair of nerves are given off to the jaws, (Pl. XVIII, fig. 22 *j n*) may be regarded as the anterior ganglion of the ventral cords. There are, therefore, according to the above reckoning, nineteen pairs of ganglia connected with the ventral cords.

The ventral cords are placed each in the lateral compartments of the body cavity, immediately within the longitudinal layer of muscles.

They are connected with each other, rather like the pedal nerves of Chiton and the lower Prosobranchiata, by a number of commissures. These commissures exhibit a fairly regular arrangement from the region included between the first and the last pair of true feet. There are nine or ten of them between each pair of feet (Pl. XIX, fig. 26). They pass along the ventral wall of the body, perforating the ventral mass of longitudinal muscles. On their way they give off nerves which innervate the skin.

In Peripatus novæ zealandiæ, and probably also in P. capensis, two of these nerves, coming off from each pair of ganglia, are distinguished from the remainder by the fact that they are provided with numerous nerve-cells, instead of being composed of nerve-fibres only, like the remaining commissures (Pl. XIX, fig. 26 *g co*). In correlation with the nerves given off from them to the skin the commissures are smaller in the middle than at the two ends.

Posteriorly the two nerve-cords nearly meet immediately in front of the generative aperture, and between this aperture and the last pair of feet there are about six commissures passing between them (Pl. XV, fig. 8). Behind the generative aperture the two cords bend upwards, and, as is shown in fig. 8, fall into each other dorsally to the rectum. The section of the two cords placed dorsally to the rectum is solely formed of nerve-fibres; the nerve-cells, present elsewhere, being here absent.

In front of the ganglion of the first foot the commissures have a more dorsal situation than in the remainder of the body. The median longitudinal ventral muscle here gradually thins

out and comes to an end, while the commissures pass immediately below the wall of the pharynx (Pl. XVI, figs. 14, 15). The ventral cords themselves at first approach very close to each other in this region, separating again, however, to envelope between them the pharynx (Pl. XVIII, fig. 22).

There are eleven commissures in front of the first pair of legs (Pl. XVIII, fig. 22). The three foremost of these are very close together, the middle one arising in a more ventral position than the other two, and joining in the median ventral line a peculiar mass of cells placed in contact with the oral epithelium (fig. 14). It is probably an organ of special sense.

The ventral cords give off a series of nerves from their outer borders, which present throughout the trunk a fairly regular arrangement. From each ganglion two large nerves (figs. 8, 22, 26) are given off, which, diverging somewhat from each other, pass into the feet, and, giving off branches on their way, may be traced for a considerable distance within the feet along their anterior and posterior borders.

In front of each of the pair of pedal nerves a fairly large nerve may be seen passing outwards towards the side of the body (fig. 22). In addition to this nerve there are a number of smaller nerves passing off from the main trunk, which do not appear to be quite constant in number, but which are usually about seven or eight. Similar nerves to those behind are given off from the region in front of the first pair of legs, while at the point where the two ventral cords pass into the œsophageal commissures two large nerves (fig. 22), similar to the pairs of pedal nerves, take their origin. These nerves may be traced forwards into the oral papillæ, and are therefore to be regarded as the nerves of these appendages. On the ventral side of the cords, where they approach most closely, between the oral papillæ and the first pair of legs, a number of small nerves are given off to the skin, whose distribution appears to be to the same region of the skin as that of the branches from the commissures behind the first pair of legs.

From the œsophageal commissures, close to their junction with the supra-œsophageal ganglia, a nerve arises on each side

which passes to the jaws, and a little in front of this, apparently from the supra-œsophageal ganglion itself, a second nerve to the jaws also takes its origin (Pl. XVIII, fig. 22 *j n*). These two nerves I take to be homologous with a pair of pedal nerves.

Between the nerves to the jaws and those to the oral papillæ a number of small nerves take their origin. Three of these on each side pass in a dorsal direction and one or two in a ventral one.

The Supra-œsophageal Ganglia.—The supra-œsophageal ganglia (figs. 8 and 22) are large, somewhat oval masses, broader in front than behind, completely fused in the middle, but free at their extremities. Each of them is prolonged anteriorly into an antennary nerve, and is continuous behind with one of the œsophageal commissures. On the ventral surface of each, rather behind the level of the eye, is placed a very peculiar protuberance (fig. 22 *d*), of which I shall say more in dealing with the histology of the nervous system.

A number of nerves arise from the supra-œsophageal ganglia, mainly from their dorsal surface.

In front are the immense antennary nerves extending along the whole length of each antenna, and giving off numerous lateral twigs to the sense organs. Near the origin of the antennary nerves, and rather on the dorsal surface, there spring a few small twigs, which pass to the skin, and are presumably sensory. The largest of them is shown in Pl. XVII, fig. 19 A. About one third of the way back the two large optic nerves take their origin, also arising laterally, but rather from the dorsal surface (Pl. XVII, fig. 19 D and E). Each of them joins a large ganglionic mass placed immediately behind the retina. Nearly on a level with the optic nerves and slightly nearer the middle dorsal line a pair of small nerves (fig. 19 D) spring from the brain and pass upwards, while nearly in the same line with the optic nerves and a little behind them a larger pair of nerves take their origin.

Behind all these nerves there arises from the line of suture between the two supra-œsophageal ganglia a large median nerve

which appears to supply the integument of the dorsal part of the head (Pl. XV, fig. 8; Pl. XVI, figs. 11—14 *d n*).

Sympathetic System.—In addition to the nerves just described there are two very important nerves which arise near the median ventral line, close to the hind end of the supra-œsophageal ganglia. The origin of these two nerves is shown in the surface view (fig. 22 *s y*, and in section in fig. 11). They at first tend somewhat forwards and pass into the muscles near the epithelium lining the groove on each side of the tongue. Here they suddenly bend backwards again and follow the grooves into the pharynx.

The two grooves are continuous with the two dorsal angles of the pharynx; and embedded in the muscles of the pharynx, in juxtaposition with the epithelium, these two nerves may easily be traced in sections. They pass backwards the whole length of the pharynx till the latter joins the œsophagus. Here they at once approach and shortly meet in the median dorsal line (fig. 16). They can only be traced for a very short distance beyond their meeting point. These nerves are, without doubt, the homologues of the sympathetic system of Chætopods, occupying as they do the exact position which Semper has shown to be characteristic of the sympathetic nerves in that group, and arising from an almost identical part of the brain.[1]

Histology of the Nervous System.

Ventral Cords.—The histology of the ventral cords and œsophageal commissures is very simple and uniform. They consist of a cord almost wholly formed of nerve-fibres, placed dorsally, and a ventral layer of ganglion cells (figs. 16 and 20).

The fibrous portion of the cord has the usual structure, being formed mainly of longitudinal fibres, each probably being a bundle of fibres of various sizes, enveloped in a sponge-work of connective tissue. The larger bundles of fibres are placed

[1] Vide Spengel, 'Oligognathus Bonelliæ, Naples Mittheilungen,' Bd. iii, pl. iv, fig. 52.

near the inner borders of the cords. In this part of the cord there are placed a very small number of ganglion cells.

The layer of ganglion cells is somewhat crescent-shaped in section, and, as shown in figs. 16 and 20, envelopes the whole ventral aspect of the fibrous parts of the cord, and even creeps up slightly on to the dorsal side. It is thicker on the inner than on the outer side, and increases considerably in bulk at each ganglionic enlargement. The cells of which it is composed are for the most part of a nearly uniform size, but at the border of the fibrous matter a fair sprinkling of larger cells is found.

The tracheal vessels supplying the nervous system are placed amongst the larger cells, at the boundary between the ganglionic and fibrous regions of the cords.

With reference to the peripheral nerve-stems there is not much to be said. They have for the most part a similar structure to the fibrous parts of the main cord, but are provided with a somewhat larger number of cells.

Sheath of the Ventral Cords.—The ventral cords are enveloped by a double sheath, the two layers of which are often in contact, while in other cases they may be somewhat widely separated from each other. The inner layer is extremely thin and always very closely envelopes the nerve-cords. The outer layer is thick and fibrous, and contains a fair sprinkling of nuclei.

Supra-œsophageal Ganglia.—In the present state of our knowledge a very detailed description of the histology of the supra-œsophageal ganglia would be quite superfluous, and I shall confine myself to a description of the more obvious features in the arrangement of the ganglionic and fibrous portions (Pl. XVII, fig. 19 A—G).

The ganglion cells are in the first place confined, for the most part, to the surface. Along the under side of each ganglion there is a very thick layer of cells, continuous behind, with the layer of ganglion cells which is placed on the under surface of the œsophageal commissures. These cells have, moreover, an arrangement very similar to that in the ventral

cords, so that a section through the supra-œsophageal ganglia has an obvious resemblance to what would be the appearance of a section through the united ventral cords. On the outer borders of the ganglia the cells extend upwards, but they end on about the level of the optic nerve (fig. 19 D). Immediately dorsal to this point the fibrous matter of the brain is exposed freely on the surface (fig. 19 A, B, &c., *a*). I shall call the region of fibrous matter so exposed the dorso-lateral horn of white matter.

Where the two ganglia separate in front the ganglion cells spread up the inner side, and arch over so as to cover part of the dorsal side. Thus, in the anterior part, where the two ganglia are separate, there is a complete covering of ganglionic substance, except for a narrow strip, where the dorso-lateral lobe of white matter is exposed on the surface (fig. 19 A). From the point where the two ganglia meet in front the nerve-cells extend backwards as a median strip on the dorsal surface (fig. 19 D and E). This strip, becoming gradually smaller behind, reaches nearly, though not quite, the posterior limit of the junction of the ganglia. Behind it there is, however, a region where the whole dorsal surface of the ganglia is without any covering of nerve-cells.

This tongue of ganglion cells sends in, slightly behind the level of the eyes, a transverse vertical prolongation inwards into the white matter of the brain, which is shown in the series of transverse sections in fig. 19 E, and also in the vertical longitudinal section (Pl. XVIII, fig. 21), and in horizontal section in Pl. XVIII, fig. 22.

On the ventral aspect of each lobe of the brain there is present a very peculiar, bluntly conical protuberance of ganglion cells (Pl. XVIII, fig. 22), which was first detected by Grube (No. 10), and described by him as "a white thick body of a regular tetrahedral form, and exhibiting an oval dark spot in the middle of two of the faces." He further states that it is united by a delicate nerve to the supra-œsophageal ganglion, and regards it as an organ of hearing.

In Peripatus capensis the organ in question can hardly

be described as tetrahedral. It is rather of a flattened oval form, and consists, as shown in sections (Pl. XVII, fig. 19 c and D, D), mainly of ganglion cells. In its interior is a cavity with a distinct bounding membrane: the cells of which it is composed vary somewhat in size, being smallest near the point of attachment. At its free end is placed a highly refractive, somewhat oval body, probably forming what Grube describes as a dark spot, half embedded in its substance, and kept in place by the sheath of nervous matter surrounding it. This body appears to have fallen out in my sections. The whole structure is attached to the under surface of the brain by a very short stalk formed of a bundle of cells and nervous fibres.

It is difficult to offer any interpretation of the nature of this body. It is removed considerably from the surface of the animal, and is not, therefore, so far as I can see, adapted to serve as an organ of hearing.

The distribution of the white or fibrous matter of the ganglia is not very easy to describe.

There is a central lobe of white matter (fig. 19 E), which is continuous from ganglion to ganglion, where the two are united. It is smaller behind than in front. On its ventral side it exhibits fairly well-marked transverse commissural fibres, connecting the two halves of the ganglion. Laterally and somewhat ventrally it is prolonged into a horn (fig. 19 D, E, *b*), which I propose calling the ventro-lateral horn. In front it is placed in a distinct protuberance of the brain, which is placed ventrally to and nearly in the same vertical plane as the optic nerve. This protuberance is best shown in the view of the brain from below given in Pl. XVIII, fig. 22. This part of the horn is characterised by the presence of large vertically-directed bundles of nerve-fibres, shown in transverse section in fig. 19 D. Posteriorly the diameter of this horn is larger than in front (fig. 19 E, F, G), but does not give rise to a protuberance on the surface of the brain owing to the smaller development of the median lobe behind.

The median lobe of the brain is also prolonged into a dorso-lateral lobe (fig. 19, *a*), which, as already mentioned, is freely

exposed on the surface. On its ventral border there springs the optic nerve and several pairs of sensory nerves already described (fig. 19 D, E) while from its dorsal border a pair of sensory nerves also spring, nearly in the same vertical plane as the optic nerves.

Posteriorly where the dorsal surface of the brain is not covered in with ganglion cells the dorso-lateral horn and median lobe of the brain become indistinguishable.

In the front part of the brain the median lobe of white matter extends dorsalwards to the dorsal strip of ganglion cells, but behind the region of the transverse prolongation of these cells, into the white matter already described (p. 234), there is a more or less distinctly defined lobe of white matter on the dorsal surface, which I propose calling the postero-dorsal lobe of white matter. It is shown in the transverse sections (fig. 19 F and G, *c*). It gradually thins away and disappears behind. It is mainly characterised by the presence on the ventral border of definite transverse commissural fibres.

The Skin.

The skin is formed of three layers.

1. The cuticle.
2. The epidermis or hypodermis.
3. The dermis.

The cuticle is a layer of about 0·002 mm. in thickness. Its surface is not, however, smooth, but is everywhere, with the exception of the perioral region, raised into minute secondary papillæ, the base of which varies somewhat in diameter, but is usually not far from 0·02 mm. On the ventral surface of the body these papillæ are for the most part somewhat blunt, but on the dorsal surface they are more or less sharply pointed. In most instances they bear at their free extremity a somewhat prominent spine. The whole surface of each of the secondary papillæ just described is in its turn covered by numerous minute spinous tubercles. In the perioral region, where the cuticle is smooth, it is obviously formed of two layers which easily separate from each other, and there is I believe a similar

division elsewhere though it is not so easy to see. It is to be presumed that the cuticle is regularly shed.

The epidermis, placed immediately within the cuticle, is composed of a single row of cells, which vary, however, a good deal in size in different regions of the body. The cells excrete the cuticle, and, as shown in fig. 32, they stand in a very remarkable relation to the secondary papillæ of the cuticle just described. Each epidermis cell is in fact placed within one of these secondary papillæ, so that the cuticle of each secondary papilla is the product of a single epidermis cell. This relation is easily seen in section, while it may also be beautifully shown by taking a part of the skin which is not too much pigmented, and, after staining it, examining from the surface.

In fig. 32 a region of the epidermis is figured, in which the cells are exceptionally columnar. The cuticle has, moreover, in the process of cutting the section, been somewhat raised and carried away from the subjacent cells. The cells of the epidermis are provided with large oval nuclei, which contain a well developed reticulum, giving with low powers a very granular appearance to the nuclei. The protoplasm of the cells is also somewhat granular, and the granules are frequently so disposed as to produce a very well-marked appearance of striation on the inner end of the cells. The pigment which gives the characteristic colour to the skin is deposited in the protoplasm of the outer ends of the cells in the form of small granules. An attempt is made to show this in fig. 32.

At the apex of most, if not all, the primary wart-like papillæ there are present oval aggregations, or masses of epidermis cells, each such mass being enclosed in a thickish capsule (fig. 31). The cells of these masses appear to form the wall of a cavity which leads into the hollow interior of a long spine. These spines when carefully examined with high objectives present a rather peculiar structure. The base of the spine is enveloped by the normal cuticle, but the spine itself, which terminates in a very fine point, appears, as shown in fig. 31, to be continuous with the inner layer of the cuticle. In the perioral region the outer layer of the cuticle, as well as the

inner, appear to be continued to the end of the spines. Within the base of the spine there is visible a finely striated substance which may often be traced into the cavity enclosed by the cells, and appears to be continuous with the cells. Attached to the inner ends of most of the capsules of these organs a delicate fibrillated cord may be observed, and although I have not in any instance succeeded in tracing this cord into one of the nerve-stems, yet in the antennæ, where the nerve-stems are of an enormous size, I have satisfied myself that the minute nerves leaving the main nerve-stems and passing out towards the skin are histologically not to be distinguished from these fibrillated cords. I have therefore but little hesitation in regarding these cords as nerves.

In certain regions of the body the oval aggregations of cells are extremely numerous; more especially is this the case in the antennæ, lips, and oral papillæ. On the ventral surface of the peripheral rings of the thicker sections of the feet they are also very thick set (fig. 20 P). They here form a kind of pad, and have a more elongated form than in other regions. In the antennæ they are thickly set side by side on the rings of skin which give such an Arthropod appearance to these organs in Peripatus.

The arrangement of the cells in the bodies just described led me at first to look upon them as glands, but a further investigation induced me to regard them as a form of tactile organ. The arguments for this view are both of a positive and a negative kind.

The positive arguments are the following:

(1) The organs are supplied with large nerves, which is distinctly in favour of their being sense organs rather than glands.

(2) The peculiar striæ at the base of the spines appear to me like the imperfectly preserved remains of sense hairs.

(3) The distribution of these organs favours the view that they are tactile organs. They are most numerous on the antennæ, where such organs would naturally be present, especially in a case like that of Peripatus, where the nerve passing to

the antennæ is simply gigantic. On the other hand, the antennæ would not be a natural place to look for an enormous development of dermal glands.

The lips, oral papillæ, and under surface of the legs, where these bodies are also very numerous, are situations where tactile organs would be of great use.

Under the head of negative arguments must be classed those which tell against these organs being glandular. The most important of these is the fact that they have no obvious orifice. Their cavities open no doubt into the spines, but the spines terminate in such extremely fine points that the existence of an orifice at their apex is hardly credible.

Another argument, from the distribution of these organs over the body is practically the converse of that already used. The distribution being as unfavorable to the view that they are glands, as it is favorable to that of their being sense organs.

The Tracheal System.

The apertures of the tracheal system are placed in the depressions between the papillæ or ridges of the skin. Each of them leads into a tube, which I shall call the tracheal pit (fig. 30), the walls of which are formed of epithelial cells bounded towards the lumen of the pit by a very delicate cuticular membrane continuous with the cuticle covering the surface of the body. The pits vary somewhat in depth; the pit figured was about 0·09 mm. It perforates the dermis and terminates in the subjacent muscular layer. The investigation of the inner end of the pit gave me some little trouble.

Transverse sections (fig. 30) through the trunk containing a tracheal opening show that the walls of the pit expanded internally in a mushroom-like fashion, the narrow part being, however, often excentric in relation to the centre of the expanded part.

Although it was clear that the tracheæ started from the expanded region of the walls of the pit, I could not find that the lumen of the pit dilated into a large vesicle in this part,

and further investigation proved that the tracheæ actually started from the slightly swollen inner extremity of the narrow part of the pit, the expanded walls of the pit forming an umbrella-like covering for the diverging bundles of tracheæ.

I have, in fig. 30, attempted to make clear this relation between the expanded walls of the tracheal pits and the tracheæ. In longitudinal sections of the trunk the tracheal pits do not exhibit the lateral expansion which I have just described, which proves that the divergence of the bundles of tracheæ only takes place laterally and not in an antero-posterior direction. Cells similar in general character to those of the walls of the tracheal pits are placed between the branches of tracheæ and somewhat similar cells, though generally with more elongated nuclei, accompany the bundles of tracheæ as far as they can be followed in my sections. The structure of these parts in the adult would, in fact, lead one to suppose that the tracheæ had originated at the expense of the cells of pits of the epidermis, and that the cells accompanying the bundles of tracheæ were the remains of cords of cells which sprouted out from the blind ends of the epidermis pits and gave rise in the first instance to the tracheæ.

The tracheæ themselves are extremely minute, unbranched (so far as I could follow them) tubes. Each opening by a separate aperture into the base of the tracheal pit, and measuring about 0·002 mm. in diameter. They exhibit a faint transverse striation which I take to be the indication of a spiral fibre [Moseley ('Phil. Trans.,' 1874, Pl. 73, fig. 1) states that the tracheæ branch, but only exceptionally.]

Situation of the tracheal apertures.—Moseley states (No. 13) that the tracheæ arise from the skin all over the surface of the body, but are especially developed in certain regions. He finds "a row of minute oval openings on the ventral surface of the body," the openings being "situate with tolerable regularity in the centres of the interspaces between the pairs of members, but additional ones occurring at irregular intervals. Other similar openings occur in depressions on the inner side of the conical foot protuberance." It is difficult in preserved

specimens to make out the exact distributions of the tracheal apertures, but I have been able to make out certain points about them.

There is a double row of apertures on each side of the median dorsal line, forming two sub-dorsal rows of apertures. The apertures are considerably more numerous than the legs. There is also a double row of openings, again more numerous than the legs, on each side of the median ventral line between the insertions of the legs. Moseley speaks of a median row in this position. I think this must be a mistake.

Posteriorly the two inner rows approach very close to each other in the median ventral line, but I have never seen them in my section opening quite in the middle line. Both the dorsal and ventral rows are very irregular.

I have not found openings on the ventral or dorsal side of the feet but there are openings at the anterior and posterior aspects of the feet. There are, moreover, a considerable number of openings around the base of the feet.

The dorsal rows of tracheal apertures are continued into the head and give rise in this situation to enormous bundles of tracheæ.

In front of the mouth there is a very large median ventral tracheal pit, which gives off tracheæ to the ventral part of the nervous system, and still more in front a large number of such pits close together. The tracheæ to the central nervous system in many instances enter the nervous system bound up in the same sheath as the nerves.

The Muscular System.

The general muscular system consists of—(1) the general wall of the body; (2) the muscles connected with the mouth, pharynx, and jaws; (3) the muscles of the feet; (4) the muscles of the alimentary tract.

The muscular wall of the body is formed of—(1) an external layer of circular fibres; (2) an internal layer of longitudinal muscles; (3) a layer of transverse fibres.

The layer which I have spoken of as formed of circular fibres is formed of two strata of fibres which girth the body somewhat obliquely (Pl. XVIII, fig. 25). In the outer stratum the rings are arranged so that their ventral parts are behind, while the ventral parts of the rings of the inner stratum are most forward. Both in the median dorsal and ventral lines the layer of circular fibres become somewhat thinner, and where the legs are attached the regularity of both strata is somewhat interfered with, and they become continuous with a set of fibres inserted in the wall of the foot.

The longitudinal muscles are arranged as five bands (vide fig. 16), viz. two dorsal, two lateral, and three ventral. The three ventral may be spoken of as the latero-ventral and medio-ventral bands.

The transverse fibres consist of (1) a continuous sheet on each side inserted dorsally in the cutis, along a line opposite the space between the dorsal bands of longitudinal fibres, and ventrally between the ventro-median and ventro-lateral bands. Each sheet at its insertion slightly breaks up into separate bands. They divide the body cavity into three regions—a median, containing the alimentary tract, slime glands, &c., and two lateral, which are less well developed, and contain the nervous system, salivary glands, segmental organs, &c.

(2) Inserted a little dorsal to the transverse band just described is a second band which immediately crosses the first, and then passes on the outer side of the nervous cord and salivary gland, where such is present, and is inserted ventrally in the space between the ventro-lateral and lateral longitudinal band.

Where the feet are given off the second transverse band becomes continuous with the main retractor muscular fibres in the foot, which are inserted both on to the dorsal side and ventral side.

Muscular system of the feet.—This consists of the retractors of the feet connected with the outer transverse muscle and the circular layer of muscles. In addition to these muscles there are intrinsic transverse muscles which cross the

cavity of the feet in various directions (Pl. XVIII, fig. 20). There is no special circular layer of fibres.

Histology of the muscle.—The main muscles of the body are unstriated and divided into fibres, each invested by a delicate membrane. Between the membrane and muscle are scattered nuclei, which are never found inside the muscle fibres. The muscles attached to the jaws form an exception in that they are distinctly transversely striated.

The Body Cavity and Vascular System.

The Body Cavity, as already indicated, is formed of three compartments—one central and two lateral. The former is by far the largest, and contains the alimentary tract, the generative organs, and the mucous glands. It is lined by a delicate endothelial layer, and is not divided into compartments nor traversed by muscular fibres.

The lateral divisions are much smaller than the central, and are shut off from it by the inner transverse band of muscles. They are almost entirely filled with the nerve-cord and salivary gland in front and with the nerve-cord alone behind, and their lumen is broken up by muscular bands. They further contain the segmental organs which open into them. They are prolonged into the feet, as is the embryonic body cavity of most Arthropoda.

The Vascular System is usually stated to consist of a dorsal heart. I find between the dorsal bands of longitudinal fibres a vessel in a space shut off from the body cavity by a continuation of the endothelial lining of the latter (fig. 16). The vessel has definite walls and an endothelial lining, but I could not make out whether the walls were muscular. The ventral part of it is surrounded by a peculiar cellular tissue, probably, as suggested by Moseley, equivalent to the fat bodies of insects. It is continued from close to the hind end of the body to the head, and is at its maximum behind. In addition to this vessel there is present a very delicate ventral vessel, by no means easy to see, situated between the cutis and the outer layer of circular muscles.

Segmental Organs.

A series of glandular organs are found in Peripatus which have their external openings situated on the ventral surface of a certain number of the legs, and which, to the best of my belief, end internally by opening into the lateral compartments of the body cavity. These organs are probably of an excretory nature, and I consider them homologous with the nephridia or segmental organs of the Chætopoda.

In Peripatus capensis they are present in all the legs. In all of them (except the first three) the following parts may be recognised:

1. A vesicular portion opening to the exterior by a narrow passage.

2. A coiled portion, which is again subdivided into several sections.

3. A terminal section ending by a somewhat enlarged opening into the lateral compartment of the body cavity.

The last twelve pairs of these organs are all constructed in a very similar manner, while the two pairs situated in the fourth and fifth pairs of legs are considerably larger than those behind, and are in some respects very differently constituted.

It will be convenient to commence with one of the hinder nephridia. Such a nephridium from the ninth pair of legs is represented in fig. 28. The external opening is placed at the outer end of a transverse groove placed at the base of one of the feet, while the main portion of the organ lies in the body cavity in the base of the leg, and extends into the trunk to about the level of the outer edge of the nerve-cord of its side. The external opening (*o s*) leads into a narrow tube (*s d*), which gradually dilates into a large sac (*s*).

The narrow part is lined by small epithelial cells, which are directly continuous with and perfectly similar to those of the epidermis (fig. 20). It is provided with a superficial coating of longitudinal muscular fibres, which thins out where it passes over the sac, along which it only extends for a short distance.

The sac itself, which forms a kind of bladder or collecting

vesicle for the organ, is provided with an extremely thin wall, lined with very large flattened cells. These cells are formed of granular protoplasm, and each of them is provided with a large nucleus, which causes a considerable projection into the lumen of the sac (figs. 20, 29 *s*). The epithelial wall of the sac is supported by a membrana propria, over which a delicate layer of the peritoneal epithelium is reflected.

The coiled tube forming the second section of the nephridium varies in length, and by the character of the epithelium lining it may be divided into four regions. It commences with a region lined by a fairly columnar epithelium with smallish nuclei (fig. 28 *s c* 1). The boundaries of the cells of this epithelium are usually very indistinct, and the protoplasm contains numerous minute granules, which are usually arranged in such a manner as to give to optical or real sections of the wall of this part of the tube a transversely striated appearance. These granules are very probably minute balls of excretory matter.

The nuclei of the cells are placed near their free extremities, contrary to what might have been anticipated, and the inner ends of the cells project for very different lengths into the interior, so causing the inner boundary of the epithelium of this part of the tube to have a very ragged appearance. This portion of the coiled tube is continuous at its outer end with the thin-walled vesicle. At its inner end it is continuous with region No. 2 of the coiled tube (fig. 28 *s c* 2), which is lined by small closely-packed columnar cells. This portion is followed by region No. 3, which has a very characteristic structure (fig. 28 *s c* 3). The cells lining this part are very large and flat, and contain large disc-shaped nuclei, which are usually provided with large nucleoli, and often exhibit a beautiful reticulum. They may frequently be observed in a state of division. The protoplasm of this region is provided with similar granules to that in the first region, and the boundaries of the cells are usually very indistinct. The fourth region is very short (fig. 28 *s c* 4), and is formed of small columnar cells. It gradually narrows till it opens suddenly into the terminal section (*s o t*), which ends by opening into the body cavity, and constitutes the most

distinct portion of the whole organ. Its walls are formed of columnar cells almost filled by oval nuclei, which absorbs colouring matters with very great avidity, and thus renders this part extremely conspicuous. The nuclei are arranged in several rows.

The study of the internal opening of this part gave me some trouble. No specimens ever show it as rounded off in the characteristic fashion of tubes ending in a cul-de-sac. It is usually somewhat ragged and apparently open. In the best preserved specimens it expands into a short funnel-shaped mouth, the free edge of which is turned back. Sections confirm the results of dissections. Those passing longitudinally through the opening prove its edges are turned back, forming a kind of rudimentary funnel. This is represented in fig. 29, from the last leg of a female. I have observed remains of what I consider to be cilia in this section of the organ. The fourth region of the organ is always placed close to the thin-walled collecting vesicle (figs. 28 and 29). In the whole of the coiled tube just described the epithelium is supported by a membrana propria, which in its turn is invested by a delicate layer of peritoneal epithelium.

The fourth and fifth pairs are very considerably larger than those behind, and are in other respects peculiar. The great mass of each organ is placed behind the leg, on which the external opening is placed, immediately outside one of the lateral nerve-cords. Its position is shown in fig. 8.

The external opening, instead of being placed near the base of the leg, is placed on the ventral side of the third ring (counting from the outer end) of the thicker portion of the leg. It leads (fig. 27) into a portion which clearly corresponds with the collecting vesicle of the hinder nephridia. This part is not, however, dilated into a vesicle in the same sort of way, and the cells which form the lining epithelium have not the same characteristic structure, but are much smaller. Close to the point where the vesicle joins the coiled section of the nephridium the former has a peculiar nick or bend in it. At this nick it is firmly attached to the ventral side of the foot by

muscles and tracheæ, and when cut away from its attachment the muscles and tracheæ cannot easily be detached from it. The main part of the coils are formed by region No. 1, and the epithelial cells lining this part present very characteristically the striated appearance which has already been spoken of. The large-celled region of the coiled tube (fig. 27) is also of considerable dimensions, and the terminal portion is wedged in between this and the commencing part of the coiled tube. The terminal portion with its internal opening is in its histological characters exactly similar to the homologous region in the hinder nephridia.

The three pairs of nephridia in the three foremost pairs of legs are very rudimentary, consisting, so far as I have been able to make out, solely of the collecting vesicle and the duct leading from them to the exterior. The external opening is placed on the ventral side of the base of the feet, in the same situation as that of the posterior nephridia, but the histological characters of the vesicle are similar to those of the fourth and fifth pairs.

Generative Organs.

[The sexes are distinct, and the average size of the females appears to be greater than that of the males.

The only outward characteristic by which the males can be distinguished from the females is the presence in the former of a small white papilla on the ventral side of the 17th pair of legs (Pl. II, fig. 4). At the extremity of this papilla the modified crural gland of the last leg opens by a slit-like aperture.

The generative orifice in both sexes is placed on the ventral surface of the body, close to the anus, and between the two anal papillæ, which are much more marked in small specimens than in large ones, and in two cases (of females) were observed to bear rudimentary claws.

1. **The Male Organs.** Pl. XX, fig. 43.

The male organs consist of a pair of testes (*te*), a pair of

prostates (*pr*) and vasa deferentia (*vd*) and accessory glandular tubules (*f*).

All the above parts lie in the central compartment of the body cavity. In addition, the accessory glandular bodies or crural glands of the last (17th) pair of legs are enlarged and prolonged into an elongated tube placed in the lateral compartment of the body cavity (*a g*).

The arrangement of these parts represented in the figure appears essentially that which Moseley has already described for this species. The dilatations on the vasa deferentia, which he calls vesiculæ seminales, is not so marked; nor can the peculiar spiral twisting of this part of the vas deferens which he figures (No. 13) be made out in this specimen. The testes are placed at different levels in the median compartment of the body cavity, and both lie on the same side of the intestine (right side).

The arrangement of the terminal portions of the vas deferens is precisely that described by Moseley. The right vas deferens passes under both nerve-cords to join the left, and form the enlarged tube (*p*), which, passing beneath the nerve-cord of its side, runs to the external orifice. The enlarged terminal portion possesses thick muscular walls, and possibly constitutes a spermatophore maker, as has been shown to be the case in P. N. Zealandiæ, by Moseley.

In some specimens a different arrangement obtains, in that the left vas deferens passes under both nerve-cords to join the right.

In addition to the above structures, which are all described by Moseley, there are a pair of small glandular tubes (*f*), which open with the unpaired terminal portion of the vas deferens at the generative orifice.

2. **Female Organs.** Pl XIX, fig. 33.

The female organs consist of a median unpaired ovary and a pair of oviducts, which are dilated for a great part of their course to perform a uterine function, and which open behind

into a common vestibule communicating directly with the exterior.

Ovary.—In the specimen figured the following is the arrangement:

The ovary lies rather to the dorsal side in the central compartment of the body cavity, and is attached to one of the longitudinal septa separating this from the lateral compartment. It lies between the penultimate and antepenultimate pair of legs.

The oviducts cross before opening to the exterior. The right oviduct passes under the rectum, and the left over the rectum. They meet by opening into a common vestibule, which in its turn opens to the exterior immediately ventral to the anus. It has not been ascertained how far this arrangement, which differs from that observed by Moseley, is a normal one. The young undergo nearly the whole of their development within the uterus. They possess at birth the full number of appendages, and differ from the parent only in size and colour.

Notes on additional Glandular Bodies in the Legs [Crural Glands].

1. They are present in all except the first.

2. They open externally to the nephridia (Pl. XVIII, fig. 20), except in the fourth and fifth pairs of legs, in which they are internal.

3. A muscular layer covers the whole gland, consisting, I believe, of an oblique circular layer.

4. The accessory gland in the male (fig. 43, *a g*) is probably a modification of one of these organs.

[The structure and relations of these glands may be best understood by reference to Pl. XVIII, fig, 20. Each consists of a dilated vesicular portion (*f gl*) placed in the lateral compartment of the body cavity in the foot, and of a narrow duct leading to the exterior, and opening on the ventral surface amongst the papillæ of the second row (counting from the internal of the three foot pads (fig. 20 p).

The vesicular portion is lined by columnar cells, with very large oval nuclei, while the duct is lined by cells similar to the epidermic cells, with which they are continuous at the opening.

In the last (17th leg) of the males of this species, this gland (vide above, note 4) possesses a slit-like opening placed at the apex of well-developed white papilla (Pl. XIV, fig. 4). It is enormously enlarged, and is prolonged forward as a long tubular gland, the structure of which resembles that of the vesicles of the crural glands in the other legs. This gland lies in the lateral compartment of the body cavity, and extends forward to the level of the 9th leg (Pl. XV, fig. 8, and Pl. XX, fig. 43). It is described by Professor Balfour as the accessory gland of the male, and is seen in section lying immediately dorsal to the nerve-cord in fig. 20, *a g*.]

PART III.

The Development of Peripatus capensis.

[The remarkable discoveries about the early development of Peripatus, which Balfour made in June last, shortly before starting for Switzerland, have already been the subject of a short communication to the Royal Society ('Proc. Roy. Soc.' No. 222, 1882.) They relate (1) to the blastopore, (2) to the origin of the mesoblast.

Balfour left no manuscript account or notes of his discovery in connection with the drawings which he prepared in order to illustrate it, but he spoke about it to Professor Ray Lankester and also to us, and he further gave a short account of the matter in a private letter to Professor Kleinenberg.

In this letter, which by the courtesy of Professor Kleinenberg we have been permitted to see, he describes the blastopore as an elongated slit-like structure extending along nearly the whole ventral surface; and further states, as the result of his examination of the few and ill-preserved embryos in his possession,

that the mesoblast appears to originate as paired outgrowths from the lips of the blastopore.

The drawings left by Balfour in connection with the discoveries are four in number: one of the entire embryo, showing the slit-like blastopore and the mesoblastic somites, the other three depicting the transverse sections of the same embryo.

The first drawing (fig. 37), viz. that of the whole embryo, shows an embryo of an oval shape, possessing six somites, whilst along the middle of its ventral surface there are two slit-like openings, lying parallel to the long axis of the body, and placed one behind the other. The mesoblastic somites are arranged bilaterally in pairs, six on either side of these slits. The following note in his handwriting is attached to this drawing:

"Young larva of Peripatus capensis.—I could not make out for certain which was the anterior end. Length 1·34 millimetres."

Balfour's three remaining drawings (figs. 40—42) are, as already stated, representations of transverse sections of the embryo figured by him as a whole. They tend to show, as he stated in the letter referred to above, that the mesoblast originates as paired outgrowths from the hypoblast, and that these outgrowths are formed near the junction of the hypoblast with the epiblast at the lips of the blastopore.

In fig. 40 the walls of the mesoblastic somites appear continuous with those of the mesenteron near the blastopore.

In fig. 41, which is from a section a little in front of fig. 40, the walls of the mesoblastic somites are independent of those of the mesenteron.

Fig. 42 is from a section made in front of the region of the blastopore.

In all the sections the epiblast lying over the somites is thickened, while elsewhere it is formed of only one layer of cells; and this thickening subsequently appears to give rise to the nervous system. Balfour in his earlier investigations on the present subject found in more advanced stages of the

embryo the nerve-cords still scarcely separated from the epiblast.[1]

We have since found, in Balfour's material, embryos of a slightly different age to that just described. Of these, three (figs. 34, 35, 36) are younger, while one (fig. 38) is older than Balfour's embryo.

Stage A.—The youngest (fig. 34) is of a slightly oval form, and its greatest length is ·48 mm. It possesses a blastopore, which is elongated in the direction of the long axis of the embryo, and is slightly narrower in its middle than at either end. From one end of the blastopore there is continued an opaque band. This we consider to be the posterior end of the blastopore of the embryo. The blastopore leads into the archenteron.

Stage B.—In the next stage (fig. 35) the embryo is elongate-oval in form. Its length is ·7 mm. The blastopore is elongated and slightly narrowed in the middle. At the posterior end of the embryo there is a mass of opaque tissue. On each side of the blastopore are three mesoblastic somites. The length of the blastopore is ·45 mm.

Stage C.—In the next stage (fig. 36) the features are much the same as in the preceding. The length of the whole embryo is ·9 mm.

The following were the measurements of an embryo of this stage with five somites, but slightly younger than that from which fig. 36 was drawn.

Length of embryo	·74 mm.
„ blastopore	·46 „
Distance between hind end of blastopore and hind end of body	·22 „
„ „ front end of body and front end of blastopore	·06 „

The somites have increased to five, and there are indications of a sixth being budded off from the posterior mass of opaque tissue. The median parts of the lips of the blastopore have come together preparatory to the complete fusion by which the blastopore becomes divided into two parts.

[1] 'Comparative Embryology,' vol. i. p. 318.

Stage D.—The next stage is Balfour's stage, and has been already described.

The length is 1·34.

It will be observed, on comparing it with the preceding embryos, that while the anterior pair of somites in figs. 35 and 36 lie at a considerable distance from what we have called the anterior end of the embryo (*a*), in the embryo now under consideration they are placed at the anterior end of the body, one on each side of the middle line. We cannot speak positively as to how they come there, whether by a pushing forward of the anterior somites of the previous stage, or by the formation of new somites anteriorly to those of the previous stage.

In the next stage it is obvious that this anterior pair of somites has been converted into the præoral lobes.

The anterior of the two openings to which the blastopore gives rise is placed between the second pair of somites; we shall call it the embryonic mouth. The posterior opening formed from the blastopore is elongated, being dilated in front and continued back us a narrow slit (?) to very near the hind end of the embryo, where it presents a second slight dilatation. The anterior dilatation of the posterior open region of the blastopore we shall call the embryonic anus.

Lately, but too late to be figured with this memoir, we have been fortunate enough to find an embryo of apparently precisely the same stage as fig. 37. We are able, therefore, to give a few more details about the stage.

The measurements of this embryo were:

Length of whole embryo	1·32 mm.
Distance from front end of body to front end of mouth .	·32 „
„ embryonic mouth to hind end of embryonic anus	·52 „
„ from hind end of embryonic anus to hind end of body	·45 „
Length of embryonic anus	·2 „
„ part of blastopore behind embryonic anus . .	·2 „
Greatest width of embryo	·64 „

Stage E.—In the next stage (figs. 38 and 39) the flexure of the hind end of the body has considerably increased. The

anterior opening of the blastopore, the embryonic mouth, has increased remarkably in size. It is circular, and is placed between the second pair of mesoblastic somites. The anterior dilatation of the posterior opening of the blastopore, the embryonic anus, has, like the anterior opening, become much enlarged. It is circular, and is placed on the concavity of the ventral flexure. From its hind end there is continued to the hind end of the body a groove (shown in fig. 39 as a dotted line), which we take to be the remains of the posterior slit-like part of the posterior opening of the blastopore of the preceding stage. The posterior dilatation has disappeared. The embryo has apparently about thirteen somites, which are still quite distinct from one another, and apparently do not communicate at this stage with the mesenteron.

The epiblast lying immediately over the somites is, as in the earlier stages, thickened, and the thickenings of the two sides join each other in front of the embryonic mouth, where the anterior pair of mesoblastic somites (the præoral lobes) are almost in contact.

The median ventral epiblast, i.e. the epiblast in the area, bounded by the embryonic mouth and anus before and behind and by the developing nerve-cords laterally, is extremely thin, and consists of one layer of very flat cells. Over the dorsal surface of the body the epiblast cells are cubical, and arranged in one layer.

Measurements of Embryo of Stage E.

Length of embryo	1·12 mm.
Greatest width	·64 „
Distance from front end of embryonic mouth to hind end of embryonic anus	·48 „
Greatest length of embryonic mouth	·16 „
Length between hind end of embryonic mouth and front end of embryonic anus	·29 „

These measurements were made with a micrometer eyepiece, with the embryo lying on its back in the position of fig. 38, so

that they simply indicate the length of the straight line connecting the respective points.

This is the last embryo of our series of young stages. The next oldest embryo was 3·2 mm. in length. It had ringed antennæ, seventeen (?) pairs of legs, and was completely doubled upon itself, as in Moseley's figure.

The pits into the cerebral ganglia and a mouth and anus were present. There can be no doubt that the mouth and anus of this embryo become the mouth and anus of the adult.

The important question as to the connection between the adult mouth and anus, and the embryonic mouth and anus of the Stage E, must, considering the great gap between Stage E and the next oldest embryo, be left open. Meanwhile, we may point out that the embryonic mouth of Stage E has exactly the same position as that of the adult; but that the anus is considerably in front of the hind end of the body in Stage E, while it is terminal in the adult.

If the embryonic mouth and anus do become the adult mouth and anus, there would appear to be an entire absence of stomodæum and proctodæum in Peripatus, unless the buccal cavity represents the stomodæum. The latter is formed, as has been shown by Moseley, by a series of outgrowths round the simple mouth-opening of the embryo, which enclosing the jaws give rise to the tumid lips of the adult.

For our determination of the posterior and anterior ends of each of these embryos, Stage A to E, we depend upon the opaque tissue seen in each case at one end of the blastopore.

In Stage A it has the form of a band, extending backwards from the blastopore.

In Stages B—D, it has the form of an opaque mass of tissue occupying the whole hind end of the embryo, and extending a short distance on either side of the posterior end of the blastopore.

This opacity is due in each case to a proliferation of cells of the hypoblast, and, perhaps, from the epiblast (?).

There can be no doubt that the mesoblast so formed gives rise to the great majority of the mesoblastic somites.

This posterior opacity is marked in Stage C by a slight longitudinal groove extending backwards from the hind end of the blastopore. This is difficult to see in surface views, and has not been represented in the figure, but is easily seen in sections.

But in Stage D this groove has become very strongly marked in surface views, and looks like a part of the original blastopore of Stage C.

Sections show that it does not lead into the archenteron, but only into the mass of mesoblast which forms the posterior opacity. It presents an extraordinary resemblance to the primitive streak of vertebrates, and the ventral groove of insect embryos.

We think that there can be but little doubt that it is a part of the original blastopore, which, on account of its late appearance (this being due to the late development of the posterior part of the body to which it belongs), does not acquire the normal relations of a blastopore, but presents only those rudimentary features (deep groove connected with origin of mesoblast) which the whole blastopore of other tracheates presents.

We think it probable that the larval anus eventually shifts to the hind end of the body, and gives rise to the adult anus. We reserve the account of the internal structure of these embryos (Stages A—E) and of the later stages for a subsequent memoir.

We may briefly summarise the more important facts of the early development of Peripatus capensis, detailed in the preceding account.

1. The greater part of the mesoblast is developed from the walls of the archenteron.

2. The embryonic mouth and anus are derived from the respective ends of the original blastopore, the middle part of the blastopore closing up.

3. The embryonic mouth almost certainly becomes the adult mouth, i. e. the aperture leading from the buccal cavity into the pharynx, the two being in the same position. The em-

bryonic anus is in front of the position of the adult anus, but in all probability shifts back, and persists as the adult anus.

4. The anterior pair of mesoblastic somites gives rise to the swellings of the præoral lobes, and to the mesoblast of the head.[1]

There is no need for us to enlarge upon the importance of these facts. Their close bearing upon some of the most important problems of morphology will be apparent to all, and we may with advantage quote here some passages from Balfour's 'Comparative Embryology,' which show that he himself long ago had anticipated and in a sense predicted their discovery.

"Although the mesoblastic groove of insects is not a gastrula, it is quite possible that it is the rudiment of a blastopore, the gastrula corresponding to which has now vanished from development." ('Comparative Embryology,' vol. i, p. 378.)

"Tracheata.—Insecta. It (the mesoblast) grows inwards from the lips of the germinal groove, which probably represents the remains of a blastopore." ('Comparative Embryology,' vol. ii, p. 291.)

"It is, therefore, highly probable that the paired ingrowths of the mesoblast from the lips of the blastopore may have been, in the first instance, derived from a pair of archenteric diverticula." ('Comparative Embryology,' vol. ii, p. 294.)

The facts now recorded were discovered in June last, only a short time before Balfour started for Switzerland; we know but little of the new ideas which they called up in his mind. We can only point to passages in his published works which seem to indicate the direction which his speculations would have taken.

After speculating as to the probability of a genetic connection between the circumoral nervous system of the Cœlenterata, and the nervous system of Echinodermata, Platyelminthes, Chætopoda, Mollusca, &c., he goes on to say:

"A circumoral nerve-ring, if longitudinally extended, might

[1] We have seen nothing in any of our sections which we can identify as of so-called mesenchymatous origin.

give rise to a pair of nerve-cords united in front and behind —exactly such a nervous system, in fact, as is present in many Nemertines (the Enopla and Pelagonemertes), in Peripatus and in primitive molluscan types (Chiton, Fissurella, &c.). From the lateral parts of this ring it would be easy to derive the ventral cord of the Chætopoda and Arthropoda. It is especially deserving of notice, in connection with the nervous system of the above-mentioned Nemertines and Peripatus, that the commissure connecting the two nerve-cords behind is placed on the dorsal side of the intestines. As is at once obvious, by referring to the diagram (fig. 231 B), this is the position this commissure ought, undoubtedly, to occupy if derived from part of a nerve-ring which originally followed more or less closely the ciliated edge of the body of the supposed radiate ancestor." ('Comparative Embryology,' vol. ii, pp. 311, 312.)

The facts of development here recorded give a strong additional support to this latter view, and seem to render possible a considerable extension of it along the same lines.]

[The editors of the present memoir intend to prepare for publication a complete monograph of all the species of Peripatus known, with figures in extension of the materials for that purpose collected by the late Prof. Balfour. They would, therefore, feel extremely obliged for the loan of any specimens, especially of species from the West Indies. Such specimens would be most carefully preserved from injury, and returned after inspection and comparison. Any such should be sent to Mr. A. Sedgwick, Trinity College, Cambridge.]

List of Memoirs on Peripatus.

(1) M. Lansdown Guilding.—"An Account of a New Genus of Mollusca," 'Zoological Journal,' vol ii, p. 443, 1826.

(2) M. Andouin and Milne-Edwards.—"Classific des Annélides et description le celles qui habitent les côtes de France," p. 411, 'Ann. Scien. Nat.,' ser. i, vol. xxx, 1833.

(3) M. Gervais.—"Etudes p. servir a l'histoire naturelle des Myriapodes," 'Ann. Scien. Nat.,' ser. ii, vol. vii, 1837, p. 38.

(4) Wiegmann.—'Wiegmann's Archiv,' 1837.

(5) H. Milne-Edwards.—"Note sur le Peripate juluforme," 'Ann. Scien. Nat.,' ser. ii, vol. xviii, 1842.

(6) Blanchard.—"Sur l'organization des Vers," chap. iv, p. 137—141, 'Ann. Scien. Nat.,' vol. viii, 1847.

(7) Quatrefages.—"Anat. des Hermelles, note on," p. 57, 'Ann. Scien. Nat.,' ser. iii, vol. x, 1848.

(8) Quatrefages.—'Hist. Nat. des Annelés,' 1865, Appendix, pp. 675-6.

(9) De Blainville.—'Suppl. au Dict. des Sc. Nat.,' vol. i.

(10) Ed. Grube.—"Untersuchungen üb. d. Bau von Peripatus Edwardsii," 'Archiv für Anat. und Physiol.,' 1853.

(11) Saenger.—"Moskauer Naturforscher Sammlung," 'Abth. Zool.,' 1869.

(12) H. N. Moseley.—"On the Structure and Development of Peripatus capensis," 'Proc. Roy. Soc.,' No. 153, 1874.

(13) H. N. Moseley.—"On the Structure and Development of Peripatus capensis," 'Phil. Trans.,' vol. clxiv, 1874.

(14) H. N. Moseley.—"Remarks on Observations by Captain Hutton, Director of the Otago Museum, on Peripatus novæ zealandiæ," 'Ann. and Mag. of Nat. History,' Jan., 1877.

(15) Captain Hutton.—"Observations on Peripatus novæ zealandiæ," 'Ann. and Mag. of Nat. History,' Nov., 1876.

(16) F. M. Balfour.—"On Certain Points in the Anatomy of Peripatus capensis," 'Quart. Journ. of Micr. Science,' vol. xix, 1879.

(17) A. Ernst.—'Nature,' March 10th, 1881.

On a Morphological Variety of Bacillus Anthracis.

By

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With Plate XXI.

In my Report of 1881 to the Medical Officer of the Local Government Board, reprinted in this Journal, January, 1883, I have mentioned the occurrence, in artificial cultivations of Bacillus anthracis, of peculiar torula-like cells connected with the Bacillus anthracis. I have stated there (this Journal, January, p. 37) that observing the growth of the threads of Bacillus anthracis in "cell specimens," it is found that the growing ends of the threads are occasionally seen in connection with a row of torula-like cells, i. e. spherical or elliptical cells closely placed so as to form a complete chain. The torula-like cells are larger than the cubical cells, which I there described as constituting the elements of the Bacillus anthracis.

I have since followed this change more in its details, and propose in the following pages to describe the results of my observations.

Every cultivation made of Bacillus anthracis in the pork broth or the mixture of pork broth and gelatine in test-tubes or flasks, as described in the above-named paper, when exposed to a temperature of 20°—25° C., shows some, although not many, of the torula-like variety. But I have succeeded in

obtaining at will a copious crop of this variety, by sowing the ordinary typical Bacillus anthracis of an artificial cultivation on to the surface of the solid gelatine pork broth, contained in test-tubes or flasks plugged with sterile cotton wool (see my former paper), and keeping these cultures at ordinary temperature of the room, i. e. about 15—20° C. The growth under these conditions proceeds slowly, and taking out a sample of the growth after two or three days, we find the astonishing fact that almost the entire growth, or the greater majority of it, consists of the torula variety. What one finds is this—spherical or slightly elliptical cells, of a diameter varying between 0·0013 and 0·0026 mm., or more, isolated, or in small groups, or more commonly in longer or shorter chains (see figs. 1, 3, and 6). Some chains are composed of cells which are twice the diameter of the cells of a neighbouring chain. In the fresh state these cells possess a distinct membrane, a clear contents, and one or two granules. In dried and stained specimens the cells are stained deeply as a whole.

Some of the cells show constrictions dividing them into two, three, or even four small cells, or they are in a state of GEMMATION, like a true torula, a smaller or larger knob protruding either in the long axis of the chain, or, what is not at all uncommon, to the side of it. In consequence of this, and also in consequence of the division of the largest cells into three or four daughter cells, we find often attached to the side of a chain one or more cells (see figs. 1, 3, and 4). When dividing into four, we obtain a form similar to a sarcina.

Besides these chains we meet with long rows of cells, of which the majority or minority are spherical and large, the remainder elliptical, and approaching more or less the ordinary thin, rod-shaped elements of the typical bacillus. When staining such a specimen, we notice this fact, that the staining does not extend uniformly over the whole chain, but that the deeply stained spherical or elliptical cells are separated from one another —or rather connected with one another—by a very faintly stained thin, longer or shorter bridge. Fig. 2 illustrates this point.

It is noticed at the same time that the torula cells of the same chain are of various sizes, some being twice and three times as big as others (see fig. 2). Whether small or large they may show division or gemmation into two or three, or four. The most interesting forms of chains are those which contain here and there one or two big torula cells amongst a long series of small cells, the former resembling a sort of sporangium. And, indeed, such big cells may contain two, three, or four granules.

As the cultivation proceeds many of the torula chains are gradually converted into the thin typical threads of Bacillus anthracis; here and there in the thread a more or less elliptical cell is still recognisable, or a series of them, so as to denote the way the thread has originated. In fig. 4 the transition is very well shown. Some of the threads that have become typical threads of Anthrax bacillus, when followed up to their growing end, still show in a very marked manner the torula nature of their cells. Although when one examines these preparations, one is at first inclined to assume that there exist in these cultivations two distinct kinds of organisms, viz. (*a*) typical threads of Anthrax bacillus, and (*b*) a kind of torula—so great is the contrast between the two; still, when examining carefully the specimen, all doubt soon disappears, since one finds not only all intermediate forms, but the two kinds along the very same thread. I have shown such specimens to many of my friends well able to judge on these points, and I had no difficulty whatever in demonstrating to them the correctness of my views, although at first sight they were hardly inclined to admit it.

In the accompanying illustrations I have selected some of the more pronounced forms, and the reader will have no difficulty in recognising the transitions between the two forms. When the torula-like cells of the chain become elliptical, and when these still more elongate, a thin typical thread of Bacillus anthracis will be the result.

As the cultivation proceeds, say after several weeks, the number of the chains and cells of the torula variety gradually

diminish, and the greater amount of the growth is composed of the typical smooth, thin threads, with here and there an indication in them of a spherical or elliptical torula-like cell.

I am indebted to my friend Dr. George Maddox for some photographs which he made of some of the specimens; the figures 6, 7, and 8 are exact copies of them. This gentleman has kindly furnished me with an abstract of a description by Dr. Antoine Magnin (translated by Dr. Sternberg, of Boston) of certain unpublished observations by M. Toussaint, according to which this experienced observer has seen the Bacillus anthracis growing in a "Ranvier's" moist chamber at 37—40° C., undergo changes, by which the protoplasm in some becomes collected into larger or smaller spherical or elliptical sporangia, each of them yielding several "spores." The membrane finally bursts, and the "spores" are freed. Toussaint has seen these spores undergo germination and elongation into typical Anthrax bacilli. There can be no doubt that some of my torula-like cells correspond to Toussaint's sporangia. I have repeated Toussaint's experiments, but could not see the freeing of the "spores." I do not consider the granules present in the cells to be spores; I have only been able to see that the protoplasm of the cells divides into two, three, and four; but each of these is capable of growing up into a large spherical or oval cell. These granules are not spores in any sense, at least not in that sense in which are the bright, highly-refractive oval corpuscles that appear in the threads of Bacillus anthracis, and in other kinds of bacilli when they are supplied with sufficient amount of oxygen. The granules stain well in anilin dyes, the typical spores do not. The small cells derived by gemmation and division from our larger spherical cells, are identical in structure and in value with their parent cells, but the typical bright oval spores of Anthrax bacillus are, as is well known, altogether different structures from the parent cells.

In our case the oval or spherical cells, no matter whether large or small, are capable of division and gemmation, and

hereby are capable of producing daughter cells; further, the spherical or oval cells in our case become ultimately elongated, so as to contribute to form the typical rods and threads of Bacillus anthracis. These rods give origin, by transverse division, to cubical cells, such as are noticed in the ordinary Bacillus anthracis after staining with anilin dyes.

We see, then, that the Bacillus anthracis shows two distinct morphological varieties, viz. one: the typical bacillus, the other: a torula-like form; the cells are spherical or elliptical, and capable of gemmation like a real torula cell, or of dividing into two, three, or four new cells like a true schyzomycetes. The spherical and elliptical torula-like cells elongate, and are transformed into ordinary typical bacillus. The torula-like cells are not spores, nor do they form sporangia.

An interesting fact that I observed is this, that as time goes on the torula variety, in chains as well as in threads, undergoes the same degeneration which I observed in ordinary cultures of Bacillus anthracis in fluid media, and have described in my paper printed in the January number of this Journal, 1883, viz. the protoplasm gradually disintegrates into granules and these gradually dissolving, leave the empty sheath behind. Of a formation of real spores there is nothing to be seen in these cases; such a culture has lost all power of infection. This is another proof that no spores are formed by the torula cells.

The torula-like cells, while intact, are physiologically just as poisonous as the ordinary Bacillus anthracis, since guinea-pigs and rabbits invariably die of typical anthrax when inoculated with them.

The bacillus found in such animals is always the ordinary Bacillus anthracis.

Note on the Foregoing.

By

E. Ray Lankester, M.A., F.R.S.

My friend, Dr. Klein, kindly suggests that I should add to his paper a note calling the reader's attention to the series of varieties of Bacterium rubescens which were described and figured by me in vol. xiii, p. 408 (1873), and in vol. xvi, p. 27 (1876) of this Journal, since they offer an interesting parallel to the important fact discovered by Dr. Klein.

Dr. Klein's observation establishes that Bacillus anthracis is, so far as form is concerned, a "Protean species," and once for all demonstrates the error of those who, like Koch, have arrived at the conclusion that the forms of Bacteriaceæ are fixed and breed true.

I have enumerated in the papers above referred to the possible variations of form known at that date as occurring amongst Bacteriaceæ. The particular variety of Bacillus anthracis now described by Dr. Klein adds a new form to the list there given.

Using the term "plastid" to denominate the unit of structure of the Bacteriaceæ, I have pointed out four categories under which their form-characters may be grouped, viz.:

A. Shape of the plastids.

B. Substance of the plastid; relation of protoplasm and cell wall in each plastid.

C. Distribution of colour (when present) in each plastid.

D. Mode of aggregation of the plastids.

Under heading A we have the following possibilities: 1. Spherical; 2. Biscuit-shaped; 3. Bacillar; 4. Filamentous; 5. Acicular; 6. Serpentine; 7. Spiral; 8. Helicoid.

Under heading B we have as possibilities—

1. Plastids clean; or, on the other hand,

2. Plastids glœogenous (producing a jelly-like cell wall)

And again as alternatives—

3. Plastids homogeneous (no cell wall distinguishable from contents); or,

4. Plastids loculate (unilocular or moltilocular).

Under heading c we have the colouring matter—

1. Diffuse; or,

2. Locular (confined to the substance within the loculi).

Lastly, under heading d we have as varieties in the mode of aggregation of the plastids the following, which undoubtedly is not an exhaustive list:—1. Linear (thread-like); 2. Stellar; 3. Globose; 4. Massive; 5. Arborescent; 6. Catenular; 7. Reticular; 8. Tesselate.

Dr. Klein's new form of Bacillus anthracis consists of plastids of spherical shape, and each plastid is unilocular and clean; the plastids are in catenular aggregation. But a special kind of catenular aggregation is exhibited by Dr. Klein's growth, inasmuch as there is an irregularity in the size of the aggregated spherical plastids. At approximately regular recurrent intervals large spherical plastids are interposed between series of smaller ones. Such an arrangement of spherical plastids aggregated in chains is familiar to botanists in the case of the Alga Nostoc, and hence this variety of catenular aggregation in the Bacteriaceæ may be conveniently registered as "nostocoid catenular aggregation."

The commonly known twisted filaments obtained in cultivations of Bacillus anthracis, and figured in fig. 4 *a* of Dr. Klein's plate, are linear aggregates of very short bacillar plastids. It will be found most convenient to separate these very short cylindrical plastids from the longer forms (twice or many times as long as they are broad) under the name "micro-bacilli"—the longer cylindrical plastids, such as are commonly observed in early stages of a growth of Bacillus subtilis, being called "bacilli" as heretofore. Short rods built up by linear aggregation of micro-bacilli are not to be confused with homogeneous macro-bacilli consisting of one elongated plastid. The twisting of the linear aggregates into a rope, shown in fig. 4 *a*, introduces to us the

necessity of an additional series of terms descriptive of the grouping of the aggregates; that is to say, we require terms describing the combinations of the second order formed by the union of those combinations of the first order, which we have called "aggregates of plastids." Of such "combinations of aggregates" the present may be described as "funicular."

Thus, in a systematic way we should give the following description of fig. 4 *a*:

"Plastids.—Shape, microbacillar; substance, homogeneous, clean; colour, wanting.

"Aggregates of plastids.—Linear.

"Combinations of aggregates. — Funicular, the filaments twisted and recurrent."

Note on a Pink Torula.

By

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Some time ago I examined for my colleague at St. Bartholomew's Hospital, Dr. W. J. Russell, F.R.S., a sample of distilled water contained in a water bottle, through which 25 cubic feet of London fog air had been passing every hour for twenty-four hours. In this water were present, besides numbers of soot and dirt particles, large numbers of mycelia, or what appeared to be the mycelium of penicillium and mucor.

There were also present bacilli in the shape of longer or shorter, apparently smooth threads, and also a few ordinary torula cells, Saccharomyces cerevisiæ. With this water I inoculated a few test-tubes plugged with sterilised cotton wool containing neutral, or slightly acid pork broth, such as I used for other cultivation purposes (see my paper in this Journal, January, 1883), and placed them in the incubator at 32° C. After several days there was present in the test-tubes a fair amount of a whitish, or rather colourless nebulous sediment, which, when examined under the microscope, was composed of the most exquisite threads singly and in spiral bundles of the above bacillus. There were also present some short bacilli of the above kind; they were all non-moving. The bundles of spirally convoluted threads were identical with the typical cable-like bundles of Bacillus anthracis, and it would have been very difficult to recognise a difference at first sight; but they were not, of course, anthrax bacilli, as was soon ascertained by experiment. Besides these bacilli there were pre-

sent in the culture numerous cells of the yeast Saccharomyces cerevisiæ. The cells are oval, consisting of a limiting membrane and a homogeneous, highly refractive protoplasm, and in it at one place one or two vacuoles. In some of them there was one large bright corpuscle present at one side of the protoplasm. In some the protoplasm appears slightly granular. The cells are of different sizes, some twice as big as others. Their sizes are as follows: the big cells 0·009 mm. by 0·01 mm., the small ones 0·005 mm. by 0·008 mm. The small ones are evidently young forms, since they could be seen to sprout out, and to become constricted off from bigger ones.

As regards the process of reproduction, it appeared to me to be that of gemmation only. Hereby large groups of cells, some chain-like, were formed, which groups by enlargement become soon confluent into larger masses.

The pork broth, kept at 32° C. for several weeks, became so concentrated, that when taken out of the incubator and allowed to cool, almost solidified. Keeping it in this state at the ordinary temperature of the room, it was noticed, after some days, that the growth appeared on the surface of the nourishing material in the shape of minute whitish spots or flat droplets, which, as they gradually enlarged, assumed a distinct pinkish colour. The enlargement in breadth and thickness proceeded in a few days so far that the whole surface of the almost solid nourishing material became covered with a pinkish film, in which, however, the individuality of the droplets could still be recognised. Under the microscope these pink droplets are composed entirely of torula cells of exactly the same nature and size as those above described. They are, no doubt, the same organisms, as will appear also from other facts presently to be mentioned.

The cells themselves do not possess any colour when looked at under the microscope, singly or in a thin layer, but they appear of a pinkish tint when viewed as a group, or in a thick layer.

I have sown out from this layer of pink torula cells on to boiled white of egg, solid gelatine, and mixture of gelatine and

pork broth, used in my experiments on Anthrax bacillus. With the egg I have not obtained any satisfactory results, but with the gelatine and the mixture of gelatine and pork broth I have obtained beautiful crops. The sowing was done with the point of a capillary glass tube on to the free surface of the nutritive material (contained in flasks or test-tubes, plugged with sterilised cotton wool); and after an incubation of about four days, the vessel being kept at ordinary temperature of the room, there appeared the first signs of the growth having taken root, in the shape of a minute pinkish droplet; this gradually spread in breadth and thickness. The very interesting fact observed with this increase was this: the masses growing downward into the nutritive material remained colourless, whereas those spreading on the free surface were pink, both being composed of exactly the same torula cells.

The thicker the layer became, the deeper the pink tint. The gelatine does not become liquified by the growth, and in this respect it differs from a growth of micrococci, bacteria, or bacilli.

Sowing the pink torula into the depth of fluid nutritive material, such as pork broth, and keeping it at the bottom of the fluid, it is noticed that no matter whether growing at ordinary temperature of the room, or in the incubator at 30—35° C., it remains colourless, and when of considerable amount, appears like a whitish precipitate at the bottom of the fluid.

Sowing this colourless torula on to a free surface, it again gives origin to pink growth. But also in the same tube the at first colourless torula, i. e. while growing at the bottom of the fluid, may, when reaching the free surface, give origin to the pink growth.

Another interesting fact I have observed is this, that when a copious growth of pink torula has made its appearance on the surface of the solid nourishing material (gelatine), and this nourishing material is made fluid, so that the pink growth sinks to the bottom, and the material is again allowed to solidify: it will be observed that the pink mass retains its colour, that is to say, that the torula, once pink does not loose its colour when removed from the free access of air. But the

new increment of the mass at the bottom of the now solid nourishing material is not pink, but colourless.

Schröter (Cohn's 'Beiträge zur Biologie d. Pflanzen,' ii Heft. p. 112) mentions in a footnote that he observed occasionally on discs cut from a potato, mucous droplets of a pinkish colour, which, when examined under the microscope, were seen to consist entirely of torula cerevisiæ. The cells were not coloured.

My friend Professor Lankester informs me, that when carrying on his researches on Bacterium rubescens (see this Journal, New Series, vol. xiii) in the laboratory of the Botanic Garden, Oxford, he observed a pink torula which spontaneously made its appearance in a test-tube containing Pasteur's solution.

Observations on Saprolegniæ.

By

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With Plate XXII.

The Saprolegniæ are a family of Fungi characterised especially by their aquatic habits and correlated delicate structure; they are for the most part Saprophytes, flourishing on the decaying bodies of animals or plants in water, though several are now known as parasites on living members of both kingdoms, in which they cause profound destructive changes, sometimes ending in the death of the host.

To the latter parasitic forms belong Saprolegnia de Baryi and S. Schachtii, found in the cells of Algæ and Hepaticæ respectively, according to Walz[1] and Frank,[2] and especially the Saprolegnia of the "salmon disease," according to Professor Huxley.[3]

Generally described, the Saprolegniæ consist of a thallus,[4] bearing reproductive organs of two kinds—zoosporangia and oogonia, with or without accompanying antheridia. The thallus consists of long, branched, tubular hyphæ, of which the main portions are free or "extramatrical;"[5] shorter,

[1] 'Bot. Zeitg.,' 1870, p. 537.

[2] 'Krankheiten der Pfl.,' erste Hälfte, p. 383.

[3] This Journal, 1882, p. 311.

[4] It seems almost necessary to preserve the general name Thallus here, as De Bary has done in his recent memoirs, although the Saprolegniæ are accepted as Fungi, chiefly on physiological grounds.

[5] De Bary, 'Beiträge zur Morph. u. Phys. der Pilze,' 4th ser., 1881. This

rhizoid-like branches penetrating the tissues from which the fungus radiates.

De Bary has lately pointed out that the "intramatrical" portion of the thallus does not spread far in the tissues, and no extension of the fungus occurs by outward developments from its internal branches; the hyphæ outside, however, continually send downward prolongations, which take root and spread slightly in the attacked tissues, and thus the area of "intramatrical" hyphæ becomes extended.[1] Hence, if zoospores, &c., are prevented from again attacking the matrix from without, the Saprolegnia thallus does not extend far within the tissues —an important distinction between these fungi and those which, like Pythium, &c., attack a host at one point and send ramifications in all directions inside the tissues. The difference is, roughly speaking, analogous to that between a banyan tree and a bamboo, in so far that the former extends its area of feeding ground by sending down prolongations from its outer branches to root afresh in the matrix or earth, while the latter extends itself under the surface by means of underground shoots, which protrude here and there further from the parent stock.

The tubular hyphæ forming the thallus vary much in diameter, partly according to position, main stems and branches being thicker than secondary and tertiary ones; their cellulose walls are very thin and transparent, and enclose protoplasmic and oily contents. These latter are, as a rule, very coarsely granular, causing the thallus branches to appear dense and opaque, usually with a yellowish hue.

Septa occur very rarely in the tubular branches, but are always found separating the zoosporangia and the sexual reproductive organs from the purely vegetative portions of the thallus.

The Saprolegniæ have usually been stated to be a-nucleate in common with other fungi.[2] This, however, is not the case; publication is now the most important authority for the morphology of the group.

[1] Loc. cit., pp. 95 to 97.

[2] Luerssen, 'Med. Pharm. Bot.' 1879, B. 1, p. 72, and Sach's 'Lehrbuch,' iv ed.

Schmitz has described nuclei in these and allied fungi, as well as in many others of the lower cryptogams.[1] I have lately also found nuclei of a very definite character in the mycelium of an allied fungus, and shall show that a perfectly definite division of nuclear masses occurs in the zoosporangia of Achlya and Saprolegnia. It is at least certain that the Saprolegniæ can no longer be regarded as devoid of nuclei, and the same probably holds good for all but the very lowest cryptogams; according to Schmitz the Phycochromaceæ and Schizomycetes.

The reproduction of the Saprolegniæ takes place by means of asexual zoospores, produced in long zoosporangia; and sexual (at least morphologically they must be considered so) oospores, produced in oogonia, with or without accompanying antheridia. The details concerning both these kinds of structures may be deferred for the moment.

With this introduction, the immediate object of the present essay may be entered upon; that is, to describe some observations made during the past summer and autumn on species of Achlya and Saprolegnia, the two most important genera of the group. These observations are not all equally valuable or new (although some of the facts were observed before I was aware that others had discovered them),[2] but they have been made quite independently of the literature,[3] and are thus of some service as confirmatory evidence to those who wish to study the subject further.

[1] 'Sitzber. d. niederrhein Gesel. in Bonn,' 1880. Quoted also in the appendix to the recent Engl. trans. of Sach's 'Textbook.'

[2] I must take this opportunity of thanking Prof. De Bary, not only for giving me material, but also kind advice and references in connection with this work.

[3] The now copious literature consists chiefly of the following, among others:—Pringsheim, several papers in 'Jahrb. für wiss. Bot.,' i, ii, and ix. De Bary, Jahrb. f. wiss. Bot.,' ii. Walz, 'Bot. Zeit.,' 1870. Cornu, 'Ann. de. Sc. Nat., S. v.,' vol. xvi.

The most important memoir, from a general morphological point of view, is De Bary, 'Beiträge zur Morph. u. Phy. d. Pilze,' 1881.

See also Huxley on "Saprolegnia in Relation to Salmon Disease," this Journal, July, 1882.

I shall adopt the simple plan of describing what I have seen and drawn, together with methods employed, leaving more general conclusions until afterwards.

1. Achlya polyandra.—Masses of débris of "meal-worms" on which this species had been grown some months previously, and which had been kept in a cool cellar during the interval, were placed, together with a fresh meal-worm in a large deep glass beaker perfectly clean, with a considerable quantity of boiled and filtered water, and a glass plate over the top; the whole stood in a well-lighted room at the ordinary (summer) temperature. In the course of two or three days, during which the water was several times replaced, the floating grub was seen to be developing pale, cottony filaments in all directions, on and in the water around. These filaments proved to be slender, straight tubes, filled with hyaline protoplasm in which numerous large granules were scattered, especially in the larger specimens; the walls became distinctly coloured blue in Schultz's solution, and in H_2SO_4, after treatment with iodine, the protoplasmic contents becoming yellow in the former reagent.

After a considerable mass of these radiating tubules had become developed, certain of them were found to bear zoosporangia. The development of a zoosporange was observed many times in the following manner:—A broad glass slip being placed in the water under the whole growth of Achlya, the attacked meal-worm was lifted up bodily, and transferred to the stage of the microscope; plenty of water being carefully added to the specimen, the upper, more or less floating branches, could be easily observed with a Zeiss D with a little care. The great advantage of this or a similar method is that the Achlya goes on growing almost undisturbed, and fresh water can be continually added as evaporation goes on. If a higher power is needed, it is very easy to place a small piece of very thin, perfectly dry and clean glass, so as to float on the flooded object, and remove it dexterously afterwards. These very delicate cryptogams will not grow in a normal manner under the pressure of an ordinary cover-slip, if continued,

and the above method (or that of suspending a small specimen grown on a fly's leg in a drop of water under a cover-slip) is advantageous in many ways. I may add, however, that with care observations may be made with Zeiss E without any cover-slip at all on favorably situated portions.

The Zoosporangium is simply the terminal portion of a branch spreading freely in the water; this becomes slightly dilated into a club-shaped body, into which very granular protoplasm collects, giving the young zoosporange a dull grey appearance, easily detected with a good hand lens. The apex of this body remains blunt, and the walls are not thickened During the course of about an hour after the protoplasm has slowly accumulated, the following changes occur:—(1) A thin septum becomes formed at the base of the dilated portion, separating its dark grey, non-vacuolated, coarsely granular protoplasm from the more sparsely granular, vacuolated contents of the rest of the branch; and (2) an aggregation of the contents of the zoosporange around numerous centres takes place. To take an instance actually observed (Pl. XXII, fig. 1). The gradual swelling and filling of the zoosporange (*a*) was completed by about 11 a.m.; at 11.15 (*b*) the septum had become formed, and the protoplasmic contents were already denser and showing signs of aggregation at many centres; at 11.45 this had proceeded so far that no doubt of the existence of a multitude of small semi-detached masses could be entertained (fig. 1 *c*).

And now followed a most remarkable phenomenon. At about twelve o'clock the appearance shown at *c* was replaced by one similar to that shown at *b*; this had occurred in many previous examples, and puzzled me exceedingly, and I was accordingly prepared to watch the exact sequence of events in this instance. The protoplasm at 11.50 was distinctly divided up into a large number of nearly globular independent masses, slightly compressing one another. No membrane could be detected around any of these, though, as subsequent investigations showed, a sort of watery-looking, clear boundary stood between the masses. At one to two minutes after 12 the masses and their clear boundaries became indistinguishable;

this fading away of the granular appearance took place so quickly that it might almost be termed sudden, and the protoplasmic mass was now in a condition apparently like that before the division, except that its granules were smaller and probably more numerous, and the mass seemed more translucent than before. During the next three minutes a large number of small, clear, equidistant areas (E) made their appearance in the now almost hyaline, fine-grained protoplasm, and a curious, pale, watery look had replaced the dark grey appearance of the earlier stages. These bright vacuole-like spots seemed approximately equal in number to the masses which preceded them (D), and one could well believe that the clear spaces form at points corresponding to the centres of the former bodies; this, however, could not be decided. In three or four minutes after the last condition was figured, the almost sudden reappearance, so to speak, of the rounded or polygonal solid masses occurred (F), as if the contents had gone back to the state figured at D. This time, however, the separation of the masses became more evident, and at ten minutes past 12 the apex of the sporangium gave way suddenly, and the whole mass of separated blocks of protoplasm suddenly flowed out into the surrounding water, and remained at the mouth of the zoosporangium as a spherical clump of protoplasmic globules (figs. 3 and 4).

The following peculiarities concerning these bodies and their exit were noticed. At the moment before the apex of the zoosporangium bursts the isolated, though closely packed, masses of protoplasm showed slight amœboid movements, and during the rapid expulsion were actively changing their form; the instant they reached the exterior, however, they all became strictly spherical, if free, slightly polygonal if compressed by neighbouring ones (fig. 4). The whole process of exit and rounding off only occupies a few seconds, and in a well-grown mass of Achlya dozens of zoosporangia may be emptying their amœboid contents at the same time; in such cases, also, the presence of this phenomenon can be detected with a hand-glass, the heads of globules being quite distinct in a good light.

The future behaviour of these zoospores—for such they must be considered—may be described, as before, from what was observed in a given example. Careful examination of one of the more loosely attached specimens on the outer portions of the spherical group (figs. 3 and 4) convinces the observer that an extremely delicate envelope becomes developed at the periphery of the resting globular zoospoore (fig. 5 *a*), and the body remains in this condition for some hours.

The specimen referred to was drawn at 1 p.m., and remained in this condition until about 4 p.m. Soon after this there were signs of change going on in the neighbouring specimens, and this particular globe was carefully watched. At 4.14 a slight protuberance made its appearance at one side, rapidly increased in size during the next minute or two (fig. 5 *b*—*d*), and a clear space (*e*) was then seen separating the delicate envelope from the granular, slightly amœboid protoplasmic contents, which were, in fact, becoming withdrawn to pass through to the outside. A minute afterwards the whole of the protoplasm was outside, except a minute papilla, which slipped out forthwith (fig. 5 *f*, *g*), and the mass commenced to writhe slowly in an amœboid manner outside the very delicate empty envelope, in the side of which could be seen the minute pore through which the zoospore had slipped out. At 4.18, the moment of complete exit, a clear spherical vacuole was seen at one side of the zoospore (*g*); the latter then quickly acquired a reniform shape, and from the sinus (corresponding to the hilus of the kidney), two minute cilia with knobbed tips were observed to spring forth, quickly grow in length, apparently at the expense of the knobs at their ends, and begin to wave slowly about. The zoospore now (4.25) commenced to swing perceptibly as the lashing of the cilia became more vigorous; nevertheless it did not move away for some time; at 4.35, however, the zoospore was freely and rapidly moving about, and at once disappeared from the field.

This process, described as faithfully as possible in one case, was repeated by the contents of the rest of the spheres composing the globular mass at the mouth of the zoosporangium

(fig. 4), and all were nearly in the same stage of development at the same instant; consequently the exit of the zoospores from the spherical envelopes can be readily observed when the critical time is carefully watched.

The mass of empty envelopes remain behind, appearing as an exceedingly delicate network (fig. 6), and even simulating parenchyma of great tenuity, mutual pressure causing the spheres to become polygonal. Here and there the minute pore can be observed as a dark spot in the side of the envelope, and sometimes a zoospore escapes much later than its companions.

There is little more to be said of the zoospores. Some time after their exit they come to rest, round off, and each at once commences to put forth a simple tube (fig. 7), having first lost its cilia and vacuole, and acquired several brilliant granules, which become arranged around the periphery. If the germination occurs on a proper matrix, such as a meal-worm, fly, &c., the tube enters and commences to grow into a rhizoid-like portion, a new thallus becoming developed from outside. On glass, &c., the tube soon reaches the end of a limited growth, its contents fade, and the whole dies.

Among other abnormalities in the course of phenomena such as the above, mention should be made of one which is not uncommonly met with, and which I have drawn at fig. 8. In certain of the zoosporangia the completely separated zoospores remain behind, rounded off, and form their delicate membranous envelopes while still in the cavity; not only so, they germinate in this position, each pushing a short tube through the sporangium wall (fig 8, a B) before emptying its contents on the exterior. In the example figured, the apex of the sporangium became open in the usual manner at length. The figure B represents part of A under a higher power and twenty-three hours later. These "Dictyuchus" forms were obtained from specimens of Achlya polyandra which had remained about six hours in the same water on a slip of glass; towards the end of the period the fungus was obviously passing into a state of inanition. As fig. 8 a B shows, the sporangium becomes filled with an apparent tissue of extreme delicacy—the

empty membranes of the zoospores—and the name "Dictyuchus" was given to express the net-like structure thus produced. Whether the genus "Dictyuchus" exists on a firmer basis than this I do not know.[1]

With respect to the sexual reproductive organs of this Achlya, my observations cover a considerable field; as before, the description applies strictly to what I have seen. The oogonia and antheridium branches become produced in large quantities when the cultivated Achlya is allowed to remain quite still, floating on the surface of abundance of water; their presence is soon detected with a good hand lens, and further examination gives the following information concerning them.

The oogonia arise as globular or nearly pear-shaped swellings of the ends of very short branchlets, developed at nearly equal intervals along the course of a vigorous branch (fig. 9); the short branchlet is usually much smaller in diameter than the parent twig, but resembles it in possessing thin walls and coarsely granular protoplasmic contents, and in its cylindrical shape. The balloon-like terminal swelling receives a large supply of protoplasm, which accumulates in it as a yellowish-grey dense mass, and then becomes shut off from the pedicle by a thin, sharply-marked septum (fig. 10). In the pedicle, which is about as long as the longer diameter of the oogonium, the remaining protoplasm is much more watery and poorer in granules; the latter is inserted sharply, as it were, into the parent branch, and there is no septum at the base—the cavity of the two remains continuous throughout. In vigorous specimens (fig. 10) the granules often seem to be arranged in rows, embedded in the layer of transparent protoplasm lining the cylindrical cell walls. The groups of Oogonia, marked by their yellowish-grey contents while young, present a striking object (fig. 9), like groups of berries developed in racemose order; the pedicles are usually slightly curved in various directions. As well shown in fig. 9, the oogonium-bearing branches may be of various orders, very

[1] De Bary, loc. cit., p. 94, says this abnormality occurs in other species.

commonly secondary and tertiary. Since the thallus has accumulated much material, and the asexual reproductive organs have been for the most part emptied when the oogonia arise, it is usual to find empty zoosporangia terminating the main twigs. The production of lateral branches from beneath the sporangia is characteristic of Achlya, and that such may bear oogonia is sufficiently demonstrated by fig. 9 B. Such is the typical mode of development of the oogonium. Before proceeding to describe the changes which its contents undergo, we may examine the mode of origin and growth of the so-called "antheridial" branches.

These are long slender tubes, springing from the main branches from points either close to the oogonia (fig. 10) or at greater distances apart, or even from separate branches. The diameter of the tube is commonly less than that of the pedicel, but may equal it: within its thin walls are finely granular, watery protoplasmic contents, not always easily distinguished. As seen in fig. 12, the "antheridial branch" arises as a simple tube; it often begins to form branches soon after its origin, and these spread in all directions, curling and waving as they do so. In this manner they become wrapped or coiled around objects, such as neighbouring branches or oogonia, with which they come in contact (fig. 9 B). It is in this coiling of the antheridium branch about an oogonium that the first stage of a proper sexual process has been recognised by earlier observers.

During the development of the coiling antheridial tubes above described, the granular, yellowish-grey contents of the oogonium, undergo certain changes, which result in their complete transformation into the egg-cells or oospheres. A clear, almost watery spot appears in the centre of the mass (fig. 11 *a*), and slowly increase in bulk as the dense grey granular protoplasm recedes to the walls; in this latter are large, fatty-looking granules, which seen from the surface (fig. 11 *b*) are in slow but evident motion. This retirement of the protoplasm to the sides is followed by another process; a collection of the whole mass into two or more clumps, which

then slowly round off as naked oospheres (fig. 12), consisting of the fatty protoplasm only, suspended in the oogonium cavity, which appears otherwise empty. This collecting of the protoplasm to form the eggs, or oospheres, is a remarkable process in more respects than one; it takes place slowly, and occupies several hours altogether. I will confine my description to one case observed.

An oogonium was favorably situated for observation from above, and at about 12 noon had attained the stage figured at fig. 11 *a*. The coarsely granular protoplasm aggregating on the walls, was in a state of continuous slow-flowing motion, quite distinct to one observing a given granule from the upper side (fig. 11 *b*); this specimen was watched carefully from this time forward till nearly 5 p.m., and underwent changes which were figured as follows (fig. 14):

For a long time the mass on the walls slowly heaved and flowed, without its lateral continuity becoming broken. At about 1.30 to 2 o'clock, however, the surface view showed that the dense layer was breaking up into more distinct masses; and at 2.35 oblique, broad bands of fatty granules represented the connection between two large masses aggregated at the sides (fig. 14 *f*). On watching the uppermost of these bands, the slow breaking up and passage over to either side of the granular protoplasm was distinctly observed (fig. 14 *g*, *h*). About 5 or 10 minutes before 4 the whole of the protoplasm was thus collected into two equal lumps, still somewhat flattened on one surface to the walls of the oogonium, and standing on opposite sides (fig. 14 *i*, *k*). The next five minutes were occupied in the collection of a few scattered granules, the raising up of the centre of each lump from the wall, and its ultimate withdrawal altogether towards the centre of the oogonium (fig. 14 *l*). During the latter process, the egg-masses were distinctly amœboid; each had its surface alternately raised into lumps and smoothed off again, and in some cases small particles of the protoplasm became detached and taken up again.[1] There is not the slightest doubt as to the

[1] This detachment of protoplasmic masses occurs still more decidedly, ac-

accuracy of these observations; the amœboid motion continued for some time, then slowly ceased, and at 10 minutes past 4, the two perfectly spherical oospheres lay obliquely in the oogonium, mutually in contact, as shown in fig. 14. The oospheres in this condition are apparently ready for "fertilization," and the following phenomena occur.

One or more of the antheridial tubes, coiled closely about the oogonium (figs. 10, 11, &c.), while the above described processes have been going on, begins to send a tubular process through the oogonium wall, at or about the time when the oospheres are smooth and rounded off; the tubular process thus sent into the cavity of the oogonium (fig. 12) has been termed the "fertilising-tube." It is a direct prolongation of the "antheridial branch," and contains finely granular protoplasm; it grows for some time in the cavity of the oogonium, coming in contact with the oospheres—even running on their surfaces. I have never seen it enter an oosphere, nor have I seen it open at the end or emptied of contents. Whether anything passes from it to the oospheres cannot be decided; but De Bary gives such strong reasons for doubting that any fertilising process whatever occurs, and supports his conclusions by so many examples and so much observation that it would be presumptuous to attempt to decide the question without devoting at least equal energies and time to the task. So far as my observations go, they decidedly fail to supply evidence for the view that anything is emptied from the tube into the oogonium or oosphere. Before offering any further remarks on this subject, it will be convenient to describe the remaining observations made on other species.

Achlya apiculata is the name by which Professor De Bary designates a species not yet (I believe) described; my observations on this form are not yet sufficient to enable me to do more than depict the formation of the zoospores. I have never seen the Oogonia or Antheridia.

cording to De Bary, in Saprolegnia ferax; he thinks it due to the throwing off of water. May not the bodies, however, be of the nature of the "Polar cells" thrown off from the animal ovum preparing for fertilization?

The zoosporangium (fig. 15) differs somewhat in shape from that of A. polyandra, the apex especially being more pointed. In a specimen carefully watched for some hours, the sporangium was at first filled with very finely granular grey protoplasm, and two or three large vacuoles remained below, abutting on the somewhat swollen-looking septum; the tube below the septum contained many and large vacuoles, the nets or bridles between which were slowly streaming. Such being the condition of affairs at 9.30, the only observed difference at 9.50 was that the vacuoles had disappeared from the zoosporangium, and the fine-grained protoplasm reached close up to the now more sharply-marked septum. About 10 o'clock the tip of the sporange appeared brighter and marked by faint longitudinal striæ (fig. 15 *c*), and a slight tendency to the formation of brighter areolæ seemed evident in the protoplasm. At 10.10 this was distinctly marked (fig. 15 D); the protoplasm arranged itself slowly into polygonal masses, each with a brighter central part. This stage lasted for nearly ten minutes, the division lines becoming brighter and sharper, until the blocks stood nearly isolated, and then, quite suddenly, at 10.20, the separation lines disappeared and the blocks fused together, and a uniform grey, granular mass (E) resulted as before. This particular sporange was not observed further, but in fig. 16 are drawings of what was seen in another specimen from the same cultivation. At 10.35 the breaking up into the preliminary blocks was nearly complete (*a*, fig. 16) and very distinct; the hard, sharp division lines disappeared quite suddenly about two to three minutes later, and then the evenly granular protoplasm became marked out into bright areas (fig. 16 *b*) by small vacuole-like points. These increased slowly in size, and at 10.45 the sporange presented a peculiar lustrous aspect, the granules appearing remarkably sharp and black in the bright, watery-looking matrix. It seemed also that there were relatively more vacuoles than preliminary divisions: this difficult point could not be decided. At 10.50 the second series of division planes were established (fig. 16 *c*). I could not satisfy myself that each vacuole occupied the centre of one of the blocks, though such was un-

doubtedly true sometimes; it seemed that in some cases a large block became further cut up into smaller ones. This process proceeded very rapidly, and by 10.53 the zoospore masses (fig. 16 *d*) were finally isolated, and slipped out in the next two or three minutes. The further fate, &c., of the zoospores need not be described in detail; they behave essentially as before.

Saprolegnia.

The observations on this genus will be confined to the forms of Saprolegnia ferax (Pringsheim), and the following descriptions, &c., will refer particularly to that called S. monoica in the sense of the above author.

Through the kindness of Prof. De Bary, I was enabled to infect "meal-worms" and house-flies with S. monoica, and in two or three days had excellent cultivations floating in abundant water as before. The methods of observation, &c., need not be detailed; they are practically the same as those described for Achlya.

Fig. 17 shows the various stages of development of the zoosporangium and zoospores; the segmentation of the protoplasm takes place as before, and need not be further described. At the completion of the second segmentation, the masses of protoplasm behave in a manner quite different from those of Achlya, however, since, instead of simply slipping out of the apex of the zoosporangium and then rounding off, they acquire two terminal cilia at once, and pass off as actively moving zoospores (fig. 17 *f*). Each zoospore is a top-shaped mass of finely granular protoplasm, with two very long cilia actively waving at its pointed (forward) end, and with a sort of zone of three small vacuoles around its broader part (figs. 17 *g*, 19 *a*). In this condition it moves rapidly from the point of exit, coming to rest (*h*) after some minutes. With care it is quite possible to watch a zoospore through all its changes. Fig. 19 shows the phases actually seen in the case of a zoospore emitted from the zoosporangium in fig. 17. It became free about 9 a.m., and moved actively for ten minutes, rounding off and losing its cilia and vacuoles in an instant (c f. fig. 19 *a*, *b*).

In this quiescent condition it remained for some hours unchanged, excepting that an envelope was gradually formed on its surface. At 2.30 p.m. the contents of the little sphere came out (*c* and *d*) as an amœboid, naked mass, which gradually acquired a kidney-shape and a large vacuole, and developed two lateral cilia; these latter, as before, arose as two minute knobbed processes, which slowly increased in length and began to wave, causing the body of the zoospore to swing more and more, and at length (about 3 p.m.) to move away. This particular specimen was then lost. But I observed another (*f* to *i*) for nearly an hour and a half; it was just coming to rest (*g*) about 4 o'clock, and had commenced to germinate before 5 p.m., growing very rapidly (*i*) and then dying. In another case (fig. 18) I followed the second more closely. It escaped from the envelope (*a*, *b*) about 2.10 p.m., and swarmed as a kidney-shaped spore (*c*) for nearly half an hour; it then lost its cilia, writhed two or three times in an amœboid manner (*d*), and suddenly became rounded off (*e*) as a naked spore. This was at 2.55 p.m. At 3.15 (*f*) it began to germinate, by throwing out a slender tube, which had reached a considerable length by 4 o'clock (*g*), when the whole was dying.

In the normal condition of affairs such a germinal tube enters the body of the insect, and continues the life-cycle. In some cultivations one often finds bright white clumps of germinating zoospores (fig. 20) lying at the bottom of the water; these result from numerous zoospores coming to rest about the same time, falling quietly through the still water, and, again, germinating almost simultaneously.

There is little more to be said concerning these processes. The zoosporangia of this Saprolegnia vary in shape within wonderfully wide limits; some are almost as broad as long, others nearly tubular, while pyriform, top-shaped, and irregular specimens of all kinds occur. In cultivations, allowed to starve from want of renewed water, &c., imperfect and distorted sporangia reach a certain stage of development, and then, acquiring very thick walls, remain in a resting condition, springing into activity again when the conditions of the

environment improve. I have drawn one or two specimens of such dormant branches of the thallus of Sapr. monoica at fig. 21. I have not yet obtained oogonia of Saprolegnia monoica, and must refer to the literature;[1] drawings of the ripe oospores are given at fig. 22, but no attempt to produce them by cultivation on my part have yet succeeded.

The immediate object of this paper, to describe accurately a few careful observations in the hope that they may help to stimulate others to pursue the subject, is now ended; but it may be well, before concluding, to call attention to some general conclusions which have been drawn lately, and on which such observations as the above throw light.

Apart from the question as to whether the Saprolegnia be regarded as true Fungi or not, they may certainly be considered as forming a distinct group of parasitic and saprophytic organisms inhabiting water, and multiplying by means of zoospores and oospores as above described.

As respects the asexual mode of reproduction, by means of zoospores, all observers are now fairly in accord as to the main facts. With respect to the processes of incomplete segmentation preceding the formation and escape of the zoospores from the sporangium, it appears to be best explained as a phenomenon of nuclear division, in which the cell plate first formed becomes used up again. Büsgen,[2] who observed a similar process in several other cases, draws attention to Strasburger's discovery,[3] that in the development of pollen grains and spores it sometimes happens that a "primary cell plate" is first formed, and then disappears, as if its materials were used up again. This certainly appears to explain the phenomena of the division, &c., inside the sporangium of Saprolegnia; but why should the protoplasm make so many tentative efforts, so to speak, before once more growing out as a thallus? Why

[1] The development of oogonia, &c., in this form is very fully given by De Bary, 'Beitr. z. Morph.,' &c., iv.

[2] "Die Entwicklung der Phycomyceten-sporangien," Jahrb. f. wiss. Bot., B. xiii, 1882.

[3] 'Zell-bildung und Zell-theilung,' ed. iii.

should the protoplasmic masses, once having become zoospores, still hesitate (if the word may be permittted) before growing on, and, having rested awhile, again become zoospores, but of a different kind?

It might be suggested that the entire series of phenomena should be connected and looked at in some such way as the following:

1st. The zoospore masses are formed, excreting a clear intercalary substance (primary cell wall of Strasburger), which they then take up again.

2nd. A more energetic separation follows, resulting in complete isolation, passage out, and removal to a distance. This active phase, though more energetic and lasting than the preceding, is in its turn superseded by a resting state, and the protoplasm excretes the substances for a membrane.

3rd. After the period of rest the protoplasm once more moves actively (having left its membrane behind) as a still more energetic zoospore—at least it moves for a longer period—which in its turn comes to rest, but only for a short time prior to germination.

4th. It then, having formed certain brilliant granules and a cell wall, throws out a germinal tube at the expense of its contents; this soon dies if no proper matrix be at hand.

Unfortunately this restatement of the matter does not seem to help us. One can dimly see that the little protoplasmic zoospore undergoes processes of activity and rest—possibly partial exhaustion—and it is not absurd to conceive that something is gained by an active vacuolated stage.

In Achlya—the above applies to Saprolegnia[1]—the first and second stages occur as before, only the second stage seems to be less energetic, and the amœboid bodies only succeed in reaching the mouth of the sporangium. The third and fourth stages are much the same.

In the "Dictyuchus" form the second stage is still more

[1] De Bary, however, says that both zoospore stages may become abnormally suppressed, and the germinal tube be formed at once on leaving the sporangium; this increases the difficulty. Loc. cit., p. 94.

abbreviated; it consists merely in complete isolation. The resting globules germinate in sitû in the zoosporangium.

That we are brought face to face here with a profound problem in its simpler forms is obvious. Perhaps the only light it affords us as yet is the suggestion once more of the exceedingly complex nature of the changes proceeding in the simplest piece of protoplasm. It appears somewhat significant that the second form of zoospore—the reniform one with lateral cilia—is that most constant. This is the only form in the nearest fungoid allies of the Saproleginæ, and must probably be regarded as the most ancestral form; nevertheless it is not easy to suggest how or why the other zoospore was acquired.

With respect to the "sexual reproductive organs" of this group, much has been written and many theories advanced since Alexander Brown and Pringsheim first described them and their relations. The antheridia were first believed to pour granular matter into the oogonium amongst the ova (oospheres). Then Pringsheim discovered that the oospheres in certain cases become normal oospores without the appearance of antheridia. Certain small antherozoid-like bodies were then believed to be set free and find their way into the oogonia amongst the oospheres. Meanwhile other observers denied that the "antheridia" either formed antherozoids, or that the tubes sent into the oogonium emptied anything into its cavity.

The discussion seems to have been somewhat in this state when Cornu,[1] in 1872, described the process of fertilisation, &c., as consisting neither in the formation and entry of antherozoids, nor the emptying of granules between the oospheres, &c., but in the passage of protoplasmic contents from the antheridium through the fertilising tube and into the substance of the oospheres. This view has been accepted somewhat widely.

De Bary seems to have maintained for some years that, in some cases at least, no passage of material takes place through

[1] 'Ann. d. Sc. Nat.,' 5th ser., t. xv.

the tube—at any rate, not as protoplasm; but that the tube remains closed at the end, and never enters the substance of the oosphere; on the contrary, the antheridium either remains coiled round the oogonium, or the tube which it sends into the cavity simply touches or pushes the oospheres. He thus thought that the fertilising influence must pass through the closed walls of the tube which remains closed.

Pringsheim, in 1874,[1] again examined the question, and came to the conclusion that Cornu was wrong, and that where the tube comes in contact with the oosphere it remains quite distinct from it, however closely applied. Thus no slow passage over of protoplasm into the substance of the oosphere occurs, and hardly any, if any, contents of the antheridium disappear. Pringsheim further came to the conclusion that in some cases, since the oospheres become ripe oospores without any antheridial branches coming near them, the phenomenon must be considered one of parthenogenesis.

De Bary's lately published views have been already referred to. He finds, after prolonged and exact researches, that not only does no observable passage of anything take place through the fertilisation tubes; not only does the naked oosphere clothe itself with a membrane (thus indicating that it no longer requires fertilisation) without the contact of the tube, but that normal, ripe oospores are produced habitually in some forms without an antheridium branch ever being formed at all. Such cases De Bary considers not "parthenogenetic," in Pringsheim's sense, but apogamous.

One more point may be shortly adverted to. It appears as said to be a constant phenomenon in certain forms, perhaps in all, that the masses of protoplasm forming the oospheres throw off smaller or larger portions of their substance during their amœboid movements preceding their final rounding off as smooth oospheres; if these detached masses of protoplasm are to be regarded as of the nature of the "polar cell"

[1] 'Jahrb. f. wiss. Bot.,' B. ix.

observed to be thrown off by animal ova prior to fertilisation,[1] may not the hypothesis thrown out by Balfour apply also to the cases observed by De Bary?

De Bary shows that these protoplasmic bodies are taken up again, and that such oospheres as have again absorbed the thrown-off bodies, become ripe oospores, capable of germination after a period of rest without being fertilised. Balfour suggested that the "polar cells" are thrown off to prevent parthenogenesis, i. e. to prevent the egg dividing up and developing an embryo which has not benefited (in Darwin's sense) by receiving protoplasm from a distance; the further development of the non-fertilised oospores (ova) of Saprolegniæ may be possible because the "polar cells" are again absorbed? Here, however, the proper limits of the present essay have been passed.

[1] C f. Balfour's 'Comparative Embryology,' p. 58.

On Double Staining Nucleated Blood-Corpuscles with Anilin Dyes.

By

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The usefulness of the process of staining tissues with several dyes has been abundantly proved. The general effect aimed at is the staining of each separate part in a different colour, so that for the purposes of histological demonstration each shall be distinct and clear. In the use of certain of the most commonly employed and easily manipulated dyes, e. g. hematoxylin and picrocarmine, it is believed that a definite effect may be always calculated upon when they are used in combination. With anilin stains, however, the results arrived at appear to differ very materially if the methods of employment are made to vary in even a very slight degree, and this has been one of the causes of the restricted use of very beautiful staining colours. It has been shown by several experimenters that with combinations of anilin colours, there is a tendency at any rate for certain dyes to pick out and stain different parts of a tissue; but I think I am right in believing that no certain result has hitherto been expected, except in the case of a very few combinations.

There is no doubt that unless more definite results be obtained with combinations of these dyes, the hope that with them it will be possible to pick out each element of a tissue in a different colour, and each kind of morbid growth in a similar manner, will be long deferred.

The following notes were made during a series of experiments, the object of which was to find out the best combination of anilin

dyes for double staining, as some of the methods recommended had, in my hands, turned out very unsatisfactorily. During the year 1881 I had, at the request of Dr. Vandyke Carter, devoted a considerable time to the preparation and investigation of the organs of patients who had died of "spirillum fever," and also of those of animals which had been experimentally inoculated with the fever virus. We wished to demonstrate in these tissues and organs the presence of spirilla as had been done with ease in the blood. We used, according to the advice of Professor Koch, several anilin dyes in turn with anything but certain results. The indefiniteness, therefore, of the staining struck me much, and induced me to think that something might be done in the way of obtaining more satisfactory combinations. The demonstration of the bacillus in tubercle has brought into prominence the manipulation of combinations of these dyes, and the fact that since the first process of Koch was introduced, large numbers of modifications of it have been brought forward, notably by Ehrlich, Baumgarten, Ermengen, Gibbes, and others, point to the conclusion that a considerable amount of work remains to be done before the subject is fully developed. These experiments in staining the nucleated corpuscles were undertaken, as I have said, with a view of finding out whether, with such definite elements of tissue, certain results could be obtained by staining with certain anilin dyes in solutions of definite strength in regular sequence.

Method of experiment.—After trying several methods, the one I adopted was as follows:—Blood from various animals —frogs, newts, &c.—was spread in thin layers upon ¾-inch cover-glasses and allowed to dry in the air. Certain of the anilin colours were then chosen as the first or primary colours; these were fuchsin, eosin, rosein, and rosanilin, in aqueous or dilute spirit solution, as red dyes; iodine or malachite green, as green dyes; and methyl violet, Hoffman's violet, or gentian violet, as violet dyes. All of these were in aqueous or dilute spirit solution. With each primary colour was included a separate series of experiments. Several drops

of the first solution were allowed to remain upon the dried blood for about five minutes, and were then washed off with a gentle stream of water from a wash-bottle. The cover-glass was then dried in the flame of a spirit-lamp and allowed to cool. When ready for the second dye, a small quantity was dropped upon the cover-glass and allowed to remain the same length of time. A second washing with a stream of distilled water followed until the washings were all but colourless; drying and mounting in Canada balsam concluded the process. Treatment with alcohol and clove oil previous to mounting in several instances quite vitiated my result, and so I gave up that method of dehydration and clearing. In cases where I thought it possible to employ three dyes, the third was used in a manner almost exactly similar to the one described above. The method of fixing the corpuscles with osmic acid did not in the least improve the staining of the corpuscles. The chief precaution which appeared to be necessary was not to allow the blood to coagulate, and to place it under conditions of rapid, but natural, drying, e.g. in the sun's rays.

Arrangement of the Combinations of Colours:

Series A.—Primary colour, red; tried with orange, yellow, green, blue, violet, and brown.

1st Dye.	2nd Dyes.
Substances used.	Substances used.
Red—	Yellow—Anilin Primrose.
Fuchsin—Lake.	Orange—Tropæolin, or Aurin.
Rosein—Crimson.	Green—Iodine Green.
Eosin—Pink.	Blue—Methylen Blue.
	Violet—Hoffman's.
	Brown—Bismarck.

Series A.

Red and orange. (Eosin and aurin.)

This combination was unsuccessful, as the solution of aurin had to be made with absolute alcohol, it being such a very in-

soluble substance, and entirely replaced the eosin, which was a saturated watery solution. The whole of the corpuscles were stained a deep orange. So far I have been able to do little with aurin as a dye, its great insolubility causes every solution speedily to deposit crystals.

Red and yellow. (Fuchsin and anilin primrose.)

Fuchsin, a salt of rosanilin, is a fine lake dye, partly soluble in water, freely soluble in dilute spirit. Anilin primrose, a penetrating yellow dye of the colour of picric acid, almost insoluble in water, and only partly in methylated spirit, from which it quickly deposits crystals. After some difficulty in obtaining a good specimen the corpuscles were found to have stained thus. The nucleus, a yellowish red, not unmixed crimson, the remainder of the coloured corpuscles a light yellow, and the colourless corpuscles a light red. The combination was not a good one, as the yellow proved a very difficult dye to turn out, but, judging by results, it had the greater affinity for the protoplasm of the corpuscles, and less for the nucleus.

Red and green. (Rosein and iodine green.)

Rosein, similar to fuchsin, but of a more deep crimson, is partly soluble in water, very soluble in dilute spirit. Iodine green is freely soluble in water; an excellent combination. The coloured corpuscles were stained a bright red, with bluish-green nuclei. The colourless corpuscles were easily made out to be of three varieties in the blood (of newt): 1, entirely stained in green; 2, partly stained in green and partly yellowish red, the nucleus green, and the surrounding protoplasm of the other colour; 3, the large masses of nuclei-like bodies, said to be developing colourless corpuscles, were deeply stained in green.

The relation in size of the nuclei of the colourless corpuscles to the stroma is a very variable one. Sometimes the nucleus appears to occupy nearly the whole cell, at others, perhaps, not more than a fourth or fifth of it. It would be possible to describe a larger number of different kinds of colourless corpuscles than the above if their reaction to the dyes were alone

considered, but no doubt the effects of the staining fluids were not constant.

Red and blue. (Fuchsin and methylen blue.)

Certainly one of the most successful combinations. The methylin blue was used as a saturated solution in absolute alcohol. In this case, as in most of the others, the blue was used second to the fuchsin, and vice versâ, with similar results. In these specimens the nuclei of the coloured corpuscles were deeply stained blue. Of the remainder of the corpuscle, a light greenish hue with the edge a bright pink, or where the staining had been less deep the whole of the corpuscle, except the nucleus, was stained pink with an edge of a deeper but similar colour. The staining of the colourless corpuscles was peculiar; some were a light bluish green. These were irregular and branched; others had a deeper purple colour with unstained spots (vacuoles?); and a third variety appeared to be stained in two colours; in these a large central mass looked like an immense nucleus. Other varieties might be mentioned, as in the last combination, but as it is possible that the size, amount of granules, and the staining are, except in the three varieties, mere differences in amount not in kind, it is scarcely necessary to mention them. For purposes of demonstrating the divisions, irregularities in shape and in varieties of colourless corpuscles, I am strongly inclined to recommend staining with methylen blue and fuchsin as the best combination possible.

Red and blue, 2nd combination. (Fuchsin and soluble anilin blue.)

Nuclei stained red, as well as the colourless corpuscles; stroma a light blue. It is as good a combination as the last.

Red and violet. (Eosin and methyl violet.)

With these colours there appeared to be a mixture of the dyes in the corpuscle, and the nuclei were not distinct.

Red and brown. (Fuchsin and Bismarck Brown.)

Bismarck brown is an anilin dye of considerable utility; it is partially soluble in water, more so in water with a few drops of glycerin added to it, and easily soluble in dilute

methylated spirit. The solution used was 2 per cent. in dilute spirit. The corpuscles stained easily and in a fairly regular manner, the nucleus a deep red, the stroma a fine brown. The colourless corpuscles pinkish red. In a few cases I noticed a mixture of the colours in the corpuscles. In using Bismarck brown, a dye much employed on the Continent, I find it best to immerse the specimens in it for twenty to thirty hours, and then they will retain their colour even if passed slowly through the dehydrating and clearing fluids.

Red and brown, 2nd combination. (Eosin and vesuvin.) Vesuvin and Bismarck Brown are said to have the same chemical formula, and are probably identical.

The vesuvin was used in a strong aqueous solution. The corpuscles easily stained with eosin, nuclei and colourless corpuscles a deep pinkish colour, and the stroma of the coloured corpuscles a light pink. After double staining with vesuvin the stroma stained a light yellowish brown, leaving the nuclei and the colourless corpuscles stained as before. A very successful combination.

Series B.—Primary colour green; tried with brown, red, orange, yellow, blue, and violet.

1st Dye.	2nd Dyes.
Substances used.	Substances used.
Green—Iodine or Malachite Green.	Brown—Bismarck.
	Red—Flamingo or Ponceau.
	Orange—Aurin and Anilin Orange.
	Yellow—Anilin Primrose.
	Blue—Bleu de Lyon.
	Violet—Methyl Violet.

Series B.

Green and brown. (Iodine green and Bismarck brown.)

The colourless corpuscles and the nuclei of the coloured corpuscles distinctly green; the stroma brown.

Green and red. (Iodine green and flamingo.)

Flamingo, a deep brownish red, soluble in water partly, but freely in dilute spirit. The latter solution was the one employed. The nuclei of the coloured and the whole of the

colourless corpuscle showed a deep bluish green, and the stroma was coloured pink.

Green and red, 2nd combination. (Malachite green and ponceau.)

Malachite green is freely soluble in water, and was used in aqueous solution. The nuclei of the coloured corpuscles stained green and the stroma a light pink. The colourless corpuscles of the same shade as the nuclei. On keeping the specimens it was found that the green dye almost entirely disappeared.

Green and orange. (Malachite green and fluorescin.)

The nuclei of the coloured corpuscles stained a light yellowish green, as did also the colourless corpuscles. The stroma stained a yellow colour. The green stain was very temporary.

Green and orange. (Malachite green and aurin.)

Entirely unsuccessful.

Green and yellow. (Iodine green and anilin primrose.)

The colourless corpuscles and the nuclei of coloured corpuscles stained green; the stroma of the latter yellow.

Green and blue. (Iodine green and Bleu de Lyon.)

Double staining quite unsuccessful. The Bleu de Lyon was employed in a dilute spirit solution.

Green and violet. (Malachite green and methyl violet.)

Combination not good. The nuclei stained a very light purple, as did also the colourless corpuscles, whilst the stroma was a pinkish yellow. The green and violet apparently mingled in staining the nuclei.

Series C.—Primary colour, violet; tried with brown, red, orange, yellow, green, and blue.

1st Dye.	2nd Dyes.
Substances used.	Substances used.
Violet—	Brown—Bismarck.
Methyl Violet.	Red—Flamingo, Eosin, Anilin Scarlet.
Gentian Violet.	Orange—Tropæolin.
Hoffman's Violet.	Yellow—Anilin Primrose.
	Green—Iodine Green.
	Blue—Methylen Blue.

Violet and brown. (Hoffman's violet and Bismarck brown.)

An excellent combination. An aqueous solution of the violet was used, and a dilute spirit solution of the Bismarck, brown. The result showed excellent double staining. The nuclei of the coloured corpuscles and the colourless corpuscles were stained a reddish brown, and the stroma a light brown. The brown evidently stained the whole of the corpuscle stroma, and nucleus, but met the violet in the nucleus, and together stained it the colour it presented.

Violet and red. (Hoffman's violet and flamingo.) Flamingo is a mixture of rosanilin and Bismarck brown.

The nuclei and stroma stained two shades of the same colour, probably a mixture of the two used, mauve.

Violet and red, 2nd combination. (Gentian violet and anilin scarlet.)

Unsuccessful.

Violet and red, 3rd combination. (Gentian violet and eosin.)

Nuclei of coloured corpuscles, deeply stained red, with the colourless corpuscles a similar colour with their nuclei. Stroma a light pink.

Violet and orange. (Hoffman's violet and tropæolin.)

Tropæolin used in 1 % watery solution. Double staining entirely failed.

Violet and yellow. (Gentian violet and anilin primrose).

Strangely enough the corpuscles were stained two shades of green.

Violet and blue. (Methyl violet and methylen blue.)

Certainly one of the best combinations tried. The methyl violet is a very pink dye, and the blue a very deep blue. The latter stained the nuclei, the former the stroma.

Results.—From my experiments I draw the following conclusions as to double anilin staining of the nucleated corpuscles. It seems reasonable to look upon such corpuscles as made up of only a few varieties of tissue, and as such I have spoken of them.

1. The only entirely successful combinations were the following:

Rosein and anilin green.
Fuchsin and methylen blue.
Fuchsin and Bismarck brown.
Eosin and vesuvin.
Iodine green and Bismarck brown.
Hoffman's violet and Bismarck brown.
Anilin violet and methylen blue.

2. The green dyes were not at all permanent. I have proved this with both malachite and iodine greens.

3. Even with the above successful combinations the results varied in a most extraordinary manner, whilst the circumstances of the staining operation and the solutions appeared to be unvaried, the very greatest care being required to produce a constant result. One thing necessary for success was certainly that the solutions should be quite fresh. This is likely to prove a great objection to the general introduction of anilin dyes into use. The simple method of dehydration employed, of course, could not be employed in the preparation of tissues, although it does for blood, sputa, &c.

4. The result was materially affected by the time each dye was allowed to remain in contact with the blood.

It is worthy of note that according to the evidence of competent authorities, various chemically diffused anilin dyes have been sold under the same commercial name; and so, both in the preceding notes and also in the annexed table, it should be said that the anilin dyes used were obtained from Messrs. Hopkins and Williams, Hatton Garden, W.C. The following table (drawn up August, 1882) includes the dyes used in above experiments:

I am much indebted to Mr. Meldola, of Messrs. Brooks, Spiller & Co., for valuable information as to the chemical composition and relations of many of the above anilines, and of the commercial names, &c., of others. This information he kindly furnished at the request of Dr. Russell.

Classified List of the Chief Anilin Dyes, with their Solubilities in Water and in Spirit.

Brown.	Red.	Orange.	Yellow.	Green.	Blue.	Violet.
Bismarck—partially sol. in water; sol. in dilute spirit.	Eosin, Pink—freely sol. in water.	Aurin—insol. in water; partly sol. in strong spirit; more so in absolute alcohol.	Fluorescin, Greenish Yellow—insol. in water; sol. in spirit, the solution being beautifully fluorescent.	Iodine Green, Blue Green—freely sol. in water or spirit.	Soluble Anilin Blue—freely sol. in water.	Hoffman's Violet—freely sol. in water and in dilute spirit.
Vesuvin—sol. in water.	Anilin Scarlet—insol. in water; freely so in methylated spirit.	Anilin Orange—ditto, ditto.	Anilin Primrose—only partly sol. in meth. spirit.	Malachite Green, a less Blue Green—freely sol. in water and in spirit.	Bleu de Lyon—insol. in water; freely so in strong spirit.	Methyl Violet, the Red predominating—sol. in water partially; freely sol. in spirit.
Chrysoidin—sol. in water.	Flamingo, deep brownish red—partly sol. in water; freely so in meth. spirit.	Tropæolin, in Deep Yellow Glistening Scales—partly sol. in water; more so in meth. spirit.			Methylen Blue, a very Deep Blue—freely sol. in water and in spirit.	Gentian Violet, the Blue predominating—freely sol. in water.
	Ponceau,[1] deep red crimson—partly sol. in water; freely in dilute spirit.	Phosphin, Yellowish Orange—partially sol. in water; more so, but not freely, in spirit.			China Blue—freely sol. in water.	Tyrian Blue, near to Violet—sol. in water.
	Rosanilin—partly sol. in water; freely sol. in dilute spirit.	Saffranin—sol. in water and in spirit.			Serge Blue—ditto.	Spiller's Purple—soluble in spirit.
	Fuchsin—partly sol. in water; sol. in dilute spirit.				Blue Black—freely sol. in water.	

[1] Ponceau is a mixture of rosanilin and phosphin.

Some recent Researches on the Continuity of the Protoplasm through the Walls of Vegetable Cells.

By

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Having been for some time engaged in investigating the subject of the continuity of protoplasm through the walls of vegetable cells, it was with no small degree of interest that I read Dr. Elsberg's paper[1] in the hope of finding something that would be of value for my research. In this, however, I was disappointed, and having carefully gone over his paper, and worked through his methods, I resolved to publish my results, believing it to be of extreme importance in a subject such as he treats of, that his statements should, if correct, receive every confirmation and support, or if any mistake had arisen, that such mistakes should as quickly as possible be rectified.

There are several points in his paper that I should like to touch upon before giving my own conclusions.

As Dr. Elsberg at the outset admits that he is not a botanist, it is perhaps not surprising to find a want of accuracy in his botanical terminology. Thus, he talks of leaf parenchyma cells as "presenting blunt polygons separated from one another by a shining rim of cellulose," meaning, I suppose, polygonal cells with thin cell walls. He then proceeds to rechristen protoplasm, and proposes to substitute for

[1] 'Quart. Journ. Mic. Sci.,' No. lxxxix, Jan. 1883.

it the certainly not euphonious name bioplasson. To this it may be answered that, although the word protoplasm conveys in some ways an unsatisfactory idea, yet its persistent and wide use, would warrant its being retained, rather than that scientific terminology should be burdened with another new word, when the value of the original word has been perfectly well defined, and has for the biological student a perfectly clear meaning.

As far as I can judge Dr. Elsberg appears to confuse reticulate arrangement with reticulate structure, for he uses the same expression, "reticulated living matter" for both. As examples of such reticulated living matter, he gives Zygnema cruciatum; the description of which he quotes from Sachs—only altering the words "primordial utricle" for parietal sac."[1] Other examples are: young cells of Zea mais, Fritillaria imperialis, and Vicia faba; hairs of Tradescantia virginica, and Cucurbita. All these are of course examples of reticulate arrangement of protoplasm, and have nothing to do with the structure of the protoplasm itself.

Passing on to where he treats of the analogy between animals and plants, his terminology again becomes somewhat confused, in his endeavours to carry the analogy too far; for comparing the fact, that just as the animal cell is limited by its layer of cement substance, so is the plant-cell limited by its layer of cellulose, he proposes to commemorate "Schleiden and his cell doctrine," by making the word cellulose subserve for the limiting membrane of both the animal and the plant-cell. It is quite obvious, however, that this is impossible, for the term cellulose is a name applied to a definite chemical substance with definite properties, and does not necessarily carry with it the idea of a limiting membrane at all. Cement substance, so far as I am aware, does not, for example, give a blue colour with iodine and sulphuric acid, nor furnish gun-cotton when acted upon with nitric acid.

We now come to the most important part of the paper, where Dr. Elsberg treats of the perforation of the cell wall.

[1] Sach's 'Text-book of Botany,' 1882, p. 46.

The first experiments were made upon Nierembergia (printed Norembergia) gracilis.[1] He took pieces of the flower—whether calyx, corolla, stamens, or pistil does not transpire—treated them with a 2 per-cent. solution of silver nitrate for half an hour, or with a ·5 per-cent. solution of gold chloride for forty minutes, washed, exposed to daylight, and examined. The silver nitrate preparations when seen from the surface, showed the cell walls stained dark brown, and demonstrated that every here and there were interruptions in their continuity. I have unfortunately been unable to obtain flowers of this plant, and have in consequence not had any opportunity of making this observation for myself, but I should like to point out that such pronounced and frequent interruption is quite opposed to our present knowledge, and certainly to the results I myself have obtained. It is much more probable that the walls were pitted, and that the pit membrane being thin escaped observation. The same may be said of the figure of the hair of Nierembergia, the transverse walls of which are probably pitted in a similar manner to those of the walls of Athæa hairs, a figure of which occurs in Sach's Text-book.[2] If Dr. Elsberg's figure is drawn to scale it can scarcely be wondered at, if he has made a mistake, for his magnifying powers have not been sufficiently high. Very frequently the pit membrane is so thin that without very careful preparation, it cannot be recognised under the highest powers, and in many cases the only way to bring out such a membrane is to stain the protoplasm and leave the membrane unstained, or to stain and swell the membrane itself with Schultz solution (Chlor. Zinc Iod.).

But it was from the study of sections of the petiole of Ficus elastica, when treated with silver nitrate, that Dr. Elsberg has obtained his most conclusive results.

He gives a drawing of one of his preparations, and it is its appearance, and the appended description of it, which, perhaps, forms the most startling part of the whole paper; for we are

[1] "Nierembergia gracilis," Hook, 'Bot. Mag.,' 58, 3108.

[2] Loc. cit., p. 43.

told that "what has been sometimes described by authors, especially in growing tissues, as 'intercellular spaces,' and "middle lamellæ" in the cellulose were revealed to be, in a number of instances, accumulations and filaments of living matter wedged in between the plant cells." Since it is impossible to understand how an intercellular space (if Dr. Elsberg really means space) can be an accumulation of anything, one must proceed to deal with the question of the middle lamella being an accumulation of living matter, &c. It is an undoubted fact that the substance of the middle lamella resembles protoplasm in many of its properties. Thus, like protoplasm, it resists the action of strong sulphuric acid in cases where it has attained to any pronounced degree of development, and, like it again, dissolves in strong potash or in Schultz's mixture; and it is very noticeable that many reagents which are used as special stains for the protoplasm will also stain the middle lamella. But whatever view be taken as to the nature of the middle lamella and the thickened cell wall, and no matter whether one accepts the intussusception theory of Nägeli,[1] or the apposition theory of Schmitz[2] and Strasburger,[3] it is quite certain that at the time of its first formation the cell wall is essentially cellulose, and is thickened by deposits of cellulose substance. If protoplasm in any way enters into the constitution of, or forms an integral part of, that structure which we recognise as cell wall, it is, to say the least of it, hard to imagine, even on the well-nigh exploded intussusception theory, that such large quantities of protoplasm should be present, not only to replace the structure which we are accustomed to regard as middle lamella, and as such consisting of altered cell wall, but even large areas on either side of it, in such proportion as

[1] Nägeli, "Die Stärkekörner."
Nägeli and Schwendener, "Das Microscop," &c.

[2] Schmitz, "Sitzber. d. niederrhein Ges. in Bonn," 1879 and 1880.

[3] Strasburger, "Bau und Wachstum," Leipzig, 1882. In connection with this subject, cf. also Schimper, "Ueber das Wachstum der Stärkekörner," 'Bot. Zeit.,' 1881, 186; and Mayer, "Ueber die Structur der Stärkekörner," 'Bot. Zeit.,' 1881, 844.

to cause the wall to consist as much of protoplasm as of cellulose.

It would rather be expected that if protoplasm does perforate the substance of the cell wall, such perforations would assume the form of fine threads, of such a degree of tenuity that they could only be recognised with great difficulty, involving very careful preparation and the use of very high powers. This subject has already been ably dealt with by Strasburger,[1] whose ideas have received confirmation from Tangl's[2] researches published in his work, on the Structure of the Endosperm Cells of Strychnos, Phœnix, and Areca, and from the results I myself have obtained in the pulvini of Mimosa, Robinia, and Amicia.[3] In my later work, which will shortly be published by the Royal Society, I shall be in a position to show that, as far as my investigations have as yet progressed, there has not only not been the least suggestion of the presence of large quantities of protoplasm in the cell wall, but also that no examples of reticulate arrangement have been met with in those cases where perforation actually takes place.

I now propose to give the experiments which were made with a view of testing Dr. Elsberg's results.

Unfortunately the name of the grass he investigated is not given. I examined in detail two grasses, viz. Poa nemoralis and Bromus maritensis. In each case it was apparent that when mounted in dilute glycerine a distinct network structure could be made out in the chlorophyll grains. The boundary line of each grain was badly defined, and it was very hard to recognise with any certainty whether the reticulate appearance was confined to the immediate substance of the grain, or whether it extended beyond these limits. Indeed, in some

[1] 'Ueber den Bau und das Wachstum der Zellhäute,' p. 246.

[2] Pringsheim, 'Jahrb.,' vol. xii, p. 170.

[3] 'Quart. Journ. Micr. Sci.,' Oct., 1882; 'Roy. Soc. Proc.,' Nov. 11th, 1882. See also the conclusions arrived at by Russow from the callus reaction given by the closing membrane of the nits of Phloem parenchyma. 'Sitzber. der Dorpat Naturfor.,' 1882, p. 350, and 'Bot. Central,' viii, 1883, p. 271, also 'Strasburger Sitzber. d Niederrh Ges.' 4 Dec. 1882, p. 12.

instances, it certainly appeared to extend from the chlorophyll grain into the general cell protoplasm.

In order to see whether any abnormal appearances had been brought about by the action of the dilute glycerine, some pieces of the blade were mounted in expressed cell sap and examined. As the thickness of the blade was, however, too great to allow of satisfactory observations being made with high powers, a small piece was teased out before mounting; and since it was found, that during the teasing process and rupture of the tissues, no observable alterations had taken place in the cells, when such a preparation was compared with an uninjured one, in subsequent experiments teased out preparations were made use of.

When such a preparation is mounted in expressed cell sap and examined, it becomes apparent that the outline of each chlorophyll corpuscle is quite defined and distinct, and that little or nothing can be made out of the reticulate structure. I was also quite unable to observe any network in the general cell protoplasm. If, however, dilute glycerine, or simply water, be run under the cover glass, the corpuscles will be seen to gradually swell up, and, in so doing, to display more and more distinctly a reticulate structure. The outline also becomes more and more diffuse, and one almost begins to make out that the network appears to extend beyond the grain into the protoplasm. I am of opinion, however, that this is not the case.

The structure of the unaltered chlorophyll grain, and the action of reagents upon it, can be much better followed in thin leaves with large grains. I found, for instance, Selaginella uncinata very good material. And just as the chlorophyll grains in uninjured cells of ærial plants will swell in the way I have described when treated with water, so will those of water plants when the cell becomes broken into or otherwise injured, e. g. the chlorophyll grains of Chara, Vallisneria, and Elodia. In each case a reticulate appearance is first produced, which, upon prolonged treatment, gives way to a granulation, and is followed at length by complete

disorganisation. The fact, however, appears worthy of notice, that whatever light the action of reagents may throw upon the constitution of chlorophyll corpuscles, yet that pronounced reticulation of structure and diffuseness of outline are not observable in grains which are normal and unaltered.

My results then agree with those of Dr. Elsberg in so far as the reticulate structure of chlorophyll grains is concerned, but I have been unable to trace any reticulation in the protoplasm itself. Since I was unfortunately unable to obtain any Nierembergia material it only remains for me to deal with Ficus elastica. When transverse sections of the petiole were examined in dilute glycerine I could make out the reticulation in the chlorophyll corpuscles, but, as in the case of the grass, was quite unable to see anything of the kind in the protoplasm. Thin transverse sections, treated for half an hour with a 2 per-cent. solution of silver nitrate, washed, exposed to daylight, and mounted in glycerine, exhibited a structure somewhat similar to the figure drawn by Dr. Elsberg, viz. that on the cut surfaces of the cell walls were a number of exceedingly small, darkly-stained patches, separated from one another by light and unstained narrow areas. The reduction appeared not to have taken place uniformly all over the section, being, for instance, specially pronounced just under the epidermis.

The most obvious questions that arose were: Are these dark patches confined to the surface of the section, or are they present, as one would naturally suppose, in the entire thickness of the wall, and thus admit of being seen at any focus? Secondly, what is their nature? Do they consist of stained cellulose.

Now, the epidermal and cortical parenchyma cells being freely pitted, it is easy to focus to any determinate depth by fixing upon any given pit. I examined carefully in this manner several thin and well-prepared sections, but was unable to see any staining whatsoever below the free surface. On the contrary, my observations led me distinctly to the conclusion that the black patches were granules resting upon the

cut surface, and that the substance of the wall itself was quite free from them.

I proceeded to make several experiments to test the truth of this conclusion. When sections of the petiole are cut in water a considerable amount of latex escapes from the injured surface and runs over the section, and it seemed not impossible that the latex mechanically deposited on the cut surfaces of the cell walls had reduced the silver. In order to expel the latex as much as possible before cutting the sections, a short piece of the petiole was taken and fitted into a bored india-rubber cork, which was then tightly fastened into the shorter limb of a manometer tube. The manometer was filled with mercury, with the exception of a short length next the cork, which contained water. Then, under a pressure of about 50 inches of mercury, a current of water was rapidly driven through the petiole tissue, and the latex was almost entirely expelled. Sections of the petiole however, showed the same granular appearance as in the first instance, thus proving that the latex had not been the cause of the reduction. Indeed, direct observation of sections of fresh petiole showed that little or no reduction had taken place in the laticiferous cells.

It was still possible, however, that some of the contents of the other cells might have been smeared over the walls either in the act of cutting or of escaping when cut. Thin sections of the petiole were cut, some of which were vigorously shaken with water in a test-tube. They were both treated together with silver nitrate. In the shaken-up sections there was much less reduction than in the others. I then resolved to try alcohol material where coagulation and hardening of the protoplasm would occur, and there would be a greater propability of getting a clean surface. In order to make out whether the action of the alcohol would interfere with the reduction of the silver, sections of fresh petiole were cut in water, and then treated with absolute alcohol. Having been washed with water, and treated with silver nitrate in the usual manner, they were examined, and it was seen that they exhibited the granulation quite as well as fresh sections. Thus having

established that alcohol did not interfere with the reduction, I cut sections of petiole which had been for twenty-four hours in absolute alcohol. The sections were washed and manipulated as before. However, hardly any reduction was found to have taken place on the cell walls, although the cell-contents themselves exhibited well-marked reduction. This, again, suggested that escaped cell-contents were the cause of the granulation.

To make this quite plain I took sections which were well reduced, and were in every way satisfactory preparations, and with a camel-hair brush freely brushed their surfaces. Nearly all the granules disappeared from the surface of the cell wall. In the cell lumen they were still numerous where the contents had not fallen away, but the cut surfaces of the walls themselves were quite clean and bright, and so was the entire thickness of the cell wall.

Again, having taken sections of fresh material, I brushed some well before the treatment with silver nitrate. Others I did not brush. The former showed no reduction. The latter exhibited well-marked granulation in the usual manner.

If the reduction be allowed to take place in diffused daylight the granules are small. If in sunlight they are large. Consequently, one may vary at pleasure the size of the granules, and therefore the size of the meshes of the reticulum.

I think these experiments have sufficiently established that the appearance described by Dr. Elsberg is simply due to the fact that granules of reduced silver are deposited on the cut surfaces of the cell walls, and that no staining occurs in the substance of the wall itself. The whole appearance of protoplasmic continuity can be brushed away by mere mechanical means, and the size of the granules can be varied at will. The reduction of the silver in the cell wall is caused by some of the cell contents which have escaped from the cell lumen.

The gold chloride preparations are not nearly so successful as the silver nitrate, for much less reduction occurs. I was unable to detect any network in the protoplasm, nor could I

trace any perforation of the cell wall by protoplasmic filaments.

The identification of the substance causing the reduction now simply resolves itself into a micro-chemical investigation. That it was a very powerfully reducing agent was evident from the fact that gold chloride, silver nitrate, osmic acid, and chromic acid were all reduced by the cell contents of a very great number of the parenchymatous cells. When tested with alcannin it was shown that the presence of resin was confined to the laticiferous cells and to the cuticle, and there being no oil globules in any of the cell contents, with the exception of the latex, it was probable that the reaction with osmic acid had not been caused by oil or fat. The chromic acid reaction pointed to tannin. Sections were therefore treated with ferric chloride, when the cells occupying the same position as those which had especially reduced the osmic, chromic acid, &c., were turned a brown-green colour, thus proving conclusively the presence of tannin.[1]

In order to examine the distribution of the tannin cells, transverse and longitudinal sections were treated with chromic acid. They are shown to be present in the tissue in very great numbers, and are especially abundant just under the epidermis (at the very place where the greatest reduction of silver nitrate occurs), and are arranged around the vascular bundle, being also dotted about irregularly in the tissue. In longitudinal section they are shown to be arranged in rows, end to end, and their cell contents exhibit a fine reticulation.

A longitudinal section of alcohol material treated with chromic acid shows that tannin has escaped over the cut surface, and thus gives confirmation to the other results. In the reduction experiments I placed along with the Ficus sections, sections of material such as the endosperm of Phœnix dactylifera, where I knew that perforation of the cell wall

[1] The tannin occurring in these cells is evidently not of the same character as gallotannic acid which gives a black colour with ferric chloride. It is probably related to catechu-tannic acid, which also gives a brown-green when treated with the above-mentioned reagent.

did occur, and obtained no satisfactory results whatever. Two years ago I made a number of experiments with gold chloride and silver nitrate, and was forced to conclude that they were unsatisfactory for botanical research. In my later work also I have tried many modifications which have met with no success as far as their use for studying the perforation of the cell wall by protoplasm is concerned, and I can only add that the experiments made in connection with Dr. Elsberg's paper have fully confirmed my previous conclusions.

Before leaving the subject I should like to make a few remarks upon a more important paper, viz. Professor Frommann's "Beobachtungen über Structur und Bewegungserscheinungen des Protoplasma der Pflanzenzellen."[1]

The only part of his paper that I shall venture to comment upon is that which deals with the perforation of the cell wall and the subject of protoplasmic continuity. His results in this direction may be summed up in his own words, in which he claims to have established "that protoplasmic nets pass from one cell to another, and connect neighbouring cells with one another by means of either smaller or larger gaps and crevices in the membrane." This structure was especially clearly seen in the epidermal and hypodermal cells of the leaves of Rhododendron ponticum and Dracæna Draco, but the leaves of Aloë arborescens, Crocus, Hyacinthus, and Mentha were also investigated.

I propose to give several quotations from Professor Frommann's paper which will serve to illustrate the exact nature of his statements, and at the same time make it quite clear, which of these I wish to deal with, and to criticise.

On page 9, when treating of the epidermal and hypodermal cells of Rhododendron ponticum, we find: "The intercellular spaces contain nets and granules;" and further on—"The partition walls, however, do not always completely shut off neighbouring cells from one another, but are pretty frequently interrupted by gaps and crevices which are generally

[1] 'Beob. über Structur und Beweg. d. Protoplasma der Pflanzenzellen,' Jena, 1880.

very narrow, so that only reticular threads or one or two series of meshes find room in them, but they sometimes attain greater breadth."

On page 10, when speaking of the mode of formation of the cell wall from the protoplasm—"And this view is also supported by the occurrence here and there observed of chlorophyll grains and coloured portions of nets, not only in the crevices, but also in the substance of the partition walls into which they appear as it were forced."

On page 11 the following statement occurs:—"In the latter (i. e. the cuticle) chlorophyll corpuscles are deposited here and there."

The next quotation, on page 17, refers to Dracæna Draco: "The partition walls separating the epidermal cells from one another and from the subjacent cells may, for a short distance, lose their brilliancy, but interruptions of continuity are more frequent which either appear isolated, or three or five of them on one partition wall; are partly very narrow, partly wider; may reach the diameter of a chlorophyll grain, and are traversed by isolated threads, or by narrow reticular bands, frequently showing isolated thickened threads and nodes, by means of which neighbouring cells are in connection."

Again, page 21: "Narrow cracks and wider crevices, reaching the diameter of a chlorophyll granule, or of a nucleus, may occur with varying frequency."

On page 29, referring to the epidermis of Crocus and Hyacinthus: "The threads of the neighbouring reticular layers are sunk into the membrane, while in places where cracks and crevices appear on the partition walls, the threads extend through them, and connect the nets of neighbouring cells with one another."

Lastly, on page 38: "But continuous roundish or band-shaped lamellæ are also deposited in those layers of membrane which shut off the epidermis cells towards the outside, and may appear on their surface, and be enclosed in their texture, and as further in them as well as in the thicker partition walls chlorophyll grains also occur, it can admit of no doubt that

reticular protoplasm may enter into the structure of cell membrane to a greater or less extent."

Briefly stated, the principal facts involved in these statements are : that open passages of a very appreciable size are of very frequent occurrence in the common cell wall. That chlorophyl corpuscles and protoplasmic reticula occur embedded in its substance. That the intercellular spaces may contain granules and nets. That these nets and reticula of protoplasm may be traced into the cell wall, and are particularly clearly defined in the case of epidermal cells, running from the cell lumen out into the cuticle.

With all deference to Professor Frommann, I cannot but think that every one of these statements would be received with some surprise by almost any botanist who is at all acquainted with the histology of tissues.

I have investigated in as careful a manner as possible the leaves of Rhododendron ponticum and Dracæna Draco, in order to give Professor Frommann's results a fair test. Transverse and longitudinal sections, as well as sections parallel to the leaf surface, were examined in water, in cell sap, and in dilute glycerine. Both fresh material and that preserved in picric acid and absolute alcohol, were made use of. Iodine, Chlor. Zinc. Iod., and hæmatoxylin, which latter Schmitz[1] so successfully employed in his researches on the structure of protoplasm, and the nucleus, were used as staining reagents. Professor Frommann used expressed cell sap, sugar solution, and dilute glycerine as fluids for mounting his preparations, and employed methyl green as a stain ; but since he expressly states that the staining due to this reagent was confined to the nucleus, and did not affect the nets and reticula, one must conclude that most of his observations were made upon preparations which were simply mounted in the fluids before mentioned.

I do not intend to enter into detail with regard to the subject of the intimate structure of the protoplasm. Suffice it to say that in a great measure my results agree with those of Pro-

[1] Schmitz, 'Sitzber. d. niederrhein Ges. in Bonn,' 1879.

fessor Frommann, although I am unable to see the nets and reticula with anything like the clearness with which he describes them in his paper, and figures them in his drawings. There is, however, a distinct reticulation in the cells of both Rhododendron and Dracæna, especially in the former. This reticulation is shown much more clearly by staining with hæmatoxylin, tissue that has been preserved in picric acid, and afterwards washed with alcohol. In Rhododendron very many of the cells, and especially the pallisade-parenchyma cells, contain tannin, and in these the reticulation is especially evident. I have frequently found this to be the case with tannin cells in general. Treatment with chromic acid, osmic acid, dilute potash, or dilute nitric acid will generally bring out a reticulate structure, although at present I am unable to give any explanation of the phenomenon. My attention was first drawn to the fact when investigating the tannin cells which occur in the pulvinus of Robinia pseudacacia.

As far as regards the reticulate structure, I can, therefore, in the main bear out the statements of Professor Frommann, whose results certainly accord with those of Schmitz[1] and Strasburger;[2] both these investigators having established that a reticulation can be observed in the protoplasm and the nucleus. The chlorophyll grains in the same way exhibit reticulation, but I should like to point out, as I did in the case of Dr. Elsberg's research, that they are much swollen and somewhat disorganised by the action of dilute glycerine. Nevertheless, after the most careful preparation with picric acid and absolute alcohol and subsequent staining, they still exhibited a distinct reticulate structure, and agree fully with the description given by Pringsheim[3] of the structure of chlorophyll grains in general.

As to the occurrence of chlorophyll grains in the cell wall, it is scarcely necessary to state that after very careful examination no such case was observed. Were I to attempt to explain

[1] Schmitz, loc. cit.

[2] Strasburger, loc. cit.

[3] Pringsheim, 'Lichtwirkung und Chlorophyll function,' 1881, p. 28.

Professor Frommann's mistakes in this direction I might suggest with regard to the occurrence of chlorophyll grains in the cell walls that he was looking down through the thickness of a cell wall upon a chlorophyll grain that had got into a pit; and in the case of the grain in the cuticle, I can only put forward the explanation that he was viewing a chlorophyll grain in the guard cell of a stoma through the cuticle of an epidermal cell.

There is the simpler view, that during swelling, and also by mechanical means, some of the protoplasm was carried on to the cut surface of the cell wall; and fig. 4, Plate I, which represents a subepidermal cell of Dracæna, certainly gives some colour to this idea, although I put it forward with some diffidence. Numerous preparations, treated and stained in various ways, showed no sign of there being either granules or nets, or, finally, any protoplasmic structure whatsoever in the intercellular spaces.

Now, as to the subject of holes, gaps, and crevices in the cell wall. At the outset I cannot but feel that Professor Frommann was somewhat unfortunate in taking for his investigation such small-celled tissue as occurs in the leaves of Dracæna and Rhododendron, and especially so as regards the epidermal cells. In both these leaves, and particularly in Rhododendron, the epidermal and parenchyma cells are very freely pitted, and it is quite evident that what Professor Frommann has taken for open passages between the cells are in reality pits, each of which is closed by its own pit membrane. In Plate II, figs. 4 and 5, he gives a drawing of the so-called holes, but even a cursory examination of carefully prepared and thin sections treated with Chlor. Zinc Iod., or otherwise appropriately stained, will at once convince one that in every case a closing membrane is present, and that the pits are not open. As I have mentioned in the earlier part of this paper, the occurrence of open pits in living cells would be quite opposed to our accepted ideas of cell structure and cell mechanism. In his descriptions, however, Professor Frommann gives passages which suggest that he has noticed, but not identified, the pit-

closing membrane. For instance, on page 9 he observes "short threads crossing from one side of the membrane (i.e. the membrane of the cell wall bordering a gap) to the other;" and again, page 17, "a somewhat stouter and more strongly refractive thread not unfrequently unites the portions of membrane bordering the gap in a bridge-like manner, and crosses the threads which pass through it." On page 11 I may quote a passage which shows the want of accuracy in his botanical terminology, for he describes as middle lamella "the layers immediately below the cuticle, which are double or three times as thick as the partition walls between the epidermal cells."

The last point I have to deal with is the question of the perforation of the cell wall, and the possibility of following protoplasmic structures into its substance. Tangl[1] has shown that it is impossible to see anything of the protoplasmic threads in the cell walls of the endosperm cells of Strychnos, Phœnix, and Areca, by direct observation in such fluids as dilute glycerine, and even by ordinary staining. In each case a special mode of preparation must be employed. I can fully bear out his statements, and, indeed, in some of my most striking examples of the occurrence of protoplasmic filaments in the cell wall, it was quite impossible to see anything of them when examined in the usual manner. I was unable to observe anything of the kind in the epidermal cells of Dracæna and Rhododendron, but I think that some satisfactory explanation can be given of the appearances which Professor Frommann describes. If sections of Dracæna be examined in dilute glycerine, what appears to be a reticulate structure can be distinctly observed, both on the upper, and the side walls of the epidermal cells. The outlines of the walls bounding the cell lumen are not well defined, and, as Professor Frommann says, the protoplasm seems to gradually merge, as it were, into the cell wall, as he endeavours to represent in fig. 25, plate I. Indeed, the whole appearance is most striking. If, however, excessively thin and exactly transverse sections

[1] Pringsheim, 'Jahr.,' l. c.

be treated with Chlor. Zinc Iod. the walls swell; the boundary bordering on the cell lumen becomes more distinct; and definite granules can be recognised deposited in the substance of the swollen wall. I made several experiments to test the nature of these granules. When the sections are warmed in dilute potash solution, the granules appear to become somewhat aggregated together, and signs of commencing solution can be recognised. When boiled with a 5 per-cent. solution of potash they are totally dissolved, the substance of the cell wall is left quite clear, and its limits sharply defined. When sections are treated with ether, and afterwards with boiling alcohol, considerable solution takes place, attended with a clearing up of the structure. These reactions appear to indicate that the granules consist of wax, and, as De Bary[1] has shown, the presence of wax is of frequent occurrence, not only on the surface of the cuticle, but even embedded in the substance of the cuticularised layers of the cell wall. The presence of these granules appears to explain, in a satisfactory manner, both the diffuseness of outline and the appearance of reticulation.

In Rhododendron, in the same way, an appearance of striation approaching to reticulation occurs. When examined in Chlor. Zinc Iod. it becomes apparent that very great cuticularisation of the epidermis has taken place, the cuticularisation extending even to the transverse walls. It is also apparent that the striation is confined to the cuticularised layers, and is separated from the cell lumen by a thin layer of cell wall, which still gives the cellulose reaction. If treated with a solution of potash, the cuticularised portion still shows striation, while the rest of the cell wall becomes clear and transparent. Examination of thin sections mounted in glycerine supports these observations in every way. I need only refer to Sach's 'Text-book'[2] to show that the occurrence of striation in the cuticularised layers of the cell wall of epidermal cells is a perfectly well known phenomenon. His figure of the epi-

[1] 'Vegl. Anat.,' Leipziz, 1877, p. 87.

[2] Sach's, loc. cit., p. 35.

dermal cell of Ilex aquifolium presents a case in point. I used in this investigation one of Zeiss No. 1 microscopes, Oculars 2 and 4, Objectives D and F, and a microscope of Hartnack's with two very excellent Objectives, No. 10, and an Immersion, known as "Foyer 1 m/m" (No. 13?). Oculars 2 and 4.

Review of Recent Researches on Spermatogenesis.

By

J. E. Blomfield, M.A.,

Since the publication of my two former papers on the subject of Spermatogenesis (vide this Journal January, 1880, and July, 1881) some accounts of the researches of other observers have been published in foreign periodicals, which go far to support the attempt made in the papers referred to, to establish a general plan of Spermatogenesis which should be applicable to the animal kingdom, or, at any rate, to a large part of it. While our knowledge of the formation of the ovum was fairly perfect, observations on the development of the corresponding sexual element, though by no means deficient in number, yet were wanting in completeness and wideness of scope. For instance, in such an animal as the frog, many had observed the process for a short time and drawn conclusions as to how this was conducted from data which were necessarily incomplete; since the observations to be of any value in such animals which procreate once in the year, must be continued during the whole period from the time of procreation till the next crop of spermatozoa is ready.

The publications now referred to, tend strongly to prove the truth of the views expressed in my former papers, and confirm the conclusion that the process as found in Mammals is closely similar to that of Mollusca. This conclusion was, at the time, somewhat speculative since it was founded on interpretations of the drawings of other observers different to those which they themselves had imposed upon their observations.

The first papers of which it is proposed here to give an ac-

count are by M. Duval, who has chosen for the investigation of the general phenomena of Spermatogenesis the snail, with other Pulmonate Gastropods, and the frog. Both of these animals were also chosen by myself, though I took my earliest type from the lower divisions of the animal kingdom, in the shape of the earth-worm, an animal which exhibits the phenomenon of Spermatogenesis, as found in the majority of the Vermes, with singular clearness and facility for study.

M. Duval's first communication on the subject is published in the 'Rev. de Science Nat.,' tome vii, June, 1878, in which he treats of Spermatogenesis in Pulmonate Gastropods, and takes Helix as the typical representative of the class. Had I been aware of the existence of this paper when I published my researches over the same ground I should have referred to it then, but I did not discover its existence till too late.

M. Duval commences by showing the importance of extending observations which apply to animals whose time of procreation is limited to a definite period of the year, over sufficient time to embrace the whole process, not as many have done, making observations only at one time of the year, and then drawing conclusions from observations which are, of course, imperfect. Taking the condition of the ovotestis as it exists in the winter, it is found to contain a few bundles of spermatozoa and some free spermatozoa, while its wall is lined with indifferent cells, to which he gives no particular name, but which obviously correspond to the testicular and ovarian epithelia. In the spring certain of these cells are seen to enlarge, some rapidly, so that it soon becomes obvious that they are destined to form the ova, while others grow more slowly, and never attain the same size. These he calls male ovules, and they form the starting-point from which the spermatozoa are evolved. These male ovules consist of granular cells containing one well-marked nucleus. The first change they undergo consists in the development of other nuclei by endogenous formation, which, by their further multiplication by division, form the immature spermatozoa or spermatoblasts. And here is the first point where his observations differ from

mine ; I did not observe any endogenous formation of nuclei, and thought that the large multinucleated mass arose by division of the nucleus. These large multinucleated masses are found during the late spring and early summer months, when the male ovules have given place to mulberry-like masses (grappes) adherent to the walls of the gland. These bodies consist of several pyriform cells, each one containing one nucleus, or a nucleus in the act of dividing into two, which are united by strands of clear substance to a central mass containing a large, well-marked nucleus. This central mass he calls the " mother cell," and its nucleus " the principal nucleus."

He gives no name to these cell groups, but in my paper I proposed to call them sperm-polyplasts. It is best to have some name for such cell masses, as they are of very frequent if not universal occurrence in the development of the spermatozoa of various members of the animal kingdom. The term blastophor or blastophoral cell was applied by me to the body, which takes no part in the formation of the spermatozoa, but plays the part rather of a support for the others, which M. Duval calls " mother cell," a term objectionable on account of its having been applied to quite different factors of the process by other authors. The term " spermatoblast " is used by me for the young condition of the cells which actually and individually develop each into a spermatozoon. These terms may seem to many to be unnecessary, but any one who has studied the subject will find that the variety of names in use, and the manner in which one author applies one term to one thing and another applies the same term to a totally different, do not conduce to clearness, and if the process is to be generalised to one or more plans, definite names are necessary to point definite stages in the course of events.

The next stage concerns the formation of the spermatozoa from the spermatoblast, which is (according to Duval) brought about in a more complicated manner than that described by me. In each spermatoblast, which has much the shape of a racquet, appears in the region of the handle, a minute spot exhibiting great affinity for staining reagents, which he calls the " cepha-

lie corpuscle." This gradually enlarges until it has assumed the shape and size of the head of the mature spermatozoon, that is, more or less pear-shaped. At the same time, or directly after, the tail of the spermatozoon appears in an unexplained manner in the substance of the spermatoblast, while the spermatoblastic nucleus disappears, so that the group of spermatoblasts have given place to a bundle of spermatozoa united in a bundle and supported on the "mother cell." The further history concerns only the "mother cell" and principal nucleus (my blastophor) which, according to M. Duval's observations and my own, undergoes fatty degeneration and disappears.

M. Duval's next communication is contained in the 'Rev. de Science Nat.,' Sept., 1879, in which he gives an account of the two kinds of spermatozoa which since the time of Von Siebold have been known to be present in Paludina. This paper need not detain us longer than to mention that he finds that the two kinds of spermatozoa develop on the same plan as those of the snail, and that they are veritably two different kinds of spermatozoa, a fact which some naturalists have disputed.

The next publication from the pen of this writer is in the 'Rev. de Sci. Nat.,' Sept., 1880, on the "Spermatogenesis of the Common Frog," which also formed part of the subject of my second paper; and on comparing the two the similarity of the figures are striking, though the interpretations drawn from them are not quite in agreement.

He commences his description by showing the condition of a testicular crypt during the winter, when the spermatozoa are seen to be united into bundles, which bundles are arranged in a radial manner, with remarkable regularity around the walls of the sac. The wall of the sac itself is formed of fibrous tissue and cells, and of the latter two kinds can be distinguished, one the "male ovules," the other smaller and more granular, embedded firmly among the male ovules, which constitute the source from which the male ovules are derived. This condition of things obtains throughout the winter till the spring, when the spermatozoa are discharged and changes

begin in the "male ovules" for the preparation of next year's crop of spermatozoa. These changes consist in the multiplication of the nucleus till large multinucleated masses are produced, comparable to those found in the snail. By the end of the spring these have reached a considerable size, and the next step consists in an arrangement of the nuclei around the periphery of the mass, while the centre part is free from them, and undergoes a kind of liquefaction, thus giving rise to a vesicular body. As this body has grown from the original "male ovule," it has carried the smaller granular cells which surrounded it on its surface towards the lumen of the sac, where they may be seen resting on the protoplasm of the vesicle. Each vesicle is now composed of a mass of uninucleated bodies, which are the spermatoblasts, and the next stage is the transformation of the spermatoblasts into spermatozoa. The exact mode in which this is brought about, M. Duval is unable to state; but reasoning from what he found in the snail, he thinks it probable that the process is the same, and that a "cephalic corpuscle" is produced while the spermatoblastic nucleus is dissolved and the filament is formed from the plasma: but the existence of the "cephalic corpuscles" he is unable to determine.

The immature spermatozoa are so arranged that the tails point to the interior of the vesicle, while the heads are arranged radially around the concave inner face.

When the spermatoblasts have nearly changed into mature spermatozoa dehiscence of the vesicle takes place, and the spermatozoa are carried back to the wall of the sac, bringing the granular cells with them, where they become arranged in bundles, and the same condition of things is found as that which formed the starting point.

The granular cells, which have been carried back, undergo an increase in size, and become "male ovules;" destined to reproduce the crop for the next year; but in teased preparations M. Duval finds that often a bundle of spermatozoa is attached to one of these granular cells, an attachment which he regards as quite accidental, though, at one time, he was tempted to see a

connection between this cell and the "mother cell" with its "principal nucleus" of the Mollusca. Further examination caused him to abandon this view, as it was possible to find distinct gradations between this cell and the "male ovules;" and here is the chief point where his observations and mine disagree. Undoubtedly these granular cells do exist and give rise to the "male ovules" (spermatospores) forming part of the testicular epithelium, but, according to my view of the matter, the cells which are carried back with the young spermatozoa are distinct from these and are, as it were, left behind during the formation of the vesicle, being homologous and analogous with the blastophoral cell of the Mollusca, and, like that, undergoing a process of fatty degeneration. For, if a testis be taken in the spring, at the right time, after the discharge of the spermatozoa there will be found in the testicular sacs a number of cells whose nuclei are disintegrated into coloured granules, whose surface is marked with striæ, representing the points of attachment of the spermatozoa, and whose substance contains many fat granules, obviously representing the degeneration of the supporting cell. This view of the existence of the blastophoral cell and explanation of its origin and function will be seen to receive considerable support from the study of the spermatogenesis of a group in many ways allied to the Amphibia, viz. the Selachians, to which I will now proceed.

The most recent account of the development of the spermatozoa in the Selachians is contained in the 'Journal de l'Anat. et de la Physiol., 'No. 4, 1882, in a paper by M. G. Hermann.

The testis of the Selachians is remarkable for its simplicity and points of resemblance to the ovary, the primitive germinal cells, forming the germinal epithelium, sink into the subjacent tissue in chains or groups (cordons de Pflüger) in the same way as they do in the ovary; but instead of forming Graafian follicles they make up the ampullæ in which the germinal cells, male ovules, or spermatospores undergo changes which result in the production of a crop of spermatozoa, and when this process is over, the whole follicle or ampulla atrophies

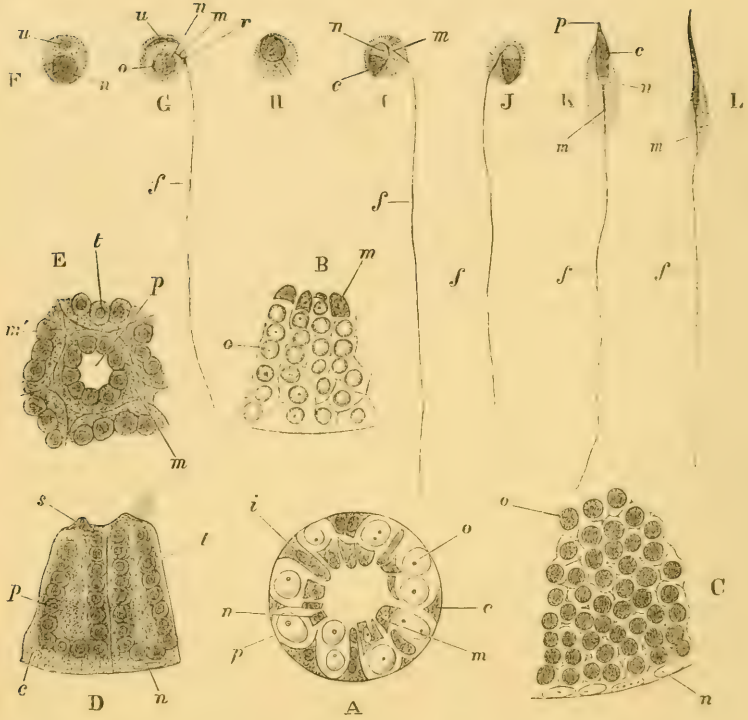

FIG. 1.—Spermatogenesis of Selachians. A. Transverse section of a very young testicular ampulla of Scyllium. *o*. male ovules of Hermann (spermatospores, Blomfield). *i*. Prismatic cells with nuclei in course of division. *m*, *n*. Small cells resulting from division of the prismatic cells. B. Portion of a similar transverse section at a later stage. *o*. Nuclei of the male ovules now constituting multi-nucleate polyplasts and increased in number by division of the prismatic cells. *m*. Terminal nuclei bounding the lumen of the ampulla. C. Similar piece at a later stage. *o*. The nuclei no longer arranged in rows. *n*. The basilar nucleus of Semper (blastophoral cell of Blomfield). D. Section showing two sperm-polyplasts or mother-cells longitudinally cut at a stage immediately preceding the conversion of the spermatoblasts (*t*) into spermatozoa. *p*. Axial protoplasm of the polyplast. *c*. Basilar portion of the polyplast. *n*. Basilar nucleus (*c* and *n* form the blastophoral cell). E. Transverse section of similar cells. *m'*, *m*. Intercalary processes of the protoplasm of the polyplast. F to L. Conversion of a spermatoblast (final stage of division of the nucleus and protoplasm of an original mother cell) into a spermatozoon. *n*. Nucleus. *u*. "Corpuscle precurseur." *o*. Cephalic nodule. *m*. Middle-piece. *f*. Flagellum.

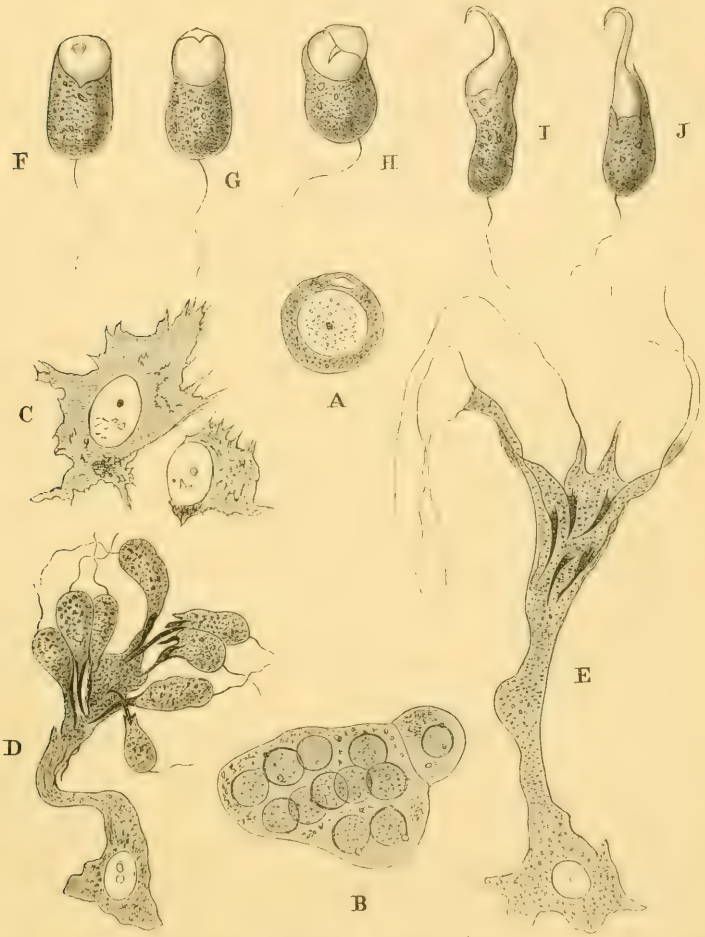

FIG. 2.—Spermatogenesis of the rat. A. Seminiferous cell of Renson (spermatospore, Blomfield). B. Multinuclear cyst of Renson (sperm-polyplast, Blomfield), resulting from the growth and division of the nucleus of A. C. Fragments of the "cellules de soutien" of Renson (blastophoral cell of Blomfield) as seen in a teased preparation. D. First phase of the supposed penetration (Renson) of the nematoblasts (young spermatozoids) into the "cellule de soutien" (blastophoral cell). E. Later phase of the same. F. to J. Development of a spermatoblast (nematoblast, Renson) into young spermatozooid, occurring, according to Renson, *before* its connection with the "cellule de soutien" or blastophoral cell.

forming a degenerated tissue much in the same way as the Graafian follicle forms the corpus luteum.

In a young ampulla formed in this way two kinds of cells are found, the first are large, round, and granular, possessing a large, well-defined nucleus. These are the male ovules; the second kind are smaller and more irregular and fill up as it were the interstices left between the male ovules. The whole ampulla is continuous with the upper part of the cordon de Pflüger, which becomes its excretory duct. The second kind of cell above mentioned is separable into two categories; some are prismatic in shape, situated on the basement membrane and squeezed in between the male ovules and obviously form male ovules, thus increasing the number of these bodies and the size of the ampulla, while the others are placed more on the internal surface of the male ovules and are often seen in the act of division, in fact it is by their division that the first few cells of the mulberry-like body soon to be described are produced.

In a section of an ampulla (fig. 1, A), rather older than the one we have just described, the male ovules, now more properly called sperm-polyplasts, are arranged radially round the periphery of the ampulla; and but few of the first kind of small cells alluded to above are left, having been converted into male ovules, while the second kind are seen more distinctly conical in shape and placed at the end of the polyplast next to the lumen of the ampulla. The polyplast itself now (fig. 1, B) consists of four, five, or six, the number varying according to age, nuclei arranged radially in a series and terminated by one of these conical cells. When the activity of this terminal conical cell is as it were exhausted in giving rise to these several cells, the cells themselves commence to multiply by division, and the outlines between each polyplast become indistinct from the mutual pressure exerted, but each group is well marked by an elongated nucleus which now comes into prominence at the base of each conical mass (fig. 1, C, *n*) or polyplast, which is called by Semper the basilar nucleus, and is regarded by him as the first formed nucleus, an observa-

tion confirmed by M. Hermann. It is needless to point out the identity of this basilar nucleus and its plasma with my blastophoral cell of Helix and the Frog.

The ampulla is now filled with polyplasts (called mother cells by M. Hermann) each consisting of 30 or 40 more small cells and a basilar nucleus towards its peripheral end; and now commences the process to which M. Hermann limits the term Spermatogenesis, viz. the conversion of the spermatoblasts into spermatozoa; but before describing this change we must lodge a protest against the restriction of the term to this portion of the change undergone by a cell in giving rise to spermatozoa. Surely it should be applied to the whole of the changes which are undergone when a germinal cell gives rise to its crop of spermatozoa, as oogenesis is understood to refer to the phenomena presented by an ovarian epithelial cell in becoming a mature ovum.

The next change that the spermatoblasts undergo is one of position (fig. 1, D), something similar to what occurs in the frog, only, instead of forming a vesicle, they form a sort of tube filled with granular protoplasm. When this is completed, the change into spermatozoa commences.

The first event (fig. 1, F to L) is the appearance in the spermatoblast of what appears to be a local consolidation of the protoplasm composing the spermatoblast which M. Hermann calls "corpuscle precurseur." It has but a short existence, and, as far as could be made out, no connection with any part of the mature spermatozoon (*u*). Before this has vanished a small projection becomes visible at one point of the nucleus (*o*), bearing, however, no relation to the position of the "corpuscle precurseur." This is the (1) "nodule cephalique," which is destined to form the head of the spermatozoon. It seems to be a thickening of the membrane of the nucleus. While the cephalic nodule is being formed, (2) a rod or bar (*m*) appears in the substance of the spermatoblast, which is in connection, by its central end, with the nucleus, and by its distal end with a fine leaf-like strand of plasma, which is situated at the end of the spermatoblast,

next the hollow of the tube and extends into (3) the flagellum or caudal filament (*f*).

The bar forms the middle piece (Mittel Stück, of Schweigger Seidel), while the caudal filament helps to complete the tail of the spermatozoon. The caudal filament would appear to be formed out of the plasma which fills up the hollow of the tubular polyplast. The further changes consist in a growth of the cephalic nodule over the rest of the nucleus in the form of a cap, and the elongation of the middle piece, which becomes freed from the cell body while the caudal filament remains attached to its distal extremity.

The ampulla now consists of a mass of tube-like polyplasts, each with its basilar nucleus, and each built up by a bundle of spermatozoa, whose tails project towards the lumen of the ampulla. As the next step, these are discharged, and now nothing remains except the blastophoral cell. Each blastophoral cell consists of plasma holding a basilar nucleus towards its base, and a body, which arose during the later development of the spermatozoa, placed about the middle of cell, and called by Semper "the problematic body."

Nothing is known about this body. It does not appear to consist of fat, but possibly it is connected with the atrophy of the blastophoral cells and ampulla which now takes place. (See Semper's 'Arbeiten,' 1875.)

We now pass to the description of Spermatogenesis in Mammals, contained in a paper by Dr. George Renson in the 'Archiv de Biologie,' tome iii, fascicule ii, 1882, according to which in its main outlines the process is strikingly similar to what we have before described in other classes.

The paper in question commences with a resumé of the work of previous observers, especially V. Ebner, Sertoli, and V. la Valette St. George. A few pages are devoted to descriptions of methods which are those in ordinary use, and then the author commences the process as seen in the Rat, an animal which all observers acknowledge to be the best for observation.

He first describes the elements which are seen in a prepara-

tion of the teased testis treated with osmic acid, (1) large, rounded cells, with one, two, or three nuclei (fig. 2, A). These are the seminiferous cells of Sertoli. (2) Multinucleated masses (fig. 2, B), to which he applies the word Kysts. The protoplasm is collected round each nucleus, so that they are rather to be regarded as collections of separate cells. (3) Nematocysts (fig. 2, F to J), or cells in which the nucleus has undergone changes to form the head of the spermatozoon. Each is provided with a filament. (4) Large oval nuclei (fig. 2, C), surrounded by masses of hyaline protoplasm, which are really the basal portion of the "cellules de soutien," to be mentioned immediately.

By means of $\frac{1}{3}$ % of alcohol of Ranvier, he found another element, which he regards as extremely worthy of attention. These are large cylindrical cells with expanded bases, containing in the expanded end a large smooth nucleus, and supporting at the other the nematocysts or immature spermatozoa. He identifies these cells as the cellule de soutien of Meckel, and the cellule fixe of Sertoli, as well as part of the spermatoblast of V. Ebner, who applied this latter name to the cell under consideration, taken together with the nematocysts supported on its central end (fig. 2, D and E).

As regards the origin of these cellules de soutien, and their connection with the nematocysts, our author thinks that they arise independently from cells placed next the lining membrane of the seminal tubule, and that they come into connection with the nematocysts as they grow towards the centre of the tubules supporting them, and by their growth pushing them as mature spermatozoa into the lumen. He gives as a reason for the distinctness of the two that very young nematocysts are not found in connection with the cellules de soutien, but, viewing the subject in the light which we have gained from the study of Spermatogenesis in other classes, I think that any one will allow that it is extremely probable that there is a closer connection between them than our author has allowed, and that the "cellules de soutien" are homologous and analogous with the body which has been described in the foregoing papers as

mother cell with basilar nucleus, or mother cell with principal nucleus or sperm-blastophor of my nomenclature.

The author then describes a series of sections showing the relations which the above elements bear to one another.

In a typical section next to the membrana propria are found two kinds of cells, the germinative cells, whose origin is doubtful, and irregular cells, containing a smooth large nucleus, which is the basal portion of the cellule de soutien. The germinative cells (my spermatospores) multiply by division to form the multinucleated masses, which he calls Kysts (my sperm-polyplasts); the nuclei, with a small portion of protoplasm, then become free, constituting the seminiferous cells (my spermatoblasts), and then the nucleus of each seminiferous cell begins to undergo changes to form the spermatozoon, when the term nematocyst is applied to it. When this state is reached, they become attached to and supported on the "cellule de soutien," where they remain till they become mature spermatozoa. After throwing off the spermatozoa, the "cellule de soutien" appears to undergo fatty degeneration in its central part, while its peripheral part remains and forms the "stellate cells," interspersed among the germinative cells, which were described by Sertoli and Meckel.

While these changes have been going on, the germinative cells have given rise to seminiferous cells, which, embedded between the radial projections of the cellules de soutien, are ready for the next crop of spermatozoa, and thus the process is continued.

The origin of the "cellules de soutien" is not given; but if we assume that the germinative cell and the cellule de soutien both arise from the division of a primordial testicular cell (a spermatospore), we can bring these observations into agreement with the generalised process as described by me.

It remains to describe the exact changes which a nematocyst undergoes in becoming a spermatozoon. First, a caudal filament is protruded, and at the pole of the nucleus opposite to this point a thickening of the membrane takes place, near the nucleus in the accessory corpuscle. The nuclear thickening

spreads till it occupies a hemisphere of the nucleus; then a swelling is seen at the pole of the hemisphere, which form the "baton terminal" (Spitzenknopf of Meckel), a formation quite independent of the accessory corpuscle which some observers have believed to have formed this structure. The hemisphere continues to thicken till it forms a hood (capuchin, Kopfkappe), while the caudal filament becomes united to the nucleus by a granule at the opposite pole. The protoplasm of the nematocyst forms a hyaline tube, in which can be seen the caudal filament. The nucleus becomes flattened, the nuclear thickening with the baton terminal disappears, but its lower limit is marked by a circular line, and the mature spermatozoon is formed.

The formation of the spermatozoon, as here briefly described, has many points of similarity with that above mentioned in Selachians. In both there is a body formed (corpuscle precurseur, Selachians, accessory corpuscle, Mammals), which takes no share in the process. In both a caudal filament is protruded, in both a thickening of the nucleus forms the head, and in both there is a consolidation of the plasma, which may be regarded as forming the middle piece; and here let me make a few remarks on this portion of the spermatogenetic process.

It is now well determined that the nucleus forms the head of the spermatozoon, and the plasma of the spermatoblast gives rise to the tail, but does the whole of each take part in the process? It seemed to me in the cases which I selected for study that the head was formed by simple elongation or change of shape of the nucleus, while drawing out of the plasma gave origin to the tail; but it is more than probable that in many cases the process is more complicated than this, and possible that it differs in different animals. The similarity of the process in Selachians and Mammals would seem to suggest that it is conducted on one plan, which plan would seem to be that a portion of the nucleus—possibly the membrane and the chromatin—form the head, while a caudal filament forms the extreme portion of the tail, the intermediate part having origin in solidification or modification of the sub-

stance of the spermatoblast. Further researches on this point are needed, especially with reference to the constitution of a nucleus disclosed by the researches of Flemming.

These remarks would be incomplete without reference to the observations of M. Sabatier, who in 'Comptes rendus,' 94, 1882, pp. 172-3, has recorded his observations on Spermatogenesis in Salmacina (one of the Serpulidæ). In this worm he finds that a spermatospore or mother cell becomes covered by multiplication and budding of nuclei with a mass of clavate cells (protospermatoblasts) which do not themselves give rise to spermatozoa, but become detached, and then, by nuclear multiplication and budding, produce a crop of spermatoblasts (deutospermatoblasts), from which the spermatozoa originate. He uses these observations to explain the process of the formation of testicular ampullæ in the lower Vertebrates, and to complete its comparison with the formation of a Graaffian follicle, as suggested by Balbiani.

According to this latter observer an ampulla is formed by a central cell surrounded by smaller ones. The central cell represents the female part, and takes no share in the formation of spermatozoa, while the smaller ones represent the male part of the primordial indifferent cells. This state of things is reversed in the Graaffian follicle of the ovary, in which the central cell (the female portion) undergoes development at the expense of the surrounding epithelial cells of the follicle representing the male element. This central cell of the ampulla could, on M. Sabatier's view, be represented by the spermatospore, the cells lining the ampulla would be the protospermatoblasts, and the cells which we have called spermatoblasts would be deutospermatoblasts.

While this paper was being written a notice appeared in the 'Zoologische Anzeiger' for the 19th Feb., 1883, referring to researches made by Max v. Brunn on the double form of spermatozoon found in Paludina, to which, in conclusion, I must make a brief reference. He finds that the formation of the head does not take place endogenously and without the nucleus, as stated by M. Duval, but that it is produced as described by

myself in Helix by division of the nucleus. He accounts for the discrepancy in the observations by supposing that the former observer obtained deceptive appearances by a too prolonged action of osmic acid. The object with which his observations were made was to assign some explanation to the striking fact of the presence of two kinds of spermatozoa—the hair-like form and the worm-like form—in Paludina. He found that only the small hair-like form was concerned in the fertilisation of the ovum, in which process the other larger form took no part, and, as far as he was able to determine, this latter had no function at all. Having failed to find any physiological reason for the presence of this form, he was obliged to fall back on morphological explanations, and the hypothesis he offers is that this large form represents a disturbed or arrested development of a spermatozoon; that the cell from which it arises is, as it were, a female cell, and that the testis, as seen in Paludina, is a transition form to the hermaphrodite glands of Pulmonates and Opisthobranchs, in which the distinction between male and female cells is at an early stage impossible.

Note on a Minute Point in the Structure of the Spermatozoon of the Newt.

By

G. F. Dowdeswell, M.A., &c.

The general structure of the spermatozoon of the water Newt (Triton cristalus) has recently been well and accurately described by Dr. H. Gibbes in this Journal.[1] It is to a point therein, which has hitherto escaped notice, that I wish here to call attention.

The spermatozoon consists, as described (loc. cit.) of the "body," to which is attached a fine, narrow, translucent membrane, bordered by what is usually termed "the filament," which takes its origin from what may be called the neck, the upper or thickest extremity of the body. This membrane and "filament" evidently consist of protoplasm, being highly contractile; in the fresh state rhythmical waves of contraction may be seen passing up them, and producing that remarkable appearance of spiral rotation, which in similar cases was often a source of perplexity to microscopists. The "body" also appears to be protoplasmic, both behaving in the same manner towards reagents, but the upper and thickest part, "the neck" —the "elliptical body" mentioned by Gibbes—appears to be of somewhat different constitution, as in some cases it stains much more deeply and readily than the rest of the body. Surmounting this, forming a cap as it were, is a long, finely tapering conical head, which, as already shown (loc. cit.), is of materially different constitution to the other parts, being apparently less stable, swelling up readily when treated with water, and being easily altered and destroyed by other re-

[1] Vol. xix (1879), p. 487.

agents. It stains more readily than the body and membrane, but not so deeply as what I have termed the neck. Towards the extreme end, from tapering very regularly, the head becomes somewhat abruptly more constricted for the last few

Fig. 1.

micromillimeters of its length, and is here, in unstained preparations, more highly refracting than the rest, its substance appears more dense; probably this end portion is solid and the remainder hollow (of which preparations stained with carmine present very much the appearance), and shows a double contour. At the extreme point of this head there is a minute barb (see woodcut, fig. 1). In successful preparations it may be very distinctly seen and readily measured, and this even when unstained. I have already[1] referred to its existence, and on further examination of better preparations do not find it so ultra minute as I at first thought it. It is indeed of very appreciable magnitude, being in breadth about 1·5 mic. m. (0·0015 mm.), and in length 2·0 mic. m. (0·002 mm.), though obviously accurate measurements of such objects are difficult, for to detect the actual termination of an impalpably fine point is not always possible.[2]

Such a determinate and remarkable structure as that here

[1] See this Journal ante, vol. lxxxii, N. S., 1882.

[2] In such measurements I have found great advantage in the use of a cob-web micrometer, admirably constructed by Messrs. Ross, which has the second web, which is usually fixed, movable; this both saves time and promotes accuracy, as in the usual form (with only one web movable) it is almost impossible, by means of the mechanical stage, to bring an object into exact contact with the fixed web, which is done at once with ease and certainty by the second movable one. Having now used this a good deal, I certainly prefer it to any other plan; whatever arrangement is adopted, however, it is necessary to determine the value of the scale of the eye-piece with a stage micrometer, as the least variation in the conditions of the instrument, as e.g. slightly turning the screw collar of the objective, appreciably alters their relations.

described cannot be supposed to exist without some purpose recent researches at once suggest that this is to attach the spermatozoon to, and enable it to penetrate into the ovum in the early stages of fertilisation, as has been shown to occur by Fol and others; and we should expect to find a similar formation in other spermatozoa. In those, however, which I have hitherto examined I have not detected it, and the structure of many, as that of the toad, and of most mammalia, does not appear to admit of its existence.

To prepare the spermatozoa for the examination of this object the first essential is to get them as nearly as possible in contact with the cover-glass and flat upon it; this requires some care, to avoid their drying, by which they are materially altered. They may be preserved by several methods, either by treating for twelve to twenty-four hours with a concentrated solution of picric acid, a dilute solution of chromic acid, by Dr. Klein's method with a 5 per-cent. solution of ammonium chromate, by iodine, by silver nitrate, or by osmic acid or gold chloride; the latter are convenient as being quicker. I have myself most usually employed picric acid. For staining I have found glycerine magenta[1] the best method, as it stains all parts as strongly as desired. To show the general structure alcoholic carminate of ammonia is the most satisfactory, but it does not stain the barb deeply. Other aniline dyes I have not found answer so well. If it be intended to examine the preparation with a homogeneous, or "oil"-immersion objective, it should be mounted in Canada balsam, the objective having, as is generally known, no advantage, and, indeed, being inferior to dry glasses for objects between which and the cover-glass there is either a film of air, or of any fluid the refractive index of which is much different from that of glass.

The use of glycerine as a mounting fluid for preparations stained with any of the aniline dyes is at best trouble-

[1] ℞ Magenta cryst., 1 part; glycerine, 200 parts; alcohol, 150 parts; aq., 150 parts; immerse the preparation in the solution for from two to four minutes according to the depth of colouring required, and then wash.

some,[1] and sooner or later, to my experience, the staining runs, and the preparation is spoiled. Solutions of acetate of potash or chloride of calcium I have not found satisfactory; the form, even of such resistant objects as bacteria, in some cases becoming materially altered by these reagents. With Canada balsam, even when dissolved in chloroform or turpentine, I have not found the preparations fade, as has sometimes been said to be the case, and as we should have expected; nor, if they are sufficiently washed in alcohol and passed through oil of cloves, will they run; the risk, however, both of fading and running may be entirely obviated by using benzine as a solvent for the balsam, or by employing it undiluted and liquified by warmth.

In examining this structure I have employed the $\frac{1}{24}$th homogeneous immersion of Messrs. Powell and Lealand, which having the very high numerical aperture of 1·38° gives, with admirable light and definition, an amplification of about 3400 diameters, with an eye-piece of $\frac{3}{4}$ m. focal length; the barb, however, in a suitable preparation, may be readily seen and examined with a good $\frac{1}{8}$th objective. Even with much lower powers, as e. g. the $\frac{4}{10}$th P. and L. I have recognised it, dependent however much upon the method of illumination employed: for, as is generally recognised, good illumination will show an object with a much lower power than is requisite in the ordinary way. The best means of this as yet available is the direct light of the flame of a paraffin lamp turned edgewise to the observer, whether with or without a substage condenser. This was recommended by Dr. L. Beale thirty years ago, and is now again frequently adopted. Light reflected from any mirror is in some way inferior to direct light, and this not owing to the double reflecting surface of ordinary mirrors, for I have tried them silvered on the upper surface without any material advantage.

[1] The method is, add an equal bulk of glycerine to the aqueous solution of the aniline dye used, stain somewhat more deeply than requisite, mount on slide with cover glass in the staining fluid, which is to be gradually replaced as the water evaporates by plain glycerine.

On the Existence of Spengel's Olfactory Organ and of Paired Genital Ducts in the Pearly Nautilus.

By

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and

A. G. Bourne, B.Sc.

A MALE and a female specimen of Nautilus pompilius were purchased a few years since by one of us for the Zoological Museum of University College, London. Leisure and opportunity for the study of these specimens have recently been afforded, and we propose to briefly report here on two interesting additions to knowledge which our observations have yielded.

The specimens were in an excellent state of preservation for the purpose of dissection, though not fit for histological study. The male was not purchased as such, and its sex was not recognised until it was withdrawn from its shell and the circum-oral tentacular apparatus examined.

With the exception of the specimens reported on by Van der Hoeven no adult males of Nautilus have been carefully examined, that referred to by Keferstein (in Bronn's 'Classen und Ordnungen des Thierreichs,' Weichthiere) being an immature specimen. A description and figure of the circum-oral tentacular apparatus of the male Nautilus and a comparison of this with the corresponding region in the female will form the subject of a memoir by Mr. Bourne in another publication. Here we shall confine ourselves to two points.

Spengel's Olfactory Organ.

The extremely important observations of Spengel on the olfactory organ of Mollusca ('Zeitsch. wiss. Zoologie,' vol. 35) lead to the conclusion that there is very generally if not universally present in the Mollusca an olfactory organ placed near to or in relation with each gill, and that this organ receives its nerve from the "visceral loop" or commissure, which, sometimes short, sometimes long, in some molluscs twisted, in others straight, joins to one another the pair of so-called "visceral" ganglia.

We adopt the name "osphradium" for this molluscan organ of smell, proposed by Professor Lankester in his article "Mollusca," in the 'Encyclopædia Britannica.' The osphradium is thus distinguished by its name from all other organs presumed to have the olfactory function, whether placed on the head, lips, tentacles, or elsewhere. The osphradium of molluscs is Spengel's olfactory organ, it lies near the gill and tests the respiratory medium.

Spengel was unable to find an osphradium in Cephalopoda. He appears not to have had the opportunity of examining Nautilus, where a well-developed pair of osphradia exist, although we have not been able to detect their representatives in Sepia or Octopus.

The osphradia of Nautilus are in the form of a pair of teat-like papillæ placed upon the body-wall of the subpallial chamber, a little to the outer side of the muscular attachments of the anterior pair of gills, one corresponding to the right gill and the other to the left gill (woodcut, figs. 1, 2, *olf.*).

These papillæ have been seen and figured by previous writers (Van der Hoeven, Keferstein), but no suggestion as to their significance has ever been made. We were led to infer from their position that they represented Spengel's olfactory organ, and proceeded to test that hypothesis by an examination, firstly, of their microscopic structure, and secondly, of their nerve-supply.

The inquiry into their microscopic structure was entirely negative. Our specimens were not sufficiently well preserved

to enable us to say whether the epithelium of these papillæ is specially modified or not.

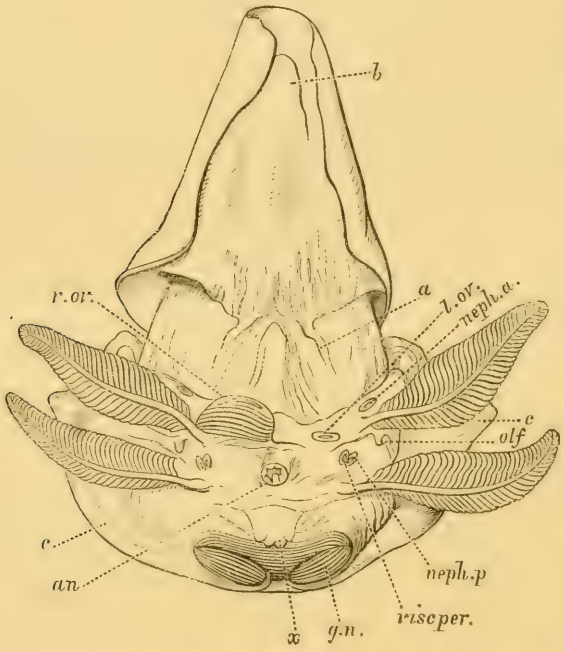

Fig. 1.—View of the postero-ventral surface of the body of a female Nautilus pompilius, as seen when the mantle-skirt is reflected. Drawn from the object by Mr. A. G. Bourne, and reduced to one half the natural diameter. *a*. Muscular band from the foot (siphon) to the body-wall. *b*. Valvular ridge of the siphon. *c*. The reflected border of the mantle-skirt. *an*. Anus. *x*. Peculiar median group of post-anal papillæ of unknown significance. *g. n*. Nidamental gland. *r. ov*. Right oviduct's aperture. *l. ov*. Left oviduct's aperture. *neph. a*. Aperture of the left anterior nephridial sac (in front of the left anterior gill-plume). *neph. p*. Aperture of the left posterior nephridial sac (in front of the left posterior gill-plume). *visc. per*. Left aperture of the viscero-pericardial sac. *olf*. The left osphradium (Spengel's olfactory organ).

With regard to the second test we obtained very satisfactory evidence. The basi-branchial papillæ of Nautilus possess precisely that nerve-supply which is characteristic of the molluscan

osphradium, viz. they are innervated by nerves arising from the visceral commissure.

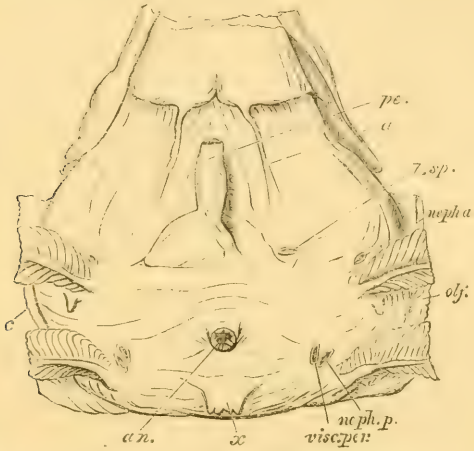

FIG. 2.—View of the postero-ventral surface of the body of a male Nautilus pompilius, as seen when the mantle-skirt is reflected. The gills and the foot are cut short. Drawn from the object by Mr. A. G. Bourne, and reduced to one half the natural diameter. *a*. Muscular band from the foot (siphon) to the body-wall. *c*. The reflected border of the mantle-skirt. *an*. Anus. *x*. Peculiar median group of post-anal papillæ of unknown significance. *pe*. Penis-like opening of the right sperm-duct. *l. sp*. Aperture of the left sperm-duct. *neph. a*., *neph. p*., *visc. per*., and *olf*., as in Fig. 1.

The nervous system of Nautilus is represented in fig. 3. In the Cephalopoda, as in some other forms (most Pteropoda, some Gastropoda), the visceral and pleural ganglia are not separated from one another, but form a continuous nervous band (*pl. visc.*). Nerves to the mantle (*m.*) proceed from the pleural portion of this transverse band, whilst a large visceral nerve (*n. visc.*) proceeds from each of the contiguous visceral portions of the same band, which represent the visceral ganglia. These large visceral nerves give off each a genital ganglion (*gen.*) in the neighbourhood of the genital ducts, and then, taking a superficial course, divide each into two branches, one supplying the anterior the other the posterior branchia of its side.

From the portion of the nerve between the anterior and posterior branchial nerve is given off the nerve to the olfactory

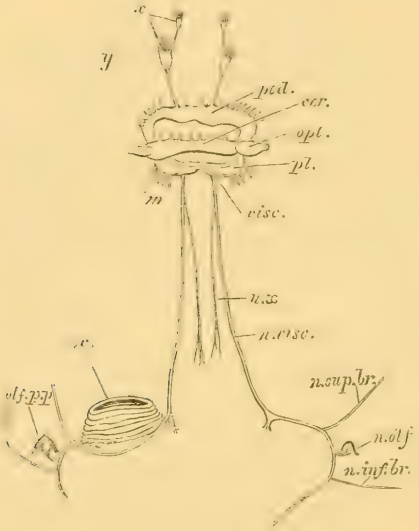

Fig. 3.—Diagram of the nervous system of Nautilus pompilius. Drawn from the object by Mr. A. G. Bourne. *cer.* Cerebral ganglion. *ped.* Pedal ganglion. *opt.* Optic ganglion (resting on the cerebral). *pl.* Pleural ganglion. *visc.* Visceral ganglion. *x* and *y*. Ganglion-like enlargements on nerves passing from the pedal ganglion to the infero-median lobe of the inner circlet of circum-oral tentacular lobes. *m.* Nerves from the pleural ganglion to the mantle. *n. visc.* The genito-branchial nerve, or chief visceral nerve (of the left side). *n. x.* Nerve accompanying the vena cava which lies between this and the similar nerve of the right side. *gen.* The left genital ganglion. *n. sup. br.* Anterior branchial nerve. *n. inf. br.* Posterior branchial nerve. *n. olf.* Olfactory nerve entering the left osphradium. *ov.* The oviduct (right side). *olf. p. p.* The right osphradium.

papilla or osphradium. This nerve supply is closely paralleled in such Gastropoda as Haliotis.

An examination of Octopus showed that in that Cephalopod a similar distribution of visceral nerves obtains, but the branch on each side corresponding to that which supplies the olfactory papilla in Nautilus simply ramifies beneath the skin, there

being no papilla or prominence of any kind in Octopus corresponding to the osphradium of Nautilus.

The paired Oviducts and Sperm-ducts.

By all previous writers (Owen, Valenciennes, Van der Hoeven, Huxley, Keferstein, Woodward, Macdonald) the genital ducts of Nautilus have been described as unpaired. A single oviduct is said to be present in the female, and a single sperm-duct ending in a penis-like process in the male. Jhering, in an article in the 'Zeitschrift für wiss. Zoologie,' vol. xxix, p. 589, in which an attempt is made to discuss the homologies of the excretory organs and genital ducts of Mollusca, says: "We are prevented from assuming that the relations (of these two sets of organs) is the same in the Cephalopoda as in the Lamellibranchia, were we otherwise inclined so to do, by the fact that in Nautilus only a single oviduct exists. Until we have the developmental history of Nautilus before us, there is no prospect of any progress in this direction."

Without endorsing Jhering's statements in any way upon other points, we may point out that, without any reference to the developmental history of Nautilus, the supposed fact that in Nautilus only a single oviduct exists is demonstrated to be no fact at all.

In the female Nautilus (fig. 1) we find on the postero-ventral wall of the body overhung by the mantle-skirt no less than nine apertures. The central aperture is the anus (*an.*). The two pairs right and left of this, just in front of the hinder gill-plumes, are the two openings of the great viscero-pericardial chamber (*visc. per.*) and of the two posterior nephridial sacs (*neph. p.*) respectively. Right and left, in front of the anterior gill-plumes, we find the pair of apertures appropriate to the anterior nephridia (*neph. a.*). Nearer the middle line is placed on the right side the great oviducal aperture, with plaited lips (*r. ov.*), and at the corresponding point left of the middle line is placed the aperture, which we have marked *l. ov.*

It is curious that this aperture has been overlooked by every student of the Nautilus excepting Keferstein. At the same time Keferstein failed to apprehend its true significance.

Keferstein showed that the aperture (*l. ov.*) leads by a duct into the "pyriform appendage," originally described by Owen as lying in close connection with the ventricle of the heart. The nature of this pyriform appendage neither Owen nor any subsequent observer was able to divine. Keferstein first showed that it communicates with the exterior by means of the aperture (*l. ov.*), discovered by him.

But this aperture is the exact left-hand representative of the large oviducal aperture (*r. ov.*). This suggested to us the inquiry as to whether the relations of the "pyriform appendage" are such as to favour the supposition that it is a rudimentary left oviduct. We find that they are; and we conclude that in Nautilus the left oviduct is reduced to a rudimentary condition, becomes constricted at the point where it joins the ovary, and so ends blindly as "the pyriform appendage," whilst still opening to the exterior by the left genital pore (*l. ov.*). The relation of the right and left oviducts to the ovary, to the ventricle of the heart, and to one another, is shown in the diagram (fig. 4.) Whether the ovary is a medium structure, or whether, on the other hand, the pyriform appendage represents an aborted ovary, as well as a rudimentary blindly ending oviduct, is a matter for further inquiry.

Our conclusion as to the nature of the aperture (*l. ov.*), and the pyriform appendage of the female, was greatly strengthened by our discovery of a precisely similar disposition of parts in the male.

The same number of apertures is present in the male Nautilus (fig. 2) as in the female. Instead of the right oviduct with plaited mouth, we have a right sperm-duct produced into a large penis-like structure (*pe.*). The aperture marked *l. sp.* in fig. 2 has not hitherto been noticed, nor has the pyriform appendage been observed hitherto in a male Nautilus. We find that, just as in the female, the left aperture (*l. sp.*) leads into a "pyriform appendage," which ends blindly. As

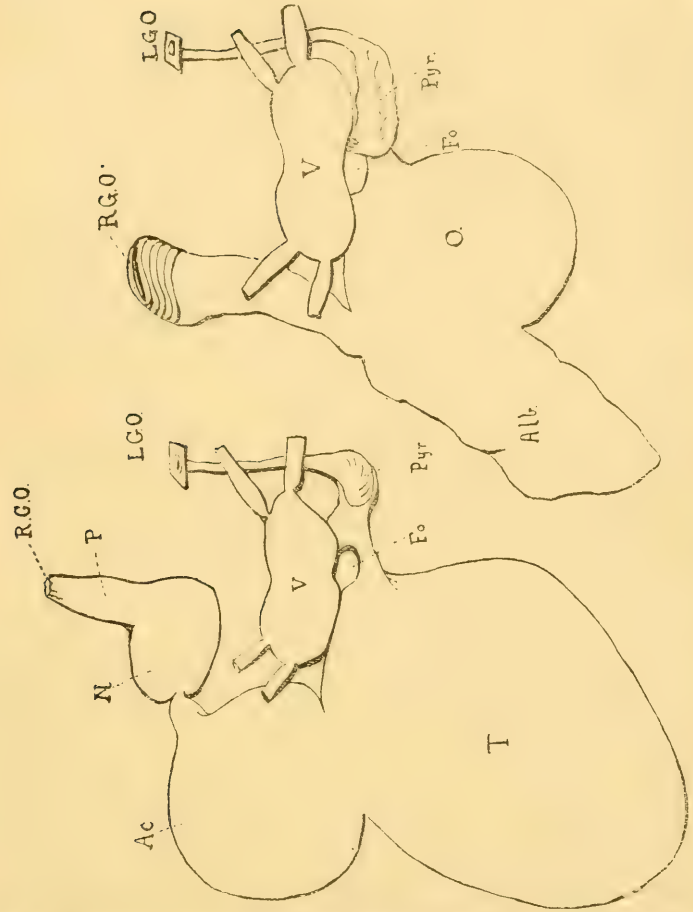

Fig. 4.—Diagrams of the male and female generative organs of the Pearly Nautilus, to show the relation of the rudimentary duct of the left side to the testis and ovary respectively, and of the cardiac ventricle to the organs of both sides. Drawn by Mr. A. G. Bourne. *T.* Testis. *O.* Ovary. *Ac.* Accessory gland of the male apparatus. *Alb.* Albuminiparous gland of the female apparatus. *N.* Needham's sac in the male, in which the spermatophores are formed. *P.* Penis (in the male). *R. G. O.* Right genital orifice. *L. G. O.* Left genital orifice. *Pyr.* Owen's pyriform appendage, attached by membrane to *V.*, the cardiac ventricle with its four branchial veins, and also to the testis or the ovary. *Fo.* Foramen in the membrane, which attaches the pyriform appendage to the ventricle and to the testis or ovary. The foramen places two portions of the viscero-pericardial sac in free communication with one another.

shown in the diagram (fig. 4), the relations of these parts in the male is (as in the female) such as to leave little doubt that we have in them a rudimentary condition of the left spermatic duct and its external opening. Whether the pyriform appendage in any way represents also a rudimentary testis, must remain for the present uncertain.

The significance of the occurrence of paired genital ducts in Nautilus is not so great as it would be were it not the fact that both in the Octopoda and in Ommastrephes among Decapoda, the female genital ducts are paired, and both equally well developed, although the sperm-duct of the males is (with the solitary exception of Eledone moschata, recorded by Keferstein) single.

The single oviduct of those Cephalopoda which have but one, and the single sperm-duct also, is, in all cases, that of the left side; whilst in Nautilus the right oviduct and the right spermduct is large and functional, the left being that which is rudimentary.

Nevertheless, on account of its archaic character, and of the great significance of the primitive Cephalopod structure in relation to the morphology of Mollusca generally, any divergence in Nautilus from the condition obtaining in other forms has possibly and even probably a special significance. Such divergence may be the remnant of an ancestral condition, and in so archaic a form as Nautilus, is not readily to be dismissed as "an adaptation" peculiar to that form.

Hence the doubt which might have arisen through Nautilus as to the primitive arrangement of the molluscan genital ducts is removed. They are now shown to be paired ducts, as in Chiton, and as in Lamellibranchs.

On the Ancestral Form of the Chordata.

By

A. A. W. Hubrecht,

Professor of Zoology at the University of Utrecht.

With Plate XXIII.

An all important question was raised in biology when the Law of Development came to be recognised as the only true explanation of the facts as they lie before us. This was the question: "From what invertebrate stock are the Vertebrata evolved, and which amongst the Invertebrates at present living approaches most closely in its organisation to this primitive parent stock." In 1868 the solution appeared to have been found when Kowalevsky's splendid researches concerning the development both of Amphioxus and of the Ascidians could be compared side by side. The Tunicate larva was for the time being proclaimed to be the missing link, to be of all Invertebrates the closest approach to the much-looked-for parent form.

Since then the aspect of things has changed and later investigations, more especially those of Dohrn and of Ray Lankester, have rendered it nearly certain that the Tunicata must, on the contrary, be looked upon as degenerate Vertebrates which can be hardly of much use in helping us to the failing clue.

Dohrn, Semper, Hatschek, Leydig, Kleinenberg, and Eisig are amongst the foremost who have suggested, and most brilliantly expounded and argued, that the Annelids offer the greatest number of points of resemblance with the Vertebrates; that they and the Arthropods have descended together with the Vertebrata from a primitive type, distantly agreeing in

shape with Polygordius, and that the only postulate which this assumption necessarily implies, is the old idea of Geoffroy St. Hilaire that the ventral side of Annelids and Arthropods is homologous with the dorsal side of the Vertebrata.

These naturalists explain the difference in situation of the mouth and œsophagus, with respect to the cerebral ganglion, by divers subtle hypotheses, which, however, generally disagree with each other. Their views are nevertheless rapidly gaining ground, although the school of Gegenbaur and Haeckel has never been reconciled to them. Gegenbaur looks upon two lateral cords such as are present in Nemertines as a very primitive arrangement, from whence might at any rate be derived the ventral nerve-cord of Annelids and Arthropods. Harting ('Leerboek der Dierkunde,' 1874) was inclined to accept the possibility of a similar dorsal coalescence out of which a spinal cord might take origin. Balfour and myself felt strongly inclined to choose this side in the contest—he in once more tracing the outlines of a similar explanation ('Development of Elasmobranch Fishes'), I in recapitulating the facts, such as they had made themselves known to me in the organisation of certain Nemertines, in which, indeed, a tendency towards approximation of the lateral cords on the dorsal side was unmistakable ('Verh. der Kon. Akad. van Wetenschappen,' Amsterdam, 1880). When Balfour, in the second volume of his 'Comparative Embryology,' made himself a definite advocate of this view in opposition to the combatants for the Annelidan affinities, it may be safely inferred that many of the younger naturalists paused to reconsider the claims of both hypotheses.

The great difficulty which is encountered in any attempt to point out a definite group amongst invertebrates most closely related to the primitive Vertebrata is the total absence of anything resembling so important and so early-formed an organ as the Vertebrate Chorda dorsalis. Attempts to find anything like it amongst the Annelids, even amongst Polygordius and its archaic allies have proved either futile or barren.

I will at present attempt to point out in what group of

invertebrate animals we do indeed find an organ which, in my estimation, ranks equal to the vertebrate notochord, and thus supplies the much-desired transitional form by which the Chordata are allied to the lower Metazoa, and in fact to such forms as have neither the much specialised organisation of the segmented animals (Arthropods and Annelids), nor require to be turned upside down before their homology with the lowest Vertebrata is admissible.

That I venture to state this hypothesis before I am able to bring into the field a well-arranged host of facts in its support, must be ascribed to my desire to induce such fellow-workers in biology as have more leisure and better occasion for investigating the numerous problems it suggests than I have, into taking up a question which cannot be but looked upon as of the highest significance for modern morphology.

According to my opinion the proboscis of the Nemerteans, which arises as an invaginable structure (entirely derived, both phylo- and ontogenetically, from the epiblast), and which passes through a part of the cerebral ganglion, is homologous with the rudimentary organ which is found in the whole series of Vertebrates without exception—the hypophysis cerebri. The proboscidian sheath of the Nemerteans is comparable in situation (and development?) with the chorda dorsalis of Vertebrates.

After this brief statement of my hypothesis I will enter into a short discussion of its different details.

It is not my intention to consider the numerous modifications of structure which the hypophysis cerebri presents in different adult Vertebrates, nor its glandular appearance, the connection into which it enters with blood-vessels, &c.; but I wish to restrict myself to the comparison of its very first ontogenetic stages, in which it may be presumed most purely to reproduce its ancestral character.

We find the hypophysis originating as an invagination from the epiblast, arising either independently on the outer surface

(this, according to Dohrn's interesting researches, is the case in one of the lowest of the vertebrate scale—Petromyzon), this invagination at the same time being directed towards the anterior termination of the notochord, and lying in its direct continuation (figs. 1 and 2), or (as is the case in the higher Vertebrates) not directly on the outer surface, but on that portion of the epiblast which has become the stomodæum (fig. 6). In the latter case it arises as a median dorsal outgrowth from the mouth-cavity, directed towards that portion of the under surface of the brain where, between Prosencephalon and Metencephalon, the infundibulum travels downwards, this being at the same time the limit up to which the notochord extends forwards under the brain. The fact that an outgrowth from the brain thus grows downwards to meet this epiblastic invagination sufficiently indicates that in ancestral generations, where the hypophysis was a less rudimentary organ, some sort of connection existed between it and the cerebral thickening of the central nervous system.

The constant presence in all Vertebrates of an organ so rudimentary as the hypophysis, and about the significance of which no plausible explanation has as yet been offered, has already been insisted upon above.

Both facts are in favour of regarding it as a very ancient structure, which was once of great importance, and had a different and at the same time a more definite physiological value.

In tracing this ancestral significance, the relation to the brain and the somewhat less direct but, nevertheless, unmistakable relation to the notochord must not be lost sight of.

We will now consider the ontogenetic and phylogenetic history of the Nemertine proboscis. In the lower Platyelminths the researches of v. Graff, lately crowned by his brilliant monograph, have brought to light the different stages through which retractility of a portion of the tactile anterior extremity of the body, in which urticating elements are present, leads to the appearance of a definite proboscidian structure, which

obtains a special musculature, and which finally (in Graff's Rhabdocoel family of the Proboscida) has definitely become a proboscis, which is directly comparable to that of Nemertines, situated like it above the intestine, internally (externally when everted) clothed by the direct continuation of the outer layer of epiblast, and serving tactile and at the same time—through its nematocysts—aggressive purposes.

The proboscis of Nemertines is thus directly related to this important structure of the lower flat-worms, as was already noticed in Gegenbaur's 'Grundzüge' (1870). We find urticating elements largely developed in the proboscidian coating of Palæo- and Schizonemertini, whereas in the Hoplonemertini the tactile significance may perhaps have come to predominate, if we judge from the extremely complicate arrangement and massive development of nervous tissue in the proboscis of these forms, which, moreover, is here provided with a central stylet-shaped armature.

As to the ontogenetic development of the Nemertean proboscis, the great majority of authorities are in accordance that it develops as an invagination from the epiblast, which commences at the anterior extremity and gradually pushes backwards. Extensive details as to its successive developmental stages are, however, not yet to hand, only the principal fact above mentioned being generally accepted.

It is highly important to notice that in this backward course the proboscis takes its way between the two anterior thickenings of the lateral nerve-cords, which in Carinella constitute the simplest Nemertean brain, and in other genera become more or less subdivided, the right and left halves being united by a thick commissure (fig. 3), ventral in relation to the proboscis, and by a thinner one dorsal to it. In all cases the proboscis passes through the ring of nerve tissue thus formed; in all cases the proboscidian sheath reaches forwards to the level of this nervous commissure, through which the proboscis passes (fig. 4).

If we may look upon the spinal cord and brain of vertebrates as a dorsal coalescence of lateral trunks similar to those of the

Nemertines (as was already advocated in my paper "zur Anatomie und Physiologie des Nervensystems der Nemertinen," Amsterdam, 1880), then the double proposition above enunciated necessarily leads to the conclusion that the spot just mentioned corresponds to that part of the vertebrate brain where the hypophysis (proboscis) bends upwards towards the central nervous apparatus and where the notochord (proboscidian sheath) terminates, i.e. the region of the primitive fore-brain. This proposition at the same time implies the homology between the vertebrate fore-brain and part of the nervous lobes of Platyelminth ancestors.

It remains to be further inquired into—and the facts as they lie before us are very suggestive in this direction—whether perhaps the distinction between the two pair of lobes as they are present in most Nemertines may not have been perpetuated in the vertebrates, these superior lobes (after dorsal coalescence of the two halves of the nervous system) becoming the fore-brain, the inferior ones the equivalents of mid- and hind-brain. The following two points are in favour of such an interpretation: (1) the nerves for the higher sense organs, eyes,[1] and olfactory (?) pits start from the superior brain lobes; (2) the strong nerve which on both sides supplies the anterior (respiratory, M'Intosh!) region of the œsophagus, and for which in a former paper I have proposed the name of N. vagus, takes its origin in the inferior lobes (figs. 3 and 5).

Upon the dorso-median coalescence of these inferior lobes and of the lateral stems, above the intestine and the proboscidian sheath, the latter must have become severed anteriorly from its connection with both nerve-system and proboscis. Might not the fact of the anterior end of the notochord being bent upwards in several of the lower Elasmobranchs (cf.

[1] It is of course understood that the ectodermal eyes of Nemertines are not directly comparable to the myelonic vertebrate eye. However, it is important that Graff has already succeeded in demonstrating true cerebral eyes in other Platyelminths ('Monogr. der Turbellarien')!

Gegenbaur, 'Das Kopfskelet der Selachier,' pl. ix, figs. 1 and 2) be interpreted as a reminiscence of this connection?

A further character which is common to the two epiblastic invaginations, known as hypophysis and as proboscis respectively, is the shifting of their external opening. Amongst Nemertines examples are found which form a parallel to the larger bulk of Vertebrates (fig. 6) where the hypophysis does not arise (as in Petromyzon) independently on the outer surface, but where it is an invagination directed upwards from the roof of the mouth cavity. Both in Malacobdella and in Akrostomum (a genus of Hoplonemertini, instituted by Grube, in which I place, for example, M'Intosh's Amphiporus bioculatus and Amph. hastatus, and of which I have myself examined several specimens) the opening for the proboscis is not independently situated at the anterior extremity, but is found on the dorsal wall of the intestinal tract, just inside the mouth (figs. 7—10). I have the strongest reason to believe, upon which I will not here further enter, that this is a secondary modification, and that the separate opening is the original state of things, phylogenetically related to the separate proboscis of certain Rhabdocoels.

The facts here advanced may justify us in looking upon the Platyelminth (*s. str.* Nemertean) proboscis as the homologue of the vertebrate hypophysis, as was implied in the first part of our proposition.

The proboscidian sheath in Nemertines is a cavity closed on all sides and lined by an epithelium. It is situated in the median dorsal line, above the intestine, just inside the muscular body wall, to which it is more or less firmly attached. Muscular fibres serve to a large extent towards the thickening of the tube we are considering.

It terminates in the immediate vicinity of the anus, and reaches forwards to just in front of the cerebral ganglia, which in Schizo- and Palæonemertini are situated at a short distance in front of the ventrally situated mouth. In the Hoplonemertini

the mouth has travelled forwards till close to the tip of the head, the intestinal tract thus passing beyond the proboscidian sheath anteriorly. In certain other Nemertines the proboscidian cavity does not extend through the whole length of the body posteriorly. This is, for example, the case in that genus which must be regarded for several reasons as the least differentiated, primitive type—the genus Carinella. It is only in the anterior region of the body that the proboscis and the cavity surrounding it are found, the latter situated as usual above the intestine. Here, too, the mouth is found ventrally, the opening for the proboscis terminally. One other genus—Drepanophorus—deserves special mention, in so far as the proboscidian sheath has the bulk of its cavity increased by lateral thin-walled sacs, metamerically placed, one above each lateral cæcum of the intestine, and communicating with the cavity of the sheath by narrow perforations of the muscular tissue of its wall. Nemertes carcinophila is said both by M'Intosh and Barrois to be without a special proboscidian sheath. Barrois found the proboscis much reduced (according to him as an effect of parasitism) and floating in the general body-cavity. Not having examined this species myself, and not having either ever met with a general body-cavity in other Nemertines, I would venture to suggest the necessity of a careful re-examination of this species, which might prove to be not without importance for the prôblem we are considering.

The type according to which the proboscidian sheath is built up is very similar throughout the whole group, although the muscular elements in its wall may increase in number (fig, 16) and become more complicately arranged, or its size may be considerably reduced. It is capable of a very considerable increase in width corresponding to the movements, the rapid retraction, or the mode of coiling up of the proboscis it encloses. It is, moreover, filled with a fluid, containing corpuscles characteristic in shape, and in one case—Cerebratulus urticans—characteristic in chemical properties, viz. by the presence of hæmoglobin. This fluid is in no way connected with the fluid circulating in the longitudinal and transverse blood-vessels.

The dorsal blood-vessel takes its course beneath the proboscidian sheath, between it and the intestine; in many instances it is enclosed in the muscular wall of the sheath in the foremost part of the body, above the œsophagus. The possibility of a comparison with the subnotochordal rod of Vertebrates ought to be considered.

The inner epithelium lining the cavity of the sheath is very marked and everywhere present; it is least conspicuous in Carinella, owing perhaps to the considerable extension which the sheath had undergone in all specimens that have hitherto been examined in view to this point.

This being the general arrangement of the proboscidian sheath, it now concerns us to examine what is known about its development in the embryo. The data available are very scanty, and in some respects contradictory. Barrois describes it in certain species of Lineus as developing from the reticulum, the mesoblastic tissue between the epi- and hypoblast, and gradually extending backwards at the same rate as the developing proboscis pushes it in that direction. In Amphiporus the development of the proboscidian sheath was studied by the same observer, and according to his description there is a remarkable divergence from the development in Lineus.

In Amphiporus the proboscidian sheath is not formed gradually, travelling slowly backwards along the median dorsal line, but the sheath suddenly appears all round the whole length of the proboscis. It is here formed out of the fatty mass, which also gives rise to the digestive tube.

Tetrastemma, another Hoplonemertine, corresponds closely, according to the same observer, with Amphiporus just described.

Salensky, who has lately given a very short account ('Biologisches Centralblatt,' 1883) of the development of Nemertes (Borlasia) vivipara, ascribes a mesoblastic origin to the proboscidian sheath. Nevertheless, he noticed what appeared to him to be a connection between the first origin (Anlage) of the œsophagus and that of the proboscis. As he

has postponed giving the details of this connection to a later publication we cannot at present judge of its significance.

Hoffman is the only other author who gives any details about the formation of the proboscidian sheath. According to his account of sections made of Tetrastemma, a portion of the proboscis is split off from the dorsal surface of the alimentary canal. The muscular proboscidian sheath is mesoblastic in origin. This observation, which can hardly be brought to agree with the epiblastic origin of the proboscis, noticed above, might perhaps allow of a different interpretation. As a simple suggestion I would advance, that perhaps Hoffmann may have mistaken the formation of the inner portion of the proboscidian sheath (so often confused with the proboscis !) for that of a part of the proboscis itself.

Hypoblastic formative elements internally would then coalesce with mesoblastic derivates, more especially muscular elements, exteriorly applied to the former and constituting together the proboscidian sheath, i.e. the wall of the proboscidian chamber.

Such an interpretation would appear to be more acceptable than the coalescence of a tubiform derivate of the hypoblast, with an invagination of the epiblast travelling backwards, the fusion of these two giving rise to the definite, cylindrical, eversible proboscis. Balfour, in his 'Comparative Embryology,' is not inclined to accept Hoffmann's statements without further confirmation.

Still this observation, if it may be interpreted as proposed, would be of importance and its repetition much to be desired. This and Barrois' description above cited appear to open the prospect that embryology may eventually succeed in demonstrating for the proboscidian sheath, or for one of its constituent parts, a hypoblastic origin.

If such be proved to be the case, not only its situations but also its development would correspond to that of the notochord of the lower Vertebrates. Still, considering in how many cases the origin of the notochord in Vertebrates is apparently mesoblastic (this phenomenon being

considered as secondary, the hypoblastic origin as the primary, or ancestral arrangement), it cannot be considered as absolutely necessary, that in the other offshoot, the Nemertines, the hypoblastic origin of the proboscidian sheath should first be demonstrated before any homology between notochord and proboscidian sheath may be accepted. In Nemertines as well as in Vertebrata the mesoblastic origin of the proboscidian sheath might be a secondary condition. In that case much value could not be attributed to special cases of coincidence in the embryological data, and it would be the most primitive representatives of both groups which would more especially be likely to furnish evidence of a conclusive character. However, we must here wait for more circumstantial evidence before being justified in further advancing in the domain of speculation. Yet, with respect to this question, it must not be overlooked that the fact of the cavity of the proboscidian sheath carrying peculiar corpuscles, and of its being a closed sac lined by an epithelium, tends far to give it the general character of a body-cavity, a cœlomic diverticulum, which is indeed situated dorsally and longitudinally, but which by those general characters would lead us to expect a derivation from the archenteron, rather than a schizocœlic origin in mesoblastic tissues.

If we were inclined to accept the mesoblastic derivation as primary, and wished to picture to ourselves a possible common origin of notochord and proboscidian sheath in the common ancestor of Vertebrates and Nemertines, we should have to postulate as a still more primitive arrangement an axial thickening of the mesoblastic tissues, which became more solid in the one and hollowed out by the proboscis in the other. This would clash both with the hypoblastic origin of the vertebrate notochord and with the phylogenetic significance of the hypophysis.

We have now to consider certain aspects of the suggested homology between notochord and proboscidian sheath.

There is no doubt that the fully developed notochord of a Vertebrate is a structure of an entirely different character from the proboscidian sheath of a Nemertean. The one is a solid,

rod-like organ, the other a hollow tube. However, at the very earliest stages of its formation the notochord of the primitive vertebrates (cf. Hatchek's 'Development of Amphioxus') possesses a central groove, which is a derivate of the archenteron, and which only secondarily, in accordance with the further differentiation of the tissues of the notochord, obliterates.[1]

The ulterior difference in histological structure of the one, a cellular tissue eminently vacuolar, and of the other: the cellular lining and fluid contents of a tube, the cavity of which does, as a rule, not obliterate, is, however, no serious objection to their eventual homology. In more than one instance modern morphology recognises solid cellular strings to be homologous with others containing a cavity inside them.

The different degree in which muscular tissue takes part in the constitution of the proboscidian sheath (figs. 16—18) must of course not be overlooked, the more so as it is entirely absent in the notochord and its envelopes. However, in Nemertines this muscular tissue can be shown to be most closely related to the function of the proboscis, and in fact to be sometimes exceedingly reduced. For this reason its importance as a point of comparison must not be over-rated.

All these differences are in the last instance due to the different significance in the animal economy which has in the two groups been attained by this organ. In the Vertebrates this central rod-like structure, sustaining the mesoblastic somites in their progressive development, has a significance as a temporary axis, round which these processes take place. Its important character as a primitive, i.e. as an ancestral organ, is recognised notwithstanding, or rather just because of, its gradual disappearance in the adult forms of the higher groups, where its

[1] I must not omit to call attention to certain papers in the volume for 1882 of the 'Archiv für Anatomie und Physiologie.' They came into my hands after the completion of my MS. The one is by Lieberkühn, "Ueber die Chorda der Säugethieren;" the other by Braun, "Entwickelungsvorgänge am Schwanzende der Säugethieren." Both naturalists have succeeded in demonstrating that in different regions of the body the notochord is at first a hollow, tubiform structure (fig. 11). Braun found the same in birds. Kölliker, Strahl, and others have lately come to similar results!

significance as a sustaining axis has been replaced by that of the vertebral column.

That in a far distant ancestor of the vertebrates it may similarly have been subservient to the lodging of a retractile proboscis, tactile in function, appears to me to follow from a careful consideration of the relations between the hypophysis and the notochord, and between the first-named rudimentary organ and the brain.

An important fact, which in conclusion I must make mention of, is a phenomenon which I have repeatedly observed in the posterior portion of the proboscidian sheath of different species of Cerebratulus, very long Nemertines, where the sheath reaches down to the posterior extremity of the body. Whereas in young specimens of this species the sheath was a hollow tube down to the very end, in older and larger specimens the aspect of things had changed. In the posterior extremity of the body the cavity was here nearly filled up by a continuous cellular tissue with distinct nuclei (fig. 18), sometimes even entirely obliterated. This cellular tissue is sometimes apparently glandular, the arrangement in some cases even such that it must be interpreted as a set of radial acini, by which the surface is considerably enlarged. Future investigations will have to decide whether the cases of evident obliteration may be interpreted as a step towards real solidification of that part of the tube which is comparatively of the smallest value for the general function in connection with the expulsion of the proboscis. This change of function and histological appearance is only present in the more primitively organised groups, which rarely make use of their proboscis; in the more highly specialised Hoplonemertini, where the proboscis is in constant play, and the development of the muscular elements in the proboscidian sheath vary considerably, it was nowhere found.

Apart from the argument which can be derived from the nature of this cellular coating the significance of this phenomenon will have to be carefully inquired into. Along such a line of development we might picture to ourselves the eventual

conversion of a hollow proboscidian sheath into a solid notochord, the more so as functionally the proboscidian sheath in Nemertines may already be looked upon as an axis, around which the other organs symmetrically arrange themselves as they do around the notochord in Vertebrates. It must at the same time be borne in mind that the muscular coating in this posterior portion is found to be considerably reduced and replaced by a more or less homogeneous and comparatively thin sheath.

Having thus far considered the arguments which are at present available for insisting upon the homology between proboscis and hypophysis, on the one hand, and between proboscidian sheath and notochord on the other, it now remains to inquire whether there are points in the anatomy of Nemertines, beyond those just now exposed, which either corroborate or weaken the evidence hitherto advanced in favour of the suggestion that the Nemertines, more than any other known group of Invertebrates, resemble the ancestors of the Protochordata.

I need hardly insist upon the fact that I do not advocate any direct relation between existing Nemertines and existing Vertebrates; my argument goes no further than the attempt to show that the general plan of structure of a Nemertine is more in accordance with that of a vertebrate animal than is, for example, that of the Archiannelida, and that the link connecting Cœlenterate ancestors with Vertebrate descendants has most probably comprised forms in which two lateral nerve-cords were present, ultimately coalescing dorsally, and in which an epiblastic proboscis served for purposes which have either been given up or have been replaced by others when the animals gradually exchanged the Platyelminth for the Chordate type.

Simultaneously with this passage from the Cœlenterate type to the Chordate the highly important processes must have been gone through which lead to the formation of a body-cavity, separate from the archenteron with which, as embryology teaches us, certain diverticula are originally in open communication, ultimately becoming constricted off and developing into the splanchnic and somatic layers, which have the body-cavity between them.

The brilliant researches of Lang on Gunda segmentata, and of Hatschek on the 'Development of the Amphioxus,' must here in the first instance guide us; and anybody having carefully perused those important contributions, and having compared them with each other, must have been struck by the great probability of the view advocated by Lang, that the alimentary diverticula of these Platyelminths are the fore-runners of the arrangement of the cœlom in the higher enterocœlous worms, and that through this link a glimpse is gained at the road along which Annelids may have developed out of an ancestral Platyelminth stock.[1]

On the other hand, the stages in the development of Amphioxus, where a double set of lateral diverticula of the archenteron is present (fig. 12), which ultimately become converted into the mesoblastic somites, appear to be of very great importance, in so far as they render it highly probable that in the ancestry of vertebrates certain forms with metamerically placed alimentary cæca must have obtained, of which the larval stage of Amphioxus is the reminiscence. In the remaining vertebrates the primitive alimentary diverticula giving rise to the cœlom are reduced to two. This appears to be an ulterior simplification. An attempt to explain this simplification, and to bring the process of the formation of the cœlom in Amphioxus under the same head with that in the other vertebrates, has at the present moment not yet been made. It is, however, sure to be made some day by the leading authorities on the subject. For the present it may suffice to point out that the ulterior development of the mesoblastic somites in the bulk of the vertebrates re-establishes the homology with the more primitive processes in Amphioxus.

For us this larval stage of Amphioxus is all the more interesting, because it must lead up to Platyelminths, corresponding with Gunda in the presence of alimentary cæca, metame-

[1] It must here be noticed that Lang has only very lately ('Biologisches Centralblatt,' May, 1883) emitted serious doubts concerning his own propositions. It remains to be seen whether future investigations will not tend to confirm his original suggestive hypothesis rather than these doubts.

rically placed and of a general internal metamerisation, but differing from Gunda in such important respects as the presence of the forerunners, both of the hypophysis and of the notochord, two structures no trace of which is found in the salt-water Triclades. Such Platyelminths must needs have resembled the present Nemertines more than anything else.

Here the important question at once thrusts itself upon us: Has the formation of a cœlom already been arrived at in the Nemertines or not? i.e. have these animals a body-cavity developed out of and separated from the primitive digestive tract or not? Although I have formerly, when attempts were made to bring the Nemertines under the so-called Parenchymatous Flat-worms, combated those attempts, and endeavoured to show that the regular arrangement of digestive and generative cæca, the development of muscular septa between them, &c., went contrary to it, yet now that our ideas about the significance of a true body-cavity as an ultimate derivate of the archenteron have of late years gained so considerably in clearness and definition, I should hesitate to affirm that any such body-cavity is present in Nemertines, and would be inclined to answer the question proposed above negatively.

Both in the more highly differentiated Hoplonemertini and in the more primitive Schizo- and Palæonemertini, I have met with numerous instances in which all the space which remained free between the muscular body-wall on the one hand, and the intestinal, generative, proboscidian, and circulatory cavities on the other, was one unbroken mass of connective tissue.

Sometimes, more especially around the œsophagus, occurred what were apparently fissures and cavities in this tissue. They were not lined by an epithelium (are perhaps in communication with the vascular system?), and could best be compared to a true Schizocœlom (Huxley), i.e. fissures in a mesoblastic tissue.

All this makes me very much inclined to look upon the alimentary diverticula of the Nemertines in the same light as

Lang does upon those of Gunda; incipient cœlomic sacs, comparable to those of the larval stage of Amphioxus.

A question very difficult to answer is this: How do these alimentary diverticula eventually come to exchange their function and significance to such an extent? If they were originally acquired with a view to an enlargement of the digestive surface, they must in the course of time, as they became constricted off, have lost this function, and in its stead have developed powerful layers of epithelial muscular tissue in their walls, which then represent the successive myomeres, and which finally supplant the original muscular body-wall (Hautmuskelschlauch), itself never divided into myomeres, and originally derived from the epiblast.

Traces of this epiblastic muscular sheath, primitively enveloping the myomeres, which secondarily spring from the alimentary diverticula, appear to be found in certain Vertebrates, externally to their general musculature.

It remains for the present unsolved what were the leading factors in this important transformation, the general outlines of which we have here only touched upon.

We have now to compare Nemertines and primitive Vertebrates under another important head: foremost œsophageal diverticula and their relation to respiratory functions and sensorial (?) apparatus. Here, too, I do not wish to enter into a thorough discussion of the subject; an enumeration of the chief points may suffice for the present.

A special respiratory apparatus in the form of external branchiæ has never been met with in Nemertines. In a very early stage of embryological development, however, two lateral diverticula, situated in the very foremost portion of the œsophagus in front of the mouth, bud out from its wall (Bütschli, Barrois, and others), and are in this stage directly comparable to similar diverticula which arise in the same region in the Balanoglossus larva, and there give rise to the first pair of branchial slits (figs. 14 and 15),

In Nemertines these diverticula become constricted off from their point of origin—the œsophagus—and entering into connection with invaginations from the epiblast, which bring about a free access of the external sea-water, they become converted—at least in the large section of the Schizonemertines—into an apparatus which I have proved to be subservient ('Zur Anatomie u. Physiologie der Nemertinen,' p. 28) to a process of cerebral respiration, in which oxygen is carried to the nervous system itself, the cellular elements of which are in this subdivision profusely provided with hæmoglobin.

I am not prepared to say that in the great subdivision of the Hoplonemertini, where the central nervous apparatus is no longer provided with hæmoglobin, but where, on the contrary, the circulating fluid is, these diverticula, which continue to develop in the same way out of the œsophagus, are also—and in the first place—subservient to a respiratory process. I am rather inclined to believe that in this group the cephalic grooves—as the epiblastic invaginations travelling inwards to meet the hypoblastic diverticula in question are called—remain more especially adapted for sensiferous purposes, probably of olfactory nature. The way in which the complicated organs in the adult, the so-called side organs, develop, remains quite the same: an outgrowth from the œsophagus coalesces with an ingrowth from the epiblast, the principal difference being that the connection with the brain-lobes is no more so intimate, and that the apparatus is connected with the brain by a special set of nerves. In some species it continues to be situated behind the brain, in others it becomes placed in front of the central nervous apparatus.

It appears to me that these facts are not without significance. However, I refrain for the present from a further discussion, and would merely wish to point to an interesting detail in the development of Amphioxus lately brought to light by Hatschek's researches. It is the presence in the anterior region of the œsophagus, in front of the mouth, of two lateral hypoblastic diverticula, differing in their nature and in their further development, both from the archenteric diverticula (mesoblastic somites), and from the branchial outgrowths of

the œsophagus. These two diverticula, originally symmetrical, become constricted off from the hypoblast, and in their further development they have a different fate, the left one communicating with the exterior by a ciliated opening, which appears in the epiblast, the right one forming an epithelial lining in the præoral body-region. The left one was looked upon by Kowalevsky as a special sense-organ of the larva.

Although I am not prepared for the present to furnish any evidence in this direction, I would call attention to the similarity in development between these structures and the cephalic diverticulum of Nemertines. Considering the amount of degeneration which in several respects Amphioxus appears to have undergone, it does not appear impossible that the left lobe is really a temporary olfactory organ, the right one having entered upon other functions, and having lost its original significance.

These œsophageal diverticula of Amphioxus would stand about in the same relation to the posterior paired outgrowths of the œsophagus which ulteriorly give rise to the branchial slits of this animal, as would the two primary larval diverticula of Balanoglossus, giving rise to the first pair of gill-slits, to the following ones successively appearing behind them. In Nemertines only one corresponding pair of respiratory diverticula is encountered, and they may remain in connection with those portions of epiblastic ingrowths which form the primary constituents of a sensorial (olfactory?) apparatus in certain of the higher differentiated genera in the way we have above traced.

The far reaching significance of our starting point has obliged us to throw a rapid glance at the principal points in which Nemertines allow of a certain degree of comparison with Vertebrates, and it would lead us too far off if we were to follow this up for the secondary, less important points, or for those which are at present not fully enough known to allow of any fruitful comparison. Among the latter I count the excretory and the generative apparatus. Do the closed generative sacs

of Nemertines arise as part of the cœlom (cf. Lang, 'Gunda segmentata')? What is the morphological significance of the generative ducts which establish a direct communication between these sacs and the exterior, and which are recognisable on the outer surface as a double set of symmetrical pores? Is the paired nephridium provided with internal openings or is it not? These and other questions will have to be diligently studied and solved before comparison can extend itself in the domain of these organs.

With respect to the vascular system, it is not unimportant that in Nemertines it is on the whole a closed system of vessels, sometimes carrying corpuscles charged with hæmoglobin, sometimes colourless, and giving off a system of transverse connecting vessels, which link together the three longitudinal stems. These transverse vessels do not give off any capillaries, and are metamerically placed with unbroken regularity, one for each internal metamere (intestinal diverticulum). If indeed the suggested homology might prove to hold good between these diverticula and the mesoblastic somites of Amphioxus, the significance of this regular disposition, one for each of the transverse subdivisions of the body, corresponding in a general way to the arrangement of the aortic arches in vertebrate embryos could not be overlooked.

In conclusion I would point out that the speculations and suggestions contained in the last pages ought to be distinguished from the contents of the first part of this article. They have not in any way contributed to the formulating of the hypothesis there brought forward; they are merely the sequel in a train of thoughts which, starting from a comparison of such important and primitive organs in both Vertebrates and Nemertines as are the nervous system, the hypophysis and the notochord necessarily extended itself to other structures and organs occurring in both groups.

With respect to these, we must await more thorough investigations before pushing our speculations further.

The Renal Organs (Nephridia) of Patella.

By

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The existence of two renal organs in Patella was first pointed out by Professor Lankester in 1867,[1] and he stated at the same time that he believed he had discovered, by careful dissection, a minute orifice leading from the pericardium into the left and smaller of the two. In 1877 Von Jhering, in a paper on the "Morphology of Molluscan Kidneys,"[2] confirmed the account given by Professor Lankester, at the same time emphasising the fact that the two renal sacs are distinct, as had already been stated by Lankester, who says, after describing the two renal papillæ with their openings to the exterior, "These two orifices represent two renal organs, as in Lamellibranchs." Von Jhering was unable to find any pericardial openings. In April, 1881, Professor Lankester and Mr. A. G. Bourne[3] examined fresh limpets as to the pericardial orifice of the kidneys. They found that injections from the pericardium passed sometimes into the right and sometimes into the left renal sac, but that there was only one orifice leading into a narrow subanal tract belonging to the organ of the right side. Professor Lankester suggested to me some time ago that I should endeavour to settle definitely this question of the reno-pericardial pore in Patella by cutting a series of sections through the parts, and so tracing their relations

[1] 'Ann. and Mag. Nat. Hist.,' 3rd series, vol. 20, 1867.

[2] 'Zur Morph. der Niere der Mollusken Zeitschr. f. w. Zool.,' Bd xxix.

[3] "On the originally bilateral character of Renal Organ of Prosobranchia," 'Ann. and Mag. Nat. Hist.,' vol. vii, 1881.

to one another. My investigations were commenced in the Zoological Laboratory of University College, London, under the supervision of Professor Lankester, and were subsequently completed at Naples in the winter 1882-83.

I injected several specimens of Patella vulgata from the south coast of England with gelatine solution coloured with Berlin blue by means of a fine-pointed pipette inserted into the pericardium of the animal in the fresh state, and after hardening I cut out the piece to be examined, stained it whole, and cut it embedded in paraffin. I frequently found the injection in the cavities of both kidneys, and soon became convinced that each kidney had a separate communication with the pericardium; but the difficulty of getting complete series of sections with the tissues well preserved, in which the channels of communication could be satisfactorily traced by means of the injection, was so great that I was unable to discover the exact character and relation of the canals leading from the pericardium to the cavity of the kidneys. At the Naples Zoological Station I have used for injection simply the cold solution of Berlin blue in water, which has many advantages over the gelatine solution; it penetrates more easily, and is seen in sections as a blue line along the sides of the cavities which it has reached, while the gelatine has the two disadvantages of solidifying before the injection is complete, and contracting during the process of hardening. After many trials I have succeeded in obtaining complete series of sections from injected and uninjected specimens, in which the two canals of communication can be traced through their whole length.

The species of Patella which I have used at Naples, and which is most common there, is P. coerulea; its shell is much flatter than the P. vulgata of the English coast, and it does not attain such a large size; but I have found no differences between the organs of the two species.

When a Patella is removed from its shell in the fresh state the dorsal surface appears of a deep black colour, which is due to the presence of pigment in the superficial epithelial cells. This pigment can easily be washed off. When this is done, and

the roof of the mantle cavity cut away, the superficial parts of several organs are seen.

The pericardium occupies the left half of the posterior border of the mantle cavity. Projecting from this border on the right are three papillæ with apertures at the ends; the central one is the anal papilla, and on each side of it is the orifice of a renal organ. The left organ is small, extending from the right border of the pericardium as far as the rectum, while its extent from before backwards is the same as that of the pericardium. The right renal organ is distinguished by its darker colour, and extends round behind the left organ and part of the pericardium, and over the greater part of the dorsal surface, except a small portion where the liver is visible. On dissection the cavity of the right kidney is found to extend under the visceral mass from the right side of the animal as far as the median line, forming here a flat sac between the muscle of the foot below and the genital gland above; it also sends a prolongation under the rectum and left kidney, between these and the liver, which extends to the wall of the pericardium.

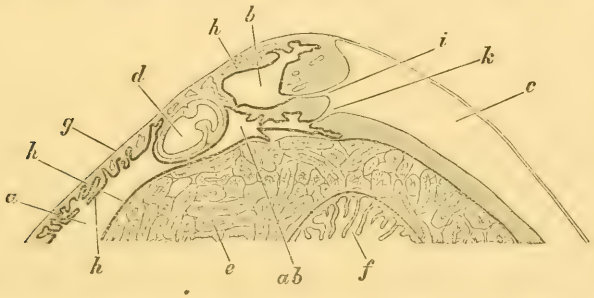

Diagram of a transverse section of Patella passing through the two renal organs, the rectum and the pericardium, so as to show the two reno-pericardial canals and their pores. *a*. Main cavity of the larger nephridium. *ab*. Sub-anal tract of the larger nephridium. *b*. Cavity of the smaller nephridium. *c*. Pericardium. *d*. Rectum. *e*. Liver. *f*. Stomach. *g*. Integument. *h*. Black line representing the renal epithelium. *i*. Reno-pericardial pore of the smaller nephridium. *k*. Ditto of the larger nephridium.

The canals which lead from the pericardium in passing to the right to reach the renal cavities ascend slightly, so that in

a series of sections the end opening into the latter is first met with when the series begins from the anterior end of the prepared piece of tissue. The actual opening of the canal into the main cavity of each kidney is seen in such sections with the injection in the canal and in the aperture. A triangular piece of tissue is seen before each opening, which lower down joins with the tissue of the projection, which contains the canal, and therefore forms a sort of valve open towards the kidney and closed towards the pericardium. The part of the canal in the immediate neighbourhood of the aperture into the kidney is lined by cellular epithelium, which consists, apparently, in some places of more than one layer of cells. There are in many sections indications that this epithelium is ciliated, but whether it is so or not I could not definitely determine. Towards the part of the canal communicating with the pericardium the epithelium disappears; it may pass into a flat epithelium lining the whole interior of the pericardium, but my sections do not show this clearly.

I have been able to discover and study these canals in series of sections prepared without injection, and in these the epithelium is seen much more clearly.

With regard to the position of these canals, it is to be observed that the projections towards the cavities of the kidneys, through which they run, lie close to the external surface of the body, that surface which forms part of the floor of the mantle cavity; this is especially true of the canal belonging to the left renal organ. In the second place, they are not far distant from the external apertures of the kidneys, as is shown by the fact of the sections in which they are seen including a portion of the mantle cavity.

It is difficult to understand how it can have come about that the right kidney of Patella communicates with the pericardium by passing under the intestine instead of over it, unless the intestine at one time passed through the pericardium; in this case it could in passing out again during ancestral history, and becoming separated from the pericardium, have passed between the two openings of the kidneys; but if the pericar-

dium had always been above the intestine as in Chiton, and had then passed to the left to take up the position it has in Patella, the channels of communication which attach the kidneys to the pericardium would have both been above the intestine. It may be suggested that the original condition in the ancestors of Patella was similar to that still obtaining in Haliotis and Fissurella where the intestine is surrounded by the ventricle and pericardium.

The structure of the kidneys in Patella resembles that of other Molluscs. The external orifice leads into a central flattened cavity lined throughout by the secreting epithelium which is characteristic of renal organs throughout the Mollusca. This central cavity sends off numerous diverticula which branch and ramify and form a spongy tissue beneath the integument on the dorsal surface, and to a less extent in the other regions bordering the kidneys. These glandular diverticula alternate with large irregular lacunæ containing blood; the wall of the diverticulum consists of the secretory epithelium supported by a thin layer of nucleated, fibrous, connective tissue, and from the latter trabeculæ of the same tissue pass off here and there and stretch across the blood-lacunæ to reach the wall of a neighbouring diverticulum.

These trabeculæ occasionally contain BANDS OF MUSCULAR FIBRES. This is true of both the small kidney of the left side, and that of the right, so that both can be contracted to a certain extent.

The minute structure of the secreting epithelium is somewhat difficult to make out; when a portion of either kidney is teased on a slide in the fresh state and examined in sea water, a number of loose spherical vesicles are seen scattered over the field; these are of various sizes and contain a varying number of small dark coloured concretions soluble in potash; they are almost all ciliated. When the edge of a piece of renal tissue is looked at similar cells are seen forming a regular ciliated epithelium; when the surface of the same epithelium is observed, the outline of the cells is seen to be polygonal. The great quantity of concretions present makes it impossible to decide

whether there is but one layer of cells or more than one. A nucleus is often visible in the cells. In sections the epithelium is often badly preserved; its cells seem to be very delicate and easily destroyed, and the presence of the concretions here also obscures the cell-outlines. When the epithelium is well preserved and stained it has the following appearance. Near its base the nuclei of the cells are seen surrounded by the concretions, and the vertical boundaries of the cells are also visible. Towards the cavity of the gland the epithelium terminates in a number of clear rounded projections; in sections I have never been able to discover the cilia. It is evident that the rounded projections are the vesicular cells still attached; it is these which drop off so easily and are seen free in teased preparations. Their remains can also be often seen in sections in the lumen of the gland, and the process of secretion is evidently effected by these cells becoming detached in the renal cavities, and then breaking up and allowing the concretions to escape. But there is evidently another layer of cells in the epithelium beneath the vesicular projections, cells which contain concretions but are not yet vacuolated, and which show a nucleus; these take the place of the mature secretory cells when they fall into the lumen, and go through the same course of development as these. Von Jhering has observed the stages of this development in the kidney cells of Tethys,[1] in preparations of the fresh kidney. I have not been able to trace the process in this way in Patella. Very often the renal epithelium in sections does not show any vesicular projections, these having disappeared in the course of preparation; but the projections present variations in size and prominence which probably correspond to variations in secretory activity.

The structure of the two renal organs in Patella is exactly the same; the difference in colour which they present to the naked eye is due to the fact that the urinary concretions are more numerous in the right kidney than in the left. The cavities of the two kidneys do not communicate.

I have also cut some series of sections through the nuchal

[1] Von Jhering, 'Morph. Jahrbuch,' Bd. 2.

papillæ of Patella, in order to test the accuracy of the figure which Spengel has given of their structure.[1] There is little doubt that part of each of these papillæ consists of a sensory organ homologous with that which exists on the attachment of the gill in Haliotis. A nerve from the visceral commissure can be traced up to each papilla, as Spengel describes it, and in sections a nervous ganglion is seen beneath the epithelium, as in his figure. Besides this ganglion there is to be seen in the sections a cellular structure, oval in shape, surrounded by connective tissue, and divided by trabeculæ into compartments of various size. This may be the branchial rudiment, but it lies beneath the epithelium, and is not a special development of it.

[1] "Die Geruchsorgane, etc., der Mollusken," 'Z. f. w. Z.,' Bd. xxxv.

A Rare Form of the Blastoderm of the Chick, and its Bearing on the Question of the Formation of the Vertebrate Embryo.

By

C. O. Whitman, Ph.D.

With Plates XXIV and XXV.

In the summer of 1878, while in Leipsic, I obtained a blastoderm of the chick, which presented at least one unusual and very remarkable feature. The egg had been kept in an incubator, at a temperature of 37° to 38° C., for eighteen hours. After cutting away a portion of the shell and removing as much of the white of the egg as could be done without injury to the embryonic disc, the remainder of the egg, while still in the shell, was carefully dropped into a bowel of nitric acid (10%). The embryonic portion was then freed from the coagulated white by the aid of a feather, and, after fifteen minutes' immersion, was carefully cut around by sharp scissors and floated into a watch-glass. The vitelline membrane was then removed by the aid of pincers, and the yolk detached by gently shaking the blastoderm in the watch-glass. The acid was next turned off and replaced with distilled water, several times renewed. After being thus thoroughly washed from the acid it was stained in an aqueous solution of carmine, then passed through several grades of alcohol, and finally mounted in balsam. The whole process was accomplished without causing any distortion or wrinkle, and without the loss of any portion of the blastoderm, as the preparation still shows.

The following topographical measurements were taken from the mounted preparation by the aid of the camera lucida:

Blastoderm 9·12 mm. long, 8·04 mm. wide.

Marginal notch ("Randkerbe," Rauber) ·46 mm. deep.

Area pellucida 2·4 mm. long, 1·76 mm. wide.

Distance from the anterior end of the area pel. to the corresponding margin of the blastoderm					3·56mm.
,,	posterior	,,	,,	,,	3·16 ,,
,,	right side	,,	,,	,,	2·8 ,,
,,	left side	,,	,,	,,	3·48 ,,

Primitive streak 1·04 mm. long; the distance between its fore end and the anterior border of the area pellucida 1·2 mm. Distance of the marginal notch from the posterior end of the primitive streak 2·2 mm.

The blastoderm is subcircular, and the marginal notch is situated on the right side instead of at the hind end of the main axis.[1] The area pellucida has an irregular pyriform outline, with the smaller end directed backward. It is placed a little excentrically, being a little nearer the right side than the left.

In the pellucid area two regions are easily distinguished; one, which is sometimes called the embryonic shield (*s*), is pyriform, occupies the central and rear portion of the field, and bears the primitive streak; the other is less opaque, peripheral in position, and crescentic in shape. The horns of the crescent are not quite symmetrical, the left being the longer, so that the hind end of the shield lies nearer the right than the left side of the pellucid area. The shield shades off very gradually into the clearer crescentic region.

The primitive streak is very well marked, and shows a well-defined primitive groove. In front of the primitive groove a linear opaque area is seen to vanish a little before the front margin of the shield, and appears to be a continuation of the primitive streak itself. This linear area is the "kopfortsatz" of Kölliker ("Axenplatte," Kupffer), and represents,

[1] The main axis passes to the left of the primitive streak, but parallel with it.

according to Dursy and Balfour, the commencement of the notochord.

The primitive groove does not end at the hindmost point of the area pellucida, but curves to the right near where it leaves the shield, and PASSES ON THROUGH THE AREA OPACA, TERMINATING IN THE MARGINAL NOTCH. The primitive streak cannot be followed beyond the inner edge of the area opaca. Not the slightest trace of a falciform expansion[1] of its posterior end could be detected. No head-fold has yet appeared to mark the limit of the prolongation of the primitive streak.

The only exceptional feature of this blastoderm is the continuation of the primitive groove to the marginal notch; and I believe this is the first time that such a feature has been recorded. Balfour, His, Rauber, and others have expressly stated that they have never been able to trace this groove to the margin of the blastoderm. Rauber, His, Balfour, and Pander have observed cases in which a marginal notch was present, but not a single instance where the groove extended to the notch. Although this is probably the first time that such a peculiarity has been described, I cannot say that it is the first time it has been observed. Indeed, I happen to know that one other similar case was found only a few days before I obtained the one here described. It was my friend, Dr. A. Böhm, the present assistant of Professor Kupffer, who found the first case. What use he has made of his preparation I cannot say, but I am quite certain that he has never published any account of it. I have long deferred the publication of this paper in the expectation that Dr. Böhm would make known his discovery, and in the hope of obtaining another specimen myself for sections. As neither of these events have been fulfilled, I have decided to describe the blastoderm in my possession.

As to the fact that the primitive groove extends to the notch, there is no room for a shadow of doubt. That arm of the groove which passes through the area opaca could be seen with the naked eye even before the blastoderm was cut from

[1] See Kupffer, 27, 28, 29, 30.

the yolk, and the same is still true in the mounted preparation. By transmitted light I can see with the unaided eye the primitive streak and the external arm of the primitive groove as a clear line leading from the notch to the base of the streak. The inner arm of the groove is just barely recognisable under the same conditions. The external arm is a little more than twice the length of the inner arm, and forms with the latter an angle of about 110°. In the blastoderm found by Dr. Böhm, the posterior portion of the groove formed a straight line with the anterior, and thus the marginal notch fell directly behind the primitive streak.

What now is the meaning of this continuity between the primitive groove and the marginal notch? It is certainly a deviation from the avian type of development, of such rare occurrence that it must be admitted to be an anomaly; still it is, if I am not mistaken, an anomaly for which comparative embryology furnishes a very satisfactory explanation. It is a well-known fact that the embryos of all Amniota are formed near the centre of the blastoderm, while those of all anamniotic vertebrates have their origin at the margin. It is now generally believed that the central position has been derived secondarily from the marginal, and this belief has given rise to various speculations in regard to the meaning of the primitive streak and its relation to the embryo. Intimately connected with these questions is another relating to the mode of formation and the growth in length of the embryo.

That these questions are not very easy of solution is evident from the fact that, after half a century of patient research, a decade or more of plodding "microtomization," and the industrious accumulation of embryometrical tables as the fruit of sections made in every nameable plane, the most eminent authorities in embryology cannot agree in their interpretation of the primitive streak, nor even in an opinion as to the part it plays in the formation of the embryo. When Kupffer and Rauber differ as to what constitutes the blastopore of the chick; when Goette, Kölliker, His, and Waldeyer affirm that the primitive streak is directly concerned in the formation of

the trunk of the embryo, while Dursy and Balfour assert that it entirely atrophies, or at most (Balfour) forms only the "tail swelling" and a part of the ventral wall of the post-anal gut; and when His and Rauber maintain that the embryo is formed by a retrogressive concrescence of the two symmetrically placed halves of the germinal ring, while Balfour and others contend that there is no such concrescence, and that the lengthening of the embryo is entirely by intussusceptional growth, it might seem like presumption on my part to offer any suggestions on these disputed points. It is far from my intention, however, to enter into an elaborate consideration of all these questions, and I have no conclusions to present which will take me very far from the plain highway of observed fact.

As to the meaning of the primitive groove, I think the blastoderm I have described furnishes a very strong confirmation—not to say verification—of a theory that originated with Balfour and Rauber; namely, that this groove represents a portion of the blastopore. It was this theory that conducted Rauber to a correct interpretation of the marginal notch. No one had ever discovered any connection between the notch and the primitive groove, or suggested any such relation between the two structures. The notch was of rather rare occurrence, and therefore treated as an irregularity that required no explanation. Its position directly behind the primitive groove appeared to Rauber to fit in with the opinion that this groove extended originally to the very edge of the blastoderm, precisely as it still does in Elasmobranch Fishes; and hence he regarded it as "the ideal hind end of the groove." This conjecture is raised to the dignity of an observed fact in the present case, and its verification is complete, provided the blastoderm here considered represents an atavistic form. Comparative embryology must be our guide in a question of this nature.

All the questions that cluster about the primitive streak are only so many special sides of a more general question, namely, How is the embryo formed? On the decision of this question hangs that of all the rest. Two different views have

been put forward, which may be conveniently distinguished as the differentiation theory and the concrescence theory. His and Rauber are the chief exponents of the theory of concrescence, and appear to have arrived at similar conclusions independently of each other. His developed his view in connection with investigations on the embryology of Osseous Fishes, and shortly afterwards extended it to the Elasmobranch and, with some reserve, to the Chick. With reference to the Salmon, His announced his conclusion in the following words:

"The structural basis (Uranlage) of the body, then, is a flat ring, which has its maximum width and thickness at the future head end, its minimum at the opposite tail end. The two lateral halves of the ring approach and unite retrogressively [successiv] as symmetrical body-halves" (No. 20, pp. 19, 20). In opposition to Oellacher, His maintains that the head end is to be regarded as a fixed point, and that the axial concrescence of the two halves of the ring begins at this end and advances towards the tail end, which is thus the last formed part of the embryo. The entire ring is thus brought together along the axial line, and this process goes hand in hand with the epibolic expansion of the blastoderm.

The Elasmobranch embryo is formed in a similar manner, but with this difference, that only a posterior portion of the ring takes a direct part in forming the embryo, so that the process of building up the embryo is completed long before the final closing in of the yolk by the blastoderm.

In his excellent memoir, entitled 'Primitive Streak and Neurula of the Vertebrates,' Rauber has given a brief survey of vertebrate embryology, and summarised the more important results reached by others and himself, with a view to making clear their theoretical bearings. Rauber claims that the Avian embryo arises, in the main, by a longitudinal concrescence of the two halves of the germinal ring ("Keimring"), in the same manner as the Piscian embryo; and further, that this process underlies the formation of all vertebrate embryos.

In the case of the Chick, both His and Rauber have called attention to a lunula-shaped area that appears, after about six

hours' incubation, in the posterior half of the area pellucida; and both agree that this area is continuous at its base with the area opaca, or germinal ring. According to Rauber, the cells constituting the lunula, by a centripetal movement, arrange themselves along each side of the longitudinal axis of the future embryo, and thus give rise to a "bilateral string," the so-called primitive streak. The streak represents simply the united edges of a small portion of the blastoporic rim ("properistome," Haeckel), and this mode of origin of the embryo is called "stomatogenic." The streak, groove, and marginal notch are regarded as so many "phenomena of conjunction." The area opaca is held by Rauber to be homologous with the germinal ring of the Fishes, and here, as in the Elasmobranch, a posterior "embryoplastic" portion (about one third of the entire ring) is distinguished from the anterior non-embryoplastic ("peri-embryonal") portion (No. 34).

His distinguishes two concentric rings in the area opaca, or "ring-area." "The ring-area of the embryonic disc accordingly now consists of an inner broad and opaque germinal-wall portion and a thin transparent margin (or secondary marginal rim); both are distinguishable with the naked eye" (No. 22, p. 165). These two zones of the area opaca are plainly seen in the blastoderm I have described. The inner thicker zone is the one which contains the embryoplastic material.

From the above citations it is evident that His and Rauber are in perfect accord on the main question; and, while both claim that the vertebrate embryo arises by the concrescence of the homotypical halves of the germinal ring, neither has anywhere intimated that intussusceptional growth could not go on at the same time. On the contrary, Rauber has expressly stated it as a self-evident fact that such growth is a concomitant of the process of concrescence (No. 39, p. 56).

In opposition to the view taken by His and Rauber we have the differentiation theory put forward by Balfour. In the second volume of his 'Treatise on Comparative Embryology' Balfour has summed up his latest conclusions on this

question, and stated at some length his objections to the concrescence theory. Balfour takes the Elasmobranch embryo as a type, a case which His regards as the best illustration of his own view. "The Elasmobranch embryo," says Balfour, "arises from a differentiation of the edge of the blastoderm, which extends inwards from the edge for some little distance. This differentiation is supposed to contain within itself the rudiments of the whole embryo, with the exception of the yolk-sack; and the hinder extremity of it, at the edge of the blastoderm, is regarded as corresponding with the hind end of the body of the adult. The growth in length takes place by a process of intussusception, and till there are formed the full number of mesoblastic somites it is effected, as in Chætopods, by the continual addition of fresh somites between the last-formed somite and the hind end of the body" (p. 254).

The "His-Rauber view" is introduced as "a somewhat paradoxical view," supported by three not very forcible arguments, and a number of minor arguments not worth mentioning. These three arguments are stated to be—(1) the continuity between the thickened edge of the blastoderm and the medullary folds; (2) the embryometrical investigations of His; and (3) some of the phenomena of double monsters studied by Rauber. Balfour says that the first argument affords no support for either theory, and the second appears to prove his own theory of growth. Rauber's view of "pluriradial development" is passed over without a word of comment, presumably because it was supposed to be without special importance to the discussion.

Balfour's objections to the concrescence theory may be summarised as follows:

1. The medullary groove closes behind earlier than in front, and the groove does not terminate behind in an acute angle, as might be expected if the embryo were formed by the coalescence of the edges of the blastoderm.

2. The formation of the neurenteric canal would make it impossible for any further increase in length by concrescence.

"If, therefore, His's and Rauber's view is accepted, it will have to be maintained that only a small part of the body is formed by concrescence, while the larger posterior part grows by intussusception."

3. The blastopore in Amphioxus is not coextensive with the neural groove, for it is nearly closed before the groove appears.

4. According to His and Rauber, "the whole of the dorsal, as well as of the ventral wall of the embryo, must be formed by the concrescence of the lips of the blastopore, which is clearly a reductio ad absurdum of the whole theory."

5. According to Kupffer (No. 31) the epibolic growth of the blastoderm in Clupea and Gasterosteus is equally rapid on all sides until the equatorial line of the egg is passed; and Balfour considers this to be "absolutely inconsistent with the concrescence theory."

These five arguments probably include all there is of importance to be said against concrescence. Although some of them may claim to be based on comparative embryology, not one of them, nor all of them together, are broad enough to cover the ground embraced in Balfour's theory of the origin of vertebrates. No attempt is made to discuss the two opposed theories on the basis of the now generally received view, that the ancestral form of the vertebrates was an Annelid; and it seems to me surprising that an advocate of this view should leave the Annelids almost entirely out of consideration. Balfour makes the Elasmobranch embryo the point of departure, and evidently because the primitive type of development has probably suffered less modification here than in the amniotic vertebrate. On similar grounds we may ask, Why not make the Annelid type of development our starting-point, since the mode of development may be presumed to have been conserved in its greatest purity in those animals that have made the smallest departures from the ancestral form? If there is any truth in the supposed genealogical relationship between vertebrates and Annelids we have certainly a right to expect some fundamental agreement in their modes of development.

share the opinion with some others that such an agreement does exist, and in it I find one of the most conclusive evidences of genetic affinity. This agreement consists not only in the metameric division of the embryo, which is the only point of agreement alluded to by Balfour, but also in the formation of the embryo by concrescence of the two halves of the embryonic ring.

Accordingly I hold that Balfour has left out of consideration one of the most important elements of the problem. This is a criticism from a general point of view; but, as it serves to make clear the standpoint from which I propose to consider the question at issue, it comes properly enough before the consideration of the above-named objections, to which we may now pass.

There is nothing in the first objection which has not been anticipated and answered in a general way in the writings of His and Rauber, and I need not here repeat their statements. It may be worth while, however, to call attention to another way of meeting the supposed difficulty. It is now quite clear that the primitive groove and the medullary groove must not be confounded, and it is equally clear that the concrescence theory must be able to account for them both in every instance, in order to maintain itself. But is it possible to keep up the distinction between them in all cases? What, for example, can be called the primitive groove of the Elasmobranch embryo? It has been said that here there is no such structure, and by some the medullary groove has been called primitive groove. There is some danger of confusion on this point, and I believe this confusion underlies the objection we are here considering. If we break loose from all mental pictures suggested by the word groove, and adhere strictly to the definition of the primitive groove as the plane of junction of the lips of the blastopore—a definition to which most embryologists will certainly assent—then it follows, on the concrescence theory, that something of this nature must be recognised in the Elasmobranch embryo. Looking at the question from this standpoint, it is plain that the medullary groove is not identical with the

primitive groove. The primitive groove is simply "a phenomenon of conjunction," as Rauber terms it; but it differs from the medullary groove in not being confined to a single embryonic layer. Both fall in the same median plane, but one is primary, the other secondary; one divides the entire embryo into two homo-typical halves, the other only marks the middle line of the neural plate. These are, of course, no new facts; but I have been obliged to state them in order to make clear my meaning. Now, if we bear in mind that the primitive groove is only a seam that marks the incomplete coalescence of two germ bands or halves of the germinal ring, and that the medullary groove simply marks the axial relations of two folds on the surface of these bands, we may avoid the confusion that is sure to arise when this distinction is not kept in view. Regarding this distinction as the only one of fundamental importance, I hold that all disputes in regard to the presence or absence of a primitive groove do not affect the main question of concrescence. The plane of junction may be marked by a narrow surface groove or only a faint line, or the coalescence may be so complete that no plain indication of its position can be detected. In all Amniota the two grooves are not contemporaneous in origin, and one is obliterated as the other takes its place; but in Elasmobranchii they are coincident, a circumstance that does not alter the fundamental distinction before insisted upon.

The aim of these remarks, if it need be stated, has been to show that the manner in which the medullary folds close is not of primary importance in deciding between the theories of differentiation and concrescence. It is possible, and perhaps not improbable, that the formation and closure of the medullary folds are determined in part by the same causes that concur in bringing together the two halves of the germinal ring; but the two closures are nevertheless quite distinct, both from a morphological and physiological stand-point. The concrescence theory undertakes to account for the conversion of the germinal ring into a bilateral embryo; but it is not within its scope to explain peculiarities in the closure of the medullary folds, since

this is only a special closure which follows the main or somatic closure.

Balfour's first objection is open to criticism from still another point, since it is based on a feature which he himself has elsewhere declared to be exceptional. In his 'Monograph on the Development of the Elasmobranch Fishes,' p. 84, he says:—"The only feature in any respect peculiar to these fishes is the closing of the medullary canal, first commencing behind, and then at a second point in the cervical region. In those vertebrates in which the medullary folds do not unite at approximately the same time throughout their length, they appear usually to do so first in the region of the neck."

The introductory phases of concrescence in the Elasmobranch are well shown in figs. 1, 2, and 3, copied after His; and they appear to me to meet the first objection with reference to the angle of coalescence.

The second objection claims that concrescence subsequent to the establishment of the neurenteric canal would be a simple impossibility. At first sight this appears to be a fatal argument against the concrescence theory. Since the general importance of this canal was first insisted on by Kowalevsky, a similar structure has been discovered in the Birds by Gasser, and in the Reptiles by Kupffer and Benecke; and indistinct traces of it in Mammalia have been reported by Lieberkühn. There is still much difference of opinion, not only in regard to the meaning of this structure and its relation to the allantois and archenteron, but also in respect to its position. Balfour places it just in front of the primitive streak; Kupffer and Benecke contend that it is situated at the posterior end of the streak; Strahl says it arises near the middle; and finally, Braun finds several independent canals that appear one after the other. Strahl's recent paper (No. 47) is the only one that offers any assistance in meeting Balfour's argument. In Lacerta agilis Strahl finds a small inconspicuous primitive streak, at the centre of which the neurenteric canal first makes its appearance. But the point of chief interest here is that this canal, according to Strahl, travels slowly back-

wards, the fore part closing as the hinder part opens further back, until ultimately the canal is found in the extreme hind end of the tail.

That part of the primitive streak that lies originally behind the canal does not atrophy, but forms the tail and allantois; while that part that lies before this point is employed in forming the medullary tube, the chorda, and the alimentary canal. If Strahl's statements in respect to the change of position of the neurenteric canal are correct, concrescence beyond its point of origin is not an impossibility. It is quite possible that this movement of the canal may explain, what would otherwise be difficult to understand, the occurrence of several apparently independent canals reported first by Braun.

The chief difficulty in the case of Amphioxus has already been considered by Rauber in two papers that appeared in 1877 (Nos. 39, 41), and I do not readily understand why Balfour should bring up the same point several years later without alluding to its previous notice. In considering this objection it must be borne in mind that Amphioxus is no longer entitled to the rank of "Urwirbelthier," but is rather to be classed among the prodigal sons of the vertebrates, to use a metaphor of Prof. Dohrn. Why, then, should "clear evidence" be expected from this source rather than from more respectable types that have not lost their senses by hiding in the sand? If degenerative simplicity of structure warrants such expectation it would hardly appear ridiculous to appeal to our more "degenerate cousins," as Lankester calls the Ascidians. The mere fact that the blastopore nearly closes before the appearance of the medullary groove cannot be accepted as proof that the blastopore is not coextensive with the groove, as Rauber has plainly shown. Rauber's remarks on this subject are substantially as follows:—In order to understand the case of Amphioxus we must not start with the last stage of the blastopore, when it has been reduced almost to minimum dimensions, but with the highest stage of its existence, when it has its widest expanse. It is about this time that it appears to lie on the dorsal side of the earlier equatorial line of the egg.

Its diameter gradually diminishes as the Gastrula assumes a more spherical form. Then the double-walled sac elongates in the direction of the axis of the blastopore, and becomes flattened on the dorsal side, at the rear end of which is seen the remnant of the blastopore. The lateral edges of the flattened dorsal surface rise up as medullary folds, which enclose at their posterior ends the blastoporic remnant. Considering the matter on the basis that the blastopore has closed up gradually on the dorsal side, it appears not at all improbable that we have here a conjunctive form of embryo formation. The investigations of Kowalevsky thus interpreted in harmony with the concrescence theory enabled Rauber to anticipate what has since been verified by observation. Hatschek,[1] who has paid special attention to the closure of the blastopore, says, "I came to the conclusion that the original broad Gastrula mouth belongs entirely to the later dorsal region, and that one point in its rim marks the hind end of the body." And again, "The closing of the Gastrula mouth begins at its anterior edge, the hind edge remaining unchanged throughout. The concrescence of the edges takes place in a line that forms the later dorsal line. The hindmost remnant of the Gastrula mouth remains then for some time as a small dorsal opening at the hind end of the dorsal surface."

The reduction to absurdity claimed in the fourth objection appears to be based on inadmissible premises; and as soon as these are stripped of exaggeration the absurdity vanishes.

To say that, according to the view of His and Rauber, the line of concrescence must be coextensive with the whole of the dorsal as well as the whole of the ventral wall of the embryo is inaccurate in both particulars. The inaccuracy with respect to the dorsal wall is comparatively unimportant, since only the foremost end of the Elasmobranch embryo undergoes no concrescence, but with respect to the ventral wall the statement is plainly hyperbolical, and herein lies the whole

[1] Hatschek, "Studien ü. Entw. d. Amphioxus," Claus' 'Arbeiten,' vol. iv, part. i, pp. 28, 31, 1881.

absurdity. Now, there is no difference of opinion as to the fact that the final closing in of the yolk takes place "at some little distance behind the embryo" in the case of the Elasmobranch. A glance at Balfour's fig. 30 B ('Compar. Embryol.,' p. 52) will show the extent of the line of concrescence. It will be seen, at the utmost, this line can only be said to extend from the region of the head along the dorsal side to the tip of the tail, from this point forward on the ventral side to the umbilical stalk, and from this stalk backward along "the linear streak" which connects the embryo with the edge of the blastoderm. The closure of the space (*yk*) will complete the line of concrescence. It will be seen that there is an important ventral portion of the body, extending from the umbilical cord to the head, which is in no sense of the word formed by concrescence. If we take into consideration the entire yolk, as Balfour certainly does, it is plain that the line of concrescence is considerably less than half of the entire circumference. When we remember that among our invertebrate vermian relatives the cases are not at all rare in which this line is much more than half the circumference of the egg, we must admit that nature delights in just such absurdities as Balfour has pointed out.

The fifth objection presents the same difficulty that we meet with in the development of the Bird, where, owing to the presence of an enormous quantity of food-yolk, the process of concrescence has undergone such extreme modification that the only constant outward manifestation of it is the primitive groove. But the connection of the primitive streak with the area opaca, the relations of thickness between different parts of the blastoderm at successive stages, the occasional appearance of a marginal notch, the extension of the primitive groove to the notch in rare cases, the occurrence of a neurenteric canal, and other considerations of a comparative embryological nature, appear to me to outweigh the objection, and compel us to recognise here the same principle of embryonic formation that characterises the more primitive forms of the vertebrate stock.

Rauber has shown that the phenomena of double monsters may be explained in perfect harmony with the theory of concrescence. A single illustration must here suffice to show the application of the theory to such cases.

Figs. 10 and 11 are diagrammatic representations of double formations of the Osseous Fish. In Fig. 10 are seen two independent formations (A and B), separated by a small portion of the germinal ring (i). Further development leads to the condition seen in fig. 11. The concrescence of the lateral halves of the germ-ring carries A and B forward, and brings the portions e e together, thus producing a monster that is double in front and single behind. The intermediate portion (i) now forms parts of the mesial sides of A and B.

The forms here diagrammatically represented have been selected with a view to show that the Vertebrates and Annelids exhibit the same ring-type of development.

Figs. 1—3 = early stages of the Elasmobranch (after His). The manner in which the two halves of the ring are brought together to form the embryo is well illustrated in these figures.

Figs. 4—6 = Salmo (after His). Here the line of concrescence does not reach to the hind edge of the ring, the two marginal lobes (m l) seen in figs. 1—3 being here represented by a single median lobe. Although the process of concrescence is thus partially disguised, it becomes evident enough by comparing successive stages as represented in fig. 6.

Fig. 7 = normal form of the blastoderm of the Chick.

Fig. 8 = atavistic form of the blastoderm of the Chick, differing from the normal form only in the extension of the line of concrescence to the marginal notch (m n).

Fig. 9 = Clepsine, showing that in a case of admitted concrescence the relations of the embryo to the germ-ring are completely analogous to those seen in the blastoderm of the Fish or the Bird.

Figs. 10, 11 = double formation in the Osseous Fish (after Rauber).

Fig. 12 = ring-stage of Euaxes (after Kowalevsky). The

two halves of the ring coalesce in the same manner as in Clepsine.

Let us now consider the two theories of embryo-formation in a more general way. The theory of concrescence undertakes to give a rational explanation of a very large body of phenomena; while that of differentiation cannot even make the slightest pretension to anything of the kind. What is differentiation except an undefined mode of growth and development? It is not a mechanical explanation, but simply a vague name for unknown processes. This theory, even when defended by the sagacity of Balfour, has never offered a suggestion by way of explaining the germinal ring, and the uniform relations which this ring sustains to the embryo. It cannot tell us why all the so-called "phenomena of concrescence" are situated behind the embryo, instead of before it; nor can it give any explanation of the general nature of these phenomena, except when it goes a begging by calling in the aid of the concrescence theory. On the other hand, the theory of concrescence makes known a law of formation which may, with reason, be said to hold good throughout the vertebrate group, and their nearest invertebrate allies. So far as these allies are concerned, the law is an established fact, about which there is not, and cannot be, any controversy; and among the lower vertebrates the appearances are unquestionably in its favour. Assuming that the immediate ancestor of the Vertebrata was a segmented worm, it is evident that our theory of the formation of the embryo should include both groups of animals. The fundamental agreement, which we should naturally expect to find, appears first of all in the formation of a germinal ring, composed of two symmetrical halves, which coalesce along the median neural line from before backward, thus producing a bilateral embryo. So far there is essential agreement in the two great classes of segmented worms, the Chætopods and Leeches.

I think no exception to this statement can be justly taken on the ground that the germ-bands in some cases contain neuroblastic as well as mesoblastic elements. But as Balfour has laid considerable stress on this very point, and

has even gone so far as to doubt the accuracy of my observations on Clepsine, on the strength of theoretical convictions, it seems necessary to give it a moment's consideration. Balfour remarks: "Till more evidence is brought forward by Whitman or some other observer in support of the view that the so-called neuroblasts have any share in forming the nervous system, they must, in my opinion, be regarded as probably forming, in conjunction with the mesoblasts, two simple mesoblastic bands. Kowalevsky has, moreover, briefly stated that he has satisfied himself that the nervous system in Clepsine originates from the epiblast—a statement which certainly could not be brought into harmony with Whitman's account" (No. 8, vol. i, p. 289). With reference to Clepsine, Kowalevsky remarks: "I preserved only several stages in weak chromic acid, and from sections of these I could only convince myself later of the origin of the nervous system from the upper layer."[1] This is all he has said on this point; and I will now show that, if we do not go behind the verbal statement itself, it does not even require to be brought into harmony with my account, since it is precisely what I have claimed. The four rows of neuroblasts in each germ-band lie, at the outset, at the surface, and must therefore be considered a part of the epiblast, although a specialised part. It is simply a precocious differentiation of the edge of the epiblast, by which epidermal and neural elements become distinctly marked at an unusually early stage. In the course of the epibolic growth of the ectoderm the epidermal portion progresses somewhat more rapidly towards the lower pole than the germ-bands, and thus sweeps over the neural portion. But it seems to me plainly a matter of little importance whether the neural portion looses its surface position during the epiboly, or immediately after the concluson of the concrescence of the germ-bands; and I confess that I do not see wherein this view requires "any special support." At the time Balfour penned the above criticism, he evidently was not aware that my observations on the origin of the nervous system in Clepsine were but little more than a corroboration of those of an eminent Russian embryologist. I take this opportunity to express my regret for the same oversight on my own part. It was Professor Metschnikoff who first determined the precise origin of the nervous system of Clepsine.[2] The accord between Metschnikoff and myself extends not only to the facts observed, but also to the interpretation of these facts. The chief distinction between Clepsine and other Articulata and Vertebrata with respect to the germ-lamellæ lies, according to Metschnikoff, in the single fact that "the epidermal layer separates very early from the basis of the nerve system." The association of neuroblastic with mesoblastic elements in the germ-bands is then a feature which presents no serious difficulty to the comparison before instituted between Chætopods and Leeches.

[1] Kowalevsky, 'Embryol. Stud. an Würmern u. Arthropoden,' p. 3.

[2] Metschnikoff, 'Beiträge zur Entw'gesch. einiger nied. Thiere.' Mélanges Biologiques du Bull. de l'Acad. imp. des. Sci. St. Petersbourg,' vii, 6, 1871.

The second great feature common to the embryos of both Annelids and Vertebrates is the metameric division of the body. In the Annelids the division into somites follows closely in the wake of concrescence, and the two processes are fundamental and invariable in sequence. Now, when we see that the vertebrate embryo arises from a germinal ring, and that soon after the formation of the embryo begins, the metameric division sets in and progresses in the same direction as in the Annelids, there is certainly good ground for inferring that the phenomena are fundamentally the same in both groups of animals. Since, in the one case, concrescence and metameric division are conceded by all to be the two grand formative processes associated in invariable sequence, it is difficult to believe that, in the other case, the second process has been preserved, while the first in order, and perhaps in importance, has been entirely suppressed.

The law set up by the elder Milne-Edwards, according to which fresh somites are intercalated between the last formed somite and the hind end of the body, does not appear to me to hold good in the case of embryos that arise by concrescence. In Clepsine it is perfectly clear that the hindmost segment is the last formed segment; and the investigations of Kowalevsky, Hatschek, and others appear to demonstrate the same for the Chætopods. The germ-bands lengthen at the expense of proliferating cells; and the additions are made to the hind extremities, so that these are always the youngest portions of the embryo. The theory of intercalation can therefore be upheld only by supposing that the proliferating cells represent the hind end of the body, which they do only prospectively. But there is still another objection to this theory, which was pointed out by Kölliker.[1] In Clepsine the somites are not added one by one as fast as the material is furnished by the proliferating cells. The metameric division begins only after the materia. for the greater number of somites is already present, so that no one of the successively formed somites, except the ultimate, can be regarded as the youngest portion of the embryo.

[1] Kölliker, 'Observationes de prima insectorum genesi.' Zürich, 1842.

If the theory of concrescence is applicable to the vertebrates, it is evident that we cannot regard the two ends of the embryo as equally old portions, which are gradually pushed in opposite directions by the interpolation of fresh somites successively budded off from the hind end. The discovery of two "Polzellen des Mesoderms" at the extreme hind end of the embryo of Amphioxus by Hatschek, supports the opinion that the lengthening and the metameric division of the vertebrate embryo take place in fundamentally the same manner as in the Annelids.

The error of the differentiation theory does not lie in the assumption of intussusceptional growth, but in excluding the concomitant process of concrescence. The formation of the vertebrate embryo does not afford such conspicuous evidences of concrescence as that of the Annelid embryo; but even in the more doubtful cases of the Osseous Fish and the Bird, both His (No. 22) and Kupffer (No. 31) have shown that there is a general movement of the embryoplastic material which must, in my opinion, be regarded as a disguised form of concrescence. No other explanation of the phenomena can claim to bring them into relation with that form of development still preserved in the nearest invertebrate relatives of the vertebrates.

According to the concrescence theory, it will not do to regard the primitive streak as analogous to the "linear streak" behind the Elasmobranch embryo, as has been done by Balfour. The primitive streak lies within the germinal ring, and is continuous with it at the hind border; the "linear streak" is located much further back, being entirely behind the embryoplastic portion of the ring. Another important difference, not alluded to by Balfour, consists in the fact that the primitive streak arises even before the medullary folds; while the "linear streak" appears after the embryo is formed, and even after the process of constriction has narrowed the connection of the embryo with the yolk to a slender cord, the umbilical stalk. Nothing analogous to this "linear streak" appears in the normal blastoderm of the Chick. In the exceptional form of the blastoderm which I have described, the streak connect-

ing the primitive groove with the marginal notch may be considered analogous to the "linear streak" of the Elasmobranch, while the marginal notch corresponds to the anterior angle of the "yolk blastopore," marked *yk* in Balfour's figure.

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(3) "On the Disappearance of the Primitive Groove in the Embryo Chick," 'Quart. Journ. of Mic. Sci.,' vol. xiii, 1873.

(4) 'Elements of Embryology,' part. i, London, 1874. By Foster and Balfour.

(5) "A Comparison of the Early Stages in the Development of Vertebrates," 'Quart. J. Mic. Sci.,' vol. xv, 1875.

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(31) "Die Entw. des Herings im Ei," 'Jahresb. d. Kommission z. wiss. Untersuchung d. deutschen Meere," Berlin, 1878.

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On the Development of the Pelvic Girdle and Skeleton of the Hind Limb in the Chick.

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With Plates XXVI & XXVII.

The investigations described below were undertaken at the suggestion of the late Professor Balfour, with a view to finding out, through a study of the development of the pelvic girdle in the chick, what are the homologies of the pubis in birds with that of other Vertebrata.

In connection with this question one or two other points, which appear to me of some importance, have presented themselves.

The histological development may be briefly considered at the outset. On the fourth day of incubation the limb is merely a local exaggeration of the Wolffian ridge, consisting, like it, of a mass of rounded mesoblastic cells, very closely aggregated together. The epiblast forms a thickened cap round the free end of the limb. No differentiation into cartilage or muscle is yet visible.

The first trace of the skeletal parts appears on the fifth day. The mesoblastic tissue of the limb is now differentiated into an axial, or more condensed, and surrounding, or less condensed, region. Both parts consist of the same rounded cells as before. They only differ in the degree of concentration of the cells. These features are shown diagrammatically in fig. 1.

The differentiation of tissue goes on more rapidly in the

skeleton of the limb than in the girdle, and more rapidly in the axial than in the superficial regions of both skeletons. Its main features are almost the same as those described by Strasser[1] in the developing cartilage of the newt. On the sixth day, or thereabouts, the cells begin to be compressed in the direction of the long axis of the cartilages. This happens especially in the tibia and fibula. Dark irregularly-shaped masses—the "prochondral elements" of Strasser—appear among the cells. They are apparently derived from the metamorphosed cells, for one occasionally meets with forms that appear intermediate, in which the protoplasm has become opaque and stains deeply, while the nucleus is still visible. I take the prochondral elements to be cells which have retrograded still further and lost their nuclei.

Rather later, on the sixth or seventh day, the prochondral elements have almost disappeared from the central part of the cartilage. Their place is taken by a homogeneous, slightly-staining matrix, by means of which the cells gradually become widely separated from one another. Still later the cells take on the crescent shape of adult cartilage cells.

Morphology.—Since chicks of the same day vary so much in their degree of development I have taken the length of the hind limb as the standard of their age. The following table shows roughly to what number of days of incubations these lengths correspond :

Length of hind limb.	Number of days of incubation.
0·06 in.—0·1 in.	5—6
0·12 in.—0·2 in.	6—7
0·2 in.—0·25 in.	7—8
0·25 in.—0·3 in.	8—9
0·5 in.—0·8 in.	9—10
1·5 in.—3· in.	14—20

The Pelvic Girdle on its first appearance (length of hind limb 0·06 in.—see fig. 1) is seen in transverse sections to form one mass with the skeleton of the limb. It consists of two slight

[1] H. Strasser, "Zur Entwicklung der Extremitätenknochen bei Salamandern und Tritonen," 'Morph. Jahrbuch,' Band v, 1879.

outgrowths of the proximal part of the femur, one being directed upwards, the other inwards, each, however, hardly extending beyond the limb itself. The future cartilage is only just distinguishable from its surroundings of indifferent mesoblastic cells, since the two tissues pass quite gradually into one another.

The next stage (length of hind limb 0·12 in.) is seen in longitudinal section in fig. 2. The series of sections shows the same perfect continuity of the girdle and femur that existed at first. We can distinguish in the girdle a blunt dorsal prolongation—the beginning of the ilium—an acetabular region behind the obturator nerve and a downward process in front of it, which is obviously to become the pubis. As we go inwards in the series of sections these two outgrowths, the ilium and the pubis, disappear, and the central or acetabular region is prolonged a little way inwards, being bounded in front by the obturator nerve. The nerve does not appear in the same sections with the pubis and ilium, but in the figure it is represented as viewed from the outside, the girdle being supposed to be transparent. At this stage the nerves are remarkable for their large size in proportion to the skeletal parts. The obturator nerve coming off from the crural plexus is at this time by far the most important of its distal branches.

In the next stage (length of hind limb 0·15 in.—see fig. 3) we can clearly distinguish three elements in the girdle, meeting in the broad acetabular region, which passes on without a break into the femur. The region of its junction with the latter is shown diagrammatically in the figure, but the cartilage of the femur is continuous with that of the girdle, as are the three elements of the girdle with one another. The ilium has grown forwards, arching over the crural nerve, and has given off a slenderer pointed process backwards. The ischium is directed almost vertically downwards, but also slightly inwards, being, as a whole, situated nearer to the middle line of the body than are the other elements. The main point of interest is the double nature of the pubis, the anterior branch of which points directly forwards and slightly outwards, while the posterior is directed

downwards and slightly forwards. The obturator nerve passes between the posterior branch of the pubis and the ischium. A series of twelve longitudinal sections has been combined to produce the figure, which is therefore diagrammatic only in so far as it represents as existing in two dimensions what really exists in three. The other figures, in which the whole girdle is represented, were drawn in the same way.

The study of the stages described above shows that the early development of the Pelvic Girdle of the Chick is similar to that of the limb-girdles of Elasmobranchs [1] in two points: (1) the skeleton of the limb is developed continuously with the girdle; (2) the parts of the girdle which are in the immediate neighbourhood of the skeleton of the limb are first developed, and the dorsal and ventral outgrowths appear later.

In the next stage (length of hind limb 0·17 in., see fig. 4) the posterior branch of the pubis has grown more than the anterior, and is curved backwards. Its proximal half, however, retains the direction which the whole posterior branch had in the earlier stage, and from this we may conclude that the change of form results from a growth, rather than from a rotation backwards of the whole cartilage.

A transverse section (see fig. 5) of about the same stage shows that the girdle is still continuous with the femur. In the latter, the cartilaginous matrix has begun to be formed internally, while the peripheral parts (a region of which is cut through in the middle of the limb) and the girdle still consists of the condensed tissue described above.

A later stage is shown in fig. 6 (length of hind limb 0·2 in.). The most striking feature here compared with the preceding stage is the large development of the posterior part of the ilium. The ischium has become distally expanded, and the posterior branch of the pubis is larger still in proportion to the anterior branch.

About this time the femur begins to be separated from the girdle by an intervening tract of tissue which has not gone so

[1] F. M. Balfour, "On the development of the skeleton of the paired fins of Elasmobranchii," 'Proc. of Zoological Society,' 1881.

far on the way to becoming adult cartilage. At first the whole structure progresses uniformly, except that the girdle always lags a little behind the femur, but passes off gradually into it. The "prochondral elements" and a small quantity of cartilaginous matrix exist across the future line of division, which, however, develops no further, but retrogrades into the fibrous tissue of the joint.

Fig. 7 represents a further advance of the girdle towards the adult form.

In later stages, no important changes take place. The anterior branch of the pubis, which is always rather behind the rest of the girdle in histological development, becomes more and more proportionately insignificant, and forms at last the pectineal process of the pubis. The posterior branch of the pubis becomes very slender. Both it and the ischium grow more and more backwards, passing through the stage permanent in such forms as Apteryx (where they are much curved, and their long axes are inclined at an acute angle to the long axis of the ilium) to the stage found in the adult fowl, where the pubis and ischium—except the most proximal portions of them—are straight, and point directly backwards, so that the long axes of all three bones are parallel to one another.

Ossification begins comparatively late, i. e. later than in the limb. For a long time there is a cartilaginous continuity of the three elements round the acetabulum. The bones gradually grow up to and surround the acetabulum. Cartilage remains also at the free ends of the bones for a long time. A day or two before hatching (see fig. 18), the acetabulum is surrounded by bone, except for a small region of its front wall, continuous with the likewise cartilaginous anterior branch of the pubis. The position and relations of this latter element, together with the fact of its remaining cartilaginous so long, remind one to some extent of the cartilage found in a similar situation in the Crocodile embryo after the rest of the girdle has ossified. According to Hoffmann,[1] this cartilage is homo-

[1] C. K. Hoffmann, "Beiträge zur Kenntniss des Beckens der Amphibien und Reptilien," 'Nied. Archiv f. Zoologie,' Band iii, 1876.

logous with the Pubis, while he calls the bone generally known as the pubis the epi-pubis. But since the acetabular regions of each bone always remain cartilaginous longer than the other parts, and since this cartilage is replaced in the adult by a bony process of the Ischium shutting out the pubis (Epi-pubis of Hoffman) from the acetabulum, I should be more inclined to agree with the older view that Hoffmann's pubis is merely a part of the ischium. This seems to me quite consistent with his own account of the ossification. He says: —(loc. cit. p. 186) "Die Verknöcherung dieses vorderen Acetabularfortsatzes des Sitzbeines fangt zuerst an der dem Sitzbein angrenzenden Partie an und schreitet so allmählig dem vorderen Fortsatz des Iliums zu, erreicht diesen aber erst bei ganz ausgewachsenen alten Thieren." The fact that the pubis is moveable in the crocodile is quite sufficient to account for its being shut out from the acetabulum.

So far as I know, the only literature bearing directly on the subject of the development of the pelvic girdle in birds is a paper by Bunge.[1] According to him, the pubis and ischium are at first situated with their long axes in a position vertical to the vertebral column, and later become rotated backwards, thus taking on the adult form. This statement has been generally accepted, but I am unable to agree with Bunge's other conclusions. He has omitted to mention the primary continuity of the femur and girdle and the existence in the embryo of an anterior branch of the pubis which becomes the pectineal process. Speaking of the pectineal process in the adult, he only says that his account of the development proves that it is a part of the ilium, and he therefore retains the name "Spina iliaca" given it by the older anatomists. He also concludes that the avian pubis is homologous with the pubis of Reptiles. He describes the pubis as originating independently of the other elements of the girdle and beginning to fuse with them about on the eighth day. I find that the pubis is absolutely continuous with the girdle at the earliest,

[1] A. Bunge, "Untersuchungen zur Entwickelungsgeschichte des Beckengürtels der Amphibien, Reptilien, und Vögel," Dorpat, 1880.

and all other cartilaginous stages. But, since it lies in a somewhat different plane from the rest of the girdle, their junction is only visible in a few sections. In most, the region of junction is not cut through, as appears in fig. 10, which represents a section taken from the series out of which fig. 4 was compounded. I think that Bunge must have been misled by the frequent occurrence of such sections, and so have overlooked the few in each series in which the junction is really visible, such as that represented in fig. 11. Fig. 12 again represents a single section, showing the complete continuity of pubis and girdle. It is the ossification alone which gives rise to any want of continuity in any part of the girdle.

Homologies of the pubis in the different Vertebrate groups.—In the pelvic girdle of Ornithorhynchus (see fig. 17) a large process, whose length is about three quarters of that of the pubis, projects forwards from the region in front of the acetabulum, in bony continuity with the pubis. The same process is found in a somewhat reduced form in Echidna, and is still more reduced in many Marsupials and higher Mammals. Sometimes it is entirely absent. In embryo birds (see fig. 15), the process is found in about the same proportionate condition of development as in Ornithorhynchus. In the adult, it becomes much reduced, or is absent. Sometimes, as in the Ostrich, the ilium takes a small share in its formation, but this appears to be a secondary condition. It is the pectineal process of the pubis.

In the Dinosaurs, as described first by Marsh,[1] the embryonic condition of birds and the adult form of Ornithorhynchus is preserved in the almost equal development of the two branches of the pubis, the anterior being shorter and more massive, and the posterior longer and more slender (fig. 16).

The homologies in these cases seem clear, and have been generally recognised.

Turning to the reptiles, it is easy to compare the pubis of

[1] O. C. Marsh, "Principal characters of American Jurassic Dinosaurs," 'American Journal of Science and Arts' (Silliman), vols. xvi and xvii, 1878 and 1879.

Lizards with that of Chelonia. In both Lizards and Chelonia the pubes are directed forwards from the acetabulum, and form a symphysis. The angle at the symphysis is generally much greater than that in Mammals. In some Chelonia it is even greater than 180°. In both Lizards and Chelonia a process is given off from the outer side of the pubis. In the latter group it is often very large (see fig. 13), and is directed forwards, outwards, and somewhat downwards. In Lizards it is not so large, but still considerable, or it may be absent. It is generally directed outwards and downwards; but in some forms, such as Cyclodus (see fig. 14), it curves backwards and slightly inwards. In this case we could hardly compare it with the process found in Chelonia were it not for the many intermediate forms existing between these two extreme types. The process in question is the processus lateralis pubis. In Crocodiles it is absent.

In the Urodela the pubes are generally represented by an unpaired cartilaginous plate, not clearly marked off from the ischium, which is often ossified. Rarely the pubis itself has a superficial ossification. The pubic cartilage in Cryptobranchus is oblong, with a median process in front bearing the epipubis, and the anterior angles of the oblong are slightly produced. In Salamandra maculosa these angles form short broad processes, which may be compared with the processus lateralis pubis of Chelonia.

We have, then, in reptiles two branches of the pubis—the processus lateralis and the main body of the pubis—which two branches it is possible to derive from the condition found in Urodela. Also in Dinosaurs, Birds, and Mammals we have the pectineal process and the main body of the pubis. The splitting of the pubis into two branches is more complete—i. e. it approaches nearer to the acetabulum—in the higher forms.

There is every probability that the two branches correspond in some way in all these types. Two theories on the subject are obviously possible. Either (1) the processus lateralis of reptiles is the pectineal process of the pubis in Dinosaurs,

birds, and Mammals, and the pubis itself is in both cases homologous, or (2) the pubis of reptiles is the pectineal process, and the processus lateralis is the pubis of the higher forms. The first is the view apparently assumed by Huxley.[1] Supposing it to be true, the processus lateralis, in becoming the pectineal process, has retained the forward and outward direction which it has in the Chelonia. In Dinosaurs the downward direction is also seen. The pubis itself has become rotated backwards. The mere fact of its pointing forwards in reptiles and backwards in Dinosaurs, Birds, and Mammals, is no reason whatever against the theory of its being homologous in the two cases, for it is generally believed that the whole girdle has rotated in Mammals through an angle of about 90° from the position it occupies in reptiles. This would completely account for the altered position of the pubis. The fact that the angle formed at the symphysis of the pubes has generally become more acute in Mammals is a natural consequence of the transition from the crawling flat-bodied reptiles to the higher walking forms, in which the body is more laterally compressed.

In birds the case is somewhat different. The fact of the two primary sacral vertebræ being situated, as Gegenbaur[2] has shown, at a very short distance behind the acetabulum may indicate that the girdle has been rotated backwards to some extent from the reptilian position. The pubis may thus have come to point vertically downwards or very slightly backwards, as in the embryo bird. The adductor muscles passing from the pubis and ischium to the femur in reptiles are to a great extent replaced in birds by large muscles, which act as flexors of the thigh and adductors of the leg. It is evidently advantageous for these muscles to arise high up, and for their points of origin to be as rigid as possible. These advantages are attained by the disposition of the bones in the adult bird's pelvic girdle, the

[1] Huxley, "On the Pelvis in Mammalia," 'Proceedings of Royal Society,' vol. xxviii, 1879.

[2] Gegenbaur, "Beiträge zur Kenntniss des Beckens der Vögel," 'Jenaische Zeitschrift,' Band vi, 1871.

form of which, therefore, may be accounted for in this way. The pubis, being placed lowest, loses its functional importance as a point of support for muscles and becomes very slender. Its middle portion may even abort altogether, as sometimes happens in ducks and other swimming birds.

So far it appears quite possible to explain the facts by the first theory.

Turning to the second, we have to imagine a somewhat different process. The processus lateralis of reptiles, in becoming the pubis of the higher forms, has retained the position which it had already come to occupy in some Lizards (see fig. 14), and has increased in extent and functional importance. In Mammals it goes so far as to form a new symphysis, while in birds the backward direction of the bone is very much exaggerated. The part corresponding to the reptilian pubis at first retains its original situation and almost its original dimensions, as the anterior branch of the pubis in Dinosaurs, the embryo bird, and Ornithorhynchus. It gradually dwindles into the subordinate position of the pectineal process.

This theory again, accounts for all the known facts, and it agrees, better than does the former view, with the relations of the pubis in Dinosaurs. Marsh[1] found that the anterior branch of the pubis in the Stegosauria and Ornithopoda, e. g. Laosaurus, passed forwards and inwards, ending in a broad spatulate free extremity. In the Theropoda and Sauropoda, e. g. Atlantosaurus, no posterior branch of the pubis existed, but the bone which evidently corresponded to the anterior branch in Laosaurus formed the symphysis. Judging from this fact there seems no doubt that the anterior branch is homologous to the reptilian pubis. I think there can also be no doubt of the homology between it and the anterior branch, which, however, no longer forms the symphysis in birds and mammals.

These conclusions may be tabulated as follows:

[1] O. C. Marsh, "Classification of Dinosaurs," 'American Journal of Science' (Silliman), 1882.

Reptiles.	Dinosaurs.	Embryo Bird.	Birds.	Mammals.
1. Pubis	Anterior branch of pubis ('pubis' of Marsh)	Anterior branch of pubis	Pectinal process of pubis	Pectinal process of pubis.
2. Processus lateralis pubis	Pubis ('post-pubis' of Marsh)	Posterior branch of pubis	Pubis	Pubis.

The development of the skeleton of the limb has been described by Gegenbaur.[1] Rosenberg,[2] has supplemented Gegenbaur's accounts by his discovery of the fifth metatarsal, and quite recently Baur[3] has published a paper on the Tarsus of Birds and Dinosaurs. The results of my work on the development of the bird's tarsus agree with Baur's in almost every detail, so that I will give only a short account of it.

In a five days' chick (see fig. 1) the tissue of the limb is condensed axially into a single mass, about three times as long as it is broad, and extending through the proximal half of the limb. The skeleton is produced by the subsequent elongation and segmentation of this mass.

On the sixth day (length of hind limb 0·14 in., see fig. 8) we can recognise all the chief elements of the skeleton, though they are completely continuous. The tarsus forms a broad transverse band, continuous with the tibia and fibula above, and with the metatarsals below. Five metatarsals are present, the first and second being rather closely united. The third is the longest and the fifth the shortest. No cartilaginous

[1] C. Gegenbaur, "Vergleich.-Anat. Bemerkungen über das Fussskelet der Vögel," 'Archiv für Anat. und Phys.,' 1863; and "Untersuchungen zur vergleichenden Anatomie der Wirbelthiere," i Heft, 'Carpus und Tarsus,' 1864.

[2] A. Rosenburg, "Ueber die Entwicklung des Extremitäten-Skelets bei einigen Wirbelthieren," 'Zeitschrift f. wiss. Zoologie,' 1873.

[3] G. Baur, "Der Tarsus der Vögel und Dinosaurier," 'Morphologisches Jahrbuch,' Band viii, Heft iii, 1882.

matrix has yet appeared, but the "prochondral elements" are visible in the femur, tibia, and fibula.

Soon after—when the limb is 0·17 in. long—separate elements begin to appear in the tarsus. Of these there are three, two in the proximal row and one in the distal. The tarsus is still continuous throughout and continuous also with the tibia, fibula, and metatarsals. But in these three centres, as well as in the tibia, fibula, and metatarsals, the differentiation of tissue has gone further. The outlines of the various parts are indistinct. They all pass gradually into one another by means of the general groundwork of condensed tissue formed by the tarsus. The knee-joint is, however, developed at this stage.

A little after this stage, the first metatarsal, which does not keep step with the others in histological development, begins to split off from the tarsus and soon lies at some little distance from it. Baur describes the first metatarsal as originating quite independently and never coming into any connection with the tarsus.

The phalanges next begin to appear. When the limb is about 0·2 in. long, they are marked off by constrictions from the metatarsals, but are cartilaginously continuous with them. Later, when the limb is 0·3 in. long, the phalanges are marked off by intervening tracts of condensed tissue with no matrix in it. The tip of each toe at this period and for some time to come consists of a mass of condensed tissue such as always precedes cartilage (see fig. 9). This appears to be the growing point of the cartilage. From these facts it seems that the phalanges are produced by a lengthening and subsequent segmentation of the original distal cartilages of the limb, so that these cartilages represent the skeleton of the digits as well as the metatarsals.

On the eighth day (length of hind limb 0·27 in.—see fig. 9) all the elements of the tarsus are at their most distinct and independent stage, though they are still united with one another, with the tibia and fibula, and with the metatarsals by the condensed tissue of the groundwork of the tarsus.

Later, the distal and proximal parts of the tarsus become

separated, and the two proximal elements fuse together. Next, the proximal part begins to fuse with the tibia, which has grown more than the fibula, so that the latter no longer reaches the tarsus. The posterior lower edge of the tibia first becomes continuous with the proximal tarsal cartilage, while the anterior face of the latter gives off an upward process, the so-called "ascending process of the astragalus," which fits into a groove in the tibia, and remains for a long time separate from it. At about the same time the distal part of the tarsus fuses with the metatarsals, first with the second, next with the fourth, and lastly with the third. All these processes take place while the tarsus is still cartilaginous.

Morse[1] describes, in the tarsus of the embryo bird, an intermedium, which at first projects upwards between the distal ends of the tibia and fibula. Later, the tibiale and fibulare fuse behind it, while the tibia extends so as to cover the whole proximal surface of the tarsus, and the intermedium remains fitting into a groove on the anterior face of the tibia. It has a separate centre of ossification, but becomes anchylosed with the tibiale and fibulare, forming what is called the ascending process of the astragalus.

Both Baur and myself fail to find a separate origin for the intermedium. Baur describes the ascending process as an outgrowth from the tibiale, in which view I am inclined to concur. But the deviation of our views from that of Morse may, perhaps, be explained by the fact that while Baur worked only at the chick, duck, sparrow, pigeon, and blackbird, and I only at the chick, Morse investigated some aquatic birds—the tern, penguin, petrel, gull, &c.

In conclusion, I have to thank Dr. Gadow for his kindness in giving me help and advice during the course of my work.

[1] E. S. Morse, "On the Identity of the Ascending Process of the Astragalus in Birds with the Intermedium," 'Anniversary Memoirs of Boston Society of Natural History,' 1880.

The Development of the Mole (Talpa Europea). The Formation of the Germinal Layers, and Early Development of the Medullary Groove and Notochord.

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With Plates XXVIII, XXIX, XXX, XXXI.

In the following paper I propose to commence with a description of the fully-segmented ovum, leaving the details of the segmentation for a future communication; thence to trace the growth of the blastodermic vesicle and the ultimate formation of the hypoblast, epiblast, and mesoblast of the embryo; to follow the early stages of the development of the medullary canal and notochord; and finally to touch upon the phenomenon of the inversion of the layers in certain mammals, and to endeavour to show that in the mole there exists in development an intermediate condition between the inverted type, of which the guinea-pig is an example, and the normal type as exemplified by the rabbit.

Owing to the difficulty of keeping moles alive and the still greater difficulty of observing their breeding habits when in captivity, I have found it impossible to determine the exact age of any embryos, and am obliged to fix their relative age in accordance with their size, and what appears to me to be the course of their development.

Under these circumstances it will be convenient to divide

embryonic life into periods which I propose shall be regulated by the following conditions:

Stage A. The period of the development of an ovarian into a fully-segmented ovum.

Stage B. The further development of the fully-segmented ovum prior to the formation of mesoblast. That is to say, the stage in which the hypoblast and epiblast are definitely formed.

Stage C. The formation of the mesoblast; and,

Stage D. The formation of the medullary groove and notochord, and the structure of the neurenteric canal.

Stage A.

I have been hitherto unable to satisfy myself as to the details of the process of segmentation in the ovum of the mole, but have been fortunate enough to obtain a fully-segmented ovum It was found at the upper end of the uterus, and its structure is as follows;

It is formed of a number of distinct cells, each surrounded by a cell-wall, and containing a nucleus. The cells are arranged in two layers (vide fig. 1), which I propose to name (1) the outer layer (*o. l.*), and (2) the inner mass (*i. m.*). The cells of the outer layer are more or less cubical in form, and are placed side by side in a single row. The main portion of each cell is composed of hyaline protoplasm, but along its inner border the protoplasm is finely granulated.

The cells of the inner mass are slightly smaller than those of the outer layer. They are irregularly polygonal in shape, and the protoplasm of which they consist is filled with large and small granules, rendering the cells opaque and dense.

The single row of outer layer cells closely invests and completely surrounds—except at one point—the inner mass of segments. At this point (*bl.* of B.), however, there is a break in the continuity of the outer layer, and here one of the granular cells of the inner mass projects on to the surface.

The ovum is surrounded by a thick membrane, the zona radiata (*z.*), which is in its turn enclosed in an irregular layer of hyaline gelatinous material (*m. c.*) derived from the uterus.

The zona is radially striated, and its outer edge has a granular appearance, which I have reason to believe, from an examination of ovarian ova, is due to the irregularity of its surface caused by the pressure of the follicular epithelium upon it while still in the ovary.

There is no albumen deposited round the ovum during its passage down the Fallopian tube, as is the case with the rabbit's ovum.

The ovum within the zona measures ·15 by ·17mm., while the inner mass measures ·1 by ·12 mm. in diameter; the outer layer being about ·05 mm. thick. The zona is ·01 mm. and the outer coat ·014 mm. in thickness.

The size of segmenting ova vary somewhat, but as a rule, while in the Fallopian tube, they measure between ·08 and ·1 mm. in diameter. This fully-segmented ovum shows a considerable increase on that size, and this is probably due to the absorption by it of nutritive material present in the uterus. It was examined first of all while fresh, but the details of its structure were rendered more apparent by treatment with silver nitrate. The figure was drawn after treatment.

The structure of the fully-segmented mole's ovum as described above is identical with that of the fully-segmented ovum of the rabbit which van Beneden has described (Nos. 4 and 5). According to this author the result of the first division of the ovum of the rabbit is the formation of two cells, the one of which is smaller and more granular than the other. The product of these two cells can be distinguished from one another throughout the process of segmentation, and Beneden finds the cells derived from the granular segment become involuted within those derived from the larger hyaline segment, and two layers are thus formed which he terms "entoderm" and "ectoderm" respectively, according to what he considers is their respective fate.

Further, the point where the involution took place remains open in the fully-segmented ovum, and gives rise to the gap in the outer layer, which is called by Beneden the "blasto-

pore," and compared by him to the blastopore of other Vertebrata.

The fully-segmented ovum is therefore considered by this author to be comparable to the gastrula stage of other vertebrata.

I have hitherto been unable to confirm the account given by Beneden of the segmentation, but am by no means therefore disposed to conclude his careful descriptions are inaccurate. At the same time it appears to me obvious, from the subsequent development of the mole, that his views of the homologies both of the two layers of segments and of the "blastopore" are incorrect.

In the first place, the so-called "entoderm" segments will be found to give rise to the greater part of the epiblast of the embryo; and in the second place, the structure of the primitive streak will be seen strongly to confirm Balfour's opinions (vide Nos. 1 and 3) of the homology of that organ with the blastopore of lower types.

Had Beneden's interpretations been correct, however, and had the inner mass really been entodermic, the fully-segmented mammalian ovum could not even then be compared to the gastrula condition of Amphioxus; for whereas the enteric cavity of the latter is within the entoderm cell mass, that of the former is eventually found to be outside those cells, and between them and the ectoderm.

Up to this point in the development no differentiation of the segmentation spheres into epiblast and hypoblast has yet taken place, and there is indeed, as I will show later, no evidence of any differentiation until some considerable time after the completion of segmentation.

The structure of the mammalian ovum at the close of Stage A is therefore seen to be, as far as we can now tell, entirely unlike that of any other animal; and until we have some knowledge of the steps by which mammals were evolved, it appears to me useless to attempt to draw any homologies.

It may be interesting to note that although the earliest conditions of mammalian development cannot be compared with

those of other animals, yet the further development proceeds (up to a certain point) the more strikingly similar these conditions become, and the usual rule that embryos of various animals differ from one another less in their earlier than in their later stages of development is therefore here reversed.

In concluding this section I would draw attention to the facts treated fully below (1) that the central position of the inner mass of segmentation spheres in both the rabbit and the mole is merely temporary, and that subsequently these cells, with the exception of a very small number, form a portion of the wall of a vesicle, the "blastodermic vesicle."

(2) That the so-called blastopore (Beneden) cannot be similar to the blastopore present in Amphioxus, and has merely a secondary origin, its existence being caused by the temporary involution of a portion of the wall of the blastodermic vesicle.

Stage B.

The Blastodermic Vesicle and the Formation of the Hypoblast and Epiblast of the Embryo.

The conversion of the fully-segmented ovum into the so-called blastodermic vesicle takes place shortly after the appearance of the ovum in the uterus. It is due partially to a flattening-out of the cells of the outer layer, and partially to the conversion of certain of the cells of the inner mass into outer layer cells.

The result of these changes is a vesicle the wall of which is composed of, for the most part, a single row of flattened cells, the much attenuated zona radiata surrounding the whole.

In the course of its growth the vesicle becomes so large that the wall of the uterus in the region where it is placed is distinctly swollen.

It is clearly impossible for the delicate-walled ovum to expand in the form of a vesicle, and distend the uterine walls by virtue of the growth of its cells; it must be therefore concluded that it obtains some support. This support is rendered from within.

The vesicle contains a transparent fluid, the nature of which I am only sufficiently conversant with to say that, after treatment with alcohol a white precipitate is present in the vesicle.

It is equally evident that this fluid can only have been obtained from the uterus, and that it is present within the vesicle at a very considerably greater pressure than in the uterus itself. Such a condition is caused by means of the cells of the wall of the vesicle; they secrete the fluid within the vesicle, this function being performed against a pressure which is greater on their inner than on their outer side, exactly as the cells of the salivary glands are known to act.

The uterine fluid is secreted by glands, present in great numbers in the uterine tissue, and is poured through their open mouths into the cavity of the uterus (vide fig. 51). There is every probability it has nutritive qualities, since it is thence taken up into the cavity of the embryonic vesicle, which eventually functions as a yolk-sac, in the walls of which embryonic blood-vessels ramify.

A specimen showing an early condition of this change of the segmented ovum into a vesicle has been drawn in optical section in fig. 2. It differs mainly from fig. 1 in that a crescent-shaped cavity (*bl. cav.*) exists between the inner mass and outer layer, this being the cavity of the blastodermic vesicle.

Van Beneden's blastopore has entirely disappeared, and I have no evidence to offer as to the position which it originally occupied; although there is good reason to believe, from a comparison of the development of the rabbit and mole with animals which exhibit the phenomena attending the inversion of the layers, that Beneden's statement is correct, viz. that the inner mass remains attached to that side of the outer layer where the gap was originally placed.

The appearance of the cells has altered but little; the outer layer cells are slightly more granular, while the cells of the inner mass are somewhat smaller and less granular than were those of the fully-segmented ovum.

The size of the two ova are different, the specimen from which

fig. 2 was drawn being smaller than the fully-segmented ovum and not larger, as would have been expected.

I can only attribute this condition either to the variation in size of different ova of the same age, of which fact I have abundant evidence, or to the effect of the preserving fluid, although in both instances the objects were treated with silver nitrate and preserved in glycerine.

However that may be—and this is the point which I wish to emphasise—the size of the inner mass in fig. 2 is relatively smaller than that in fig. 1, the diameter of the ovum (fig. 2) being ·12 mm., and that of the inner mass ·06 mm.

The ovum rapidly enlarges, and in fig. 3 the relation in size of the whole vesicle to the remnant of the inner mass is represented in an early stage of the development of the blastodermic vesicle.

The vesicle in this specimen is ·31 mm. and the inner mass ·04 mm. in diameter.

This increase in size is due to some extent, without doubt, to the flattening out and multiplication of outer layer cells (*vide* figs. 16—19); but I believe that up to this point in stage B the cells of the inner mass also contribute to that end.

I have been unable clearly to substantiate this opinion by means of sections, but the size of the inner mass in this specimen bears out my views; it is ·02 mm. less in diameter than the inner mass in the specimen figured in fig. 2, and ·07 mm. less than the inner mass of the fully-segmented ovum. Further, I have made measurements of a considerable number of specimens of a similar age, and have found this ratio to be almost uniformly constant.

The structure of the wall of the vesicle and of the inner mass at this stage is seen in figs. 16—19.

The vesicle wall is formed of much flattened polygonal cells closely attached to the zona radiata, which bounds them on their outer side.

The cells contain a large nucleus situated in the centre, and causing it to bulge towards the cavity of the vesicle.

The nucleus in section appears to be of oval form, while in

a surface view (vide fig. 4) it is seen to be rounded. The oval shape in section is due to it being flattened out, and it is for this reason also that the nuclei of the outer layer appear in a surface view larger than those of the inner mass (fig. 4).

The inner mass is solid, more or less rounded in form, and is attached on one side to the wall of the vesicle. The cells of which it is made up are always, after treatment with picric acid, closely adherent to one another, and are sharply marked off from the cavity of the vesicle (vide figs. 17 and 18).

The specimen drawn in fig. 19, however, was treated with silver nitrate and preserved in weak glycerine, afterwards being transferred to spirit, embedded, and cut into sections; in it the cells are much more loosely held together, and in another specimen I have, which was similarly preserved, the same appearance presents itself.

The irregularly-rounded cells of the inner mass, which are very considerably smaller than either the cells of the inner mass in the fully-segmented ovum, or of the specimen drawn in fig. 2, are composed of granular protoplasm, and many of their nuclei exhibit the modifications attending cell division.

As the vesicle continues to enlarge the inner mass also now increases in size, changes its shape, and becomes flattened out along the side where it adjoins the outer layer; and further, the cells of which it is now composed become differentiated into two layers.

The differentiation occurs in the following manner:—Certain of the cells bordering the blastodermic cavity become separated off from the main portion of the inner mass, and form a single layer of cells bounding the mass on its inner side.

This layer is the hypoblast.

The hypoblast is, therefore, derived from cells which result from the multiplication of the inner cell mass present in the fully-segmented ovum.

Figs. 20 to 23 adequately represent these changes as they take place; the cells here and there along the lower border of the inner mass become more flattened than their fellows, and stain more deeply with hæmatoxylin (fig. 20); gradually a

continuous layer exhibits these phenomena (fig. 21), and then become separated from the remainder of the mass (fig. 22).

The remaining cells of the inner mass increase in number, and assume a columnar form, at the same time becoming separated by a narrow cavity in the centre from the outer layer. The cells of the latter layer in the region of the cavity also increase in number and become thicker than their fellows (fig. 23).

The further development of the hypoblast may be stated in a few words; it extends laterally by virtue of the multiplication of its cells, which at the same time become considerably flattened. Later on, as may be seen in fig. 28, the cells are again more rounded, and, indeed, at different stages during the formation of the layers, they assume various proportions. It is to be noted that this layer, after being once completely separated off from the inner mass (vide fig. 23), remains separate until the mesoblast is formed, and increases, therefore, wholly by the division of its own cells.

The hypoblast eventually completely surrounds the whole of the blastodermic vesicle.

The changes which take place in the remaining portion of the inner mass and in the outer layer adjoining it are somewhat more complicated.

1. The inner mass increases in size and its columnar cells, arranged in a double row, form an hemispherical plate, the edge of which rests upon and is continuous with the cells of the outer layer. In consequence of this the narrow cavity mentioned above assumes considerably greater proportions; it is bounded below and at the sides by the plate, and above by the outer layer; at the same time it becomes partially filled up by branched stellate cells, which are derived from the cells of the outer layer.

This cavity may be termed the secondary cavity of the blastodermic vesicle in contradistinction to the cavity which is formed at the close of stage A, and which also arises, although at the opposite side, between the outer layer and inner mass.

Fig. 5 is a drawing of an ovum at this stage of growth.

The opaque inner mass is seen attached to the wall of the vesicle, and in the centre of the mass a lighter coloured space indicates the presence of the secondary cavity.

The relations of these parts are, however, more clearly seen in fig. 24, which represents a section through the centre of the inner mass of the ovum drawn in fig. 5. The single row of long columnar cells (fig. 23) has given place to a double row of more cubical and broader cells which are continuous with the cells of the outer layer at the circumference of the plate.

The hypoblast lies free below the inner mass and stretches out laterally beyond the area of the latter.

The cells filling up the secondary cavity are stellate, and are connected with both the outer layer and inner mass by means of protoplasmic processes; the size and general appearance of the cells and of their nuclei, however, as well as the manner in which they stain with hæmatoxylin, leaves little room for doubt in my mind that they are derived from the former (outer) layer.

2. The plate of cells now changes its form and becomes flattened out and applied closely to the zona above, the stellate cells within the secondary cavity and the outer layer cells above uniting with it, and the secondary cavity is obliterated. The structure resulting from these changes is the epiblast plate of the embryonic area.

Reference to figs. 7, 25, 26 and 27, will, I think, substantiate this view.

Fig. 7 is a surface view of the inner mass represented in section in fig. 25. The section is cut along the line of the greatest diameter of the mass, and shows the commencement of the process of the flattening out of the plate.

The flattening occurs in the first place along one side, the secondary cavity being there much shallower, while elsewhere it is as deep as before. This arrangement gives rise to the appearance seen in fig. 7, in which the light, crescent-shaped area at one side of the inner mass is the deeper portion of the cavity (compare figs. 7 and 25).

In all the sections of this inner mass only a few cells were to

be seen in the secondary cavity, and in the section here figured there were none present. I have, however, never found this to be the case in any other specimen, and imagine they must have been displaced during the process I then used of cutting and mounting sections.

The flattening process afterwards extends all round the edge of the inner mass and the cavity is throughout much shallowed (fig. 26), cells are present within the secondary cavity, and are seen in this section becoming incorporated with the plate of columnar cells. The edge of the plate, as I before mentioned, is continuous with the cells of the outer layer, and at a slightly later stage, when the plate is completely flattened out, it occupies the position until then held by that portion of the outer layer which overlay the inner mass.

At this stage the two layers are indistinguishable from one another, but wedge-shaped cells can be observed in the upper portion of the plate (fig. 27 *t c*) which on account of their shape, the direction of the long axis of their oval nucleus, and the position they occupy appear to me without doubt to have been derived from the cells of the outer layer.[1]

Up to this point in the development the blastodermic vesicle lies free within the cavity of the uterus, and can be obtained therefrom without difficulty by merely slitting up the uterus with scissors and transferring the ovum upon the point of a scalpel to a watch-glass containing the hardening reagent. This method is, however, no longer possible when the ovum attains a very slightly older stage. It then becomes still further enlarged and its walls project into the widely open mouths of the uterine glands. I find no actual attachment between the two, and have not been able to distinguish any outgrowths from the zona such as Bischoff described for the rabbit and dog (Nos. 6 and 7).

The only method which I have found to enable me to obtain the fresh vesicle entire, is to sink the uterus, after being cut open, with the ovum in *situ*, slowly in a vessel of salt so-

[1] In support of this view see below, an account of a stage in the formation of the epiblast of the rabbit.

lution, it then becomes possible to separate the two without damage.

Figs. 8 and 9 represent very faithfully the appearance of ova of this age obtained by the above method.

The wall of the vesicle is bulged out here and there into papillæ where it projected into the mouths of the glands. The elongated condition is due to the fact that when the uterus was slit open the vesicle fell into that shape in which it received the greatest amount of support from the surrounding tissue.

If it is not desired to examine the vesicle in a fresh state it will be found advantageous to harden the embryo within the uterus, and to dissect it out afterwards which is an easy matter.

In order to show the position and condition of the uterine glands, I have drawn in fig. 51 a transverse section through that region of the uterus from which the ovum represented in fig. 8 was obtained.

It will be seen that only on the free non-mesometric side of the uterus are there any widely open mouths of glands; while upon reference to figs. 8 and 9, only that side of the ovum around the embryonic area is seen to be prolonged into papillæ-form projections, and as the embryonic area lies against the non-mesometric side of the uterus in the mole we may conclude the projections lie in the mouths of the glands. A portion of the epithelium of the uterus abstracted from the latter is drawn in fig. 52; it is seen to be prolonged into hollow finger-like processes which line the uterine glands.

Transverse sections of the embryonic area of this embryo (fig. 28) show that it is formed throughout of two layers of cells, epiblast and hypoblast.

One of the prolongations of the vesicle wall has been cut at one side of the embryonic area (*pl*) and another is shown in fig. 29. They are seen to be formed wholly of epiblast, the hypoblast not being extended into them.

Of the hypoblast layer in the area I have nothing to add to the account already given; the epiblast, however, has under-

gone a slight change since we last examined it, inasmuch as it now consists for the most part of a single row of columnar cells, which at the sides of the area gradually become less and less columnar and eventually merge into the flattened epiblast cells of the wall of the vesicle. This change is, however, temporary, since in sections of older embryonic areas the epiblast is again two layers deep (figs. 33, &c.).

Fig. 30 is a transverse section of the area drawn in fig. 10; it is very similar to fig. 28; but the edges of the embryonic area in this case appear to end abruptly, the wall of the vesicle having been torn away owing to its close attachment to the uterus.

The condition of the ovum is now considerably changed from what it was when the blastodermic cavity first appeared; it may be divided into two areas, the embryonic and non-embryonic areas. The embryonic area is throughout composed of an outer thickened layer of columnar epiblast cells which has been derived partially from a portion of the inner mass and partially from outer layer cells, and an inner layer of somewhat rounded hypoblast cells derived entirely from cells of the inner mass. The non-embryonic portion of the ovum may in its turn be divided into two regions.

First, the region immediately surrounding the embryonic area which is formed of two layers, an outer of flattened outer layer cells now known as epiblast cells, continuous with the epiblast of the area, and an inner of flattened hypoblast cells continuous with the same layer in the embryonic portion of the ovum.

Secondly, the region situated at the opposite pole of the ovum to the embryonic area, where a single row of flat epiblast alone exists.

Historical.—The details attending the formation of the epiblast has given rise to a considerable amount of discussion. According to Edward van Beneden (No. 5) the fully-segmented ovum of the rabbit develops into the blastodermic vesicle by a multiplication and a flattening of the outer layer cells, the inner

mass remaining the same in size, and attached to the outer layer in the region of the now-closed "blastopore." Subsequently the inner mass flattens out and splits up into two layers, the lower of which forms the hypoblast and the upper the mesoblast of the embryonic area, the epiblast being formed solely by the multiplication of the outer layer cells, which become at the same time columnar and arranged in a single row.

Beneden, therefore, has not found a stage in which two layers only exist throughout the area.

Rauber (No. 21), in a previously written paper, finds three layers present in the embryonic area of a rabbit before the formation of the primitive streak; the outer of these (my outer layer) he calls the "Deckschicht," and states that it early disappears, while the middle layer alone forms the epiblast and the lower the hypoblast of the area; a two-layered area being thus formed.

Kölliker, in a recent elaborate paper (No. 16), traces the fate of the three layers, which he also finds in common with Rauber and Beneden, and declares, in accordance with the views of the former author, that the outer layer gradually disappears, the middle forming the epiblast and the lower the hypoblast of the embryo. The details of the gradual disappearance of the Deckschicht occupy much of this paper. Professor Kölliker has never seen the cells of this layer assume a columnar form, as Beneden asserts is the case, and by means of nitrate of silver staining he satisfies himself they gradually become broken up, and eventually disappear altogether.

Lieberkühn (No. 19) gives an account of the formation of the epiblast in the dog and mole which is very similar to my own, in that he considers it is formed of the greater portion of the inner mass, together with that portion of the outer layer cells which originally overlaid it. He also draws attention to the cavity which appears, according to him, within the inner mass of cells in the mole, and which he suggests may be comparable to the segmentation cavity of other animals.

Hensen (No. 12) for the rabbit, and Schäfer (No. 25) for the

cat, also describe a two-layered stage of the embryonic area prior to the formation of the primitive streak.

Summary.—With regard to my own work I hold that the blastodermic vesicle increases in size, not merely on account of the increase in number and the flattening of the outer layer cells, as Beneden believes, but by the migration of inner mass cells to the exterior. This view is supported by the fact that the inner mass decreases in size during the early development of the vesicle. I have also satisfied myself of the existence, both in the mole and rabbit, of a stage in which the embryonic area is composed of only two layers, the epiblast and hypoblast.

The hypoblast I have shown to be derived from the cells of the inner mass—a fact which all the observers above mentioned are agreed upon.

The epiblast I believe to be formed, as does Lieberkühn, of the remaining portion of the inner mass, after the hypoblast has been detached, together with that portion of the outer layer which overlies the inner mass.

In the mole this includes also certain cells which we have seen are derived from the outer layer, and which at one time lie in a cavity between that layer and the inner mass.[1] In the rabbit, however, no such cells exist, and I believe that the epiblast is formed of inner and outer layer cells.

With reference to the development of the epiblast in the rabbit I may saythat since working at the question under the supervision of the late Professor Balfour (No. 3), I have examined more embryos, and have been fortunate enough to obtain good sections of the embryonic area of a rabbit embryo of six days four hours old, which appear to me to be conclusively in favour of the view we were then inclined to accept. Fig. 49 represents a section through this area; in it the epiblast plate is seen to be composed of two entirely different kinds of cells—(1) a lower more or less columnar or rounded cell, and (2) an upper flattened or wedge-shaped cell. The latter cells invariably occupy a position on the outer side of the plate, across

[1] In a previous paper (No. 11) I erroneously described these cells as being derived from the inner mass.

which they form an almost continuous layer, and they are distinctly darker stained than are the deeper placed, more columnar cells. They are generally wide at the top, ending below in a wedge-shaped base, which grows downwards between two of the columnar cells lying beneath. Some of the cells are, however, more flattened, possessing no downward prolongation, and some are more columnar, having little or no expanded upper surface; indeed, there are cells in all stages of transition, between the flattened outer layer cells of the previous stage and the columnar cells of the future epiblast plate (vide fig. 49, *t. c.*).

Kölliker's valuable paper contains most careful descriptions and drawings, which, however, appear to me to be capable of a very different interpretation from that put forward by him; in fact, they appear to me to be strongly confirmatory of my own views. He states that the large nucleated plates which are visible in surface views of young areas split up in older embryos into small polygonal areas without nuclei. Now, I would venture to suggest that the disappearance of the nuclei of these large outer layer plates can be fully accounted for by their migration downwards among the cells of the inner mass (vide fig. 49): and the apparent breaking up of the large cells may be explained by the actual appearance on the surface of the epiblast plate, of the polygonal ends of the columnar cells of which it is now composed.

Stage C.

The Formation of the Mesoblast.

The middle germinal layer has two distinct sources: in the first place it arises from the epiblast and hypoblast at the hind end of the embryonic area, in the structure known as the primitive streak; and, secondly, from the hypoblast alone in the anterior region of the area in front of the primitive streak.

The Primitive Streak Mesoblast.

The primitive streak originally appears at the hind end of an area similar to the one represented in fig. 10, its presence being shown in surface view by a slight opacity.

Fig. 31 is a longitudinal section through such an area, along the middle line. The anterior portion of the area consists of a layer of columnar epiblast, and a somewhat flattened layer of hypoblast: at the hind end, however, a passage perforates the blastoderm and surrounding it the epiblast and hypoblast become continuous with one another, forming the wall of the perforation. The opening is wider below than above, and owing, I believe, to the curved condition of this specimen, was not visible from the surface. The whole length of the area is not drawn in the figure, and the portion anterior to the spot at which the reference letters *ep.* are placed, was bent back, and underlay the hinder portion of the area.

The cells forming the wall of the passage give rise to the first mesoblast cells, which are thus derived from epiblast and hypoblast conjointly; they extend in front and laterally for a short distance only as a thin sheet lying free between the two primary layers, while posteriorly they form a thicker layer and are united with the epiblast in the middle line.

From this point the primitive streak extends backwards, the embryonic area itself enlarging in that direction.

Figs. 11 and 12 are surface views of two areas, in which the primitive streak represented by the dark shading is well defined. In the former, which is the younger of the two, the opaque band extends about half way across the oval area, spreading out behind into two short horns; and down the centre of the band a lighter streak may be seen, which is caused by a groove in the epiblast, and is the well-known primitive groove.

At the front end of the primitive groove there is distinct evidence in section of the involution of the epiblast, although no actual perforation of the blastoderm exists. This I consider

is the point where in the earlier specimen the blastoderm was perforated (fig. 31), the increased size of the area being due hitherto to a growth backwards.

Fig. 12 represents the most advanced condition of the primitive streak. The embryonic area is pyriform, and the primitive streak is considerably longer than in the former specimen (fig. 11), and extends relatively further along the area; it is more opaque, and ends behind in a dark rounded mass or knob.

I could distinguish no primitive groove by an examination of the surface, and am obliged, therefore, to rely chiefly upon sections to determine the relations of the growth. Near the front end of the streak there is here also distinct evidence of an involution of the epiblast, although there is no actual perforation; and I am inclined to believe this point is identical, both with the front end of the primitive streak in fig. 11, and with the point where the perforation exists in the younger embryo (vide fig. 31). It is a curious fact, however, that the extent of the area anterior to the front end of the primitive streak appears to be less in this area than in the younger one (fig. 11), while the length of the primitive streak in fig. 12 is greater than that in the older embryos (figs. 13—14).

The presence of the involuted point at the front end of the streak appears to me to favour the view that this structure has not grown forwards, while the addition of the pyriform hind end is an argument in favour of its backward growth.

The reduction in size of that portion of the area anterior to the primitive streak may possibly be due to curvature, but this I am unable definitely to decide.

The eventual reduction in length of the primitive streak is more easily comprehensible, and is doubtless due to the widening out of the end knob, this structure having disappeared in older embryos.

I have frequently observed in surface views a darkly-shaded spot at the front end of the primitive streak, which is spoken of as the node of Hensen, and find that it corresponds with the spot where the three layers unite. It may also be seen some-

times when there is no other superficial evidence of the existence of the primitive streak, but in these cases I have invariably found by sections that a primitive streak does exist, but that the mesoblast to which it has given rise is so uniformly distributed everywhere except at the front end, that it is only there apparent.

The structure of the primitive streak is different in different parts, to illustrate which I have figured sections (figs. 33—36) through various regions of the blastoderm drawn in fig. 12.

The first section (fig. 33) is taken through the anterior portion of the primitive streak. A plate of columnar epiblast cells extends across the area; it is thinner at each edge, but of uniform thickness elsewhere, except in the middle line, where a keel-like ridge is formed. The upper half of the keel is wide and joins the epiblast, with the cells of which it is continuous, and the lower portion projects into a mass of cells below, but has no connection with them. These underlying cells I will deal with later, and will in this place merely draw attention to the fact that the lower borders of the cells of the keel are sharply marked off from them, and that these somewhat oval cells lying below the keel of epiblast are entirely different, both in shape and character, from the cells above them.

The second section is taken close behind the first; it passes through the front end of the primitive groove, and is, I believe, in an analogous position to the point immediately behind the perforation existing in the embryo, of which fig. 31 is a longitudinal section.

The epiblast is curved in the middle line constituting the primitive groove, and from the cells of this portion of the epiblast, mesoblast is produced.

Immediately below the primitive groove there is no layer of hypoblast to be distinguished, and here mesoblast is produced from hypoblast cells. Laterally all three layers are distinct, but in the middle line they may be said to combine with one another, and in this region, therefore, the middle layer is formed from both epiblast and hypoblast. The former does not here extend beyond the boundary of the embryonic area.

Between the two sections described above, the cord of cells (fig. 33) joins the front end of the mass of cells formed by the union of the epiblast and hypoblast in the middle line; and where this junction occurs there is distinct evidence of an involution of the epiblast layer.

From the front end of the primitive streak a tongue-shaped cord of mesoblast cells is projected forwards into the mass of cells underlying the epiblast in that region, and gives rise to the lighter shaded prolongation of the primitive streak seen in fig. 12.

I have been unable to find any complete perforation of the blastoderm at this stage of growth, although in a somewhat younger embryo and in an older one in which the medullary groove is formed, there is no doubt that it exists. I have, therefore, either missed the section in which the perforation occurred in this embryo, or it has been closed up by the rapid production of mesoblast which at this stage takes place.

Although I have only seen a complete perforation of the blastoderm in one embryo during the primitive streak stage, I have invariably found at the front end of the primitive streak evidence of an involution of the epiblast; on this account, as well as for reasons which will appear in the sequel, I conclude the front end of the primitive groove is the spot where the perforation of the blastoderm seen in fig. 31 occurs at an earlier and later stage. The cord of cells described in the first section is the front wall of the perforation seen in fig. 31, and the tongue of mesoblast projecting forwards is homologous with the anterior growth of mesoblast also seen in the younger embryo.

This statement is supported by a study of sections of an area but slightly older than that drawn in fig. 11, and somewhat younger than the one we have been considering. In this area there was no layer of cells underlying the epiblast at the anterior end of the primitive streak, and the behaviour of the forward growth of mesoblast from the front end of that structure could be more definitely determined. At the front

end of the primitive streak, at a point relatively similar to that drawn in section in fig. 34, the epiblast was involuted in the middle line and a deep pit formed which opened below into mesoblast, which is budded off from the lips of the ingrowth. At this point the epiblast, mesoblast, and hypoblast were united in the middle line, but in front of it an axial rod of mesoblast projected forwards for a short distance distinct from both epiblast and hypoblast, but soon becoming attached to, and indistinguishable from, the hypoblast. In this condition it may be spoken of as a thickened axial rod of hypoblast, and as such it extends forwards for some sections, gradually becoming reduced in size and eventually giving place to the single row of rounded hypoblast which elsewhere existed below the epiblast in front of the primitive streak.

The third section (fig. 35) demonstrates the structure of the area throughout the remainder of the primitive streak in front of the end knob. It is similar to fig. 34 except that (1) the hypoblast forms a complete layer across the whole of the area, and is nowhere combined with the layer of mesoblast; (2) the primitive groove is not present, and the number of epiblast cells concerned in the formation of mesoblast is greater than before, and (3) the mesoblast extends laterally, lying freely between the epiblast and hypoblast, outside the limits of the area. This is a typical section through the middle of the primitive streak of all the specimens I have examined, and it may be generally stated that throughout this region the epiblast only gives rise to mesoblast.

In the knob at the hind end of the primitive streak (fig. 36) the three layers are again seen to be closely combined, the hypoblast being indistinguishable from the mesoblast, and the epiblast throughout nearly the whole breadth of the area giving rise to mesoblast cells; there is also a much greater mass of the latter layer extending some distance beyond the limits of the area, which in this region is very narrow.

The junction of hypoblast and mesoblast does not appear to occcur in this region in all specimens, although the embryo

from which this section was taken is not singular in exhibiting such a relation between the two.

The Hypoblastic Mesoblast.

A single layer of rounded hypoblast cells similar to those represented in section in fig. 30 is present throughout the lighter shaded anterior portion of the area drawn in fig. 11. At a somewhat later stage, however, these rounded cells in the region on each side of the thickened axial hypoblast, in front of the primitive streak, give rise to cells from which they are themselves indistinguishable; gradually the hypoblast situated anteriorly follows suit, and eventually the whole of that portion of the area in front of the primitive streak consists of a plate of epiblast below which lies a mass of cells several layers deep. These cells are rounded and appear throughout as do the lateral masses of cells below the epiblast in fig. 33.

Fig. 32 represents a section through the anterior region of the area drawn in fig. 12, a glance at which will, I think, prove the origin of these cells from the hypoblast.

It appears to me that the continuity of the intermediate layer with either of the primary layers is a safe guide as to the origin of the former—by continuity, I mean such relations as are shown at the node of Henson (fig. 34), where the boundaries of the three layers cannot be distinguished;—and if this be true I imagine there can be little doubt as to the origin of the mass of cells above described.

At a later period of development these cells become split up laterally into two layers, a lower single layer of flattened hypoblast and an upper layer of mesoblast several rows deep. This differentiation takes place from behind forwards, as does the original formation of this layer. These relations are seen by comparing figs. 32 and 33; in the former there is no trace of a separation of the cells into hypoblast and mesoblast, while further back (fig. 33), several cells (*hy*) along the lower border of the mass are more flattened, their nuclei more elongated, and they stain more deeply with hæmatoxylin than do the remainder of the cells;

these become hypoblast cells. In the axial line no such change occurs, and the mass of cells existing there is continuous behind (by means of the axial rod described on p. 430) with the front end of the primitive streak, and continuous laterally with both the hypoblast and the mesoblast. Fig. 42, although it is a section through a considerably older embryo, represents these relations fairly accurately.

The axial mass of cells eventually gives rise to the notochord. The lateral mesoblast may be called hypoblastic mesoblast in accordance with its origin, and to distinguish it from the mesoblast of the primitive streak. The lateral masses of hypoblastic mesoblast adjoin posteriorly the mesoblast of the primitive streak, and it does not appear to me to be possible, with the existing methods of discrimination, to determine the exact extent of either layer; roughly, however, we may say that the front end of the primitive streak is the boundary line.

At the stage of development now reached the embryo may be compared with that of Amphioxus, as far as its structure is concerned in front of the primitive streak; two masses of mesoblast are formed from the hypoblast laterally and the axial hypoblast thickens and gives rise to the notochord. The latter is similar to the median diverticulum of the enteric cavity of Amphioxus, and the lateral masses of mesoblast to the mesoblast of the united diverticula on each side in that animal; the lateral diverticula do not, however, appear, but the median one is, as we shall see, formed later.

With regard to the embryonic vesicle it is much larger than in the previous stage, and no longer projects into the mouths of the uterine glands, but is exceedingly closely applied to the uterine epithelium, so closely that some of the latter is generally pulled away from the uterus when the ovum is obtained whole. Fig. 53 is a section of a portion of the vesicle wall which is formed of flattened epiblast only, and of the uterine epithelium to which it is closely adherent.

Historical.—Various accounts have been given by different observers as to the origin of mesoblast in mammalian embryos.

Beneden (No. 5) describes that portion of the inner mass which remains after the hypoblast is separated from it, as mesoblast, and states that it retreats to the hinder end of the embryonic area, becomes secondarily united with the epiblast, and gives rise to the mesoblast of the embryo.

Rauber (No. 21), Kölliker (No. 16), Hensen (No. 12), and Lieberkühn (No. 19), argue that the mesoblast arises first in the primitive streak. Kölliker considers that the epiblast alone gives rise to it, and that after being formed in the primitive streak it spreads, eventually, over the whole embryonic area, and also supplies the mesoblast of the area opaca. From this author's statement I gather he considers the primitive streak arises first in the end knob (Endwuldst), and extends from thence forwards.

Lieberkühn differs from Kölliker, and agrees with Hensen, in that he derives the mesoblast of the primitive streak from both epiblast and hypoblast; while Hensen differs from the other observers mentioned, in considering a certain amount of the mesoblast of the area opaca to be formed in sitû from hypoblast.

Summary.—My own observations lead me to differ entirely from Beneden as to the formation of mesoblast, and to agree with the other observers mentioned above in concluding that it is first formed in the primitive streak. I cannot, however, accept Kölliker's statement, that the epiblast is alone responsible for its production, and that it is first formed in the hind knob. I consider that the hind knob is formed some time after the first portion of the primitive streak, and that the formation of the latter takes place from before backwards instead of from behind forwards, as this observer states; my main reason for this being the universal presence at the anterior end of the primitive streak of an indication of the involution of the epiblast.

Again, I agree most distinctly with Hensen and Lieberkühn in regarding the epiblast and hypoblast as the originators of mesoblast at the front end of the primitive streak, but I must differ from them, and from Kölliker (loc. cit.) and Schäfer

(No. 26), in believing that the primitive streak mesoblast supplies the whole of the embryonic area.

With regard to this point my results are in entire agreement with those of Balfour and Deighton, expressed in their account of the development of the chick (No. 2), who consider that the anterior portion of the mesoblast is derived as two lateral plates from the hypoblast, while the axial hypoblast gives rise to the notochord.

Further, the similarity of the origin of the epiblast, hypoblast, and mesoblast of the embryo and of the notochord in the mammal is so strikingly similar to the relations of the same organs in Amphioxus that Kölliker's (loc. cit.) statements as to the dissimilarity of the germinal layers of mammals with those of other animals appears to me to require some modification; and Repiachoff's recently expressed opinions (No. 24) that there is no homology between the germinal layers of higher Vertebrata and Amphioxus, receives no support from what is known of mammalian embryology.

Finally, it is very generally believed that mammals are descended from animals which possessed a large yolk sac, and it is stated that the blastodermic vesicle is a remnant of this yolk sac. If this be true (and as far as we know there seems to me to be no reason to doubt it), the primitive streak of mammals is homologous with the same structure in birds, and the existence of such an arrangement, together with the presence of a complete neurenteric canal (which I shall describe later) in the mammal, is another instance of the morphological facts which led Balfour (No. 1) to conclude that the primitive streak was homologous with the true vertebrate blastopore.

The views as to the relations of the layers at the front end of the primitive streak will be more advantageously noticed in the following section.

Stage D.

The Medullary Groove, Notochord, and Neurenteric Canal.

The main differences in the superficial appearance in an embryo of this stage of growth are:

1. The disappearance of the hind knob of the primitive streak and the widening out of that portion of the area.

2. The great enlargement of the area in front of the primitive streak, and

3. The appearance in the latter portion of the area of a broad, light-coloured band, the limits of which are at first vague, but which gradually become more emphasised.

This is the medullary groove which first arises near the anterior end of the primitive streak, and from there extends forwards.

Fig. 13 represents an embryonic area, in which a shallow medullary groove is formed; the extent of the groove is faintly indicated in front; at the sides it is more definitely marked off; while behind it abuts upon the anterior end of the primitive streak, and terminates abruptly.

At the junction of the medullary groove and primitive streak a deep pit is visible; this is the dorsal opening of the neurenteric passage, which, however, does not appear completely to perforate the blastoderm at this stage.

The primitive streak extends from the hind end of the medullary groove to the edge of the blastoderm, spreading out there into two horns.

In fig. 14 the medullary groove is more distinctly indicated; a tongue-shaped band extends anteriorly from the front end of the groove towards the edge of the blastoderm in that direction, and is the anterior end of the thickened axial mass of cells underlying the epiblast.

The primitive streak meets the opposite end of the medullary groove, and sends a forward prolongation between its divergent walls.

There was no indication, as far as I could see from the surface, of a dorsal opening to a neurenteric canal in this embryo. The growth from the front end of the primitive streak is similar to what has already been noticed in younger embryos (fig. 31 and p. 431).

Fig. 15 is a drawing of the hind end of the medullary groove of a still older embryo. Here the walls of the hind end of the groove, hitherto widely separate, have joined each other, and have enclosed within the groove the front end of the primitive streak, and with it the hinder dorsal opening of the neurenteric passage. From the front end of the primitive streak a prolongation is sent forward similar to that seen in fig. 14.

The walls of the groove are now distinct.

The structure of the groove of such an embryo as that drawn in fig. 13 is represented in transverse section in fig. 43. The plate of epiblast is thin where it is grooved, on each side, however, becoming about double the thickness, and then gradually thinning off until it is only a single layer deep at the edge of the area; here it is curved upwards, and thus indicates the commencement of the amnion.

The cells underlying the epiblast in this region are divided in the same manner as we have seen are those of a much younger embryo (stage c, p. 434), into (1) lateral plates of mesoblast and hypoblast, and (2) an axial mass of cells, the commencing notochord showing no differentiation into those layers.

It will be noticed, however, that the axial cells are considerably more isolated from the lateral masses than heretofore, although still continuous with the latter. The lateral mesoblast is thick, and at the edge of the area becomes divided into two layers, which are the future somatic and splanchnic mesoblasts.

These relations remain the same throughout the medullary groove, excepting that at the posterior end the axial notochordal cells become thicker and join a forward growth from the front end of the primitive streak, while at the anterior end the groove widens, and all the cells underlying the epiblast

come into closer relations with one another. Fig. 42 represents the latter condition in a somewhat similar embryo.

Anterior to the medullary groove the lateral hypoblast and mesoblast are not yet separated, and a continuous mass of undifferentiated cells underlies the epiblast plate.

Behind the groove the primitive streak occasions changes identical with those already described (p. 438).

The mesoblast throughout the embryo projects beyond the limits of the area, and is there split into somatic and splanchnic layers. The relations of the neurenteric canal I will describe in detail in another place (on p. 440); for this specimen I will only say that at the junction of the primitive streak and medullary groove a deep pit is formed by the involution of the epiblast in the middle line; the pit is widely open above, but enters a mass of mesoblast below, and is there, as far as I could see, entirely obliterated.

The groove now deepens, forcing the notochordal cells underlying it further downwards, and in this way the latter, while remaining connected with the hypoblast, becomes separated from the lateral masses of mesoblast. Such relations are shown in fig. 44, which is a transverse section through the medullary groove of an embryo slightly older than fig. 13, taken from the same relative position as the section in fig. 43.

This is, however, the deepest portion of the medullary groove, and only in this section and those immediately on each side of it do the relations hold which are here figured. Both anteriorly and posteriorly the groove is more shallow, and the axial hypoblast is continuous with both lateral mesoblast and hypoblast.

The structure of the remainder of the embryo is identical with that described above for fig. 13.

The amnion in this embryo is completely formed over the hind end of the primitive streak, although not so far advanced at the front end of the area.

In describing the next embryo I will give an account of the structure of the neurenteric canal.

The arrangement of the layers at the front end of the primi-

tive streak and the structure of the neurenteric canal at this stage of growth will readily be understood by a glance at the drawing of the surface view of an embryo (fig. 15), and comparing it with the diagrammatic longitudinal section in fig. 50 and the transverse sections in figs. 37 to 41. The latter are taken from an embryo of an age between that of fig. 13 and fig. 14, and the walls of the medullary canal do not yet enclose the front end of the primitive streak, although the latter is already placed between them.

The longitudinal section is taken from a younger embryo.

The dorsal hinder opening of the neurenteric canal (figs. 15 and 37) is formed by an involution of the epiblast in the middle line at the head end of the primitive streak, and the separation of the lips of the involuted layer.

The passage so formed enters the mass of mesoblast cells, budded off from epiblast and hypoblast in this region, as it does in the embryo of which fig. 13 is a drawing; but it does not end there, it travels forwards almost parallel to the plane of the layers, and is seen eight sections further forward (fig. 38) as a canal within the axial mass of mesoblast, which we have invariably seen to be projected anteriorly from the front end of the primitive streak. There is no doubt the cells surrounding the canal at this point are mesoblast cells; they are continuous with the epiblast in the middle line and with the lateral mesoblast, and there is a distinct layer of hypoblast below them (compare fig. 38, and fig. 50 immediately in front of *p. sk'*); gradually, however, the canal dips downwards, and as this prolonged cord of mesoblast joins anteriorly the axial hypoblast, the walls of the canal also there, some sixteen sections in front of fig. 37, become hypoblastic (vide fig. 39). Here the lateral mesoblast does not join the thickened axial hypoblast, which is continuous with the lateral hypoblast only.

Three sections further on the axial cells become continuous with both lateral mesoblast and hypoblast. The lower wall of the canal now shows signs of becoming thinner (fig. 40), and five sections beyond this, that is, twenty-four sections from the hind opening, it becomes divided in the median line, and the

neurenteric canal opens below to the cavity of the vesicle (fig. 41). The arrangement of the layers at the front end of the primitive streak may be shortly described, therefore, as follows:—The epiblast and hypoblast meet and form the hind wall of the dorsal opening of the neurenteric canal; from the front portion of this wall a tongue of mesoblast is projected forwards, separated from the underlying hypoblast, but united with the lateral mesoblast and with the epiblast in the middle line; it then joins the thickened axial hypoblast, and becomes freed from the lateral mesoblast (fig. 39), while anteriorly to this point the axial cells are continuous with both lateral hypoblast and mesoblast.

With regard to the structure of the remainder of the embryo, the medullary groove is shallow and wide, and throughout its length the axial hypoblast causes a swelling upwards of the bottom of the groove. The lateral hypoblast and mesoblast join the notochordal cells throughout the region where the latter exist.

The notochord is formed of cubical or columnar cells, and is alternately in the form of an arch and a tube throughout the whole length of the medullary groove (vide figs. 40 and 41). Beyond the groove it becomes more flattened out (fig. 42), and the arrangement there is similar to that described for fig. 13.

This tubular form of the notochord appears to be very transitory, as I have not met with it in any other embryo except in that drawn in fig. 14, in which it is not either so definite or so continuous.

The sections through the area represented in fig. 14 show a slightly different arrangement. Fig. 47 is taken through nearly the same region as are the sections from which figs. 43 and 44 were drawn. The medullary groove is much the same as is represented in fig. 43, but the notochord is less substantial, and the single row of somewhat cubical cells of which it is composed form an arch whose cavity opens into that of the vesicle below. It will be obvious that the thickness of the notochordal cells is much less than in fig. 41, and that the arch

is not so completely tubular. The lateral mesoblast is not continuous with the axial hypoblast (notochordal) cells in the middle and deeper portion of the groove, but such is the case both further forwards and backwards.

Immediately beyond the anterior end of the groove the flattened epiblast is thickened to form the medullary plate, and below it the arched notochordal cells form a complete tube, the structure of which is similar to that already described excepting that the lower wall of the tube is thicker.

Fig. 46 is a section through this region of fig. 14, the dark streak seen in surface view being accounted for by this thickened mass of notochordal cells; it is the only portion of the notochord in the anterior region which remains thickened at this stage of growth. The relations of the layers at the hind end of the area are the same as are described on p.

At a stage slightly older than that represented in fig. 14 the medullary groove is still deeper. A section (fig. 45) taken through about the same region as are those drawn in figs. 43 and 44 demonstrates this. The epiblast now exhibits a further change, that portion of it forming the walls of the groove are for the most part but slightly thicker than heretofore, but immediately on each side it becomes suddenly considerably thicker, and then gradually becomes thinner again towards the boundary of the area, where it is turned up to form the commencing amnion.

The mesoblast is here completely separated from the axial cells, being rounded off at the sides bordering the medullary groove, and at the edges it is split to form splanchnic and somatic layers. The hypoblast is continuous across the area, the axial portion exhibiting no increase in thickness to that situated laterally; the former being forced by the deep medullary groove into a bow projecting into the vesicle below.

In front of the point from which the section is taken, the groove first becomes narrower, and then more shallow and wider, the notochordal cells becoming at the same time thicker and continuous with the lateral mesoblast. In front of the groove the relations are similar to what were described for fig.

14 (p. 442), excepting that the lateral cells below the epiblast are not in such numbers as before, and the axial cells, instead of being in the form of a tube, as in fig. 46, are only one row deep, and are arranged as an arc, the bay of which opens below (vide fig. 48).

Behind the section (fig. 45) the groove also becomes shallower, and the notochordal cells thicker, terminating, as in former specimens, in the front end of the primitive streak; the latter is, however, now cut off from the remainder of the streak, and lies within the medullary groove, into which the dorsal pore of the neurenteric canal now opens.

The section of the groove in fig. 45 exhibits evidence in both epiblast and hypoblast of an advanced growth on those drawn in either figs. 43, 44, or 47, although the measurements of the area are almost exactly similar to the one represented in fig. 14, from which fig. 47 was taken.

I do not propose to trace the development beyond this point, but may briefly say, that after the stage just described the medullary groove becomes much deeper, the epiblast of its wall being thick, while the epiblast over the lateral portions of the embryo is composed of only one layer of cubical cells. The groove deepens first about the hinder portion of the anterior third of the groove, and from there extends backwards and forwards.

The further development of the notochord takes place in the same direction. The flattened notochordal cells seen in fig. 45 become slightly more rounded as the lateral hypoblast and mesoblast sink to the level of the bottom of the groove, and then the lateral hypoblast grows inwards, and a small bunch of cells are isolated and lie between it and the now closed neural canal.

The amnion is first formed, as I have stated, over the hind end of the primitive streak, and from there grows forwards a considerable distance before the head is covered by the anterior fold.

To recapitulate, we may conclude it is probable that the region of the area in which the main portion of the

embryo is formed is derived by a forward growth. The hind knob of the primitive streak is lost, and the pyriform hind end of the area becomes shortened and widened. The medullary groove appears first as a wide shallow groove in the region adjoining the head of the primitive streak, from which point it extends forwards.

The changes undergone by the axial hypoblast are somewhat complicated. At first the cells of which it is composed are numerous, they then become fewer, and are arranged first as a flattened then as an arched plate, which may or may not be completely closed in to form a tube. Later on the arched plate becomes flattened out again by the deepening of the groove, and the notochord is represented by a thin layer of flattened cells which, as the lateral mesoblast and hypoblast sink down to a level with the bottom of the groove, become again more cubical in form. Eventually the lateral hypoblast grows in from the sides and the axial cells are separated off as a notochord.

The isolation of the axial cells from the lateral mesoblast takes place, as does the separation of the notochord from the hypoblast, from about the middle of the embryo backwards and forwards.

The same may be said for the medullary groove, which is first formed from behind forwards; its conversion into a canal takes place from about the middle towards the hind and front ends.

The neurenteric canal is complete, opening at first dorsally at the head end of the primitive streak, and between the latter and the medullary groove, but eventually becoming enclosed within the groove and opening at the bottom of its hinder end.

The canal travels forwards in an anterior growth of mesoblast from the head of the primitive streak, and enters a thickened axial mass of hypoblast, from which it opens downwards to the cavity of the vesicle.

The amnion is first formed over the hind end of the embryo, and only at a considerably later period envelops the front end by a separate formation.

Historical.—The arrangement of the layers at the front end of the primitive streak has been described by Hensen (No. 12), Schäfer (No. 26), and recently by Lieberkühn (No. 20).

According to the former, the axial cells below the medullary groove at its posterior end are thickened and join the primitive streak at the node of Hensen, this portion of the primitive streak being composed of epi-, hypo-, and mesoblast fused together.

Schäfer describes a similar arrangement in a somewhat different manner. According to him the axis of the embryo in this region is "occupied by a continuous column of cells, which inseparably connect the epiblast and hypoblast, and, traced from behind forwards, would appear to be chiefly epiblastic in origin."

This author does not appear to believe that the hypoblast takes part in the formation of the primitive streak, and he therefore considers, I imagine, that the latter organ begins where the hypoblast lies free below the mesoblast.

Neither of these observers described any canal perforating the blastoderm at this point.

Balfour, however, in his 'Comparative Embryology' (No. 3), has expressed his belief that the axial cord of cells described by Schäfer is the rudiment of the neurenteric canal of Lacertilia and birds.

Lieberkühn agrees with Hensen as to the arrangement of the layers at the front end of the primitive streak, and further finds a canal present in the mesoblast, which grows forwards from the front end of the "node." He states that the canal arises in the mesoblast, and does not open dorsally through the epiblast, but that it is prolonged forwards, and opens below through the hypoblast.

The notochord he believes to be formed from mesoblast, which secondarily becomes united with the hypoblast. This author also compares the neurenteric canal, such as he finds in mammals, with that of birds and lizards, and declares they are essentially different, inasmuch as in the latter the canal arises

as an inpushing of the epiblast, and connects the neural tube with the gastric cavity.

Kölliker (No. 17) agrees with Lieberkühn as to the mesoblastic origin of the notochord.

Summary.—My own work indicates that a complete neurenteric canal is formed similar to that in birds and lizards, first of all by an inpushing of the epiblast; secondly, the canal is conducted to the hypoblast within a tongue of mesoblast, which grows from the anterior end of the primitive streak; thirdly, the canal enters the axial hypoblast, and opens below to the cavity of the vesicle; and fourthly, the dorsal opening of the neurenteric canal is eventually enclosed within the walls of the neural tube.

With regard to the notochord, it appears to me evident that it is an hypoblastic structure, since it arises from an axial mass of cells, which are themselves derived from the primitive hypoblast.

My observations are at variance with Schäfer's, in that I find no continuous layer of mesoblast in front of the medullary groove, such as he describes, but a mass of undifferentiated cells, whose development shows that they are of hypoblastic origin, and that they split up laterally into sheets of hypoblast and mesoblast, while axially they remain undifferentiated, and give rise to the notochord.

Further, that the differentiation of the mass of cells which gives rise to the notochord takes place, as does the first formation of the medullary groove, from behind forwards, but that the separation of the notochord from the hypoblast takes place first of all somewhat anterior to the middle of the embryo, in the region where the medullary groove first deepens, and where the lateral mesoblast first forms protovertebræ, and that from that point the notochord is separated off both backwards and forwards.

Finally, that the derivation of the notochord from hypoblast is still further evidence of the incompatibility of Repiachoff's views (loc. cit.) with the facts of development.

Comparison between the Early Stages of Development of the Mole and Mouse, &c.

Until a few months ago there had been no satisfactory explanation of the manner in which the extraordinary phenomenon of the inversion of the layers in the Guinea-pig had been brought about, although the fact that such an inversion really existed had been described many years ago by Bischoff (Nos. 8 and 9), Reichert (No. 23), and Hensen (No. 12).

Kupffer (No. 18), Selenka (No. 27), and Fraser (No. 10), have, however, recently worked at the development of the Field Mouse, House Mouse, and Rat, and have found that the position of the layers in these animals is also inverted. Further, they each discovered the method by which the inversion was accomplished, and at the same time Hensen (Nos. 13 and 14) arrived at somewhat similar results for the Guinea-pig.

From these papers and from that of Spee (No. 28) I gather it is probable that the fully-segmented ovum of these various animals is similar to that of the Mole.

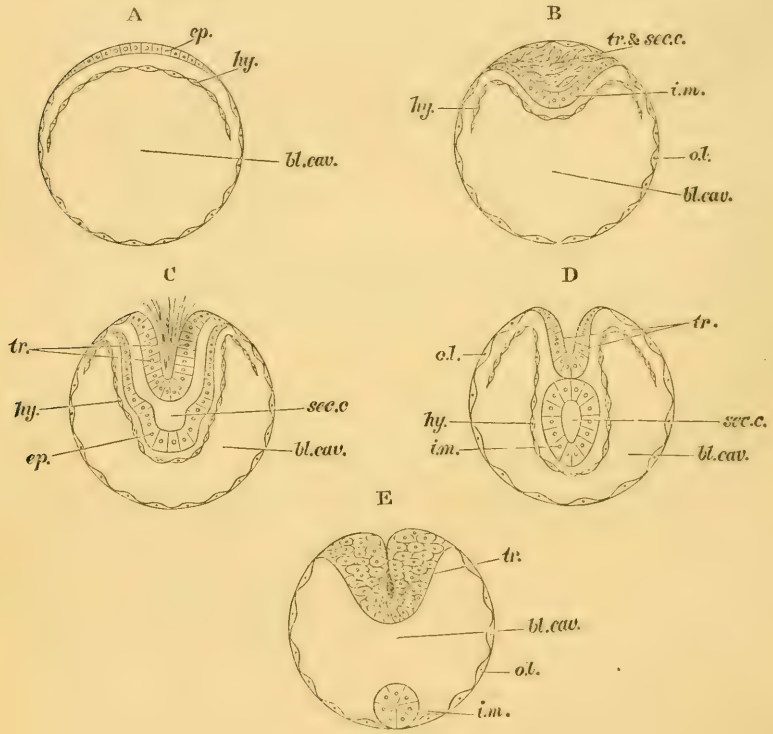
The changes which take place after segmentation are, however, somewhat different in each, and show a gradually increasing difference from the normal type to that one most specialised, viz. the Guinea-pig; while the phenomena exhibited during the development of the mole supply the connecting link between the two types.

These facts have not been, as far as I know, hitherto brought forward, and I venture to think merit some attention.

In the Field Mouse a blastodermic vesicle of flattened outer layer cells is formed, at one place on the circumference of which a solid inner mass is attached.

A layer of hypoblast is formed on the lower side of the inner mass, and the two shortly after flatten out; a thickening of the outer layer then takes place above the inner mass, and the flattened plate, with the hypoblast on its inner side, becomes involuted within the vesicle, and in this way an arched plate is formed, the circumference of which rests upon the outer layer

cells. The cavity of the arch (the secondary cavity) is filled up more or less with cells derived from the outer layer, and thus a condition is arrived at remarkably like the stage of development in the Mole represented in the woodcut, Fig. B, and on Plate XXIX, fig. 24.



Explanation of Woodcut.

Diagrammatic representation of:—A, ovum of Rabbit; B, of Mole; C, of Field Mouse (after Kupffer); D, of House mouse (after Selenka); E, of Guinea-pig. A, B, C, and D are at a similar stage of development. E is at a much earlier stage, before the formation of a secondary cavity.

bl. cav., blastodermic cavity; *ep.*, epiblast; *hy.*, hypoblast; *i. m.*, inner mass; *o. l.*, outer layer; *sec. c.*, secondary cavity; *tr.*, rudimentary träger.

There is, however, a difference in the future development.

The plate of cells in the Mole flattens out again, while in the Field Mouse it becomes further involuted within the vesicle, and the lower middle portion becomes the epiblast of the embryo, while the lateral portions form the amnion (Fig. C). In this manner the secondary cavity is surrounded by inner mass and outer layer cells, and into this cavity the embryo projects.

In the common House Mouse a layer of hypoblast is first formed below the rounded inner mass; next above the latter the outer layer cells become thickened and involuted within the vesicle, carrying with them the solid inner mass.

A cavity is subsequently formed in the latter, and it elongates until it nearly reaches the opposite pole of the vesicle, to which it was originally placed.

Thus the cells of the inner mass alone line the secondary cavity in this case, and into it the developing embryo projects (Fig. D).

The Rat develops similarly to the House Mouse, a secondary cavity forming in the inner mass after it is involuted.

In the Guinea-pig, however (Fig. E), the solid inner mass appears to become attached to the opposite pole of the ovum at a very early stage in the development of the blastodermic vesicle, and the outer layer does not become involuted, if observations made by Dr. Wilson and myself be trustworthy, until a considerably later period. A secondary cavity is eventually formed within the inner mass, and into it the embryo projects.

Thus a complete series of conditions may be traced in these various animals between the inverted and normal types. In the Rabbit the solid inner mass flattens out and remains on the surface of the vesicle; in the Mole it is first formed into a curved plate, which subsequently becomes flattened out and lies on the circumference of the vesicle; in the Field Mouse it flattens out first on the surface and then becomes and remains involuted; in the House Mouse it becomes involuted before becoming flattened; and in the Guinea-pig the inner mass remains attached at the opposite pole of the ovum before it becomes a vesicle, and an involution of the outer layer secondarily takes place.

A consideration of these facts, together with an examination of the conditions attending the later stages of development in some of these animals, leads me to believe that the difference in the development of normal animals and those in which the so-called inversion of the layers takes place is one of secondary importance, and, in fact, that no such fundamental differences exist as was supposed by the older observers; the temporary inversion of the layers which occurs in the Mole connecting the two types very closely.

I do not propose here to enter into a more detailed discussion of the points noticed above, or to attempt to compare the later stages of development; the only points which have immediate bearing upon my present work are—

(1) The explanation of the existence of the secondary cavity and the cells situated within it in the Mole; and (2) the fact that in the inverted types the epiblast of the embryo is formed entirely of inner mass cells.

The former may be considered as inversion phenomena of a temporary nature; while with regard to the second point the conditions of development appear to me to be sufficient explanation of the difference.

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The Tongue of Ornithorhynchus paradoxus: the Origin of Taste Bulbs and the parts upon which they occur.

By

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With Plate XXXII.

I am indebted to Professor Moseley for kindly giving me a very perfect tongue of Ornithorhynchus. This animal has never been brought alive to Europe, and therefore it must be unusually difficult to procure the tissues in a condition favorable for histological investigation. Professor Moseley obtained the specimen from which the tongue was taken in 1874, and the animal was one of those mentioned on page 263 of the 'Notes by a Naturalist on the Challenger.' The organ was hardened in chromic acid, and subsequently in spirit, and I found it in excellent condition during my work upon it last Christmas and again at Easter. There appears to have been some slight alteration of the most delicate tissues, due to the time that unavoidably elapsed before the organ could be hardened, together with the heat and jolting of a journey by coach. However, this change was not so great as to prevent me from arriving at definite conclusions with regard to these tissues, and it is very unlikely that the terminal organs of the gustatory nerves (to which I allude) could have been made out perfectly, except by work upon the fresh specimen.

General Account of the Tongue.—The size and shape of

the organ are shown in figures 1 and 2, which are drawn of the natural size. There is an obvious division into an anterior and a raised posterior part.

The Anterior Division is only free from the floor of the mouth for about one third of its own length, and therefore the movements of the tongue must be very limited.

The upper surface is covered anteriorly with large papillæ directed backwards, becoming gradually smaller posteriorly, where little more than a rough appearance can be detected by the naked eye. The sides and lower surface of the free part are perfectly smooth, and the large papillæ terminate abruptly at the lateral and anterior limits of the upper surface. Posteriorly, however, the smaller papillæ are continuous on to the sides of the organ, and appear to be present upon the mucous membrane of the floor of the mouth. There is no trace of a raphe in the anterior part.

Posterior Division.—The anterior surface of this division is continuous with the upper surface of the anterior division, and the former overhangs the latter, making with it an angle of 60° (fig. 2). This surface is 11 mm. broad where it joins the anterior division, and above this its lateral contours slope upwards, forwards, and inwards, to the most anterior part of the posterior division. Here the superior and anterior surfaces are continuous, and at this point are two large horny papillæ or teeth (deserving this latter name as much as the maxillary teeth of Ornithorhynchus, which are also epithelial).

The internal sides of the teeth slope inwards, and appear to be continuous ; the apices are broad and chisel-like. The bases are surrounded by small hair-like papillæ, which spring from the teeth themselves. Posteriorly the upper surface presents a small and shallow pit in the middle line, and from the anterior margin of the pit a linear raphe is continued for a short distance, disappearing as a slightly-marked groove. A fold is formed at the junction of the mucous membrane of the floor of the mouth with the side of the posterior part of the tongue. This fold first appears at about the middle of the side, and runs backwards and upwards, and on nearly reaching the pit on the

upper surface it turns suddenly forwards and is lost. There is a groove in front of the fold and overshadowed by it. This fold may be considered as the posterior limit of the whole tongue. The fold and groove, the pit, and the whole of the upper surface of the posterior division, appear quite smooth (although really papillate for the most part), while the lateral surfaces and the anterior overhanging surface appear rough from the presence of small and generally hair-like papillæ. Just behind the teeth are two deep grooves (figs. 1 and 2, *a b o*), directed obliquely to the long axis of the organ. These contain the structures which bear the taste-bulbs, but the former cannot be seen from the surface. There is also another gustatory structure in each groove in front of the fold (figs. 1 and 2, *p b o.*), but in this case the convex surface which bears the bulbs can be seen. I have omitted to give dimensions in this general description because the size of the parts described is given in figures 1 and 2.

Histological Account of the Tongue.—For the purposes of this description it is convenient to divide the organ into an anterior and posterior part; but the former in this case should include the anterior surface of the posterior part and the horny teeth. These two regions are histologically very distinct. The anterior region (1) contains exclusively tactile and mechanical papillæ, while the posterior region (2) bears the gustatory structures, together with papillæ of probably mechanical function, and different in structure from many of those on the anterior region.

1. The Anterior Region.—The upper surface of the tongue at the tip (and for about 19 mm. behind the tip in the middle line, and rather more at the sides) is covered by large papillæ (fig. 1), and presents many points of difference from the surface of the more posterior parts. This region is, therefore, conveniently subdivided into (A) an anterior subregion of large papillæ (easily recognisable in fig. 1) and (B) a posterior subregion of small papillæ (including the horny teeth).

A. The Anterior Subregion.—The papillæ, which form an irregular fringe (one or two deep) on the sides and front of

the papillate surface on the tip of the tongue, project horizontally or slightly downwards. They have swollen rounded ends and constricted bases, and the superficial layer of cells is not cornified. They are especially large in front, and resemble the ordinary type of fungiform papilla. These papillæ contain especially large medullated nerves, which are accompanied by blood-vessels. Behind these papillæ, in front and at the sides, occur others of a conical shape, with constricted bases and fine recurved cornified apices. Their anterior surface is convex, and the cornified layer of the apex extends downwards upon it for a short distance, while the posterior surface is less convex, and the cornified cells descend almost to the superficial epithelium of the tongue. There are many rows of these papillæ, and they become gradually lower, broader, and more scale-like posteriorly, with a sharp crescentic ridge of cornified cells on their apices, directed transversely to the long axis of the tongue. The arrangement is very regularly imbricate. The layer of cornified cells is now much thicker, and forms the important part of the anterior and posterior surfaces, while the shape gradually assumes that shown in longitudinal section in fig. 6, and is singularly like that of the teeth of a rasp. This form passes on into the posterior subregion. The anterior papillæ are about 1 mm. in height, while posteriorly they decrease till at the limits of the subregion they are not more than ·3 mm. in height. The epithelium is very simple in structure, a stratum corneum being entirely absent, and the superficial cells being fusiform with distinct nuclei. The layer appears to represent the rete Malpighii only. Occasionally little isolated groups of cornified cells occur at some depth beneath the surface, and surrounded on all sides by non-cornified cells. Papillary processes are absent in front, where the papillæ are thickly placed, occasionally present behind, as very long and narrow upgrowths between the less numerous papillæ.

The epithelium of the papillæ is always penetrated by secondary papillary processes, which are sometimes very long and narrow. There are traces of the existence of a few hair-like papillæ between those of ordinary type at the posterior limits

of the region, but they are never numerous or distinct. Connective-tissue corpuscles are often found between the cells of the epithelium, having intruded from the mucosa; but this phenomenon is better marked in another part of the tongue (although otherwise similar), where it will be described in detail. The smooth epithelium beneath the tip of the tongue is similar to that just described, with few papillary processes. Its lowest cells contain pigment granules.

This epithelium must be highly sensitive, as the mucosa beneath it is richly supplied with tactile end-organs, to be described in connection with the papillæ. This anterior subregion is the most glandular part of the tongue, and in the region of the tip the gland-tubes occupy far more space than the muscle-fibres (fig. 3). The gland-tubes ramify between the muscle-fibres in the whole thickness of the organ for a distance of 5 mm. from the tip. Posteriorly to this point they are not found at the lower surface, but at the posterior limit of this subregion they form a layer more than 2 mm. thick beneath the upper surface, becoming slightly thicker in the posterior subregion, where they finally disappear about 10 mm. in front of the junction with the overhanging surface. The gland-tubes, which end cæcally without dilatations, are very large, and take an independent course for long distances among the muscle-fibre bundles, not branching frequently. They are not united together into any distinct gland (figs. 3 and 4). The cells have suffered a little by the time that elapsed before the organ could be hardened, but they are easily recognisable as belonging to the "mucous" type of Klein. They are transparent tall columnar cells, staining very slightly in picro-carmine and borax carmine, deeply in logwood. The walls of the ducts are for a short distance composed of several layers of cells continuous with the lower cells of the superficial epithelium. The lumen of the duct is very narrow during the passage through the epithelium to the surface, but it rapidly expands below, and is at once continuous into a gland-tube of usual structure. The opening on the surface is very slightly funnel-shaped. The ducts very commonly run in little groups of three

or four, and penetrate the epithelium close together. The ducts open freely on the lower surface of the tip over the 5 mm., where the gland-tubes occur; while above they open comparatively rarely between, but very abundantly on the papillæ, and especially towards the lower part of the anterior slope (fig. 4). At this point the great majority of openings are found. Posteriorly in this subregion, and in the posterior subregion itself, the gland-ducts only open between the papillæ. It seems very likely that the papillæ are rendered sticky by the glandular secretion, and that the aquatic larvæ, &c., which form this animal's food, are thus caused to adhere to them. It is not likely, however, that the larvæ are thus captured. A sticky secretion would be of little use in the mud at the bottom of ponds, and the tongue has such limited powers of movement, and is set so far back (the tip is 13 mm. from the anterior margin of the lower bill), that it is not probable that it can even be protruded. The prey is caught by the bill, and the animal is known to rapidly vibrate the lower bill in the water like a duck, by which means the mud would be washed away through its lateral grooves. The prey is thus held by the ridges between the grooves and the flat surface of the upper bill, and probably crushed to some extent by the peculiar (and, I believe, undescribed) smooth, ridge-like horny teeth, which are situated at the inner ends of the grooves (two in each jaw). During all these processes the food is far from reaching the posterior part of the singularly inflexible mouth. Hence the importance of these large, adhesive, and (as will be shown) highly tactile papillæ on the anterior part of the tongue. By their means the food can be drawn backwards to the more effective and corrugated teeth, to be thoroughly crushed. Hence the importance of this excessive development of glandular tissue at this particular part of the tongue.

The imbricated arrangement, and sharp points and ridges of the papillæ, would also be of great importance in retaining small insects, &c., which were caught by the adhesive anterior papillary slopes. Thus an insect attempting to escape would be met by the hard corneous posterior surfaces of the papillæ

in front, whose inclination backwards would prove a further obstacle. Thus the anterior surfaces are tactile and adhesive, while the posterior are chiefly of mechanical use.

The tactile end-organs mentioned above form a new terminal organ, apparently nearly allied to Pacinian corpuscles. The shape is oval or fusiform, and the poles are often slightly flattened. They are very small, as is seen by the highly magnified fig. 5 (405 diameters). The corpuscle is surrounded by a laminated investment formed of 6—8 extremely thin concentric layers. There was doubtless an intervening fluid between these capsules during life, as they are now found in a very collapsed and crumpled condition. The number of capsules is very uniform, together with the general appearance and size of the corpuscles. Between the capsules occur a few relatively large oval granular nuclei (fig. 5). As in Pacinian corpuscles the capsules constitute the chief mass of the bodies. There is an axial, longitudinally, striated fusiform mass, and an examination of transverse sections with high powers ($\frac{1}{18}$ oil immersion of Zeiss) showed that this also contains a central column of different structure. A single medullated nerve-fibre terminates in each body, losing its medulla on entrance, the axis cylinder being continuous with the spindle. The corpuscles are extremely common in the papillæ; it is quite usual to find three in a section of a single papilla, and more than once I have seen five (fig. 4). Corresponding to this abundance of end organs the papillæ are richly supplied with medullated nerve-fibres. The corpuscles always occur close to the lowest layer of the epithelium, and never any distance below this. They are never situated in the secondary papillary processes although they may be close to the bases of these. The long axis of the body is nearly always parallel to the lower surface of the nearest epithelium. Groups of two or three bodies are very common, and sometimes a nerve-fibre appears to pass through one body into another, although in some instances careful examination shows that such is not the case when two corpuscles are arranged in a line, with their apposed ends almost in contact.

These tactile end organs are very common beneath the epithelium of the lower surface of the tip, and are rarely found between the papillæ of the upper surface of this subregion. They do not occur in any other part of the tongue, and are not found in the posterior papillæ of this subregion where the cornified layer is much developed and the functions are purely mechanical. They disappear about 4 mm. from the posterior limits of the anterior subregion.

It is very probable that there are also nerve endings in the epithelium of the papillæ, for it is very common to see fibres continued from the mucous membrane into the epithelium, especially at the apex of a secondary papillary process. If these fibres are nervous (which cannot certainly be made out in this specimen) they are probably bundles of primitive fibrils. Blood-vessels are very abundant in the tactile papillæ.

B. The Posterior Subregion.—The transition between this part of the tongue and that just described is shown in fig. 6. It is seen that the rasping papillæ are continuous from the one on to the other without any change of structure except an increase in thickness of the corneous epithelium. The gland tubes where present open between, never on, the papillæ, and are wider just before they perforate the epithelium than is the case anteriorly. No tactile end organs appear to be present, and the functions of this subregion must be exclusively mechanical. Between the widely separated papillæ already described, occur abundant simple pointed papillæ (figs. 6 and 7), which are usually much worn down. In all the points mentioned there are traces of a transition from the anterior to the posterior subregion, but a distinct difference between the two is seen in the structure of the epithelial layer. At the point of contact the epithelium of this subregion forms a thinner layer (fig. 6), and this is also true of all other parts of the tongue where there is a similar change of structure. This fact only holds for the contact, since there may be great variation in the thickness of both kinds of epithelium distally to this point. The structural difference is of greater importance, and can be detected even in an unstained section of the tongue

without the use of a lens. The epithelium of the posterior subregion is thus seen to be far denser and to possess an obvious division into three layers, of which the median one is much darker than the other two. The use of staining reagents and moderate powers shows far greater complexity, and the various layers of cells behave very differently with different reagents. Looking at many results it seems that there are four distinct layers, best shown by aniline black and picro-carmine. Beginning from below the stratum Malpighii (1) stains light slate colour in aniline black, light red in picrocarmine; darker at the lowest layer of cells with both reagents. The upper fusiform cells of the stratum Malpighii form a layer (2) staining deeply in both fluids. Then follows a layer (3) of very long thin cells whose outlines are difficult to make out, staining yellow in picrocarmine and straw-colour in Aniline black. The uppermost layer (4) stains deeply for the most part, and the outlines of the cells become distinct and the form less attenuated except at the surface. Logwood gives the same results, but in this case it is possible to see the nuclei of the cells of layer 3, which are usually long and thin, but sometimes almost spherical, following the shape of the cells themselves. In patches of variable extent the cells of layer 3 are continued into 4, not often reaching the surface. The cells of layer 3 are shown by their behaviour with reagents to be cornified, and the most remarkable thing about the epithelium is the fact that in upward succession cornified cells again become noncornified except in rare and isolated spots. However, these cells are not cornified to the same extent as those of the horny teeth and the papillæ. This epithelium is shown in figs. 6 and 7, and it is seen to be thicker in the overhanging surface (fig. 7), where layer 4 contains more cornified cells in larger patches. In all cases the cornified layer (3) is continued upwards into the papillæ of the subregion, but corneous cells are also derived directly from layer 4. Close to the point of transition into the anterior subregion the cells above as well as below 3 stain especially deeply, and at the junction itself 3 ceases altogether, while

2, and the deeply stained cells above, coalesce and disappear after persisting for a short distance (fig. 6).

The oral mucous membrane which is continuous with the sides of this part of the tongue, has a very thick simple epithelium with fine papillæ.

The overhanging surface is almost identical in structure with the rest of the subregion with which it is continuous, and the papillæ are of the same structure. The rasp-like papillæ on the horizontal surface are directed backwards, and these being continuous on to the overhanging surface (which is directed forwards at an acute angle) are there directed forwards. Hence in antero-posterior movement of one surface upon the other these papillæ would work in opposite directions and would form very efficient agents of attrition, being greatly aided by the very numerous pointed papillæ. That this is the true function of the subregion is rendered likely by the situation of these two surfaces at the level of the four most effective teeth. The food after being crushed by the teeth would be forced inwards and further rubbed down between these surfaces, the two horny tooth-like papillæ no doubt assisting in the operation by friction against the horizontal surface below. The greater thickness of the epithelium on the overhanging surface is doubtless due to the greater length of horizontal surface rubbed against it.

In favour of this view of the action of the subregion is the great wear shown by the epithelial surface and the papillæ (especially the more numerous simple pointed ones); and the fact that the two surfaces are almost apposed in a tongue preserved in the Oxford Museum, while the horny teeth were directed downwards towards the surface beneath. In another specimen which I was enabled to examine through the kindness of Professor Moseley, the teeth were almost in contact with the horizontal surface below, while in this also the overhanging and horizontal surfaces were nearly apposed. It is probable that the chisel-like summits assist in scraping off particles that are entangled among the papillæ. The teeth and anterior part of the horizontal surface may also be rubbed

against the roof of the mouth, which here is very dense and presents transverse curved ridges. No glands open upon the overhanging surface. In working at this surface a singularly difficult structure was met with which I mention, as it seems likely that others might have a similar experience. At one point only, a series of specimens showed successive oblique sections of a single hair which penetrated to the mucous membrane. The epithelium round it was much modified by the presence of the hair, so that it was long before I could be sure that the structure was accidental. A hair (probably one of the animal's) must have been arrested at this surface and the end become inserted in some slight cavity between the cells. Thus the hair gradually worked through the whole thickness of the epithelium. It was very interesting to note that the superficial epithelial layer (4) was cornified for a considerable distance round the foreign object and due to its presence. There was a hollow at the surface filled with fragments of foreign objects, and probably partially derived from the hair itself. The contour of the hair was rough and frayed where it passed through the epithelium, and most of its course was greatly twisted. These were indications of its gradual passage along lines of least resistance, and of the great friction caused.

The two horny teeth which form the boundary between the anterior and posterior regions (here classified with the former) are covered, except at their apices, with a thick layer of cornified cells (fig. 7). These cells are, however, different from those of the corneous layer (3) of the overhanging and other surfaces, the difference being especially seen in their behaviour with logwood. The peculiarity belongs to the latter, and the superficial cells of the horny teeth are normal cornified cells. Towards the apex a few large rounded deeply staining cells are sometimes seen in the cornified layer. They may represent isolated unaltered cells continued from the layers below, as they are sometimes seen in lines extending from the apex of a papillary process. I do not feel certain as to their correct interpretation.

The layer beneath is made up of very granular polygonal

cells which seem to be partially cornified. These are again transitional into the ordinary fusiform cells of the rete Malpighii. This extremely granular cell is certainly a transition into a corneous cell similar to that described in the mechanical papillæ of Perameles (see this Journal for January, 1883). Here, however, the cell is very finely and densely granular, instead of the coarse type observed in Perameles. There is an exactly similar transition through polygonal finely granular cells to corneous cells, even better marked in the maxillary teeth which I hope to describe on a future occasion. Secondary papillary processes enter the rete Malpighii from below, and in the axis of the horny papillæ these processes are long and fine, and from the summit of each a line of granular cells extends to the very apex. Thus there is no cornified investment at the centre of the apex, and that which covers the sides of the papilla terminates in a sharp-edged corneous ring. The edges are kept sharp by the constant wear of the softer central epithelium. There is reason to believe that the maxillary teeth are rendered uneven in a similar manner, i. e. by the wear caused in parts by the presence of very long papillary processes, with lines of soft cells extending from their apices. Similar lines of cells can be detected in the hard investment of these lingual teeth as well as in the maxillary teeth (fig. 7, *l c*), but in these cases the cells are completely cornified, and their arrangement in lines is only recognisable by a looser connexion between them. Small secondary papillæ cover the upper surface of the base of the two horny papillæ.

2. The Posterior Region is covered with a thick simple epithelium resembling the rete Malpighii. The complex epithelium of the overhanging surface ceases at the rounded angle which separates it from the side of this region, but below, in the slight groove which separates the posterior part of the tongue from the floor of the mouth, the epithelium remains complex. The same structure is continued upwards along the shallow groove in front of the fold (*f*, figs. 1 and 2), while the fold itself is covered with simple epithelium. Similarly at the sides thin complex epithelium occurs only at the junction

of the tongue with the oral floor, while the latter is covered with a thick simple layer with long papillary processes, exactly like that of the fold (*f*, fig. 10). All over the tongue of this animal there is a great tendency for the subepithelial elements to penetrate between the cells of the epithelium. This is especially true of the interpapillary processes of the shallow groove, between the left gustatory area and the fold. Here in many cases great masses of connective-tissue corpuscles make up a considerable bulk of the interpapillary process, as is seen in fig. 9. Outlying corpuscles have processes which extend toward the mucosa, indicating the direction from which the intrusion took place. These have been often described before, but I believe never to such an extent as is here figured. The corpuscles never ascend above the lowest layer of the complex epithelium, corresponding to the rete Malpighii. The fine pointed papillæ are always much bent as they pass through the complex layer, while they are quite straight in the simple epithelium (compare fig. 8, *f p* and fig. 10, *f′ p′*). The complex epithelium ascends along the groove in front of the fold, and is continued into the pit (fig. 1). Here it is extremely thin (·08 mm.), while the simple epithelium in front of the pit is many times as thick (over ·5 mm.). So also behind the pit the epithelium becomes simple and comparatively thick. Thus the convex upper surface and sides of the posterior region, covered with simple epithelium, are completely surrounded by the dense complex epithelium prolonged backwards from the lower part of the overhanging surface, skirting the sides of this region and rising along the groove until it meets in the pit. Glands are only found in association with the gustatory areas and beneath the epithelium of the pit; the former are serous, while the latter appear to be mucous, and are very numerous, with few openings into the pit. It is probable that there are more numerous openings posteriorly, beyond the limits of the tongue in my possession, as I inferred from other specimens that a groove is continued backward from the pit, ending in a depression in front of the epiglottis.

The whole of the sides and convex upper surface of this

posterior region are covered with fine hairlike papillæ (fig. 8, *fp*, &c.). These are stoutest and longest at the sides, shortest, smallest, and most crowded in the raphe (fig. 1). In a horizontal section, taken between the anterior gustatory organs, I calculated that there are over 500 of these papillæ to the square millimetre. This is probably a fair average for the whole surface.

The roof of the mouth exactly fits this convex part of the tongue, and the former is covered with dense epithelium, presenting minute ridges against which the fine papillæ must rub.

This is the only part of the tongue upon which gustatory areas occur, there being two pairs, an anterior and posterior. The anterior pair (*a b o*, figs. 1 and 2) are situated on the convex surface behind the horny teeth. All that can be seen from the surface is an oblique furrow, but it is shown by sections that the bottom of this furrow is invaginated upwards into a ridge which bears the taste-bulbs over the whole of its circumference (fig. 8). The lips of the furrow are surrounded by comparatively stout and short papillæ, of which the axial upgrowths of mucosa are continued from the tissue enclosed between the superficial epithelium and its prolongation downwards to form the furrow. The inner walls of the furrow are formed of corneous cells continued from the papillæ encircling the opening. This corneous layer ceases below where the furrow becomes expanded to contain the ridge. One of the most interesting things about this whole organ is that the furrow can almost certainly be closed by the contraction of smooth muscle-fibres arranged as a sphincter. Smooth muscle-fibres are very difficult to identify with certainty in a section, but I have no doubt of their presence and arrangement. So effective is this closure that I have been entirely unable to detect a sign of the organ in some specimens, by examination of the surface with a lens. Of course the stout papillæ would meet during approximation of the lips, and act very effectively in preventing the entrance of particles. Smooth muscle-fibres are probably present in the posterior organs, but they cannot be nearly

so effective, nor is there the same necessity for such protection. Beneath the sphincter are cells with a meridional arrangement which must act as a dilator muscle (fig. 8).

The posterior gustatory areas (*p b o*, figs. 1 and 2) are very similar, but there is less need for protection in a deep furrow, because of the posterior position and situation in a slight groove overhung by a fold. Hence the gustatory ridges rise to the surface (figs. 10 and 11), and bear some resemblance to an ordinary circumvallate papilla, but the bulbs are placed on the upper surface and sides, as in the anterior organ, and unlike any gustatory area yet described in Mammalia (except the isolated bulbs on the fungiform papillæ). This description only applies exactly to the right posterior area, for there was a great lack of symmetry in this specimen. The left area appeared to be rudimentary, and was only represented by a slight ridge at the bottom and rather on the anterior side of a furrow, with few bulbs, and these often placed beneath the epithelium or only partially embedded in it. Some, however, were situated normally, and possessed pores. I am unable to state certainly that this lack of symmetry is abnormal, but it is very probable that this is the case, considering that the anterior areas entirely resemble each other. The structures accompanying the gustatory ridges, in all cases (even the rudimentary left posterior ridge), are the same as those of other Mammalia. The serous gland-ducts open as usual into the spaces round the ridges, and this type of gland is not found elsewhere in the tongue. The structure of the gland-cells did not seem to be identical with that of the usual serous type, but this is probably due to post-mortem changes, especially as the mucous glands have also undergone alteration. The ducts of the serous glands sometimes contain nuclei and the débris of cells. Non-medullated nerve-fibres almost fill up the centre of the ridge and radiate outwards to end in the bulbs. I was surprised to find no indications of ganglion-cells (as described in Perameles), although minute ganglia occur on the large nerve-branches. Beneath the posterior gustatory areas a tissue resembling adenoid tissue occurs in rather large amount (fig. 10), and traces of it can be found

beneath the anterior areas. The taste-bulbs are, as far as I have observed, entirely unique in being developed at the ends of long papillary processes, up which the nerve-fibres can be seen streaming from the central nervous mass, sometimes accompanied by capillary blood-vessels.

Sometimes the external surface shows indications of lobation, the convexities corresponding to the bulbs; but this is uncommon. In rare cases a papillary process may divide and end in two bulbs. Gustatory pores are present, and are singularly like those of normal bulbs. The outline of an exceptionally long and distinct pore is given in fig. 12, from which its length and diameter are easily calculated. The ordinary length of the pores in this animal is not more than half that shown in fig. 12. I never saw any protrusion of cells or processes from the pore. There appear to be rather under 500 bulbs to the square millimetre of surface. The bulbs are oval or fusiform, and their sides rise gradually from those of the papillary processes, of which they are the expanded ends. The structure of the wall is of great importance in the organogeny of the gustatory termination. At the same time it was singularly difficult to be certain as to interpretations, owing to changes that had taken place in these delicate structures. However, after comparing immense numbers of sections, I can confidently assert that many of the elements are not modified epithelial cells, but are altogether subepithelial in origin. There are seldom traces of the meridional arrangement of cells that characterises ordinary bulbs. The elements also differ in being packed loosely, and in being very heterogeneous.

In many bulbs I have detected the yellow stain that results from the disintegration of blood in a capillary. Many of the cells look as if they might have scaled off the sides of the oval chamber, and thus have been added to elements intruded from below. Such cells are fusiform in shape, sometimes thick, sometimes attenuated, but they always stain differently from the surrounding epithelium. Other cells have many processes and resemble connective-tissue corpuscles, although they may be nerve terminations. Others resemble small multipolar gan-

glion cells, while some are spherical. In all the nuclei are very distinct, and the elements, as a whole, differ from the surrounding epithelium in staining much more deeply, and in their loose, irregular arrangement. If the looseness is due to shrinking this proves a difference of structure, as the epithelial cells, exposed to the same conditions, have not shrunk away from one another. The nerve-fibres enter the bulb, and do not terminate in a group at the basal pole in the usual way, but are seen running between the cells of the bulb in various places.

There is more certainty of the nerve-fibres passing to each bulb in this case, with the easily found papillary upgrowth as a guide to the bulb, and containing its special fasciculus of nerve-fibres. I was enabled by teasing to isolate one undoubted terminal cell with the nerve-fibril still attached to it. It is shown in fig. 14, and the fibril is seen to branch before termination. The shape is very simple and fusiform, with no peripheral process. There are traces of a nucleus and of an axial line continued from it along the cell. The general appearance of a bulb is shown in fig. 13, in which the elements are seen to be separated by considerable intervals, probably due to shrinkage. There is no doubt that the epithelium has been penetrated by the bulb, and not merely reflected over it. This is proved by the non-continuity of the lowest layer of columnar cells over the bulb (fig. 13). But this intrusion of the bulb does not mean, as in other mammals, that the elements are formed from the modified epithelial cells, although these may be present. A further conclusive proof that the bulb is essentially subepithelial in nature is found in the fact that isolated bulbs in the abnormal and rudimentary left posterior organ occur beneath the epithelium, others partially embedded in it, and others, again, arranged in the usual manner with gustatory pores.

This peculiar form of bulb has an important bearing upon a theory as to the origin of taste-bulbs suggested by me in the January number of this Journal (the "Tongue of Perameles").

The Origin of Taste-bulbs.—From observations upon the tongue of Perameles I was led to infer that the usual mammalian bulb was developed from a group of interpapillary

epithelial cells. At the same time I concluded that this method of development was of comparatively recent date in Perameles, while the singularly complete accesssory apparatus suggested that some other and more primitive form of terminal organ had not long been supplanted, and had probably coexisted with these perfect additional structures. Arguing entirely à priori the suggestion was made that the primitive type of bulb was papillary in position and subepithelial in structure, and had gradually given way to a bulb that was interpapillary and epithelial.

At the time of this suggestion I had little hope that such a primitive bulb would ever be seen. It seemed probable that the stage had existed, but that it was incapable of direct proof.

The very next tongue I worked upon—that of the highly ancestral Ornithorhynchus—supplied a bulb that was at once papillary and subepithelial. The new bulb, although in some cases retaining its original position beneath the epithelium, has usually ascended and acquired epithelial cells, and has finally penetrated the surface as a gustatory pore. This latter was a structure which I had not expected to appear until a later stage. Nevertheless, in these new bulbs and their arrangement we can see a cause why this should not be the permanent type.

It is obvious that a subepithelial end organ, specialised for gustatory stimuli and raised until it is in actual relation with the exterior through an aperture, must be extremely sensitive. In fact it is probable enough that such an end organ is too sensitive for the purpose, especially when continual friction and the mechanical effects of accidental particles are taken into account. Evidence of this is seen in the entirely unique protection afforded to the more exposed anterior gustatory ridges, a protection which must seriously interfere with their efficacy. It is therefore probable that a less delicate form of terminal organ arose, which could be brought into closer relations with the stimuli. This I believe was the cause of the change of type, and not increased sensitiveness, except in so far as this is caused by greater exposure. At the same time

the bulbs of Ornithorhynchus are, in their arrangement on the summits of the ridges, more exposed than those of any other animal. But this is made up for in one case by the sinking of the whole ridge till it only communicates with the outside by a deep and narrow chink (fig. 8), and in the other by the position and relation to adjacent structures (fig. 10).

The Origin of the Gustatory areas of Mammalia.—Omitting the fungiform papillæ, which seem to be primarily tactile (as they are here), and to have acquired bulbs comparatively recently—the gustatory areas are either of the circumvallate or foliate type. The former is by far the commoner type, but foliate areas are not so rare as is generally supposed.

They were first discovered in rodents, but there are indications of them in many orders, and I find them well developed in Marsupials (I have found them in Phalangista with many furrows whose sides were crowded with bulbs). Thus the two types appear to have arisen together, as we find them both represented in the lowest order in which they occur. It seems to me that the ridges of Ornithorhynchus are intermediate between the two. In both cases the bulbs become confined to the sides (changing their mode of origin also) as the areas become more exposed. A circumvallate papilla is then produced by the shortening of the ridge until it becomes a subcircular elevation. At first the base of the papilla would be constricted as it now is in Perameles. Then the sides would become straight, and the vallum very deep and narrow (Phalangista), and finally the vallum becomes wide and shallow and of very little value for protection, as in most higher mammals. Conversely the ridge lengthens, rises to the surface, and two furrows of a foliate organ are produced, over both sides of which the terminal organs would spread. Just as the circumvallate papillæ of marsupials present traces of this origin, so their foliate organ (as far as I have seen it) consists of a less number of furrows than other Mammalia, and with a less regular arrangement.

As a conclusion to these hypotheses, it is well to remember that Ornithorhynchus cannot show us the exact ancestral form

of any stage, but it is of immense value in affording suggestions as to what the stage has been. This remarkable animal is doubtless a direct descendant of a type which would give us sure knowledge as to the origin of many peculiarly mammalian features. But individual specialisation has accompanied the long course of descent, so that even in this lowest of living mammals, structures which are characteristic of the class, and of which we might fairly expect to see the origin, are assumed as it were—used as the raw material for further structural modification. Upon this subject I hope to write on a future occasion and to give details; I mention it now to show the uncertainty of interpretating the origin of a structure from data of Comparative Anatomy only. And yet such data sometimes afford valuable suggestions, capable of verification, and often of a kind that could not be given by any other study.

Observations upon the Fœtal Membranes of the Opossum and other Marsupials.

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With Plate XXXIII.

In 1834 and 1837 Professor Owen published in the 'Philosophical Transactions' and the 'Proceedings of the Zoological Society' descriptions of the fœtal membrane of the kangaroo. Quite recently his observations have been confirmed by Professor Chapman,[1] of Philadelphia. They are given in full in the 'Comparative Anatomy of the Vertebrates,' and are to be found, in abstract, in Balfour's 'Comparative Embryology,' vol. ii. It is surprising that no additions have been made to our knowledge of these forms during the long period intervening between Owen's observation and the present time, even when one is aware of the extreme difficulty of obtaining females during the period of gestation.

Professor Owen established the following as the distinctive features of the Marsupial fœtal membranes: (1) A large subzonal membrane with folds fitting into the uterine furrows, but not adhering to the uterus and without villi. (2) A large and vascular yolk-sac partly flattened out over the inner surface of the subzonal membrane, and supplied by an artery and two veins. (3) An allantois of comparatively small size, not attached to the subzonal membrane, with a blood

[1] 'Proc. Acad. Nat. Sciences of Phila.,' 1881.

supply of two arteries and one vein. (4) An amnion closely investing the embryo and reflected over the base of the yolk-sac and allantoic stalks.

One sees at once that Professor Owen's observations, valuable as they are, still leave us in doubt as to the real relationship existing between the fœtal and maternal blood currents, which, after all, is the main question. By what process does the embryo, with little or no food yolk to draw upon, support life during the short but rapid period[1] of intrauterine growth, extending not over seventeen days in the opossum, and thirty-eight days[2] in the kangaroo?

My own observations partly confirm and partly contradict those of Professor Owen; they show that not only does the yolk-sac in the Marsupials perform the functions of the allantois in the placental Mammals, but that the method is the same, namely, by means of vascular villi developed upon the subzonal membrane over the attached or chorionic portion of the yolk-sac.

In the early part of March I had the good fortune to receive from one of my students[3] a female opossum (Didelphys Virginiana), which was found to be in an early stage of pregnancy. After opening the animal I found that each horn of the uterus had a single swelling an inch and a half long and an inch in diameter. Upon laying one of these open, eight embryos were seen, lying in a row, partly enveloped in one or two long furrows. These furrows would extend along the lower internal wall of each uterus; if the animal were in its natural position they would then be horizontal—a fact the importance of which will appear later. The fœtuses varied considerably in development, some being nearly twice as large as others. In the larger embryos there were two visceral clefts, the fœtal circulation was completely established, the fore limb was comparatively well developed, with the position of the toes faintly

[1] Rev. Dr. Bachman, 'Proc. Phila. Acad.,' April, 1848, p. 46. This writer's statements have been confirmed by several observers.

[2] Owen, 'Comp. Anat. of the Verts,' vol. iii, p. 718.

[3] Mr. Robert Speir, of South Orange, N. J.

outlined, the hind limb was still bud-like. The tail extended somewhat beyond the hind limb; the cup of the eye was backward in development, presenting a horseshoe appearance, like that of a chick in the third day. Altogether by a comparison of the older embryos with some newly born opossums found upon another female, I conjecture that the embryos were about eight days old, and that the short period of intrauterine development was about half over. A fœtus of median size was detached by a slight pressure of the needle, and the subzonal membrane was found to be about 10 mm., or $\frac{3}{8}$ of an inch in diameter. Through this membrane the embryo could easily be seen. There was an opaque disc-like area on the subzonal membrane, and this was found to correspond to the partially adherent yolk-sac, which was spread over about one third of the inner surface of the membrane. When a portion of this area was seen in profile a large number of minute villi were at once noticed upon the surface of the subzonal membrane, which was smooth elsewhere. The yolk-sac, as in the kangaroos described by Professor Owen, had the figure of a cone, the base attached to the subzonal membrane and the apex at the umbilicus. At the edge of the area the yolk-sac was folded back upon itself, as in fig. 1 (woodcut). The umbilical stalk was wide. The attached area was covered with capillary vessels, and circumscribed by the sinus terminalis; this united near one edge of the disc, to form a single vitelline vein (Pl. XXXIII, fig. 1), and the vitelline arteries were either double or branched close to the embryo from a single trunk. They were difficult to distinguish.

The allantois was found in the various embryos in all stages of development, two of which are represented in Plate XXXIII, figs. 1 and 4). It arises, as in the Placentalia, just behind the umbilical stalk, and the mesoblast and hypoblast could be readily distinguished. In later stages it was a small sac with a wide stalk. In the embryos which were examined no blood-vessels could be detected, but they undoubtedly develop at a later period. Compared with the yolk-sac the allantois was extremely small, nor was it in contact with the subzonal mem-

brane at any point. Still, no opinion could be formed as to its subsequent relations, for its development is evidently very rapid, and the embryos were in an early stage of growth.

The greatest interest naturally was directed to the villous area of the subzonal membrane. This could be separated with ease from the subjacent portion of the yolk-sac, revealing the rich capillary network of the latter. At this point a careful drawing of the fœtus was made (Plate XXXIII, fig. 1), magnified about five diameters, and representing the proportions as nearly as could be done by the eye. The membrane was composed of a single layer of polygonal epithelial cells. When seen from above the villi appeared as rings of thickened epithelium of all sizes in profile (fig. 3); they were seen to be composed of a single layer of columnar cells, some of which were produced into minute processes. The villi varied considerably in height; they were hollow, and beneath them was a layer of flattened epithelium; whether the latter was derived from the subzonal membrane or had been torn off from the yolk-sac could not be ascertained. A portion of the villous area near the sinus terminalis, containing one of the vitelline arteries and a section of the vena terminalis (Plate XXXIII, fig. 2), shows that at this period there was no especial enlargement of the capillary vessels near the villi; in fact, none of the latter showed any trace of vascularity. Two facts, however, indicated that this would appear in a subsequent stage:—1. The villi were apparently beginning a similar line of development to that which they pursue over the attached allantoic area in the Placentalia. 2. The villous area in each fœtus was in close contact with the uterine furrow, whereas the remainder of the subzonal membrane floated free in the uterine cavity. The word "attachment" would be incorrect in this connection, but the union with the uterine wall was sufficiently close to prevent separation, even when there was considerable motion in the water in which the uterus was placed.

By an unfortunate blunder in the laboratory one horn of the uterus containing the embyos in sitû was thrown away, so

that no satisfactory examination of the adjacent uterine wall could be made.

Although energetic efforts were made, no other pregnant females were captured,[1] so that my observations upon the opossum terminated at this period. I was quite convinced, however, that older Marsupial embryos would show vascular villi upon close examination.

The opportunity of completing and confirming the above results was due to the generous assistance of Professor Wilder, of Cornell University, and Professor Chapman, of the Jefferson Medical College of Philadelphia. The former most kindly placed at my disposal a quantity of Marsupial material, which he had procured from Australia; the latter allowed an examination of his valuable kangaroo fœtus. To both of these gentlemen I wish to express my hearty acknowledgments.

Among Professor Wilder's material was a fine fœtus, which will be referred to as Specimen 2, because, although it was labelled "Removed from an Australian Marsupial," the memorandum giving the generic name was lost. The fœtus was evidently not that of a kangaroo, but probably belonged to one of the smaller Australian Marsupials. Its external structure[2] as well as the character of its membranes left little doubt of this.

Specimen 2 is drawn natural size in fig. 5, and was believed to be in a somewhat advanced period of intra-uterine life. The embryo had well-developed fore limbs, in which the fingers were all distinct; the hind limbs, although much smaller, showed the division of the toes. The eyes were in a rudimentary condition, but the ear-pits could be plainly seen, while

[1] All writers upon this subject refer to the difficulty of procuring females during the period of gestation. The Rev. Dr. Bachman (loc. cit.) at one time in the course of three days procured thirty-five opossums, not one of which was a female. Audubon mentions a still greater proportion of males. At ordinary periods the sexes are equally numerous.

[2] Characteristic features of advanced Marsupial embryos are the large size of the tongue, the disproportion between the fore and hind limbs, the large mouth, and wide nostrils. In the case of Specimen 2, the subsequent examination of the kangaroo was further confirmation of the fact of its being a Marsupial.

the mouth was large, with a much-protruding tongue. The tail was quite long. As a whole, the embryo in size and appearance resembled closely that of the oppossum at birth, except that the snout was shorter, suggesting that the embryo belonged to one of the short-faced genera—Chironectis, Petaurus, Phalangista, or Phascolarctos.

Owing to the rupture of the subzonal membrane, as well as the removal of a portion of the yolk-sac, the precise relations of the membranes were difficult to determine. As far as they could be made out the whole was surrounded by a subzonal membrane, within which the yolk-sac was flattened out over a larger area than in the case of the opossum, a fact which was quite consistent with the advanced age of the embryo. The missing portion of the yolk-sac was largely within the sinus terminalis, so that the extent and character of the attachment of the yolk-sac to the subzonal membrane could not be satisfactorily ascertained. The latter was carefully examined, and soon a number of low villi were discovered upon it without the aid of the glass; they were distributed over an area to which a highly vascular portion of the yolk-sac was adherent, which was, however, just without the limits of the sinus terminalis; what their distribution was within the limits circumscribed by the sinus terminalis could not be be followed, owing to the removal of the latter. In fig. 5 their position is indicated by a number of dots (*v*); as the figure represents the inner view of the yolk-sac, the villi were of course upon the lower surface, their position being more plainly shown in the woodcut (fig. 2, *v*). The villi are shown in fig. 6 as they appeared in profile under a low objective. They were considerably lower than the subzonal upgrowths of the opossum, so that the term villus cannot be given them very accurately. Upon separating the subzonal layer from the yolk-sac, the former was seen to be composed of somewhat flattened cells, which, over the summits of the villi (fig. 6, *b*), had a truly squamous character, being quite transparent. The separation of the subzonal membrane did not leave the surface of the yolk-sac smooth as in the opossum, but covered with apparently

solid papillæ, derived from the yolk-sac epithelium. Each of these was found to be provided with a single dilated capillary vessel branching over its summit (fig. 6, *a*). These papillæ, therefore, with their subzonal caps, recalled at once the simplest form of allantoic villi, which Professor Turner represents[1] as consisting of a vascular core raised upon the surface of the allantois and covered with a layer of pavement epithelium derived from the subzonal membrane.

The allantois in Specimen 2 consisted of a highly vascular sac, with quite a long narrow stalk, which was attached to the embryo just behind the umbilical stalk; its distal surface was covered with capillary vessels ramifying in all directions; the number of main blood-vessels supplying the allantois was not ascertained. This allantois was proportionately larger than in the advanced kangaroo fœtus described by Professor Owen; in other respects it had the same appearance. A more important difference was seen in the fact that this sac had a disc-like area of attachment to what was apparently a portion of the subzonal membrane composed of pavement cells. This feature, if confirmed by later observations, is a highly important addition to our knowledge of the fœtal membrane of the Marsupials. Unfortunately, owing to the torn condition of the subzonal membrane, this point cannot be considered by any means certainly established.

The uncertainty which existed as to the generic and specific character of Specimen 2, and the difficulty of a positive determination of the relation of its membranes, made an examination of the kangaroo fœtus very desirable. According to Professor Chapman's record, the mother was killed fourteen days after impregnation. The embryo was, however, in an earlier stage of development than that of the opossum;[2] the visceral clefts were still very distinct; the fore limb was elongated, but the hind limb was a mere bud. The yolk-sac, however, was spread over the inner surface of the subzonal membrane very much as

[1] 'Journal of Anatomy and Physiology,' vol. xi, p. 34.

[2] H. C. Chapman, 'Proceedings of the Philadelphia Academy,' 1881, part iii, p. 469.

Fig. 1.

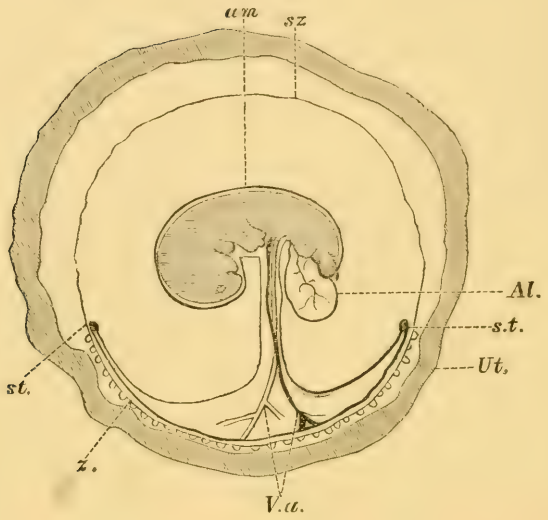
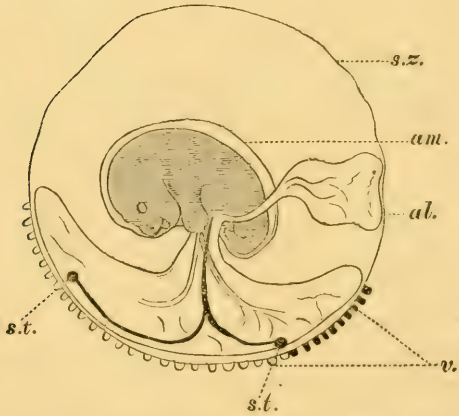


Fig. 2.



Diagrams showing the relations of the foetal membranes.—Fig. 1 represents the actual relations of the membranes at the middle period in the opossum and the kangaroo. The shaded ring (*ut.*) represents the wall of the uterus in section, showing how the villous area of the subzonal membrane (*sz.*) is in contact. Fig. 2 shows how in Specimen 2 a portion of the yolk-sac forms an attached villous area beyond the sinus terminalis; the dark villi are those actually observed, the remainder are supposed to have been present when the membranes were complete. The supposed adherence of the allantois (*al.*) to the subzonal membrane is also shown. *st.* Sinus terminalis. *am.* Amnion. *Va.* Vitelline artery and veins. *z.* Villi of younger specimen. *v.* Vascular villi of older specimen, the black ones observed, the unshaded ones inferred.

in the older opossum embryos, while the allantois was a small sac supplied by two arteries.

The line of attachment of the yolk-sac to the subzonal membrane was marked, as described by Professor Chapman, by the sinus terminalis, and this, as in the opussum, seemed to give rise to a single vein near the edge of the disc; there was a single artery. The fœtus therefore closely resembled the earlier opossums, and it was very gratifying to discover minute villi all over the attached area of the yolk-sac, thus confirming the previous observations. These villi were so minute that it is not at all surprising that they were overlooked by previous observers. I have not yet had an opportunity of examining them closely; their external size and appearance was similar to those found in Specimen 2, although they were in an early stage of development. Beneath them the disc formed by the yolk resembled closely that of the opossum, and it was quite evident that at a later period they would resemble in internal structure those found in Specimen 2.

I think we may now regard the following facts, in respect to the fœtal membranes of the Marsupials as fairly well established.

1. That the yolk-sac at an early stage spreads over the inner surface of the subzonal membrane, forming a disc-like chorion, which in the kangaroo and opossum is bounded by the sinus terminalis. This chorion may become extensive in the later stages. The subzonal epithelium then gives rise to hollow conical upgrowths of columnar cells. From the epithelium of the yolk-sac there arise papillæ, which become vascular, while the subzonal cells become very much flattened. The rudimentary villi thus formed, in the early oposssum and kangaroo embryos, are thickly distributed over the area surrounded by the sinus terminalis, but in other forms they may extend beyond this area.

2. The allantois arises in the same way as in the Placentalia at quite an early stage of development, and soon becomes vascular. In the kangaroo, if it unites with the subzonal membrane at all, it is only in the later period of gestation. In the

opossum it develops rapidly, so that a brief union with the subzonal membrane before birth is not improbable. In the unknown Marsupial (see Specimen 2) this union seems actually to have taken place.

3. The amnion, as in the Placentalia, in all cases invests the embryo.

4. One or two long furrows are formed along the lower internal border of the uterus in the kangaroo and opossum. In close contact with one of these in the opossum is placed the villous chorionic disc of each of the numerous fœtuses; the remaining portions of the subzonal membrane are free. The embryo is undoubtedly retained in this position throughout intra-uterine life. During this period the opossum is known to keep remarkably quiet, so that the uterus is little disturbed, and is most of the time in a horizontal position.[1] The presence of fœtal villi is strong evidence by analogy of the presence of minute crypts on the inner wall of the uterus.

It is an undoubted inference from the above facts that in the early stages of Marsupial development the vessels of the yolk-sac not only are the channels for conveying the maternal nutriment to the fœtus, but that this function is performed by capillaries distributed in low villi, and separated from the maternal structures, whatever the arrangement of the latter may be, by an extremely thin layer of subzonal epithelium. It is evident that these villi are altogether similar in structure to those which are found over the allantoic chorion of the pig;[2] the difference is merely one of degree. The rudimentary mechanism is sufficient to support the rapid growth of the embryo opossum, which at birth is completely equipped with all the necessary respiratory and digestive apparatus acquired during an intra-uterine period barely exceeding two weeks.[3] This

[1] The fact noticed by several observers, that the females are found in plenty immediately after the birth of the young, would seem to indicate that they had been in hiding for some time.

[2] See Turner, loc. cit.

[3] The feebleness of the young at birth has been exaggerated. The opossum young weigh from four to five grams, and in their bent position are one half

could not be effected if the absorbent villous area were shifting about from one part of the uterus to another. This fixity of position must have been an important step towards the establishment of an allantoic placenta.

Although we may now be reasonably certain of the early condition of the fœtal membranes in the Marsupials, it must be borne in mind that all the latter part of their history is still a blank, and that the allantois in the later stages may enter into very important relations with the subzonal membrane. Balfour, with his usual discernment, suggested a probable condition among the primitive Placentalia,[1] in which the yolk-sac and allantois shared the placental function. I think it is not improbable from the evidence given by Specimen 2, and from the rapid growth of the allantois in the opossum, that this condition may yet be found among the Marsupials. The fact that no fœtal membranes are brought forth at birth has, I believe, been correctly attributed to the very tortuous vaginal passage through which the young pass in their descent.

The evolution of the placenta is an interesting subject of speculation, which it is tempting to review, now that we have more light upon the functions of the yolk-sac.

(1.) In the low reptilian forms which preceded the Mammals there was undoubtedly a substitution of the viviparous for the oviparous mode of reproduction, by the gradual reduction of the food yolk and the retention of the embryo in the uterus. The whole character of Mammalian development points unquestionably to the former presence of a mass of food yolk in the ovum, and there is every reason to suppose that the loss of this source of supply was gradually and pari passu compensated by the substitution of the maternal nutrition, so that the embryos were partly nourished by the yolk, partly by a feeble absorption of

an inch long. All the bodily functions are fully in action, the fore limbs are strong and provided with claws, the young are taken in the mouth of the mother from the valva and placed in the pouch, probably close to one of the nipples, the grasping of which is instinctive. They will retake the nipples after removal from the pouch and exposure for several hours.

[1] 'Comparative Embryology,' vol, ii, p. 216.

nutriment from the uterus through the contiguous umbilical vessels, the allantois retaining solely its reptilian functions.

(2.) With the diminution of food yolk came an increasing absorption of maternal nutriment through the chorion of the yolk-sac, upon which villi gradually appeared. The Marsupials may fall into this or the following class.

(3.) A condition in which the allantois and yolk-sac shared the placental function.

(4.) The primitive Placentalia (Balfour), in which the yolk-sac formed a large false chorion, and the allantois formed a small discoid placenta, and in which the maternal parts were not deciduous.

I hope during the spring of 1884 to be able to follow out the membranes of the opossum to the later stages. At present, owing to the hurried preparation of this paper, some valuable drawings have been omitted, and the study of the kangaroo was not so complete as I desired, nor have I been able to refer to all the authorities upon the subject, as I hope to do in a later paper.

May 14*th*, 1883.

Observations on the Genus Pythium (Pringsh.).

By

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With Plates XXXIV, XXXV, XXXVI.

Among the numerous species and genera of fungi which have become known to science of late, there are perhaps none more important from a biological point of view — unless Bacteria be excepted—than the minute, and for a long time ill-understood organisms comprising the genus Pythium, founded by Pringsheim in 1858 as a group subordinate to the Saprolegniæ. During some recent investigations which I have lately made for the purpose of obtaining a clearer insight into certain obscure processes in the vegetable cell, I had the good fortune to obtain material which afforded an opportunity for a study of these plant-devouring fungi, under exceptionally favorable circumstances. It appears worth while, therefore, to describe these observations, not only because they embrace important facts of general biological interest, but also because the organisms concerned have been apparently almost ignored in England.[1] Whether they are to be regarded as morphologically of such importance as they are believed to be may remain an open question until they have been further examined.

[1] They are not mentioned, for instance, in Cooke's 'Handbook of British Fungi;' and the 'Micrographical Dictionary,' 3rd edition, 1875, has a very insufficient note on the genus.

One great difficulty experienced by those who have attempted to define the taxonomic limits of these and similar organisms, comprising the Saprolegniæ, &c., is to determine whether they are Fungi or Algæ; but since true parasitic members of the group are now known to infest land plants, as well as green Algæ, we may safely put this difficulty aside when their want of any trace of chlorophyll or starch-forming substances are also borne in mind. After all, and the opinion may be abundantly supported, the exact limitation of the lower Algæ and Fungi is of comparatively small importance, since it may be considered certain that they pass into one another at one or more points.

The name Pythium was given by Pringsheim to a group of Saprolegnia-like organisms, because he found that the process of formation of the "swarm-spores" differed in this newly discovered type[1] from that of Saprolegnia generally; De Bary soon afterwards described new species of the genus,[2] and other observers gradually added to the list. For many years, however, great confusion seems to have existed between Saprolegnia, Achlya, Pythium, and other groups, and it was not until a comparatively recent date that something like order was arrived at. This has been accomplished by continuous and careful observation of the development of individual forms, and much is due to the indefatigable labours of De Bary, whose last monograph[3] is a model for all morphologists.

Several of the known species of Pythium are parasitic on living plants, though others appear to be habitually saprophytes. In 1874, however, Hesse discovered a species[4] which seems to be almost omnivorous, attacking living and dead plants of widely different kinds, and which can be grown on animal substances as well. I shall commence by examining this species in some detail, not only because it is one of the

[1] 'Jahrb. f. wiss. Bot.,' B. i.

[2] 'Jahrb. f. wiss. Bot.,' B. ii, and literature quoted below.

[3] 'Beitr. z. Morph. u. Phys. der Pilze,' R. iv, 1881.

[4] 'Pythium De Baryanum, ein Endophytischer Schmarotzer,' Halle, 1874.

largest and most vigorous forms, but also on account of the ease with which it may be obtained and cultivated, and further, on account of its importance as a parasitic enemy of food and other plants. It is, moreover, in every sense typical.

Pythium De Baryanum.[1]

In almost any sowing of ordinary cress (Lepidium sativum), certain of the seedlings after three or four days of growth especially if kept moist may be observed to become weak at that part of the stem which joins the root, and to bend sharply over. In many cases the general rotting and death of the seedling follow. An examination of such a diseased seedling shows the part of the stem nearest the ground to be in a rotten state. Instead of being turgid, semi-translucent, and of a pale greenish colour, the affected part is seen to be much contracted; its cells turn brown or yellow, and become unfit to support the weight of stem, &c., above it. At fig. 1 is a drawing of a seedling in this condition, straightened out and with the soil washed from the roots. The line may be taken to illustrate the level of the soil at the region where the damage occurs.

This rotting tissue, and parts of the stem immediately above it, are seen under the microscope to be full of very delicate hyphæ, branching in all directions in and between the cells. Closer examination shows that the thin-walled, cylindrical hyphæ are confined to the parenchymatous tissues, and avoid the still young fibro-vascular bundles forming the more central parts of the hypocotyledonary stem of the seedling. It is in consequence of the flexible axial portion being no longer supported by the turgid parenchyma, that the "top-heavy" seedling bends sharply over at the injured spot.

More minute examination shows that the hyphæ run through the tissues, especially in a longitudinal direction, and if observation is particularly directed to the portions which are

[1] Literature:—Hesse, loc. cit., 1874; De Bary, 'Bot. Zeit.,' 1881; and 'Beiträge z. Morph. u. Phys. d. Pilze,' 1881, R. iv.

still only partially injured, the hyphæ are seen to bore through the cell-walls, cross the cell-cavities, and send out secondary branches in all directions, which repeat the same processes in their turn.

If such a piece of "infected" tissue be placed in water in contact with a healthy seedling of cress for 12—24 hours, the latter will be found firmly attached to the former by means of hyphæ which have grown across the interval and commenced to bore their way into the healthy tissues. These penetrating hyphæ not only enter any stomata in the epidermis of the attacked plant, but make perforations through the cell-walls as before (fig. 2). Similar events follow if a portion of the infected tissue be placed on the clean, cut surface of a potato (fig. 3), or on portions of many other plants. These facts will be referred to later, when we are concerned with the mode of action of the hyphæ. Once inside a suitable tissue, the mycelium makes it way through all the thin walls as already described. After some hours the tissues thus attacked become reduced to a mere mass of pulp, and the well-nourished mycelium begins to form its reproductive organs, at the same time developing new ramifications in the surrounding water. The rapidity of these processes, and the extent to which they go on, are dependent on a number of conditions, apart from the nature of the host plant. Amongst these, temperature and the abundance of oxygen are the chief. All being favorable, certain branches of the mycelium become swollen at the apex into pear-shaped bodies, large quantities of protoplasm passing into them. Each of these, having attained a maximum size and become nearly globular, becomes cut off by a septum from the rest of the branch, and persists as a nearly spherical thin-walled cell (fig. 5).

This is a "conidium"—a simple dilation of the hypha, rich in protoplasm, and capable of germinating at once (in fresh water) after separation from the parent branch. These conidia are formed in immense quantities at the ends of the numerous branches of the mycelium; not only free in the surrounding water, but also in the destroyed tissues (fig. 2).

In any vigorous cultivation they become formed at an early stage, and are scattered around in large numbers as development proceeds.

Besides these terminal conidia, however, there usually appear numbers of interstitial conidia (fig. 4), each of which arises as a simple swelling on the course of a hypha, which having received much granular protoplasm, becomes at length cut off by a septum on either side. In fig. 4 are shown the various changes of the slowly moving vacuoles, &c., noticed in such a body, observed for some time at intervals during development.

Each kind of conidium acts as a simple asexual reproductive cell. If fresh water containing oxygen be added to the specimen, the conidia soon put forth processes which develop forthwith into new extensions of the fungus; this happens whether the conidium be free or still attached (figs. 5 and 6). If allowed to remain undisturbed, no germination occurs; the terminal conidia drop off and remain dormant, and the interstitial ones become free by the decay of the remnants of hyphæ on either side. Under proper conditions their vitality is maintained for months,[1] ready to be called forth in a few hours when fresh water is added; the older conidia usually show a better developed "exospore" than those which have not been kept.

Germination consists simply in the extrusion of the "endospore" into a simple tube (from one or two points) into which the protoplasmic contents pass, until all is used up in the formation of the germinal tube; the latter grows quickly by apical growth, enters a suitable nidus by boring through the cell-walls, or, if none such is present, soon decays. It is noteworthy that the formation of septa originates when the reproductive organs commence to be developed; in its young stages, the mycelium, though copiously branched, consists of a continuous series of tubes.

[1] De Bary says that drought and frost are withstood by these conidia, 'Bot. Zeit.,' 1881, No. 33, p. 524.

Hesse[1] and De Bary[2] both describe the formation of zoosporangia in this species; I have not succeeded in obtaining zoospores, and can only refer to their descriptions. The zoosporangium arises, according to these observers, in exactly the same way as a terminal conidium, and is not to be distinguished from it by outward characters, until its further development. This takes place by the lateral outgrowth of its wall into a kind of beak, into which the contents pass; the end then swells up quickly into a gelatinous vesicle which receives the protoplasm, and this at once becomes divided up into zoospores. These processes, as figured by Hesse, are very similar to what occurs in Pythium proliferum, in which form I have studied them very carefully; nevertheless the differences are sufficient to distinguish them. De Bary points out that the conidia only produce zoospores if sown at once,[3] and immediately on separation, in fresh water. My failure to obtain the zoospores may possibly be attributed to this. Besides the asexual forms of reproductive bodies, however, this species possesses sexual organs of a simple and typical character, and from the comparative ease and certainty with which the process of fertilisation may be studied in these fungi, an accurate knowledge of the essentials of the sexual process can be hoped for with some success.

In a well nourished cultivation of Pythium de Baryanum, the appearance of the sexual organs usually occurs in from forty-eight to sixty hours at latest, and enormous numbers sometimes arise, after the crop of conidia has considerably advanced. If a piece of cress-seedling, thoroughly infected with the fungus, be placed in fresh water in a watch-glass or open vessel, the oogonia and antheridia are commonly produced in a few hours: a favourable portion attached to the lower side of a thin cover-slip in a suspended drop of water, kept in a damp chamber—allows every step of the development

[1] Loc. cit., p. 19.

[2] 'Bot. Zeit.,' 1881, p. 523.

[3] "Zoosporen sah ich diese Conidien nach mehr als höchstens wenige Tage langer Aufbewahrung nie bilden," 'Bot. Zeit.,' 1881, p. 524.

of sexual organs, and of the sexual act to be followed on one specimen.[1] I will describe the different phases as observed on such a cultivated example.

From a branch which had developed into the surrounding water, an apical swelling was formed exactly as for a terminal conidium: much dense granular protoplasm accumulated in this, and then a septum formed below. Soon afterwards, a lateral protuberance arose from the hypha immediately below the septum; this rapidly developed as a somewhat club-shaped branchlet, also filled with protoplasm, which curved upwards towards the large spherical terminal body. The first formed, conidium-like sphere is the oogonium; the smaller, clavate body, the antheridium. In the case described—a very common one in this species—the antheridium, having become separated by a septum from the common parent hypha, pushed aside the oogonium as its apex came in contact with it (fig. 9). The antheridium does not, however, always arise immediately beneath the oogonium; it may even spring from a different branchlet (fig. 8), and other cases occur.

On an oogonium favourably situated for examination I made the observations illustrated at fig. 10. This specimen was continuously watched from a little before eight o'clock in the morning till three o'clock in the afternoon, drawings being made at intervals, when the progress of events was marked by changes of special interest or importance.

At 8.15 a.m. the oogonium and antheridium had been completely formed, and in contact for some time, the apex of the latter being closely attached to the oogonium wall, and having already commenced to send a short process into it. The contents of the antheridium were bright and less dense than those of the oogonium, with several large brilliant granules scattered inside; a firm septum marked off this upper part of the antheridium from the rest. At a period just preceding

[1] In many cases the mycelium grows down, across the cavity of the damp chamber, and, carrying water with it, spreads on the glass slip below: excellent preparations of the sexual organs in all stages may thus be obtained.

the hour named, the contents of the oogonium were coarsely granular, and marked by many small oily particles, giving the whole a yellowish-grey appearance. At 8.15 these contents were beginning to contract towards the centre of the oogonium, strings and bands of protoplasm being left attached to the inner wall. This contraction occurred slowly, but was distinctly attended with amœboid movements. During the next three quarters of an hour this withdrawal of the coarsely granular fatty protoplasm slowly continued, and at 9 a.m. the condition of affairs was that figured at fig. 10, *b*. The beak-like process (which had already commenced to be formed in *a*), sent by the antheridium through the oogonium wall, now became more distinctly evident; the protoplasm inside the antheridium also seemed to me to have become paler and more transparent.

The protoplasm of the oogonium was still anchored by radiating threads to the walls, and seemed to contain fatty globules; its slow amœboid movements still continued. These movements became still more decided during the next hour; and at 10 o'clock (fig. 10, *c*) the central mass of the oogonium had become almost spherical and free from the walls. Its fatty and granular contents were also arranged into rather angular blocks, formed by the gradual flowing together of the smaller globules and granules. In this condition the naked mass may be looked upon as an egg, or oosphere, ready for fertilisation; for, although the tube from the antheridium extended right through to the mass in question, I could at this time detect no passage of substance through it. The brilliant, refractive granules in the body of the antheridium were observed to be distinctly undergoing slow changes of position, however, and the amœboid movements of the oosphere were carrying it round the inside of the oogonium. That this was a period of activity, or excitement, so to speak, preceding the passage of the contents of the antheridium through the tube into the oosphere, was amply demonstrated by what followed. Another point, which became clearer afterwards, was noticed: the oosphere did not comprise the whole of the

protoplasmic contents of the oogonium, part being left between it and the walls.

Shortly after the stage just described—the period of excitement, so to speak—culminated in the passage of the contents of the antheridium-tube towards the oosphere, and by 10.55 (cf. fig. 10, *d*) this process was fairly commenced. Close and careful observation of the contents of the antheridium and its "fertilising tube" convinced me that the large, brilliant granules were gradually being carried through the tube into the oosphere by means of a slow, more or less continuous, current of protoplasm.

There is not the slightest doubt of the accuracy of this observation. A particular granule just about to enter the tube at 10.55 (fig. 10, *d*) was observed to pass slowly into the tube, and disappear at the other end (fig. 10, *e*) in less than five minutes. The motion was not rapid, but consisted of a gradual, steady streaming, as a comparison of figs. 10, *d* to *f*, shows. In some cases the granules appeared to melt away in the tube. Meanwhile, the granules in the antheridium were slowly accumulating at its upper part, each to be carried down the tube in turn. At 11.10 (fig. 10, *f*) two distinct vacuoles, separated by streaming protoplasm, appeared in the antheridium; and by 11.30, three or four others had been formed. At this time, also, nearly all the remaining bright granules were aggregated at the entrance of the tube (fig. 10, *g*), and a comparatively rapid passage of these through the tube occurred during the next five minutes (cf. fig. 10, *g* and *h*). The slow revolving motion of the oosphere was still taking place; but the tube was plunged further into the substance of the oosphere at 11.30, for instance, than at 11.10, as shown in figs. 10, *g* and *f* respectively.

At 11.45 the last three of the large granules began to pass over (fig. 10, *i*) in the final flow of protoplasm, and the vacuoles now became very large. The remaining hyaline protoplasm was still, however, slowly streaming towards the mouth of the fertilising tube, and much of it passed through during the next three quarters of an hour, the quantity left in the antheri-

dium at 12.30 being very small (fig. 10, *k*). The oosphere, during the last-named stages, slowly came to the centre of the oogonium (fig. 10, *k*), and then ceased to revolve. Meanwhile a very delicate skin had been formed over the now smooth exterior of the oosphere. This was first detected about 11.30 (fig. 10, *g*), but had become much more evident at 12.30 (fig. 10, *k*). At 3 o'clock p.m. the antheridium contained practically nothing except a trace of granular matter; all its remaining protoplasm had passed over into the oosphere, thus changing it into an oospore. At this hour, too, the oospore—as it must now be termed—had become clothed with a thick envelope. The process of fertilisation was completed, and the oospore was "ripe" (fig. 10, *l*). A comparison of figs. *k* and *l* shows that the protoplasm which persisted between the oosphere and the oogonium wall at 12.30 had entirely disappeared at 3 o'clock. There can be no doubt that it became used up to form the thick envelope formed in the interval around the oospore. Long after the completion of the latter, the empty antheridium and tube can still be recognised, though the ripe oospore lies loosely in the oogonium. In such condition the oospores remain resting for months, as can be seen in old material (figs. 6 and 7).

It was already known, from the researches of Hesse, that P. De Baryanum attacks many different kinds of living and dead plants, and it seems certain that the mycelium and spores of this fungus are very wide spread in European soils. Although this fungus is so omnivorous, however, there are some plants which it apparently refuses to attack, e. g. fresh water Algæ. Hesse observed it on Trifolium, Spergula, Panicum, and Zea, besides cultivating it on Camelina and Lepidium seedlings; on the other hand, he found that numerous allied plants—among others Brassica, Pisum, Hordeum—refused to be infected by P. De Baryanum. Hesse also failed to cultivate it on Potato seedlings.

The tuber of this latter plant, however, is in reality a very good medium for the cultivation of the fungus, and my researches go to show that Hesse's list of favorable host

species may be largely extended. I have cultivated this Pythium successfully on the young buds of Carrot, on cut slices of Potato and Dahlia tubes, and on the stems of ordinary greenhouse Pelargoniums.

I do not here propose to go very fully into the details of these cultivations, since it seems probable that prolonged research will yield facts of more general significance than may be safely stated at present. The following observations, however, are true so far. The mycelium of the Pythium confines its ravages to the parenchyma cells of the seedlings, stems, and tubers, and, so far as I could discover, never enters or crosses a fibro-vascular bundle; in well developed specimens, however, vigorous branches envelop the vascular bundles, and possibly obtain nourishment from the young sieve tubes. The conidia and sexual organs become formed in any invaded parts of the parenchyma, as well as on the extra-matrical branches of the mycelium. The mode of action of the mycelium seems to be always the same. It consists, speaking generally, in the absorption of the dissolved materials of the cell contents, leaving behind a series of empty bags enveloped —e.g. in the case of a cress-seedling—by the common cuticle; these remnants become more gradually the prey of Bacteria and saprophytes.

Although the above statement is true so far as it goes, there are some details of importance in the modus operandi of the parasite, which I can only touch upon here, since I hope to obtain more information during the course of experiments now in progress.

In cultivating this mycelium on slices of Potato and Dahlia tubers, for instance, I noticed particularly that the starch granules of the former, and the inulin of the latter are not directly attacked by the mycelium. This is very clear in the case of the Potato; the starch grains remain intact long after the hyphæ have destroyed all other cell contents. In the Dahlia, the difficulty consists in deciding whether the inulin sphere-crystals (precipitated by alcohol in the usual manner) have diminished in the invaded cells; I think that such is not

the case. Long after the cells had become traversed by the hyphæ, I was able to obtain sphere-crystals of inulin in quantities which seemed to me not less than in cells which were still uninjured.

Again, in the stems of pelargoniums cut in November, and into which the Pythium was allowed to grow, the starch granules could be recognised even after the Pythium had formed its conidia and oogonia.

It seems highly probable from these observations that Pythium De Baryanum—not to extend the generalization too far at present—is unable to dissolve starch grains unaided; on the other hand, it is quite certain that it absorbs material from the cell contents.

My observations so far have led to the following conclusion as to the mode and sequence of action of this parasite on the cells of the Potato and Dahlia tubers. Certain portions of the protoplasm and cell sap are directly attacked, and absorbed immediately, but of course we cannot say unchanged. The hypha grows at the expense of these, enters another cell, and leaves the starch-grains, part of the protoplasm, and the nucleus untouched.

During these processes the walls of the attacked cells turn brown, especially where the hyphal tube entered the cell, and the dead granular remains of the protoplasm and nucleus soon acquire a similar yellow-brown colour. Starch grains may often be observed embedded as in a matrix of these yellow-brown granular remnants, and, if detached from it, one notices the cavity from which the starch grain has fallen as from a mould. I have observed a similar phenomenon in potatoes invaded by Phytophthora infestans, and Prof. De Bary had noticed it in this case also. It is well known that this Pythium grows better in very young cress-seedlings than in those more advanced, and this seems to be in accordance with what is stated above; unless it is assumed that the more developed cell-wall of the advanced seedlings simply prove formidable barriers to the progress of the hyphæ, a supposition which does not seem to cover all the facts.

How is it then that at a late stage in the development of such a fungus as this Pythium, the starch grains and all traces of cell contents—even cell walls—disappear? Without entering into details, it appears at least highly probable that the remaining changes in the cell contents are effected by Bacteria, carried into the invaded tissues by the hyphæ of the Pythium; that these Bacteria reduce the rest of the protoplasm and nucleus first to a soluble mass, and then cause the dissolution of the starch grains. But facts point to the possibility that the action of the Bacteria in such cases is taken advantage of by the fungus, and that it is not till a very late period that the mycelium of the latter suffers from the dominance of the former and eventually becomes in part a prey to its companion, having meanwhile formed its well protected oospores and conidia, which lie unhurt among the rotting débris.

Pythium proliferum.[1]

The next example of this genus, the life-history of which I have thoroughly examined, is P. proliferum, discovered by De Bary on decomposing insects in water about 1860. In many respects, especially in regard to the sexual organs, &c., it resembles P. De Baryanum so closely as to be hardly distinguishable from it; important differences indeed can hardly be said to exist. It occurs, however, only as a saprophyte, and all attempts to grow it on living plants have failed, though its cultivation on dead cress-seedlings—the seedlings being killed by plunging into boiling water—is very easy. Perhaps the best distinctive character is to be found in the zoosporangia, which are formed in large numbers and retain their power of forming zoospores for long periods, and are peculiarly beaked. The mycelium resembles that of P. De Baryanum in all essential respects, ramifying in the dead tissues and finally putting forth free branches, which rapidly spread

[1] 'Pringsh. Jahrb. f. wiss. Bot.,' vol. ii, p. 182; 'Beiträge z. Morph. u. Phys. d. Pilze,' 1881, R. iv; 'Bot. Zeit.,' Sept., 1881, p. 558.

into the surrounding water and form racemose systems of branches; the ends of the main, secondary, and tertiary branches then swell up as before into pyriform or oval zoosporangia, each of which becomes cut off by a septum, and then commonly puts forth a short necklike process or beak before it separates and falls from the plant (fig. 11). The formation of this beak may, however, be deferred until after the dormant period. The branch, at the end of which such a sporangium has arisen, then frequently puts forth a lateral branchlet beneath the sporangium, the latter thus appearing to be placed laterally on the hypha (fig. 11).

The fallen zoosporangium may either remain dormant for weeks or months or germinate at once; its behaviour in this respect apparently depending simply on the fitness of the environment: germination, or the formation of zoospores, is not, however, necessarily preceded by the falling of the sporangium, as will be seen in examples to be studied immediately.

In fig. 11[1] are depicted zoosporangia which had been kept dormant many months in a cool cellar; the thick outer wall, short beak-like process, and large central vacuole are characteristic. Mingled with these one often observes oospores and empty zoosporangia (fig. 12), the necks of the latter having become prolonged and open to admit of the emission of the contents; soon after adding fresh water to sporangia in the condition shown at fig. 11[1], very many of them become thus emptied of their contents. Others, however, instead of becoming thus emptied germinate in the ordinary manner by throwing out a simple tube as in P. De Baryanum. I have carefully observed both processes.

The zoosporangium (fig. 13 *a*) was drawn at 12.30, several hours after the addition of pure oxygenated water; from the condition shown in fig. 11[1] the contents had not much changed excepting that the vacuole had become broken up into several smaller ones distributed in the active granular protoplasm; the beak, which was not formed immediately after the separation of the sporangium, now commenced to appear as a faint papilla, laterally situated near the point by which the spo-

rangium was formerly attached to the parent branch. At 1 o'clock the beak was much longer (fig. 13, *b*) and the vacuoles had entirely disappeared; and at 2 p.m. (fig. 13, *c*) the whole contents had become evacuated as zoospores, leaving the empty beak and case behind. Fig. 14 gives the results of the examination of another example. The ripe zoosporangium presented at 4.30 p.m. the appearance shown in fig. 14, *a*. At 5 5 p.m. the beak was considerably advanced in development (*b*). Soon afterwards the motion of the protoplasm was much more evident, and a peculiar stage was passed through, during which the contents partially divided up and again became granular. At 5.25 p.m. the soft end of the beak gave way, apparently to the pressure from within, and the contents flowed out (fig. 14, *c*) and immediately became divided up into five actively amœboid masses, which were soon afterwards further separated in the jelly-like envelope; during the next few minutes each acquired a vacuole and two lateral cilia, and at 5.35 (fig. 14, *d*) were transformed into five rapidly struggling zoospores, moving in jerks, and changing the form of their amœba-like bodies continuously. At 5.38 they were all free, escaping rapidly from their mucous prison and swimming about as reniform zoospores of the well-known type.

Before proceeding to describe the other mode of behaviour of these bodies, I may record the observations registered in fig. 16, on the development of the zoospore itself. The zoosporangium in this case was in the condition shown at fig. 16, *a* at 5 o'clock, having lain for several hours in fresh water. As there shown, the granular contents had become excavated by vacuoles of various sizes, and a prominent, firm beak was developed. The vacuoles had not been long formed when the figure was drawn, and were rapidly changing their sizes, numbers, and position, as the granular protoplasm became churned-up, so to speak. The rate at which the changes were proceeding at this period may be estimated by comparing fig. 16, *a*, *b* and *c*, all of which were registered within five minutes.[1] The condition shown in *b* was reached in three

[1] The plan I pursued in making these drawings was chosen after several

minutes. Two minutes later—i.e. at 5.5 o'clock—the vacuoles had almost disappeared, a number of minute bright points, slowly playing in the granular contents, probably representing them. At this stage, also (*c*), the tip of the beak became pale, diffluent, and began to protrude like a gelatinous drop. Within five minutes later a large clear vacuole appeared in the protoplasm at the end of the sporangium opposite the beak (*d*), and the pale swelling at the apex of the beak, suddenly began to be inflated like a blown-up bladder. This condition, at 5.10, ushered in the rapid changes depicted in fig. 16, *e* to *h*, all of which occurred within two minutes. The softened apex of the beak (*d*) became rapidly distended into a vesicle, into which the granular protoplasm flowed smoothly and continuously, evidently impelled from behind by the pressure of fluid in the vacuoles. These vacuoles no doubt contained some soluble material, excreted by the protoplasm, and an osmotic pressure was thus established. The details are accurately figured as they were observed: the rapid flow of the granules through the axis of the beak (*e*), the distension of the pale (cellulose?) apex into a larger and larger vesicle, becoming more and more tenuous as the contents flowed in (*f* and *g*), being very conspicuous. At length—at 5.12—the last granules passed slowly up the axis of the beak, and the former sporangium remained as an exhausted case, in the cavity of which remained a few minute granules, and a slight residue on the inner walls, no doubt representing in part excreted material. The walls of the emptied sporangium collapsed a very little, and a large number of minute Bacteria could be observed attached to the outer surface in all cases (*k*). Even as the last granules passed slowly up the axis of the beak (*h*), the slowly writhing mass of protoplasm began to divide up

different trials: since the granular protoplasm, outer walls of the zoosporangium, and the main part of the beak are practically constant in appearance, I drew a large number of outlines, and left the granules to be filled in later. My attention could thus be concentrated on the numbers, sizes, and positions, &c., of the vacuoles, zoospores, and other details; and these are accurately represented in all respects.

into separate blocks. This proceeded very rapidly to the isolation of the blocks as zoospores. In three minutes the stage shown at (*i*) was reached, the individual amœboid masses becoming quite active at 5.15, tumbling and rolling over one another meanwhile in a most comical manner. At 5.20 their movements became more active, and the cilia appeared (cf. fig. 14); and at 5.25 they were vigorously moving in the extremely tenuous vesicle, the lashing of the two lateral cilia becoming more and more rapid. One minute later, and the vesicle burst suddenly, the active zoospores flitting off at once in all directions. A distinct remnant of the lower third of the vesicle remained attached to the apex of the widely open beak (*k*). The upper parts appeared to become completely dissolved in the water.

As an illustration of the other mode of behaviour of the zoosporangia, the various stages shown in fig. 15 may suffice. Two zoosporangia, which had remained dormant for many months in a cool cellar, were each observed to put forth the pale swelling at the apex of the beak, as described above. Instead of forming the vesicular swelling and zoospores, however, the pale apex became prolonged into a tube, the vacuoles in the sporangium increasing meanwhile as the contents passed slowly forwards. To give an idea of the rate of growth of such a germinal tube, the changes at the apex were observed, as figured (*c* to *i*). The germinating sporangium or conidium (fig. 15 *c*) was drawn at 2.50; at 3.2 the apex had swollen and perceptibly elongated (*d*); fig. *e* represents the condition four minutes later; *f*, after another two minutes; *g*, after four more minutes—*i.e.*, at 3.12 o'clock; *h* was drawn at 3.20; and *i*, at four o'clock. Although it may be convenient to distinguish these germinating bodies as conidia, it cannot be maintained that any perceptible differences between them and zoosporangia are observable until germination occurs. Whether the behaviour depends on internal or external influences cannot be decided at present, though much may perhaps be said for the latter view.

As already stated, the zoosporangia may germinate either

forthwith, after separation from the parent, or after a long dormant period; but they also often emit zoospores while still attached to the parent hypha. I have carefully followed the phenomena of this process, and select fig. 17 as affording sufficient illustrations of the details. The formation of the zoosporangium requires no minute description (*a* to *d*). The zoosporangium (*e*) was completely formed, and separated by a septum as figured, at 12.25, and remained in pretty much the same condition until after 3 o'clock; at 3.35 several vacuoles were observed, slowly changing their positions in the very granular protoplasm (*f*). Shortly afterwards, the sporangium remaining attached, the beak was formed, and by 4.30 (fig. 17, *g*) was completed. During the next ten minutes the processes of formation of the zoospores figured in fig. 16 took place as already described, and the zoospores became developed in the gelatinous vesicle at the apex of the beak (*i*); the rupture of the vesicle, and escape of the reniform bi-flagellate zoospores took place as before, and at 5.5 the only remains of the vesicle were attached to the end of the empty beak (*k*).

Meanwhile, shortly after the passage of the protoplasm through the beak into the vesicle, the septum separating the zoosporangium from the hypha became protruded into the cavity of the former (*i*), and soon attained a considerable development as a vesicular swelling, in which the granular contents of the hypha were slowly accumulating (*k*). From 5.40 to 6.20 (*k* and *l*) this went on gradually and continuously, until a new sporangium had become formed in the cavity of the old one. In this instance, the new zoosporangium ceased to develop during the night; but in other examples it followed the usual course. This proliferation of the hypha is the characteristic which gave P. proliferum its excellent specific name. It is very common to find the second zoosporangium thus developed into the old cavity, where it forms the beak and large central vacuole before passing into the dormant state, behaving as before on the renewal of favorable conditions. The beak of the new sporange is not always coincident with that of the older one, and may stretch

the empty membrane of the latter in the manner shown at fig. 18—a not uncommon case.

The oogonia and oospores of Pythium proliferum were obtained in large quantities, and observed with ease; neither in the processes of development nor of fertilization did I observe any facts of sufficient importance to need description, after what has been said concerning P. De Baryanum. De Bary has pointed out that the antheridia are shorter and less curved; but whether the importance of the distinction can be insisted upon I will not attempt to decide. The ripe oospores also resemble those of P. De Baryanum very closely, and need not be further described. Figs. 19, 20, and 21 show the most important points.

Pythium gracile (De Bary)

may be selected as a further type, and I have had the opportunity to observe it closely. A form called P. gracile had already been discovered by Schenk[1] in the cells of Algæ, when De Bary found his P. reptans[2] with similar habit; both these species are either identical with Pringsheim's P. monospernum[3], or are so near that with the evidence at command they cannot be definitely distinguished. For the present, therefore, we must look upon De Bary's P. gracile as possibly taking the place of these. It has been carefully studied by De Bary[4], and now stands as one of the best known types of the genus. The general characters of P. gracile are similar to those of the other forms, except that, as the name implies, the hyphæ are more slender; correlatively, the oospores and oogonia are more delicate than in the preceding forms. I obtained oospores from an old cultivation and have depicted their structure and germination in fig. 22. The

[1] 'Verhandl. d. Phys. Med. Gesellsch.,' Würzburg, ix, 1857.

[2] 'Jahrb. f. wiss. Bot.,' ii, 1858.

[3] 'Jahrb. f. wiss. Bot.,' i.

[4] 'Bot. Zeit.,' Sept., 1881. De Bary, however, does not consider the identity of these three forms settled. C. f. also 'Beitr. z. Morph. u. Phys. d. Pilze,' R. iv, 1881. See also below, p. 510.

ripe oospore (A) differs from that of P. De Baryanum and P. proliferum, especially in entirely filling up the cavity of the oogonium, the exospore becoming closely fitted to the oogonium wall, and being indistinguishable from it, except under favourable circumstances during development, &c. After a few hours in fresh water the oospores observed commenced to germinate in the usual manner (fig. 22B), and the germinal tube either entered the substance of a favourable matrix—flies' legs, meal worms, dead cress seedlings, &c.—or proceeded to form zoospores (C) in a manner to be described shortly.

I made some observations on the particulars of growth and entry of the germinal tube into a host-plant, which are illustrated by fig. 23 (*a* to *f*). After some hours, an oospore (*a*) was seen to have germinated at some little distance from the surface of a Cress-seedling (represented by × in the figure), near which a minute unicellular alga was adherent; this was at 11.10 a.m. At 11.30 one branch of the germinal tube had grown rapidly (the others hardly elongating at all) and extended (*b*) so as nearly to touch the algal cell near the cuticle of the seedling. Half-an-hour later, as shown at (*c*), the apex of the hypha had touched and glided over the loose algal cell, becoming sharply bent at right angles in doing so, and displacing the cell somewhat from its original position. So far the observation seems to show clearly that the extension of the hypha takes place by apical growth only. The free apex refusing to attach itself to the algal cell, then became bent towards the surface of the seedling (*d*), and at 12.30 was closely appressed to the cuticle. No intercalary growth had occurred in those parts of the hypha behind the apex, as is plain from the position of the angle and loose cell in (*d*); meanwhile, however, the apex became closely pressed against the cuticle, apparently lifting the whole hypha slightly in the process, and by ten minutes past one o'clock (*e*) it was clearly making its way into the cell wall. At two p.m. the end had completely perforated the cuticle and cell wall—not drawn in *f*—and had begun to extend vigorously inside. A slight inter-

calary growth behind the apex appeared to have occurred in the interval (*c*, *d*, and *f*), but I could not be sure of this since the change might be due to a straightening out of the tube in that region. The protoplasm was by this time passing forward towards the apical portion, and only a few granules remained in the oosphere and proximal part of the hypha. Once inside the dead seedlings the fungus extends in the manner already described, and sooner or later begins to form zoosporangia.

These arise as projecting hyphæ, usually vertical or nearly so from the epidermis of the seedling, and they differ in several important respects from those already described. In fig. 24 are drawings showing the various stages witnessed in the formation of the complete sporangium. As seen at first, it was a simple perpendicular branch from the mycelium, filled with densely granular protoplasm and with a rounded apex; at 11.8 o'clock the slightly swollen rounded apex was extremely bright, and appeared capped by a hyaline dome, due to some alteration in the cellulose. During the next ten minutes this cap of diffluent cellulose commenced to swell up gradually and at 11.20 presented the appearance seen in the figure. Almost immediately after this, the finely granular contents streamed suddenly forward into the centre of a gelatinous vesicle formed by the bulging out of the diffluent swollen cap, this streaming resembled very much the rush of endoplasm often noticed in the protusion of a large pseudopodium by a vigorous amœba; the flow of granules was most rapid in the axial portion, and the last particles followed more slowly. The third figure represents the moment before this flow; in the last of the series are depicted the appearances at the instant of its occurrence. In this example the succeeding stages could not be drawn rapidly enough; they were the same as shown in figs. 25 and 26, and a good idea of the rapidity of this process is gained by comparing the time-records made; the last two stages of fig. 2 *a* were drawn at 11.23; at 11.25, the whole mass of protoplasm had passed out into the mucous globe, and was already becoming divided up into zoospores (fig. 26 *b*). At 11.32 these zoo-

spores, about thirty in number, were rolling over one another and waving their cilia (fig. 26, *d*) still enveloped by the vesicle of mucus, and by 11.37 they were fully formed very active zoospores. At 11.43 the enveloping vesicle suddenly gave way and the zoospores passed out free. Fig. 25 shows drawings of the stage when the protoplasm, having all passed out of the sporangium into the vesicular enlargement, is writhing about in an amœboid manner preparatory to its simultaneous division into zoospores; *a* and *b* were drawn at two successive minutes. These stages are between *a* and *b* of fig. 26, which may now be described.

The sudden "blowing out" of the hyaline dome into a vesicle had just been completed at 11.40 (fig. 26, *a*), having gone through the stages already described. Two minutes later the writhing mass of protoplasm, having passed through the stages figured in fig. 25, contracted towards the centre of the swollen vesicle, and rapidly divided into about nine blocks (*b*), which became separate amœboid masses during the next three minutes (*c*). At 11.50—*i.e.*, five minutes after—each mass was an active reniform zoospore (*d*); at 11.52, the very diffluent vesicle, having almost dissolved in the water, gave way and allowed the zoospores (*e*) to escape. The complete zoospore resembles those of the other species of Pythium in its possession of a reniform amœboid body, two lateral cilia from the sinus, and a bright, vacuole-like spot near the base of the cilia.

In figs. 27 and 28 are shown the details observed as to the germination of the zoospore after coming to rest. At *a* (fig. 27) is drawn a zoospore actively moving at 5.15 in a very minute drop of water. It was watched continuously, and drawn again at 5.20 (*b*) and 5.30 (*c*), when it came to a standstill, and commenced to withdraw its cilia. At 5.45 (*d*) the zoospore, having come to rest, had lost its cilia and vacuole, and had developed an envelope and several large refractive granules. From this point, however, apparently owing to a want of oxygen in the water, its changes were distinctly retrograde. At 6.5 (*e*) the granules were coarser, and at 6.35 (*f*) still larger.

At 8 a.m. next day (*g*) no signs of germination were apparent; the very large granules were now dull, and their protoplasmic matrix evidently becoming pale and disorganised. Fig. 27 (*h*) however, shows the normal course of events in another specimen: the zoospore, after about five-and-twenty minutes of active life, had come to rest (as in fig. 27, *d*), and at once protruded a short process. Within an hour after this, the stages *i* and *k* were passed through; the granules becoming used up in the elongating germinal tube, and a vacuole forming in the spore, which became larger and larger as its contents were drawn upon. Soon after the stage *k*—the tube having reached its highest state of development at the expense of the protoplasmic and granular contents, and having met with no suitable matrix to enter—the whole perished. Such zoospores, attached to the cuticle of a cress-seedling killed in hot water, germinated in the same manner, the germinal tube, however, entering the cell wall (fig. 28), and extending as a mycelium in the way described previously.

On cress-seedlings which had been killed by hot water, and which had been brought into contact with some débris containing Pythium given to me by Prof. De Bary, I observed the development of numerous zoosporangia of the typical P. gracile already described, together with a much smaller number of a second, hitherto undescribed form of zoosporangium. Attempts were at once made to separate the two forms in the following manner, by a method often successfully employed in similar cases, and which may be described in detail, because it is instructive in many ways.

A small portion of the semi-rotten cress-seedling was selected, on which a young zoosporangium of the required Pythium was observed to be preparing to emit its zoospores. This was teased with needles in the hope of removing all the zoosporangia of P. gracile. This done, the cleaned specimen was placed in contact with a freshly killed cress-seedling in a drop of pure water on a perfectly clean glass slip. As in all these experiments, every precaution was taken to avoid accidental infection, by heating the needles, forceps, &c., and of

course the seedling—having been killed by immersion in boiling water—need not be supposed to contain sources of error. After lying in contact with the prepared infective material until the desired zoospores had been observed to escape into the surrounding water, the infective mass was removed immediately. The new material now lay in a drop of water in which were the desired zoospores. After a time sufficient for contact and entry of the germinal tubes, it was quickly removed with perfectly clean instruments and allowed to remain for several hours in pure water, in the hope that it was infected by the desired zoospores and no others. In all cases—and many trials were made—the new material developed numerous zoosporangia of P. gracile before a single specimen of the desired form could be detected. Not only so: the P. gracile got the upper hand very quickly, as it had in the original infections. Nay, in many cases the desired form did not appear at all. The reason was clear: the superabundant P. gracile not only formed its zoospores more rapidly and in larger quantities, but they made better progress in the matrix, and killed off the other form in the mutual struggle for existence. In every experiment an odd zoospore of P. gracile got the start, and beat its competitor in the race, and the only result of all the care bestowed appeared to be the establishment of a purer growth of the mycelium bearing the P. gracile form of zoosporangia, prodigious quantities of which became formed in the course of a few hours.

During these cultivations I obtained enormous extramatrical developments of the mycelium of P. gracile, and, both on cress and on the young buds of carrot, was enabled to watch the development of the sexual organs with great success.

At fig. 29 are drawn specimens of the extramatrical mycelium of well-developed cultures. As shown here, the otherwise very slender hyphæ became swollen up here and there into knob-like groups of oval or rounded protuberances, into which the fine grained protoplasm became collected at length. In fig. 29 A, for example, a firm septum marks off an empty distal moiety of a hypha, from one full of protoplasm, and with

several of the protuberances or lateral outgrowths; this condition was observed at 2 o'clock, and remained substantially the same at 6 o'clock. At 9.30 next morning the change depicted A′ had occurred; the protoplasm of the hypha had become further retracted—presumably into the now more developed protuberances — and a firm septum was formed nearer the proximal portion of A, a small commencing outgrowth having become emptied of its contents also. Two days later, almost all the protoplasm was thus accumulated into the largest knob of the outgrowths. At B, a similar accumulation has occurred, and in this manner the mycelium becomes irregularly septate independently of the sporangia, conidia, or other reproductive organs. I think the protuberant outgrowths here described, must be looked upon as physiologically important for the accumulation of protoplasm which may serve either for the immediate need of the mycelium, or for the production of oogonia; since it is certain that the large protuberances may do either of two things, they may put forth ordinary hyphæ which merely continue the vegetative growth (fig. 29, D), or several of them develop into oogonia and antheridia (fig. 31), apparently at the expense of certain of their neighbours, which they deprive of contents. The above view—that the difference between a young oogonium and a mere bud or conidium, is determined by physiological, and not morphological causes—seems to be almost established when we compare the facts just described, with those figured in figs. 31 and 35, where the oogonium is distinctly beaked, as if it had begun to grow forth like a conidium, and then been impelled to behave as an oogonium.

The proper zoosporangia of this form could not be distinguished from those described before (figs. 24 to 27), and they were produced in equally enormous quantities. In fig. 30, I have drawn two abnormal types or states, one of which (*b*) was rather common in my cultivations. In (*a*), for example, the zoosporangium commenced to form, but proceeded no further than the stage figured; the contents becoming withdrawn and used up later. In the second case

(*b*), the swelling up and passage out of the protoplasm took place as usual: but the protoplasm was extremely small in quantity and very watery. Large vacuoles at once became formed, and the whole faintly granular mass soon fell to pieces, merely becoming diffused, as it seemed to me, after the bursting of the absorbent vesicle. These are undoubtedly pathological phenomena, and cannot be considered as specifically considered of the Pythium.

As to the sexual organs and process of fertilisation, little need be said here beyond calling attention to figs. 31—36. The formation and structure of the oogonia and antheridia are essentially as before; and the passage of the fertilising material through the tube (figs. 31A, 32, 33 and 34) needs no further description—it takes place exactly as before described.

The ripe oospore (fig. 36) presents the peculiar characters of P. gracile (De Bary), especially in filling up the oogonium, and I can select no distinguishing features between the two forms, unless the peculiar knot-like groups of tuberosities and the extensive extra-matrical growth, &c., be considered more important than they seem to me.[1]

It may be, however, that some confusion still exists between one or two forms with the simple zoosporangia of this type, since, as De Bary points out,[2] the P. gracile so closely studied by himself is always a saprophyte, and refused to attack Algæ, whereas the earlier forms (P. reptans, De Bary and P. gracile, Schenk) with which he seeks to identify it, were found on living Algæ. It seems probable that further research, directed to enquire whether and how far the species named are distinct, may yield important information as to the limits between parasitic and saprophytic tendencies; such research, however, is likely to be valuable only so far as it is made on carefully isolated cultures, observed during sufficiently long periods.

[1] De Bary, however ('Bot. Zeit.,' 1881, p. 570), says the oogonia and antheridia (of P. gracile) are found only in the substratum, 'Nur im Innern des Substrates, inter und intracellular.'

[2] 'Bot. Zeit.,' 1881, p. 572.

Within the cells of a large species of Spirogyra, observed this summer (1882), I found the Pythium with very delicate hyphæ figured in fig. 37. It was not present in any considerable quantity, and all attempts to cultivate it in the mass failed, as did also my endeavours to make it spread to other Algæ or cress-seedlings. I was also unsuccessful in the search for zoospores and sporangia, and am thus unable to state exactly what species it was. It is an obvious suggestion that this was probably the earlier Pythium gracile of Schenk, which was discovered in similar algal cells, and of which the sexual organs are not known. If this be the case, it is clear that De Bary's P. gracile is a different species, and this would be in accordance with his failure to cultivate that form on living Algæ—it being, so far as is known, a saprophyte only.[1] It may be considered probable, from the evidence at disposal, that the form here discovered is really Schenk's P. gracile (De Bary's P. reptans); and, at any rate, it were better to assume this for the present than to assign a new name to my Pythium until further observations are to hand.

The hyphæ of this species are very slender much branched filaments, which bore through the septa and side walls of the Spirogyra in all directions, causing the chlorophyll bands to become contracted into irregular lumps and bands, which retain their green colour however for a long time before they slowly decay. The great interest attaching to the specimens observed was, that oogonia with oospheres and antheridia were produced in the normal course of the cultivation, and it is clear that the oospheres and oospores differed considerably from those of P. gracile (De Bary), in that the ripe oospore is much smaller than the oogonium, whereas in De Bary's P. gracile the oospore entirely fills the cavity of the oogonium; the antheridial cell is also shorter and broader. The fertilising process was observed and offers nothing specially worthy of note differing from what has been described. In fig. 39 the very short antheridium, at the end of a very

[1] 'Bot. Zeit.,' Sept., 1881, p. 572.

long branch from a neighbouring hypha, was in close contact with the oogonium at 9 a.m., and the process of fertilisation was already commenced, though no tube could be detected, owing to the fatty globules of the egg coming close up to the point of contact. At 12 noon, however, the contents of the antheridium had passed over, the oospore was already clothed with a membrane and its contents presented a different appearance, and the "fertilising-tube" could now be clearly seen extending between the oospore and the point of attachment of the oogonium wall. The completely ripe oospore (fig. 38) has a very thick membrane, and, in some cases at least, a conspicuous central nucleus-like spot.

It does not seem wise to attempt any further speculations as to the relations of the three types of Pythium with which we have been engaged; but it is clear that the one just described cannot be included in P. gracile (De Bary). It is therefore either the same as Schenk's P. gracile, with which it agrees in habits, &c., or it is entirely new. This can only be decided after the discovery of the zoosporangia.

It now remains to describe all I was able to observe concerning the fugitive Pythium, the zoosporangia of which occurred mingled with those of P. gracile, as referred to on p. 507, but which I could not cultivate separately.

The zoosporangia (figs. 40 and 41) occurred as ovoid or pyriform swellings of the ends of single, thin hyphæ, which projected vertically from the cress-seedlings, considerably overtopping the sporangia of P. gracile (De Bary), with which they were mingled. Each appeared in some respects similar to those of P. proliferum already described, a resemblance which might be insisted upon, if the mycelium of this form were not so much more delicate, and if the zoosporangia were developed upon racemose branchings, as in P. proliferum. Such, however, was not the case, and I must regard this slender form as distinct for the present. The development of the sporange as a terminal swelling of the free hypha, which then becomes separated off by a septum and develops a beak as a continuation of the long axis, are sufficiently shown in fig. 40,

and, after what was said before, need not be further dwelt upon.

The development of the zoospores may be more fully described, since it affords further distinctions for separating this species. At 10 o'clock a.m. the ovoid zoosporangium was fully formed, and had developed its short vertical beak from the distal extremity or apex (fig. 41, A). It remained almost unchanged during an hour, the only recognisable changes being the movements of the numerous minute granules, and the formation of ten or twelve small vacuoles (fig. 42, 2) in the protoplasm. These soon disappeared, and the end of the beak became more transparent and its walls marked by fine longitudinal striæ. At 11.40 a.m. the granular contents passed out slowly, inflating the substance of the beak into a delicate gelatinous vesicle in the mode already described in P. proliferum. Here, also, the mass commenced to divide into zoospores, passing through similar stages, and finally becoming free (figs. 41, B-D). All these processes occupied a perceptibly longer time; in this case, however, nearly twenty minutes having elapsed between the emission of the protoplasm and the completion of the zoospores within the vesicle. At 12 o'clock the young zoospores were moving independently (D), tumbling one over the other with active amœboid movements, and soon afterwards the cilia appeared, at first short and slowly waving, then soon elongated, apparently at the expense of the knobs at their extremities, and by eight minutes past 12 the ten zoospores were rapidly flitting about. One minute later (at 12.9) the vesicle gave way, and the free zoospores escaped in the usual manner.

The gemination of the zoospore took place in the usual manner (fig. 43, A) after the swarming, and in one case I observed the entry through the cuticle of a dead cress-seedling (fig. 43, B). But the process of zoospore-formation may occupy even a longer period than above described. In the specimen drawn at fig. 42 (4-6), the separated zoospores moved in an active amœboid manner for more than an hour before the cilia were developed. Of course, that this Pythium

was struggling under unfavourable circumstances must not be forgotten—since we have seen that it apparently became driven out of the field eventually by its successful rival, P. gracile, De Bary—and such circumstances may have affected the normal course of its development, even in details. Nevertheless, there was no direct evidence to show that such was the case in the specimens figured.

Another peculiarity which renders the separation of this Pythium necessary was the behaviour of the sub-sporangial portion of the hypha. As shown in fig. 44, the end of the hypha grows through the empty sporangium, and becomes continued as a long hypha which can certainly bear a zoosporangium again at its apex, though I only once saw one such, and that not fully developed.

All things considered, it seems necessary to regard the above form as distinct from any yet described in this paper. Whether it is the form called P. ferax by De Bary[1] cannot be decided until the oospores, &c. are observed, though it appears so far to answer to the descriptions given of that species.

The last representative of this remarkable genus which I have actually studied, is P. intermedium, De Bary,[2] a form which occurs mingled with P. De Baryanum both on dead and living plants. My specimens were obtained through the kindness of Prof. De Bary, and cultivated on killed cress-seedlings as before. The sexual organs have never been observed, and its only claim to be regarded as a distinct species seems to be its habit of producing the conidia in vertical series (figs. 45, 46), and the many similarities between these conidia and those of the Peronosporeæ; facts of which advantage has been taken in comparing the two groups.[3] The chief points are as follows, according to De Bary: the conidia

[1] 'Bot. Zeit.,' 1881, p. 562. I have only once seen the zoosporangia of P. ferax, through the kindness of Prof. De Bary, and cannot decide that the two forms are identical.

[2] 'Bot. Zeit.,' 1881, p. 553.

[3] 'Bot. Zeitung,' 1881, loc. cit.

may be formed in series of four or five, the apical one always being the older, and will even become developed free in the damp air of a moist chamber. The conidium may produce either a germinal tube or zoospores on germination, much as in the Peronosporeæ: after drying, however, the conidia appear to lose their power of germinating altogether.

As to the claim of this form to be considered a species nothing further can be said, so long as the sexual organs are undiscovered.

On Budding in Polyzoa.

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With Plates XXXVII and XXXVIII.

Introductory.

Mr. Hincks, in his valuable monograph on the British Marine Polyzoa (1), after discussing the nature of the "brown body" found in the old zoœcia of Polyzoa, and its relation to the developing bud closes with these words (p. lxiii): "There seems, therefore, to be grounds (pro tanto) for desiring some further investigation of the subject." This, then, must be my excuse for offering these somewhat imperfect observations.

The investigations on Flustra carbasea were made in the month of May, 1879, whilst I was occupying the table belonging to the University of Cambridge, in Dr. Dohrn's Zoological Station at Naples. The other observations were made on species obtained from Dublin Bay during 1881-2.

Of the nature of the brown body itself I do not propose to treat, as the evidence of other observers as well as of my own studies is perfectly satisfactory in favour of its being, as Hincks says, "derived from the polypide, and is the result of its decline;" but I will limit myself solely to the origin and development of the bud.

Own Observations.

Flustra carbasea, E. and S. (The following observations, when not otherwise stated, apply to the living state only) In

most of the empty zooecia which had been previously inhabited, a brown body was observed situated towards its lower end, this was surrounded by funicular tissue ("endosarc," Joliet), which sent out irregular strands to the walls of the zooecium, some of them being connected with the band of flexible endocyst which stretched across the mouth of the zooecium. In the centre of this band, and therefore connected with the endocyst on the one hand and with the funicular tissue on the other, was situated, in the earliest observed stages, a small rounded mass of cells yellowish in colour, surrounded by a sheath of transparent cells, which together constitute the nascent bud.

The bud soon acquires a well marked central cavity (Pl. XXXVII, fig. 1), then becomes oval in form, and depends from the anterior band of endocyst. A further elongation next takes place, this process being more rapid above than below, resulting in a pyriform body, of which the upper and narrower part consists of a thin double walled sac, the outer wall being the sheath and the inner one the attenuated internal layer. The lower and wider portion consists of the thin outer sheath enclosing the active internal cells (Pl. XXXVII, fig. 2). To anticipate—the inner cells will form the external layer of the tentacular sheath, the external epithelium of the tentacles, and the internal epithelium of the alimentary canal of the new polypide, while the outer layer or "sheath" will form the inner layer of the tentacular sheath, the inner epithelium of the tentacles and the tissue which surrounds the digestive tract.

A series of somewhat complicated changes now takes place in the lower moiety of the inner layer. (It should here be premised that the outer layer is perfectly passive throughout, merely adapting itself in such a manner as to wrap itself round the active inner layer.) One side of this portion of the bud protrudes, the protrusion becomes constricted off in such a manner as to produce a blind sac, depending by the side of the remainder of the bud, the constriction is quite complete except at the uppermost point, this being the spot where the rectum will be connected with the lophophore; fig. 3, Pl. XXXVII, which is drawn from a preparation, illustrates the commence-

ment of this process. It will be noticed that the inferior portion of the area which is being constricted off is connected with funicular tissue; as a matter of fact, there is often a slight difference in the character of those cells which occupy a corresponding position in yet earlier buds. While this has been happening, the other portion, which has a plate-like form, becomes crenulated along its margin, the crenulations, which point upwards and rather inwards, increase in size and we have some twenty-two incipient oval tentacles formed. These tentacles are thus early ranged into a circular lophophore, continuous except in that region from which the above-mentioned pouch is hanging. This gives an appearance of bilateral symmetry to the lophophore, as was noticed by Allman in Paludicella (19), and Nitsche in Flustra membranacea (13) (Pl. XXXVII, figs. 4 and 5.).

The developing polypide now consists of a disc-like body (lophophore), surrounded by twenty-two oval tentacles, emarginated at one spot from which depends a cæcal pear-shaped bag—the future stomach and intestine.

A circular depression occurs towards one end of the disc of the lophophore which rapidly deepens into a rounded sack (Pl. XXXVII, figs. 6 and 7). The fundus of this sac impinges

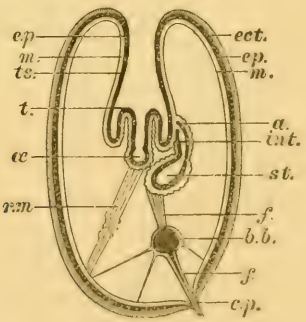

Fig. 1.—Diagram showing the relations of the developing bud in a Marine Polyzoan. *a*, anus; *b. b.*, brown body; *c. p.*, communication plate; *ect.*, ectocyst; *ep.*, epiblast; *f.*, funiculus; *int.*, intestine; *m.*, mesoblast; *œ.*, œsophagus; *r. m.*, retractor muscle; *st.*, stomach; *t.*, tentacles; *t. s.*, tentacular sheath.

upon the cæcal stomach, the two organs coalesce and their lumens become continuous by wall-absorption. This diverticulum is the œsophagus, and the polypide has now the characteristic form of the adult.

The woodcut (fig. 1) indicates the relation of the developing bud to the zooecium far better than a verbal description can do.

Very shortly after this, owing partly to absolute increase in size and also to the elongation of the tentacular sheath, the polypide has come into contact with the brown body, which, as was before mentioned, lies near to the bottom of the zooecium (Pl. XXXVII, fig. 8). The walls of the stomach, or more strictly, that portion of the stomach which forms the gastric cæcum, grow round and envelop the brown body, so that the brown body passes as a whole into the alimentary tract of the young Flustra, which now has the form depicted in Pl. XXXVII, fig. 9.

The brown body immediately commences to undergo disintegration, and, previously to passing into the intestine, the remains are whirled round and round within the globular pyloric portion of the stomach by the action of the minute cilia with which the latter is clothed (Pl. XXXVII, figs. 10 and 11). Ultimately all trace of the brown body, as such, is lost save a small quantity of fæcal matter in the intestine, and by this time the gastric glands become very apparent (Pl. XXXVIII, fig. 12).

The reason why these buds, at this stage, appear of a different colour from the ordinary marginal buds of the colony, as was first noticed by Hincks and animadverted upon by Joliet (17) (still later, see Hincks (1) pp. lvii, lxii), is probably owing to the digestion of the brown body with the concurrent development of digestive glands, the other buds gaining the whole of their nutriment directly from the parent tissues, and thus not requiring a distinct digestive apparatus.

Annulations of the stomach at this stage indicate the existence of circular muscles, the walls of the pylorus become muscular and much thicker, and, as before mentioned, its lumen is ciliated, the cæcum is a wide pouch lined with secretory cells. The intestine is swollen, while the rectum is a short very

narrow tube still retaining its primitive connection with the tentacular crown, to which it is attached about one third from the base.

While the changes described above have been taking place, the tentacles have been gradually lengthening, at first, they are short finger-like processes from the periphery of the lophophore, closed above, open below, containing within their cavities an extension of the original outer layer of the bud which here forms an epithelial lining (Pl. XXXVII, figs. 8 and 9, *a*, and also woodcut, fig. 1); not till comparatively late do cilia arise on the outer epithelium, only certain aspects of the surface of the tentacles are clothed with cilia (see Pl. XXXVII, fig. 9, *a*).

The tentacular sheath ultimately becomes continuous with that portion of the endocyst of the zooecium which surrounds the mouth of the cell as was insisted upon by Nitsche (13, p. 463).

The retractor muscles of the body and lophophore arise, as noticed by Repiachoff (15) from the peritoneal lining of the polypide.

The funiculus early becomes prominent and is probably derived from the irregular strands of funicular tissue which occur in the parent zooecium; it appears as a thickish cord stretching from the fundus of the developing polypide to the base of the zooecium, and, almost invariably, it is in direct connection with the brown body, so that it serves to direct the developing alimentary tract to that nutritive mass, thereby ensuring the better nutrition of the growing bud.

Abnormalities extremely rarely occur in which there may be two buds developed, or more than one brown body, or the polypide may not come into contact with the brown body. The second abnormality probably being the result of the third.

It is thus clear that the bud in Flustra carbasea is developed at a distance from the brown body, that it approaches the latter, envelopes it, and extracts nutriment therefrom. As was pointed out by Repiachoff (16) the same occurs in several genera of Polyzoa (Tendra, Lepralia, Membranipora,

&c.). Joliet also witnessed the ingestion of the brown body in Eucratea chelata, which passed through the alimentary canal of the developing polypide, but owing to its resistant membrane the brown body suffered no alteration; but in Lepralia granifera the very thin envelope of the brown body is destroyed, not being able to resist the action of the juices of the stomach, the movements caused by the cilia, and the contractions of the intestinal walls: thus the brown granules which it contains are set at liberty, whirled about and shortly evacuated by the rectum. Hincks, himself (l.c. p. lxii, footnote) noticed the formation of a polypide-bud quite separate from the brown body in Bugula calathus.

After the able discussion of the subject by Joliet (17), it seems quite superfluous to reopen the controversy as to the probable origin of the bud from the brown-body ("germ-capsule"); but Hincks (l. c. p. lxiii) has still left it a slightly open question. According to Smitt (and Hincks), there would be at least two modes of bud-formation amongst the Polyzoa: 1. In the old zooecia (*a*) formed quite close to the brown-body, and arising directly from it, (*b*) formed at a distance from the brown-body and not arising from it. 2. In the new zooecia, also arising de novo. From the accounts of other observers, one method of bud-formation serves in all cases, the origin in an old or a new zooecium being always from the same tissue, though they are by no means agreed as to what that tissue is. It is merely a question as to how close to or how far from the brown-body the bud shall arise.

Taking all the evidence we possess, it seems to be quite evident that the generally received account is the correct one, but that the approximation of the undeveloped bud to the brown-body may mask its real distinctness in a few instances.

A further observation on a living specimen (Naples, Sept., 1881) is represented on Pl. XXVIII, fig. 13. The bud had reached the stage of Pl. XXXVII, fig. 1; it was suspended in the anterior band of endocyst, and was connected with the parent polypide by the tentacular sheath of the latter, and probably also by some funicular tissue. In this example the

older polypide was rapidly histolysing into the brown-body; thus in this case the bud was formed before the complete degradation of the parent, and at a slight distance from it.

A prepared specimen (Pl. XXXVIII, fig. 14) indicates the origin of part at least of the bud from the endocyst of the opercular opening; the original occupant of this zooecium had scarcely commenced to decay.

Flustra securifrons, Pall.—Another prepared specimen from Naples (Pl. XXXVIII, fig. 15) shows a possible double origin for the lophophore and stomach in a young marginal zooecium. It will be seen that anterior band of endocyst has just been formed, and slung upon this is an undoubtedly epiblastic invagination or proliferation, coated by mesoblast. On one side is a mass of cells, which is continuous with what appears to be the incipient funiculus. This mass of cells, I take it, will form the future stomach and intestine; it soon ceases to exist as a distinct group of cells. I have several times noticed this stage.

Flustra papyracea, E. & S,—In new zooecia the buds may be seen to arise in close contact with the endocyst of the floor or of the wall of the cell, according to whether they may be terminal or lateral additions. Very shortly they assume a more central position, and are more or less thickly enveloped in a funicular plexus, from which latter there is every appearance of additions being made to their substance. The development of the polypide is exactly as described above.

In old zooecia the buds are developed in the anterior portion of the cell.

Pl. XXXVIII, fig. 16, shows a bud which is partly formed of columnar cells and partly of rounded. The latter appear to be produced at the expense of the funicular tissue; the former probably arose from the epiblastic layer of the endocyst.

Bugula flabellata, J. V. Thompson.—Pl. XXXVIII, fig. 17, shows a new zooecium, within which is the young bud, which has a well-marked bilobed appearance. Closely applied to the fundus of the stomach-sac is an ovary, which has been supplied ready-made to the bud. It is invested by the funicular tissue, which organically connects all the members of a Polyzoan

colony. Figs. 18 and 19 are consecutive sections of a similar bud at a later stage, and illustrating the same point. Fig. 20 is a longitudinal section of a slightly later stage, showing the œsophageal invagination inpinging upon the stomach.

Eucratea chelata, L.—In old zooecia the bud is derived from a small mass of cells, which is situated just below the hinge of the operculum, and from the first is apparently in equally intimate connection with both the endocyst and strands of funicular tissue (Pl. XXXVIII, fig. 21); subsequently it occupies a central position just above the brown-body, and then it commences to go through the characteristic development. It is this stage which has, I imagine, deceived Joliet into believing that the bud arises from the funiculus itself.

In new zooecia the bud has a similar origin, only in this case from the base of the zooecium. Pl. XXXVIII, fig. 22, shows the lophophore to be quite distinct from the digestive tract, while the latter is closely connected with the funiculus.

Alcyonidium gelatinosum, L.—A portion of the bud, at all events, arises by invagination of the endocyst. Pl. XXXVIII, fig. 23, clearly shows that both the epiblastic and the mesoblastic layers of that tissue are equally implicated.

Fig. 24 is a longitudinal section, corresponding to figs. 7, 20, 22, &c.

I have, in fact, observed the distinctness of the lophophore from the alimentary tract in the following forms:—Bugula avicularia, B. flabellata, Flustra carbasea, F. papyracea, F. securifrons, Eucratea chelata, Diachoris magellanica, Alcyonidium gelatinosum, Vesicularia spinosa.

Larval Gemmation.

The phenomenon of budding is generally supposed to take place during the embryological history of a Polyzoan. The following very brief summary of what is known on the subject is abstracted from the late Prof. Balfour's 'Elements of Comparative Embryology,' vol. i.: the sentences within inverted commas being transferred from that invaluable work.

Entoprocta.

The larval gemmation of Pedicellina is, for convenience sake, noticed a few pages further on.

Ectoprocta—Gymnolæmata.

At the stage of thirty-two segmentation spheres the archenteron is formed by the invagination and subsequent sub-division of four (Barrois) or eight (Repiachoff) middle cells of the oral surface, but it does not appear that this archenteron is ever functional, and there is every probability in favour of the view that this functionless organ gives rise to a bud, the so-called "dorsal organ" (='pharynx' of Barrois), as the archenteron in Pedicellina has been shown to do by Hatschek (see below, p. 531). It is worth noticing that "according to Hatschek it develops as a solid outgrowth of the hypoblastic walls of the mesenteron shortly before the mesenteron joins the œsophagus (fig. 129, B, *x*)," p. 244. "A nearly similar organ to this is found in the embryo of Loxosoma [Vogt, 6, and Barrois, 1*]. Here, however, it is double, and forms a kind of disc connected with two eye spots," p. 245.

The greater part of the internal organs of the larva now degenerates and forms a nutritive or yolk-mass. "The skin of the larva after these changes gives rise to the ectocyst or cell of the future polype. The future polype itself appears to originate, in part at any rate, from the so-called dorsal organ."

"The first distinct rudiment of the polype appears as a white body, which gradually develops into the alimentary canal and lophophore. While this is developing the ectocyst grows rapidly larger, and the yolk in its interior separates from the walls and occupies a position in the body cavity of the future polype, usually behind the developing alimentary canal. According to Nitsche it is attached to a protoplasmic cord (funiculus) which connects the fundus of the stomach with the wall of the cell. It is probably (Nitsche, &c.) simply employed as nutritive material; but, according to Barrois, becomes converted into muscles, especially the retractor muscles."

"Adopting the hypothesis already suggested in the case of the Entoprocta, the metamorphosis just described would seem to be a case of budding accompanied by the destrucion of the original larva."

"This view of the nature of the post-embryonic metamorphosis is apparently that of Claparède and Salensky, and is supported by Claparède's statement (see below, p. 538) that the formation of the first polype 'resembles to a hair' that of the subsequent buds," p. 249.

Dr. W. Repiachoff (14) in his study of the development of Tendra zostericola, says that he cannot with certainty say how the inner epithelium of the middle and hind gut arises, but his figures clearly show that this tissue is intimately connected with the "brown-mass." Several figures in his plate viii, indicate the occurrence of an epiblastic involution at the pole of the embryo, opposite to that where the blastopore has closed up. This invagination will form the external layer of the tentacular sheath, the outer epithelium of the tentacles and the œsophagus of the primary zooid; it is in fact the stomodæum. The pedicle of invagination of the archenteron is absorbed, the latter being the rounded body, which he calls the "brown-mass." From one end of this a U-shaped prominence is produced, which is apparently hollow from the very commencement of its formation, the remainder of the mass being solid: this is the future intestine. The outer cells of the "brown mass" differentiate into the inner epithelium of the stomach, which soon acquires a free communciation with the exterior through the œsophagus. The central residual portion of the "brown-mass" is digested within the stomach like any other food-yolk. The "brown-mass" is surrounded by a delicate membrane, the splanchnopleure. To render the above account more clear, I reproduce his fig. 7, woodcut No. 2, which compare with woodcut No. 1.

It is clear, if the above be a correct interpretation, that the initial individual of a colony, in this species at all events, passes through a development which is normal in all its essentials, nor does there appear to be any histolysis of the primary larva.

To recapitulate—omitting the purely secondary phenomena of the external form and the behaviour of the body-wall—the blastopore closes up and the pedicle of invagination forms

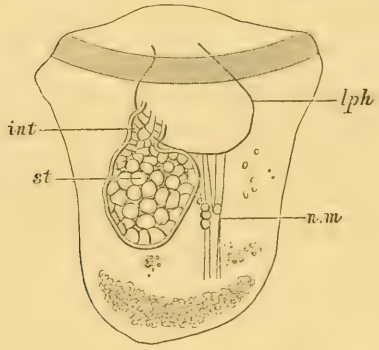

Fig. 2.—Primary zoœcium of Tendra zostericola. The tentacles, though present, are not shown. (After Repiachoff.) *lph.*, lophophore; *int.*, intestine; *n. m.*, retractor muscle; *st.*, stomach.

neither the œsophagus nor the intestine. The archenteron is at first solid; a portion of its substance is prolonged to form the intestine, which subsequently opens to the exterior outside the tentacles. The tentacular sheath and the tentacles are derived from an epiblastic depression, from the floor of which the œsophagus is evaginated, which then fuses with the stomach. The inner face of all these organs is coated with mesoblast. The details of the later development are perfectly normal.

It is possible that, in some cases, the indifferent character of the cells of the archenteron and the stomodæal invagination, have misled observers into the belief that the embryo has undergone histolysis, and that the first zooid of the colony is produced by larval gemmation, for the view of the total formation of a bud ('polypide' of authors) from the endocyst has been so firm that a well-marked involution, such as the stomodæum, would be interpreted as a bud rather than as a portion of the embryo. For myself, I am inclined to believe, with Barrois, that the occurrence of the destruction of the primitive larva is not necessarily universal amongst Polyzoa.

Ectoprocta—Phylactolæmata.

I have treated of these later on.

Other Authors' Observations on Adult Gemmation.

Entoprocta.

According to Prof. Carl Vogt, in Loxosoma phascolosomatum (6), the bud is formed by a rising of the outer cellular layer of the parent, carrying its cuticle along with it. The cavity thus produced is filled not with "cells" but with an undivided sarcodic mass, which very soon breaks up into homogeneous non-nucleated masses of protoplasm. This anomalous material at first groups itself into three masses, superiorly the hood (capuchon) or lophophore, which from the first possesses a central cavity, the vestibule; below this is a small solid mass of cells, the stomach, and inferiorly lies the pedal gland. Other differentiations of these protoplasmic masses produce the transitory pedal body between the stomach and pedal gland, the reproductive organs between the lophophore and stomach, and the general parenchyma of the body. The stomach acquires a central lumen and the intestine and rectum now make their appearance, also, by him, derived from the protoplasmic mass. They, too, are at first solid. The œsophagus is a diverticulum from the hood. The tentacles are the last organs to make their appearance, then the vestibule first opens to the outer world, and the rectum into it, and the bud becomes detached. In this form the pedal gland atrophies. The author informs us that he has tried the effect of various reagents and also section-cutting, but has "abandoned these methods, which demand so much time and care, and in the present case could give me no positive information upon points which direct observation of the living organism had failed to solve." It appears to me that the formative elements of the bud are true cells, as all other observers maintain, and that the earliest stages were incorrectly determined. The difficulties of the homologies of the parts vanish, if taking a somewhat later stage, we look upon the anterior mass with its central hollow

to be an invagination from the epiblastic cells ("hypodermal layer") at the apex of the developing bud, whilst the underlying originally solid mass of cells which have primitively proliferated from the parental stomach and the pedal gland with the other internal structures, are modifications of migrated mesoblast. The development of the œsophagus as a depression from the hood also favours the interpretation, as does Vogt's account, if we except his earliest stages.

Subsequently, Prof. M. Salensky examined the gemmation of Loxosoma crassicauda (9). He describes the first stage as consisting of a small group of cells surrounding one central one. The latter by division forms a central mass which attaches itself to the anterior end of the lengthening and pedunculated outer wall of the bud. A slight longitudinal fissure appears in the ectoderm (epiblast of the bud), which is the rudiment of the orifice of the hood. The central mass becomes hollow and forms the hood and the whole of the digestive tract. "The rudiment of the digestive tube presents itself under the form of a cul-de-sac, in which two parts can be distinguished. . . . The superior part is the rudiment of the intra-tentacular depression, the inferior part is the rudiment of the digestive tube and of the rectum. . . . The superior part appears as a sac open in front. The edges of the aperture by which the sac opens now consists of ectoderm and endoderm which are completely united," p. 21. By "endoderm" Salensky means the inner layer of the double-layered bud, which tissue, according to him, forms the inner epithelium of the alimentary tract, the intra-tentacular space and the inner surfaces of the tentacles, their outer surface being formed at the expense of the ectoderm, the tentacles themselves arising just where these two layers fuse. On p. 19, he says, "the ectoderm and endoderm have arisen from the ectoderm or the integument of the mother. This fact is so clear to anyone who observes the profile of young buds of Loxosoma, that there cannot exist any doubt as to its reality. From the analogy which exists between all the species of Loxosoma, I may affirm that the described phenomena should be common to all the species."

The history of the other organs need not detain us; it is not stated from which of the two primitive tissues they are secondarily derived.

Salensky's account presents us with fewer difficulties than that of Vogt, but while agreeing with him as to the epiblastic nature of the outer layer of cells, I would suggest that the central cell, which he thinks is of the same value but does not prove it, is really derived from the alimentary canal of the parent, and is therefore hypoblastic. It is also possible that the involution which he describes, but on which he does not lay much stress, really forms the intra-tenacular space, as his account of the formation and position of that cavity appears to me to warrant that supposition, and that his (epiblastic) endoderm developes only into the stomach and intestine.

Hincks in his abstract of this paper (10), says: "I am quite unable to harmonise the account given by the author of this portion of the developmental history with that which we have from Vogt."

It will probably be found that the harmonizing of these and other accounts is possible according to the views stated above.

Prof. Oscar Schmidt (5) has propounded the original view that the bud in Loxosma cochliaris formed parthenogenetically from an egg, and that it is therefore not a true bud but an embryo! His paper is accompanied by a plate which is too sketchy to be of any value whatsoever. Nitsche and Salensky overthrow this theory, and the latter points out that buds in which no ovaries are developed may give rise to secondary buds, thus precluding any possibility of a parallelism between the bud of a Loxosoma and the ovicell of one of the Ecto procta.

Nitsche (4) has studied the gemmation of Loxosoma Kefersteinii. In this form he asserts that the bud originates from a grouping together of one or two ectoderm cells, these divide and form a single layered ring round one central cell. This latter, which he calls the "Endodermzelle," divides into two, then into four, and altimately forms a mass of cells which acquires a central lumen, and by subsequent constriction differ-

entiates into the cavity of the hood and into the alimentary tract; the generative organs arise as a pair of lateral protuberances between the hood and the stomach; the external orifice of the hood is formed comparatively late. The muscle cells and gelatinous connective tissue of the bud are derived from two or three "Mesoderm" cells which make their first appearance when there are some half dozen "Endoderm" cells, and which are probably segmented off from ectoderm cells of the bud. He is unable to say from which layer the foot gland is derived. In this form the bud is not attached to the parent by the aboral extremity of the stem but at a spot where the body and the stem unite.

It is thus quite clear that this able investigator regards the whole bud as being derived from the epiblast of the parent.

I have cut a large number of sections to elucidate the question of the budding in Loxosoma. The form I worked at was L. tethyæ, so abundant on sponges of the genus Tethya, at Naples. Most of my specimens were killed with osmic acid and stained in picro carmine. Unfortunately my results are not so exhaustive as they might be. Pl. XXXVIII, fig. 25, shows an epiblastic down-growth from the apex of the bud, which will form the cavity of the hood; below this is a small group of cells the nature of which I am unable to state definitely; they may be mesoblastic, or they may partly or wholly be hypoblastic, for there is no reason why the closely lying hypoblast cells of the stomach should not proliferate to supply its complement towards the bud, but it must be distinctly borne in mind that I have no direct evidence at present in favour of this view. Pl. XXXVIII, fig. 26, is a slightly later stage. At a much later stage (Pl. XXXVIII, fig. 27), below the hood cavity lies the small circular stomach which contains a central cavity and which is continued into a short blind intestine which already possesses its normal curvature. I could discover no connection between the stomach and the cavity of the hood. Woodcut 3 would represent a diagram of such a stage. There is no need to point out its parallelism with a similar stage in so many other Polyzoa. The subsequent formation of an œso-

phagus, and the later development of the bud may be passed over.

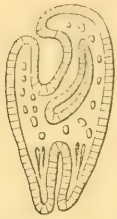

FIG. 3.—Diagram to illustrate the probable relations of the lophophore and stomach in a Loxosoma bud. The upper invagination is the lophophore cavity, the lower is the foot-gland, the compressed body within the bud is the stomach.

Uljanin (3) describes the development of the bud in Pedicellina. A protuberance of the cuticle contains some round clear cells; the outer soon arrange themselves as an epithelium, and a constriction divides off the bud from the stem; meanwhile two cavities appear in the central parenchyma; the lower and larger one he rightly regards as the stomach, the upper one he calls the "brood pouch," whereas it really is the lophophore cavity, the lumen of which is at first quite distinct from that of the stomach. There is nothing of further interest to us in his paper.

Prof. Salensky (9) has also studied the development of the bud in Pedicellina echinata, on p. 32 he says, "At the summit of the bud, several of the ectodermal cells elongate and sink within; probably these cells give rise to the endoderm." The further development of the bud follows almost precisely the same course as that which he gives for Loxosoma. As we shall immediately see, Hatschek gives a different rendering of the phenomenon, and I would point out that Salensky's figure of his earliest stage (Salensky pl. xiv, fig. 26) would very well bear the former's interpretation.

The fullest account of the budding Pedicellina is in the very careful researches of Hatschek (8), in which he shows that at the growing point of the stolon there is a single-layered tubular mass of cells lying close beneath the external epithelium,

which is continually dividing into two by transverse constriction. Of these the anterior portion separates from the posterior, and becomes connected with a solid mass of cells, which have proliferated off from the external epithelium. This latter soon acquires a lumen, and we have a pair of single-layered closed sacs occupying a distinct prominence of the stolon, which are beginning to be shut off from the general cavity of the stolon by the neighbouring fusiform mesoderm cells arranging themselves into a diaphragm. Some of the scattered fusiform cells of the stolon become cut off, and so pass into the bud; but at the junction of the primitive closed sac, with the proliferating epithelium, there is one single mesoderm cell, which by division soon forms a small rounded mass, and is apparently concerned with the formation of the generative organs. The larger anterior sac forms the intra-tentacular space, and by the involution of its walls produces the tentacles, and of its floor the œsophagus and the hind gut. A central solid invagination, which shortly becomes a hollow sac, is the rudiment of the nervous system. The posterior smaller sac is prolonged and bent upon itself, and becomes converted into the stomach and intestine, communication taking place between the invaginations which form the fore and the hind gut.

In the embryo Hatschek has discovered that a couple of cells separate themselves from the oral side of the endoderm. These form a single-layered sac, which becomes quite detached from the alimentary tract of the embryo, and is connected with a small ciliated invagination of the lateral epiblast; it also possesses a mesoderm coating. This remarkable structure is regarded by Hatschek, with great probability, as the first bud; and it will be noticed that it contains the three germinal layers of the embryo. Unfortunately, there is a gap between this stage and the earliest of his true stolon buds; but it seems pretty evident that the primitive single-layered tubular mass of cells mentioned above is the persistent structure derived from the stomach of the embryo. Assuming this to be the case, we have then in every Pedicellina-bud the three embryonic layers, each one of which gives rise to its traditional

organs, viz. the epiblast, to the external skin, the lophophore, the intra-tentacular space, the œsophagus, rectum, and nervous system; the hypoblast, to the stomach with its digestive cells, and the intestines; the mesoblast, to the muscles and general parenchyma of the body. The generative organs apparently arise from a special mass of mesoderm cells, which very early appear as a single cell, which may arise from the primitive hypoblast, or may be one of the primitive embryonic mesoderm cells. After describing the embryonic bud, Hatschek says (p. 515):—"The whole formation, which we have just studied, gives, as will be shown further on, the material for the construction of all the secondary individuals of the stock, whilst the whole of the remainder of the larva goes directly over to the primary oldest individual."

It appears that Prof. J. Reid (2) was one of the first (1845) to point out the fact that new buds form on the stems of Pedicellina echinata when the polypides die; it has also been noted by several observers since. It would be a most interesting fact if this process were found to take place when no remnant of the polypide was left. The histology and morphology of this phenomenon require to be elucidated.

Ectoprocta—Gymnolæmata

(Marine Polyzoa).

Nitsche (13) makes a distinction between the outer epithelium of the endocyst and the inner muscular layer, and he derives the outer epithelium of the lophophore and tentacles and the inner epithelium of the alimentary canal of the bud in Flustra membranacea from the former ("Epithelialschicht")—in other words, for him, the lophophore and alimentary canal of the young bud have a purely epiblastic origin. The tentacular sheath, the muscles and peritoneal lining, &c., of the polypide being derived from the inner muscular tunic of the endocyst, i. e. from mesoblastic tissue.

In Fl. membranacea all the changes in the decadence of a polyp into a brown-body can be seen; this is yet more clearly manifest in Alcyonidium hispidum. Here single

zooecia likewise very frequently lose their polyps by decay, but long before the polyps have lost their characteristic form, and have become brown-bodies, the endocyst of the upper end of this zoœcium begins to form a new polyp by budding inwardly. In the same zoœcium we very frequently find a decaying polyp, which very distinctly shows its original nature, together with a new young bud, which does not differ in the least from the polyp-buds in the zoœcium-buds at the edge of the colony. Here, also, the new polyp is formed, just as the old one, by the budding of the endocyst of the zoœcium inwardly (p. 466). By "polyp" Nitsche, of course, refers to the alimentary tract, as he accepts the dual nature of the zooecium and its contained digestive apparatus.

Salensky (9) states (p. 55) that the internal tissue of the two-layered bud is derived from the external epithelium of the endocyst of the parent, and the outer from the internal layer. Here again the lophophore and digestive tract are epiblastic structures, while the mesoblast of the bud is derived from the mesoblast of the parent.

Joliet (17) refers all the buds to his "endosarc." Under the term 'endosarc' Joliet includes "all the formations which one calls under the names of colonial nervous system, funiculus, fusiform layer of the endocyst." "It is this which constitutes the muscular tunic of the fresh-water Bryozoa, the parenchyma of the stems of the stolons of the Pedicellinæ, and of the feet of Loxosoma."

He says that it is a "provisional name," "which I shall be quite disposed to change for another more general term as soon as I shall have seen, or someone has shown me, its homologue with the ectoderm or the endoderm of allied animals or of the embryo." Surely neither alternative is necessary! It will be seen from what follows that I do not regard the funiculus as a simple structure, nor the bud as entirely derived from the funiculus, therefore I cannot class all the contents of a zoœcium, save the outer layer of the endocyst, as being formed from an homologous tissue. The tissue, as he describes it, answers in position, structure, and generally in function, to the mesoblast

of all other animals, and therefore it seems to be to be superfluous to coin a new term to express mesoblastic tissue.

Joliet asserts that in some cases the bud is entirely derived from the funiculus—Eucratea chelata, Vesicularia spinosa (young zoœcia), Beania mirabilis, Lepralia Martyi, and L. granifera; in others, the bud is apparently in intimate relation with the 'endocyst,' but always connected with a funiculus — Membranipora membranacea, M. pilosa, and possibly the old zoœcia of Vesicularia spinosa. It must be remembered that Joliet limits the term 'endocyst' simply to the external epithelium (epiblast) of the body wall. He says (pp. 221-2): "When a bud forms anew upon the endocyst of an old cell, one generally sees that it is very early provided with a funiculus, which, even then, almost attains its (proper) diameter; and ever since my attention has been drawn to this point I have never seen a bud formed under these conditions which lacked this attachment. I am thus driven to believe that the buds develop by preference upon the points of the endocyst where the strands, of which I have spoken, are fixed, and thus from their earliest state they are naturally provided with a funiculus."

Thus Joliet is driven to admit, apparently against his inclination, that in some cases the 'endocyst,' outer epithelium (epiblast), may participate in the formation of the bud, he goes on to say (p. 247): "I should almost be tempted to generalise and to say, to terminate, that in all the Bryozoa the development of the polypide is made at the expense of the pretended colonial nervous system, if the Pedicellinæ did not constitute, according to Salensky, a very serious and very striking exception. This author, in a recent work (9), seeks to demonstrate that the budding of the digestive tract, which he compares to the Polypide, is made at the expense of the endocyst. I here produce a figure certainly strongly resembling his, and in which the bud is still reduced to five cells; but these cells do not appear to me to be directly united to those of the endocyst, and have always appeared to me to have more resemblance with the fusiform cells of the parenchyma.

Even supposing that the opinion of Salensky is justified, as we shall see immediately, the tissue called nervous is directly derived from the endocyst and that in the young buds of Vesicularia spinosa the granules, at the expense of which the bud is formed, belong to the colonial nervous system, and are only the cells of the endocyst recently detached from the walls, — one may say that the two cases are closely allied."

But, as in the case of Pedicellina, he begs the question by some such argument as the following :—That, according to Salensky, the new bud arises from cells proliferated from the endocyst; that the endosarc similarly arises from the endocyst; therefore we may say that in this case the bud arises from the endosarc!

It does not appear to me that Joliet's figures illustrating the proliferation of the endosarc from the "endocyst" are perfectly conclusive. The figure of the bud of Pedicellina, which he refers to above (his pl. xii, fig. 9) really proves nothing. His fig. 1, pl. xii, is possibly more to the point, but then Hatschek (accepting his statements to be true) has disposed of this most thoroughly. The only other figure he gives us is that of the vegetative extremity of a stolon of Bowerbankia imbricata (pl. xii, fig. 2) ; it remains to be proved whether this case falls with Pedicellina, or, if it exists, what is the exact interpretation of this proliferation.

Dr. E. Ehlers (16) describes the phenomena of budding in the form he has more particularly examined, Hypophorella expansa (Ehlers). In the lateral branch from the stolon which is about to form a new animal, and which we may term the bud, he finds externally a cuticle within a nucleated blastema (kernhaltiges Blastem) ; he does not find the two layers which Nitsche describes in Flustra, but has thought, though he cannot prove it, that the outer layer which forms the cuticle may be a Syncytium, though "I have never succeeded in showing nuclei in it," corresponding to the cylindrical layer found in Flustra. The bud increases greatly in size and early assumes a form much like the adult zoœcium. The cuticle

passes into the ectocyst of the adult internally. Owing to the rapid growth there is a large central cavity, the walls are undoubtedly lined with the syncytium and with a portion of the original central blastema, while from the apex depends, icicle-like, the remainder of the blastema. This latter is to form the alimentary tract, its peritonenal lining and some of the muscles, the rest are formed as processes from the body-wall, i.e. from its inner layer. The tentacle-sheath is formed by an invagination of the tissue at the apex of the bud, so that the syncytium forms its inner lining; that is really the future outer layer of the tentacular sheath. "With regard to this homogeneous outer layer of the Hypophorella bud, in which one would like to see the homologue of a cell-layer, I must remark that I have never seen it continued into the first rudiment of the gut" (p. 109). "The endoderm appears as a separate development of the tissue of the indifferent body-wall at the spot distinguished by the above-mentioned invagination." The tentacle disc (Tentakelscheibe) or incipient lophophore is formed from the endoderm. There is very early a cavity in the formative material of the alimentary tract; this is the stomach. The tentacles grow out from the edge of the tentacle disc, at first only eight. The remaining two or three appear a little later. [It is usually stated that the permanent number of tentacles arise from the first, but in several forms, e.g. Fl. papyracea, Diachoris magellanica, &c., I have observed that four lateral ones usually appear first, the more central being the larger, but I have not yet satisfied myself as to the exact rhythm; 'lateral' has, of course, relation to the median line as marked by the mouth and anus.—A. C. H.]

As far as I can discover, Ehlers speaks of the blastema which forms the alimentary canal as "endoderm," because it does produce that structure, and naturally speaks of the remainder as mesoderm, while he really has no doubt that the outer homogeneous layer is the ectoderm. In this all critics will probably agree with him, but the exact origin of this blastema has yet to be demonstrated. I would, however, join issue with our author on one point, and that is the origin of the tentacle-disc.

On Taf. IV, fig. 34, he figures the invagination of the tentacle-sheath, and in the bottom of this depression he shows two large cells. These he imagines form the inner portion of the sheath; apparently they have the same optical character, as the incipient tentacle-disc, and from what I can make out that organ has not yet appeared. It is strange that the tentacle-sheath should so early differ in optical characters from the remainder of the alimentary tract, when it is derived, by him, from the same tissue, so I am strongly inclined to suspect that this careful observer has fallen into an error, and that the lophophore like the outer portion of the tentacular-sheath, is really an epiblastic derivative, which later on acquires continuity, as far as its cavity is concerned, with the remainder of the digestive apparatus. This correction will give morphological completeness to the whole process.

Claparède (12) derives the buds from the endocyst both in the larvæ and in the adults. In his description of the larva, he says: "From a certain spot of the endocyst an oval mass projects towards the interior, in which a cavity soon appears. This hollow structure entirely corresponds with the invaginated sac of a young Bugula-bud, the development continues henceforth in a perfect parallel with that of the bud. It is very probable that this sac arises from the primitive mouth-furrow of the larva, but I have not directly observed that it does so. I need not describe the formation of the polypide within the sac, as it resembles to a hair that of the polypides of the bud," p. 169.

From an examination of Smitt's Plates (11), it would seem that the lophophore and œsophagus are at first distinct from the digestive tract in Tubulipora serpens (pl. iv, fig. 9), and in Alcyonidium parasiticum (pl. v, fig. 13-14), and that they subsequently become united. Hincks, who is the English exponent of Smitt, clearly states Smitt's opinion that the buds are derived from the brown-body ("germ-capsule") at all events in many cases; but this view has been so fully discussed and combatted by all subsequent writers, that I need not dwell on it further.

Hincks, in his admirable Monograph (1), adds his testimony to that of Smitt, but is willing to admit that in many cases the buds may be derived from the endocyst or funicular tissue. He does not really go into the question of gemmation, nor does he give any perfectly satisfactory observations of his own, neither does he discuss the morphology of the phenomenon.

Ectoprocta—Phylactolæmata.

Freshwater Polyzoa.

Allman in his beautiful monograph (19) says: "With the exception of some peculiar forms of gemmæ (statoblasts) to be presently described, these bodies (gemmæ) always originate in the endocyst." He then goes on to describe the process of gemmation in Paludicella and in Lophopus. The figures which he gives bear out his view, but all his observations were made from living examples, and thus he has not seen the cells implicated in the process, nor verified his results by means of sections. It is thus left uncertain what exact part is played by

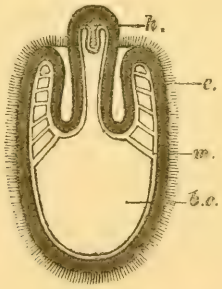

Fig. 4.—Diagram of embryo of Alcyonella, modified from Allman. *e.* Ciliated epiblast. *m.* Mesoblast. *h.* Hypoblast. *b. c.* Body-cavity.

the external cells (epiblast) and the inner network of muscular fibres (mesoblast) of the endocyst, but judging from pl. xi, figs. 5, 9, and 10—14, it would seem that the epiblast of the parent gives rise to all the alimentary organs of the bud, while the mesoblast of the mother passes into the mesoblast of the

bud. I would point out that his pl. xi, figs. 7—9, and 12—15, suggest a double origin of the alimentary organs, and that the connection between the cavity of the lophophore and the lumen of the stomach occurs comparatively late.

The account of the development of Alcyonella by Dr. Allman is unfortunately far from satisfactory, and I would venture to suggest another interpretation (fig. 4) of the stage represented in pl. xi, fig. 30, and by No. 4, fig. 5, p. 34, which is, that the polypide is developed from the remains of the archenteron of the embryo, probably by a direct conversion of the walls and of the lumen of the archenteron into those of the alimentary tract of the young polypide. The lophophore and œsophagus would be derived from the overlying epiblast. The remainder of the body wall of the embryo, consisting of epiblast and peripheral mesoblast, "becomes enveloped in an ectocyst, to constitute the cell of the adult polyzoon. The subsequent changes are produced by the gemmation of new polypides, with their proper ectocysts and endocysts" (p. 35). In other words the embryo passes over entirely into the first adult of the colony. At a very early stage, between figs. 30 and 31, a second polypide makes its appearance; there is little doubt that this second bud is constricted off from the older polypide, although Allman leaves one to suppose that it, like the former, "appears to take place in a manner quite similar to that by which new polypides are produced by gemmation from the walls of the endocystal cavity in the adult" (p. 34). It is very unfortunate that Allman should have derived the alimentary canal from the epiblast, when hypoblast already was present in the embryo.

"Plumatella fruticosa presents similar developmental phenomena; the ciliated larva, however, in this species, differs from that just described, in having its polypide single."

I do not propose to discuss the morphology of the statoblasts at present. Allman (l. c. p. 38) describes how they take their origin entirely from the funiculus. Ultimately "a young polyzoan gradually emerges and floats away. . . . At the period of its escape it possesses all the essential organization

of the adult. . . It loses no time, however, in developing gemmæ, which soon change it to the compound form of the adult" (p. 39).

Metschnikoff (21) in his studies on Alcyonella describes the formation of the bud in the embryo. Allman in his Presidential Address to the Linnean Society (24) thus narrates it. The segmentation of the egg produces "a central cavity surrounded by a double layer of cells. This constitutes the cyst of the well known Alcyonella-larva, within which two polypides subsequently make their appearance by budding. In this budding both lamina of the cyst-walls participate. The outer lamina serves for the formation of the outer epithelium of the tentacles, and the inner epithelium of the alimentary canal; while the central nervous system, which in the larva is very large, is also most probably derived from it. The inner lamina, on the other hand, forms all the muscles of the body, as well as the genitalia and the inner epithelium of the body cavity."

Nitsche (22) has also studied the budding in Alcyonella fungosa and in Cristatella mucedo. I again quote from Allman's address, p. 499, "He (Nitsche) had already shown that the wall of the cystid or zoœcium of Alcyonella consists of three different layers besides the externally excreted ectocyst or cuticula. These are an outer epithelium, an inner epithelium, and a tunica muscularis lying between the two and consisting of a structureless supporting membrane on which lie transverse and longitudinal muscular fibres. The first indication of the polypide-bud shows itself as a sac-like bulging inwards of the cystid wall. In this bulging the tunica muscularis, however, takes no part, but seems to be absorbed at the spot where the bud occurs. The polypide-bud consists therefore at this stage of a two-layered cellular sac, whose inner layer, bounding its central cavity, passes continuously into the outer epithelium of the cystid wall, while the outer layer is continuous with the inner epithelium of the cystid. Nitsche follows Metschnikoff in regarding the outer epithelium of the cystid as the outer germinal layer or ecto-

derm, the inner epithelium as the inner germinal layer or endoderm; and if we further regard the tunica muscularis as a middle germinal layer or mesoderm, we may view the young polypide-bud as composed of two concentric cellular layers, the internal derived from the ectoderm, the external from the endoderm of the cystid, while the mesoderm of the cystid takes no part in the formation of the bud. . . Folds and secondary introversions of this two-layered cellular sac give to the young polypide its definite form. . . The inner epithelium of the alimentary canal is derived from the ectoderm of the cystid, while the outer is derived from the endoderm. The two layers of the tentacular sheath have a precisely similar derivation." A detailed account of the further development is given, and on p. 501 we read, "as Nitsche suggests, we must not in the present instance lose sight of the fact that the inner layer of the bud, though arising from the ectoderm of the cystid, has fundamentally different relations from those of an ordinary ectoderm, for there proceeds from it, at the same time with the nervous substance of the ganglion, the entire epithelial lining of the intestinal tract." While the "endoderm" of the cystid behaves in all respects like an ordinary Mesoblastic tissue. What could have been the conditions which in process of time have so upset the traditional functions of the germinal layers!

Hatschek (8) describes, in Cristatella, the relations of the polypides to the colony and the increase by gemmation. In the lateral growing points he finds that there is a single-layered sac, depending within the cavity of the stolon and slung by a mesodermic layer. This sac constricts into two unequal portions: the portion constricted off becoming the inner epithelium of the alimentary tract of a new polypide, the tentacular portions being supplied by an epiblastic involution (Hatschek, fig. 3, p. 539). This process being repeated, Hatschek says that Nitsche's figures (22 and 23) do not prove his statement that in Alcyonella the inner layer of the polypide sac is derived from the ectoderm of the cystid, and goes on to say that Nitsche's figures will bear his (Hatschek's) inter-

pretation of the similar process in Cristatella. This continually constricting sac described by Hatschek lies between the epiblast and the mesoblast of the stolon, and it is quite open to us to discuss its morphological value. If we look upon this sac as hypoblastic tissue derived from the archenteron of the embryo, the budding of these fresh-water Polyzoa would present no difficulty. Allman describes the initiatory steps of the formation of a colony. If we accept a different (i.e. a hypoblastic) origin of the internal epithelium of the alimentary tract of the earliest polypides than that which Allman indicates, then the two accounts mutually assist one another.

Reinhardt (25) states that in Alcyonella fungosa and Cristatella mucedo: "After segmentation the mass is converted into a gastrula by invagination; the gastrula-mouth closes and the segmentation cavity disappears." . . .

"The cystid (in Cristatella) consists, as in Alcyonella, of an ectoderm, a median layer (the tunica muscularis), and an entoderm. Thus, Hatsckek must be wrong when he names the inner layer of the bud mesoderm, and his description of the budding is inexplicable by comparison with the above-mentioned details, though these may perhaps correspond with his second unknown process of budding. The bud develops by a thickening of the ectoderm into which the entodermic cells are pushed; there is no indentation of the former. The tunica-muscularis is very early formed; the cavity of the tentacle-sheath is separated later from the alimentary canal, and the lophophore is formed by an invagination into this tentacle-sheath. The later development of the buds corresponds with that described by Nitsche in Alcyonella." (From the English abstract.)

Reinhardt clearly gives us the three germinal layers, it is difficult to understand him perfectly as he gives no figures, but, accepting his statements, we apparently have an embryo in which the alimentary canal has a retarded development, an embryo which is, in fact, all body-cavity, such an embryo can easily result from an ordinary enterocoelous form, such as Argiope, Sagitta, &c., by an exaggeration of the cœlomic

diverticula and a simultaneous arrest in the formation of the permanent alimentary canal. The following diagram (fig. 5) will sufficiently explain my meaning. This would make the body-cavity of these forms an enterocoel. The musculature of the body-wall appears to develop prior to the formation of these diverticula.

This author brings into harmony the observations of Allman, Metschnikoff and Nitsche, for we have only to concede that the epithelial lining of the body-cavity of the embryo (cystid) in my interpretation of Allman, (which, by the bye, was made before I had seen the accounts by Nitsche and Reinhardt), is derived from archenteric diverticula; an earlier or later development of the tunica muscularis between the two layers, is really of little consequence.

Fig. 5.—Diagram representing a possible degradation in the formation of the alimentary tract from an originally enterocoelous larva.

Salensky (9) states that his own observations on Paludicella have convinced him that the superior layer of the zooecium gives rise to the lophophore and the internal epithelium of the polypide, while the inferior layer is transformed into the interior layer of the zoœcium, the tentacular sheath, and, at the same time, into the muscles. He says (p. 56):—"It is impossible not to remark the interesting analogy existing between these two layers and the embryonic layers of other animals." . . . . "It is acknowledged by several recent embryological researches that the endoderm of many animals forms from the ectoderm, sometimes as a thickening of this latter, sometimes as an invagination."

This "analogy," which other authors have remarked, must not be relied on as giving any true insight into the nature of the phenomenon of budding, for we cannot look upon the epiblastic layer of the endocyst as being morphologically

equivalent to the embryonic cells (segmentation spheres) of larvæ in the blastula stage. Moreover, the invaginated or grown-over hypoblast cells of the gastrula stage are not derived from the epiblast (ectoderm). Before this difference in position these two layers are usually optically, and they certainly are morphologically and physiologically, quite distinct—apparent optical similarity can never constitute morphological identity. The fundamental difference between the epiblast and the hypoblast is shown by the usually very early distinction between these two layers. For instance, the two layers are often practically distinguishable in the stage of eight segmentation spheres, and even in some cases the first segmentation furrow marks their distinctness. We must, therefore, disallow the use of the term "endoderm" for that mass of cells derived from the epiblast of the parent zooecium, which is supposed to give rise to the alimentary canal of the new polypide.

We can agree with Salensky when he continues:—"From its position and from the formations which it produces, the inferior layer of the zoœcium presents a great resemblance to the mesoderm." He might have added that they are one and the same.

This method of bud-formation he believes to be common to the whole group of the Polyzoa.

A. Hyatt, in his elaborate memoir on the 'Sub-order Phylactolæmata' (20), scarcely alludes to the phenomenon of gemmation. On p. 221 he says:—"The free part of the endocyst of the cell on the abdominal side, bringing forth true buds." And on p. 218 he gives a sketchy account of how "the statoblastic polypide begins to multiply by the process of budding. An internal swelling of the endocyst, on the lower side, in the vicinity of the bases of the anterior retractor muscles, first shows the position of the coming polypide. This elongates into a little hollow sac with a thickened rim, upon the upper edge of which, in the Hippocrepian Polyzoa, a slight notch is formed by the duplication and pushing out of its sides into two loops joined along the centre" (the lophophore). . . . "A

transverse constriction of the body of the little sac draws the line between the œsophagus and the stomach; and the subsequent deepening of this constriction divides off the internal cavity, establishing the cardiac and pyloric valves." The figures which Mr. Hyatt gives are most unsatisfactory, nor does he appear to have checked his observations by means of sections. The minute outlines which he gives of Cristatella ophidioidea (Hyatt) will equally well bear Hatschek's interpretation. The incipient bud of Fredericella regina (Leidy) (pl. vii, fig. 5, v) is made to depend from the endocyst, but we are not informed as to the significance of the two layers which he there depicts. The figure of Plumatella arethusa (Hyatt) may have any interpretation. The only point which is quite clear is that Hyatt believes that the polypide buds are entirely derived from the endocyst of the parent.

Dumortier (18) made some interesting observations on Lophopus crystallinus. He states that he has seen balls of mucus floating in the general fluid of the body become attached to the body-wall. "I have said that these globules appear to be of the nature of mucus, for, besides that they are formed by the stomach, an eminently mucous organ, their substance does not permit the supposition that they are endowed with any organised tissue" (p. 49). "The adventitious bud once disposed of, it soon establishes a focus of irritation in that place, which will excite the development in the interior of its mass, and a protuberance at the exterior so as to make a bump." Dumortier states that the alimentary tract is developed from this ball of mucus, while the tentacles are developed from the body-wall. The tendency of his statements is towards the view of the hypoblastic origin of the digestive portion and an epiblastic origin of the lophophore, though, of course, this view of the case could not present itself to him (1836).

General Conclusions.

In all cases of budding in the animal kingdom, so far as I am

aware, it has been shown that representatives of the three primary germinal layers enter into the bud, and there form corresponding tissues, but, strangely enough, the Polyzoa form an apparent exception to this rule, as the buds are said to arise solely from the endocyst (Nitsche, &c.), or from the endosarc (Joliet). Assuming the generally received opinion of the nature of these tissues, in neither case would the bud have any hypoblast in its composition. It is inconceivable to me how a bud could originate unless it possessed an offshoot from all the essential organs of its parent: that is to say, the bud should possess a portion of the parental epiblast, mesoblast, and hypoblast; for how could either the epiblast or the mesoblast suddenly depart from its ancestral traditions and take upon itself the function of digestion? It is conceivable that, in process of time, the method of gemmation should be considerably modified, but hardly that one of the most important of the three primary embryonic tissues should not be represented at all. Embryologists are fully conversant with variations in the development of organs, and with the masking of the origin of certain organs, as in the case of "precocious segregation," but they nevertheless have firm faith in the essential "conservatism" of the layers themselves.

The question now before us is: Are the three germinal layers represented in the buds of Polyzoa? The following are my reasons for answering this in the affirmative.

Nitsche and others, as we have noticed above, would derive the whole of the bud from the endocyst—that is, from the epiblast and peripheral mesoblast. Joliçt, in combating this view, points out that in Eucratea chelata and in all the Cheilostomata which he has studied he has reason to believe that the bud is really formed on some portion of the endosarc, and not on the endocyst. In Hypophorella, Ehlers, the bud "is produced on the funiculus in the centre of the cell, as in Eucratea. In many cases it is developed at the very base of the zoœcium, immediately over the communication plate or septum and the orifices through which the connective threads pass, and therefore probably in connexion with the endosarc. I [Hincks]

have observed it in this position in the young cell of Beania mirabilis; and in this species Joliet has convinced himself that the polypide is actually derived from the endosarcal cord. In the rudimentary zooecium of Victorella pavida the forming polypide seems to me [Hincks] to be enveloped in the endosarcal plexus, and to be (in all probability) produced by it. . . . It may be, as Joliet suggests, that the authors who have referred it to the endocyst have not been sufficiently alive to the distinction between these two tissues. It may be that the function is to some extent shared by the endocyst" (Hincks, pp. l, li).

Joliet thus clearly believes in the endosarcal origin of the bud. This 'endosarc' is, by him, derived from the endocyst. In his use of the term 'endocyst' one must not understand both layers, but only the outer. This seems to be clear from Joliet's account, and from his deriving migratory cells also from the growing end of the outer layer. The inner layer of the endocyst (peripheral mesoblast or somatopleure) is composed, according to all authors, of fusiform cells—i. e. cells similar to the characteristic cells of Joliet's endosarc. It is thus certain that Joliet would consider the buds of the Polyzoa as composed solely of mesoblastic tissue, or possibly of some modified epiblast as well.

We have seen that in Flustra carbasea the tentacles and the mouth area arise from one mass of tissue, and from the latter an invagination takes place forming the mouth and œsophagus (Stomodæum); whereas the stomach and intestine arise from another mass of tissue. These two closed sacs (Stomodæum and stomach) later on unite to form a continuous tube. It was this well marked double origin of the digestive tract which first led me, when in Naples, in 1879, to study the question of Polyzoan gemmation. I have already enumerated some of the forms in which I have since seen the same phenomenon.

The resemblance of the above to the formation of similar structures in the embryos of so many animals is most striking, and seems to suggest that we have here to deal with an epi-

blastic derivative which forms the outer layer of the tentacular sheath, the outer epithelium of the tentacles, the mouth area, and the lining of the œsophagus; and with a hypoblastic derivative which occupies itself with the inner lining of the stomach and intestine. We may safely assert that the outer layer of the incipient polypide is mesoblastic as it develops into the inner layer of the tentacular sheath, the inner epithelium of the tentacles (somatopleure), and into the investing sheath of the alimentary canal (splanchnopleure), as well as into the muscles of the future polypide. Nitsche and Hatschek show for the Phylactolæmata, and the latter for Pedicellina, that the nervous system is derived from an epiblastic invagination. There are no observations for the Gymnolæmata, as to the ganglion, but it is in such close contact with the lophophore that we may safely assume its origin from that body. This would, of course, give it an epiblastic derivation.

Prof. G. J. Allman was, I believe, the first to promulgate the view that the zooecium and the polypide are distinct individuals, at all events this statement is very generally accepted; but it seems rather incredible that generations of individuals solely composed of a digestive canal and its appurtenances, such as muscles and nerve ganglion, segmental organ, and possibly generative organs, should live within the body cavity of one persistent individual which lacks these organs and only possesses a body wall, funiculus (?) and body cavity.[1]

[1] The analogies which have been drawn between this supposed phenomenon, and the undoubted cases of physiological and structural differentiation amongst the Hydrozoa, will not really hold good: for in these the buds, though still connected, are all external, and their specialisation can readily be accounted for; whereas, in the other case, each successive internal so-called bud develops within the body-cavity of its parent in such a manner as to have precisely the same relations as if it really was its alimentary tract, and not a bud. It is not easy to conceive how this could come about, nor is it rendered any easier if we yet farther follow the distinguished author of this view, and regard the zooecium as the host not only of a nutritive polypide, but also of male and female individuals; for Prof. Allman suggests that even the testis and the ovary are, save sexually, aborted polypides!

It is impossible to regard the body-wall and the alimentary canal of the Entoprocta as distinct individuals, and their gemmation resembles, in its essentials, that of those animals which can multiply by budding (e.g. Ascidians). The budding of some of the Phylactolæmata, too, does not necessitate this strange commensalism. Why, then, should it only occur amongst the Gymnolæmata?

Let us admit that the previous inhabitant of a zoœcium dies away altogether, but before doing so gives rise to a bud in a normal manner, which bud is primitively located on the oral wall of the zoœcium of its parent. The future history of the bud would present no startling peculiarities if its growth were to take place in two directions; if some of the epiblastic and mesoblastic portions of the bud tended to form the body-wall of the new Polyzoan: as it is already provided with an ectocyst there would be no need to form a new one, so the new body-wall would simply be applied to the dead cyst. Meanwhile, an epiblastic involution depends into the body cavity of the newly-formed individual, carrying down with it the hypoblastic derivation from the parent, both being coated with a mesoblastic sheath. This is the structure which has been regarded by authors as a whole bud, and which has been variously termed "bud," "zooid," "polypide," and "polyp," but which I make bold to say is merely a portion of the new bud. (It will be noticed that in the preceding pages I refer to this structure under the generally received terminology, I purposely do so to prevent any confusion.) I have already detailed the future history of this part of the bud, so it would be superfluous to repeat it again here, and that of the body-wall has no especial interest.

It might be objected that the funicular tissue extends throughout the entire colony, and that it does not die with the temporary inhabitants of the zoœcium; assuming this to be the case, there is nothing to prevent this tissue being enclosed by the body-wall of the growing bud, without its being a primitive portion of that bud, after being thus enclosed it would serve to connect the new member with the rest of the colony, and

by this means the bud would be engrafted into the life of the whole, for, undoubtedly, without being histologically nervous, this tissue can transmit stimuli, and it certainly possesses other important functions. It is difficult to conceive of portions dying and being renewed de novo, besides, having such undifferentiated functions it would à priori have greater vitality and be less likely to die with each individual, especially as it is all the time protected from external damage by the walls of the zoœcium.

I have shown that in Eucratea chelata the bud partly arises from the endocyst, and therefore we must be cautious in accepting Joliet's statement as to the universality of the origin of the buds from the endosarc.

We, have, however, just seen that Joliet and Hincks lead one to imagine the possiblity of different tissues, the endocyst and the endosarc (funicular tissue) being implicated in the gemmation of certain forms, and my own observations very strongly incline me to this view. Hatschek's beautiful investigations are very clear as to the complex origin of the bud, and practically prove that all the three germinal layers are concerned in the budding of Pedicellina and Cristatella.

To recapitulate:—In the Entoprocta, Hatschek's observations prove the process of gemmation to be normal in Pedicellina. My own on Loxosoma indicate that no real anomaly exists in that form.

The discrepancies of most observers, combined with the errors of some in their interpretation of the phenomena in Pedicellina, will allow us some latitude in dealing with the generally received views on the budding in Loxosoma.

In the Phylactolematous Ectoprocta, Hatschek's account of Cristatella gives a clue as to what will possibly prove to be the characteristic method of gemmation in the group, and it is one which has every morphological probability.

The absence (?) of statoblasts in Paludicella may perhaps be accounted for by supposing that, compared with the true Phylactolæmata, this form is a late immigrant into fresh water, and that it still retains most of the structural characteristics of

the Marine Ectoprocta. If this be the case, it is probable that the mode of gemmation in this Polyzoan will be found to resemble that in the latter rather than that in the former.

The Gymnolæmatous Ectoprocta present us with the greatest difficulty, and it must be remembered that we have here to deal with a highly specialised and at the same time degraded group—the degradation being mainly caused by the sessile habits and by the secretion of a strong protective covering, resulting not only in the loss or diminution of certain organs, such as a muscular body-wall, nervous system, sense organs, excretory ogans, &c., but also in the simplication of certain tissues. This is especially noticeable in the body-wall and in the mesoblastic tissues generally, the tendency apparently being for these tissues to lose their distinctive cellular character and to form syncytia or even plasmodia; for the vagrant protean funiculus is more comparable with a plasmodium, in which the fusiform cells described by Joliet are immersed, than with an ordinary cellular tissue.

In many forms of this group both the endocyst and the funiculus appear to take part in the gemmation. I would again draw attention to the marginal buds of Flustra (Pl. XXXVIII, fig. 16) and Bugula flabellata (Pl. XXXVIII, fig. 17), in the latter of which the ovary lies in such close contact with the fundus of the developing stomach that it suggests something more than a secondary attachment. The ovary (shown by Huxley to be developed from the funiculus in Bugula avicularia), as is well known, passes ready formed into some buds imbedded in certain funicular tissue. Might we not assume that the stomach tissue also has a similar origin? Indeed, some still earlier buds exhibit a very close connection between the stomach and the funiculus. In most of the forms enumerated on p. 523 I have seen the stomach intimately united with the funiculus in early buds, and, though I have not yet been able to prove that the stomach mass does absolutely and entirely arise from the funicular tissue, yet the evidence in favour of that view is, to my mind, very strong.

There is, however, a certain amount of direct evidence that a portion of the bud is derived either from an invagination or from a proliferation from the outer layer of the endocyst—in other words, from the epiblast of the parent organism (see Pl. XXXVIII, fig. 23, &c.).

Every one will agree that the bud contains mesoblastic elements directly derived from the parent.

Assuming, then, that the digestive tissue of the bud is derived from the funiculus of the parent, a new construction must be put upon this important organ of the Polyzoa, necessitating a hypoblastic origin for a part at least of this much discussed tissue. I would venture to suggest that, at all events in the Gymnolæmata, a portion of the cord is indirectly derived from the archenteron of the embryo which initiated the colony. This derivative may be plasmodic rather than cellular, and probably is more or less clothed with degenerate mesoblast. If subsequent investigations can demonstrate this, then the anomalous character of Polyzoan gemmation will be taken away, and the phenomenon reduced to a more normal method.

Whatever value the suggestions just put forth may possess, this paper will at least indicate the lines upon which this question must be approached in the future.

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The Structure and Relations of Tubipora.

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With Plates XXXIX and XL.

Amongst a most valuable series of specimens collected at Zanzibar, by Mr. G. Gulliver, M.A., M.D., late Naturalist to the Transit of Venus expedition, was a fine specimen of Tubipora purpurea, which had been carefully and admirably preserved in strong methylated spirit. Having the good fortune to have this material at my disposal and likewise some fine dried specimens in the Oxford University Museum, I undertook, at the suggestion of Professor Moseley, a renewed examination of both the hard and soft parts in this important and interesting genus. My main objects were (1st) to examine and record the varieties of the structures known as the "infundibuliform tabulæ" in the dried specimens; (2nd) to discover the true meaning of these tabulæ by sections made through spirit specimens of the corallites; and (3rd) to clear up, as far as possible, certain of the other doubtful points in the anatomy and histology of the genus. For the first of these I found that a lump of Tubipora musica given to me by Professor Moseley, afforded the most fruitful results. My second object was fully achieved upon the specimen of Tubipora purpurea brought from Zanzibar by Mr. Gulliver. The same specimen also gave me some excellent results in studying the anatomy

of the polyps, but at the same time many important histological details must remain doubtful until some further material can be procured in which the polyps have been killed in the fully expanded condition.

Brief history of our knowledge of Tubipora.—The first mention I can find of Tubipora is by Aldrovande, (1) who, in 1648, describing it under the name "Pseudo-corallium rubrum calamites," considered it could not be a true coral, because, as he asserts, it does not adhere to rocks. "Hujusmodi coralium caret cortice in fundo Maris crescit non autem saxis adhæret more aliorum coraliorum." In 1651 Bauhin (2) described it under the name "Coralliis affine alcyonium rubrum." Subsequently, it was referred to by Imperato (9) Rumphius (23) and Tournefort (25), the last named being the first to give it the common name "organ-pipe coral." "Tubularia est plantæ genus, ferè lapidem ex pluribus tubulis constans, organi musici aemulum." Pallas, (21) writing in 1776, was the first to give it the name Tubipora. He seems to have made a much more accurate examination of it, and to have been thoroughly convinced of its animal nature. In his definition, he refers to the tabulæ in these words: "Tubuli articulati, siphunculo continuo ad orificium stellato communicantes." The first good figure of the coral was published by Ellis and Solander (6) in 1786, and this was improved upon by Lamouroux (13) in 1821, both of whom figure and describe the tabulæ. The first attempt to give a figure of the polype was made by Quoy and Gaimard (22) in 1833.

Coming down to more recent times, our literature is still very meagre. Percival Wright, (26) in 1869, first discovered and described the formation of the skeleton of the tubes by the fusion of spicules, and also described many details of the anatomy of the soft parts which were previously unknown. In 1874 G. von Koch (10) published his dissertation on the anatomy of the organ-pipe coral, but he seems to have been unacquainted with the paper previously published by Wright on the same coral. He gave figures for the first time of the mesenteries, muscles, transverse sections through polyps in various regions,

&c., but he overlooked entirely the tabulæ and the "siphonoglyphe."

A. Anatomy of Tubipora.

Throughout this memoir I shall employ the following terms: The encrusting lamina attached to stones, &c., from which the young colony springs, I shall call the "stolon." The individual tubes I shall refer to as the "corallites," the laminæ connecting the tubes together I shall call the "platforms" (Brücke of v. Koch). The inner tubes, funnel-shaped tabulæ or flat tabulæ, in whatsoever form they occur I shall call the "tabulæ." The points at which the platforms meet the corallites I shall call the "nodes," and for the ciliated groove on the ventral side of the stomodæum I shall use the term I have elsewhere proposed for it (8), namely, the "siphonoglyphe."

I. The Skeleton of Tubipora.—The hard parts of the "organ-pipe coral" have already been described by several authors from the time of Pallas, but as many points still remain obscure and others entirely undescribed, I propose to give here a further account of them from the examination of a large number of different specimens belonging to different species.

In the Oxford Museum there is a specimen of a young colony of Tubipora purpurea growing upon a piece of a madreporarian coral. The corallites are seen to spring from a flat lamina, the stolon (fig. 1, *st*) which, creeping over the surface of the support, gives origin as it goes to new corallites (fig. 1, *a a*). The presence of this stolon in the young colonies of Tubipora seems to have been overlooked by previous authors. This may be accounted for by the fact that as the colony increases in size the stolon ceases to grow; the colony, however, continuing to increase in size by the origin of new corallites from the platforms (fig. 1, *b b*), soon completely hides the stolon and the area to which it is attached. Moreover, when the colony dies and is broken off the stolon remains attached to the rock to which it was attached, so that none of the large pieces in our museums (as far as I have been able

to observe), nor the pieces offered for sale by the dealers possess the stolon at all. The stolon does not follow all the fluctuations of its support, but in many places may be seen to skip over large crevices. This point is, I think, of some importance when we compare the stolon of Tubipora with the creeping network of tubes from which the corallites of Syringopora spring, the two being, I consider, homologous. That the stolon should cease to grow at an early stage in the growth of the colony is not to be wondered at, as its function (namely, that of giving origin to new corallites) is completely taken up by the platforms situated in the more peripheral regions of the colony.

The individual corallites then, originating either from the stolon or from a platform, pass up through a varying number of platforms towards the periphery. They are usually straight, but occasionally I have observed them deviating considerably in their course. Their power of growth is not unlimited; in one case I have traced a single corallite passing through as many as seventeen platforms, but the average number does not exceed twelve or thirteen in Tubipora musica. Towards the termination of the corallites the walls become thin, lose their deep red colour, and end in pale jagged edges. In the course of the growth of the colony these free ends of the dead corallites become covered over by neighbouring platforms, and lost to view.

The platforms are formed as outgrowths from the lips of the growing corallites, in a manner which will be more fully described when I come to describe the soft parts of the animal. At first they are exceedingly thin, and their skeleton composed only of a few scattered spicules in the mesoderm. Consequently in dried specimens the young platforms are entirely lost.

As the platform becomes older it increases in thickness, and the spicules unite together to form a firm lamina. Each of these older platforms may be seen in section to be really composed of two delicate laminæ, between which numerous canals ramify in all directions (fig. 2).

Some of the most important and interesting structures in the

skeleton of Tubipora are the tabulæ. These may consist either of simple flat partitions in the cavity of the corallite (fig. 6), or they may be concave or convex, or cup-shaped (fig. 6); they may be in the form of long drawn out funnels, or in the form of axial tubules within the corallites (fig. 3), or assume much more complicated shapes and forms. They were first referred to by Pallas (21), and subsequently figured by Ellis (6) and Lamouroux (13). These authors called them the "siphunculi," and seem to have considered that their normal if not their only condition was that of hollow tubes open at both ends. Curiously Professor Nicholson has only recently fallen into the same error, for he says (20, p. 221): "The axial tube itself, so far as I have seen, is always open along its entire length. . . ." As, however, every intermediate condition can be found between axial tubes open at both ends and simple flat partitions exactly similar to the tabulæ of the Favositidæ, I shall call them throughout the "tabulæ."

The simple flat tabula is a condition which is very frequently met with, but very often the tabula is not complete, but stretches only part of the way across the cavity of the corallite.

Sometimes only a small strand could be found, reminding one of the "tongue-shaped" tabulæ of the Favositidæ (Nicholson, 19, p. 41), and in a few instances the tabulæ were still further reduced to mere spiniform projections of the walls of the corallite, reminding one of the condition found in Pachypora.

Complete tabulæ were also found slightly convex or concave, as in Michelinia and other Favositidæ; others were cup-shaped (fig. 6), and others were funnel-shaped with the narrow end drawn out to a fine point.

A very common condition, however, is that in which the tabula takes the form of an axial or inner tube, bulging at the nodes and giving off a varying number of short tubules to the platform (fig. 3) or, as Ellis (6) puts it, "siphunculis continuis geniculatis, ad genicula radiatis."

Frequently, however, the condition is much more complicated. One tabula, starting from a node, is drawn out into the shape of a long funnel, and passing downwards is entirely

enclosed by a similar tabula proceeding from the next lowest node, and the two pass some distance down the corallite as a tube within a tube (fig. 4). This condition, which was first discovered by Professor Moseley, (18) is of great importance in the consideration of the relations between Tubipora and Syringopora. In one instance, I have observed the two tabulæ, after passing some distance down the corallite apart from one another, fuse together to form one tube, as they do in Syringolites.

Another example[1] I have met with is of some interest, from its giving a superficial resemblance to certain Zoantharian corals. A tabula, in the form of an axial tube, completely closed above by a convex tabula, gives off eight delicate tubuli to the platform at the node. When viewed from above, this has exactly the appearance of a central solid columella connected to the wall of the corallite by eight septa. (See wood-

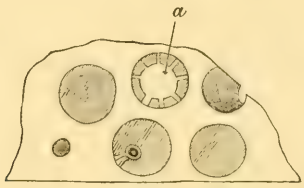


Fig. 1.—Diagram of septiform tubulæ of Tubipora. (*a*), axial tabula from which eight processes radiate.

cut, fig. 1.) When we remember that in many instances the presence of septa-like structures is the only reason for placing certain fossil forms amongst the Zoantharia, examples such as the one just mentioned showing structures which might have been mistaken for septa and columella, had Tubipora been known only in a fossil condition, become of importance.

It would be, however, an endless task were I to attempt to describe the varieties of tabulæ met with in Tubipora, and I think it is only necessary for me further to mention that the conditions met are often of the greatest possible complication. In fig. 5 is represented a condition illustrating this statement;

[1] This example, and many others illustrating the varieties of the tabulæ of Tubipora, may be seen in the Oxford University Museum.

but many more could have been figured showing features quite as complicated as this.

Before leaving the tabulæ, I may mention that at first I had some difficulty in determining the exact shapes of the tabulæ. They are frequently exceedingly delicate, crumbling at the slightest touch or blown away by the slightest breath. The only satisfactory way of exposing them is to carefully file away the wall of the corallite until a small hole is formed, and then, with a fine pair of forceps, break away the parts of the wall which have been thinned by the filing process.

When the corallites or platforms of Tubipora are examined with a hand lens, the coral is seen to be covered by numerous round holes (fig. 2), and thin transverse sections reveal the fact that these holes completely penetrate through the walls of the corallites and the platforms. These perforations have already been described and figured by Professor Nicholson (20, fig. 2), and, as he points out, they are not always in the form of simple tubuli, but are very often branched. The examination of thin sections reveals the fact that the corallites are built up of a number of spicules, which are so firmly bound together that it is impossible to separate them without injury. Both Professor Wright, who first discovered this, the true nature of the skeleton, and Professor Nicholson, speak of the spicules as being "fused" together. I think the employment of this word is likely to lead to misunderstanding, The spicules are not really fused together, but firmly bound together by means of minute serrations fitting into minute serrations, just like the membrane bones of the skull. The sutures between the spicules can always be seen (fig. 9, *Sp.*), and I have no evidence at command to prove that they are ever obliterated. In the walls of the corallites the sutures have a tendency to run across—that is to say, in a direction parallel with radii drawn from the centre of the corallite, and similarly, the longest axes of the spicules are usually disposed in the same direction. This fact is of some importance, as occasionally individual spicules will project out radially into the cavity of the corallite in a manner exactly similar to the so-called "septa" of Syringopora.

Towards the free end of the corallite the walls become much thinner, and above this, in spirit specimens, perfectly free spicules can be found scattered in the mesoderm. I have nothing further to add to the excellent description of these free spicules given by Professor Percival Wright (26).

The tabulæ consist of a simple network of spicules, the extent to which the spicules are joined together being much less than it is in the wall of the corallite (fig. 7).

II. Anatomy of the soft parts of Tubipora purpurea. —When a transverse section is made through the wall of a polype of Tubipora from which the skeleton has been removed by decalcification, it is seen to be composed of three principal layers (figs. 9, 10, and 12). Externally there is an ectoderm (Ep_2) composed of a single row of oval cells situated with their longest diameter parallel with the layer of mesoderm upon which they rest; internally there is an endoderm (Ep_1 composed of two or three rows of spherical cells, and between the endoderm and ectoderm a mesoderm composed of a homogeneous jelly-like matrix containing a few scattered mesoderm cells (*c*) and fibres (*f*); and in this mesoderm are seen a number of large spaces (*s s*) occupied by the spicules before decalcification. The ectoderm is composed all over the surface of the polypes of a single row of oval cells in which, even after prolonged staining in borax carmine, I have been unable to discover any nucleus. In places, especially in the older parts of the polyps, the ectoderm is entirely destroyed by parasites of which endless different kinds both animal and vegetable may be found (see figs. 9 and 10 *d a*.[1]).

Where the ectoderm is invaginated into the corallite (fig. 8.*) its character changes. In the first place the cells are considerably smaller in size. Whilst outside their longest and shortest diameters are respectively ·03 and ·02 mm., in the invaginated portion they are never larger than ·01 by ·003 mm. In the second place, there are two or three rows of cells instead of only one, and cells of the most superficial row by being elongated

[1] For a description of the Foraminifera infesting Tubipora see Carter (3).

vertically and closely approximated, give the appearance of a columnar epithelium. It is impossible to say from the study of non-living material only whether these cells are really ciliated, but I am inclined to think that they are not, as in some places I could distinguish a delicate membrane, like a thin cuticle, covering the free edges of these cells. In the above description of the histology of this portion of the ectoderm, I have described what I believe to be the true nature of it after a careful examination of numerous sections; but as the cells are here so very small, a renewed examination of specimens specially preserved for histology is desirable.

The ectoderm covering the tentacles is composed of two or three rows of cells the most superficial of which is distinctly ciliated. These cilia are probably in the living animal long and powerful and produce currents by their action which bring food to the polype.

The mesoderm consists of a homogeneous matrix in which may be found cells and fibres. The cells are usually pyramidal in shape but sometimes spherical or bipolar (fig. 12, *c*). The angles of the cells are usually drawn out into long processes lying in the matrix. Fibres are seen spreading through the matrix in various directions just as described and figured by Kölliker (11) in other Alcyonarians. In the actively growing mesoderm, such as is found in the young platforms, groups of small cells may be seen, which, budding off from the ectoderm, sink into matrix of the mesoderm. These groups of cells give rise to the spicules in the following manner. At first a small calcareous particle is seen lying in the midst of these cells, and as this increases in size the cells become more and more flattened around it until only a delicate membrane with two or three nuclei can be seen covering the spicule. After a time even this membrane disappears and the spicule lies freely in the matrix.

The endoderm consists of a layer of loose spherical cells varying considerably in size and appearance (fig. 12, Ep_1). The cells which lie next to the mesoderm are the smallest and youngest, and they stain well in hæmatoxylin and borax

carmine ; the more peripheral cells are much larger, and after even prolonged soaking in various staining fluids retain their peculiar brown colour. I have noticed the same peculiarity in the endoderm cells of the Gorgonidæ. These cells are moreover filled with highly refracting bodies, which make it a matter of great difficulty to determine whether they possess a nucleus or not. In Alcyonium the endoderm cells are constantly being shed, especially when the colony is in a sickly condition, and then they exhibit a slow and irregular amœboid movement. From the similarity that exists between the endoderm cells of spirit specimens of Alcyonium and Tubipora I am inclined to think that in the latter genus also they exhibit amœboid movement in the living condition.

The tentacles, eight in number, stand, in the retracted condition of the animal, side by side in front of the stomodæum. They are not withdrawn into tentacular pouches at the side of the stomodæum as they are in Paragorgia, Sarcophyton, and certain other Alcyonarians, nor introverted as they are in Corallium (Lacaze-Duthiers) and Heliopora (Moseley), but they simply remain, as they are withdrawn, in front of the stomodæum and parallel with one another (fig. 8, *T*).

Each tentacle is provided with 14—16 pinnulæ on each side, arranged in a single series, but both the number and arrangement of these pinnulæ varies in the different species. Each tentacle is covered, as previously described, by a ciliated ectoderm, and internally an irregular cavity communicating with the general body-cavity is lined by endoderm (fig. 8). Between the ectoderm and endoderm there is a thick layer of mesoderm which contains a number of scattered spicules as first described by Prof. Wright (26).

The stomodæum is, in the retracted condition, thrown into a number of folds, as it is in so many other Alcyonarians. Heliopora (Moseley), Pennatula, &c. (Marshall), (fig. 8, Stom.). Its epithelium is columnar and ciliated, the cilia over the general surface of the stomodæum being very small and difficult to see in spirit specimens. At first I had some difficulty in finding any trace of the siphonoglyphe owing to the

numerous and close folds into which the stomodæum is thrown, but after a careful examination of a number of sections, the characteristic long cilia and the thickened epithelium on the ventral side of the stomodæum were found (figs. 8 and 9, *Si*), and I have no doubt that when the polype is fully expanded a siphonoglyphe is present of the characteristic shape and nature.

The stomodæum is held in position by eight mesenteries which bear the powerful retractor muscles (figs. 8 and 9, *r. m.*). The muscular bundles exhibit the same arrangement as in other Alcyonarians (von Koch 10, fig. 6), being placed in all cases on the side of the mesentery which faces the ventral side of the polype. The protractor muscles (fig. 8, *pm*) are exceedingly delicate structures, consisting of but a few parallel fibres situated on the parts of the mesenteries in front of the stomodæum. The remarkable difference in size between the retractor and protractor muscles may be accounted for by the fact that strong muscles are required to suddenly retract the polypes, when irritated, and to drive out the water contained in the body-cavity at the same time, whereas the expansion of the polypes is always in Alcyonarians a very slow process and is probably aided, to a considerable extent, by the ciliary action filling all the cavities of the polype with water and thus helping to drive the polype out of the tube.

The ova are attached to the sides of the dorsal and dorso-lateral mesenteries immediately below the termination of the stomodæum (fig. 8, *ov.*). Each ovum is enclosed in a capsule and attached by a short stalk to the side of the mesentery. I have never seen the stalk of an ovum attached to the mesenterial filament as von Koch (10) describes it to be, nor can I find more than exceptionally that an ovum is attached either to the ventral or ventro-lateral mesenteries (conf. von Koch 10, fig. 7).

There are only two mesenterial filaments, the dorsal ones, as in the siphonozooids of Pennatulidae (Kölliker) and Sarcophyton (Moseley), and these extend for a considerable distance

down the tube (fig. 10, *M. F.*). Each mesenterial filament consists (fig. 10, *M. F.*) of an enormously thickened, columnar, ciliated epithelium supported by a portion of the mesoderm of the mesentery, but my specimens were not sufficiently well preserved to allow me to enter into any further details of their histology.

Formation of the Platforms.—Professor Wright, in his description of Tubipora, says: "I think it is pretty evident that the external tabulæ (i. e. platforms) are formed in the first instance as flattened offshoots from the upper edges of the tubes." I am able entirely to confirm this view, as many of the polypes in my spirit specimen exhibit the earliest indications of a platform in the form of a thin rim spreading out from the lip of a polype. This thin rim, as it increases in size, either meets and fuses with other similar rims proceeding from neighbouring polypes, or else it simply surrounds the lips of the adjacent polypes and fuses with them (fig. 11). At first the young platforms are quite pale, but soon delicate pink spots may be seen scattered over their surface, and as the platform increases in size and thickness these spots unite together into a delicate network, and eventually the whole surface assumes, to the naked eye, a deep red homogeneous colour. An examination of sections through these young platforms shows that at first the rim consists of a fold of ectoderm containing a thin lamina of mesoderm; subsequently, however, as the lamina of mesoderm becomes thicker, canals lined by endoderm are pushed into it, and soon ramify in its substance, forming the canal systems of the platforms (vide von Koch 10, fig. 10).

The ectoderm of the young platform is of the same nature as the ectoderm of the invaginated portion of the retracted polype (vide supra, p. 563, fig. 8.*), consisting of a number of small cells arranged in more than one row, and giving the appearance of being in a condition of rapid multiplication and growth; the mesoderm, too, does not contain the characteristic pyramidal cells and fibres of the other parts of the polypes, but contains numerous groups of small round cells, which have sunk down into the matrix from the ectoderm.

Even in exceedingly young platforms small white thickened spots may be seen on the upper surface, and these are young buds. The first sign of a young bud is a proliferation of endodermal cells on the upper side of the cavity of one of the canals of the platform; this is followed by an invagination of the ectoderm above it, which soon takes the form of a wide bag with a narrow mouth. Around this bag eight lobate folds of the canal with its thickened endoderm grow up, the thin laminæ of mesoderm remaining as the eight mesenteries. Subsequently a communication is established between the ectodermic invagination and the canal, but I have been unable to trace the growth of the bud further.

Formation of the Tabulæ.—As Stewart (24) suggested some years ago, the tabulæ are formed by a shrinking of the endoderm and its accompanying lamina of mesoderm away from the calcareous wall of the corallite, and the reformation of spicules upon it (fig. 10). As the corallite increases in length and the polype recedes farther and farther away from the lower parts of the tube the calcareous wall becomes thicker and thicker. This increase in thickness of the wall of the corallite is accompanied probably by a certain loss of vitality of the mesoderm, and this causes the thin strands (fig. 12) connecting the ectodermic and endodermic mesodermic laminæ to break, and consequently the endoderm and endodermic lamina of mesoderm shrink towards the axis of the tube. Having shrunk, the mesoderm forms a fresh layer of spicules, which, uniting together, form the tabulæ of the dried coral. Having undergone one process of shrinking, it is quite possible for it to undergo a second and to form a second deposit of spicules; in this manner the condition in which two axial tubes are found may be accounted for. At the nodes the endoderm runs out in the form of canals into the platforms, and these canals, when the shrinking occurs, would have a certain restraining action, and hence the bulging of the axial tubules at the nodes as described above (fig 3), and the formation of the delicate radial tubules figured in woodcut, fig. 1, and in figs. 3, 4, and 5.

B. The Relation between Tubipora and Fossil forms.

If it be borne in mind that the only known living forms at all allied to Tubipora (De Blainville (5), von Koch (10), Hickson (8)) are Cornularia and Clavularia, the former possessing no skeleton at all, and the latter but a few scattered spicules, it is evident that a long series of intermediate forms, some of which must have possessed skeletons suitable in every way for geological preservation, must have become extinct. Formerly it was considered that the extinct Syringopora was a near ally of Tubipora, and the older naturalists, such as Ellis (6) and Cuvier ('Regne Animal'), placed them in the same family; and this view is held now by such authorities as Zittel ('Handbuch der Palaeontologie'), G. von Koch(10), Moseley (17), and others. Certain eminent palæontologists, however, have recently maintained, on grounds which I can hardly consider to be entirely satisfactory, that Syringopora is not really allied to Tubipora. Dr. Lindstrom (14) places Syringopora amongst the Rugosa and Verrill, Nicholson and others place it amongst the Zoantharia perforata. The renewed examination I have made of the skeleton of Tubipora, carried on side by side with the examination of the soft parts, leads me to believe that the view of the older naturalists is the correct one, and that Syringopora is really an Alcyonarian closely allied to Tubipora.

When Professor Nicholson published his book on 'Tabulate Corals' (19) he seems to have considered that the position of Syringopora amongst the Zoantharia perforata was definitely settled, for he says (p. 213): "As to the recent genus Tubipora it seems unnecessary to enter into any detailed discussion, as the known facts as to the internal structure of Syringopora render any direct affinity between the two out of the question." More recently, however, he has published a paper which specially discusses the relationship between these genera (20), and he urges the following three differences between them as being of special importance:

"(*a*) In the first place there is the very important and remarkable difference in the minute structure of the calcareous skeleton in the two types in question. In Tubipora the corallum is made up of fused calcareous spicules, which are so disposed as to give rise to a universally distributed system of minute canaliculi or tubuli, which open on both the outer and inner surfaces of the skeleton by well-marked apertures. The size of these tubuli is comparatively so great that it is quite impossible that their presence could be overlooked in thin sections of Syringopora, if they really existed in this genus. On the other hand, the skeleton of Syringopora, as regards its minute structure, is quite compact, and shows no signs whatever, either of being penetrated by a system of tubuli, or of being formed by the fusion of ectodermal spicules." It is difficult to see why this difference should be considered of any great morphological importance. The size of the pores or "tubuli," as Professor Nicholson calls them, varies considerably in the different regions of the corallite, being at the younger ends much larger than they are at the older ends, so that it is evident that as the corallite grows older the tubuli have a tendency to be filled up, and a still further continuation of this process would make the wall of the corallite quite aporous. I have no evidence to prove that the complete filling up of these perforations in the walls ever does occur in Tubipora, but should an example be found in which this has occurred I should certainly not consider it sufficient reason for the formation of a new genus or even a new species. That the skeleton of Syringopora "shows no signs of being formed by the fusion of ectodermal spicules" is not to be wondered at, as we possess no means of studying either the development or the growth of the skeleton of this form, since the delicate growing ends would be broken down and destroyed; and even in recent genera (such as Corallium, Lacaze-Duthiers), in which the skeleton is known by an examination of its growth to be composed of fused spicules, no evidence of them can be seen in thin transverse section through the hard parts.

The second difference urged by Professor Nicholson as being

of importance is that "(*b*) True tabulæ are always present in Syringopora, and in all the typical forms of the genus have the character of a series of invaginated cones, which gives rise centrally to an axial tube. In no specimens that I have ever seen can there be recognised any similar series of funnel-shaped tabulæ in Tubipora. I cannot, in fact, recognise that any true tabulæ are present in Tubipora, so far as my own observations enable me to come to a conclusion on this point, and, as already stated, I do not regard the axial tube of Syringopora as being formed in the same way as the somewhat similar looking structure in Tubipora, or as being really homologous with it." The description I have given in this paper of the tabulæ of Tubipora proves, I think, that the difference between Syringopora and Tubipora is only one of degree and not one of kind, and I cannot see that there is any evidence to prove that the tabulæ in the two are not homologous. There are perfectly flat tabulæ in Tubipora, as in some examples of Syringopora, and there are cone-shaped tabulæ fitting one into another as in Syringopora (fig. 13, *it*). In fact, the only striking difference between the two in respect to the tabulæ is that in Tubipora they are more sparsely scattered in the corallites, and are more frequently of the form of axial tubes, open at both ends.

The third point of difference urged by Professor Nicholson is that (*c*) "the corallites of Syringopora are provided with a well-developed septal system, of which absolutely no traces can be recognised in Tubipora. Moreover, the septa of Syringopora are not mere marginal plicæ, such as form the 'pseudo-septa' of Heliopora, but they are in the shape of vertically-arranged rows of spines, which may be well compared with the septal spines of such undoubted Zoantharians as Porites." In answer to this the third and last objection of Professor Nicholson I must point out that in some cases, as I have mentioned above, spicules do project into the corallites of Tubipora, giving an appearance in transverse section exactly similar to the individual septal spines of Syringopora, and that in many cases the septal spines of Syringopora are exceedingly sparse and reduced in size to a minimum, so that when a specimen of

Syringopora is examined with spines in this rudimentary condition and compared with a specimen of Tubipora, no difference of importance can be distinguished between the two genera in this respect.

These, then, are the three principal objections to the relationship between Tubipora and Syringopora, and, as I have endeavoured to show, none of them is by any means insuperable. When this is borne in mind, and the numerous points considered in which the two genera resemble one another, I think that the Zoantharian affinities of Syringopora must at least be considered very doubtful.

The corallum in both Syringopora and Tubipora consists of a number of tubular parallel corallites separated from one another by spaces, which are bridged over by hollow tubular processes in Syringopora or platforms containing a network of canals in Tubipora. In both genera new buds are formed on these connections between the corallites, a striking similarity which has been quite recently dwelt upon by G. von Koch (10 *a*), in a paper which came into my hands since my plates were sent to the lithographer.

I have drawn in fig. 13 *a* a corallite springing from one of the tubular connections between the corallites in Syringopora, and if this be compared with the corallites springing from the platforms in fig. 1 *a*, or in von Koch's fig. 20, the striking similarity between the two genera in this respect will be seen.

When the surface of a corallite of Syringopora is examined with a lens it is seen to be covered with a number of small pits which bear a striking resemblance to the mouths of the perforations of Tubipora (fig. 2, *h h*), and this pitting can be seen quite as plainly and distinctly on the inner side as on the outer side of the corallites. Although these pits do not penetrate the walls in Syringopora as this coral is presented to us after centuries of fossilisation, yet I think they afford some confirmation of the opinion that its corallites are really of the same nature, i. e. spicular, as in Tubipora. This opinion is, moreover, still further confirmed by an examination of the

tabulæ of Syringopora, for they are found to be exceedingly friable and perforated by numerous small holes, just as they are in Tubipora.

The corallum of Syringopora springs from a prostrate network of tubes; in Tubipora it springs from a prostrate stolon, which, as I have shown, contains a network of canals. The prostrate network of tubes in Syringopora was in contact with rocks and stones at certain places; the stolon of Tubipora is in contact with its support only in certain places, and at others rests upon no support, so that I think we have sufficient evidence to assume that these two structures are really homologous.

The presence of tabulæ in Syringopora, which was formerly considered to be a strong point in favour of the Zoantharian affinities of the genus, has become, since our knowledge of the structure of Tubipora has increased, an argument in favour of its Alcyonarian affinities. In addition to the striking similarity in shape between the tabulæ of Syringopora and Tubipora, the general shrunken appearance they have in the former genus, as shown in the numerous figures given by Professor Nicholson (19, 20), goes to prove that they are due in this genus too to a shrinking of the mesoderm, and that consequently they are in every respect homologous.

A very great difficulty, however, that is found by Professor Nicholson and others in regard to the Alcyonarian affinities of Syringopora, is the undoubted relationship which exists between this genus and the family Favositidæ, which family is considered by them to be undoubtedly Zoantharian, and closely allied to Porites. But the evidence in favour of the Favositidæ being really Zoantharians is by no means conclusive, for it seems to rest entirely upon the presence, in some cases only, of spiniform septa in the corallites.[1] The presence of tabulæ is no longer evidence against their being Alcyonaria, as Professor Moseley has shown that Heliopora, which possesses tabulæ, is undoubtedly an Alcyonarian and Tubipora, also is now known to possess true tabulæ. In fact, when we remember that tabulæ

[1] In Stenopora septal spines are absent.

are occasionally found in Tubipora, exactly similar to the flat tabulæ of Favosites, the convex tabulæ of Michelinia, the lappet- or tongue-shaped incomplete tabulæ of Pachypora, and the funnel-shaped tabulæ of Syringolites, and that tabulæ are quite unknown amongst the Poritidæ—the family which is supposed to be most nearly related to the Favositidæ—we must look upon these structures as evidence, if any, in favour of the Favositidæ being Alcyonarians. But the evidence in favour of the Alcyonarian affinities of the Favositidæ does not come entirely from the side of Tubipora, for, as Professor Moseley (17) has pointed out, there is strong evidence of close relationship between Heliopora and Favosites.

Professor Moseley also points out that the signs of Favosites forbesi being dimorphic is also in favour of this affinity; and this point becomes of greater importance as our knowledge of the Alcyonarians increases. Kölliker has pointed out that in such closely allied genera as Heteroxenia and Xenia, the former is dimorphic, the latter is not. Again, in such closely allied genera as Paragorgia and Briareus the former is dimorphic and the latter is not; and I have just been informed by Mr. S. Ridley, who has been investigating the Alcyonarians brought back by H.M.S. "Alert," that the genus Melitodes is also dimorphic, whilst, as far as I am aware, the genera most nearly allied to it are not dimorphic. Thus, a tendency for various genera to become dimorphic seems to be a characteristic feature of the Alcyonaria, a feature, moreover, which, as far as I am aware, is entirely unknown amongst the Zoantharia, and consequently the evidence bearing upon the question afforded by Favosites forbesi, which was apparently dimorphic, is not without considerable weight.

Thus, I think that taking all things into consideration, the evidence at our command tends to prove that the Favositidæ are really Alcyonarians, and that Syringopora is also an Alcyonarian allied to Tubipora.

c. Remarks on the Zoological position of Tubipora.

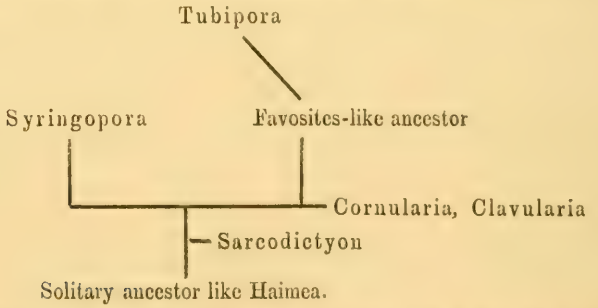
In De Blainville's 'Manuel d'Actinologie,' 1834, Tubipora is placed in the same family, namely, "les Tubipores," with Cornularia and Clavularia. In modern text-books of zoology there is a tendency to place the genus Tubipora in a separate family, and to classify Cornularia and Clavularia with the Alcyonidæ. Von Koch (10 and 10 *a*), however, agrees with the older view, and he says (10 p. 6): "Von den lebenden Formen stehen ihr wohl die Cornulariden am nächsten, und scheint es, dass diese Familie einen sehr ursprünglichen Zustand der Octokorallen repräsentirt." I am inclined to agree with von Koch and the older naturalists, and have elsewhere (8) proposed that Tubipora should be included with Cornularia, Clavularia, Sarcodictyon, and allied forms, in one family, which may be called the Stolonifera.

In order to arrive at any conclusion as to the grouping of a family of animals it is necessary to take into consideration the lines upon which the phylogeny of that group probably proceeded, and consequently, I propose to give certain speculations concerning the phylogeny of Tubipora, to which I have been led in the course of my investigation.

As I pointed out above, there is every reason to suppose that a long series of intermediate forms between the recent Tubipora and a Cornularia-like ancestor must have become extinct. As long as the walls remained unsupported by skeletal structures as they are in Cornularia, it was a matter of impossibility for the corallites to attain any great length. When, however, owing to the formation of a skeleton the corallites increased in length, communications between the individual corallites would be of immense value to the colony for keeping up a continuous and sufficient circulation. If we suppose that the corallites stood as near to one another on the stolon as the polyps do in the recent Cornularia, fusion of the walls of adjacent corallites and the formation of pores after the manner of the mural pores of Favosites would be not impossible but even

probable. Having formed communications of this nature between the individual corallites, every intermediate condition between this and the condition in which the pores are drawn out into the form of hollow tubular communications or perforated platforms would be of advantage to the colony as affording more room for the development and growth of buds; and, indeed, as Professor Nicholson (19) has shown, every intermediate condition can be found between the mural pores of Favosites and the tubular communications of Syringopora or the platforms of Chonostegites.

Previously, however, to the Cornularia-like ancestor there must have been a solitary ancestor similar to Haimea or Hartea. The genus Sarcodictyon (Gosse, 7) consists of simple polyps united together only by very delicate tubular threads. From a condition of the stolon so simple as this there may have been in the course of evolution two principal variations. Either the thread-like communications increased in number and size and underwent various anastomoses, forming a retiform stolon like Syringopora, or else the threads may have become broader by the growth of coenenchym, and eventually formed a lamellar stolon containing a network of canals, as in Cornularia and Tubipora. Thus arranged in a tabular form the phylogeny of the Stolonifera might be represented as follows :



Before concluding this paper, I must acknowledge my indebtedness to Professor Moseley for much valuable aid and advice, to my sister, Miss A. W. Hickson, for the most excellent and valuable drawings represented by figs. 1 and 2, and to

my sister, Miss C. M. Hickson, for the careful drawing of the spicules of the tabulæ of Tubipora, represented by Fig. 7.

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On the Malleus of the Lacertilia, and the Malar and Quadrate Bones of Mammalia.

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With Plate XLI.

The apparent absence of a quadrate bone in Mammals has given rise to a large number of theories, differing in details, but all, or nearly all, tending to discover this bone in the chain of ossicula auditus of these animals. A recent work, published by my friend Professor P. Albrecht,[1] departs, however, from the view ordinarily taken, and, as I believe, advances our knowledge considerably on the subject. I have been led by the study of this memoir to a discovery which I believe to be of considerable interest, of which it is the object of the present communication to give an account.

Before passing to the consideration of my own observations I will briefly review the state of the question. This cannot be better accomplished than by reproducing the excellent recapitulative tables given by M. Albrecht.

[1] P. Albrecht, "Sur la valeur morphologique de l'articulation mandibulaire, du cartilage de Meckel et des Osselets de l'ouïe, avec essai de prouver que l'écaille du temporal des Mammifères est composée primitivement d'un squamosal et d'un quadratum" (Mayolez, Bruxelles, 1883).

Table I.[1]

Theories published up to the present time on the Morphological Differences between the Mandibular Articulation of the Lower Gnathostomous[2] Vertebrata and the Mammalia.

Mandibular Articulation of the Lower Gnathostomous Vertebrata.	Mandibular Articulation of Mammalia.	
Quadrato-articular articulation	Huxley[3] . .	Squamoso-mandibular articulation.
	Gegenbaur[4] .	Dentary articulation.
	v. Kölliker[5] .	Ditto, ditto.

[1] P. Albrecht, op. cit., p. 249.

[2] E. Haekel, 'Anthropogenie,' Leipzig, 1874, p. 425.

[3] Huxley, 'A Manual of the Anatomy of Vertebrate Animals,' London, 1871, p. 84. Wiedersheim, 'Lehrbuch der vergleichenden Anatomie der Wirbelthiere,' Jena, 1882, T. i, p. 139 and 155.

[4] Gegenbaur, 'Grundzüge der vergleichenden Anatomie,' 2e Auflage, Leipzig, 1870, p. 662.

[5] V. Kölliker, 'Entwicklungsgeschichte des Menschen und der höheren Thiere,' 2e Auflage, 1879, p. 486.

Table II.[1]

Theories on the Development and the Morphology of the Ossicula Auditus of Mammalia.

	Mandibular Arch.	Hyoidean Arch.	Auditory Capsule.
Reichert[2]	Malleus, Incus	Stapes.	
Günther[3]	Malleus, Incus, Stapes		
Gegenbaur[4]	Malleus (Articular). Incus (Quadrate)	Os lenticulare (Symplectic). Stapes (Hyomandibular)	
Huxley[5]	Malleus (Quadrate)	Incus (Hyomandibular) Os lenticulare, Stapes.	
W. K. Parker[6]	Ditto	Ditto.	
W. K. Parker and Bettany[7]	Ditto	Incus (Hyomandibular)	Stapes.
Salensky[8]	Malleus, Incus, Stapes		
V. Kölliker[9]	Malleus (Articular). Processus gracilis (Angular) Incus (Quadrate)	. . . . .	Stapes.
Wiedersheim[10]	Ditto	. . . . .	Ditto.

[1] P. Albrecht, loc. cit., p. 250.

[2] Reichert, "Ueber die Visceralbögen der Wirbelthiere," 'Müller's Archiv,' 1837.

[3] Günther, 'Beobachtungen über die Entwicklung des Gehörorgans,' Leipzig, 1842.

[4] Gegenbaur, loc. cit., pp. 662 and 663.

[5] Huxley, loc. cit., and 'Proc. Zool. Soc. London,' 1869.

[6] P. Albrecht, loc. cit., p. 250.

[7] Parker and Bettany, 'The Morphology of the Skull,' London, 1877.

[8] Salensky, "Zur Entwicklungsgeschichte," 'Zoologischer Anzeiger,' Leipzig, Jahrgang ii, p. 250.

[9] V. Kölliker, loc. cit., pp. 471—473, 475—478, 480—487.

[10] Wiedersheim, loc. cit. See also Salensky, 'Morpholog. Jahrbuch,' vol. vi, p. 415; and Fraser, 'Philos. Transact.,' 1883, p. 901.

TABLE III.[1]

Theories on the Morphological value of the Incudo-mallear and the Mandibular Articulations of Gnathostomous Vertebrates.

Articulations.	Gegenbaur, v. Kölliker.	Huxley, Parker and Bettany.
Incudo-mallear of Mammalia.	Quadrato-articular.	Hyomandibular-quadratic.
Mandibular of Lower Gnathostomous Vertebrata.	Quadrato-articular (viz. Incudo-mallear).	Quadrato-articular (viz. Malleo and Articular).
Mandibular of Mammalia.	Squamoso-dentary.	Squamoso-articular.

From the contents of these tables it follows :

1st. That according to the anatomists who have preceded M. Albrecht, the lower gnathostomous Vertebrata have their jaws hinged by means of a quadrato-articular articulation, whilst Mammalia are provided with an entirely different kind of articulation, concerning the exact nature of which these anatomists are at variance.

2nd. That according to the same authors the Promammalia[2] must have possessed originally a quadrato-articular articulation of the lower jaw, but that they have lost it (Huxley, Parker, and Bettany), or at least have given up its use in the act of mastication (Gegenbaur and von Kölliker), and that at the same time their quadrate (Huxley, Parker, and Bettany), and perhaps also the articular (Gegenbaur and von Kölliker) and angular elements (von Kölliker) of their mandible have become included in the chain of the ossicula auditus.

After this statement M. Albrecht[3] continues :

1st. That he is persuaded that all of the ossicula auditus of Mammalia are represented by homologues in the Amphibia, and by all the ossicula auditus of the Sauropsida, and that they correspond to the suspensorium of fish. The facts may be stated as follows :

[1] P. Albrecht, loc. cit., p. 12.

[2] E. Haeckel, 'Natürliche Schöpfungsgeschichte,' Berlin, 1872, p. 538.

[3] P. Albrecht, loc. cit., p. 251.

	Fenestra tympanica. Albrecht.	Interfenestral Chain, Albrecht, extending, without interruption, from one Fenestra to another	Fenestra ovalis.
Mammalia.	Membrana tympanica.	Malleus + Incus + Os lenticulare + Stapes.	Membrana ovalis.
Sauropsida.	Ditto.	Columella auris.	Albrecht. Ditto.
Amphibia: Urodela.	Ditto.	Ditto.	Ditto.
Amphibia: Anoura.	Ditto.	1st ossicle + 2nd ossicle + 3rd ossicle + 4th ossicle.	Ditto.

Further :

Mammalia.	Mandible.	Extramandibular portion of Meckel's Cartilage and Interfenestral Chain.	Otic Region of Cranium.
Sauropsida.	Ditto.		Ditto.
Amphibia.	Ditto.		Ditto.
Pisces	Ditto.	Suspensorium.	Ditto.

Thus, since the quadrate does not form any part at all of the interfenestral chain of Sauropsida, it cannot enter into the composition of that of Mammalia.

2nd. That it is not possible to understand (especially on the theory of Gegenbaur, v. Kölliker, and others) how the Mammalia could have acquired an articulation of the lower jaw different from that of other gnathostomous Vertebrata, and that, therefore, he is of opinion that the glenoid cavity of Mammalia ought still to be found in the quadrate bone.

M. Albrecht has in his hands at the present time the skull of a new-born child, in which the squamous portion of the temporal bone is divided into two parts, viz.:

a. The squamous portion of the temporal bone, properly so called, the homologue, in his opinion, of the squamosal of Sauropsida.

β. The zygomatic portion bearing the glenoid cavity, and thus homologous, with the quadratum of the Sauropsida.

Other cases of the complete separation of the squamosal and the quadrate are mentioned in literature, and are cited by M. Albrecht. In addition this distinguished anatomist states that he has observed traces of a squamoso-quadratic suture in several

skulls of apes in the Royal Belgian Museum of Natural History, and that he will shortly publish an account of them.

To sum up, M. Albrecht's arguments lead, on the whole, to the following two conclusions:

1st. The quadrate cannot form part of the interfenestral chain of bones of Mammalia.

2nd. One of the two bones (the zygomatic portion) formed by the division of the so-called squamosal is doubtless the homologue of the quadrate of Sauropsida.

I shall examine these two statements successively, in order to show whether the results of my own observations tend to confirm or invalidate M. Albrecht's conclusions.

I.

Can the quadrate form a part of the interfenestral chain?

It is evident that if there were found simultaneously existing in the same animal a mandible composed of six normal elements, a true quadrate, and a malleus, it would immediately follow that it was impossible that that quadrate could form any part of the chain of ossicula auditus, for—

1st. It could not be confounded with the malleus, because there would already be one there.

2nd. It would be still more impossible to identify it with the remainder of the interfenestral chain, because it would be situated outside the malleus, and would not touch any of the remaining ossicula.

Everything depends, therefore, on the discovery of a malleus in the condition described above. Now, I have found in several Lacertilia (*Leiolepis guttatus*, *Ctenosaura pectinata*, *Uromastix spinipes*, *Lophyrus dilophus*, *Basiliscus vittatus*) a small bone which appears to answer the question. I shall endeavour to prove that it has really the morphological value of a malleus.

a. Firstly, it has the form of a malleus; it being possible to distinguish in it:

a. A capitulum.

b. A cervix.

c. A manubrium.

d. A processus gracilis.

β. It has the same connections; that is to say:

a. It is applied along the tympanic membrane in such a manner that the manubrium is parallel to the membrane.

b. It is united besides to the remainder of the interfenestral chain by means of a cartilage attached to it in the region of the cervix.

c. It lies in contact with the quadrate in exactly the same manner as that in which the malleus of Mammalia is in contact with the quadrate of M. Albrecht.

γ. It is connected with the articular element of the mandible by means of a malleo-articular ligament, which M. Albrecht identifies with the extra-mandibular portion of Meckel's cartilage.

δ. There is little doubt, there appears to me, that this malleus is identical with that described by Peters as existing in the crocodile,[1] and with the "suprastapedial extrastapedial (manubrium)" of Mr. W. K. Parker.[2]

This being admitted, the first of the naturalists cited has demonstrated the continuity of the malleus with Meckel's cartilage, as in Mammalia,[3] an observation confirmed by the English naturalist. This result, therefore, is in support of the opinion expressed in δ.

ε. The malleus of Mammals serves for the insertion of a small muscle (tensor tympani), the origin of which is in the otic region of the cranium. The same occurs in the case of the malleus of Lacertilians.[4]

[1] W. Peters, 'Monatsberichte d. K. p. Akademie d. Wissenschaften zu Berlin,' 1868, p. 592.

[2] W. K. Parker, 'Phil. Trans. Roy. Soc.,' London, part. ii, 1879, pl. 43, fig. iii and vi.

[3] W. K. Parker, 'Nature,' July 13th, 1881, p. 253.

[4] W. K. Parker, 'Phil. Trans.' (v. supra), fig. vi, st. m. (the so-called "stapedius" of the author).

To sum up, then, I believe that I have discovered in Lacertilia a real malleus, the homologue of that of Mammalia, and with that fact as a starting-point it appears that the conclusion may be formed that the quadrate of Mammalia is not to to be sought for amongst their ossicula auditus. In this respect, therefore, M. Albrecht's theory receives confirmation, although it will be necessary that it should be slightly modified. Instead of the statement with regard to reptiles :[1]

Columella = malleus + incus + os lenticulare + stapes, the matter must stand thus :

Columella = incus + os lenticulare + stapes.

II.

Is the quadrate of M. Albrecht really the homologue of the quadrate of Sauropsida ?

Such is the second question to be considered. Before going further it will be well to consider what are reasons given by M. Albrecht in favour of his interpretation. They are three in number.

1st. The quadrate of Mammalia, since it could not find place in their interfenestral chain, must be to be found elsewhere.

2nd. The glenoid cavity ought still be found in the quadrate bone.

3rd. There are a certain number of instances known in which the squamous portion of the temporal bone of man, or of the primates, is divided into two parts.

a. The squamous bone properly so called.

β. The zygomatic portion.

It appears to me that two further methods of inquiry should be employed to make certain whether the quadrate of M. Albrecht is a real quadrate or not. These are :

1st. The examination of its connections.

2nd. The study of its development.

[1] P. Albrecht, loc. cit., p. 15.

Leaving aside the latter question, to which M. Albrecht proposes to return shortly, the attempt will be made to demonstrate the correctness of his views by the former method.

Nevertheless, before this interesting subject is commenced, it is indispensable to determine the morphological value of the malar bone of Mammalia, since this determination will be of the greatest utility in the sequel.

In a recent work[1] M. Albrecht has proved that the malar bone of Mammalia ought to be considered as formed of three parts, which he calls

1st. A premalar.
2nd. A postmalar. .
3rd. A hypomalar.

} See the Figs. 1, 2, 3, 4, below.

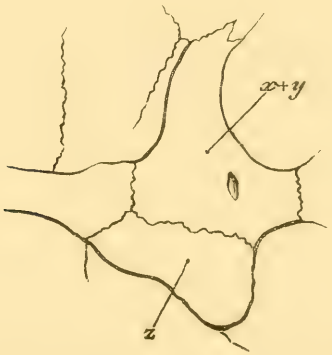


Fig. 1.—Diagram of the malar bone divided into two parts by a horizontal suture. (The Japanese bone.) (After P. Albrecht).

$x + y$. Post-malar (Posterior postfrontal, Albrecht.) (Postfrontal, Dollo). + Premalar (Anterior postfrontal, Albrecht.) (Jugal, Dollo). z. Hypomalar. (Quadrato-jugal, Albrecht).

[1] P. Albrecht, "Sur le Crâne d'une Idiote de 21 ans," 'Bull. Soc. Anthropologie d. Bruxelles,' T. I., p. 163.

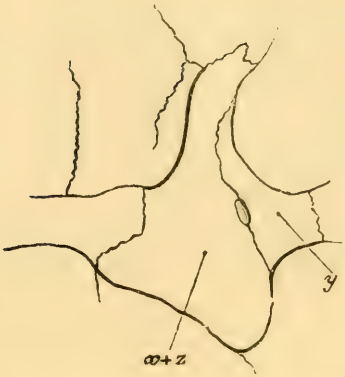


Fig. 2.—Diagram of the malar bone divided into two parts by a vertical suture. (The right malar bone of the cranium of Albrecht's idiot.) (After P. Albrecht.)

y. Premalar (Anterior postfrontal, Albrecht.) (Jugal, Dollo). $x + z$. Postmalar (Posterior postfrontal, Albrecht). (Postfrontal, Dollo.) + Hypomalar (Quadrato-jugal, Albrecht).

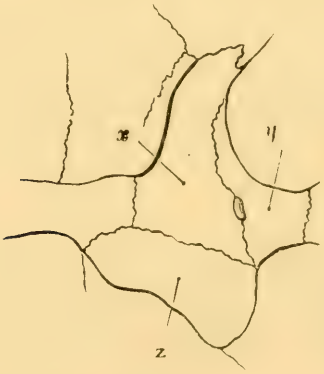


Fig. 3.—Diagram of the malar bone divided into three parts, constructed from the bipartite malar with the horizontal suture (the Japanese bone), and the bipartite malar with the vertical suture (right malar of Albrecht's idiot). After P. Albrecht.

x. Postmalar (Posterior postfrontal, Albrecht) (Postfrontal, Dollo). *y*. Premalar (Anterior postfrontal, Albrecht.) (Jugal, Dollo.) *z*. Hypomalar (Quadrato-jugal, Albrecht).

FIG. 4.—Diagram of the normal malar. (After P. Albrecht).
$x + y + z$. Postmalar (Posterior postfrontal, Albrecht.) (Postfrontal, Dollo). + Premalar (Anterior postfrontal, Albrecht.) (Jugal, Dollo). + Hypomalar (Quadrato-jugal, Albrecht).

By comparison with the Sauropsida M. Albrecht arrives at the following interpretation of these parts:

Premalar—Anterior postfrontal.

Postmalar—Posterior postfrontal.

Hypomalar—Quadrato jugal.

Lastly, in his opinion a small isolated bone discovered in a young Cynocephalus appears to represent the jugal, which is usually co-ossified with the supramaxillary bone.

I regret that I cannot agree with his conclusions, for in fact:

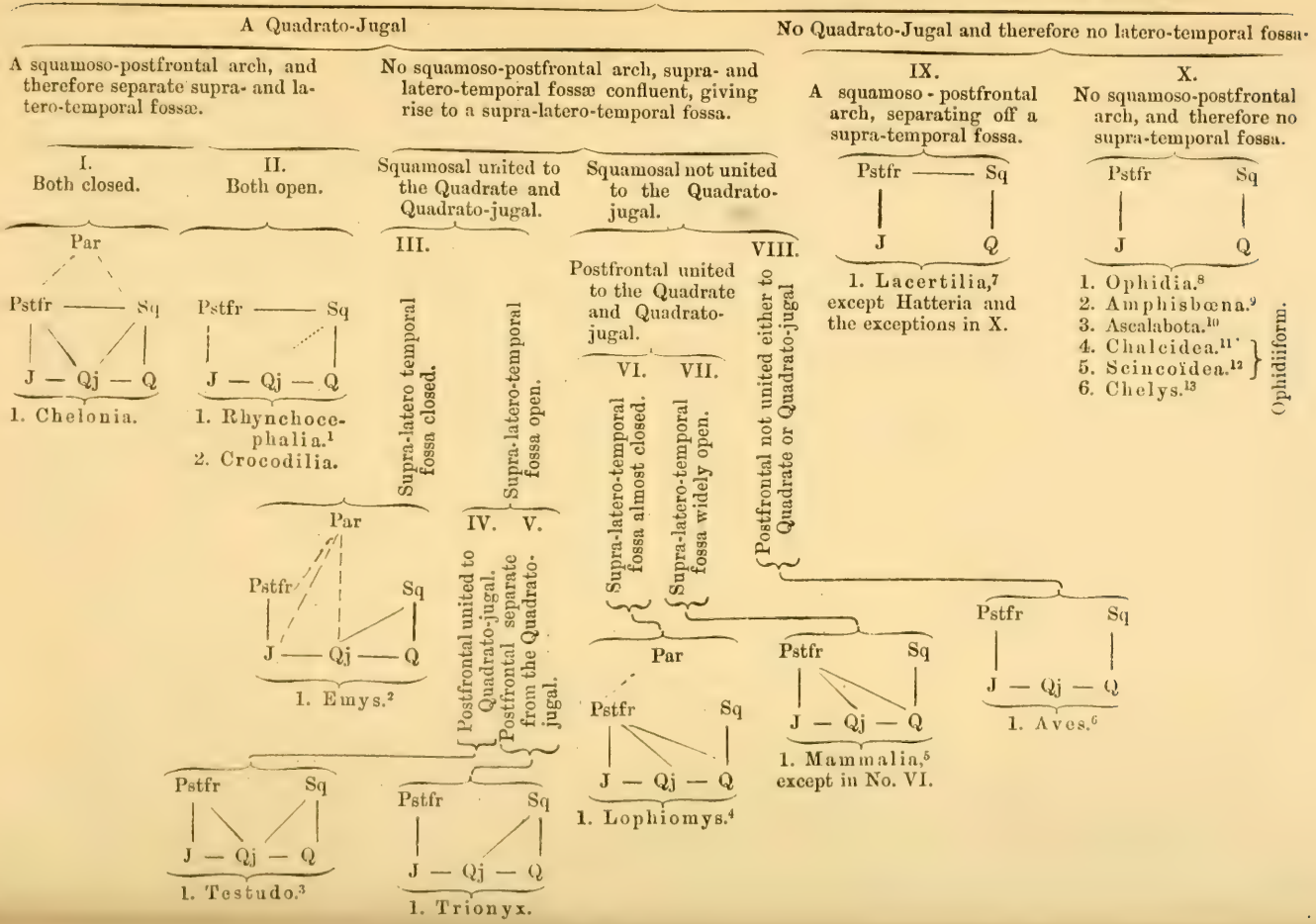
1st. The bone of the Cynocephalus appears to me to be simply a wormian bone, which has appeared at this spot as if for the very purpose of leading to the construction of a theory.

2nd. In M. Albrecht's comparison, the jugal is excluded from taking part in the contour of the orbit, an arrangement of which I know no other example, not even in the Lacertilians with the double postfrontal.

3rd. Why should it be desired to assign to the human subject two postfrontals, when in the Sauropsida this structure is only to be met with amongst the Lacertilia, and even amongst these an exception rather than the rule?

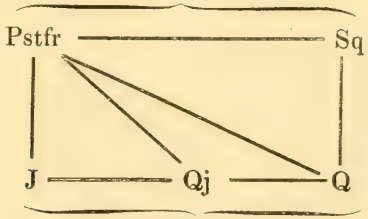
(Continued on page 592.)

Supra-Temporal and Latero-Temporal Fossæ of Existing Sauropsida and Mammalia.



(Notes to the tabular statement on page 590.)

[1] I observe the following arrangement in Rhynchosuchus Schlegelii :-



Rhynchosuchus Schlegelii.

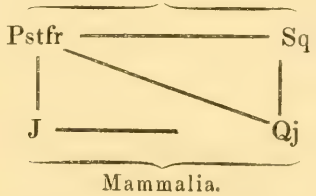
This fact will be made use of in a moment.

[2] Cuvier, 'Ossemens fossiles,' 1836, pl. 239, figs. 9—12.

[3] Cuvier, loc. cit., figs. 17—20.

[4] A. Milne-Edwards, "Mémoire sur le type d'une nouvelle famille de l'ordre des Ronguers," 'Nouvelles Archives du Museum de Paris,' 1867, p. 66 and pl. vii, figs. 1, 2, 3, 4.

[5] The formula, as well as the preceding one, assumes that the quadrate of M. Albrecht is really a quadrate. Otherwise we have :



Mammalia.

that is to say, the structure of a Chelonian, in which the supra-temporal fossa has remained open, and in which the quadrate has been withdrawn and transformed into an auditory ossicle.

[6] T. H. Huxley, 'A Manual, &c.,' p. 282.

[7] T. H. Huxley, 'A Manual, &c.,' p. 220.

[8] T. H. Huxley, 'A Manual, &c.,' p. 233—238.

[9] T. H. Huxley, 'A Manual, &c.,' p. 230.

[10] T. H. Huxley, 'A Manual, &c.,' p. 225.

[11] T. H. Huxley, 'A Manual, &c.,' p. 228.

[12] T. H. Huxley, 'A Manual, &c.,' p. 228.

[13] Cuvier, loc. cit., figs. 21—34.

4th. M. Albrecht's [1] former interpretation, viz. that the jugal when it is not ossified by a particular centre is ossified either by the supra-maxillary or by the quadrato-jugal, appears to me even less capable of defence, for among the living Sauropsida possessing a quadrato-jugal Hatteria is the only one in which this bone is fused to the jugal. In Hatteria we have to deal with an exception. Therefore, why should it be desired to render the condition in the human subject a matter of exception rather than to bring it within the rule? In other words, it appears to me more rational to assign to Man a separate quadrato-jugal, such as occurs in Birds, Chelonians, and Crocodilia.

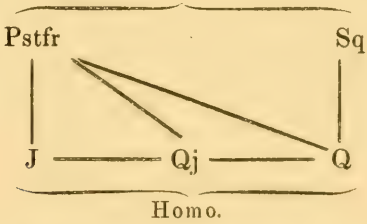
The explanation which follows seems to me a more correct one, because by its application all the objections we have just passed in review disappear.

Premalar = Jugal.

Postmalar = Postfrontal.

Hypomalar = Quadrato-jugal.

This, if M. Albrecht's quadrate be admitted, gives us the formula:



Let us attempt to discover in the Sauropsida an analogous arrangement, in order to better justify our theory.

If a skull of Hatteria be examined, it is seen that three temporal fossæ can be distinguished,[2] a latero-temporal, a supra-temporal, and a post-temporal. I have discussed the variations of the latter at length in another memoir,[3] and it

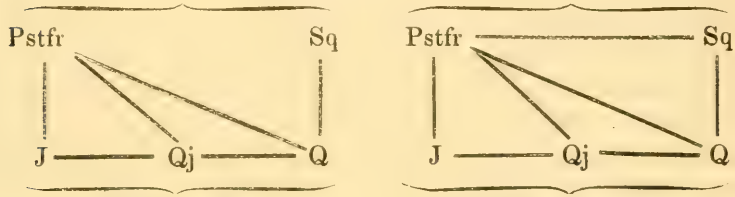
[1] P. Albrecht, 'Sur la valeur morphologique de l'articulation mandibulaire, &c.' Hypomalar = Jugal + Quadrato-jugal.

[2] T. H. Huxley, 'A Manual, &c.,' p. 220.

[3] L. Dollo, "Quatrième note sur les Dinosauriens de Bernissart," 'Bull. Mus. Roy. Hist. Nat. Belg.,' t. ii, p. 242.

is not therefore necessary to return to the subject here. Let us study the variations of the two former; they are comprehended in the table on page 590.

Let us now compare the formula given for man with that of Rhynchosuchus Schlegelii.



Homo sapiens. Rhynchosuchus Schlegelii.

Only two things are necessary to pass from one to the other, viz.:

1st. The disjunction of the squamoso-postfrontal arch.

2nd. The closing together of all the other bones under consideration.

Now, the former condition is very common amongst the Sauropsida. It is to be observed, as has been already stated, in the Ophidians, the Amphisbœnidæ, the Ascalobotidæ, the Chalcidea and Ophidiiform Scincoïdea, the Chelonia, and in Birds; whilst the latter is by no means rare amongst the Chelonia.

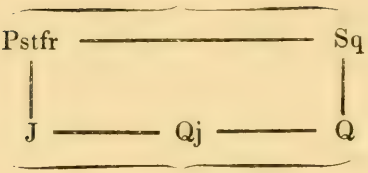
In short, my explanation of the malar bone of Mammalia avoids entirely the objections raised by M. Albrecht's theory, and exhibits a simple combination of the known phenomena in Lizards:

Malar = postfrontal + jugal + quadrato-jugal.

Before resuming the discussion concerning the quadrate, I may again express my opinion, based on the facts which follow, that—

1st. The Lacertilia and Ophidia possessed originally an ossified quadrato-jugal.

2nd. That therefore there existed originally in all the Sauropsida a latero-temporal fossa bounded thus:



Sauropsida.

My reasons are as follows:

1st. The ancient Reptiles (Ichthyosauria, Plesiosauria, Dinosauria, &c.) possessed an ossified quadrato-jugal

2nd Amongst the existing Sauropsida, Birds, Crocodiles, and Chelonians, as well as Hatteria, possess one still.

3rd. This latter animal represents amongst the Lacertilia a primitive type, as it is closely allied to the Triassic Rhynchosaurus.

4th. The quadrato-jugal exists still in a ligamentous condition in the Ophidia and Lacertilia, and this is certainly an indication of rudimentation since in all the Chalcideæ and ophidiiform Scincoidea the squamoso-postfrontal arch likewise becomes ligamentous

Let us now consider by what peculiarities in its connections the quadrate of Sauropsida is distinguished, and then examine whether these peculiarities are also to be found in M. Albrecht's quadrate.

The quadrate of Sauropsida may be in relation—

1st. At its proximal extremity with the squamosal, the parietal, and the parotic process (Iguana).

2nd. At its proximal extremity again with the malleus (Uromastix).

3rd. At its distal extremity with the mandible and the pterygoids (Iguana).

4th. At its distal extremity again with the quadrato-jugal (Crocodile).

5th. Occasionally with the postfrontal (Rhynchosuchus).

6th. Lastly, with the tympanic membrane, which is inserted into it in a posteriorly placed concavity, which might be termed the tympanic concavity (Iguana).

Now, all these relations, excepting the one with the pterygoid, occur in the case of M. Albrecht's quadrate. If, then, it is borne in mind that it is solely because of the enormous development (compared to that in the Sauropsida) of the alisphenoid in man that the separation of the quadrate and pterygoid is due, it will be admitted that M. Albrecht had good grounds for holding the quadrate of the lower gnathostomous Vertebrata as homologous with the zygomatic portion of the squamous part of the temporal bone of Mammalia.

Summary.

To resume:

I. I believe that I have discovered in Lacertilia a true malleus homologous with that of Mammalia, and that this circumstance allows of a modification of the table of homologies given by M. Albrecht[1] in the following manner.

	Interfenestral Chain.
Fishes in general ...	..... Suspensorium.
Teleosteans	.....Symplectic... + Hyomandibular.
Sauropsida.....	M. tymp. ... Malleus + Columella.....M. ovalis.
Mammalia	M. tymp. ... Malleus + Incus + Os lenticulare + Stapes.....M. ovalis.
Amphibia { Urodela.	M. tymp. ... Columella ..... M. ovalis.
Amphibia { Anoura.	M. tymp. ... 1st ossicle + 2nd ossicle + 3rd ossicle + 4th ossicle ..... + M. ovalis.

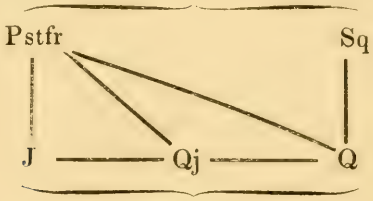
II. I believe that I have determined the morphological value of the malar bone of Mammalia. It is as follows:

Malar = postfrontal + jugal + quadrato-jugal.

[1] P. Albrecht, 'Sur la valeur morphologique, &c.,' p. 253, m.

Albrecht has also communicated to me his idea that the Malleus is nothing else than the symplectic of Teleosteans. The grounds on which he supports this opinion are, that according to Stannius the extra-mandibular portion of Meckel's cartilage fixes itself upon the symplectic. I am the more disposed to admit the correctness of this homology because, in the Lacertilia, it is to be clearly observed that the Malleus (in regard to which M. Albrecht agrees with me) is the intermediate bone between the quadrate and the columella (hyomandibular), just as in the osseous fishes the symplectic is interposed between the quadrate and the hyomandibular.

This may be expressed in the following formula :



Homo.

relations which are to found amongst the Sauropsida also.

III. Lastly, I have by the study of the connections of the bones materially strengthened M. Albrecht's theory, which holds that the zygomatic portion of the squamous part of the the temporal bone of Mammalia is the homologue of the quadrate of the lower gnathostomous Vertebrata.

In conclusion, it is my duty, which is very agreable to me, to thank my learned friend, Professor P. Albrecht, for the liberality with which he has placed at my disposal the wood blocks which he has used in the illustration of his two recent memoirs, and all the more so because they are to be used to attack one of the theories of the distinguished anatomist himself.

Notes on Echinoderm Morphology, No. VI. On the Anatomical Relations of the Vascular System.

By

P. Herbert Carpenter, M.A.,
Assistant Master at Eton College.

I DREW attention in my last note[1] to the striking variations in the results of observations upon the vascular system of the Echinoderms, which have been made by different Continental naturalists. The leading member of the French School, Professor E. Perrier, asserts positively that the so-called "heart" of the Echinozoa is an excretory gland, which communicates with the exterior through the madreporite, and is entirely free at its inner end, no vessel proceeding from it to join an oral ring; in fact, it is not a part of the blood-vascular system at all. Professor Perrier had arrived at this conclusion as the result of his own observations upon Urchins and Starfishes, together with those of Apostolidès upon the Ophiurids. He totally denies the existence of the radial blood-vessels described by Ludwig in the Asterids; and as regards the Urchins he, like Hoffmann, is able to find but one vascular ring around the mouth. This is described as connected with the water-tube (stone-canal) and the radial vessels supplying the tentacles, and also as the ring in which the ventral or internal marginal vessel of the intestine originates. "Il y a donc bien réellement communication entre l'appareil vasculaire intestinal et le prétendu appareil aquifère."[2]

[1] This Journal, vol. xxii, pp. 371—386, October, 1882.

[2] 'Sur l'appareil circulatoire des Oursins,' 'Comptes rendus,' 1874, t. 79, pp. 1128—1132.

This question of the communication or independence of the water-vascular and blood-vascular systems is one of fundamental importance in the morphology of the Urchins, and indeed of all Echinoderms. Hoffmann, Agassiz, and Perrier have expressed their belief in the former; while the more recent work of Teuscher and Koehler seems to indicate that the water-vascular and blood-vascular systems of the regular Urchins, at any rate, have no communication with one another, except through the spongy tissue of the Polian vesicles.

I have pointed out in previous notes[1] that Ludwig's observations upon the Stellerids led him to regard the so-called heart as a plexiform portion of the vascular system, connected both with an oral and with an aboral blood-vascular ring, the former giving rise to radial trunks which lie between the water-vessels and the ambulacral nerves. I have, as I have said before, considerable faith in the accuracy of Ludwig's observations; and this led me to suggest the possibility that the connection of the "heart" or "central plexus" with a *second* oral ring, other than that of the water-vascular system, might have been overlooked by the French naturalists. I little thought, however, that, as regards the Urchins, a few months would bring a striking confirmation of this suggestion in the complete work of Mons. Koehler himself, from whose earlier writings, unaccompanied by figures, it was difficult to gain a clear conception of his results.

He has recently published an elaborate memoir,[2] enriched with seven beautifully executed plates, which illustrate the minute anatomy of the Urchins in a manner that has never before been attempted. Working, like Hoffmann and Perrier, by the injection-method, he has been able largely to extend the results of his predecessors; and he has also added very considerably to our histological knowledge. His observations have not been limited to the regular Urchins, but have been

[1] This Journal, vol. xxi, 1881, pp. 170—180; vol. xxii, 1882, pp. 372—375.

[2] "Recherches sur les Echinides des Cotes de Provence," 'Ann. du Mus. d'Hist. Nat. de Marseille,' Zoologie. Mémoire No. 3, pp. 1—167, pl. i—vii.

extended to the Spatangidæ, which appear to present certain anomalous characters, as yet but imperfectly understood.

It will be remembered that Perrier, like Hoffmann, could only find one vascular ring round the mouth of an Echinus; and he described it as connected, not only with the water-tube and radial water-vessels, but also with the ventral vessel of the gut. The results of his injections of the so-called heart led him to assert that it is "très nettement terminé vers le bas et qu'il n'en sort aucun canal. Il ne peut donc être question d'un vaisseau inférieur parallèle au canal du sable et aboutissant à l'un des deux cercles vasculaires que l'on suppose exister autour de l'œsophage."[1]

Koehler finds, however, that by inserting his cannula into the lower end of this organ, which, like Perrier, he calls the "ovoid gland," he is able to inject a vessel lying alongside the water-tube, but totally distinct from it. He calls it the "glandular canal," and finds it to be connected with an oral ring in which radial vessels originate. These are not the water-vascular ring and radial water-vessels, which can be injected from the water-tube altogether independently of those described by Koehler. He says that the glandular canal can be readily followed with the aid of a lens, right up from the lantern to the apex of the ovoid gland. He speaks of it as containing blood, and has frequently found coagulum both in it and in its ramifications over the peripheral part of the ovoid gland. When this glandular canal is carefully injected, the fluid enters the circumoral ring before mentioned, together with the internal marginal vessel which is connected with it, and not with the water-vascular ring as formerly described. The injection also passes into arborescent ramifications within the Polian vesicles; but if pressure is used, it fills these organs and enters the water-tube and radial vessels.[2]

This second oral ring seems to have been previously seen by

[1] "Recherches sur l'appareil circulatoire des Oursins," 'Arch. de. Zool. exp. et. gén.,' t. iv, 1875, p. 613.

[2] Op. cit., pp. 65-70.

Teuscher,[1] who failed, however, to distinguish the radial blood-vessels in the ambulacra, confounding them with the so-called perineural spaces. The two vessels are readily visible on the inner aspect of the ambulacra, each sending branches to the ampullæ, and may be injected with different materials; while their distinctness, though not apparent in Teuscher's section of the ambulacra, is quite evident in that figured by Koehler.

According to Teuscher, the water-vessels of the ambulacra ascend the outside of the lantern to join the ring at its summit, just as Perrier described them; while the blood-vessels pass over the actinal membrane towards the mouth, ascend along the pharynx, between it and the interpyramidal muscles, and join the superior of the two rings, in which the intestinal vessel originates. Koehler, however, also working by the section method, has been unable to find these radial vessels described by Teuscher on the pharynx, and his injections have not led him to suspect their presence.[2] In fact, he says distinctly that at the edge of the peristome "les vaisseaux ambulacraires, de doubles qu'ils étaient, deviennent simples et forment alors les cinq branches qui montent sur la face-externe de la lanterne, et vont aboutir au cercle périœsophagien inférieur" (*i.e.* water-vascular ring).

It is noteworthy, however, that in the Spatangids either of the two oral rings, internal or external, may be injected from the corresponding radial vessel, and Koehler says distinctly that each of these rings sends branches into the ambulacra.[3] This leads to the suspicion that each radial vessel of an Echinus communicates directly with a corresponding vascular ring, just as was described by Teuscher, and that the water-vascular and blood-vascular systems are distinct, at any rate in the peristome and ambulacra.

Koehler's memoir would have been still more valuable than it is, had he entered more fully into a comparison of his results

[1] "Beiträge zur Anatomie der Echinodermen," iv, 'Echinidae. Jen. Zeitschr.,' Bd. x, pp. 520—521, Taf. xx, fig. 6.

[2] Op. cit., pp. 70, 73.

[3] Op. cit., p.p. 93, 94, 100.

with those of other workers, especially Teuscher and Ludwig, both in this group and in other Echinoderms. He entirely ignores the fact that blood-vascular and water-vascular systems, consisting respectively of oral rings and radial vessels, have been described as distinct and totally independent in Asterids, Ophiurids, and Crinoids, apart from Teuscher's work on the Urchins. He continually speaks of the ambulacral vessels as being "double," meaning that both of them belong to what Perrier called the respiratory portion of the vascular system, that, namely, which communicates with the exterior by the water-tube. The so-called absorbent portion of the vascular system consists of the intestinal vessels, the ventral one of which is connected with the superior oral ring.

Koehler's position, therefore, involves the curious anomaly that Echinus has two oral rings, both containing blood, but the one is "absorbent" and the other "respiratory;" while there are also two sets of radial vessels, both, however, belonging to the respiratory system, and connected exclusively with one ring (water-vascular, auct); whereas in Spatangus, as he himself describes, each oral ring is connected with a corresponding set of radial vessels. This is also the case in all the other Echinoderms; and I cannot but think that the great difficulties involved by the presence of the lantern in Echinus may have caused the connection of the smaller radial vessels with the superior oral ring to have escaped Mons. Koehler's notice. He gives no figure showing their union with the water-vessels proper before joining the inferior or water-vascular ring, which suggests the possibility that the supposed union is an inference and not an observed fact. But if an inference was necessary, it would surely have been more natural to suppose that a character which has been described by four different observers in Asterids, Ophiurids, Crinoids, and Spatangids, is also to be found in Echinus.

The next point to be considered is the connection of the so-called heart or ovoid gland with the vascular system, which has been so positively denied by Perrier. The glandular canal discovered by Koehler which rises from the superior oral ring

(blood-vascular, auct) is frequently described by him as continued upwards through the ovoid gland by means of its excretory canal to the level of the madreporite. He says, for example, that it is "en communication avec la glande ovoïde et lui permet de reçevoir le sang en assez grande quantité." Further on he speaks of "l'interposition sur un certain point du trajet des vaisseaux d'un organe glandulaire, destiné sans doute à débarrasser le sang de produits inutiles et à les laisser s'échapper au dehors à travers la plaque madréporique."[1] There can, therefore, be no question about the intimate relation of this organ with the blood-vascular system of the Urchins, just as in other Echinoderms, as described by Ludwig, Teuscher, myself, and others.

This relation, however, has been denied by Perrier and Apostolidès, who suppose the ovoid gland not only to open by its excretory duct into the sinus beneath the madreporite, as described by Koehler, but also to be entirely independent of any vessels whatever. Their observations on Asterids and Ophiurids are in direct conflict with those of Ludwig; but Koehler's discoveries in the Urchins furnish a strong argument in favour of Ludwig's views.

The minute structure of the ovoid gland in Urchins and Spatangids is briefly described by Koehler in the following terms:—"A reticulum of connective tissue, supporting cellular elements that undergo a peculiar degeneration, the final result of which is the formation of numerous pigment masses." This network of connective-tissue fibres is most regular near the periphery of the gland, the fibres being more numerous and also better defined towards the centre. It encloses alveolar spaces, within which are groups of from one to four naked masses of protoplasm, of an irregular stellate form. Each contains a nucleus, which is of variable size. It occurs in all stages, from a finely granular condition to one in which it is little else than a mass of brown pigment-granules, surrounded by a thin protoplasmic envelope, and in the peripheral region the alveoli sometimes contain nothing but these pigment groups.

[1] Op. cit., pp. 72, 102

These cells were supposed by Perrier, and also by Apostolidès, in the case of the Ophiurids, to be grouped into columnar acini, as in the case of compact glands like the liver. Koehler was at first inclined to take the same view of their arrangement, but subsequent investigations led him to give the description of the structure of this organ, which has been summarised above. Several so-called lacunæ are visible in sections through the gland. Some of them correspond to the numerous anastomosing canaliculi which unite into its excretory duct. But others, especially those in the peripheral and lowest parts of the gland, are filled with coagulum. These represent the lumina of the vessels which ramify on its surface, and are connected with the "glandular canal" rising from the oral blood-vascular ring. The structure of the upper part of this canal is essentially similar to that of the gland itself; but towards the peristome the trabecular structure becomes less marked, and lacunar spaces lined by an epithelium relatively more prominent. Various transitional stages may be found between the cellular elements of the ovoid gland and the epithelial cells lining the lower portion of the glandular canal, and Koehler regards the former as a modification of the latter.

The glandular canal and parts of the ovoid gland of the Urchins thus consist of numerous small vessels with an epithelial lining, while the cells of other parts are more irregularly arranged among fibres of connective tissue. This is not so very different from Ludwig's account of the same organ in Asterids and Ophiurids, the correctness of which has been questioned by Perrier and Apostolidès; and it would seem, therefore, that the designation "central plexus" is not so very inapplicable after all. "Plexiform gland" would, perhaps, be better; but any appellations based upon its form, such as "ovoid gland" (Perrier) or "piriform gland" (Apostolidès), are inconvenient when applied to other types of Echinoderms, such as the Crinoids, in which the so-called ovoid gland breaks up into a number of loosely-connected lobules. The organ in question is most certainly not a heart, and may very probably have something to do with the production of the brown

pigment-bodies, which are so familiar to all workers on Echinoderms.

Koehler makes little reference to the observations of Geddes,[1] who found amœboid corpuscles containing brown pigment-granules in the perivisceral fluid, and considered them as respiratory in character. These cannot be very different from the pigmented "cellules à protoplasma irrégulier émettant de fins prolongements," which Koehler found in his osmic-acid preparations of the ovoid gland; while the larger pigment masses, devoid of a nucleus but surrounded by a thin protoplasmic envelope, which Koehler found in the ovoid gland, seem to have been also met with by Geddes in the intestinal vessels. He also describes them as occurring at certain times in the ambulacral vesicles; and he speaks of these yellow-brown granules as being developed at the expense of the nuclei of their epithelial lining, which recalls the relation of similar granules to the nuclei of cells derived from the epithelium of the so-called glandular canal described by Koehler. The Polian vesicles have essentially the same structure as the "ovoid gland," and are evidently the source of many of these pigmented cells. The colouring matter of the latter contains iron, and undergoes changes of tint when exposed to the atmosphere, so that it is doubtless of a respiratory nature, as supposed by Geddes, though Foettinger's discovery[2] of hæmoglobin-tinted corpuscles within the water-vessels must not be forgotten.

Considering that the production of these pigment granules involves the destruction of the cells of the ovoid gland and Polian vesicles, Koehler regards these organs as excretory in function, as had been previously done by those who denied the connection of the ovoid gland with the vascular system, though positively asserting its communication with the exterior by a duct. It is likely enough that this organ is one of the factories of respiratory pigment, the necessary material reaching it

[1] "Observations sur le fluide périviscéral des Oursins," 'Arch. de zool. exp. et. gén., t. viii, pp. 483—496, pls. xxxvii, xxxviii.

[2] "Sur l'existence de l'hémoglobine chez les Échinodermes," 'Arch. de Biol.,' vol. i, pp. 405—412, pl. xvii

from the oral blood-vascular ring through the "glandular canal," and the fluid being forced on by the contractions of the ventral intestinal vessel which joins the oral ring. One would wish, however, for something more than the mere assertion of Koehler that a liquid is secreted by the gland. He describes a coagulum found in its excretory duct as the "coagulated secretion" of the organ.[1] But he finds the same coagulum in the vessels ramifying on the surface of its lower portion and in the internal vessel of the intestine.[2] If the one is a glandular secretion, why not the other too? They are both described in exactly the same terms, and are evidently of the same nature as the coagulum so often found in the blood-vessels of other Echinoderms. I have met with it over and over again in the radial, and also in the intervisceral vessels of Crinoids. Is this a coagulated secretion too? Not even Professor Perrier himself, who has recently published a brief note[3] "On the Organisation of Crinoids," has ventured to assert that the so-called ovoid gland of these creatures communicates with the exterior, though he will not admit the existence of any intervisceral vessels connected with it. But as regards the Echinozoa, I would still ask for proofs, based not merely on the results of injection, but also on the section method, that the "excretory duct" of the ovoid gland communicates with the exterior, and not with an aboral ring in which the genital vessels originate, as described by Ludwig in the Stellerids. The direct communication with the exterior of the blood-vascular as well as of the water-vascular system would, if established beyond dispute, be a somewhat important morphological fact; and before accepting the apparently well-grounded conclusions of Koehler and Perrier respecting the regular Urchins, one would like to know what Ludwig has to say on the subject. This will appear, I hope, ere many months are past.

[1] Op. cit., pp. 98, 99.
[2] Op. cit. pp. 77, 90.
[3] 'Comptes rendus,' t. xcvii, No. 3, pp. 187—189.

In the Spatangids, as in the Urchins, Hoffmann[1] was only able to find one vascular ring around the mouth, that of the ambulacral system. He described the ventral vessel as not originating in this ring, but as communicating with it by a special connecting branch; while he was unable to differentiate the water-tube from the so-called "heart," of which, however, he recognised the glandular nature.

Teuscher[2] satisfied himself of the existence of another vessel than the water-tube at the side of the gullet, but failed to make out its connections in the peristome. It corresponds to the "glandular canal" of Echinus. Working downwards from the madreporite, he found the "heart" or gland with the water-tube close to it. The latter is readily recognisable by its lining of columnar epithelium, and lies closer to the gland than in Echinus. At the lowest part of the gland, where the water-tube dilates slightly, Teuscher finds "seine innere dem Herzen anliegende Wand immer stark verdünnt. . . . Der Steincanal nachdem er am Herzen vorübergegangen ist, wird von einem oder zwei feinen Gefässen begleitet." It then passes downwards underneath the diverticulum to reach the gullet. There is some obscurity in this description, as there also is in that given by Koehler, but the two partially interpret one another. In Spatangus, as in Echinus, Koehler found two vascular rings round the mouth, each with radial extensions into the ambulacra. But he describes the communicating branch from the internal marginal vessel as connected with both rings. "Simple sur presque toute sa longueur, elle se bifurque à son extrémité et chacune des deux branches se jette dans un des cercles peribuccaux." This is improbable, to say the least of it, for in no other Echinoderm are the visceral vessels known to communicate with the water-vascular ring. Doubtless, however, it does not surprise Koehler, who does not distinguish between blood-vascular and water-vascular systems. But as this point is important, one regrets that he

[1] "Zur Anatomie der Echinen und Spatangen," 'Niederl. Arch. de Zool.,' Bd. i, pp. 10—112, Taf. iii—x.

[2] Loc cit., pp. 531—534.

did not illustrate it by another of his admirable figures. In any case, however, he is to be credited with the discovery of the blood-vascular ring in Spatangus, though not regarding it in the same way as most other authors would

In accordance with his peculiar views he described the water-tube as double along the whole length of the gullet, meaning that there are two canals, as stated by Teuscher. One of these, that farther from the gullet, is somewat irregular and sinuous in form, being lined by large cells with voluminous granular nuclei and masses of pigment, while the more uniform vessel, closer to the gullet, is lined by small epithelial cells. "Au point où l'œsophage se termine, le canal sinueux s'amincit peu à peu et cesse d'être distinct; il se confond avec le deuxième canal qui reste dès lors unique et continue son chemin jusqu'à l'extrémité de la courbure inférieure et de la jusqu'à l'organe d'excrétion." After the disappearance of this sinuous pigmented canal the more uniform one remains of the same character until it reaches the diverticulum. Its lumen then becomes divided up by partitions, and its cellular lining consists of larger elements with granular nuclei, so that it passes gradually into the so-called excretory organ. Koehler speaks of it as the water-tube[1], not only communicating with the gland, but in perfect structural continuity with it; and it is doubtless the one described by Teuscher as the water-tube between the gland and the gullet. But I much doubt its really belonging to the water-vascular system; and it appears to me to correspond to the "glandular canal" of Echinus, which likewise connects the gland with the oral blood-vascular ring.

Towards the apical extremity of the gland, where its characteristic parenchyma becomes less developed, two special canals differentiate themselves. 1. The "madreporic canal," more centrally placed, often containing coagulum, and the first to appear at the tapering upper end of the gland, which it resembles in structure. 2. A more peripheral one lined by a regular columnar epithelium. The glandular tissue finally

[1] Op. cit., pp. 92—99.

disappears, and these two canals pass up side by side towards the apical pole.

The results of Koehler's injections lead him to identify the second canal with one or two canals which pass from the surface of the gland on to its supporting mesentery, where they lose themselves in a large and irregular network in the interstices of the connective tissue. According to Koehler, therefore, the two vessels which together make up the stone-canal at the level of the gullet, each communicating with one of the peribuccal rings, soon fuse into a single canal that terminates in a gland placed at the extremity of the diverticulum, while the intestinal vessel communicates through its connecting branch with both the peribuccal rings. "The fusion of the two systems is thus as complete as possible, and it would be difficult to admit the existence of a distinction between a water-vascular and a blood-vascular system.

To this I would remark: (1) The fact that each peribuccal ring sends a separate branch into the ambulacra, does not look like a more complete fusion of the two systems than exists in Echinus; where Koehler, though I believe wrongly, describes both the radial vessels as communicating only with the water-vascular ring. He cannot surely mean that each ring in Spatangus communicates with both the radial vessels. As regards the connecting branch joining both the peribuccal rings, I should wish, as I have pointed out above, for more distinct proof than a mere assertion.

(2) Koehler regards the organ which is commonly called the stone-canal of Spatangus, as homologous with the glandular canal of Echinus, on account of its relations to the excretory gland. It appears to me, however, that Teuscher's identification of the canal with columnar epithelium, found by him at the apical pole, as the water-tube, is more correct than Koehler's view of what is evidently the same structure. A canal of this kind would not be likely to end in a vascular network in the mesentery; and I cannot but think that Koehler, who does not mention Teuscher's opinion, is here in error. Both authors agree in its peripheral position as

regards the gland, and also that it is not continuous, independently of the "glandular canal" with either of the two canals arising from the peribuccal rings. I will assume, though Koehler nowhere says so, that the more sinuous pigmented canal which "disappears" higher up, starts from the water-vascular ring; while the other, which eventually becomes glandular, is connected with the blood-vascular ring as in Echinus. If this be the case, it would seem that the Spatangids, like the Crinoids, and possibly also the Ophiurids, have an interrupted water-tube arising from the water-vascular ring; though indirect communication between the two is effected by the body-cavity into which both tubes open by their inner ends. This seems to me much more probable and in better accordance with the morphology of Echinoderms generally than the position taken up by Koehler. According to him the "madreporic canal" of Spatangus is to be considered as the water-tube, though it lacks the characteristic columnar epithelium. It is further comparable in every respect, as Koehler himself admits, to the so-called excretory duct of the ovoid gland in Echinus, and therefore, also in other regular Echinoderms. In both Asterids and Ophiurids this "excretory duct" is said by Ludwig to join an aboral ring in which the genital vessels arise; and certain points in Koehler's memoir lead me to think that the same may be the case in the Urchins. Various facts mentioned by Hoffmann, Perrier, Teuscher, and Koehler, more especially by the two latter, who are the only ones to figure sections of the ambulacra, also indicate that the system of perihæmal spaces originally derived from the cœlom, which have been described under various names in the Asterids and Ophiurids, really occur in the Urchins too. But on this point, as on many others, we shall doubtless be enlightened by Ludwig himself.

I have already alluded to the recently published note by Professor Perrier, "On the organisation of Crinoids." He, of course, uses the somewhat inappropriate term "ovoid gland" in speaking of the central plexus, but says nothing whatever about its having any communication with the exterior. This is not very surprising, for the water-pores of a Crinoid

are scattered about over the ventral surface of the disc, and not collected into a single plate as in most Echinozoa. He does not seem to believe in the connection of the central plexus with the intervisceral blood-vessels, which has been described by Ludwig and myself. For he says, "Les vaisseaux qui paraissent en partir ne sont autre chose que les ramifications de la glande, se terminant d'ordinaire par des renflements ayant l'aspect de culs-de-sac. Ces ramifications courent au milieu des innombrables trabécules du tissu conjonctif de la cavité générale, qui peuvent eux-mêmes parfois prendre l'apparence de vaisseaux." In the early larva, the central plexus is "un corps fusiforme plein, allant du cercle oral au pédoncule dont il continue le cordon axial. Ce corps n'emet aucune ramification: il ne saurait par conséquent être question à ce moment d'appareil vasculaire. Le corps ovoïde s'implante chez la Comatule adulte sur l'un des planchers horizontaux de l'organe cloisonné."

Professor Perrier does not definitely name the species which has afforded him material for his observations. But, as he says that they have principally been made on young individuals, and on Pentacrinoid larvæ, it is tolerably evident that the common Ant. rosacea of the English Channel and the Mediterranean has been the subject of his researches. I do not know whether he has ever made preparations of Ant. eschrichti, or of any species of Actinometra or Pentacrinus. But unless he has done so, he appears to me to be somewhat rash in denying the conclusions reached by other workers who have had these opportunities, on the strength of observations made on a single species.

Some years ago Professor Perrier, who had worked by one method only, was led not only to deny the existence of a particular canal in the arms of Ant. rosacea, which had been described by Dr. Carpenter, but also to predict that no one else would find it. It has, however, been described by Greeff, Teuscher, Ludwig, myself, and finally by Perrier himself; and I cannot help suspecting, therefore, that he has been again misled by the limited nature of his observations. The mere

fact that any one fails to demonstrate the existence of a certain anatomical structure is no proof of the non-existence of that structure. Two instances already mentioned, viz. the connection of the ovoid gland in the Urchins with an oral ring, and the existence of both cœliac and subtentacular canals in the arms of Crinoids are cases in point. Another, which will be noticed immediately in more detail, is that Professor Perrier, working with young and fresh material, has seen the connection between branches from the axial cords of the arms and the muscle-fibres, which I have long sought for in vain in spirit specimens.

Both Ludwig and myself have found that certain anatomical points are not easily demonstrated in Ant. rosacea, whereas they are much more evident in the larger Ant. eschrichti. I have five series of sections through the disc of this species; and have also cut four other species of Antedon, four of Actinometra, and three of Pentacrinus, together with Promachocrinus, Rhizocrinus, and Bathycrinus. I venture to think, therefore, that I am on the whole in a better position than Prof. Perrier for forming a judgment respecting the anatomical relations of the central plexus. In one respect, however, my opportunities have been inferior to his. All my work has been done on material which has been some time in spirit; and though this affects anatomical structure but little, it makes a vast amount of difference in histological work. Prof. Perrier, on the other, hand has had access to an abundant supply of fresh Ant. rosacea of all ages; and his statement that the histological structure of the "ovoid gland" of this type is identical with that of the same organ in other Echinoderms[1] must therefore be received as authoritative. But when he says that the intervisceral blood-vessels described by Ludwig and myself as originating in this organ are merely ramifications of the gland ending in apparently blind dilatations, I must totally disagree with him. That the walls of the central plexus are of a glandular nature must be apparent to everyone who has examined a section of it. But I have also

[1] As described by himself, Apostolidès, or Koehler?

no doubt whatever as to the connection of its cavities with those of the chambered organ, and through it, with the vascular axis of the stem in stalked Crinoids. Ludwig[1] has given excellent figures in illustration of these points; and my own observations have repeatedly demonstrated their accuracy, not only in Ant. rosacea, but in other genera and species. The chambered organ is an enlargement at the top of the vascular axis of the larval stem, which Perrier describes as continuous with the ovoid gland. But he nowhere mentions the vessels contained in this axis which expand into the cavities of the chambered organ above, just as in the stalked Crinoids; and he does not appear to consider these chambers as part of any blood-vascular system. If, as he seems to imply, they are disconnected from the ovoid gland in the adult, how does he explain the connection of the latter with the axis of the larval stem?

It will be noted that Prof. Perrier tacitly admits the connection of apparently vascular structures with the ovoid gland, though he speaks of them as its ramifications and as seemingly blind. I am well aware of their apparent blindness, but it is simply due to the impossibility of any single section showing more than a very small portion of their winding course. This is a difficulty familiar to all workers. But a careful study of a good dissection, or of a moderately thick transparent section, especially with a binocular, or an accurate plotting out on paper of a series of thin sections by means of a camera, will reveal much that is totally unrecognisable in other ways. The diagrammatic figures which I have given of transverse and longitudinal sections through the disc of Actinometra[2] were made by thus plotting out with a camera.

The intervisceral blood-vessels of this and other types have no glandular structure whatever, as they should on Professor Perrier's theory. They are simple tubes as described by Ludwig, and lined by an epithelium which is more delicate than that within the extensions of the body-cavity into the

[1] 'Zeitschr. f. wiss. Zool., Bd. xxviii, Taf. xiv—xviii.

[2] This Journal, vol. xxi, Pl. xii, figs. 14, 15.

arms. They often contain coagulum, and with a little practice may be readily distinguished from connective tissue.

Professor Perrier gives us hardly any information respecting the relations of the upper end of the ovoid gland. He admits its connection with an oral ring in the Pentacrinoid, but he does not say whether the functions of this ring are water-vascular, blood-vascular, or the two combined; and when he says that the gland gives off no ramifications, he must have forgotten Dr. Carpenter's description of its subdivision "into diverging branches, of which one passes to each ray." This is perhaps a stage which has not come under Professor Perrier's observation. The radial branches of the ovoid gland develope into the genital vessels which form a plexus beneath the ambulacra of the disc, and eventually extend into the arms and pinnules. Professor Perrier does not mention this plexus, though it cannot well have escaped his notice; nor does he enter at all into the question of the ventral termination of the ovoid gland in the adult. I regret his silence the more, as this is especially a point on which more extended observations are wanted. Both Ludwig and myself have experienced considerable difficulty with Ant. rosacea; but I have found A. Eschrichti, A. carinata, Pentacrinus decorus, and Promachocrinus kerguelenensis much more favorable subjects of study. In the first-named species the ventral branches of the central plexus end in a spongy organ with well-defined limits, which has somewhat the appearance of a lymphatic gland. It is especially developed between the mouth and anus, and is connected both with the oral blood-vascular ring and with the genital vessels of the rays. An organ of essentially the same character, though less prominent, occurs in Ant. rosacea; and I had hoped for some account of it from Professor Perrier, who, unfortunately, does not mention it. I trust, however, that in the complete memoir which he is preparing he will remedy this omission. Meanwhile I am searching for this spongy organ in as many different species as possible, and

[1] 'Proc. Roy. Soc.,' vol. xxiv, p. 221.

propose eventually to describe the comparative anatomy of this portion of the vascular system in the various types of Crinoids. At present, I would emphasise two points strongly, viz. the connection of the central plexus with the oral ring and genital vessels above, and with the vascular axis of the stem at its other end, which does not communicate with the exterior, as the corresponding (?) part of the ovoid gland is said to do in the Echinozoa.

It will be evident from what has been written above that, so far as the vascular system is concerned, I am inclined to adopt Ludwig's views rather than those held by Professor Perrier and his colleagues. The French author, however, is returning good for evil, and sides with me in one, if not both, of the two cardinal points wherein I disagree with Ludwig, viz. the nervous system of the Crinoids, and the homologues of their basal plates in Starfishes. It is with the first of these questions only that I am now concerned. For the past six years I have been continually advocating my father's view respecting the nervous nature of the fibrillar envelope of the chambered organ of the Crinoids, and its extensions into the rays and arms. Ludwig, however, expressed his total dissent from this doctrine; and it has consequently been ignored or dismissed with the briefest possible mention in the various German text-books on comparative anatomy. I have reason to believe that a few teachers have assented to it; but, so far as I know, Professor Perrier is the first continental worker on Echinoderms who has publicly adopted it. This is the more important, as he formerly expressed his inability to do so; and he has been able to strengthen it in two important points. For he has not only seen the ultimate branches of the axial cords, which altogether escaped the notice of the German observers, but he has also traced a connection between some of them and the muscle-fibres through the intervention of stellate cells; while he has followed others into the tentacles, and describes them as entering the papillæ borne by these organs. I have lately found that these ramifications of the axial cord occur in the stem of Pentacrinus and Bathycrinus, and that they are greatly

developed in the arms of the latter, bipolar cells being intercalated in their course.

I have also seen these bipolar cells in Ant. Eschrichti, which type has further yielded me another important piece of evidence. The existence of a fibrillar plexus, derived from the axial cord, within the connective tissue forming the perisome at the sides of the brachial ambulacra has long been known to me. But until about a year ago I searched in vain for any similar structure on the disc. At last, however, I succeeded in following this plexus from the arm-bases down on to the disc. It is extensively developed among the sacculi at the sides of the ambulacra, and forms an annular network in the connective tissue occupying the lip, but of course much further from the mouth than the subepithelial ring discovered by Ludwig. I detected this plexus first in a specimen which had been stained with borax-carmine, and subsequently found it also, though less readily visible, in hæmatoxylin preparations. Last of all, on looking over the remains of my earliest sections, made in Professor Semper's laboratory at Würzburg in the winter of 1875-76, and stained with Beale's carmine, I was able to make out traces of the same parambulacral network which had originally escaped my notice.[1] I have likewise found it in the disc of A. antarctica, and even of A. rosacea; and I have no doubt that the action of gold-chloride or osmic acid on fresh material would bring it out in a more striking manner.

I am strongly inclined to believe that extensions of this plexus are in direct connection with the fibrils of the subepithelial band, which is regarded by Ludwig as the sole nervous apparatus in the Crinoid organisation. In fact, some histologists who have seen my preparations have expressed themselves as having no doubt that this is the case. I hope, however, to obtain some still better sections than those upon which this opinion was based before finally adopting it as my own. In

[1] Preparations illustrating this structure were exhibited at the meeting of the Zoological Society on December 19th, 1882, and will be figured in the "Challenger" report.

the minute details of the disc structure of the Crinoids there is still very much to be worked out; and having plenty of material I trust in the course of time to be able to add considerably to our present knowledge, both of the nervous and of the vascular systems.

Recent Researches upon the Origin of the Sexual Cells in Hydroids.[1] A Review.

By

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The question as to the place of origin of the sexual products of Hydroids is one upon which very various opinions have of late years been current. The ectoderm and endoderm have, in turn, been put forward as giving rise to either, or both, eggs and spermatozoa.

Kleinenberg, in speaking of Hydra, and F. E. Schultze, of Cordylophora, state that both products are derived from the ectoderm, a result with which Weissmann agrees.

Grobben has observed the same origin in Podocoryne carnea, and F. E. Schultze in Sarsia tubulosa. The Hertwigs have shown the same ectodermal origin of both elements in numerous medusæ; and lastly, Ciamician has shown the same origin in Tubularians, Weissmann having arrived at the same conclusions even before Ciamician's publications.

On the other hand Weissmann has clearly demonstrated both products to have an endodermic origin in Plumularia, Sertularella, and Eudendrium.

[1] Weissmann, A. "Observations sur les cellules Sexuelles des Hydroides." 'Bibliothèque de l'école des Hautes Etudes, Section des Sciences Naturelles,' xxiv, No. 3.

Varenne, A. De. "Sur l'origine des spermatozoides chez les Hydraires," 'Comptes Rendus,' xciii, pp. 1032—1034. "Développement de l'œuf de la Podocoryne carnea," 'Comptes Rendus,' xciv, pp. 892—894. 'Recherches sur les Polypes hydraires, (Reproduction et Développement),' Paris, 1882, 8vo, (104 pp., 10 pls.).

The spermatozoa have been stated in some cases to arise from the ectoderm while the ova arise from the endoderm. E. von Beneden has shown this to be the case in Hydractinia, Fraipont in Campanularia, and Weissmann in Gonothyræa. These various modes of origin have been described as existing in the same family.

Weissmann's recent observations advance our knowledge a considerable step in a slightly different direction; he has shown that there are a large number of species, of genera, and even of families, in which the generative products do not originate in reproductive individuals—gonophores—but in the parenchyma of the trophosome, the cœnenchyme of Milne Edwards and Haime, the cœnosarc of Allmann, and that they afterwards migrate to a "maturing bud" (gonangium). Such an origin Weissmann terms cœnosarcal, in contradistinction to a blastoidal origin; and he would recognise two types of Hydroids—cœnogenous (cœnosarcogenous), and blastogenous.

The whole process especially with regard to the formation of the gonangium and the migration of the sexual elements into it is a remarkable one, and we may cite Weissmann's observations upon Plumularia echinulata (Lam.).

Both varieties of sexual cells form in the endoderm of the cœnosarc, usually in the trunk of the colony, often at the base of the lateral branches. At the time that the sexual cells appear, there is no trace of gonangia. These form in the cœnosarc in the neighbourhood of sexual cells. The sexual cells arise in a similar manner in both sexes, ova and spermatospores (sperm mother-cells) arising by metamorphosis of ordinary endoderm cells: this Weissmann has observed with great certainty.

The gonangia develope with perfect regularity from below, upwards, so that we can determine beforehand the precise spot where a gonangium will later on develope.

Where a gonangium is about to develope the first change takes place in the ectoderm, which in the trunk consists of several layers of cells.

The cells of the outer layers which have primitively an irregular polygonal form become elongated and placed perpendicularly to the plane of the basal lamella, at the same time changing in character, losing their granules and becoming clear. Such a modification takes place just above a group of sexual cells.

The modified ectodermal cells now form a rounded tubercle which becomes nipped at the base by a circular groove and will now penetrate the perisarc. The perisarc in fact is gradually eaten away by a chemical action. That there is no mechanical pushing and gradual thinning out of its substance can be seen by tracing the parallel striæ which stop short at the edges of the window which is formed. This eating away of the perisarc is a most remarkable process. The perisarc is a chitinous substance and only dissolves with the greatest difficulty in concentrated acids or alkalies.

Experimenting upon Plumularia echinulata, Weissmann found that the perisarc entirely resists the action of sulphuric and hydrochloric acids for five days, as well as that of caustic potash: it did, however, dissolve in the latter at the end of a month.

Numerous organic bodies resist the action of strong acids or alkalies while they are attacked by weaker solutions, Weissmann has therefore tried all stages of dilution. In a ·1 per cent. solution of potash the perisarc had not, however, completely dissolved at the end of a fortnight.

There is a further curious point; even while these cells are dissolving the outer layers of perisarc they do not attack the youngest (innermost) layer which they push before them. This young layer, which Weissmann terms the cambium layer, present differences both chemical and physical from the older layers, it stains more strongly and more readily with carmine,[1] and, moreover, is soft and elastic, as may be seen during the

[1] I have observed a similar difference in the chitinous layers of the lens of the central eye of a young Limulus, one portion although here occupying not an internal but a central position, stains deeply when treated with borax-carmine while the remainder remains unstained.

further growth of the gonangium. After the formation of this circular window in the perisarc, the ectodermic tubercle passes outwards, and the endoderm commences to grow into its lumen and to line its walls.

The gonangium is now ready for the penetration of the sexual cells. The entrance is partly passive, the result of displacement and growth, but partly active, resulting from the actual migration of the ova or spermatospores.

The time of the descent of the sexual cells is not the same in both sexes. In the males this takes place when the endoderm sends its prolongation into the ectodermic tubercle, the mass of spermatospores then glides slowly upwards towards the opening in the perisarc. In the females the corresponding movement often only takes place somewhat later.

In certain gonangia the peripheral portion retires from the perisarc, and the cellular mass of a gonangium separates as a blastostyle, properly so called, upon which develope the true gonophores. While the blastostyle is growing in length there forms at the spot where the ovules are, a cul-de-sac, which becomes a gonophore; the latter separates more or less from the blastostyle, and, finally, remains attached by a short pedicle only.

In the interior of the gonophore the endoderm growing more rapidly than the ectoderm becomes plicated, and the ovules come to lie in the niches so formed.

As soon as the eggs arrive at maturity fertilisation takes place, and at the same time the endodermic tube slowly retires from the gonophore at its extremity, and during the maturity of the first gonophore a second is formed. Whence come the ova in the second gonophore? Are they derived from the cœnosarc or from the blastostyle? Weissmann supports the latter hypothesis. A third gonophore may be formed, but such rarely happens.

Weissmann describes similar processes in Plumularia setacea and Sertularella polyzonias and S. gayi.

In Gonothyræa Loveni Weissmann states that the sexual cells do not arise in the cœnosarc, but in the gonophores.

Weissmann also describes the process in Eudendrium ramosum where the sexual cells are cœnosarcal in origin.

It is important to note that in no case have sexual cells been seen to arise in the hydranth, but always in the trunk of the colony.

De Varenne has come independently to somewhat similar conclusions, but he goes further and describes a cœnosarcal origin where Weissmann had not observed it.

He shows that in all the forms which he has studied Campanularia flexuosa, Plumularia echinulata, Sertularia pumila, Gonothyræa Loveni, Podocoryne carnea and Obelia geniculata, the ova and spermatozoa alike develope in the cœnosarc of the trophosome, and, moreover, originate from endodermal cells, whether they are matured in fixed sporosacs, in medusoids which remain attached (Gonothyræa Loveni), or in medusoids which have a free existence; whether they remain in obvious connection with the endoderm or migrate so as apparently to lie in the ectoderm.

De Varenne believes that previous observers who have put forward the ectoderm as the place of origin of the generative products of either sex have been misled by the fact that in many cases, although actually originating as stated above, such cells migrate even after they have reached the gonangium. The endoderm reforms itself beneath the migrated ova or spermatozoa, a new homogeneous membrane (Stützlamella) is secreted by the newly-formed endodermal cells, which might easily be mistaken for the original structureless lamella, the latter as well as the ectodermic layer having become reduced on account of the pressure exercised by the developing egg or spermatozoa to an extremely thin layer, which, however, remains outside the sexual products.

It has been usual to consider the gonophores, whether these remain fixed (sporosacs) or become free-swimming medusoids, as the sexual persons, the trophosome polyps being asexual persons, but if the observations above recorded are true and should be proved to have the universal application that De Varenne seems to consider they have, the view now usually

held that there is a true alternation of generations among the gymnoblastic and calyptoblastic hydroids must be abandoned. Asexual buds and sexual generative products both arise in the trophosome, the latter, however, become collected into specialised buds which may, to the obvious advantage of the species, become detached and actively locomotor, and even acquire a higher organisation than the fixed trophosome.

On the Osteology and Development of Syngnathus Peckianus (Storer).

By

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With Plates XLII and XLIII.

It is now some time since I began the study of Syngnathus, recognising the fact that little, if anything, had been done towards elucidating many points in the anatomy and development of the grotesque group of the Lophobranchs. Other matters have prevented as rapid work, and as close application to the subject in hand, as would have been wished for, and even now the work is exceedingly incomplete. Nevertheless it has seemed to me to be wise to publish the observations as far as completed, inasmuch as I have been able to throw some light on certain points, and to correct some mistakes made by authors who have preceded me.

The material for study was obtained at Beaufort, N.C., where it was quite abundant among the seaweed near the shore. The presence of the brood-pouch on the under surface of the postabdominal region of the male renders the collection of young stages comparatively easy, but the intermediate stages between the newly hatched young and the adult were less easily obtainable, and I have been unable to bridge over this gap in my observations. The material under observation may be divided into five stages, the relative characters of which may be described as follows:

A. Length 3—4 mm.: cartilages not quite fully formed.

B. Length 6—7 mm.; cartilages fully formed; considerable amount of yolk still present.

C. Length 8—9 mm.; yolk-sac very much diminished in size.

D. Length 10—11 mm.; yolk completely absorbed; ready to leave the brood-pouch.

E. Adult.

I. Development and Structure of the Cranium.

In Stage A (Pl. XLII, fig. 1) the head is round and small, with the lower jaw bent up, and closely applied to the under and anterior surface of the cranium, there being as yet no differentiation of the upper jaw and snout. Below the medulla oblongata (M. O.) the notochord (N.C.) bends abruptly downwards, and becomes ensheathed on either side by the parachordal cartilages, which are continued forward to unite with the trabeculæ cranii (Tr. Cr.), the extremity of which is somewhat bent upwards, and as yet, as it were, within the cranium, reaching no farther forwards than a point between the eyes. The auditory capsules (Au.) are formed in cartilage, and are apparently united to the parachordals, but possess no rudiment of the semicircular canals, though two otoliths are plainly visible.

In Stage B (fig. 2) the snout makes its appearance, but it is as yet bent upwards, and lies closely applied to the front of the cranium, owing to the upward flexion of the trabeculæ mentioned above, which has increased considerably. At their coalesced extremities, and articulating with their sides, is on either side a cartilage (E. Pa.), whose signification will be discussed when treating of the visceral arches. When the skull in this stage is viewed from above (fig. 4), the nares (Ol.) may be seen lying on the upper surface of the snout, slightly anterior to the eyes. They have a thickened margin, and behind them, extending down from the centre towards the sides of the snout or rostrum, are two cartilaginous bars (Na. C.),

while above these is a cartilaginous plate (Tg. Cr.) extending upwards to between the eyes, and produced to a point between the nares, forming the tegmen cranii. This and the two nasal rods have apparently no connection at present with the coalesced trabeculæ, but are apparently independent cartilages: the tissue lying between the nares and the extremity of the trabeculæ is somewhat fibrous. The auditory region is now more fully developed, and the semicircular canals are represented by fibrous bands. The parachordals posteriorly have extended upwards and around the nervous cord to form the occipital region of the skull.

Stages C and D are very slightly different. They present an increase in size from Stage B, and the fibrous semicircular canals have become cartilaginous. The snout has increased somewhat in length in C, and still more in Stage D, the growth taking place at first by an increase in length of the horizontal portion of the cartilaginous "rostral plate," as the elongated coalesced trabeculæ may be called, and, latterly, by a straightening out of its bent up end, which even in Stage C (fig. 5) forms a right angle with the horizontal portion. The posterior region of the skull is almost completely encased in cartilage, its very summit only being of dense membrane; more anteriorly the membrane becomes thinner, and extends further down the sides of the skull.

At the base of the skull, and extending from its posterior portion forward for a considerable distance in the median line, is a dense membrane (fig. 15, Pa. S.), identical in appearance with that roofing in the cranial cavity, and also with the membrane surrounding the notochord at this stage: this is the commencement of the formation of a bone, which from its position must be the parasphenoid. At the sides of the vertex of the anterior portion of the cranium proper, and extending back nearly to its posterior portion, are two similar condensations of tissue (Fr. S.). They consist, as is seen on section, of plates lying in the connective tissue enclosing the cranial cavity, one on either side, and at the centre of each plate and perpendicular to it a ridge passes along its entire

length, projecting out into the integument. Still more posteriorly only one such plate of membrane is seen, which occupies a median position at the vertex. These appear to be the membranous rudiments of the frontals and dermo-supra-occipital.

The Adult.—In the adult cranium the cartilage persists to a great extent, although surrounded almost completely by bone. No enchondroses, as in the higher vertebrates, appear; ectostoses and parostoses form the cranium. One point noticeable at the first glance is the elongation of the occipital regions, and the compactness of the region immediately behind the orbits; separating these two portions there is a membranous space, closed in by the parasphenoid.

The occipital region ossifies below as the basi-occiptal (fig. 9, B. O.). Posteriorly this is round, forming an articular facet for the first vertebra, but anteriorly it becomes flattened out, and expanded into a thin plate—being, in fact, fan-shaped. Posteriorly, on section, the rapidly diminishing notochord is plainly visible, and on either side of this are the parachordal cartilages. More anteriorly the chorda does not appear, and the parachordals unite to form a single plate. Below it is deeply grooved for the reception of the parasphenoid, which underlies it, and is almost enclosed by it. Laterally it articulates with the exoccipitals, and more anteriorly with the pterotics.

The exoccipitals (Ex. O.) are well-developed bones, forming the postero-lateral floor of the skull, and extending somewhat upwards upon its sides to articulate with the epiotics, and in front with the pterotics. Above them on either side is a parosteal bone (fig. 8, S. Tp.), upon whose homologies I am undecided. I am inclined, however, to consider it homologous, to a certain extent at any rate, with the supra-temporal of Amia.

Above, at the vertex of the skull, is the supra-occipital (S. O.), a large bone, extending forward nearly to the sphenotics. It appears to consist of two portions; (1) a parostosis, which may be termed the dermo-supra-occipital, and which de-

velops apparently in a manner similar to what occurs in the placoid scales of the body, from the membranous plate already spoken of; and (2) an ectosteal portion, which may be termed the auto-supra-occipital. The supra-occipital is somewhat narrowed in front, projecting between the posterior portions of the frontals.

Laterally from this bone we have on either side the parietals (Pa.), small bones which do not meet in a sagittal suture, but are separated throughout their entire length by the supra-occipitals, as in the pike and the salmon. Anteriorly they articulate with the frontals. The name dermo-epiotics, which Bridge has suggested for these bones in Amia,[1] is here very applicable, for they directly overlie the epiotics, appearing like a parostosis formed upon these bones.

The pterotics (Pt. O.) extend far forward to a point just behind the sphenotics, and form, along with the pro-otics and sphenotics, the articulating surface for the hyomandibular. They descend pretty well to the base of the skull, their posterior parts overlapping the anterior lateral portion of the basi-occipital, and articulating with the exoccipitals. They form the lateral boundaries of the membranous space in front of that bone. Anteriorly they articulate with the pro-otics.

These bones (fig. 9, Pr. O.) bound the membranous space in front, and extend forward nearly to the anterior limit of the postorbital region of the cranium. They extend only slightly upwards on the sides of the skull to articulate with the pterotics and sphenotics, and unite across the middle line of the skull anteriorly, but are separated posteriorly, leaving a space closed in only by the parasphenoid. At the sides in this region are the sphenotics (Sp. O.). There seem to be no distinct alisphenoids, the part of the skull having the usual relations of these bones being ossified by the pro-otics. There is apparently also no basisphenoid.

The frontals (fig. 8, Fr.) are membrane bones of comparatively large size, extending from the parietals posteriorly to

[1] T. W. Bridge, "The Cranial Osteology of Amia calva," 'Journ. of Anat. and Phys.,' vol. xi, 1877.

slightly in front of the ectethmoids anteriorly. They are somewhat club-shaped, broadening out posteriorly. On viewing them from the surface they appear to be unsymmetrical, one forming a projection which fits into a corresponding indentation on the other. This want of symmetry is apparent rather than real, for a section (fig. 11, Fr.) shows it to be caused by an overlapping, the portion of bone overlapped being equal to that which overlaps it. Opposite the sphenotics, from the inner surface of each frontal, a process passes down, which articulates with the front edge of the ascending or pro otic process of the parasphenoid.

The parasphenoid (fig. 9, Pas.) is a long parostosis, extending from the basi-occipital nearly to the anterior extremity of the cranium on its under surface. Behind it is round, and almost enclosed in the basi-occipital, lying in a deep groove in that bone. More anteriorly it widens out to form a partial floor for the membranous space in front of the basi-occipital, the cartilaginous trabeculæ lying immediately above it. At the anterior limit of the postorbital region of the skull (fig. 10) it sends up on either side a process, somewhat triangular in shape, when viewed laterally. These articulate with the pro-otics, and along their anterior edges with the descending processes of the frontals. These processes may be termed the ascending or pro-otic processes of the parasphenoid. The space bounded laterally by these two bars, above by the frontals, and below by the parasphenoid, serves for the passage of the orbital muscles and nerves. Anterior to this, in the orbital region, the parasphenoid becomes rectangular, and finally triangular (fig. 11, Pa. S.); the apex being directed upwards, having attached to it the lower edge of the interorbital membrane. In front of the orbit it articulates on either side with an ectethmoid, and still more anteriorly (fig. 12) becomes convex, being deeply grooved on the under surface, in which groove lies the vomer (Vo.), almost enclosed, and presenting a similar appearance to the parasphenoid when lying in the groove in the basi-occipital.

There is no orbitosphenoid, the passage for the orbital muscles and nerves being very large, appearing, in fact, almost

as if the whole anterior wall of the cranial cavity were wanting. The interorbital septum (fig. 11, I. S.) is merely membranous, as in the Siluroids and Cyprinoids.

Immediately in front of the orbits, on either side, is an ectethmoid (fig. 8, Ect. E.), the ossification of the preorbital process of the ethmoidal cartilage. They extend down the sides of that cartilage, and articulate below with the parasphenoid, and above with the frontals.

At this point or slightly anterior the frontals terminate, the remainder of the rostrum being formed by the ethmoid, with the vomer lying along its under surface. There appear to be no membrane bones occupying the position of the nasals of most fishes.

The ethmoid (fig. 12, Eth.) consists posteriorly largely of cartilage surrounded by a certain amount of ectosteal bone. In the cartilage, on either side, is a canal, in which the olfactory nerves and vessels run, passing to the olfactory capsules, which form deep indentations in the sides of the cartilage. Immediately in front of the orbits the cartilage appears to consist of two portions, an upper, originally the tegmen cranii, and a lower, the coalesced trabeculæ, and between these the olfactory nerves and vessels at first run. This distinctness of the two parts obtains, however, only for a short distance, the two halves soon uniting and becoming indistinguishable, the olfactory structures becoming enclosed in a canal. In front of the olfactory organs the cartilaginous part of the ethmoid rapidly diminishes, there being a nearly corresponding increase in bone. In this a canal appears, which passes towards the surface as one traces it forwards. I take this to be the continuation of the main slime canals, which also traverses the frontals. Anteriorly the ethmoid becomes thinner, but remains nearly of the same breadth, and its cartilaginous portion entirely disappears; still more anteriorly (fig. 13) it becomes almost scale-like, the vomer (Vo.), which hitherto has been round and small, now becoming larger and triangular, and forming the greater part of the thickness of the rostrum in this region, and this relation persists to its extremity.

Summary.—The most noticeable feature in the configura, tion of the embryonic cranium is the bending up of the facial portion against the front of the skull, this being due to the bending up of the coalesced trabeculæ cranii at their extremity. As development proceeds this bending up does not diminish-but the mouth becomes carried forward by the growth of the horizontal portion of the trabeculæ, and it is not until Stage D is arrived at that the elongation of the snout is dependent upon the straightening out of the rostral cartilage.

In the adult the first thing to be noticed is the forward extension of the occipital region and the compactness of the pro-otic region. The absence of any cartilaginous sphenoid bone, the wide opening for the passage of the orbital muscles and nerves consequent upon this, the absence of an osseous inter-orbital septum as in the Siluroids and Cyprinoids, the want of nasals, and the structure of the ethmoids, are also points of considerable importance.

II. The Visceral Skeleton.

In the youngest stage observed (A) most of the visceral arches were apparently fully formed, and consisted of seven cartilaginous bars, some, however, being more differentiated than others, the first three already showing a specialisation into their future parts.

The first postoral or mandibular arch consists, on either side, of a well-developed mandibular portion (fig. 1, Mck.), bounding the gape below, extending forwards and upwards, and curving slightly inwards towards its fellow of the opposite side. Articulating with the proximal extremity of each of these Meckelian cartilages is a single rod-shaped portion (Pt. Qu.), extending backwards and slightly upwards, but lying quite free in the tissue of the prævertebral portion of the skull, except for the articulation with Meckel's cartilage. Subsequent development shows this to be the rudiment of the pterygo-quadrate portion of the jaw, and hence it may be denominated the pterygo-quadrate cartilage.

The second postoral or hyomandibular arch is represented by

a curved rod (H. M.), extending from the skull downwards and forwards towards the proximal extremity of Meckel's cartilage, with which it appears to articulate. Anteriorly it lies below, and almost parallel to, the pterygo-quadrate cartilage, and becomes somewhat broader posteriorly. It presents as yet no differentiation into the hyomandibular and symplectic portions, to which this segment of the arch corresponds, nor in fact does it so separate afterwards, the line of division between the two being indicated merely by the articulation of the second segment of the arch, i. e. hyoid portion. This consists at present of a rod lying below, and inclined towards, the upper cartilage, which it meets a little in front of the dilated portion.

The remaining four arches constitute the branchial arches (Brs.), present at this early stage to the same number as in the adult. No azygos, median hyoid cartilage could be detected at this stage, nor is there a median branchial cartilage.

In Stage B (figs. 2 and 3) the mandible has become bent up upon the skull, and the pterygo-quadrate cartilage retains the same characters as in the preceding stage. The hyomandibular has, however, undergone some modifications. It has become distinctly angulated, the symplectic portion (Sym.) extending forward parallel with the axis of the skull, while the hyomandibular (H. M.) moiety is bent up almost at right angles to it, slightly posterior to the eye, and articulates with the lateral under-surface of the auditory capsule. Near the anterior border of this portion is an oval foramen, and at the angle of junction with the symplectic a separate cartilaginous centre appears, uniting the hyoid to the hyomandibular portion of the arch, and representing the interhyal. The hyoid arch (Hy.) extends from this downwards towards the median line, and represents the combined ceratohyals and hypohyals. About half way from the extremity of each portion of it is a process extending upwards towards the symplectic, which is notched to receive it. A median hyoid rod (G. H.) is now easily seen, extending from in front of the hyoid cartilages back to the branchial region. It is not quite straight, but curves slightly to the side, and at the junction of the hyoid and hyomandibular

turns abruptly upwards towards the base of the skull, parallel to the first branchial arch. This is probably the genio- or basihyal.

The branchial arches (Brs.) remain the same practically as in the last stage, and throughout show very little differentiation. Another cartilage, however, makes its appearance at this stage, which, though not at present connected with the visceral cartilages, eventually unites with them. It consists (fig. 2, E. Pa.), on either side, of a cartilaginous rod, articulating with the sides of the rostral cartilage, near its anterior extremity, and extends backwards and slightly downwards. It is the commencement of the ethmopalatine cartilage.

The principal change to be noted in Stage c (fig. 5) is the growth of the pterygo-quadrate cartilage. It has now grown upwards and expanded at the extremity with an anterior and posterior process. The anterior is clearly connected with the ethmo-palatine by a band of connective tissue, and represents the pterygoid portion, while the posterior one has no connections, but probably is the future metapterygoid. Another point is clearly noticeable at this stage, which helps in no small degree to indicate the homologies of the cartilages. The symplectic does not meet the basal portion of Meckel's cartilage; the only cartilage articulating with this being the quadrate portion of the pterygo-quadrate. In previous stages this meeting and articulation seems to exist, or, at all events, the two cartilages are almost in contact, but now their want of union can be clearly seen.

The growth of the pterygo-quadrate constitutes again the most noticeable feature when we examine Stage D. In this it is seen that the pterygoid process has grown so far forward, and the ethmopalatine so far backward, that they are now separated only by a very small portion of connective tissue. The growth has been mainly, however, on the part of the pterygoid process. The metapterygoid process has grown backwards only a short distance, and at this stage does not form a buttress to the hyomandibular, as in the salmon. In the preceding stage, but more clearly to be seen in this, one can

notice on section a dense, somewhat irregularly-shaped membrane (fig. 15, Inf. O. S.) lying on the outer surface of the symplectic. It appears to correspond to a membrane bone in the adult, which I have named the infra-orbital.

It will be well to compare the cartilages of the young Syngnathus with those of a typical Teleost, and for this purpose no better choice can be made than the salmon, which has been so admirably worked out by Prof. Parker.[1]

On comparing Parker's fifth stage with my Stage D, the resemblance will at once be seen. The hyomandibular and symplectic elements are not separated. The former is broad and stout, tapering towards the point of articulation with the interhyal, and a little below this there is a slight bend. In Syngnathus the bend is greater and a little further back, being exactly at the point when the interhyal articulates, i. e., at the point where the two elements meet; and with this greater angulation there is a consequent elongation of the symplectic element. The quadrate element in the Salmon presents the same relations as in Syngnathus, but its metapterygoid portion is much larger, and lies upon the symplectic, forming a buttress to it, while the pterygoid process is shorter.

The relations, however, of the pterygoid and palatine portions will be more readily recognized on examining an earlier stage of the Salmon. It will then be seen that the palatine portion is originally distinct from the pterygo-quadrate, consisting of a rod bounding the gape above, and extending from the trabeculæ almost to the angle of the mouth. Anteriorly it is large and stout, tapering gradually posteriorly, being in fact club-shaped. This is denominated by Parker the second visceral arch, and evidently is the same as the cartilage I have described as the ethmopalatine in Syngnathus.

In Clarias capensis the palatine or ethmopalatine rod is longer than in the Salmon, and overlaps the pterygoid,[2]

[1] W. K. Parker, "On the Structure and Development of the Skull in the Salmon," 'Phil. Trans.,' 1873.

[2] W. K. Parker, "On the Structure of the Skull in Sharks and Skates," 'Trans. Zool. Soc.,' 1878.

while in a young Eel (Anguillula acutirostris) it is entirely wanting.[1] We can thus from these four types construct a scale, passing upward from Anguillula with no ethmopalatine to Syngnathus with a comparatively small one, then to Salmo, in which it is more developed, and finally to Clarias, in which it extends back to overlap the pterygoid. The splint bone, which corresponds to and ensheaths this cartilage, is the maxillary; and I regret that I have been unable to collect data from which conclusions as to the relative extent of the gape in these forms might be drawn.

Interesting comparisons can also be made between Stage D in Syngnathus and the cartilaginous skull and arches of Acipenser. In this the relative angulation of the hyomandibular is the same, and at the same point as in Syngnathus; the symplectic runs horizontally forward, but is not quite so long, and a very strong resemblance obtains between the pterygo-quadrate in the two forms. In Acipenser, as in Syngnathus, this is represented by a hammer-shaped cartilage, the basal portion or handle corresponding to the quadrate, the anterior process of the head to the pterygoid, and the posterior to the metapterygoid, which does not pass back to form a buttress to the hyomandibular. The ethmopalatine seems to be wanting.[2]

The phylogenetic significance of the teleostean ethmopalatine is apparently doubtful. Parker and Bridge considered it a structure with no representative in the Selachian jaw, while Balfour[3] points out the possibility of its being "an element, primitively belonging to the upper arcade of the mandibular arch, which has become secondarily independent in its development." This suggestion I do not think tenable, and would prefer to side with the later view of Parker and of Marshall, that it represents a præoral visceral arch, to which the lachrymal cleft and the third nerve correspond.

[1] Ibid.

[2] This description has been taken from fig. 241, in Gegenbaur's 'Elements of Comparative Anatomy,' London, 1878.

[3] F. M. Balfour, 'Comparative Embryology,' vol. ii, p. 478, London, 1881.

The only literature referring in any important way to the development of the Lophobranchii which I have been able to find is confined to three papers; of these, Calberla's[1] treats of a much earlier stage than I have been able to study, and de Quatrefages'[2] is of little or no importance, leaving only J. A. Ryder's[3] from which I could obtain any information Dr. Ryder's observations were unfortunately limited to a single specimen of Hippocampus antiquorum, which had just left the brood-pouch, and corresponds almost to my Stage D, being very slightly older. From the rather peculiar arrangement of the mandibular skeleton at this stage no little difficulty would no doubt be experienced in determining the homologies of the cartilages from a single specimen, since it is only by tracing their development that one can be certain of the signification of abnormalities. Accordingly, in Ryder's paper, there are certain statements which I am convinced are errors, partly of observation and partly of mistaken homology.

These remarks apply especially to his description of the mandibular and hyoid arches. On comparing Dr. Ryder's figure with my drawing of the cartilages in Stage C (fig. 5) it will be seen that what I have represented merely as a foramen in the hyomandibular portion of the second postoral arch is in his figure pourtrayed as a dividing line, completely separating the hyomandibular cartilage into two parallel portions, the anterior of which he terms the metapterygoid. He was dealing with Hippocampus antiquorum, while my observations were made on Syngnathus; but these two forms are very closely allied, and the arrangement of the cartilages are apparently identical, so that a statement made regarding one is no doubt tenable for the other. Accordingly, since the develop-

[1] E. Calberla, "Zur Entw. des Medullarrohres u. d. Chorda dorsalis der Teleostier u. d. Petromyzonten," 'Morph. Jahrb.,' iii, 1879.

[2] A. de Quatrefages, "Memoire sur les Syngnathus," 'Ann. des Sci. Nat..' 1842.

[3] J. A. Ryder, "A Contribution to the Development and Morphology of the Lophobranchiates (Hippocampus antiquorum, the Sea Horse)," 'Bull. U.S. Fish Comm.,' 1881.

ment shows the metapterygoid at this stage to be an entirely different cartilage, and since, by repeated observations, I have satisfied myself of the absence at this point of a chondrification distinct from the hyomandibular, and of the existence of an elliptical foramen, which, being covered by a band of muscular tissue, might easily be mistaken for a line of separation, I take exception to Ryder's identification.

Having thus started on the wrong path, the homologies continue to be erroneous. Thus the distal horizontal portion of the hyomandibular arch is termed the quadrate, whereas, from its relations and from the evident absence of articulation between it and Meckel's cartilage, it must be the symplectic.

But it is in the homologies of the two upper cartilages of the arch that Dr. Ryder errs chiefly. He says: "Above the articulation of the quadrate (i. e. the symplectic of my figures) with Meckel's cartilage a curious bent element (*x*) appears to represent the superior maxillary. Just in front of the expanded upper extremity of the maxillary lies the posterior extremity of the upper labial or intermaxillary element (*la*), which is continuous with a similar piece on the opposite side; this intermaxillary bar curves over the anterior upward bend of the rostral cartilage (*r. c.*). It contributes the skeletal boundary of the upper part of the oral opening (*m'*), and is not segmented in the median line, so as to articulate with its fellow of the opposite side, like Meckel's cartilage of the lower jaw." Now, in the first place, I differ from him in regard to his assertion that the intermaxillary bar, as he calls it, curves over the rostral cartilage. In Syngnathus these bars, as seen from a surface view (fig. 4), certainly articulate with the sides of the rostral cartilage, though when viewed from the side the turned-up extremity of the latter gives at first sight an impression of their continuity across the front of the skull. In the second place I differ as to the identification of the cartilages. The terms "maxillary" and "intermaxillary" are misnomers, the bones so denominated in the adult being, without exception, membrane bones; and further, the development of the lower cartilage, and the fact that it is the only

cartilage which articulates with the mandibular, shows conclusively that it can be no other than the pterygo-quadrate.

Other discrepancies between Ryder's paper and my own, such as the transverse segmentation of the hyomandibular arch, the existence of a distinct symplectic (which probably is my geniohyal), and the division of the hyoid element into cerato- and hypohyal portions, will be noticed at once on comparison, and certain of them may be due to the difference in the age of the forms compared.

Adult.—I must here repeat the statement made previously that intermediate stages between D and the adult were not obtained. As a consequence some of the points in the following description are merely conjectures. There is a certain amount of complication, owing to the excessive elongation of the symplectic, and the presence of membrane bones, which study of the intermediate stages can alone satisfactorily unravel.

The hyomandibular articulates with the skull immediately behind the orbit, the articular surface being afforded it apparently by the sphenotic, pro-otic, and pterotic. Its direction is perpendicular to the longitudinal axis of the skull, and it is composed of a central cartilage surrounded by ectosteal bone (fig. 10, H. M.).

Below it articulates with the interhyal (I. H.), a somewhat triangular ossicle, in which the original cartilage still persists. This forms the connecting link between the hyomandibular and hyoid element proper. The latter (Hy.) consists apparently of two portions, a cerato- and hypohyal, the epihyal being apparently absent. The elements of either side approach each other in the median line below, and are moveable upon the interhyal. Two muscles, inserted by a single tendon, pass from their distal extremities backwards, and anteriorly they are connected with the mandible. When the muscle is in a state of repose the hyoids are bent up on the under surface of the skull, and lie between the symplectics ; by its contraction their extremities are drawn downwards, and consequent upon this there is a similar downward move-

ment of the mandible, and at the same time an enlargement of the buccal cavity, whereby water for respiration is drawn in. These bones form consequently an important portion of the respiratory pump, acting as it were the parts of handles, whereby the force of the muscles is transmitted to the mandible and to the buccal cavity. The geniohyoid element does not appear to ossify and in fact has disappeared.

Extending forwards horizontally from the hyomandibular almost to the extremity of the elongated snout, is the symplectic, the cartilages of the two portions of the arch being continuous. Posteriorly (fig. 11, Sym.) the bone is oval in shape, and consists mainly of the original cartilaginous bar, the osseous portion being small. In the anterior portion of the orbital region, however, the latter becomes greater in proportion to the cartilage, and the bone assumes a hammer shaped appearance on section, the two heads of the hammer articulating with a membrane bone afterwards to be described, and enclosing with it a space. The handle is directed upwards towards the orbit. In the nasal region (fig. 12, Sym.) the handle has extended upwards to articulate with a membrane bone bounding the nasal cavity below, and the hammer shape has entirely disappeared. Anteriorly (fig. 13, Sym.) the symplectic becomes completely surrounded by the quadrate (Qu.), and its cartilage becomes entirely absorbed, its position being indicated by a round foramen in the section.

The quadrate (Qu.) extends backwards to the region of the nasal capsules. It then consists (fig. 12) of a small oval portion lying below the symplectic. Anteriorly (fig. 13), however, it enlarges and grows upwards on the outer side of that bone, and finally completely encloses it as mentioned above. The cartilage is entirely absent from the bone posteriorly, and it is only in its most anterior part that it is present.

Lying outside the symplectic, and slightly bending round so as to bound it below, there is to be seen in the orbital region a membrane bone (Inf. Or.). It bounds the orbit below, and corresponds to a certain extent with a bone, or a series of bones, in other Teleosts, which usually receive the name of infra-

orbitals. To this bone I propose to give the same name, although it is much larger, and extends farther forward, leaving the orbital region altogether, and thus loses to a certain extent the claim to the name. It is largest in the orbital region (fig. 11), and then constitutes the outer bone of the snout; more anteriorly (fig. 12), however, it becomes, as it were, thrown down towards the under surface of the snout, and gradually becomes smaller, terminating slightly in front of the posterior extremity of the quadrate.

Appearing in the series of transverse sections[1] in the nasal region, about the same time as the quadrate, is a bone (fig. 12, Mpt.), which I am inclined to refer to the pterygoid series, probably the metapterygoid. Posteriorly, as in the bones above described, no trace of cartilage is present, but near its anterior extremity it appears, and is there seen to be continuous with that of the quadrate, so that the ectosteal bone would correspond to the posterior process of the pterygo-quadrate cartilage of the young stages. It passes forward as far as the mouth, becoming gradually smaller anteriorly, just as the quadrate becomes broader. Posteriorly it articulates with the upper portion of the symplectic in its broad region. It has the appearance of a scale, separated from the symplectic by a quantity of muscular tissue. At first sight one would not be inclined to identify this bone as the metapterygoid, on account of its great separation from the hyomandibular, since it is generally described in connection with that bone to which it usually forms a buttress. From its origin, however, one would be rather inclined to imagine it having a much closer relation with the quadrate than the hyomandibular, which belongs to the succeeding arch, and this we find to be the case here. The great elongation of the symplectic has carried the quadrate far forward, and the metapterygoid has accompanied that bone, and lost all connection with the hyomandibular.

Overlapping the lower portion of the under surface of the

[1] This series, consisting of 165 sections, was prepared by Giesbrecht's shellac method, the object being first decalcified by a 3 per-cent. solution of HCl in 96 per cent. alcohol, and then stained in toto with Bismarck brown.

infra-orbital posteriorly, and the quadrate for some distance anteriorly, is, on either side, a scale-like bone (figs. 12 and 13, M.), which has no special representative in other Teleosts, and is merely a membrane bone formed in the dense integument closing in the buccal cavity below.

The anterior process of the pterygo-quadrate cartilage ossifies as the pterygoid, its cartilage apparently passing forward to become continuous with the ethmo-palatine. Anterior to the pterygoid bone, bounding the mouth above, is the palatine, an ectosteal bone developed upon the ethmo-palatine cartilage, and on the outer side of this is a splint-bone, which bounds the gape above, and which, from its relation to the ethmo-palatine cartilage, must be the maxillary. The premaxillæ appear to be wanting, the maxillæ bounding the gape.

My sections of the mandibular region not being very good, I am unable to make any statements concerning the ectosteal bones of the mandible. The opercular bones are not present in as great numbers as in typical Teleosts. There is a very large operculum, somewhat scale-like and convex outwardly, which articulates with the hyomandibular. The præoperculum, a very constant bone in the Teleostei, here appears to be absent, or at any rate very rudimentary. A membranous suboperculum bounds the operculum below, and is continued up behind it as far as the spiracular branchial cleft; there is no interoperculum. The outer surface of all bones upon the surface is beautifully sculptured, some of the thinner ones presenting an elegant fenestrated appearance.

Summary.—In the first place, the great elongation of the symplectic is very noticeable, and as a consequence there is a wide separation of the hyomandibular and metapterygoid elements. The ethmo-palatine articulates with the sides of the anterior extremity of the ethmoid or rostral cartilage, and grows backwards to unite with the pterygoid process of the pterygo-quadrate. In the adult the elongation of the posterior mandibular region, and the concentration of the anterior portion, are well marked. The great elongation backwards of the metapterygoid and quadrate bones, the absence of any

apposition of the former to the hyomandibular, the abortion of the geniohyoid, the absence of the intermaxillaries and the preopercula, are the most important points.

During the past few years, chiefly through the investigations of Parker, Balfour, Gegenbaur, and Marshall, much light has been thrown upon the segmentation of the skull and the relations of the visceral arches. There has been an increasing tendency to refer the various subcranial arcades to the category of visceral arches, and to increase the number of segments which have become coalesced to form the cranium.

It is now well recognised that all the visceral arches behind the mouth, including the mandibular and hyoid arches as such, are merely the modification of a series of cartilaginous bars situated in the walls of the pharynx, and originally supporting branchiæ. The posterior visceral arches, to the number of five, increasing to six or seven in the Notidani, and diminishing to four in Syngnathus, still retain this original function, but it is not so with the two anterior postoral arches; these have become modified to subserve other purposes, and have lost to a large extent their original structure and appearance. The distribution of cranial nerves to these, however, resembles that of the branchial arches, the trigeminal being referable to the mandibular, and the facial and the auditory (which from embryological facts may be considered as one) to the hyoid arcade and the adjacent structures. The visceral clefts point to the same conclusion. Each visceral arch bounds posteriorly a visceral cleft; so for the hyoid arch we have the spiracular cleft, and for the mandibular the mouth, since Dohrn's late researches[1] show that this is formed by the coalescence of two hypoblastic outgrowths, the median opening only forming later. From the relations of the head cavities one would deduce the same conclusions. Balfour, who has worked them out very thoroughly in the Elasmobranchs, thus speaks of them:[2]—"As the rudiments of

[1] A. Dohrn, "Studien zur Urgeschichte des Wirbelthierkörpers," 'Mith. a d. Zool. Station zu Neaple,' Bd. iii, 1882.

[2] F. M. Balfour, loc. cit., p. 558. See also 'Monograph of Elasmobranch. Fishes.'

the successive visceral clefts are formed, the posterior part of the head-cavity becomes divided into successive sections, there being one section for each arch. Thus, the whole head-cavity becomes on each side divided into—(1) a præmandibular section; (2) a mandibular section; (3) a hyoid section; (4) sections in each of the branchial arches."

The question arises—How many are the præoral visceral arches, and what are their relations to the cranial nerves, visceral clefts, and head-cavities?

If we consider Amphioxus as an ancestral type for the vertebrates, we find that originally there were no præoral visceral arches, and the Ascidians point to the same fact. Accordingly one must suppose that the anterior cleft, which, functioned as the mouth in the ancestral forms, gradually lost that use, its function being as gradually assumed by succeeding arches, since we have strong grounds for supposing that there are in the Craniota arches in front of the mouth. As to the number of these arches we have several theories—some maintaining that there is only one, others deciding for two, and others for several. As far as our present knowledge goes, I consider that we are entitled to recognise two.

The authorities upon this subject are by no means few; from them the views of Parker and Marshall may be cited as illustrating what has been done in this line. Parker, in his paper on the skull of the Salmon,[1] seems inclined to accept the trabeculæ cranii as a præoral arch, but later rejects this theory. He says:—"When we consider . . . . that the walls of the forepart of the cranium are formed by growth from the trabeculæ, just as posteriorly the walls are formed by growth from the parachordals; that nerves are similarly emitted through the trabecular and occipital walls; when it is seen, in short, that the trabeculæ are neural in their relations, as completely as, and in similar fashion to, the parachordals, it seems impossible to resist the conclusion that the trabeculæ and the para-

[1] W. K. Parker, "On the Structure and Development of the Skull in the Salmon," 'Phil. Trans.,' 1873.

chordals must be placed in one and the same category."[1] Still later, he speaks for the presence of three præoral arches, which he names epipterygoid, ethmo-palatine, and prorhinal.[2]

Marshall's view is that only two arches are represented in the præoral region, namely, the ethmo-palatine, or lachrymal, and the olfactory.[3]

Upon what grounds is it possible to base conclusions as to this point? It seems to me that before we can come to any definite conclusion as to whether a structure is really a visceral arch, we must be able to show the presence or rudiments of an arch, a cleft, a nerve supplying that arch, and a head-cavity. Let us now apply these tests.

In front of the trigeminal nerve which supplies the mandibular arch, we have the olfactory, optic, oculomotor, patheticus; and the abducens may also be enumerated here, as it has not yet been accounted for. It has been shown by Marshall and others that of these the optic, patheticus, and abducens are probably not entitled to be ranked in the same category as the other cranial nerves, leaving only the oculomotories (iii) and olfactory (i) to be accounted for. The former is referable to the region of the ethmo-palatine cartilage, and we have also a cleft, the lachrymal, and a head-cavity, the præmandibular, the walls of which, according to Marshall,[4] become transformed with the superior, inferior, and internal recti, and inferior oblique muscles of the eye, to which the third cranial nerve acts as a supply.

All the necessary parts are present, then, for forming a firm basis on which the conclusion as to the validity of the ethmo-palatine or lachrymal arch may be vested. The olfactory arch is, however, much more complicated.

[1] Parker and Bettany. 'On the Morphology of the Skull,' London, 1877.

[2] W. K. Parker, "On the Evolution of the Vertebrates," Hunterian Lectures, 'Nature,' vol. xx, 1879.

[3] A. Milnes Marshall, "The Morphology of the Vertebrate Olfactory Organ," 'Quart. Journ. Micr. Sci.,' vol. xix, 1879.

[4] A. Milnes Marshall, "On the Head-cavities and associated Nerves of Elasmobranchs," 'Quart. Journ. Mic. Sci.,' vol. xxi, 1881.

Marshall's observations are apparently conclusive as to the segmental nature of the olfactory nerve, and the homology of the Schneiderian membrane of the olfactory capsules with the gills of the posterior arches. Dohrn's[1] researches on the pituitary body of fishes tends to refer this structure also to a visceral cleft, and there is a union between it and the nasal cavity in Petromyzon, which, however, Dohrn states is merely secondary. We have here, then, a nerve, and probably a cleft, but the remaining structures are apparently absent. As to the head-cavities in Elasmobranchs, there is only one præmandibular; and in Teleosts Ganin states[2] that this is the only one present, evidently showing that there is a tendency for these structures to disappear; and we may conjecture that there was originally a second præmandibular cavity, which has disappeared even in the Elasmobranchs.

The visceral arch corresponding to this segment, I am inclined to think, is represented by the trabeculæ cranii. The lowest type of Vertebrate presents no prolongation of the cerebral nervous system beyond the extremity of the notochord. The portion in the higher vertebrates anterior to that point is merely an overgrowth, and we must consider the pituitary region as corresponding to the extremity of the under-surface of the brain in Amphioxus. Accordingly we have a præ-vertebral portion of the cranium which is supported by the coalesced trabeculæ cranii. These structures at first are two cartilaginous bars, articulating with the extremities of the parachordals, and extending down parallel to the posterior visceral arches. Eventually they become bent upwards so as to run parallel with the long axis of the skull, unite anteriorly, and finally send up lateral outgrowths to form the side wall of the anterior region of the skull. At first they present no differences in appearance or relations from the visceral arches. They are curved so as to approximate below, and articulate with the vertebral region of the skull. This represents the

[1] A. Dohrn, loc. cit. See also 'Zool. Anz.,' vol. v, 1882.

[2] Ganin, "Ueb. die Entw. des Kopfskelets bei Knochenfische (Rhodens Gasterosteus)," 'Zool. Anz.,' No. 51.

first stage in the phylogenetic history of the group. The second stage of union is also well marked, and it is not until comparatively late in life that the commencement of the third stage, represented by the beginning of the lateral upward growth, makes it appearance; and it may be explained by the function the trabeculæ have in the adult of supporting the facial region, and protecting the olfactory sense organs. This specialization is only a degree greater than that found, for instance, in the second postoral arch, where a longitudinal division occurs, the posterior rod forming the hyoid, for the support of the tongue and aiding in the respiratory process, and the anterior one the symplectic, uniting the mandible to the skull, and serving for its support.

The relation, too, of these trabeculæ to the pituitary fossa is also important. They articulate with the vertebral region of the skull immediately behind that structure, and, in fact, have the same relation to it as the other arches have to their clefts. By being bent up, and by the atrophy of the pituitary cleft, they eventually lose their original relations, and enclose them instead of being their posterior boundaries.

The following table, which is a modification of Marshall's table,[1] itself a modification of one by Balfour,[2] will show at a glance the relations which I consider probable as existing between the various segments. As in Marshall's table, clefts, &c., have been allowed for the Notidani.

[1] A. Milnes Marshall, "The Morphology of the Vertebrate Olfactory Organ," 'Quart. Journ. Micr. Sci.,' vol. xix, 1879.

[2] F. M. Balfour, "On the Development of Elasmobranch Fishes," 'Journ. of Anat. and Phys.,' vol. xi. See also 'Monogr. of Elasmobranchii.'

Branchial Clefts.	Visceral Arches.	Head-cavities.	Cranial Nerves.
Pituitary or olfactory.	Trabeculæ cranii.	Aborted (?).	Olfactory.
Lachrymal.	Ethmo-palatine.	Præmandibular.	Oculomotor.
Mouth.	Mandibular.	Mandibular.	Trigeminal.
Spiracular.	Hyomandibular.	Hyoid.	Facial and Auditory
1st visceral.	1st Branchial.	1st Branchial.	Glossopharyngeal.
2nd ,,	2nd ,,	2nd ,,	1st Branch of Vagus
3rd ,,	3rd ,,	3rd ,,	2nd ,, ,,
4th ,,	4th ,,	4th ,,	3rd ,, ,,
5th ,,	5th ,,	5th ,,	4th ,, ,,
6th ,,	6th ,,	6th ,,	5th ,, ,,
7th ,,	7th ,,	7th ,,	6th ,, ,,

The position of the second arch articulating with the anterior extremity of the trabeculæ appears at first an insuperable objection to this theory; but when we consider that it is possible that concomitantly with the bending up of the trabeculæ and the overgrowth of the cerebrum there was a carrying forwards of this arch without any bending up, a way out of the difficulty is presented. The fact that nerves pass out through openings in the walls of the skull formed by the trabeculæ does not appear to be of any weight against the theory, since it is well known that when in the process of extension cartilage meets with a nerve or blood-vessel instead of contracting and closing or interfering with the action of that structure, a wide foramen is left through which it may pass freely. In favour of the theory this may be said, that it reduces to a natural group structures which, viewed in any other light, are exceedingly abnormal, and is what one might expect from what occurs in the persisting ancestral forms.[1]

III. The Notochord and Vertebral Column.

In Stage A (fig. 1) the notochord in the cranium is ensheathed by the parachordal cartilages. Below the medulla oblongata it bends abruptly downwards at an angle of 45°.

[1] By this expression I mean Amphioxus and Ascidians, which, though probably not in the direct genealogical line, may still be recognised as types of the direct ancestors.

Posteriorly at the tail it is somewhat bent upwards, and tapers abruptly to a point, so that the tail-fin is at this stage heterocercal.

In Stage B, on transverse section, a very tough fibrous membrana limitans externa is seen, having in fact exactly the same appearance as the membrane, in which eventually calcic material is deposited to form the parasphenoid. No cells were to be distinguished in it as described by Götte. Within this is the notochordal sheath, apparently cellular, as if the cells of the notochord forming it had not had their protoplasm displaced. In older stages a membrane similar to the membrana limitans externa forms round the spinal cord, and lateral condensations of tissue form the rudiments of the transverse processes. In the membrana limitans externa ossific matter becomes deposited, and similar depositions take place in the other membranes, resulting in the formation of the vertebral centra, and spinous and transverse processes.

In the adult the vertebræ are of the ordinary amphicœlous type, about 2 mm. in length, and presenting little variation in appearance. The anterior ones are slightly modified, chiefly in the transverse and spinous processes. In the first the latter structures are expanded at their extremities, having a wing-like shape; the spinous process is ridge-like, produced into a process posteriorly. The anterior face of the centrum is very much enlarged to form an articulating surface for the occiput, while the posterior is the same size as in the succeeding vertebræ. From the posterior edge of the base of each neural arch, a zygapophysis is given off, which passes directly backwards to articulate with the second vertebra, and serves to strengthen the articulation.

In the second vertebra there are both anterior and posterior zygapophyses, and in the third posterior ones only. In the succeeding vertebræ no zygapophyses are present; the transverse processes are straight, somewhat flattened, projecting out at right angles to the hour-glass-shaped body. The spinous processes vary slightly for some distance back, having a ridge-like appearance, and extending the whole length of the ver-

tebra. The canal for the spinal cord is larger than that structure; immediately above the cord a partition (fig. 14), downwardly pointed in the middle, projecting with the dorsal fissure of the cord, divides the canal into two portions, the lower of which contains the central nervous system. The notochord persists to a large extent. There are no ribs.

A very close relation exists between the neural and pleural processes and the dermal scales. These structures first make their appearance as stout, tough, membranous plates, having the same appearance as has been described for the membrane plates in the roof of the cranium. In these membranes ossification takes place, apparently concomitantly with that of the neural and transverse processes, and the relations found in the adult commence to show themselves. In a transverse section (fig. 14) there is to be seen, astride, as it were, of the spinous process, a plate—the dorsal scale (D. S.), and articulating with the transverse processes very closely; there is, on either side, a corresponding lateral scale (L. S.).

These latter apparently take the place of ribs, acting as protective structures to the organs within.

IV. The Paired and Unpaired Fins.

In Syngnathus the fins present are a fairly developed dorsal, weak pectorals, and a caudal. In the young stages an anal is present, which, however, does not pass beyond the stage in which the fibrillation begins, but aborts, and is entirely wanting in the adult.

In Stage B (fig. 2) the dorsal fin consists of cartilaginous rays embedded in the tissue of the body, and resting directly on the membrane surrounding the spinal cord. The portion of the fin outside the body is yet cellular, but a distinct fibrillation is present, which, increasing, results in the formation of the horny rays.

In Stage D (fig. 6) these have fully developed, their supporting rays (I.C.) still being cartilaginous. Above, these are united by a longitudinal bar, similar to what occurs in the paired fins of

Elasmobranchs.[1] This is apparently a secondary formation due to the coalescence of the extremities, since in earlier examples it is not present. Resting on this longitudinal bar, opposite the intervals between the cartilaginous rays, are oval cartilaginous nodules (B.C.), each one of whichs upports a horny ray (H. R.). These do not present any transverse segmentation To each of these nodules are attached two muscles, a posterior smaller one (Dep. M.), which by its contraction depresses the ray, and an anterior (Er. M.), attached to the other extremity of the nodule, which acts as an erector.

The cartilaginous supports for the fins appear long before there is any trace of ossification of the spinal column.

As was stated before, the tail fin is heterocercal at first, and passes through nearly the same formative changes as the dorsal. The urostylic cartilages are large, and become formed previous to the completion of the differentiation of the horny rays: they are comparable, to a certain extent, to the interspinous or supporting rays of the dorsal.

V. The Gills and Alimentary Canal.

The gills of the Lophobranchs have usually been described as "tufted," from the supposition that they consisted of tufts of filaments. Dr. Ryder[2] has corrected this mistake for Hippocampus. The diagram of a gill leaflet of Syngnathus, given on Plate XLII, fig. 7, will show their real structure. Coming off from either side of the rachis are a number of leaflets, gradually increasing in size from below upwards until near the extremity, when they suddenly decrease. Four such rows (only two are represented in the diagram) are arranged on each rachis, forming, as it were, a rectangular pyramid affixed by its apex. As Dr. Ryder says:—"There is therefore nothing at all in these structures which is not represented homologically in the fish's gill of the ordinary type, since the two series of vascular branchial appendages to each arch in Hippocampus are perfectly comparable with the bifurcated, vascular, branchial appendages of such a form as Salmo."

[1] F. M. Balfour, 'Comparative Embryology,' vol. ii, London, 1881.

[2] Loc. cit.

Dr. Ryder refers the abnormal structure of the gill to the degeneration which has taken place, evidenced by the rudimentary structure of the arches, and by the fact that the leaflets are far less numerous than in ordinary fishes. He supposes that "the reduction in number of these appendages may have called for the extension of the area of the ultimate branchial lamellæ or pinnæ." It seems to me that this degeneration of the pinnæ and arches has been due to the almost complete covering-in of the gill cavity, which is evidently an old ancestral character, since, even in Stage A, the branchial cavity is completely closed over by membrane.

The dilatation of the anterior portion of the alimentary canal, seen in Stage A, reminds one very strongly of the pharyngeal dilation in Amphioxus, and the respiratory sac in the Ascidians.

No communication between the yolk-sac and the intestine was noticeable. Von Baer states that communication exists immediately behind the liver, and Lereboullet believes that such a communication exists between the stomach and the liver, and persists until the complete absorption of the yolk. Balfour,[1] however, from his observations on the Trout and the Salmon, could not confirm these statements. I can agree with him that "all communication between the yolk-sac and the alimentary tract is completely obliterated very early." Even in Stage A the intestinal wall is seen to pass quite unbroken over the yolk-sac, absorption taking place through the cells of the wall of the intestine, or else entirely by the blood-vessels.

Near the posterior extremity of the intestine in Stage D a well-marked valve is present. In Stage A no trace of this is noticeable, but it makes its appearance in Stage B (fig. 2), where it appears as a constriction of the walls, which eventually increases, and closes off the rectum from the anterior portion of the canal.

GUELPH. June 28th, 1883.

[1] F. M. Balfour, 'Comparative Embryology,' vol. ii, p. 65.

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