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A STUDY OF THE CAUSES UNDERLYING THE ORIGIN OF HUMAN MONSTERS. (THIRD CONTRIBUTION TO THE STUDY OF THE PATHOLOGY OF HUMAN EMBRYOS.)

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INTRODUCTION.

The present communication is the outcome of a study of 163 pathological human embryos which I have collected during the past fifteen years. The first contribution[1] which I made to this subject included a report of 53, and the second[2] of 20 of these embryos. These two studies are rather anatomical in nature and do not consider the causes which produce pathological embryos, nor their relation to ordinary human monsters. A more careful study of my specimens, which have more than doubled in number during the past five years, establishes beyond doubt (1) the identity of pathological embryos and small monsters, that is, many of them would have developed into real monsters if they had not been aborted, and (2) that all of them are developed from normal ova due to external influences,—in man to a condition which I shall term faulty implantation.

From the earliest ages in the world's history the study of monsters, and the causes which produce them, has been one of the capital problems in anatomy, medicine and natural history. Supernatural causes for the production of monsters, like the influence of the gods, celestial or diabolical, or lunar influences, as expressed in their name, monster or moon-calf, held for a long time universal sway and are still believed in by many ignorant people. However, the Greek naturalists and physicians were inclined to ascribe them to natural causes, which belief was gradually displaced in the Dark Ages by the one that monsters were hybrids of bestial origin, a theory which was finally overthrown in the eighteenth century. Equally general has been the theory that maternal impressions affect the offspring and convert them into monsters. This

[1]Mall, Welch Festschrift, Johns Hopkins Hospital Reports, IX, 1900.

[2]Mall, Vaughan Festschrift, Contributions to Medical Research, Ann Arbor, 1903.

belief is of great antiquity, and is at present of world-wide distribution. It also was attacked in the eighteenth century, first by Blondel from a philosophical and then by Haller from a scientific standpoint.

All the theories can be resolved into the simple question: "Are the conditions which produce a monster germinal and therefore hereditary, or are they produced from normal germs by external influences?" The discussion on both sides of the question has been a long one, conducted during many years by the ablest masters, among whom are always included the leading anatomists of the time. However, the theory of external influences gradually gained ground during the nineteenth century, as the science of embryology was cultivated more and more. But here again we have two schools, the one believing that monsters are formed from normal germs due to maternal impressions, the other that they are due to mechanical influences. It may be noted here that the obstetricians and gynecologists of America as a class advocate strongly the theory of maternal impressions, due largely, no doubt, to their insufficient scientific education. On the other hand, we may pride ourselves over the masterful strokes of American teratologists against this theory; the experimental teratologists have produced double monsters, spina bifida and cyclopia, under the very noses of these practitioners, but they continue their futile speculations over mere coincidences.

With Meckel, who laid the embryological foundation for a scientific teratology, and the Saint-Hilaires, who made the first teratological experiments, we have the beginning of the development of the mechanical theory. It appears in a variety of forms, as, for example, that monsters are due to tight lacing which causes pressure upon the embryo, or to the contracting uterus which naturally might have the same effect. However, this theory was gradually transformed by teratologists so that now it rests upon the idea that amniotic bands constrict or compress the embryo, thus bringing about its deformity. Occasionally it has been found that monsters are

attached to the chorion through newly-formed bands of tissue, and such bands, whether present or not, are held responsible for all terata. This coincidence, as I term it, cannot be of frequent occurrence, for usually there is present an hydramnios. Nor can even the "coincidence" occur in anamniotic animals. Furthermore, no amniotic bands were ever found in any of the 169 specimens which I have studied.

In place of these theories it is my purpose to demonstrate that all monsters are produced by external influences upon normal ova which affect the nutrition of the embryos due to faulty implantation of the ovum. That the power to become a monster is present in every ovum is fully demonstrated by experiments upon a variety of vertebrates as well as by all of my pathological ova, especially those obtained from tubal pregnancies.

The changes found repeatedly in the chorion are no doubt primary; they are usually of an hemorrhagic nature, often indicating inflammatory changes in the uterus. I shall only hint at the cause for the changes in the uterus, which may interfere with the formation of the decidua, and recommend this field as a very fertile one for gynecologists and obstetricians to investigate. At any rate, the change interferes much with the attachment of the ovum, and this condition and what results from it I have termed faulty implantation.

It has been impossible, in fact it is not desirable, to discuss extensively the immense amount of excellent literature upon teratology. The whole makes one of the best chapters in medical literature to which the greatest minds of medicine have contributed their best efforts. One cannot go through these writings, many of which are comprehensive, without laying them aside with profound respect. In this study I have used many of them freely, but make, however, very few references.[3] I have also avoided technical terms as much as

[3] J. F. Meckel, Handbuch d. pathol. Anatomie, Leipzig, 1812.
Förster, Die Missbildungen des Menschen, Jena, 1865.
Bischoff, Wagner's Handwörterbuch, 1842.
Ahlfeld, Die Missbildungen des Menschen, Leipzig, 1880-1882. Part

possible, and in general have adopted Ballantyne's classification, which, in turn, is based upon Taruffi's.

I wish it were possible to thank adequately the many physicians who have contributed the specimens and who have responded so generously to my many inquiries. All data obtained from them are given in quotations under the description of the specimens, which are also properly credited to the donors. Some names will be seen repeatedly, as Miller, Boldt, Lamb, Brödel, Ballard, West and Minot. In addition, I wish also to thank my colleagues at the Johns Hopkins, who have aided me in every possible way to bring together the gynecological, obstetrical, pathological and experimental embryological evidence.

In this paper all of the embryos mentioned in the two previous ones are discussed, and brought together in Part II. The essence of the first contribution is given, and all of the second contribution is incorporated in this publication, so in a measure it may be viewed as a study of the whole collection. However, the various steps by which I came to arrange the specimens, as I have, can be understood only by consulting the two previous publications.

III, which was to be the important part, never appeared. Fortunately, however, Ahlfeld's library, which is very complete, was presented to the Johns Hopkins University. This I have consulted freely and in a way it makes up for the missing Part III.

Marchand, Missbildungen, Eulenburg's Real-Encyclopedia, 3d edition, 1897, Vol. XV.

Taruffi, Storia delli Teratologia, 8 volumes, Bologna, 1881-1895.

Hirst and Piersol, Human Monsters, Philadelphia.

Piersol, Teratology, Ref. Hndbk. Med. Sci., new edition, Vol. VII, 1904.

Ballantyne, Antenatal Pathology, 2 volumes, Edinburgh, 1904.

E. Schwalbe, Die Morphologie d. Missbildungen des Menschen und der Thiere, Jena, Pt. I, 1906, Pt. II, 1907.

PART I.

HISTORICAL AND GENERAL.

HISTORICAL.[1]

The changes found in the pathological embryos to be described in this memoir are so radical in nearly all specimens that it is almost useless to speculate regarding the fate of the embryos had they continued to grow to the end of a normal pregnancy. Could the circulation be maintained these specimens might have developed into amorphous monsters, a condition which is probable only when there is a normal twin fœtus to supply the nutrition. In only one of my specimens (No. 87) are the possibilities for such a termination present. Here on one side of the chorion there is a normal embryo of the third week and on the other side a highly developed umbilical vesicle with but a rudimentary amnion, but no real body of an embryo. In all of the other twin specimens the changes in both embryos are radical and identical, so that we could not hope to have had the one embryo dependent upon the other for its circulation and nutrition.

In general then the changes in the embryo and its membrane, due to the inflammatory action in the uterus, are so great that if the ovum is not aborted at an early date (as it usually is) it is converted into a solid mole which in the course of time is likewise expelled. A few specimens, however, are but slightly changed, and these would probably have grown into some sort of merosomatous monsters had they been retained in the uterus. From my experience I am convinced that in the study of specimens like these we have the key by which we can unlock many of the mysteries of teratology.

In my first two communications I carefully avoided all speculations on this subject, for I was well aware of the sad state this subject is in, and mere speculations would not

[1]The data here recorded are taken largely from Ballantyne's Antenatal Pathology.

help teratology out of its difficult position. However, what little progress has been made in the study of terata has been made by the embryologist and we naturally still have confidence in him. The course to be followed, therefore, is the study of early abortions, and this I have done diligently. I can, therefore, subscribe fully to what Ballantyne has recently said in his able and scholarly treatise on antenatal pathology. He says, page 77: "Now, in reference to the inquiry into the problems of teratology or embryonic pathology, let me emphasize the importance of a thorough scrutiny of the fœtal membranes and of the routine examination, microscopic as well as macroscopic, of all abortion sacs and their contents thrown off in the early months of pregnancy. What is most wanted at present are careful descriptions of monstrous embryos from abortion sacs, observations upon teratological conditions while the organism is still in the embryonic period of antenatal life. These are essential for the further progress of a knowledge of human teratogenesis, and they are at the present time the desiderata of embryonic pathology. Microscopic human monstrosities are, as a matter of fact, almost unknown."

The last sentence is hardly justifiable, for a pretty large number of young pathological embryos have been described by His, Giacomini and myself, but these do not resemble monsters at full term any more than an embryo of the fourth week resembles a new-born child. Whether the early pathological embryos are young monsters, or young monsters of so extreme a degree that they will not continue to grow, is now the most important question of the capital problem in teratology. I think that the specimens that are reported in this publication contribute to the answer of this question, but many more observations are required before the answer will be accepted by all teratologists.

The history of teratology co-exists with that of medicine and includes mythology, the vilest superstitions and scientific embryology. The medical profession have abandoned the idea of supernatural causes in the production of monsters

and have gladly exchanged the hybridity theory (cohabitation with lower animals) for the more innocent one of maternal impressions. The last notion is of great antiquity, is of world-wide distribution and is intimately related to witchcraft. It is gratifying to note that these superstitions, based upon coincidences, have been raised from medicine by the study of scientific anatomy, and the more recent work by J. F. Meckel in this direction can be ranked with that of Morgagni and Virchow. Morgagni gave the first blow to humoral pathology by giving medicine an anatomical basis, Meckel cast out devils, witches and mother's marks by placing teratology on an embryological basis, and Virchow won the third great victory for anatomy, probably the greatest contribution ever made to medicine, by giving it an histological basis. It would be inappropriate to enter any further into a discussion of teratogenesis in this publication, for in general the superstitious notions are abandoned by scientific physicians, although they may still be entertained by a few practitioners of some eminence. It is humiliating to state that these practitioners seem to reside exclusively in America, but we have every reason to hope that when scientific medical education becomes general with us they will also disappear.

Most of the great men who have contributed to the progress of medicine, from Hippocrates and Aristotle to the modern scientists, tried to ascribe the production of monsters to natural and not to supernatural processes. From the first the explanations were as satisfactory as they are to-day, for even now we barely do better than Aristotle did. However, the spread of the scientific spirit beginning with the study and practice of anatomy by all medical students has driven medical superstitions pretty well out of the medical profession. In this respect we differ from the ancients. The first scientific explanations were of a crude mechanical nature, like those due to excessive lacing, malformations of the uterus or a twin fœtus, which might injure the embryo. This notion was superseded in part by the theory of Morgagni, who maintained that monsters were due to fœtal disease. This

again received its death-blow from J. F. Meckel, who pointed out the well known fact that many structural anomalies are hereditary. This observation naturally divided terata into two groups: those which are hereditary and germinal, and those which are not hereditary but due to mechanical injury or disease. I think this line of division should be drawn much sharper than it is, but until our data can be arranged better than is now possible we are still quite uncertain regarding a large number of terata. It seems to me that many merosomatous terata (all kinds of anatomical anomalies and variations of the extremities, like polydactyly and possibly some cases of arrested development like ectrodactyly and hare-lip) are germinal and cannot be produced experimentally. Other monsters in which more or less of the fœtus is destroyed, as in iniencephaly, spina bifida, anencephaly, cyclopia, club-foot and many varieties of arrested development, are not germinal but are produced in some mechanical way which usually interferes with the nutrition of the embryo. In my notes I have been in the habit of calling those belonging to the first group as being abnormal and those to the second group as pathological. The one is germinal with a hereditary tendency, and the other is acquired and therefore not hereditary; polydactyly is inherited, cyclopia is not, although there seems to be a tendency for it to occur more than once in abortions from the same woman. However, if this is true, it may be due to the same cause in the uterus of the mother affecting the nutrition of successive ova, thus producing similar deformities in the embryos. Usually a woman who gives birth to several monsters has the varieties mixed up pretty well, the first may have hydrocephalus, the next hare-lip and the third cyclopia. Reducing it to a matter of chance, a woman who has given birth to one monster is more likely to give birth to a second one, which, however, is rarely like the first. In experimental teratology in birds and amphibia the result is the same. Here monsters may be produced experimentally with a variety of agents, even by treating the semen of toads with X-rays, but the

variety of monster can never be predicted, and if there are a number of them they are usually of mixed types.[2]

What I have to say in this publication of monsters applies only merosomatous terata which are not of an hereditary nature and are no doubt produced by agents which interfere with the nutrition of the embryo. Having taken only those monsters from which the germinal factor is excluded, it makes it necessary once more to consider some minor mechanical agents as their cause, which may be termed a modified mechanical theory.

The advocates of the mechanical theory gradually lost ground, for they had to combat the germinal theory on the one hand, and on the other they were compelled to state that mechanical influences, generally those due to lacing, caused the fœtus to become monstrous by the pressure that was exerted upon it. The theory was then modified to include primarily intra-abdominal influences like tumors, malformations of the pelvis and uterus, as well as those within the ovum itself. Gradually we see less and less weight placed upon any of these specific causes, and finally the modern advocates of the theory believe that amniotic bands and adhesions are the main influences in the production of monstrosities. It is needless to state that each advocate had his own combination of circumstances, and when all of them are taken together, with modifications and exceptions, it is practically impossible to make general statements. Suffice to say that the objections to each form of the theory appear to be sufficient to explode the whole theory, and to the bulk of physicians maternal influences seem to be as rational a cause in the production of monsters as mechanical influences, for the data of experience are about as good in the former as in the latter.

There are some rare cases of spontaneous amputation of the extremities which are said to be due to pressure of the umbilical cord. However, these cases can be separated into two marked groups, one in which there is an actual amputa-

[2]Bardeen, Jour. of Experimental Zool., 1907.

tion and the other in which there is an atrophic or rudimentary hand or foot attached. In the latter instance it seems to me that it is very irrational to hold the umbilical cord responsible for the amputation. Furthermore, the cause is possibly germinal, as may be the case in sympodia, syndactyly and ectrodactyly. The rare cases in which there is actual amputation of the extremity are more likely to have been produced by mechanical injuries during labor than by having the amputated limb caught in a loop of the umbilical cord. In fact, we must admit that we are unable to explain by any satisfactory hypothesis either congenital amputations or dislocations.

It has been noticed occasionally in merosomatous monsters that the diseased or malformed part is tied by means of bands of tissue either to the amnion or to adjacent parts of the body of the fœtus. These observations, relatively few in number, have led to the theory that the bands caused the deformity. It seems to me that, in view of the idea that many monsters are due simply to an arrest of development of some part of the embryo, that hydramnios is usually present, and that all kinds of monstrosities may be produced in lower animals (including amphibia which have no amnion), it is highly probable that amniotic bands and the like are secondary in their formation and have nothing whatever to do with the production of monsters. The more the embryological theory is tested by experimental methods the more all simple mechanical explanations suffer, and it seems to me that all of them will have to be abandoned.

It is not especially remarkable to find that when the head or face is malformed the diseased part occasionally forms a secondary attachment with the amnion; or that, as in exomphalos, where the umbilical cord is "dilated," the extruded viscera come in direct contact with the placenta, as they should, and the blood-vessels are scattered and run along the amnion to the placenta, as should also be the case when the subject is viewed from the standpoint of embryology. Furthermore, deformities of the extremities are of frequent occurrence, but

amniotic bands are rarely found, and when they are present they are often attached to the body of the embryo and not to the deformed extremity. It seems to me, therefore, that as facts accumulate it becomes clearer and clearer that the occasional amniotic adhesions found are due to the presence of the monster and are not causal in nature.[3]

Possibly I have devoted too much space to the discussion of mechanical theories in teratogenesis. What has been said is no doubt acceptable to all embryologists, and my apology is due to the fact that the influence of maternal impressions upon the offspring is still believed in by so large a number of American medical writers of note and that mechanical notions regarding embryology are entertained by physicians in general.

The great embryologists from Harvey onward explained the conditions found in monsters as due to an arrest of development, for they saw in these distorted individuals conditions which are normally found in the embryo. The embryological theory was first well formulated by J. F. Meckel, who explained the beast-like appearance of some monsters by the fact that in his development man passes successively through stages found in lower animals. To those who have accepted the doctrine of evolution this is all clear, but it remains to be shown what are the factors in development, and the effect of changes in the embryo upon the growth of the fœtus.

As has been pointed out above, we must divide monsters into two groups, those in which the proper conditions to produce them are already in the germ (are therefore inherited), and those due to certain external influences which act upon the egg after it is fertilized. It is obvious that only the second group can be considered in any experiments made upon the embryo. So, if the pathological ova I have studied

[3]Ballantyne says: "The reader may feel (and he is justified in so feeling) that, after all, experimental teratogeny has not done much for the understanding of the mode of origin of monstrosities, if it has weakened a belief in the influence of the amnion." I may add that this argument can be applied to maternal impressions as a cause equally as well.

are all due to a diseased chorion, which in turn is dependent upon endometritis, then we should find embryos tending towards club-foot, anencephaly, iniencephaly, spina bifida and cyclopia, which in fact proves to be the case. However, a large group of new monsters, known only to embryologists, make their appearance and from the very nature of the abnormality found but few of them could develop beyond the first months of pregnancy. In their study comparisons have been constantly made with normal embryos of the same size, and in this way, to a certain degree, it is possible to picture the order of events. It is found that in these specimens some tissues are more susceptible than others, and when the nutrition of the ovum is impaired it is these that are affected first. In very early stages the amnion and embryo are equally susceptible and the umbilical vesicle and chorion are the most resistant. Later it is the embryo alone, and still later the head, central nervous system and extremities. It follows then that the parts most susceptible are those most frequently found changed, or wanting, in merosomatous nongerminal monsters. In general the varieties found in my collection of young embryos correspond with those obtained experimentally by others in birds and appear much like the most common human monsters.

The Saint-Hilaires, who contributed so very much to our knowledge of teratology, were the first to study the subject experimentally. By a variety of experiments made upon the shell of the egg (*e. g.*, pricking and varnishing) the older Saint-Hilaire produced a large number of anomalies in which there were defective heads and spina bifida. His experiments were made upon eggs after development was well under way, and his results were pronounced enough to allow of comparison with human monsters. The younger Saint-Hilaire extended the experiments to include the earliest days of incubation, and found that the embryos which developed were dwarfed or were wanting altogether. In no instance were polysomatous monsters produced. At any rate, the experiments of the Saint-Hilaires show that a change in the external

physical conditions may influence and modify normal development and thereby produce a variety of merosomatous terata.

During the following seventy-five years a great amount of experimental work was done upon chicks by numerous investigators, which showed that the varieties of monsters produced were quite constant, no matter what agent is used, but no single variety could be produced with certainty. It was found impossible to experiment with precision, for a certain per cent of eggs would produce one or more varieties of deformed embryos.

EXPERIMENTAL.

Recent Work Upon the Production of Polysomatous Monsters.

The various theories regarding teratogenesis which had troubled mankind for so many centuries were finally exploded by naturalists, whose speculations gradually led them to experiment upon this subject. Anatomists and zoologists had deduced that the primary change lay in the egg about the time of fertilization, and we read in J. Müller,[1] Valentin[2] and Leuckart[3] that a double monster is due to division of the embryo-forming substance in the earliest stage of development. The experiments subsequently made by Gerlach,[4] Panum[5] and Dareste[6] upon chicks were negative in this respect, but the tradition has come down to us that polysomatous monsters are produced by a process of splitting of the primitive streak. The more recent anatomists—Fol, Rauber,

[1]Müller, Meckel's Archiv, 1828.
[2]Valentin, Handwörterbuch d. Physiologie, I, 1842.
[3]Leuckart, De Monstris, Göttingen, 1845.
[4]Gerlach, Doppelmissbildungen, 1882.
[5]Panum, Entstehung der Missbildungen, 1860.
[6]Dareste, Recherches sur la production de monstrosités, Paris, 1891.

Born and O. Hertwig—who observed the developing egg and experimented upon it, were at first inclined to the theory that the first cause in the production of monsters is due to polyspermy, but this has not been substantiated.

The first reliable and valuable observations upon the production of double monsters were made by Vejdovsky,[7] who noticed that the eggs of Lumbricus produce more monsters in warm than in cool weather, and he expressed the suspicion that they were produced by the change in temperature. Driesch[8] seized upon this idea, experimented upon sea-urchins' eggs, and found by subjecting them to high temperatures that the cells, in the two-cell stage, separated, each growing into an individual, but, however, remaining connected with each other. Driesch had already shown that when the blastomeres of these eggs are fully separated by shaking, each grows into a whole embryo, and it was now clear to him that double monsters are produced by separating the blastomeres slightly, but still keeping them close enough together so that the independent embryos grow into each other's bodies to form a double individual.

By a very different method double monsters were also produced by Loeb.[9] He subjected sea-urchin eggs to an equal mixture of sea-water and distilled water shortly after they had been fertilized. The rapid absorption of water caused many of the cell membranes to burst, and part of their protoplasm escaped, which, however, remained connected with that inside of the membrane. All this took place before the nucleus had divided. Upon returning the eggs to normal sea-water cleavage began, and one of the first two nuclei wandered into the extruded protoplasm. Each nucleus with its protoplasm then became an embryo, and in case the embryo within the egg was not separated from the extra-ovate embryo by its active movements in the blastula and gastrula stage a double monster

[7]Vejdovsky, Entwicklsg. Untersuchungen, Prag, 1890.

[8]Driesch, Zeit. f. wiss Zool., LV, 1892.

[9]Loeb, Biological Lectures at Woods Holl, 1893; Pflüger's Archiv, LV, 1894; Roux's Archiv, I, 1895; and Studies in General Physiology, Chapter X, Chicago, 1905.

or "Siamese" twins were formed. Frequently they became separated and independent animals developed. It often happened that the outflow of protoplasm was multiple, and then the three, or even more, protoplasmic drops which were formed developed respectively into triple or quadruple monsters.

These important discoveries were soon extended to the vertebrates by E. B. Wilson,[10] who experimented upon the eggs of Amphioxus, and by O. Schultze,[11] who experimented upon those of the frog. Wilson partly separated the blastomeres of Amphionus in the two-celled stage and produced a variety of double monsters which developed until the first gill-slits were formed. In the gastrula stage almost every possible transition occurred between forms slightly expanded laterally to those in which the two bodies were joined only by a slender bridge of tissue. Incomplete separation of the blastomeres in the four-celled stage gave rise sometimes to double embryos of equal size, triple embryos, one being as large as the other two, or rarely to quadruple monsters. Wilson's studies prove, he believes, "that the unity of the normal embryo is not caused by a mere juxtaposition of the cells, but they indicate that this unity is not mechanical but physiological, and point toward the conclusion that there must be a structural continuity from cell to cell that is a medium of coordination, and that is broken by the mechanical displacement of the blastomeres."

Oskar Schultze produced monsters in frogs by fixing the eggs between two glass plates, and after they had developed to the morula stage the plates were inverted. A number of the eggs righted themselves, but others grew into double embryos. Wetzel[12] extended the observations of Schultze and showed that there was a flow of protoplasm in each of the blastomeres into their upper hemispheres, which may

[10]Wilson, Jour. of Morph., 1893.

[11]O. Schultze, Verhandl. d. anat. Gesellsch., 1894, and Roux's Archiv, I, 1895.

[12]Wetzel, Arch. f. mik. Anat., XLVI, 1895.

account for the separation of the primary cells, thus laying the foundation for two embryos instead of one.

Spemann[13] has also produced polysomatous monsters from the frog's egg by tying a ligature loosely around it in the two-cell stage between the two blastomeres. Specimens in which the ligature struck the median plane of the embryo produced two-headed monsters of all grades, their development depending somewhat upon the degree of the mechanical constriction. He performed similar experiments upon Triton eggs, and in some instances found cyclopia in one or both of the heads. By broadening the anlage of the tail through splitting, a double tail may be formed, or in case limb-buds are divided one or more times two or even a cluster of limbs may be produced where but one develops normally.[14]

The experiments enumerated above, although not quite to the point in the present study, are reviewed because they show that teratogenetic problems are solved by experimental embryology and because they are very striking. If it is clear that polysomatous monsters are produced experimentally with such precision, the great variety of merosomatous terata of the experimenter must be admitted worthy of careful study. It is necessary to state this because only a small per cent of them live for some length of time, but they show a great similarity with early stages of human terata with which we are familiar. A glance at the great works of Dareste and Panum makes it clear that the deformed embryos they obtained are not easily interpreted and they could easily be pushed aside as not bearing upon the subject in question. The same criticism may be made against early pathological human embryos. That it is difficult to see any marked relation between them and monsters at the end of pregnancy caused me much confusion for a long time, but after studying a large number of deformed embryos I am finally convinced that the pathological embryos are nothing but young monsters. This conclusion is sup-

[13]Spemann, Stizungsber. d. phys.-med. Gesellsch., Würzburg, 1900; Zool. Jahrbuch, VII Supplementband, 1904.

[14]Tornier, Roux's Archiv, XX, 1905.

ported especially by the numerous investigations in experimental embryology, many of which are also at the same time investigations in experimental teratology. For this reason I shall consider briefly the recent work upon the production of merosomatous monsters.

Lithium Embryos and Nodular Forms in Chicks and in Man.

Comparative teratology gives ample testimony to explain the production of double monsters, and it is now clear how they may be produced in man. But when the study is extended to merosomatous terata great difficulties arise in making comparisons between the large number of experimental monsters in lower animals, the pathological embryos which I have studied, and finished monsters at the end of pregnancy. The endless literature upon these subjects is very difficult to blend into a continuous story on account of the various terminologies used by the different writers. However, I hope to draw some satisfactory lines through it, being guided by comparative anatomy and embryology.

The immense number of experiments performed upon the eggs of different species of animals has given the greatest variety of monsters of very irregular form, and extremely difficult to interpret properly in the light of our present knowledge of embryology. Quite recently our distinguished teratologist, Morgan, has presented this problem in a new light in his series of studies on the relation between normal and abnormal development of the embryo of the frog.[1] No doubt scientific investigations like these of Morgan will soon clear up many of the questions in teratology which have perplexed us so long. It may be noted that new kinds of monsters are constantly being produced by the experimental teratologist, one of the most interesting being that known as the lithium larva.

[1]Morgan, Ten Studies in Roux's Archiv, Vols. XV-XIX, 1902-1905.

In 1893, Herbst,[2] while studying sea-urchins' eggs, observed that the action of lithium salts upon them caused the layers of the blastoderm to invert in development. The lithium experiments were repeated by others, including Morgan, upon frogs' eggs, who found that the upper protoplasmic contents of the egg fails to move downward, which is followed by a complete inversion of the germ layers. The entire upper part of the egg sinks into its interior and forms a medullary plate which is bent back upon itself. This change in development is due to the physical and chemical action of lithium salts, for it cannot be brought about by any other means.

Very recently Stockard[3] experimented upon Fundulus with solutions of lithium chloride and produced monsters which developed into quite decent fishes, but at present it is impossible to compare them with lithium larvæ of sea-urchins and frogs. In these embryos the blastoderm is usually prevented from growing downward over the yolk, as is also the case in the frog, and therefore bulges as a cap upon the upper part of the egg. In stronger solutions of lithium this cap often constricts at its borders and finally pinches itself off from the yolk and dies. In embryos which survive, the heart beats slowly, the eyes often fail to develop, the blood is colorless and therefore appears to lack hemoglobin. These characteristics, taken with the inability to recover from the lithium effect, seem to prove that they are due to chemical causes.

At present it is difficult to compare the great variety of monsters produced in anamniotic with those obtained in amniotic animals, for the number of the latter is relatively small and their description meager. We have a series of excellent papers on the production of monsters in the hen's egg by Panum,[4] Dareste[5] and Féré.[6] These authors, however, devoted their main discussion to the teratogenic agents,

[2]Herbst, Mitth. Zool. Sta., Neapel, 1893, and Roux's Archiv, 1896.
[3]Jour. Ex. Zool., Baltimore, 1906.
[4]Panum, Entstehung d. Missbildungen, Berlin, 1880.
[5]Dareste, Recherches sur la production de monstrosités, Paris, 1891.
[6]Féré, Cinquantenaire de la Société de Biologie, Paris, 1899.

of which they used a great variety. In general, they employed variations in temperature during incubation and, although they produced many kinds of monsters, they never could predict which kind they were to obtain from a given batch of eggs. It is, therefore, very apparent why experimental teratologists did not make much headway as long as they experimented upon the chick. However, they did establish two facts: first, that monsters are produced from the hen's egg by all kinds of external influences, as varnishing the shell, placing the egg in the vertical position, change of temperature, traumatic means, shaking, magnetic and electrical influences, by gases which penetrate the shell and by a great variety of chemical poisons and toxines injected into the white of the egg. In general, any substance which either interferes with the nutrition of the egg or poisons it, causes the embryo to become abnormal, but a special kind of monster is never produced by a given teratogenic agent. In this respect the experiments upon anamniotic eggs are far more satisfactory.

Panum classified the monsters he produced into two great groups: (1) Those in which the whole embryo is involved, (2) those in which but part of it is abnormal. Under the first group there are (*a*) flattened forms, that is, the germinal area is not much changed in shape; (*b*) flattened forms with the production of red blood, *i. e.*, only the embryo is affected; (*c*) cylindrical forms, the embryo becomes abnormal in a more advanced stage; and (*d*) amorphous forms. This first group, with its four subdivisions, may possibly be compared with the great variety of irregular monsters of which the lithium larva may be considered the type. At any rate, we may say that there is an analogy. Certainly there is a similarity between the cylindrical forms, which do not live long, and the deformed fishes obtained from Fundulus by means of lithium chloride solutions. The great change which has taken place in both varieties makes it impossible for either of them to exist for a longer time. A similar form of monster is also often found in mammals, and His, in adopting Panum's terminology, has classed it with cylindrical monsters. The

pathological changes in them are so radical that their lives are also short. Amorphous forms are analogous with lithium larvæ and identical with the nodular form in man. In general, only part of the embryo continues to develop in an irregular fashion and finally the whole embryo dies. The flattened forms, with and without blood-vessels, are identical in man with the vesicular forms, that is, ova containing umbilical vesicles only, and to ova without embryos, respectively.

The merosomatous monsters in which the whole embryo is affected, total monsters as they are also called, are not likely to live long, but they are of great interest to those studying teratological problems. While they are being formed a certain number of eggs develop into partial monsters, and in man some of them grow into fœtuses which may go on to full term, and a very small number of them live on to maturity after birth.

The recent total monsters produced by Bardeen[7] in subjecting the sperm of toads to X-rays before fertilization can be explained on the same ground as are lithium embryos. The tadpoles which develop from such eggs are entirely diseased, continue to grow in an irregular manner and appear much like lithium larvæ in Fundulus or as ordinary pathological embryos in man. In all three cases the primary radical change involves the whole embryo; in Bardeen's experiment the cause affected the sperm before fertilization, in Fundulus shortly after fertilization, and in human pathological embryos somewhat later. Although the methods employed are very different, the principle involved and the results obtained are much the same.

A very large number of monsters are to be classed as total monsters. They are probably brought into existence by a variety of circumstances, all of which interfere with the nutrition and growth of the whole embryo and the changes in them are so radical that their lives are very short. In man

[7]Bardeen, Jour. Ex. Zool., IV, 1907.

the primary trouble cannot be due to the presence of poisons in the blood of the mother, to correspond with chemicals used by teratologists in producing lithium larvæ, for instance, nor to a fever, to correspond with the changes in temperature used to produce chick monsters. The process in man is quite different, being due probably to faulty implantation of the ovum, which naturally affects the growth of the embryo. This will be discussed under a subsequent heading.

Experiments With Salts of Potassium and Changes of the Embryo Especially Marked in the Heart.

In case interference in the nutrition of the embryo is not too great the growth of part of the embryo, instead of all of it, may be retarded, with an additional destruction of tissue, thus producing the partial monsters in man, which frequently develop to full term. However, there is every kind of gradation between total and partial monsters, the former often showing many indications of the latter and the latter are frequently multiple. A total monster may have spina bifida, hare-lip, anencephaly, club-foot, cyclopia, etc., and a partial monster may include several of these types. The primary affection which is to produce a pure spina bifida in man must be slight, must come at the right time, and subsequently the faulty implantation must be remedied so that the embryo may continue to grow. In order to make my standpoint clear I shall first give some data regarding the frequency of some types of monsters as well as some experiments which show that the heart is extremely susceptible, its growth being easily retarded and often arrested after the embryo is well formed.

Von Winckel[1] has given us some data regarding 87 monsters obtained from 12,378 births in Dresden, that is, there

[1]Von Winckel, Ueber die menschl. Missbildungen, Samml. klin. Vorträge, Leipzig, 1904.

was one monster in every 142 children born. He also states that there were 105 monsters in 20,000 births in Munich, or 1 to 190. The most marked deformity in each of the 87 Dresden cases number as follows:

Deformities of the head	23
Deformities of the face	12
Deformities of the neck	4
Deformities of the abdomen	7
Deformities of the back	3
Deformities of the upper extremity	9
Deformities of the lower extremity	17
Deformities of the skin	11
Deformities of other organs	1

I have compiled a similar table from Panum,[2] who gives data obtained from Otto and from Meckel. It is as follows:

	Total number of monsters.	Number of cases.
Anencephalus	618	119
Anencephalus (according to Ballantyne	325	46
Hydrocephalus	618	26
Hydrocephalocele	618	93
Hare-lip	618	77
Cyclopia	618	16
Eyes missing	618	9
Deformed upper jaw	618	3
Deformed extremities	618	115
Spina bifida	404	38

In my collections of 163 pathological embryos there are 48 which show deformities which can easily be recognized as similar or as being forerunners to fœtal monsters. In 27 of the embryos this deformity is limited to a single part of the embryo, as indicated in the table, but in 21 of them two or

[2] Panum, *l. c.*

more malformations are present. They are as follows. Total number of cases, 48.[3]

Atrophic head	24
Malformed face and neck	17
Displaced eyes	3
Deformed extremities	18
Spina bifida	12
Exomphaly	5

In a general way the deformities in these 48 malformed embryos correspond pretty well in per cent with the type of monsters as recorded by Von Winckel and Panum. As shown in the table on page 43, seven pregnancies out of every

[3]In order to make it possible to look up the histories of the embryos given in this table I add the numbers of the specimens which are included under each heading:

Atrophic Head.	Face and Neck.	Eye.	Extremities.	Spina bifida.	Exomphaly.
12	110	135	81	6	115
60	122	201	94	12	162
69	124	285	122	54	166
81	132		124	94	244
104	201		132	135	364
132	212		135	182	
135	226		142	189	
137	232		177	226	
177	246		200	251	
182	251		230	293	
189	276		232	364	
200	297		251	365	
201	330		316		
207	343		325		
212	357		343		
226	364		344		
276	365		357		
295			366		
309					
335					
341					
343					
364					
365					

100 give pathological ova of which but one-third give well formed embryo monsters, or two per cent of all pregnancies. The number of monsters which go on to full term is about .6 per cent, and it is just this group which has escaped me. The embryo and fœtal monsters form, therefore, 2.6 per cent of all pregnancies, or, in other words, three well-formed monsters are aborted in the early months of pregnancy for every one which goes on to the end of pregnancy.

It is clear that those cases in which the embryo is markedly deformed or is absent altogether, as is the case in about 100 of my specimens, cannot possibly develop for any great length of time, for without either heart or form they cannot exist. However, the second group of forty-eight embryo monsters show within themselves such radical changes that they also could not have existed much longer. In nearly all of them the heart is markedly changed, is atrophic or is wanting altogether. There are also many other changes, especially in the central nervous system, which makes it probable that they have lived as long as they could and were then finally aborted. In general, I think that the form of the monsters and their classification show clearly that they are practically identical with those that grow into fœtuses and then to full term, differing only in the degree of their changes. These are so radical in the embryo monsters that their lives are destroyed.

Teratologists have long ago observed that the heart must be affected more or less in monsters on account of the frequent œdematous condition of the tissues and of the excessive accumulation of fluids in the serous cavities and in the amnion. In fact, a large per cent of monsters have hydrocephalus and hydramnios. These conditions are seen in many of my specimens and have been observed by experimental teratologists like Panum and Dareste. However, we now have some good experiments which throw some light upon this subject.

In 1893 Loeb[4] made the brilliant discovery that the heart beat could be arrested in Fundulus by placing the eggs in a

[4]Loeb, Pflüger's Archiv, LIV, 1893.

1.5 per cent aqueous solution of potassium chloride shortly after fertilization. He found that the eggs develop in a pretty normal fashion with the exception that though the heart develops it does not beat at all. The blood-vessels develop properly as regards their course and division, but their lumina are irregular, like a chain of beads, which Loeb believes to be due to a lack of normal blood-pressure. Similar pictures may be seen in pathological human embryos, that is, only part of the vascular system is present, or the heart is atrophic but some of the blood-vessels are present, or the whole vascular system of the body is absent, with remnants of vessels in the yolk sac and in the chorion. In the last instance the vessels of the body may have been present at one time, for they should not have reached the chorion without passing first through the body. The embryos in Loeb's experiments rarely hatched, and all of them died before the sixteenth day, due to heart poisoning. Loeb thought that all of the organs—brain, eye, ear and myotomes—developed without any marked anomalies, but I do not think that his experiments were extensive enough to test this point thoroughly. However, he states that the pigmentation of the yolk sac was affected decidedly by the absence of the circulation. Under normal conditions the pigment, which is at first evenly scattered over the yolk, wanders to the blood-vessels as soon as the circulation begins and stays there, forming pictures which correspond with the branching vessels. In potassium embryos where the blood does not circulate the pigment cells are not attracted to the blood-vessels, but remain scattered evenly over the yolk.

By extirpating the heart anlage from very young frog embryos Knower[5] obtained a similar arrest in the development, but his experiments show that absence of the heart or early defects in its action produces marked abnormalities in the development of the embryos. While the earlier stages of the development of these frog embryos is normal, the later changes

[5]Knower, Anatomical Record, Amer. Jour. Anat., VII, 1907.

are arrested and strikingly abnormal. These embryos grow in an irregular fashion, become œdematous and the lymph vessels, blood-vessels and serous cavities are distended. Especially is the pronephros thus affected and the glomerulus distorted. The vascular system is much distended and very irregular, the chief vessels being laid down, though incomplete. The aorta and pronephric sinuses open into a mesenteric sac. The capillary system is imperfect or absent, blood corpuscles are relatively few and apt to be collected in the enlarged sinuses. Remarkable also is the rôle the lymph hearts play in such specimens. They continue to beat and pump the lymph containing some blood into the veins, and from their periphery it must pass again into the tissues. In connection with arrest in the development of organs the coiling of the intestines is limited to a single loop, the pancreas and liver are not normal, the subdivisions of the brain do not acquire their specific size and shape, the eyes are much aborted and the musculature is vacuolated. Knower obtained similar results from frog embryos developed in acetone chloroform, which inhibits the heart action from its earliest stages.

The recent experiment of Bardeen, in which he produced toad monsters by subjecting the sperm to the influence of X-rays before fertilization, may also bear upon the question of the importance of the heart in early development. "The eggs develop at first apparently normally or even better than the control, but beyond the gastrula stage the development begins to become retarded, and at the time of hatching, as the tail begins to grow out, marked deformities begin to appear in the larvæ." The change takes place at the time the heart begins to function, for the vascular system was barely developed in any of the embryos experimented upon. The heart is rudimentary and may have no continuous lumen. The chief arteries and veins are incompletely developed. There are but few blood corpuscles in any of the embryos, and Bardeen[6] states that it is uncertain whether the blood had

[6]Bardeen, Jour. Ex. Zool., Baltimore, 1907.

circulated at all in any of the embryos. In all of them the spaces in the tissues indicate that there is a marked œdema. The mesenchyme is increased in amount, the cells being spread apart by the fluids between them. Unfortunately for my purpose, Bardeen has not examined early stages of his monsters, nor has Loeb examined with sufficient care the later stages. If we keep Knower's experiments in mind, we may think for the present that the changes in circulation in Bardeen's experiments are primary, and the other changes, like irregular development of the nervous system, dropsy and hydrocephalus, are secondary, that is, due to the absence of the circulation. At any rate, Bardeen's description of his "X-ray toads" corresponds in many respects with those I have given of pathological human embryos in earlier publications and in Part III of this publication.

It is also clear that the necrotic changes in the central nervous system of these larvæ, as well as those in pathological human embryos, can be due to deficient nutrition, but in order to produce a finished monster the nutrition must not be impaired too much. The heart may be poisoned somewhat, which in turn may affect the central nervous system, causing histolysis and dissociation there, and the general development may be retarded and the embryo deformed, but as soon as the heart ceases to function all growth ceases and the embryos gradually disintegrate, as has been the case in about 100 specimens out of my 169.

In very rare instances human embryos continue to develop to the end of pregnancy without a heart. Such a specimen must be one embryo of duplicate twins, with a common umbilical cord through which it may receive nourishment from its healthy brother. Quite early in development its circulation must be reversed in the descending aorta, for as soon as its heart stops blood enters the body through the umbilical arteries, which passes in a reversed direction up the descending aorta. Under these conditions all kinds of curious monsters develop, ranging from a single head without a body to a kind of teratoma known sometimes as a

placental parasite. Of course, a "rescue" like this is out of the question in nearly all cases, and the life of the embryo is of short duration after its heart has ceased to beat.

Specimens similar to Bardeen's and mine have often been found in chicks by Dareste and by Panum. Panum describes quite extensively the changes which take place in chick monsters. He recognizes in his classification that flattened monsters are of two kinds: (1) Anæmic ones, in which no blood is found, and (2) those in which red blood had been found but most of the vessels are retained in the area vasculosa. In these probably the vascular anlage was destroyed very early or it began in the area vasculosa and did not develop into the embryo. The result is similar to that obtained by Bardeen, Knower and myself.

It is clear, I hope, that certain parts of the embryo are more susceptible to insults than others, and this must be admitted in order to explain why potassium stops the heart, lithium affects in a peculiar way the movement of the protoplasm in the blastomeres, and sodium produces spina bifida by arresting the movements which close the spinal canal. In order to analyze the situation we must concern ourselves mostly with simple reactions in their early stages, for they are not under way long before they become very complex and beyond our reach. Mesenchyme is less susceptible than nerve tissue, and the brain is more susceptible than the spinal cord; and so on. A susceptible tissue when affected undergoes certain morphological changes well recognized by Panum. Panum pictured to himself a kind of inflammation of the tissues, parenchymatous, due to disturbances in the nutrition of the part, but it is by no means clear that anything like inflammation in the healing of wounds takes place in embryos or in the various tissues after they have become partly necrotic. Often it is noticed that cells accumulate in portions of the embryo, and His thought that they wandered out from the blood-vessels. In my former publications I spoke of these cells as the wandering cells of His. Hertwig and Bardeen speak of necrosis, and there is every evidence that destructive

and not constructive processes are present in parts of the embryo which are becoming deformed. As this question is being investigated more and more it is clear that we must build up a pathology of our own based upon observations upon normal healing of wounds,[7] etc., in embryos, just as Morgan was compelled to study anew the development of the frog in order to interpret properly the various malformed eggs he had under consideration.

I am quite certain that in the human embryo the cells may spread from the blood-vessels into the surrounding tissues after the heart has stopped, and I am also certain that mesenchyme cells may separate and segregate, and that the cells of the central nervous system may become necrotic in part, dissolve their connections and gradually fill the central canal. It matters little what we call this process, it probably includes a series of processes, but for the present I shall apply to it the term *dissociation*. In doing this I do not commit myself as to the origin of a group of cells. In order to describe my human embryos properly I must also use the term *maceration,* and by it I mean that the process has taken place after the death of the part. When cells dissociate they are still alive, but they are on the way to meet their fate. As the tissues continue to grow their sharp borders are broken and they gradually become hopelessly confused. For the present the terms *dissociation* and *maceration* will do; in a short time it will be necessary to displace them, for experimental teratology will continue to be a fruitful field of research.

Sodium Monsters—Spina Bifida and Anencephaly.

Probably the most satisfactory chapter at present in experimental teratology is the subject of spina bifida. Under this heading, of course, is meant that kind of spina bifida which is due to a lack of closure of the neural canal and not the kind that may be produced in older embryos after the cord is

[7] See, for instance, Eycleshymer, Amer. Jour. Anat., VII, 1907.

formed perfectly and the vertebral canal remains open. However, it is easy to conceive of the second kind as a variety of the first, for in it the development went on normally, but the vertebral arches did not meet in the dorsal mid-line to produce the vertebral canal.

In 1892 O. Hertwig[1] published his remarkable article on open blastopore in frogs' eggs and its relation to spina bifida. Hertwig was experimenting upon eggs to produce polyspermy, after they had been kept for some time after maturation and found that many of these eggs developed abnormally, due to polyspermy, he believed. A part of the eggs segmented irregularly and developed in a peculiarly pathological fashion, that is the blastopore remained open much longer than it should. The further study of these specimens showed that they grew into embryo monsters with all kinds of deformity of the spinal canal, often producing quite typical specimens of spina bifida. Hertwig saw clearly the bearing of these experiments upon the general explanation of spina bifida and its relation to the blastopore. This he discusses at great length and with much ability. He was able to show the relation of his work with that obtained by Lereboullet on the pike, by Oellacher on the salmon, and by Rauber on the trout. These investigations had shown that an open spinal canal or even a total fissure of the body may result when the germ ring does not unite properly to form the body of the embryo.

It now became possible for the first time to follow spina bifida from its very earliest stages in amphibian and fish embryos up to a time when it is clear that the process is identical with that found in birds and mammals. To be sure, in the latter cases we must take the specimens as they occasionally come to us, for it is impossible at present to experiment upon mammals successfully, and in chicks the experiments are not very satisfactory. This comparison was made by Hertwig with great acumen, using the excellent

[1]Hertwig, Arch. f. mik. Anat., XXXIX, 1892.

article of Von Recklinghausen[2] upon spina bifida as a representative one for man.

About the same time Morgan and Tsuda,[3] in working upon the orientation of the frogs' eggs, subjected them to a great variety of solutions, and found that a .6 per cent solution of sodium chloride prevented closure of the blastopore. By them the nail was hit upon the head; the other investigators only obtained monsters occasionally (Hertwig incorrectly believed them to be due to polyspermy), but Morgan and Tsuda obtained them in great number. It was found that less than .6 per cent of salt did not affect the embryo and a stronger solution killed it. Successful specimens, and there were many of them, were examined from stage to stage in their development and the exact steps by which the blastopore is closed was followed. This gave them a decided advantage in study over the hap-hazard one in finding embryos already formed. The experiments were used mainly to study the orientation of the embryo in its relation to the lips of the blastopore.

The crucial experiment of Morgan and Tsuda was immediately seized upon by Hertwig[4] and employed in his experiments on spina bifida. Spina bifida could now be studied experimentally. Hertwig also found that a .6 per cent solution of common salt delayed the development of frogs' eggs, the intestines, chorda, myotomes and nervous system developing normally, but gastrulation was postponed for from twelve to twenty-four hours. As a result of this the spinal cord does not close posteriorly as rapidly as it should and permanent spina bifida follows. Often the walls of the spinal tube are thin and its lumen is small, showing that there is a general arrest of its development.

In general, Hertwig did not continue the experiments beyond the sixth day, for the salt caused marked changes in the exposed spinal cord. It seemed to be less resistant, inas-

[2]Von Recklinghausen, Virch. Arch., 105, 1886.

[3]Morgan and Tsuda, Quart. Jour. Micr. Sci., N. S. 35, 1894. Also, Morgan, Roux's Archiv, pp. 266, 269 and 293, 1902,

[4]Hertwig, Arch. f. mik. Anat., XLIV, 1905.

much as it underwent histolysis and cytolysis. The epidermis also showed changes; instead of being smooth on the outside it became rough, grew up into numerous papillomata, as Bardeen found in his X-ray larvæ, and as I have often found in pathological human embryos.

Hertwig explained Morgan's remarkable experimental production of spina bifida by assuming that the concentration of the salt retarded the growth of the cells of the egg and that the reduction of energy is unequal in different portions of the egg. Through this change differences in the rate of growth are established unlike those in the normal embryo, which naturally ended in the production of an abnormal embryo, that is, a monster. In this instance Morgan's sodium larva is the typical embryonic stage of spina bifida.

The spina bifida, although complete at first, rarely remains so, for the neural tube closes more or less, remaining open usually behind and often in front, giving quite typical specimens of anencephaly. It is clear, therefore, that this variety of spina bifida is also due to an arrest of development which could easily undergo secondary changes and produce a condition which is often found in fœtuses at full term. Hertwig concludes, properly so, I think, that every human ovum has within it the power to develop into a monster, either anencephalic or otherwise, and that it is not due to any abnormal condition of the germ, but to external influences which affect the growth of the egg. A monster is due to the influence of external substances which retard the growth of the embryo, usually one portion more than the other. For a long time teratologists have practically stated the same in recognizing that monsters usually represent arrestments of normal development. Not only is this true regarding merosomatous monsters, but every egg has within it the power to develop into a polysomatous monster, or into duplicate twins.

Later Hertwig[5] extended Morgan's experiments to Axolotl, thus making it applicable to at least six species of animals.

[5]Hertwig, Gegenbaur's Festschrift, II, 1896.

In this animal the monster lives much longer than the larvæ of frogs and toads do, and for this reason terata with spina bifida or anencephaly are obtained that resemble very much those found in man. It was found that a .5 per cent solution of NaCl produced no perceptible effect on Axolotl, that a .6 per cent solution made half of them grow into monsters, and in a .7 per cent solution all of them had spina bifida. In them it was found that the neural tube did not close regularly, and often several dorsal openings remained, some until the embryos were quite large. In frogs gastrulation was affected decidedly by the .6 per cent solution of salt; in Axolotl gastrulation remained normal in the .7 per cent solution, the change being confined to the brain and cord, but did not extend to its caudal end. The exposed cord underwent a certain amount of histolysis and cytolysis with more or less scar formation, thus resembling very much the condition found in spina bifida in man. At the conclusion of Hertwig's paper he rightly asks whether it is not possible for chemical substances in the blood, alcohol, toxines or doses of medicine, to pass from the uterus to the ovum in man and produce monsters. It is clear that he believes that monsters are not germinal and hereditary, but that they may be produced from every normal ovum through influences in its environment.

Schaper[6] has shown us, by producing anencephaly in tadpoles by mechanical means, that the rest of the animal grows normally without the presence of a brain. In fact, only the spinal cord degenerates after the brain has been removed. The experiment of Schaper has been further extended by Harrison,[7] who removed only the spinal cord, leaving the brain, before the spinal nerves are formed. In these experiments also the tadpole grows normally without a spinal cord or spinal nerves unless the operation interferes with the development of the lymph-heart, when dropsy follows. Harrison produced similar results in embryos in which the action of

[6]Schaper, Jour. Bost. Soc. Med. Sci., 1898, and Roux's Archiv, VI, 1898.

[7]Harrison, Amer. Jour. Anat., III, 1904.

the whole nervous system is thrown out by means of acetone-chloroform. The animals remain perfectly motionless and also develop dropsy, due probably to the effect of the acetone upon the heart of the animal. As I have mentioned above, Knower has shown that simple enucleation of the heart anlage causes an embryo to grow without a heart, which always has more or less dropsy, especially of the pronephros, while those in which the nervous system only has been removed are not thus affected. Therefore, when the nervous system is paralyzed by the action of acetone, which also retards the action of the heart, we must conclude that the dropsy of the embryo is due to the deranged heart and not to the damaged nervous system.

In their experiments, Panum and Dareste occasionally obtained spina bifida in chicks, not including those monsters in which the brain was deformed. Some time later Richter[8] found three cases of spina bifida among several hundred hens' eggs upon which he experimented. Otherwise these chicks were quite normal and no amniotic bands were found. This last point was considered to be of great importance, but now, since monsters are produced in animals without an amnion, it would be well, it seems to me, to relegate the amniotic theory of the production of monsters into the class into which that of maternal impressions has fallen. In Richter's cases, however, the spina bifida was more or less associated with anencephaly, and there were also specimens of exencephaly as well as a few of spina bifida occulta. In other words, the conditions here were more complicated than those found in the frog.

In my own specimens of human embryos there are at least twelve good ones of spina bifida. These are among 163 pathological ova, or about one case of spina bifida in every 200 pregnancies. Acording to Panum's table, there were 38 specimens of spina bifida among 404 monsters, or again about 10 per cent. If one monster results from every

[8]Richter, Anat. Anz., III, 1888.

hundred pregnancies, as my tables indicate, we then have one fœtus with spina bifida in 1,000 pregnancies, which is also Koch's[9] proportion. In other words, five young embryos with spina bifida are aborted early, while one goes on to full term or may live after birth.

The smallest embryo with spina bifida in my collection is 2.1 mm. long and in general appears normal. However, the brain is atrophic, is quite wide open and may be considered anencephalic. The cord below is also wide open, wider than in other embryos of this age which have been described. A similar but a little larger embryo has been described by Torneau and Martin (Fig 1, Plate I). Their embryo is 8 mm. long, apparently normal in form, with the spinal cord below wide open. Sections of the specimen showed that the spinal ganglia are present, lying on either side of the motor roots, which nearly encircled the chorda. There is also some histolysis of the cord. No. 189 is a case of complete spina bifida with marked histolysis and destruction of the superior end of the central nervous system.

The other specimens given in the footnote on page 27 show a variety of forms of spina bifida of the cord, probably the most interesting being No. 293, in which there is histolysis of the membrana reunions behind. A specimen like this may represent an early stage of spina bifida occulta. Otherwise the remaining specimens show a considerable destruction of tissues, both mesodermal and nervous, which makes them correspond more with the cases found at birth. Here the nervous tissue is quite vascular, often forming peculiar tissues, such as Von Recklinghausen[10] has pictured.

Recently Voigt[11] has described a case of cervical spina bifida in an embryo 18 mm. long, which was aborted fifty-four days after the last menstrual period. A much more satisfactory account of several specimens is given by Fischel[12] in

[9]Koch, Beiträge zur Lehre von Spina Bifida, Kassel, 1881.
[10]Von Recklinghausen, Virch. Archiv, 105.
[11]Voigt, Anatom. Hefte, XXX, 1906.
[12]Fischel, Ziegler's Beiträge, XLI, 1907.

his study of anomalies of the central nervous system in young human embryos. Fischel describes a case with multiple but irregular canal formation, which cannot possibly be viewed as a case of arrest of development. His other specimen is an embryo 10 mm. long, obtained from a woman who was perfectly healthy and aborted for unknown reasons. This embryo was apparently normal in every respect, with the exception of a well marked dilatation of the cord below just opposite the root of the leg. There was histolysis of the cord and the embryonic skin just over the hydromyelia, which Fischel believes indicates that spina bifida is preceded and caused by hydromyelia, that is, he accepts Morgagni's theory. At any rate, the great variety of malformations of the spinal cord which are grouped under the name of spina bifida cannot all be likened directly to Hertwig's spina bifida in amphibia, although in both there is considerable histolysis. Fischel's specimen, which is a very important one, shows conclusively that there is a destruction of tissues in the formation of spina bifida much the same as I have noted in the description of some of my specimens. In other words, the embryo was normal before it developed spina bifida. The relations of hydromyelia to spina bifida, and of hydrocephalus to anencephaly, have been discussed so much since the time of Morgagni, and my cases, as well as Fischel's, throw no new light upon the subject. Dropsy of the cavities and tissues of the body accompanies practically all pathological changes in the embryo, and it may be considered an effect just as well as a cause in these cases of spina bifida.

Embryo No. 6 shows an interesting condition in the lower part of the spinal cord similar to the first case described by Fischel. There is a marked vesicle coming off the cord between the motor roots of the two last spinal nerves, as may be seen in the illustrations. The lower end of the cord extends somewhat beyond the vesicle. The vertebral column ends just above the vesicle and is composed of two cartilages. Bardeen[13] has shown that the double arrangement of the last

[13]Bardeen, Amer. Jour. Anat., IV, 1905.

cartilage of the cord is of quite common occurrence among the normal embryos of my collection. I have looked through the normal embryos of about the same stage as No. 6, and in no instance have I found one with a vesicle like it attached. Among the specimens two occur with very small vesicles at the extreme tip of the cord, this being best marked in No. 22, an embryo 20 mm. long. Another embryo, 19 mm. long, No. 229, also shows this dilatation. It seems to me that in these cases there is only a slight exaggeration of the normal, while in No. 6 the vesicle is newly formed.

While the per cent of cases of spina bifida among pathological embryos and fœtal monsters is about 7 per cent of the total number of monsters in each case, it rises in anencephaly to about 20 per cent, that is, in 1,000 pregnancies there are 15 cases of anencephaly aborted very early and one case goes on to full term.

Among the embryos with changes in the brain that indicate the beginning of anencephaly there are many varieties of deformed brains that are exposed more or less. The brain may be escaping from the front of the head, the mid-brain may be exposed, or the medulla is distended and fills the whole atrophic head, as the various figures show. In most of the specimens there is a marked histolysis of the surrounding tissues as well as of those of the brain, with vascular metamorphosis of the brain tissue, as is shown in specimens like Nos. 364 and 365. In these cases we cannot speak of a simple arrest of development only, but also of a destruction of tissue, histolysis and necrosis, or parenchymatous inflammation, as Panum would call it. These specimens are discussed sufficiently under various headings further on, and under the descriptions of the embryos in the last portion of this paper.

It has been shown that typical spina bifida and anencephaly can be produced in a number of species of amphibia by Morgan's experiment, that is, by cultivating the eggs in dilute solutions of NaCl. This causes an arrest of development of the embryo, which is decidedly more marked in the central nervous system than elsewhere. There is also a more or less

marked histolysis of the cord and brain. Similar changes can be produced in birds, while in man the number of cases of spina bifida and anencephaly are at least ten times as numerous in the embryo as in the fœtus.

In man, however, the pathological changes in the embryo are very marked and complicated by an arrest of the development of the heart or by its complete destruction. In my specimens no doubt the destruction of the heart must be held responsible for the general œdema and the marked histolysis of many of the tissues, including those of the brain. It may be that the faulty implantation of the ovum affects the heart first and that the changes in the nervous system are produced secondarily, but our data are too meager to allow us to draw any conclusion regarding the sequence of events. At any rate, there must be other factors at work which make the process more complicated than it would be if there were only a simple arrest of the development of the spinal cord. The other changes are in the region of the spinal cord and canal and aid in producing the various forms of spina bifida, including spina bifida occulta, which are found in the fœtus and at birth.

In some instances, in which the individual lives after birth, the primary change must have been of a slight degree to begin with, and the faulty implantation of the ovum must have been corrected, or in case the ovum was poisoned, the disease must have been eliminated in order to allow the embryo to continue its development. However, very simple or uncomplicated cases must be very rare, for spina bifida is usually accompanied with other malformations, as is the case with most monsters.

Monsters in Which Tissues Must Have Been Destroyed —Magnesium Monsters, Cyclopia and Club-Foot.

It has been repeatedly noted by experimental teratologists that, whenever the malformation of an embryo is slight, there is a general retardation of development of the whole body which is more marked in some portions of the embryo than in others. Thus it is stated that the tail of an embryo is atrophic, or even club-shaped, a condition which cannot be brought about without some destruction or rearrangement of its tissues.

In the human embryo there are all gradations of form between typical anencephaly, atrophic head and deformed head; in fact, pure types are rarely seen. These varieties can be brought together under the general heading of atrophic heads. The tissue change in them ranges from histolysis to dissociation or even to maceration. In a general way the following table gives the frequency of variations of pathological embryos and of monsters per 100,000 pregnancies. The figures from which the table has been constructed will be found on pages 26, 27, 65 and 66, and may be considered to be fairly reliable:

Pregnancies.	Births.	Normal Embryos and Fœtuses.	Pathological Embryos.	Monsters.
100,000	81,000	12,000	7,000	615
Hydrocephalus			....	119
Deformed head			953	....
Anencephaly			574	119
Spina bifida			410	47
Face deformed			697	96
Deformed eye			123	25
Deformed extremities			697	115

The figures given under monsters are actual figures, those for hydrocephalus, etc., being from Panum, in which the total number is 618. This is practically equal to the 615 which I have obtained from various authors. My data, which are from 163 pathological ova, were multiplied by 41 to bring

them up to the total estimated number of pathological ova per 100,000 pregnancies. "Deformed heads" I have paralleled with "hydrocephalus," but I do not mean to infer that one is directly related to the other. Otherwise the subdivisions coincide. It is remarkable that in each instance there are about six of a given variety of embryonic monsters for each at full term. At any rate, the constancy of the ratios speaks volumes in favor of the genetic relation of monsters to pathological embryos.

In my collection there are five specimens of exomphalos (Nos. 115, 162, 166, 244 and 364), mostly in very young stages, in which there is an extreme degree of atrophy of the embryo. It is a question whether any of these embryos in which the atrophy is so extreme could possibly have lived much longer, for in them the body of the embryo is nearly destroyed.

In many of the specimens there is a marked distention of the central nervous system, and it would probably be more to the point if they had been classed with hydrocephalic monsters. The frequency with which hydrocephalus and dilatation of the embryonic nervous system are encountered makes it questionable whether anything is to be gained by the comparison.

As Giacomini has pointed out, the amnion is sometimes partly destroyed or is wanting entirely. More often, however, there is hydramnios, as is also the case with monsters. The excessive secretion of fluids into the cavities of the body and into the amnion is often accounted for by a supposed interference with the circulation, and this theory is supported by removing the heart in young tadpoles (Knower's experiment), which is always followed by general dropsy.

To come back to the deformed heads, in which there is not only an arrest of development, but also an actual destruction of many of the tissues, the head is more or less necrotic or stubby, often the brain is exposed, either in front or over the mid-brain, or the whole brain may be wanting. In some cases the lumen of the brain communicates directly with the

exterior of the body, the edges of the opening often being rounded, that is, there has been an attempt to repair the wound. In other instances the whole brain has been destroyed and the medulla is markedly dilated and fills the whole of the stumpy head. In such specimens the face is more or less deformed and may be adherent to the thorax below without an intervening neck. So, with destruction of tissue, there is a slow and continued growth, for if there were not the chin and thorax could not have united.

There are all kinds of deformities of the face, from a simple atrophy, in which the external features are obliterated, to atrophic jaws, deformed or closed mouth, hare-lip, absence of the neck, or ears, which are not developed, are deformed, pointed or displaced. Such changes could not take place without a marked destruction of tissue and an attempt at further growth, which is necessarily irregular. Probably the most pronounced of all deformities of the face are those associated with the eyes, and my collection contains one specimen without eyes (No. 285), one with the eyes deep in the head (No. 135), and one with cyclopia (No. 201). The last belongs to the wonders among monsters, which has interested the thinking world for centuries.

In cyclopia the eye is in the middle of the face, is often partly double, the nose is above the eye, and the cerebrum is atrophic and usually single. Teratologists are in the habit of holding the single brain as primarily responsible for the condition, and I think they are right. We have seen repeatedly that the central nervous system suffers very frequently in pathological embryos, and a slight atrophy or destruction of the front of the brain in an early stage (like No. 12) might easily end in cyclopia. We cannot admit, however, that the tendency to produce cyclopia exists in the ovum, nor that a close-fitting amnion did the mischief, as there is no evidence for these theories, and the facts are against them. If, however, the brain is malformed and the lack of correlation of growth of the parts does not push the frontal process down rapidly enough, the eyes move towards each other and unite. All this has been proved experimentally.

Ten years ago Born,[1] in making numerous experiments upon frogs' eggs, occasionally produced cyclopia by splitting the head through its sagittal plane after the medullary plate is formed and then readjusting the halves. They united at once, but in a few instances a double eye was formed. Later Spemann[2] made similar experiments, and he also produced cyclops embryos. In some of Spemann's experiments Triton eggs were ligated in the sagittal plane during segmentation, and frequently embryos resulted with double heads, one or both being cyclops. He believed that this experiment proved that the anlage for the cyclops eye was defective from the beginning and is not produced by concrescence of two anlages. Levy[3] also produced cyclops embryos by cutting off the front of the head of Triton larvæ. In the course of two weeks the two eyes approached each other and formed a double eye, but they were not fused; the pigment layer became destroyed, or at least was absent at these points. The two optic cups touched each other.

A year ago Harrison produced a new variety of cyclopia by removing the entire brain from frogs' embryos. The eyes moved to the back of the head in these specimens and appeared to unite into a single vesicle in the region usually occupied by the pineal eye. By pricking the extreme anterior end of the embryonic shield in Fundulus eggs Lewis found, in 1905, that many of the eggs developed into cyclops embryos. All stages of eyes were formed, from a double eye, and hour-glass eyes with two lenses, to oblong eyes with either two lenses or a single lens. The optic cups blended absolutely, thus proving the mode of development in these eyes. Lewis also found that in many of the embryos the brain had not been injured at all, but the prick had destroyed the nose only. This experiment shows conclusively that it is the absence of tissues between the eye anlages that allows them to come

[1]Born, Roux's Archiv, IV, 1897.

[2]Speman, Roux's Archiv, XV, 1903, and Zool. Jahrbücher, VII, Supplement, 1904.

[3]Levy, Roux's Archiv, XX, 1906.

together and unite, and that a rudimentary brain is unnecessary. The experiments of Harrison and of Lewis have not been published, and with their permission I have made this note of them.

Finally, since the above was written, the remarkable experiments of Stockard,[4] of New York, made their appearance. Stockard found by placing the eggs of Fundulus into a solution of $MgCl_2$ that 50 per cent of them develop cyclopia. In them the two optic cups wandered towards each other and united, much as was the case in Lewis' specimens in which the embryonic shield had first been pricked. The union of the two cups formed a large compound cup, which in turn derived its lens from the epidermis immediately over it in the middle line of the embryo. How the magnesium acts upon the embryo is not clear from Stockard's description. No doubt it will be found that it retarded the growth of the frontal process much as is the case in Lewis' experiments. However, the salt acted also upon the whole body of the embryos, for their development was retarded, making them smaller than usual, and their circulation was feeble, but they did not die. In them, as in Lewis' experiments, the growth of the brain was normal.

The remarkable experiments of Stockard set at rest all germinal theories of cyclopia, and prove that every egg has in it the power to develop cyclops monsters.

At any rate, these experiments, as well as the numerous pathological embryos with deformed heads and faces, prove that there is an extensive destruction and shifting of tissues in the formation of monsters. This is also well illustrated in the production of club-foot in the human embryo. It has frequently been noticed that tadpoles whose development had been arrested formed stubby or club tails and fins, a condition that corresponds well with club-shaped extremities in man. In my collection there are eighteen embryos with deformed legs or feet, ranging from the very earliest period

[4]Stockard, Roux's Archiv, XXIII, 1907.

until the fœtus is well formed. The leg bud is filled with condensed mesenchyme and is irregular in shape, sometimes being stubby on one side of the body and normal on the other. The study of the larger embryos shows that there is a kind of "inflammation" in the deformed extremity, there being an "infiltration" of cells, which is especially well marked in the tendons and around the cartilages. In general, this condition may be accounted for by a general arrest of development due to impaired nutrition. At any rate, embryos that are not developing well, experimental larvæ, and human embryos with other malformations, often have club-shaped arms, legs, fins and tails.

PATHOLOGICAL OVA.

As we pass up the vertebrate scale it becomes more and more difficult to ascertain the primary causes which produce pathological ova, and presumably monsters. In fact, the causal study of teratogenesis has been and still is one of the capital problems in medicine which is gradually being solved by anatomists. It has been stated repeatedly in this paper that the missing link to complete the chain of evidence is to be found in the careful study of aborted ova which are found to be more or less diseased. In the excellent monograph by Granville[1] we find a report of the study of forty-five aborted ova, from which he concludes that the chorion is first diseased, which naturally results in retarding the growth of the embryo. He notes that an inflammatory condition must have been present in the uterus, for the abortion of pathological ova is usually accompanied with great pain and an excess of hemorrhage.

I have been unable to obtain valuable data regarding the condition of the uterus in early abortions from pathological, gynecological or obstetrical literature. It is all clouded in

[1]Granville, Graphic Illustrations of Abortion, London, 1834.

mystery, and one finds an endless contradiction of opinions. It seems to me that a study of the norm, uterus and chorion is required before much headway can be made. In my opinion, this is possible only in some great clinic which has attached to it a first-class laboratory manned by able investigators. However, for the present, we must do the best we can with the data at our disposal. First, I shall quote from several competent recent writers.

Ahlfeld states in his treatise on obstetrics[2] that many abortions are due to endometritis, which produces inflammatory adhesions of the placenta and membranes; hypertrophy of the decidua is associated with abnormal forms of the placenta, which is followed by an arrest of the development of the embryo. Furthermore, atrophic endometritis is commonly followed by the formation of an atrophic decidua, which in turn must retard the growth of the ovum. In addition to these forms there is a condition known as hemorrhagic endometritis, due to a variety of infections. The hemorrhages which take place in the chorion or placenta are often accompanied with bacteria or may be due to nephritis, which may be followed by decidual infarctions and death of the embryo. In these cases the effused masses of blood are in successive layers of old and new clots, forming a tumor known as decidua tuberosa. In case the bleeding continues after the death of the embryo the chorion may be converted into a fleshy mole.

Ahlfeld further states that repeated abortions are due to endometritis or to syphilis, but the second abortion need not by any means be due to the same cause as the first. If due to syphilis successive abortions occur later and later in pregnancy. Syphilis, and possibly gonorrhœa, causes abnormal development of the decidua; in chronic endometritis the decidua undergoes diffuse hypertrophy. According to Virchow, syphilis causes knotty development of the decidua in case the mother is infected; in case the father is infected the primary change is found in the chorion.

[2]Ahlfeld, Geburtshilfe, 1903.

According to Williams[3] "the death of the fœtus is frequently due to abnormalities in the development of the embryo which are inconsistent with fœtal life. More often, however, it results from changes in the fœtal appendages, which interfere with its nutrition, such as excessive torsion of the cord, producing hydramnios, hydatidiform mole or syphilis. . . . Abnormalities of the generative tract likewise play an important part in the etiology of abortion. Thus developmental anomalies of the uterus, or imperfect development of the normally formed organ, may be responsible for conditions which are unfavorable for the implantation of the ovum and later for the development of the placental circulation. Chronic metritis is supposed to act in the same way. . . . The most important factor in the production of abortion is afforded by diseases and abnormalities of the decidua. In hypertrophic forms of decidual endometritis—decidua polyposa—the bulk of maternal blood brought to the placental site goes to nourish the hyperplastic decidua, while in the atrophic forms the conditions are unfavorable for the normal implantation of the ovum and the development of the placenta. More important still is the part played by chronic glandular endometritis and acute inflammation of the decidua. The former is usually acompanied by hemorrhagic changes, and is the most frequent cause of abortion in the early months.[4]

I gather from conferences with competent scientists of large

[3]Williams, Obstetrics, New York, 1903, p. 522.

[4]Marchand, writing on moles, says in Eulenberg's Encyclopedia, Vol. 15: "Abortives Ei ohne Spur eines Embryo oder mit mehr oder weniger unbekanntlichen Resten derselben. Ein sehr häufiges Vorkommniss bei Aborten, welche wohl in den meisten Fällen durch frühzeitige Unterbrechung der Ernährung infolge beginnender Lösung des Eies von der Uteruswand, Blutungen in der Decidua basalis und capsularis oder durch vorausgehende Erkrankungen der Uterusschleimhaut bedingt ist. Zuweilen findet sich ein knötchenförmiger Rest des Embryo an der Innenfläche oder ein Rest des Nabelstranges oder eine mit Flüssigkeit gefüllte Blase. Ist der Embryo nicht vollständig zu Grunde gegangen und erfolgt die Ausstossung des Eies nicht, so können anderweitige Missbildungen die Folge sein. Bei der Ausstossung findet man die Decidua basalis und capsularis mit Blutextravasaten durchsetzt (Blutmole)."

experience that "uterine scrapings after abortion rarely show signs of endometritis, although they contain many leucocytes and characteristic masses of fibrin. When the abortions from one woman are frequent she is undoubtedly syphilitic." Another argues that endometritis rarely shows the presence of inflammation, and states further that inflammation of this organ is usually confined to the cervical canal. Still another states that endometritis, which is a rare affection, is usually due to the gonococcus or sometimes to an acute infection. At this place it may be pertinent to state that pathological ova and monsters, which are quite frequently found in other mammals, cannot be due to syphilis or gonorrhœa, but are often accompanied with a peculiar kind of separation of the chorion. In such specimens a large mass of mucus and no blood encircles the ovum, and from all indications the embryo has died suddenly, for it is not deformed. It is not necessary to introduce more opinions, for they will not lead us nearer to a solution of the problem. For the present, the opinions as expressed by Ahlfeld and by Williams are the best at our disposal. Both are able scientific obstetricians, Ahlfeld being in addition a teratologist, and Williams a leading obstetrical pathologist.

It is well known that a woman who aborts a pathological ovum or gives birth to a monster will probably abort again, and runs a greater chance of giving birth to a second monster. Teratologists are inclined to read these facts in favor of the germinal origin of monsters, which may even be hereditary. Since there is no recorded case of a woman giving birth to a second polysomatous monster, while there are numerous cases in which women bore second merosomatous monsters, we can as well consider the former as "accidental" and the latter as due to some change in the uterus and not inherited through either the germ or the sperm. (Certain varieties like those of the extremities and anatomical anomalies must be excluded from this discussion, for they are known to be germinal and are hereditary.) To be sure, we cannot exclude the possibility of a certain per cent as being germinal, that is,

there was some change in either of the germs before fertilization took place. On the other hand, experimental work on amphibian, fish and bird embryos shows that monsters can be produced with ease from perfectly normal fertilized eggs. In general the methods employed by experimental teratologists is to subject the eggs to various insults which affect the nutrition and impair the growth of the embryo. If now a similar condition can be found to exist for human pathological ova which corresponds with those the experimental teratologist produces, the point is proved, that is, many merosomatous monsters may be formed by placing normal ova into an unfavorable environment. All of our experience in teratogeny, if read aright, indicates that the normal ovum got into a diseased uterus did not implant itself well, and the consequent impairment of nutrition produced a monstrous embryo. This hypothesis, which will be proved to be correct under the heading of tubal pregnancy, explains fully the presence of so many pathological embryos in multiple abortions and the apparent germinal origin of merosomatous terata like spina bifida and anencephaly.

His,[5] in the discussion of normal and abnormal embryos, is rather of the opinion that pathological embryos are due to primary changes in the germ, and that their abortion naturally takes place because such ova act as foreign bodies in the uterus. In some instances, however, he excludes the possibility of the primary cause being due to an interference with their development, such as may be brought about by deficient nutrition, lack of oxygen and mechanical influences due to the uterus being displaced. Later,[6] in a discussion of open questions in pathological embryology, he seems to be inclined to abandon the theory of the germinal origin of pathological ova altogether, for the examination of several specimens showed that the changes within them were of a secondary nature. They indicate that the embryo is in process of dying, that

[5]His, Anatomie menschl. Embryonen, II, 1882.

[6]His, Virchow Festschrift, I, 1899.

is, the tissues of an embryo as normally formed have become swollen, are disintegrating and strange cells are wandering through them. In His's opinion such changes cannot be viewed as primary, but rather as secondary conditions.

The other student of pathological embryology, Giacomini,[7] emphasizes the necessity of studying the form and structure of the decidua in normal as well as in pathological ova, for at this point mechanical and nutritive influences must occur, which are of prime importance in the production of early pathological embryos. He predicted that such a study, together with experiments upon lower animals, would ultimately explain the origin of monsters.

There is one more opinion, from the hundreds upon this subject, which I must not omit. It is from O. Hertwig,[8] in his more or less general article on the production of spina bifida in Axolotl. After stating that a .6 per cent solution of NaCl will produce spina bifida in frogs and a .7 per cent solution will produce the same kind of monster in Axolotl, he asks whether it is not possible that some similar method is employed by nature to produce spina bifida in man? Is it not possible for chemical substances in the blood—as alcohol, toxines or medicines—to pass from the uterus to the ovum and make it monstrous? Evidently he believes that the power to become monstrous is not inherited, but is due to external influences.

It is extremely difficult, if not impossible, to prove directly that the primary changes which produce pathological ova are in the chorion and not in the embryo. I find in glancing over the tables which follow, with the discussion of the individual specimens, that among 143 pathological specimens but fifteen appear to have a normal chorion, and that in thirty-five the chorion is sufficiently infiltrated with leucocytes to indicate that some inflamamtory process was present in the uterus. In all of the specimens excepting the fifteen in which the chorion

[7] Giacomini, Merkel u. Bonnet, Ergebnisse, IV, 1894.

[8] O. Hertwig, Gegenbaur's Festschrift, II, 1896.

appears to be normal all kinds of secondary changes have taken place. The mesodern is fibrous, hyaline or œdematous, the villi are atrophic, hypertrophic or missing altogether, and the syncytium is irregular or necrotic, and sometimes it has attacked and invaded the mesoderm of the chorion. The decidua when present is usually infiltrated with leucocytes, which often accumulate in great masses, or often form abscesses. All this could take place if the embryo had died and the ovum had continued to grow, but on account of the presence of a dead embryo the uterus reacts as if it had a foreign body to expel. In fact, most of these changes just enumerated probably took place long after the embryo had become monstrous, and we are no doubt treating with the primary process, much intensified by the presence of a pathological ovum. The final proof in favor of the theory that these changes are primary will be given under the discussion of tubal pregnancy.

It will be noticed that the "normal" chorion is most common in young ova, that is, before the process of destruction has been under way for a long time. In an earlier publication[9] upon this subject I was much inclined to the idea that the primary difficulty in a pathological ovum is to be sought in the embryo, but later[10] I formed the specimens into two groups: (1) Those in which the primary cause lies in the embryo, and (2) those in which it is outside of the chorion. This gradual change of my ideas is identical with that which both His and Giacomini passed through, for all of us based our conclusions upon a simple morphological study. The morphologist must be very careful in the arrangement of his sequences, and I think it is to our credit that we have been so. But now, since we have experimental teratology and a more careful study of the gynecological history of the specimens to fall back upon, it seems to me that the solution of the problem is at hand.

The ova which appear to be normal, but have within them deformed embryos, or none at all, are the ones that require

[9]Mall, Welch Festschrift, J. H. Hosp. Rep., IX, 1900.

[10]Mall, Vaughan Festschrift, Ann Arbor, 1903.

our most careful consideration, for in them we are to find the first pathological changes. In studying the villi of the chorion in these specimens I tried to remain on the safe side when I stated that they were fibrous or œdematous, and no doubt erred correspondingly when I stated that others were normal in structure. In the course of time I found that in most chorions which were markedly pathological a stringy mass of fibrin or mucus more or less rich in leucocytes was found between the villi. In specimens undoubtedly normal and containing a normal embryo this stringy mass was never found. Occasionally a stringy mass was found between the villi in ova which appeared to be perfectly normal. A good example is found in an ovum which appeared perfectly normal with the exception of a lateral pouch to it, containing an embryo four millimeters long which is slightly deformed[11] (No. 80). Sections of the villi show that they are perfect in form, and in structure, being covered with a well-developed syncytium. Between the villi there are strands of a fibrin-like mass, in which there are imbedded a number of leucocytes. Another specimen which has been described by me as a normal one contains a similar substance between its villi[12] (No. 12). In this specimen there is an unusually well developed magma reticulé and the head is underdeveloped. The neural tube is wide open at both ends, and it seems to me that its form is not quite normal. It came from a woman twenty-three years old who had been pregnant twice, aborting both times.

Two other specimens may be mentioned, one which I have also described as a very young normal ovum because I knew that the abortion had not been a natural one.[13] The woman had had a continuous hemorrhage for seven days before the abortion, and since then I have learned that the detachment of a normal ovum for a much shorter time than seven days is

[11]Mall, Johns Hopkins Hospital Reports, IX, Fig. 80.

[12]Embryo No. 12, Journal of Morph., X, 1897, Arch. für Anat., Suppl. Bd., 1897, Johns Hopkins Hospital Reports, IX, Fig. 12.

[13]No. 11, Anat. Anz., VIII, 1893, Journal of Morph., X, and Johns Hopkins Hospital Reports, IX, Figs. 14 and 15.

sufficient to cause an embryo to become monstrous. Specimen No. 250 of this communication is about as old as No. 12, only it is slightly more deformed. It had been removed with a curette from a woman who was suffering from uterine trouble. The decidua which encircles the ovum is well infiltrated with leucocytes, showing that the decidua was inflamed. These four specimens are representative. One was detached by mechanical means, one was removed by a curette on account of endometritis, and two were spontaneous abortions of ova which appeared to be normal but contained a stringy mass between the villi. This condition is usually well marked after the chorion has undergone radical changes and is well infiltrated with leucocytes, which often form into small abscesses.

In the following table I have brought together all of the pathological ova in my collection in which there is any history of the women from whom they were obtained. Positive as well as negative histories are given.

A glance at this table shows that in eleven cases the main trouble preceding the abortion was a severe hemorrhage extending over a number of days. In a second set of twelve cases the abortions were from first pregnancies in women newly married or who had been married for some time and were anxious to have children. In the third group of ten cases the women had given birth to a number of children and then began to abort, often a second or third time. The first group need not be considered further, but the second group consists of women who are naturally sterile and abort when they become pregnant. The third group of ten cases is more easily understood. The women, perfectly healthy, gave birth to one or more children and then conceived but aborted quite regularly. In these cases we must admit that the uterus was at first perfectly healthy and the ovum was normal, but later, due to a variety of infections, the uterus became "inflamed," and thereafter the fertilized ovum could not implant itself, became pathological, and later was aborted. According to the data given, seven of the mothers were healthy and twelve had uter-

No.	Condition of Mother.	No. of Child'n	Remarks.
11	Apparently normal.	Some	Hemorrhage for 7 days.
12	Married 3 years.	None	Two abortions.
32	.....	?	Hemorrhage 4 days.
58	.....	.....	First pregnancy.
70	.....	1	Great flooding.
71	Chronic cystitis and endometritis.	None	First pregnancy, gave birth to a child a year later.
87	.....	.....	Hemorrhage for 12 days.
110	Uterus large and retroverted.	9	Hemorrhage 5 days. See No. 141.
122	.....	.....	Hemorrhage 8 days.
133	Perfectly normal.	.....	Hemorrhage 8 days.
134	.....	.....	Mechanical injury to ovum.
141	Uterus large and retroverted	9	See No. 110.
142	.....	3	Hemorrhage 4 days, third abortion, one 3 mos., and one 20 mos. ago.
152	Endometritis,	Some	Third successive abortion, each time in third month.
159	Perfectly healthy father and mother. No indication whatever of endometritis.	None	Married two years. This is second abortion at third month. Repeated hemorrhage during pregnancy. Anxious to have child.
161	Purulent leucorrhœa. Had tube introduced 4 weeks before abortion.	?	
162	Not the slightest indication of uterine disease.	5	Bleeding for two weeks before abortion.
205	Syphilis suspected.	None	Married three months.
209	30 years old.	?	Three years ago miscarriage in third month and 3 months ago gave birth to a monster.
226	.....	3	.....
228	Fairly healthy.	None	First pregnancy.
230	Always menstruated regularly during pregnancy.	3	Three other miscarriages.
246	Youngest child 7 years old. Since then miscarriages.	2	Five miscarriages, all about the same size as this one.
250	.....	?	From uterine scrapings.
252	First pregnancy in an unmarried woman.	None	Continuous hemorrhage for a month.
278	Chronic endometritis.	?	From uterine scrapings,
292a	Was curetted two years ago for menorrhagia.	None	First pregnancy.
297	.....	?	From uterine scrapings.
308	.....	?	From the same woman.
325	.....	?	
330	.....	None	One other abortion in eighth month.
364	Uterine trouble.	None	First conception in a woman anxious to have a child.
395	Removed on account of eclampsia.	?	From uterine scrapings.
399	Woman a marked bleeder.	None	Married ten months.
402	Subinvolution of the uterus.	2	

ine disease. Although this division does not correspond with the above three classes, in a general way it is suggested that women who are called normal abort with much hemorrhage, while the ones with uterine disease belong to the second and third classes mentioned above. Although these data indicate that pathological embryos are due to faulty implantation of the ovum, they by no means prove it. All of the ova in the third group of ten cases could certainly not have been destined to become pathological, for they all came from women who had given birth to healthy children. They could not attach themselves successfully to the diseased uterus, and, due to malnutrition or poisons which are thrown out from inflamed surfaces, the chorion became pathological and the embryos deformed. This point is fully proved, I believe, in the study of ova from tubal pregnancies.

Twin Pregnancies.

Especially instructive and interesting are those cases in which two pathological ova were obtained from the same woman. Five such sets are found in my collection which I shall describe. The first set, Nos. 308 and 325, are from a woman who had born two children during the previous two years, and are especially valuable in this discussion. The first ovum (No. 308) appeared to me perfectly normal, and the embryo within it was not changed at all. However, the amnion was found filled with a jelly-like mass of granular magma, and this aroused my suspicion. Sections were therefore cut from the placenta at the attachment of the cord and a stringy mass rich in leucocytes was found between the villi. They were normal in form and possibly their mesoderm was fibrous in structure. Nine months later a second ovum was obtained from this woman, which was decidedly pathological. Both the chorion and the embryo were much changed, as the figures and description of the specimen will show. This case, which should be observed further, is to be explained by disease of the uterus, which began after the birth of the second child. This change had not gone far

at the time of the first abortion, but was more advanced at the time of the second abortion. (Later the woman died of pneumonia. See history of No. 308.)

The second set, Nos. 110 and 141, came from a woman who had had nine children, after which she broke down in health, about ten years ago, when she conceived quite regularly, but aborted each time. The two specimens, which are about a year apart, are much alike, no doubt due to their subjection to the same environment. The chorions are markedly changed and the embryos are macerated and very much deformed.

The third set, No. 330a and b, are twin ova from a woman who had aborted once before. These two specimens show practically the same changes in the chorions and in the embryos, as may be seen by the figures and the description.

The fourth and fifth specimens, Nos. 207 and 341, form two sets of duplicate twins. Unfortunately, no histories accompany either set of specimens. However, in each set the changes within the embryos are about the same degree, but, of course, these sets do not throw any light upon the question whether the primary change was in the germ or in its environment.

The history of the first three sets, however, speak decidedly in favor of the hypothesis that the ova were normal to begin with, and the pathological changes within them are due to the diseased condition of the mucous membrane which surrounded them. The implantation was faulty and a variety of other complications was present to interfere with the nutrition and growth of the embryo, which consequently became deformed.

Very recently Dr. West sent me two ova (Nos. 384 and 419) from a woman with an undeveloped uterus of infantile type. She had been married three years, became pregnant twice and aborted on the fifty-fourth and on the fifty-ninth days. The chorions are covered with degenerated villi, which are imbedded in and encircled by much blood. Both are markedly pathological and each contains a deformed embryo about 3 mm. long.

Unruptured Tubal Pregnancies.

It is of interest to consider together the ova obtained from tubal pregnancies, for it is through them that light may be thrown upon the question, if the embryo is pathological, whether its condition is inherited or is due to the bad environment of the ovum. In case it is the former, the per cent of pathological embryos should not be larger than those obtained from the uterus; in case it is due to the latter, the per cent should be increased.

It is stated by different writers that embryos are rarely found in tubal pregnancies, but that remnants of the chorion are often present. However, it is also stated that, in case the tube is found ruptured and much blood has escaped into the peritoneal cavity, the embryo may have been present, but could not be found on account of the great quantity of blood. On the other hand, Professor Brödel informs me that among eleven specimens of tubal pregnancies found recorded in his catalogue of human embryos nine contained normal specimens. In my own collection seven tubal pregnancies out of nineteen specimens contained normal embryos. It must be remembered that as a rule specimens were sent to us only in case the surgeons who removed them found normal embryos, which they thought we were collecting. Considering only the tubes that were sent to me unopened and excluding those which were obtained from Dr. Kelly's gynecological laboratory, I find among seven specimens two ova without embryos, four with pathological embryos and but one with a normal embryo. The other six normal embryos spoken of above were all recognized by the surgeons as "normal and valuable specimens" before they came into my hands.

Following the hint obtained by considering all of the specimens which came to me unopened, I collected all of the histories of the same kind of specimens from Dr. Kelly's laboratory. These cover a period of about ten years and are taken from the laboratory records of over 10,000 miscellaneous cases. I find that altogether 128 cases of tubal preg-

nancy were carefully described after numerous sections of them had been examined microscopically. I have excluded the reports of 82 of the specimens, for in them the tubes had ruptured before the operation. Of the 46 that remain the histories state that they were unruptured and vary from one to six centimeters in diameter. Two of the 46 contained normal embryos of the second month and five of them pathological embryos. The rest, 39 in number, contained entire ova without embryos or simply villi of the chorion in various stages of degeneration. Usually the dilated tube was found filled with blood through which were scattered villi, the chorion rarely being intact, that is, encircling the cœlom. The chorion had collapsed, leaving scattered villi, which were "degenerated," "poorly formed," or "necrotic," in different cases. Usually, it is stated in the record, "scattered villi were found in the clot; no embryo was found."

The normal embryos need not be discussed more than to mention that the amnion was very small, as is usually the case in these specimens. The pathological specimens, however, are of the same nature and degree of degeneration as those found in the specimens obtained from the uterus. A number of small specimens which were cut into serial sections contained no embryos at all; they are included among the 39 mentioned above. From my experience in searching for embryos in pathological ova I am of the opinion that a few more pathological embryos would have been found had the specimens been examined with greater care. It is unlikely that more normal embryos would have been found, for in all cases they lie in a cœlom or an amnion filled with a clear fluid. I have never found a normal embryo in an ovum which did not contain a cavity well marked by a sharp wall and filled with a transparent fluid, and therefore think it unlikely that those who made the sections for microscopical examination overlooked any normal embryos.

From my records not over seven per cent of uterine pregnancies contain pathological embryos and were the primary cause which produces them located in the germ we

would not expect a higher per cent in ova from tubal pregnancies. Instead, we find that 96 per cent are pathological and but 4 per cent normal (two in 46 specimens). Since this point is of prime importance in the causal study of terata, I have brought together all of the pathological ova I have obtained from tubal pregnancies. These have been studied with greater care, as a number of them have been cut into serial sections. Most of them will be found figured among the illustrations of this article. The following table shows that there are 14 specimens, of which seven contain pathological embryos and six are entirely free of them. Nearly all of the ova are very small, and in practically all of them the chorion is markedly affected. Generally the mesoderm of the chorion is fibrous and atrophic, the villi also showing all kinds and degrees of degeneration. Occasionally some of the villi are hypertrophic:

Number.	Dimensions of Ovum.	Embryo.	Condition of Chorion and Remarks.
	mm.	mm.	
158	12 x 6	2, Vesicular	Atrophic.
196	12 x 12	3, Homogeneous	Atrophic—Some villi are enlarged and invaded by syncytium.
298	4	None	Fibrous villi partly infiltrated with leucocytes.
324	45 x 45	3.5	Atrophic and fibrous. No syncytium.
342	30 x 20	5	Atrophic and fibrous.
348		6, Atrophic	
361	10	None	Cœlom filled with a dense magma.
367	10 x 7	None	Villi degenerated in part.
369	7 x 3	None	Villi fibrous and degenerated.
378	12	None	Villi œdematous.
396	7	2, Vesicular	Mesoderm and villi fibrous, some invasion by syncytium
Plate II, Fig. 6	8 x 6	None	
Plate II, Fig. 5	6	Vesicular (?)	
Plate II, Fig. 7	60 x 20	11	Chorion hypertrophic and embryo disintegrating.

In most instances the cœlom is filled with a dense magma and in six the embryo is entirely wanting. The embryos in the remaining seven are of the vesicular form in three, of

the cylindrical form in four, and are necrotic and disintegrating in the remaining specimens. In general, the changes in the chorion and embryo in these 14 specimens are the same as in those that are obtained from uterine pregnancies. It cannot possibly be admitted that the primary difficulty in these specimens is to be found in the embryo itself, that is, it is germinal, for the ova which become lodged in the tube are probably of an average kind, unless the unreasonable stand is taken that there is a greater tendency for abnormal than normal ova to lodge in the tube. To take this stand it is necessary to overlook altogether those cases in which tubal pregnancy is due to mechanical obstruction of, or to diverticula from the uterine tubes. The results obtained from the study of these 14 specimens are probably representative of all tubal pregnancies which are examined with great care before the tubes rupture. In the very earliest specimens there are indications of faulty implantation, due no doubt to the character of the tissue of the tube which permits of an excessive hemorrhage around the ovum (*e. g.*, No. 396). Only in rare instances does a good decidua form in the tube, which in these cases must be produced by the presence of a growing ovum. However, just in these cases a decidua develops in the uterus, although the ovum is not present there.

I have found in collecting 434 human embryos of all kinds that 163 of them, or 38 per cent., are pathological. If we consider that an abortion occurs in every fifth pregnancy, then a pathological ovum is found in every twelfth pregnancy (7 per cent in the table). If anything, this number is too high, for a number of larger normal fœtuses were not catalogued and are not included with the total number—434. If the data obtained from unruptured tubal pregnancies where the number of pathological specimens rises to 96 per cent are compared with the pathological specimens from uterine pregnancies (7 per cent), it seems to me that the argument against the germinal origin of pathological ova and monsters is overwhelming.

The relation of the chorion to the wall of the tube or to the mucous membrane of the uterus is well known for ova

two millimeters in diameter or larger. The two structures become beautifully adjusted, but in the case of most tubal pregnancies the small ova and villi float largely in a mass of blood, are not adjusted to the decidua, and, apparently, on account of impaired nutrition, degenerate. The syncytium becomes atrophic, the villi become fibrous, and often leucocytes as well as syncytial cells invade the mesoderm of the chorion. It naturally follows that when the nutrition of the ovum is impaired the most advanced growing point, the embryo, for which all is adjusted, should suffer most. Thus it happens that in many instances the chorion is not markedly changed, but the embryo is almost entirely destroyed or is wanting altogether. In a short time the ovum collapses, becomes an irregular mass, and its "rootlets," the villi, are still found scattered throughout the blood-clot, or a small heap of them are found poorly adjusted in a fold of the tube covered with changed and distorted syncytium and decidua. These conditions, found so well marked in tubal pregnancies, are also found in uterine pregnancies, but in them it is difficult to determine whether the degeneration of the chorion follows because the embryo has died suddenly or has inherited the power to become abnormal. The study of the ova from tubal pregnancies demonstrates conclusively, it seems to me, that the changes in the chorion are primary, and those in the embryo secondary, due to faulty implantation of the chorion.

Another argument in favor of the view I have advanced regarding the production of pathological embryos is obtained by studying those embryos in tubal pregnancies which were not destroyed at once, but which became well attached and grew on towards full term. I mean the fate of the 4 per cent of normal embryos found in early unruptured tubal pregnancies.

Ruptured Tubal Pregnancies.

According to Williams,[1] most of the ova in tubal pregnancy are extruded through the internal opening into the abdonimal cavity, producing a condition known as tubal abortion. He collected the cases published by various authorities, and found that in 289 cases that were carefully reported 78 per cent ended by abortion and 22 per cent by rupture of the tube. Among these there is a small per cent of normal embryos, and the fate of them has recently been studied by Von Winckel.[2] Before considering Von Winckel's report it is necessary to collect some data regarding the frequency of abortions of pathological ova and of monsters in uterine pregnancies.

Williams states that "a conservative estimate would indicate that every fifth or sixth pregnancy in private practice ends in abortion, and the percentage would be increased considerably were the early cases taken into account, in which there is a profuse loss of blood following the retardation of the menstrual period for a few weeks." I also find that Marchand[3] has collected the per cent of monsters from a number of writers; his figures are as follows:

Author.	No. of Births.	No. of Monsters.
Chaussier	22,293	132
Peuch	772	7
Schworer	39,917	88
Winckel	10,056	156
Winckel	8,149	232
	81,187	615

[1]Williams, Obstetrics, p, 539, New York, 1903.

[2]Von Winckel, Ueber die Missbildung von ektopisch entwickelten Früchten, Wiesbaden, 1902.

[3]Marchand, Missbildungen, Eulenburg's Real-Encyclopedia, Bd. 15, p. 439, 1897.

It is noticed that these data, which are given in chronological order, give an increasing per cent of monsters, indicating that the more recent ones are collected with greater care. Taken together they give pretty well, I think, an average, for no doubt slight anomalies are included only in the records given by Von Winckel. If we assume that the number of births represent only four-fifths of the pregnancies, the figures will read about as follows:

	Pregnancies.	Births.	Abortions Normal Embryos.	Abortions Pathological Ova.	Monsters at term.
Number	100,000	80,572	11,765	7,048	615
In per cent ..	100	80	12	7	.6

I find in my own records of 434[4] embryos (Catalogue Nos. 1 to 404) that 163 of them are pathological, which, when raised to the number of abortions given in the table above, gives 7,048 as the number of pathological embryos in every 100,000 pregnancies. For the present this is as near as I can

First Month.

Nos. 1 to 208— 26 normal and 33 pathological = 56 % of pathological
Nos. 209 to 404— 18 normal and 45 pathological = 71*% of pathological
Nos. 1 to 404— 44 normal and 78 pathological = 59 % of pathological

Second Month.

Nos. 1 to 208— 59 normal and 32 pathological = 35 % of pathological
Nos. 209 to 404— 46 normal and 28 pathological = 38 % of pathological
Nos. 1 to 404—107 normal and 60 pathological = 36 % of pathological

First and Second Months.

Nos. 1 to 208— 85 normal and 65 pathological = 43 % of pathological
Nos. 209 to 404— 66 normal and 73 pathological = 53 % of pathological
Nos. 1 to 404—151 normal and 138 pathological = 48 % of pathological

Total Numbers of All Months.

Nos. 1 to 404—271 normal and 163 pathological = 38 % of pathological

[4]Total number of specimens (434) catalogued under 404 numbers.

*The per cent has been fully up to 70 from Nos. 127 to 404. The low per cent (44) up to No. 127 is due to the fact that only normal embryos were at first collected.

approach the proper number and per cent, but it will do for the sake of making comparisons. It appears, therefore, that in every 100 pregnancies in cities there are seven abortions and about one monster is born at term. It may be less in country districts.

I have made no special effort to collect fœtal monsters, but find that my collection contains seven monsters which are not included in the 163 pathological specimens mentioned above.

I have been unable to collect any good data regarding the frequency of monsters in tubal pregnancy, but, according to Joachimsthal, they are very rare, and according to Leopold they are rare, while Martin and Orthmann, Ruge, Olshausen and Veit state that they are more common than in uterine pregnancies. It may be that the latter gynecologists confused early pathological embryos with older monsters, while the former did not, a line between them being difficult to draw, and, therefore, it is not frequently recognized.

Von Winckel has done us a service in collecting those fœtuses from tubal pregnancies which continued to live and were removed alive from the abdominal cavity. The fœtuses which he considers must have been derived from the 4 per cent of normal embryos I have found in unruptured tubes removed in Dr. Kelly's clinic. Ninety-six of the specimens were so markedly pathological and so far destroyed that they could not possibly have lived until the end of pregnancy. Von Winckel's cases are especially valuable to determine the fate of the embryos that must have been normal before the tube ruptured, that is, during the first weeks of pregnancy.

Von Winckel first gives the cases that have been published by others, as follows:

Date.	Author.	No.	Monsters.
1876.	Henning	150	2 and 6 compressed fœtuses.
1894.	Orillard	6 (alive)	6
1893.	Schelling	257	25
1891.	Küchenmeister ...	43	7

Date.	Author.	No.	Monsters.
——.	Harris	45	11
1901.	Sittner	126 (alive)	36
1902.	V. Winckel	13 (alive)	13
——.	Kehrer	93 (uterus bicornis)	7

It is seen in the table from Von Winckel that the number of monsters increases in per cent from year to year. However, he thinks that it is safe to say that one-half of the fœtuses in ectopic pregnancy are deformed, the most common deformity being that of the hands and feet. Von Winckel further collected 87 cases (14 his own) and found that 57 of them were much deformed and 12 were markedly monstrous. Among these there were six cases of hydrocephalus and one each of hydromeningocele, encephalocele, anencephalus, omphalocele, spina bifida, and hypospadia. In addition, the head was found deformed, 57; legs, 44; arms, 35 times; with club-feet in 12 and amniotic bands in 4 cases. The placenta was usually deformed, sometimes multiple, broad and thin or short and thick, and often very hemorrhagic.

In general, then, it is the poles of the body that suffer most, the head being deformed in 75, legs in 50, arms in 40 and the trunk in 4 per cent of the cases. It is clear that a good share of the difficulty is due to ordinary mechanical causes, but the 12 cases that were markedly monstrous could not be due to such causes alone. For them we must hold the hemorrhagic placenta responsible, which could be included under what I have termed faulty implantation. Therefore, 14 per cent of the 87 cases become monstrous, while in normal pregnancies it is but .6 per cent. However, in all of 100 total pregnancies the per cent would be as follows:

	Pregnancies.	Births Normal.	Pathological.	Monsters.
Uterine	100	80	7	.6
Tubal	100	3	96	.56

The proper per cent of real monsters was obtained from the 4 per cent of normal embryos. The 14 per cent of monsters were obtained from the 4 per 100 of normal embryos in tubal pregnancy, the remaining 96 having become pathological at a very early stage. This gives, as is shown in the table, .56 per cent of monsters for the full 100, which is only a coincidence and an improper comparison. Three of the four embryos that remain, that is, those that produce normal fœtuses, are by no means so according to Von Winckel.

I have given the data obtained from tubal pregnancy at some length on account of their bearing upon the etiology of teratogenesis. No matter how these data are considered, they all point in one direction—the number of pathological embryos and monsters is greatly increased in tubal pregnancy.

Partial or Complete Destruction of the Amnion, Leaving the Umbilical Vesicle.

One of the first changes seen in early human embryos is a destruction of this important organ, the amnion, which develops shortly after the umbilical vesicle is formed. We can now picture to ourselves pretty well the formation of the amnion from the epithelium of the chorion if we blend the observation of Selenka upon Hylobates with the very young human ovum described by Peters. About the time the ovum reaches the uterus, when it is less than 3 mm. in diameter, and after the cœlom begins to form there must be an invagination of ectodermal cells into the stem of the vesicle within. A portion of the cells of this sac gives rise to the ectoderm of the embryo and the rest becomes the epithelial lining of the amnion.

It is easy to conceive that any change in the environment which influences the growth of the ovum would first make

itself felt at the very apex of the growth, and in the early stages of development this is in the amnion, including also the ectodermal plate.

At this early stage, probably during the second week of pregnancy, the three primary layers have established themselves well in their capsule of decidua, which must give nourishment to all sides of the ovum through the syncytium and young villi. In looking through sections of young ova one cannot help but think that the syncytial cells form the aggressive elements which eat themselves through everything that comes before them and cause the mucosa of the uterus to respond at once. Hemorrhages naturally follow such an action, and, judging by the frequency free blood is found between the villi, it appears that in all cases the blood comes in direct contact with the syncytium, which then grows so much the better. This vigorous layer of cells no doubt nourishes the layer of mesodern below it, which in turn cares for the embryo, for at this time there is no vascular system to carry the food from the mother's blood to the embryonic disc. The villi which are growing so rapidly must cast some of the fluid within their mesenchyme into that of the main wall of the chorion as it splits into two layers to form the cœlom. Thus, for a time, even after the blood-vessels are formed in the umbilical vesicle, the nutrition of the embryo passes through the cœlom exclusively. The first blood-vessels to the embryo hasten the process from the umbilical vesicle, and it is not until the heart is formed and the vascular system has reached the villi that the nutrition passes in through the umbilical cord. This does not take place until the embryo is fully 1.5 mm. long, *i. e.*, in the specimens of Eternod and Graf Spee. From now on the amnion begins to expand, at first very slowly and later much more rapidly, and gradually obliterates the exocœlom. This is complete by the time the ovum is 45 mm. in diameter and the embryo is 20 mm. long.

About the time the blood-vessels are formed and reach the embryo an interference in the nutrition of the embryonic mass naturally results in the destruction of the anlage of the

amnion and embryo, leaving only the umbilical vesicle, which may be found either attached to the chorion or lying free in the cœlom.

During this period, while the cœlom is relatively very large, a disturbance in the transmission of nutritive substances from the decidua to the embryo is marked by an increase in the reticular magma of the cœlom. This delicate reticulum was first described about a century ago and is pretty well marked in normal ova. As the amnion expands to fill the cœlom the magma reticulé is gradually pushed before it and often remains for a time as a delicate layer between the amnion and chorion. When the embryo and amnion are more or less destroyed the magma reticulé gradually becomes denser and denser, encircles the umbilical vesicle and fills the cœlom. In case the amnion is still intact but does not fill the cavity of the chorion entirely, pathological ova are usually marked by a mass of dense magma in the exocœlom. This change in the structure of the magma is so well pronounced in early pathological ova that specimens which are believed to be normal on account of a perfect villous covering are at once recognized as being diseased as soon as they are opened.

The nature of the magma is not known. I have made numerous tests with Weigert's fibrin stain, but in no case did the fibers take on the color. Neither do they appear to be related to ordinary reticulated tissue, which is present in the embryonic state in the mesoderm of the chorion, for they are not connected in any way with the protoplasm of these cells.

As the magma reticulé becomes more pronounced in pathological specimens it is often converted into, or is intermixed with, a granular substance which may be termed the granular magma. In case the ovum grows to be large, as is specimen No. 115, the reticular magma is often destroyed, leaving only the granular substance, more or less mixed with fluid to fill the cœlom. In older specimens we often see the cavity of the amnion filled with a mass of granular magma, while the surrounding cœlom is filled with reticular magma. However, stained sections show this separation only in a

general way, for there is always more or less mingling of these two substances.

TABLE I.
Normal Embryos of the First and Second Weeks.

Specimen.	Embryo.	Ovum.	Menstural Age.
	mm	mm.	days
Peters*.....	.19	1.6 x .9 x .8	30
Merttens		3 x 2	21
Breuss.....		5	38
Reichert.....		5.5 x 3.3	42
Siegenbeck van Heukelom.....	.325	5.5 x 4.5	
Graf Spee.....	.37	7. x 5.5	5 wks.
No. 11.....	.8	10 x 7 x 7	41
Keibel.....	1.	8.5 x 7.7 x 6	
Eternod.....	1.3	10.8 x 8.2 x 6	34
Graf Spee.....	1.54	10 x 8.5 x 6.5	5 wks.
No. 71.....		10 x 9 x 5	40
No. 361.....		10 x 10 x 10	41
No. 250.....	2.	10 x 9 x 8	
No. 384.....	2.	16 x 13 x 10	49
No. 391.....	2.	16 x 14 x 12	42

*References are given in my article in the Johns Hopkins Hospital Reports, Vol. IX, 1900.

With this introduction to the primary changes in very young pathological ova we are ready to discuss those cases in which the amnion and embryo are destroyed, individually and in groups. In order to make this easier I have brought the specimens together in Table II, giving certain important data. Table I includes all of the normal ova I have been able to collect from the literature and is to be used for making comparisons. By comparing the two tables it is noticed at once that the pathological ova are older and larger than the normal ones. But, judging by the changes within, it is highly probable that these began some time before the second week of pregnancy. If their ages are estimated by the size of the umbilical vesicles they would range themselves from one to

TABLE II.

VESICULAR FORMS OF PATHOLOGICAL OVA.

No.	Ovum.	Vesicle.	Mens'l Age.	Amnion.	Chorion.
	mm.	mm.	days		
13	8 x 7	1.4x6		Formed	Fibrous and partly covered with villi.
304	15 x 7 x 6	2		"	Chorion normal, but decidua infiltrated with leucocytes.
158	12 x 6	2		"	Atrophic. Tubal pregnancy.
143		25x10		"	Normal.
11	10 x 7 x 7	1.5x1	41	Partial	Covered partly with villi.
396	7	2x1	50	"	Somewhat fibrous and invaded by syncytium.
134	17 x 11	3x9	33	"	Normal. Embryo infected with mother's blood.
58	20 x 18 x 12	6	71	"	Fibrous.
87	24 x 16 x 9	2.5	42	"	Normal.
24	21 x 16 x 5	2.6		Multiple	Syncytium increased.
78	36 x 33 x 13	1.6	87	"	Atrophic.
247	40 x 40 x 17	2.5		"	Nearly normal.
21	12 x 9 x 5	5.5x3.5		None	Some magma reticulé in cœlom.
130	15 x 10 x 6	4x3x1.5	14	"	Normal. See Table IV.
123	17 x 14	2x1.5	27	"	Imbedded in pus.
180	20 x 15 x 10	1.5	37	"	Fibrous. In a mucoid mass rich in leucocytes.
264	25 x 20 x 15	2.5	58	"	Fibrous. Few villi with pus.
14	30	1.5		"	Thin and fibrous. No villi.
147	30 x 27 x 20	1.	89	"	Very fibrous. Few villi.

six weeks, during which time the vesicles grow from one to six millimeters in diameter. However, if we consider each millimeter in diameter as indicating a week in age, we again get into trouble when this is compared with an arrangement according to their ages as determined by their last menstrual periods. It seems to be safer to assume that the pathological changes in these specimens began during the first and second weeks of pregnancy, and that the chorion continued to grow while the vesicles within became larger in some cases and smaller in others. Had the changes in them begun as late as the third week, when the amnion is sufficiently developed to remain and continue to grow, entirely different specimens would have been obtained, as will be shown later on.[1]

[1]The age of the embryos is given according to His. Since writing the above I have come to the conclusion that he has underestimated

Amnion formed.—One of the earliest specimens of vesicular forms in which the amnion is present but the embryo is pretty well destroyed is No. 13. There is much magma reticulé in the cœlom. It is quite clear that the double vesicle represents the amnion and umbilical vesicle, which are bound together by a mass of mesoderm. This contains two blood-vessels, which unite at the point where the mesoderm passes over into the chorion. There are no other structures of the embryo present. The tissues have become dissociated, the mesoderm cells are round, and other round cells are in the cavity of the yolk sac. In general, we have the remnants of an embryo a little younger than Eternod's with the blood-vessels reaching to the chorion.

An excellent specimen, No. 304, which was cut into serial sections with the entire chorion and decidua, is most instructive. The villi of the chorion are normal in shape and are covered with a very active syncytium. They contain remnants of blood-vessels within them, showing that at one time there was vascular connection between the yolk sac and the villi. The decidua is encircled and infiltrated with leucocytes and between the villi there is a mucoid mass rich in leucocytes, showing that the inflammatory process has reached the ovum. The cœlom is well filled with magma reticulé, in which there is imbedded the umbilical vesicle attached to the remnants of the embryo. This is partly covered with the amnion, which runs out into a stem, containing an allantois, the latter not connecting with the chorion. There are remnants of a nervous system and numerous blood islands in the yolk sac, but no heart.

This specimen corresponds well with No. 13 and gives, in

them, by about ten days for embryos less than 22 mm. long. For embryos from 22 to 33 mm. long I believe his estimations fairly accurate. To make the proper correction it would be necessary to recast all of my tables and much of the text. The reader may make them by adding ten days to the age of embryos less than 43 days old. My new data, about 1,000 in number, relating to the age of human embryos will be published in Keibel-Mall's Handbuch der Entwicklungsgeschichte early in 1909.

addition, the changes in the decidua which caused the difficulty. The equilibrium between the chorion and embryo was overthrown at about the same time as in No. 13 and the magma became more pronounced than normal. The tissues then became dissociated, and on account of lack of nutrition they began to disintegrate.

The next specimen (No. 158) which belongs to this group is of about the same age, judging by the size of the chorion. It is from a tubal pregnancy and is interesting because there are no villi upon the chorion. The main wall of the chorion is somewhat fibrous and there are but few epithelium cells upon it; these come in direct contact with the lining epithelium of the tube. The nodule within is as a double sac, partly joined by a clump of cells, which runs out into a long process containing a blood-vessel (?), but does not join with the chorion. The whole mass appears necrotic, at least it does not stain well, and probably represents the amnion and yolk sac, which come to a sudden end due to the radical changes in the chorion.

The fourth specimen (No. 143) which may be included with this group is unique, for within a normal chorion there is a double vesicle much larger than the umbilical vesicle ever becomes during development. However, the specimen had been in alcohol for a long time and the cells are mostly destroyed, due to bad preservation. The two sacs, which do not communicate with each other, are of the same structure as the mesoderm of the chorion, to which they are bound by a strong pedicle.

Amnion partly formed.—The five specimens in this group, Nos. 11, 396, 134, 58 and 87, are most interesting, and have caused me much trouble. Four of them were considered in the first communication and need only be reviewed in this place in order to make the chain of events complete.

No. 11 was first described as a normal embryo because its chorion and apparently all of its tissues were normal. The embryonic mass, however, communicated freely through a rounded and natural opening with the cœlom. Furthermore, I

had every reason to believe that the abortion was produced through mechanical means, a circumstance which I have since learned does not insure a normal embryo. When I received the specimen (1893) sketches of it were submitted to Professors Minot and Graf Spee, who discussed it quite extensively in their letters to me, and they both felt that more young specimens would have to be studied before this could be properly interpreted. Professor His, however, to whom the sections were shown, was inclined to think the embryo normal, and as such it was first published. At present it seems to me that the ovum was normal until the woman "sprained herself six days before the abortion." The sprain was followed by a flow of blood each day until the abortion occurred. Thus it happened, it seems to me, that the chorion grew large and the villi small; certainly they are not as well developed as in the other young ova given in Table I. Through some means, possibly mechanical, the amnion became torn and the ectoderm spread itself partly over the cœlomic side of the yolk sac and belly stalk. The amnion in Peters's ovum is very delicate at one point, being composed of but a layer of ectodermal cells, and in Van Heukelom's there appear to be actual openings in the amnion. With these facts before us, it is not remarkable that a break should occur at this point occasionally.

Another very valuable specimen is No. 396, which was obtained from a tubal pregnancy. Within the cœlom of the ovum there is a double sac, one of which is clearly the umbilical vesicle, and the other may represent the amnion and embryo. What is especially interesting in this specimen is the relation of the umbilical vesicle to the chorion. At a number of points they come in contact, are adherent, and the blood-vessels from the umbilical vesicle pass directly over into the chorion, from which they spread into its villi. This specimen proves that the presence of blood-vessels in the chorion is not dependent upon the development of the body of the embryo. They may grow to it in a direct way.

A third specimen (No. 134), much like No. 11, also ob-

tained from a criminal case, is equally remarkable, for we have here the amnion communicating with the cœlom. In this case the ovum had been punctured by a bougie and the cœlom filled with mother's blood. The clot is very recent, for it is composed largely of well-formed red corpuscles. A portion of it is composed of many leucocytes, and where they come in contact with the embryo the leucocytes are in a very imperfect state of preservation, showing irregularities and fragmentation of their nuclei. Fragmented leucoyctes are also found throughout the clot, in the blood-vessels of the embryo, in the chorion and yolk sac. At points in the villi leucocytic thrombi are found in the embryo's blood-vessels, which shows the effect the tissues of the embryo have upon the leucocytes of the mother. No bacteria were found in a section stained for them. On the other hand, the tissues of the embryo are well preserved, there being no evidence of extensive necrosis. However, on one side of the yolk sac the cells have desquamated.

The bougie in puncturing the chorion probably also entered the yolk sac and was followed by its collapse. In the table its dimensions are given as 9 x 3 mm., which equal about a spherical vesicle 5 mm. in diameter.

The amnion is also torn open, and within it there are fragmented leucocytes. The invagination does not include the whole embryo, for a portion of the mesoderm covering the yolk sac contains myotomes. The experiment represented in this ovum gives us much over which to reflect.

According to the woman's statement from whom this specimen was obtained, the menstrual period had lapsed five days before the abortion took place, and her mechanical interference took place but a few days earlier. It is difficult to understand this high refinement in the production of abortions, and the degree of development of the ovum and embryo indicates that the specimen dates back to the last menstrual period. Possibly morning sickness, which in such cases may precede the first lapsed period, induced the woman to pass a bougie into the uterus after the period had been overdue a couple of days.

The amnion is partly within and partly upon the stem of the yolk sac in specimen No. 58. In it the mesoderm of the chorion, villi, pedicle and sac is fibrous and abnormal in appearance. It may be that in this case a portion of the amnion broke out of the stem and the portion that remained developed into a small vesicle filled with beautiful epithelial cells. There are some blood islands within the stem of the vesicle. The cœlom is filled with jelly-like magma and the vesicle has a granular deposit within it.

The last specimen of the group is No. 87. Here the yolk sac is imbedded in reticular magma and is not connected with the chorion. It is covered with a layer of epithelium, which at one point is invaginated. Below this layer there is a thick mesoderm, in which there are numerous blood islands. The chorion is normal. On the opposite side of the cœlom there is a normal embryo of the third week with its own umbilical vesicle and cord. In this specimen we have twins, one of which is normal and the other has undergone this remarkable change, found beginning in specimen No. 11.

Multiple amnions.—The last two embryos discussed may also be classed under this head, and they therefore represent intermediate stages between the two groups. By multiple amnions I mean two or more vesicles which arose from the original amnion located in the stem of the yolk sac.

In these three specimens, Nos. 24, 78 and 247, nothing marked is found in the chorion, excepting that of No. 78, in which it is atrophic. In this specimen the cœlom was found filled with fluid; in No. 24 it was filled with a moderate amount of reticular magma, and in No. 247 with granular magma.

In No. 24 the stem of the vesicle is broad and contains blood-vessels. The endoderm of the yolk sac is well marked and from it the allantois arises and branches as it spreads, thus forming a multiple allantois. Within the stem there are also a number of sharply-defined vesicles, some of which communicate with its epithelial covering. Each vesicle appears

to be a small amnion, and this condition may therefore be designated multiple amnion. The second specimen, No. 78, is much like the one just described. Again the stem of the vesicle is encircled by a layer of epithelial cells, and within it there are a couple of vesicles lined with the same kind of cells. There are also some blood-vessels within the stem.

In the third specimen (No. 247) of this group the vesicle is detached from the chorion; it is pear-shaped and is lined with a single layer of epithelial cells. The outer layer is relatively thick, is composed of mesoderm in which are located numerous large spaces filled with blood; there are no blood-vessels in the chorion. Within this mesodermal layer there are a number of sharply-defined vesicles lined by a single layer of epithelial cells which is unlike that of the main vesicle. It is natural to conclude that the large vesicle belongs to the yolk sac and is lined with endoderm, and that the smaller vesicles form multiple amnions and are lined with ectoderm.

Table II shows that the age of the specimens (from Nos. 11 to 78) increases with the size of the chorion. The same is true regarding the last group, Nos. 21 to 147, which is also arranged according to the size of ova.

Complete destruction of the amnion.—In the specimens just discussed it has been shown quite conclusively, I think, that radical changes may take place in the amnion and embryo of very young ova when the chorion is affected. The remaining seven specimens of this group show still greater changes in the embryonic mass, *i. e.,* both the amnion and embryo are destroyed entirely, leaving only the umbilical vesicle. That it should be so, and not the opposite, is quite natural when we take the order of development into consideration. The embryo and amnion receive their nutrition in early stages from the umbilical vesicle, which in turn draws upon the fluid within the exocœlom. This in turn is acted upon by the exchange of fluid with the villi.

In these specimens (Nos. 21 to 147) it is seen that the changes in the villi are more pronounced than in those in

which the effect upon the embryonic mass was not so marked and show to what extent the yolk sac can endure hardship. The degree of change is expressed pretty well by the size and age of the ova; in the younger ones (Nos. 21, 130 and 123) the yolk sacs are simply detached, while in the older ones (Nos. 264 and 14) they are fibrous and well attached to the chorion.

The amount of magma within the cœlom tells the same story. There is some in No. 21, considerable in No. 130, much in No. 123, hard and hyaline in No. 264, and completely filled in No. 147. Thus we have in these specimens the gradual changes in the umbilical vesicle after the amnion and embryo have been destroyed. No doubt some of the earlier stages (Nos. 21 to 180) would have reached a stage similar to No. 147 had the ova not been aborted. Instead, the yolk sacs would probably have been destroyed entirely to make chorions without embryos and uterine moles. But few of them could go on degenerating for eighty-nine days, ending with an atrophic umbilical vesicle.

The first specimen of this group is composed of two vesicles with blood islands in the outer layer of mesoderm. Of course it is possible that one of these represents the amnion, but I am of the opinion that it is a dilated allantois on account of its close resemblance in structure and layers with the main vesicle. Another free umbilical vesicle is found in No. 130. However, it is uncertain whether or not this was torn away from the main embryonic mass before or after the ovum was aborted. No. 123 is from a clear case of complete separation of the umbilical vesicle from the chorion. The ovum appeared normal, but more careful observation showed that it was encircled with pus. Within the cœlom the free umbilical vesicle was found to have a large opening on one side, showing that its destruction had also begun.

No. 180 is another case of entire destruction of the embryo, leaving only the umbilical vesicle. The chorion contains villi and blood-vessels. Between them there is a slimy mass, in which there are many leucocytes and islands of

syncytium. The growth of the syncytium appears to have been violent, and it encroaches upon the mesoderm of the chorion, which at points is beginning to be fibrous. Here also the primary trouble seems to be due to the mucus and pus which bathe the villi of the chorion; they naturally cause havoc with the nutrition of the ovum.

The next specimen of this series (No. 264) is a very valuable one, for its tissues, from the embryo to the decidua, are unusually well preserved and the menstrual age is given. The chorion is fibrous and thickened, and between the villi there is mucus which is well infiltrated with leucocytes. The cœlom is very small, but 10 mm. in diameter, and is filled with a mass of hard hyaline magma.

The embryo is represented by a vesicle composed of two layers, the outer of which is much thickened at its attachment to the chorion. Here it is decidedly mesodermal in character and contains many large blood-vessels, filled with blood, which spread into the chorion. In the tissues around these blood-vessels there are many round cells which are similar to, and no doubt have come from, the embryo's blood. Many round cells are also scattered through the magma.

A similar remnant of an embryo may be seen in specimen No. 14. The mesoderm is very fibrous and extends over the vesicle within. Within the chorion there are groups of epithelial cells which are no doubt derived from the syncytium. There are a few blood islands at the base of the nodule and two other spaces lined with spindle-shaped cells. The main cavity of the nodule is lined with epithelial cells, which no doubt represents the yolk-sac cavity.

No. 147 is a specimen much like No. 14, giving, however, its menstrual history, which makes it eighty-nine days old. The chorion is fibrous and partly covered with villi and the cœlom is filled completely with magma reticulé. Lying in the magma, but detached from the chorion, there is a small vesicle one millimeter in diameter. One-half of the vesicle is composed of the single inner layer, and on the other there is an additional thick outer mesodermal layer, in which there are

numerous blood-vessels filled with blood. There are also blood-vessels in the chorion in the immediate neighborhood of the vesicle, showing that the two were connected at an earlier period in their development.

No doubt this vesicle has gradually degenerated, but has lived so long because the blood cells are more resistant than any of the other tissues of the embryo, and, therefore, could hold the yolk sac intact more or less. However, it is clear that of the structures of the embryo the yolk sac is the last to disintegrate when the chorion is affected.

Ova in Which the Embryo and Amnion Have Been Destroyed, Including Certain Moles.

Under the previous heading those specimens were discussed in which most or all of the embryo and amnion had been destroyed, leaving only the umbilical vesicle. Various stages of destruction of the umbilical vesicle were also considered. Altogether there were 19 specimens which came under this heading. Now we have a second group of 29 specimens in which the whole embryo, amnion, belly stalk and yolk sac are missing, leaving only the main wall of the chorion and its villi.

In the first group a number of the specimens showed fibrous changes in the mesoderm of the chorion with more or less destruction of the villi with other changes, such as leucocytic infiltration or mechanical injury, indicating that the primary cause for these changes is located in the environment of the ovum rather than within it.

In the second group, where the change in the embryo mass is more radical, greater changes should be found in the chorion, and, in fact, this is the case, as a glance at Table III will show. The first eight specimens of the group, from Nos. 298 to 204, inclusive, may be considered together, for in many respects they are alike. They belong to the first weeks of pregnancy.

TABLE III.

PATHOLOGICAL OVA AND MOLES WITH NEITHER EMBRYO NOR AMNION PRESENT.

No.	Chorionic Mass.	Cœlom.	Menstrual Age.	Magma.	Chorion.	Villi.	Syncytium.
	mm.		days				
298	4		3 wks.	None.		Fibrous and invaded by leucocytes.	
278	6 x 4	3 x 2.5		Recticular.	Normal.	Normal.	Normal.
154	10 x 7	7 x 4.	4 or 5 weeks.	None.	Somewhat changed.	Somewhat changed	
71	10 x 9 x 5	8	40	Some.	Normal.	Normal.	Normal.
361	10		41	Reticular.			
367	10 x 7 x 5	3		Reticular.	Normal.	Œdematous.	Atrophic.
378	12			Granular.	Normal.	Œdematous.	Netrotic.
204	14 x 12 x 8	*[11]	3 wks.	Granular.	Normal.	Normal.	Normal.
299	16 x 12 x 10	[13]		Reticular.	Œdematous.	Œdematous.	Normal.
395	17 x 10 x 7		6 wks.		Fibrous.	Fibrous.	Irregular.
181	18 x 18 x 10	[15]		Granular.	Œdematous.	Œdematous.	Diminished.
310	18 x 14 x 14	[15]		Reticular.	Hyaline.	Hyaline.	Irregular, invades chorion.
20	20 x 14 x 6	16		Reticular.	Normal.	Normal.	Normal.
255	20 x 20 x 10	15 x 6		Granular.	Invaded by leucocytes.	Fibrous.	Leucocytes in syncytium
190	25 x 22 x 12	[20]		Reticular.	Normal.	Normal.	
29	30	24		Granular.	Fibrous.	Atrophic.	Norm. Leucocytes
195	30 x 30 x 30	[24]		Reticular.	Normal.	Normal.	
243	30	[24]	?	?	Atrophic.	Atrophic.	?
358	30 x 16 x 10	81x6x16	6 wks.	Reticular.	Fibrous.	Fibrous.	
55	35 x 20 x 14	15	54	Granular.	Invaded by leucocytes.	Necrotic.	Necrotic.
185	40 x 25 x 15	[32]	7 wks.	Granular.	Invaded by leucocytes.	Atrophic.	Normal.
223	40 x 18 x 5	Absent			None.	Hypertrophic.	Increased.
280	40 x 25 x 25	10x5x5	6 wks.	Reticular.	Atrophic.	Normal.	Necrotic.
70	45 x 30 x 28	5	113	None.	Necrotic.	Hypertrophic.	Increased.
153	50 x 20 x 20		77	None.	Invaded by leucocytes	Normal.	Normal.
290	50 x 15 x 10	None	6 wks.	None.		Invaded by leucocytes.	
233	70 x 45 x 40			None.		Invaded by leucocytes.	Irregular.
82	75 x 60 x 40		279	None.	Hypertrophic.	Atrophic.	Increased.
332	120 x 90 x 65	15x10		None.	Hypertrophic.	Hypertrophic.	Necrotic.

*Numbers in brackets are estimated.

No. 298 is from a tubal pregnancy regarding which there was much doubt until the remains of the ovum were found in serial sections. The remnants of a very small ovum were found at the edge of the rupture of the tube. They are composed of fibrous villi partly invaded by leucocytes and surrounded by an irregular mass of syncytium, decidua and blood, I am inclined to believe that the case might have cured itself if it had not been treated by the surgeon.

The second specimen (No. 278) is of the greatest significance in this discussion, for we have in it the whole ovum with its surrounding decidua, which appear normal, and changes within, which show that the embryo has been destroyed. The specimen was removed by a curette from a woman suffering with endometritis. Although no inflammatory changes were seen in the decidua and chorion, the condition of the uterus may have caused the destruction of the embryo. The cœlom is found filled with reticular magma, and this is permeated by a coarse network of mesodermal cells, which are continuous with and no doubt derived from those of the chorion. In one of the sections, lying free in the middle of the cœlom, there is a small clump of epithelial cells, about 100 in number, which may have been derived from the embryo. The chorion contains no blood-vessels and in general reminds one much of Peters' ovum.

No. 71 has a history similar to No. 278, it being, however, a natural abortion from a woman suffering from endometritis. The exterior of the specimen is normal in appearance, and in sections the structure of the chorion and villi also appears to be so. Within the cœlom there is some reticular magma and a small mass, which would not stain, and appears like a mass of dried blood corpuscles. With this there is another specimen (No. 204), without any history. Its chorion is again normal, both macroscopically and microscopically, is filled with a mass of granular magma, but contains no remnant of an embryo nor amnion.

The remaining specimens of this group are all from tubal pregnancies and show no remarkable reactions. They are

valuable because they show the early changes in the chorion when its implantation is faulty. The structure of the main wall of the chorion and its villi is also more or less changed, as the table and histories of the specimens show. It is natural to read into these specimens the following history: The embryonic mass grew for some time, but was soon arrested because the chorion could not supply the proper nutrition. Soon the embryonic mass began to degenerate, and this process was only hastened by the secondary changes which were beginning in the villi. Soon the whole ovum was disorganized, as we see by the study of the specimens.

This group of specimens throws much light upon the primary cause in the destruction of very young embryos. In five it is mechanical and in two it is clearly due to endometritis, although no secondary changes are found in the chorion. I have every reason to think that this kind of abortion is much more common than is believed to be, for physicians often have told me that they "found but lost, or threw away, suspicious specimens," or that they "sought but failed to find small fœtuses in suspected abortions." No doubt curing the endometritis in such cases would favor future pregnancies, as is generally believed by gynecologists. At any rate, for our purpose, these specimens show that impaired nutrition due to faulty implantation causes destruction of the embryo without making any marked impression upon the chorion.

The first group of this series is no doubt composed of specimens of the second and third week of pregnancy, in which the whole embryo was destroyed and the ovum aborted before any change took place in the chorion. The next group includes ova of the third and fourth week, judging by their size, and by the presence of blood-vessels in the chorion of some of them.

The smaller specimens of the second group also show no macroscopic changes in the chorion, but microscopic examination tells a different story. In specimen No. 299 there is a dense magma reticulé, and the mesoderm of the chorion and the villi appear to be œdematous. Nos. 395 and 181 tell the

same story. In 310 the villi are of irregular length, their structure is hyaline with vacuoles, and they contain remnants of blood-vessels of the embryo. They are imbedded in a mass of fibrin and pus.

In No. 20 the only change found is a considerable amount of granular matter between the villi, and in No. 190 there are no changes whatever; however, the villi contain blood-vessels. In specimen No. 255 it is again observed macroscopically that the villi are atrophic, and sections show that the mesoderm is fibrous. In between the syncytial masses there are many leucocytes, which have invaded the villi and the mesoderm of the main wall of the chorion.

Up to this time the cœlom contains reticular magma in most cases, but as the specimens grow larger and presumably older first granular magma is found mixed with the reticular and finally displaces it altogether. The last four ova of the group under consideration (Nos. 29, 195, 243 and 358) are each 30 mm. in diameter, may be fully four weeks old, and are beginning to take on secondary changes. No 29 is filled with granular magma, the mesoderm of the chorion and its villi are fibrous and being invaded by the syncytial cells. The whole is encapsulated in a layer of mucus, in which there are numerous leucocytes. No. 195 shows no changes in the chorion, which, however, contains blood-vessels. Extreme changes have taken place in No. 243. It is an irregular, collapsed, pear-shaped chorion, showing the beginning of a solid mole. Sections of No. 358 show that the villi are matted together, much blood and syncytium being between them, and they are encircled by a fibrous syncytium rich in leucocytes.

Up to this time the changes in the chorion are quite equally distributed over its walls, and a change found on one part of it is also found on the other. However, the impaired nutrition may influence villi which stand side by side, some becoming smaller and disappearing while others are becoming hypertrophic. In fact, one of the best signs of the abnormality of an ovum before it is opened is this inequality of its villi. However, when an ovum is collapsed the story is entirely

different. In so doing it makes more room for itself, the various structures from magma reticulé to decidua become intermixed, and if there is any further growth it is irregular, as is shown by numerous specimens. In specimens of this sort, that is those in which the amnion is destroyed in an early ovum, the diameter of the cœlom does not exceed 24 mm. in any of my specimens, while if the amnion is retained and reaches the chorion, obliterating the cœlom, the amniotic cavity is then often over 75 mm. in diameter. In a measure this is repeating what takes place in normal development, for here the cœlom reaches its largest diameter (25 mm.) at the beginning of the fifth week. So in pathological ova, in which the amnion is absent, the ovum goes on developing, as in the normal, until the cœlom has reached its maximum size; beyond this it cannot continue to grow, for under normal conditions its further growth is due to the presence of blood-vessels in the chorion, which carry fluid to the embryo from which the liquor amnii is secreted.

In case the ovum does not collapse, *e. g.,* No. 358, the walls of the chorion become gradually thicker, the villi longer, and the diameter of the cœlom smaller. This is seen to be the case in regular order in specimens Nos. 55, 280, 70 and 223.

No. 55, an ordinary fleshy mole, contains a sharply defined cavity, which proves to be the cœlom, for it is not lined by the amnion. From its lining membrane, the chorion, the villi rise and radiate through a mass of syncytium, decidua, blood, fibrin and pus. The bulk of the syncytium is necrotic and the mesoderm of the chorion is invaded in part by leucocytes.

Another specimen (No. 185), as large as No. 55, is considered here, for it was not the outside, but the inside, of the chorion that was filled with pus. No doubt the ovum was punctured by mechanical means and filled with pus, as was the early stage (No. 134) described above. In this the leucocytes invaded the chorion from the inside, but had not entered the villi. Some of the villi are atrophic and some are œdematous; the syncytium is normal.

Nos. 280 and 223 follow in regular order No. 55. In the first the cœlom is small, contains some reticular magma and the wall of the chorion is thin. The villi are not very large, are well developed, contain remnants of blood-vessels and are covered with a mass of necrotic syncytium. The whole specimen is surrounded with mucus, blood and pus. Leucocytes have entered the mesoderm of many of the villi. The second specimen is a solid mass with its base broken off. Radiating from its base of attachment there are long villi, which encircle mostly, and partly penetrate, the main mass of the tissues composed of blood and fibrin. Between the villi there is an active syncytium more or less necrotic, which gives the picture of a cancer. The whole is covered with a capsule of pus.

The remaining specimens may be considered in two groups, solid moles and hydatiform moles. Belonging to the first group (No. 153) is a pear-shaped body composed of an inverted chorion imbedded in an organized blood clot, intermixed with villi and syncytium. There are also numerous leucocytes, which have invaded the mesoderm from its cœlom side. No. 290 is composed of decidua, mucous membrane of the uterus, blood, fibrin, pus and villi which are being destroyed by leucocytes. No. 233 is composed of an irregular mixture of villi, syncytium, decidua, blood and pus. Fresh blood is in the middle of the tissues, which, like most moles of this kind, are nourished through their centers. Its exterior is covered with pus.

To what extent a collapsed ovum may grow is shown in specimen No. 82. A large solid mass the size of a duck's egg was expelled nine months after the last menstrual period. On the end which lay in the os uteri there is an extensive ulceration of the mole; otherwise it is very compact. After it had been hardened, I cut it into two parts, which, to my astonishment, contained within a collapsed chorion, sending its folds in all directions throughout the mole. In the middle of the specimen there are large spaces along the collapsed chorion, filled with fresh blood. The opposite walls

of the chorion are in apposition throughout most of the specimen, and at points they have grown together. There is no amnion, and on this account I place the beginning of this mole back to the first month of pregnancy. The extensive ramification of the folds of the chorion shows that it must have continued to grow throughout the nine months of its existence, this being made possible by the nourishment brought to it by the fresh blood in its interior. Islands of syncytial cells are located upon the chorionic wall throughout the specimen. The syncytium shows active growth and its cells stain well at numerous points where they come in contact with fresh blood. All the syncytial masses, distant from the fresh blood, are necrotic, which is undoubtedly due to the lack of nutrition. Nests of leucocytes with fragmented nuclei are scattered throughout the specimen. The walls of the chorion are not invaded by the syncytium.

In a number of the specimens enumerated above it was noted that the villi are œdematous and hyaline, a condition which might easily end in hydatid degeneration of the villi, forming hydatiform mole. In a specimen which contains an embryo 17 mm. long (No. 357) many of the villi are quite large and have undergone hydatiform degeneration. Another specimen (No. 70) contains a small cœlom which sends radiating cavities into the walls of the hypertrophied villi. There is no amnion. In a second very large mole (No. 323) most of the villi are about 5 mm. in diameter, and some of them four times as long. They are very irregular in form, the larger ones containing cavities, some measuring 15 x 10 mm., giving all the characteristics of the cœlom. Between the villi there are numerous masses of necrotic syncytium, some blood and leucocytes, which invade the mesoderm of some of them.

Pathological Ova and Moles Containing an Amnion With the Embryo Destroyed Wholly or in Great Part.

Under the last headings specimens were described in which most or all of the embryonic mass was destroyed, leaving the chorion to outline the cœlom. Such specimens are numerous and no doubt give rise to most of the solid moles. All other specimens in which the embryo is destroyed are of necessity those in which the amnion is formed, sweeps through and obliterates the cœlom in its development and lines the chorion. It is evident when these two groups of specimens are considered that the first must arise from ova of the first month, for in them the amnion is small, and the second group, from older ova after the amnion has reached the chorion.

In general, the older the embryo is when it begins to become pathological the more resistant it is, and it follows that the younger the specimen the more easily it is destroyed. Probably this is the reason why an ovum without an embryo rarely contains an amnion. No matter how large the specimen may be, if the interior of the chorion is not lined by the amnion it is safe to say that the pathological changes in it began during the first month (probably during the first fortnight) of pregnancy. In case the disease of the ovum, which is usually due to endometritis, begins during the second month of pregnancy or later, the amnion is well formed, usually continues to develop, and reaches the chorion. Ova of this sort, which constitute the major portion of my specimens, contain embryos more or less degenerated, and at best form vesicular moles with the remnants of embryos within them. In a few of them, however, the embryos are destroyed, leaving only the umbilical cords, and in two or three specimens they were also destroyed, leaving only the chorion and the amnion.

In all of these ova the cavity of the amnion is retained, and they do not appear to develop into solid moles, but may continue to grow on indefinitely like those of the group given in Table III.

TABLE IV.

PATHOLOGICAL OVA AND MOLES WITH AMNION BUT NO EMBRYO.

No.	Ovum.	Embryo mass.	Menstrual Age.	Chorion.	Villi.	Syncytium.
	mm.	mm.	days			
369	7 x 3 x 3	Amnion only	23		Atrophic and fibrous.	Diminished.
130	15 x 10 x 6	7 x 2	14			
25	25	6 x 2		Normal.	Normal.	
37	25 x 18 x 15	2 x 2		Normal.	Normal.	
198	25	7		Fibrous.	Fibrous.	
32	30	9 x 2	82	Normal.		
159	[30] Walnut	Amnion only	70 (?)	Invaded by leucocytes and syncytium.	Invaded by leucocytes and syncytium.	Increased.
342	30 x 20 x 20	5 x 1		Fibrous.	Atrophic.	
377a	30 x 22 x 14	.5		Fibrous.	Fibrous.	
93	40	Amnion only		Invaded by syncytium.	Hypertrophic.	Increased.
334	50 x 40 x 30	Embryo 5 mm.	4 wks.	Atrophic and invaded by syncytium and leucocytes.	Atrophic.	Diminished.
257	55 x 40 x 40	14 x 2		Hypertrophic.	Fibrous.	
77	70 x 40 x 30	1 x 0.25		Invaded by leucocytes and syncytium.	Invaded by leucocytes and syncytium.	Increased.
279	100 x 60 x 60	30 x 5		Hyaline.	Normal (?)	Diminished.
379	35 x 25 x 15	Granular embryo of the 4th week	10 wks.	Fibrous.	Fibrous.	Increased and necrotic.

I shall first consider those ova in which the embryo, or embryo and cord, has been destroyed, leaving only the amnion, and will leave the ova or moles with pathological embryos to follow. There are many of them, and when they are classified in weeks they tell a continuous story.

Table IV gives the list of ova in which the amnion is retained, with such other data as I have been able to collect. Unfortunately, the data relating to the age of the specimens are very incomplete. However, it is possible to connect the specimens in a satisfactory way if we begin with those which have remnants of the embryo attached to the cord, and gradually proceed to those in which the cord is destroyed entirely, leaving only the amnion and the chorion.

In general, the size of the chorion and cord do not correspond properly with each other, showing that either one or the other has been retarded in its growth. In specimens Nos. 130 and 32, for instance, the embryo masses are of about the same size, representing cords of the second month, but one is from a small and young ovum and the other is from a large and much older one. It is fair to assume that the embryo in No. 32 was destroyed when the ovum was as small as No. 130 is at present.

Table IV gives a list of the specimens with all stages of destruction of the embryo after the amnion is well formed, leaving only a portion of the embryo, or, in extreme cases, the umbilical cord alone. In a few of the specimens the cord is also destroyed and in them the chorion is lined simply by the amnion. The villi of the smaller ova of this group appear quite normal, and for this reason I have been inclined to think that in them the primary cause lay in the embryo itself, and that in the older stages the changes found in the chorion were of a secondary nature. Further investigation, however, may reveal the same early changes in the neighborhood of the villi here as are found in the specimens in which the embryo and amnion were destroyed during the first four weeks of pregnancy (Table III). Here also the earlier specimens, those with numbers lower than 150, did

not show any signs of endometritis, but since then nearly all of the specimens collected have been hardened in formalin instead of being first washed in water or in weak alcohol, as uninstructed physicians do so frequently. Further observation with well preserved specimens will also probably show signs of endometritis in specimens of this group, less than 30 mm. in diameter.

The embryo is entirely destroyed, leaving only the amnion in a very small per cent of pathological ova. Usually the embryo continues to grow slowly in an irregular fashion, but sometimes there is a destruction of some of its parts. In most of these cases, however, the circulation has been established and the cœlom is pretty well obliterated, thus eliminating the importance of the magma reticulé. Therefore primary changes in the embryo are clearly associated with the blood and the vascular system, and this naturally affects the embryo more than it does the other structures.

I shall consider No. 37 first, because it still contains the outline of a portion of the embryo. The atrophic head of the embryo is seated upon a very small cord and these are surrounded by the amnion. The umbilical vesicle is attached to the side of the cord, but does not reach into the embryo.

The central nervous system is very rudimentary, and the heart, liver, myotomes and lower end of the body are wanting. The lower jaw is still recognizable, and from it two arteries pass over into the cord. The single vein of the cord ends blindly just below the rudimentary branchial arch. The size and degree of development of the embryo places it in the beginning of the third week, when no doubt its destruction began. The chorion, however, belongs with those of the fourth or fifth week, which indicates that the process of atrophy has been under way for a week or two.

In specimen No. 130 the embryo is reduced to a small mass of round cells showing no structure whatever. The umbilical cord is filled with its usual blood-vessels, showing that an embryo had been present at an earlier date. The whole is inclosed in a relatively small amnion, which no

doubt protected and held together what little of the embryo there was left. The cord extends to the chorion in the wall of the amnion, showing why the yolk sac, described on page 80, broke away so early. The amnion fills only half of the cœlom, the remaining portion being stuffed with a dense mass of magma reticulé.

No. 257 is similar to No. 130, inasmuch as both of them have small bodies upon the end of the cord, representing the remnants of the embryo. However, it is much older, the chorion being larger and many of the villi having undergone fibrous changes are atrophic. The body at the end of the cord is not the remnant of the embryo, but simply its continuation, with the umbilical vein running through it. Although this specimen might have passed for a normal one when examined superficially, more careful examination showed that the decidua was infiltrated with leucocytes. The chorion is lined by the amnion, which is mostly adherent and contained a clear fluid. No remains of a disintegrated embryo were found within the amnion.

Another specimen with a small remnant of the embryo is No. 342, which is from a tubal pregnancy. Attached to the free end of the cord is a bit of tissue which must belong to the embryo, with a small mound of active cells growing in it. The chorion, amnion and cord have undergone fibrous degeneration.

Nos. 25, 32 and 198 are specimens of simple ova with a naked cord projecting into the amniotic cavity in each case. In No. 198 the amnion is filled with reticular and granular magma intermingled with scattered flakes of the embryo and numerous free cells. The cord is rounded at its free end and its blood-vessels are empty. The mesoderm of the chorion, villi and cord is fibrous, with an excess of spindle-shaped cells scattered through it. Similar stages are shown in specimens Nos. 32 and 25. In both of them the blood-vessels are well filled with blood, and in the first there is an extensive wandering of blood cells into the surrounding tissue, especially at the tip end of the cord. Here they stain intensely with carmine

and suggest very much a section through an ulcerating wound. No. 25 may possibly represent a more advanced stage, inasmuch as the free end of the cord is more rounded.

To what extent a cord may grow, or at least round itself off, is shown in No. 279. The fleshy chorion is composed of villi which seem to be nearly normal, the mesoderm being somewhat hyaline in structure, with a diminished number of nuclei scattered through it. Within there is a large free umbilical cord curled upon itself and rounded at its free end. No doubt the fœtus escaped from its membranes before they were expelled. However, I was unable to determine whether this had really taken place. The blood-vessels of the large villi are well developed, indicating that at one time the fœtus present must have been pretty large. At any rate, the broken end of the cord became rounded and healed over after the fœtus had been broken off.

Specimen No. 77 shows that the free cords are gradually destroyed if the ovum is retained in the uterus long enough. In it the chorion and amnion are both more fibrous than normal. The villi are being invaded by leucocytes and syncytium, giving the secondary changes which are often seen when the mesoderm of the villi has lost its vitality. No remnants of blood-vessels are present in the villi. The cavity of the amnion contains a clear fluid, and on one side there is a small stumpy cord, about one millimeter in diameter, which attaches the amnion to the chorion.

Nos. 334 and 379 may also be considered with this group. No. 334 formed a fleshy mole, with a cavity in its center, 15 mm. in diameter, and contains the fragment of an embryo which must have been fully five weeks old when it died. The main tissue of the mole is composed of uterine mucous membrane, decidua, blood and pus, with a ramifying chorion in it. The wall of the chorion is infiltrated with leucocytes on its outside and invaded by syncytium from its inside. Had it not been for the fragment of an embryo this specimen would have been grouped in Table III. No. 379 contains a granular embryo, 10 mm. long, which readily fell into pieces upon being handled.

Nos. 77 and 334 show what may become of the chorion and amnion when they are retained in the uterus long enough. The villi are attacked on the outside by leucocytes and syncytium, the cavity of the amnion collapses, or is penetrated and filled, making the mole solid, as is the case in so many younger ova after the embryo and amnion have been destroyed.

Another specimen belonging to this group is No. 93. It came to the laboratory fresh, enveloped in its decidua, and the whole was hardened in formalin. Between the decidua and the ovum there is a layer of blood and fibrin. The main body of the mole is composed of irregular hypertrophied villi with a great amount of blood and syncytium between them. Occasionally the syncytial cells are found in the mesoderm of the main walls of the chorion. The small cavity within is lined with the amnion and is filled with blood. No embryo was found. Nos. 159 and 369 are similar specimens, since in them the cord is also destroyed entirely. No. 159 is composed of fragments of a mole, the embryo having been lost. However, the fragments are made up of mucous membrane of the uterus, large portions of the chorion and some fragments of the amnion. The mucous membrane is full of small abscesses, and leucocytes have invaded the mesoderm of the chorion and its villi. The syncytium is very active and at numerous points it also has invaded the mesoderm of the chorion and villi. The amnion is hyaline, thickened, curled upon itself, and at points its epithelial layer has proliferated, forming small mounds.

The specimens just described show the fate of ova after the embryo is destroyed, leaving first the cord and amnion and then the amnion alone. Finally the cavity of the amnion is punctured, the ovum collapses and the whole is converted into a solid mole. Specimens of this kind are rare, since most solid moles are formed from ova in which the amnion and embryos were destroyed at a much earlier date.

Pathological Embryos of the Second Week.

The preceding pages have been devoted to the discussion of those pathological ova in which the embryos were nearly or entirely destroyed, leaving only the membranes. A large number of these specimens appeared to be normal ova when examined superficially, but careful examination showed that in many of them mucus, leucocytes and pus were present between the villi. In many the chorion was thickened and more or less invaded by leucocytes and syncytium, while in others the cavity within had been obliterated completely to form typical fleshy moles. We have in them all stages of transformation between young normal ova and solid moles.

The specimens in the preceding sections are easily divided into two groups: the first, in which the embryo and the amnion are destroyed, and the second, in which the embryo is destroyed but the amnion and more or less of the cord remains. In each of these groups there are intermediate stages which may be properly considered under this heading. In the first group these changes began in very early specimens, and in some of them the destruction of the embryo and amnion was not always complete. These might properly be considered with the embryos given in Table V, but I have found it more convenient not to do so and have included in this and subsequent tables only those embryos in which the form and structure could be made out with considerable certainty. By doing this there is still a wide margin left for the imagination in linking the pathological specimens of a given week with normal embryos. After this has been done it is easier to correct errors than it is when the specimens in which the embryos have been destroyed are grouped with atrophic ones. My arrangement for the present is as follows:

(1) Normal embryos.
(2) Atrophic embryos.
(3) Remnants of atrophic embryos.
(4) Ova with amnion but without embryos.
(5) Ova with neither amnion nor embryos.
(6) Moles.

What has been said about ova with neither embryos nor amnion applies equally well to those in which the amnion is not destroyed. In this group there are also all intermediate stages present, and they can be arranged in a series, if considered alone, for if the embryo is present there is also an amnion, and adding them spoils the group. The group given in Table IV could, therefore, be scattered under the following headings, but this is not convenient, because the absence of the embryo makes it difficult to determine at what time the pathological changes in the embryo began, and, furthermore, it is easier to consider alone those specimens in which the amnion is present with more or less of the cord, including an occasional fragment of the embryo. This group blended with embryos over four weeks old would compel us to subdivide those of each week as follows:

(1) Normal embryos.

(2) Atrophic embryos.

(3) Remnants of atrophic embryos attached to the umbilical cord.

(4) Umbilical cords alone.

(5) Ova lined with an amnion alone.

(6) Moles with remnants of the amnion present.

With this brief introduction, I shall proceed to consider pathological ova in which the development of the embryo is arrested, beginning with those of the second week. In my first *Contribution* there were none belonging to this list; in the second *Contribution* there was one. I now have three new ones (including No. 12) to add.

TABLE V.

Arrested Development of the Embryo. (Second Week.)

No.	Embryo.	Chorion.	Menstrual Age.	Changes in the Chorion.
	mm.	mm.	days	
162	1.	70 x 30 x 30	81	Atrophy.
250	2.	10 x 9 x 9		Leucocytic infiltration of decidua, chorion normal.
321	2.	40 x 40 x 20		
12	2.1 Normal(?)	20 x 20	41	Small amount of mucoid mass between villi.

The embryo of specimen No. 162 is one millimeter long, with structures which would make it as old as Eternod's or Graf Spee's, given in Table I. The chorion is very thin, devoid of villi and enveloped in layers of coagulated blood. Through this but little nutrition could have come to the embryo, if it got any at all. The amnion fills the entire chorion and its cavity measures 35 x 12 x 12 mm., although the whole specimen measures 70 x 30 x 30 mm. According to the menstrual history the age of the specimen is at least fifty-three days, and if we deduct thirteen days, the age of the embryo when it first became affected, then the pathological process must have continued during forty days. Sections of the embryo show that we are dealing with a remarkable specimen, in which great changes have taken place gradually. All of the organs and tissues are dissociated, that is, they have grown in an irregular manner, each one growing by itself, not being markedly influenced by the surrounding structures. The different tissues are not of uniform structure, being mucoid in some places and necrotic in others, as is shown in the figure. The mucoid tissue runs as a column from the heart to the apex of the nodule and may have been derived from the chorda dorsalis. At the point of union between the amnion and chorion there are three elevations from the embryo mass into the cœlom. These are marked in the figure. The heart lies within a pocket of its own, which communicates with the exocœlom and is filled with blood. There are also blood-vessels filled with blood in the center of the embryo.

This interesting specimen of a dissociated embryo, that is, one in which the tissues grew in an irregular fashion, is accompanied with another excellent one, No. 250, in which these changes are just beginning. In many respects it is normal, and for this reason I have also included it in Table I as an embryo of the fourteenth day. The ovum and decidua were curetted from the uterus and came to me opened and well preserved. The presence of an excess of magma reticulé gave the hint that the specimen was not quite normal.

The chorion, villi, syncytium and decidua are beautifully developed and are normal in structure. Between the villi

there is much mother's blood and within them there is a well-developed system of capillaries filled with embryo's blood. There are numerous leucocytes in the decidua, but they do not form abscesses.

The front end of the amnion is wanting and its free ends are well imbedded in reticular magma, showing that this injury took place before the abortion was produced. The embryo is normal in form, the heart and blood-vessels well developed and filled with blood. The rest of the organs are about of the same stage at No. 391, an embryo of the fourteenth day. The tissues of the embryo and the ventricle of the fore-brain are filled with numerous small round cells with fragmented nuclei. Most of the blood corpuscles are still within the blood-vessels, but those within the tissues are perfectly normal and in no way do they seem to give rise to the strange cells in the tissues. However, it may be noted that the mesodermal cells are diminished in number in those tissues in which the round cells are present, which indicates that the one changes into the other. The primary histological change in this embryo is found in the mesoderm, which is dissociating to form some of the so-called wandering cells. Later this process affects the wall of the vascular system and the blood cells escape into the tissues, as was pointed out by His. The cells within the ventricle of the brain as well as those of the neural tube are mostly fragmented and have between them a few normal blood corpuscles. It is probable that most of these new cells arise from the dissociated nervous tissue. I shall come back to this question from time to time as I discuss specimens which have changes within them that bear upon this point. However, this much is clear: the round cells with fragmented nuclei lying within the tissues have not emigrated from the blood, but, instead, have arisen by a process of dissociation of the tissues within which they lie.

The process of dissociation, begun in No. 250, is carried to an extreme degree in No. 321. Both of the embryos are of the same size, but in the second the amnion and chorion have continued to grow. The chorion is normal in appearance and

is lined entirely by the amnion, the cavity of which is 35 mm. in diameter. It is interesting to note that this ovum has reached its maximum growth without the presence of a vigorous embryo. Ova without embryos rarely exceed 40 mm. in diameter, and in normal development the amnion reaches the chorion and obliterates the exocœlom in ova of this size. If the cavity of the amnion is to exceed 40 mm. in diameter, it is necessary to have a fairly active embryo within it to secrete the liquor amnii. As long as there is an exocœlom present, which is not obstructed by magma reticulé, it appears as if fluid of the amnion is obtained from that of the cœlom.

In this specimen (No. 321) the embryo is attached to the chorion at its middle, that is, there is no umbilical cord left. The body cavity of the embryo spreads out on the inside of the chorion, and into this the degenerated heart hangs. The dissociation of the tissues is pretty complete, as in No. 162. The outline of the brain is barely recognizable and all the tissue in the tail of the embryo is of equal density. Here the dissociation is complete. Unfortunately, the specimen had been preserved in 50 per cent alcohol for ten days before it was sent to me, and in a measure the extreme degree of dissociation may be due, in part at least, to the macerative influence of the weak alcohol. However, this could not alter the general outline of the embryo and its organs.

No. 12 is extremely interesting, for it also is probably pathological, although I have often referred to it as being normal. When it came to me I found considerable magma in the cœlom, enough to almost obscure the embryo, but on account of the general normal structure of the tissues I overlooked the excess of magma. More careful investigation of the chorion shows that there are also some fibrinous or mucoid masses between the villi. They also indicate that the specimen is not quite normal. Furthermore, there is a marked anencephaly and probably the beginning of spina bifida present. Before a definite opinion can be given regarding the normality of this specimen it will be necessary to examine with much greater care than has been done the tissues of the

embryo, and especially those of the chorion of many so-called "normal" specimens. This will be done, no doubt, in the near future.

EMBRYOS OF THE THIRD WEEK.

The specimens of the third week can be divided into three groups, representing normal embryos 16, 18 and 20 days old, respectively. In the first division there is but one specimen,

TABLE VI.

NORMAL EMBRYOS OF THE THIRD WEEK.

Specimen.	Embryo.	Chorion.	Menstrual Age.
	mm.	mm.	days
No. 12.....	2.1	18 x 18 x 8	41
Thomson	2.1	5.7	42
His (E).....	2.1	8.5 x 5.5	
Eternod.....	2.12	16.3	
His (Lg).....	2.15	15 x 12.5	40
His (SR)	2.2	9 x 8	
His (L).....	2.4	9 x 8	
Thomson	2.5	15 x 10	14
No. 318	2.5	20 x 18 x 11	42
Chiaringi	2.6	15 x 12 x 8	
His (M)	2.6	8 x 7.5	
Graf Spee.....	2.69	15 x 14	42
His (EB).....	3		42
No. 239	3	19 x 17 x 15	50
Janosik	3	8	43
His (BB).....	3.2	14 x 11	48
No. 164.....	3.5	17 x 17 x 10	
No. 186	3.5	25 x 20 x 15	17
No. 87.....	4	24 x 16 x 9	42
No. 136.....	4	14 x 11 x 6	56
Ecker.....	4		45
His (III)	4	30 x 25	51
His (Lr).....	4.2	15	
Steubenrauch (K)	4.3		52
No. 148	4.3	17 x 14 x 10	38
Wagner	4.5		20
No. 1	4.5	30 x 30	
Hensen.....	4.5		21
No. 76	4.5	22 x 20	
No. 248.	4.5	30 x 23 x 15	

TABLE VII.

ARRESTED DEVELOPMENT OF THE EMBRYO.

(Third Week.)

No.	Embryo.	Chorion.	Menstrual Age.	Changes in the Chorion.
	mm.	mm.	days	
166	2.3	40 x 40 x 40	71	Tubal pregnancy.
115	3	30 x 27 x 22	56	Atrophic.
196	3	12 x 12		Atrophic. Tubal pregnancy.
209	3	25 x 15 x 10		Atrophic.
246	3	30 x 21 x 14		Hyaline.
252	3		84	Hyaline.
292a	3.5	50 x 30 x 30	54	Fibrous.
324	3.5	45 x 45 x 22		Fibrous and atrophic.
400	3.5			
189	4	28 x 25 x 15		
228	4	60 x 25 x 25	79	Very fibrous.
244	4	25 x 15 x 15		
253	4	38 x 30 x 15		Hyaline.
302	4	25 x 20 x 15		Fibrous.
309	4	23 x 20 x 20		
399	4		4 or 5 wks	
402	4	40 x 25 x 20	6 or 8 wks	
328	4.5			Normal and covered with necrotic syncytium.

No. 166, and in it the dissociation of the tissues is complete. This is not remarkable, for the menstrual history says that it is 71 days old, showing that the pathological process has been under way at least a month. The ovum has thick walls with a large cavity within lined entirely by the amnion, which is not attached to it at any point. There are no blood-vessels in the villi of the chorion. The embryo is cylindrical in form and is attached for half its length to the amnion and then perforates it. Its organs and tissues are almost completely dissociated, there being but the faintest outline of the nervous system in its center. In the tail end of the embryo there is a blind tube, which may represent the allantois. Within a sac on one side of the body, which communicates with the cœlom, there is a small mass representing either the heart or the umbilical vesicle. Greater changes could not have taken place without obliterating the anatomy of the specimen entirely.

There are eight specimens (Nos. 115 to 400) of the second group in this series, that is, embryos which began to degen-

erate when they were 18 days old. A variety of changes are found in each specimen which are by no means of the same degree, thus permitting their discussion in regular order. It is probable that those with the least amount of change in them have been under pathological influences for less time than in those in which the tissue changes are more marked.

There are practically no changes in embryo No. 209, and for this reason it may be classed with normal specimens. However, it appears as if the chorion were atrophic, being very thin immediately over the embryo, and the amount of magma reticulé within the cœlom is greatly increased. There are some changes in the amnion, as it has become adherent to the embryo over its tail and back, and is wanting entirely over its head. There are numerous cells in the surrounding magma which may have migrated from the mesoderm. It is clear that in this specimen the primary trouble is in the chorion immediately over the embryo, which receives most of its blood-vessels at this stage. The amnion and cœlom were next affected, and had the abortion not followed the tissues and organs would soon have dissociated.

The dissociation of the tissues is well under way in embryo No. 246, the chorion of which is somewhat hyaline and the amnion greatly distended. Unfortunately, the embryo is broken. Enough of it remains, however, to show that the central nervous system is distended and partly filled with round cells, which seem to be derived from the dissociated neural tube. The heart and large blood-vessels are empty, and the liver and optic vesicles are wanting.

The changes in this specimen can be ascribed to the hyaline chorion, but it is difficult to understand how this can cause distention of the amnion and destruction of the umbilical cord. At any rate, the process of dissociation is well illustrated in this embryo. The sharp boundaries of the tissues and organs are obliterated and the cells which are liberated take on an indifferent form. With the dissociation of the walls of the blood-vessels the blood corpuscles wander out, to be added to the dissociated tissues, and convert the whole into an indifferent

mass, which barely outlines the embryo. Such a condition is found in No. 196. The tissues are nearly homogeneous, only the central nervous system and some of the large blood-vessels being recognizable on account of the increased number of nuclei in these regions.

In No. 115 the amnion is greatly distended and the embryo is spread out upon it, much as is the case in the chick in normal development. The body cavities communicate freely with the cœlom, Wolffian bodies are still visible, and the central nervous system, heart and some large blood-vessels are represented as bands of round cells.

Various degrees of dissociation of the organs are seen in different embryos, as is naturally to be expected. In No. 292a, for instance, the outline of the body cavity is very marked, it being distended and partly filled with round cells. The amnion is also greatly distended, filling the entire cœlom. There is no umbilical cord. Some of the spinal cord is still sharply outlined; otherwise the dissociation is complete.

In embryo No. 252 the dissociation is complete with the exception of the eyes, which have been converted into small black spots composed of pigment cells. The skin is also markedly thickened, the epidermis forming papillomata, as well as small lens-like bodies.

In the specimens just considered only those organs which are present in the early part of the third week were seen, there being no signs of cartilage, muscles nor peripheral nerves. In the next group of ten specimens we have clearly the remains of organs and forms of embryos to correspond with normal ones of the latter part of the third week, that is, embryos 4 and 4.5 mm. long.

This group can also be arranged in the order of the degree of pathological change. In No. 189 the central nervous system is open below throughout its whole extent. A number of motor nerve roots are developed, more in the region of the tail than elsewhere. There are no cranial nerves present. The heart is almost detached from the body, and the large blood-vessels are irregular in shape and changed entirely from

the normal type. The liver, stomach and intestine are wanting entirely, and the dissociation of the optic vesicle, chorda, allantois is almost complete. In this embryo the branchial arches and brain show the least changes in them, while the rest of the tissues have suffered most.

Specimens Nos. 302, 309 and 328 are in many respects alike and may be considered together. The walls of the brain and cord are much folded and fill the central cavity in No. 302, while in the other two they are thin, the central cavity being enlarged and well filled with round cells, which, however, do not all seem to arise from the walls of the nerve tube, for their nuclei are smaller, being similar to those of blood cells.

The tissues of these embryos are pretty well dissociated, being composed largely of round cells, within which the outlines of large blood-vessels may be seen. No. 328 has arms and legs attached, which have undergone mucoid degeneration, a well marked cavity, pleuro-peritoneal, and finally remnants of precartilages which are not fully dissociated.

In the next embryo (No. 228) the central nervous system has also shown itself most resistant. It is markedly dilated and the walls are partly dissociated. There are also small links of cells present, which remind one very much of the growing cord, connecting large masses of dissociated tissue. The ventricle is dilated and has within it a large mass of dissociated nerve cells. In the face there are two large nerve tubes extending from the brain, which no doubt represent the eye vesicles and their stalks. The embryo with epidermis intact, connects with the degenerated but apparently active umbilical vesicle, but not with the chorion. The vascular system is represented by a dissociated heart and a large blood-vessel, which extends into the umbilical cord. The rest of the tissue is a dissociated mass, more or less spotted, being composed of a variety of cells, including possibly remnants of myotomes.

Degeneration of the dissociated structures now follows rapidly. In No. 253 the embryo still shows the outlines of the pleuro-peritoneal cavity within, but the tissues have be-

come more hyaline, the round cells having diminished in number. Most of the central nervous system is fully destroyed. There are traces left of some of the large blood-vessels, the Wolffian ducts and the chorda dorsalis. No. 244 may be considered together with this specimen, for in it radical changes have also taken place. The central nervous system is still sharply defined, more so in the brain than in the spinal cord. The heart is represented as a mass of cells in front of the head. Below this there is an irregular body, probably the dissociated liver, composed of epithelial-like cells intermixed with some round cells. The rest of the tissues are of homogeneous structure, with an occasional necrotic mass. In the tail end of the embryo there are some blood spaces with blood corpuscles, some of which infiltrate the surrounding tissues. In many respects this specimen resembles No. 115, with the difference that it is larger and was probably a little older when the pathological process began in it.

Pathological Embryos of the Fourth Week.

No doubt the reader has noticed that the embryos grow more and more resistant as they become older, and this condition continues to a more marked degree in those of the fourth week. In embryos of the second week pathological changes in the chorion were followed by a partial or complete destruction of the amnion and embryo, while in those of the third week it is not always the same organ or tissue which resists the influence longest. However, the brain and heart are recognizable in most of the specimens.

When we reach the fourth week we find that the main change in the normal embryo is due to the addition of the peripheral nervous system, which is associated with a sharper delineation of the organs; they begin to assume some of their adult characteristics. A normal type with which to compare these changes is seen in embryo No. 2, which has become a standard. In my description of pathological specimens of the fourth week I shall keep this embryo constantly in mind.

The specimens of the fourth week cannot be considered in the sequence given in Table IX, for a number of them are straightened and, therefore, measure larger than others that are more advanced in development but curled up in their natural shape. This, I think, is well shown in the various illustrations. However, it is clear that Nos. 334, 285, 312, 336 and 347 are decidedly larger than the rest, about the same stage of development as the normal specimen No. 2, and, therefore, about twenty-eight days old. The rest of the specimens are younger and belong to the beginning of the fourth week. In the first and younger group some of the embryos that are straight and measure 8 mm. are a little earlier than others that are but 5 mm. long.

TABLE VIII.

Normal Embryos of the Fourth Week.

Specimen.	Embryo.	Chorion,	Menstrual Age.
	mm.	mm.	days
No. 80.....	5	24 x 18 x 8	
His (D2).....	5	20 x 15	
His (W).....	5	25 x 20	21
His (R)	5	22	
Meyer	5.25	22	18
No. 19.....	5.5	18 x 14	
No. 16.....	6	24 x 18	
No. 241.....	6	40 x 40	51
No. 245.....	6		58
Steubenrauch (I).	6		45
No. 173.....		25 x 15 x 10	54
No. 116.....	6.5	28 x 20 x 10	55
No. 2.....	7	25 x 25	52
No. 18.....	7	18 x 18	
No. 187.....	7		64
Steubenrauch (II)	7		51
His (B)	7	25 x 22	
His (Stt).....	7.75	21 x 17	57
Meyer	8	45	28
No. 221.....	8	40 x 33 x 33	
No. 208.....	8	22 x 11 x 11	49

TABLE IX.

ARRESTED DEVELOPMENT OF THE EMBRYO.

(Fourth Week.)

No.	Length of Embryo.	Dimension of Chorion.	Menstrual Age.	Changes in Chorion.
	mm.	mm.	days.	
122	5	20 x 16 x 6	65	Atrophic.
136	5	14 x 11 x 6	56	Atrophic.
150	5	35 x 30 x 10		But few villi.
291	5	?		Atrophic.
398	5			
334	5	50 x 40 x 30	4 wks.	Fibrous. Infiltrated with leucocytes and syncytium.
80	5	24 x 18 x 8		Small amount of mucoid mass between the villi.
401	5.5			
340	6			
297	6		3 mo.	
104	7	35 x 35 x 15	35	Atrophic.
379	4th wk.	35 x 25 x 15	10 wks.	Fibrous.
60	8			
110	8	46 x 30 x 30	82	Fibrous. Invaded by leucocytes.
141	8	40 x 30 x 20	78	Fibrous. From same woman No. 110 was obtained.
275	8	40 x 30 x 25	2 mo.	Fibrous.
285	8	45 x 35 x 35	72	Very fibrous. Atrophy.
289	8		4 wks.	
312	8	25 x 15 x 10		Fibrous. Invaded by leucocytes.
347	8	40 x 35 x 30		Fibrous. Infiltrated by leucocytes.
336	8	35 x 25 x 15		Hyaline and fibrous.

Specimen No. 136 contains in it a normal embryo of the fourth week, but its enveloping chorion is too small for its age. The villi of the chorion are fibrous, but contain a large number of blood-vessels which are well distended with blood. The syncytium is very active and no doubt provided well for the embryo. The only marked changes in this specimen are the fibrous chorion and the excessive amount of magma reticulé in the cœlom. Possibly the inequality of things may have brought about the abortion.

Of the remaining specimens of the first group, No. 312 shows the least amount of change in it. The embryo, however, is straight, shows three gill arches and some myotomes. The spinal cord can still be outlined in the tissue of the body, which is well filled with round cells.

Generally, in the rest of the embryos, the brain is solid, the spinal cord dilated, the blood-vessels more or less distended with blood and the remaining organs and tissues pretty well dissociated. These changes are least marked in No. 297, which, however, is a pretty typical one. The outlines of the precartilages can still be made out, and some of the peripheral nerves are present. The lower jaw is disintegrating, which naturally brings the distended medulla closer to the midventral line of the head. All these changes are more pronounced in No. 340. The dissociation of the organs is here quite complete, with some indications of growth of the mesodermal tissue, including the precartilages. In the spinal cord there is a tendency toward regeneration, provided the curious bands of cells seen here indicate it. The dissociation of the larger blood-vessels is practically complete, but their outlines can still be seen, although many round cells fill the surrounding tissue. This condition is practically completed in the embryo of specimen No. 275, in which but few of the tissues are recognizable.

In specimens Nos. 104, 110 and 141 the destruction of the embryos is pretty well under way, showing what may be the fate of embryos of this sort. The details are much the same in the different specimens, and may be summed up in the words "more advanced." That Nos. 110 and 141 are from the same woman about a year apart is especially noteworthy. The woman was suffering from leucorrhœa and in general the ova show the same changes within them. Nearly all of the villi have been destroyed, and the main walls are fleshy and are invaded by leucocytes. The embryos are dissociated and atrophied. That the process was slow is indicated by their menstrual ages. In one the amnion is destroyed entirely and in the other it is greatly dilated, nearly filling the entire cavity of the chorion. That two specimens coming from the same woman should show the same changes in them indicates that the cause of the trouble lies in the diseased condition of the uterus. There are a number of other specimens which corroborate this conclusion.

During the fourth week of development the preskeletal tissues make their appearance, and through this change the embryos naturally fall into the two groups under which I am discussing them. The mesenchyme also becomes more resistant and does not dissociate so easily. The peripheral nerves are well laid down and the premuscle tissue is making its appearance. Thus we have new conditions by which we can recognize the time at which development was arrested; these naturally influence secondary changes in the embryo. In these specimens hydramnios is nearly always found, and since the embryos usually become markedly smaller when their development is arrested it is rational to conclude that hydramnios is produced by a continued growth of the amnion and chorion when the development of the embryo has been arrested. I have found no evidence in favor of the theory that hydramnios is due to arrested development of the amnion and dwarfism of the embryo. Were this the case we should find bones and cartilages in my specimens of the fourth week. In the specimens given in Table IX we have four weeks' embryos in eight weeks' chorions, not degenerated eight weeks' embryos in eight weeks' chorions. The four good specimens (Nos. 347, 285, 336 and 334) of the end of the fourth week are of different degrees of degeneration and form an excellent continuous series. No. 347 is from an ovum in which the mesoderm of the chorion is fibrous, the syncytium being scanty and mixed more or less with pus. The changes within the embryo are well marked, the brain being dissociated and solid and the medulla and cord are dilated. The blood-vessels are well marked, dilated and filled with blood, which is just beginning to infiltrate into the surrounding tissues. The other organs are just beginning to dissociate. The precartilage is well marked, however, and the rest of the mesodermal tissue has in it many round cells which seem to have come from the blood. Many of these cells are in the pleuro-peritoneal cavity. Conditions are advanced markedly in No. 285. Here the menstrual history tells us that the pathological process has been under way for a number of weeks. The mesoderm of

the chorion is fibrous and between the few villi covering it there is much mucus rich in leucocytes. The embryo is atrophic, the tissues, however, being still active, although dissociated. The brain and head are reduced in size, the medulla is solid and fills the region of the face, and the cord is dilated and its walls are folded upon itself. The organs are more dissociated than before, the large blood-vessels and the heart being greatly dilated and filled with blood. The precartilages and peripheral nerves are well marked and the remaining tissue is pretty well filled with round cells. Most of the epidermis is intact.

The two specimens just described show to what extent an embryo four weeks old may dissociate when its nutrition is partly cut off. In the next specimen (336) the amnion did not continue to dilate as well as it did in Nos. 285 and 347, but remained clinging to the embryo, as is the case normally at this time. However, it is pretty well destroyed, being partly infiltrated with embryo blood-cells. The cœlom is well filled with granular magma, in which there are many migrating cells. In form the embryo is curled upon itself and distorted. Within the central nervous system is dilated and the walls are folded upon themselves. The liver is completely infiltrated with blood. Mesodermal tissues seem to be normal. What is especially noteworthy is the condition of the vascular system. The heart walls appear normal and the vascular system is well proportioned and filled with blood. It appears as if it had functioned until the abortion took place. The vessels from the embryo through the umbilical cord to the chorion are cut off and the enlarged omphalo-mesenteric vessels seem to take their place. The walls of the yolk sac are necrotic, but have in them large blood-vessels, which on one side spread over into the chorion. The old original circulation has re-established itself and is now connected with the chorion in this roundabout way. There are in the chorion two kinds of capillaries, degenerate ones which are connected with the umbilical vessels and new ones which communicate with the omphalo-mesenteric vessels.

The other specimen of the fourth week is the remnant of the embryo from No. 334. Here the destruction is quite complete, only fragments of a four weeks' embryo being found in a small space of a large mole. The piece shows dissociated organs of an embryo much like No. 285.

These specimens give the beginning and the end of dissociated embryos during the fourth week, with an attempt to remedy the difficulty in one specimen (No. 336), and the almost complete destruction in another (No. 334). It is probable that the primary trouble in No. 336 lay in the umbilical cord.

Embryos of the Fifth Week.

The changes in the beginning of the fifth week are quite similar to those at the end of the fourth week, for the normal development has advanced but very little. However, toward the end of the fifth week, when the anlages of the ribs appear and there is further differentiation in the mesenchyme, we also find modified pathological processes, which are quite characteristic, and are not seen in earlier stages. The first specimens, then, which are about to be described could also with propriety have been considered with those at the end of the fourth week. In these we find again the dilated and dissociated central nervous system, dissociation of the tissues and the organs, infiltration of the liver with round cells, and a dilated and gorged vascular system. All these changes are well marked in Nos. 97 and in 251. In No. 251, however, the pathological changes are so marked that it merits a special description. The chorion is well enveloped in pus, showing that an active endometritis encircled it. The head of the embryo is rounded, solid and filled with a dissociated brain. The face is practically destroyed and the brain is protruding on the dorsal side of the head. Following the sections in order down the spinal cord, it is found that in this the central canal is distended and the walls partly dissociated.

TABLE X.

Normal Embryos of the Fifth Week.

Specimen.	Embryo.	Chorion.	Menstrual Age.
	mm.	mm.	days
His (17).....	8.5	20 x 12	
No. 163.....	9	35 x 35 x 20	5 wks.
No. 388.....	9		52
No. 258.....	10	35 x 30 x 25	
Ecker	10		60
No. 88.....	10	30 x 28 x 15	
No. 389.....	10		30
His (98).....	10.3	35 x 25	
No. 109.....	11	30 x 30	54
No. 353.....	11	40 x 35 x 30	
His (Br)	11	30 x 27	61
His (97).....	11	30 x 25	
His (Rg).....	11.5	30 x 27	
No. 156.....	12	35 x 35	
His (Sl)	12.5	30 x 27	

TABLE XI.

Arrested Development of the Embryo.

(Fifth Week.)

No.	Length of the Embryo.	Dimensions of the Chorion.	Menstrual Age.	Changes in the Chorion.
	mm.	mm.	days	
97	9	30 x 30 x 15	61	Fibrous.
135	9	105 x 65 x 65		Atrophic. Infiltrated by leucocytes.
251	9	30 x 25 x 25	77	Fibrous. Abscesses.
366	9			Fibrous or hyaline.
161	10	50 x 25 x 25	83	Pus between villi.
54	11			
133	11	32 x 32 x 32	65	Normal in shape but fibrous.
288a	11	85 x 35 x 35	5 or 6 weeks	Fibrous. Invaded by leucocytes and syncytium.
343	12	45 x 35 x 25		Fibrous.
177	12			
330a	12	60 x 55 x 50	128	Infiltrated by leucocytes.
330b	12	55 x 50 x 45	128	Infiltrated by leucocytes.
348	12	50 x 30 x 25		Fibrous degeneration.

The usual changes are again seen in the vascular system, and the dissociation of the tissues and organs is well marked. The mesodermal tissues, including the precartilages and peripheral nerves, are more or less filled with round cells, which, as the epidermis is wanting, have also wandered to the exterior of the body.

Specimen No. 161 is especially interesting, for the inflammatory changes around it are no doubt due to the repeated attempts at abortion by the mother. The woman was already suffering with leucorrhœa, and it was easy for her to extend this purulent inflammatory matter into the uterus with the rubber catheter she had used. While this experiment was followed by great activity in the leucoytes and syncytium on the outside of the chorion, an equally active reaction took place within the embryo. The head end of the embryo is almost completely dissociated, but the process is less intense in the lower part of the body. In general, the changes are similar to those in the embryos described above.

More intense changes are found in embryo No. 135, in which the duration of the pathological process must have been long, judging by the changes in the embryo and the chorion. The chorion, to which we look for the primary lesions, is smooth and devoid of villi. The large amnion within is filled with a jelly-like mass which became firm after it had been treated with formalin. The atrophic embryo contains a dissociated central nervous system without a brain, the head being very small and converted entirely into a mucoid mass. The eyes have sunk deep into the tissue of the head and contain hard lenses, composed of lens fibers. The anterior end of the chorda dorsalis is much enlarged and forms a mucoid tumor, on either side of which may be seen a large cartilaginous mass of tissue. Heart and vascular system are filled with blood, which extends through their dissociated walls into the surrounding tissue, obscuring the outlines of the organs and peritoneal cavity. All this shows that development ceased a long time before the abortion took place, and that the tissues simply grew onward in an irregular fashion, that is, they dissociated.

The remaining nine embryos of the fifth week may be considered together, for in many respects they are alike. In all of them the bodies of the vertebræ are well outlined and the precartilages of some of the ribs are laid down. They may be compared with the normal embryos, Nos. 109 and 163, whose skeletons have been studied with great care by Bardeen. It may be that the first four embryos of this group belong to the fourth week, for it is certain that their development is not as far advanced as No. 163, but they are fully as large. The slight difference may be due to errors in measurements or to the possibility that pathological embryos of younger stages may simply "swell" but not develop. However, the opposite is usually observed.

Specimens Nos. 54, 133, 348, 288a, 343 and 177 show much the same changes in them. The tissues are well dissociated, with a variety of other changes in the body. In Nos. 54 and 343 the front end of the brain is missing and the ventricle communicates with the exterior of the body, as if the neuropore were open. In these two specimens, in which the cerebral vesicles have been fully destroyed, there are but few pathological changes in the rest of the body. They may be compared with No. 256 (Fig. 8, Plate III), in which the fore-brain was removed by mechanical means, that is, through rough handling, and is in every respect an anencephalic monster.

No. 133 is well dissociated, but in it, as in the rest of this group, the liver is more like the normal, showing that in later stages the liver is more resistant than it is in younger ones. No. 348 shows about the same changes, only that in addition the embryo as a whole is disintegrating. The deformed embryo from specimen No. 288a is from a mole in which the chorion was found to be collapsed. The position of the embryo is in the upper right hand corner. In No. 177 the process of dissociation has outlined the ribs into two zones, an outer and an inner, although no true cartilage is present. Back of the eyes, in the occipital region, there are two cartilaginous masses, much too well developed for an embryo

of this stage, and similar to those found in No. 135. These changes are given as examples of further development of some of the tissues after the general growth of the embryo has come to an end. In No. 343 the fore-brain is destroyed entirely and the medulla is distended. The outlines of the organs and tissues are well defined, and they are fairly well infiltrated with migrating blood cells.

The structure and form of the organs in No. 343 resemble in many respects the state of things found in Nos. 330a and 330b. These two specimens came from twin ova which were aborted 128 days after the beginning of the last menstrual period, thus making the duration of the pathological process fully nine weeks. The chorions are both fibrous, are enveloped in pus and infiltrated with leucocytes. Both embryos show practically the same changes in them as are found in several other sets of twins, which seems to me to be strong evidence in favor of the theory that the deformed embryos are due to endometritis. The changes in both embryos are very much alike and can be described together. The epidermis is intact, but the true skin is hypertrophied, and in front of the head, in the region of the deformed mouth, the epidermis shows peculiar thickenings. Both spinal cords are dilated and their walls are dissociated. The cerebral vesicles and mid-brain are nearly destroyed, the main portion of the head being taken up by the hind-brain. The large blood-vessels and the heart are filled with blood; in 330b the wall of the ventricle is infiltrated with blood cells and in 330a it is nearly destroyed by them. The tissues and organs of the embryos are well dissociated and more or less filled with round cells. Some of the liver tissue is necrotic.

In reviewing the most marked peculiarities of the pathological changes in embryos of the fifth week, it may be noted that the differentiation of the tissues has made some of them more resistant than others; the more central tissues show the least amount of change, and the extremities, head and face the most, these giving way first. The spinal cord and medulla show more resistance than the brain and do not dis-

integrate as easily. The vascular system seems to suffer more than the nervous system does, but this may be due to the character of the primary trouble in the chorion, which probably first made itself felt in the heart. It is clear that when the heart is affected and stops that the embryo is then deprived of its nutrition, and under these circumstances the brain suffers before the spinal cord. Among the tissues the precartilages and cartilages suffer least of all.

Embryos of the Sixth Week.

In the beginning of the sixth week of development the cartilages of the extremities are outlined, and at the end of the week some of the ossification centers are present. Coincidentally the peripheral nerves ramify through the body and the muscle anlages appear. Thus we have before us a highly differentiated organism, and from now on anything which affects its nutrition does not produce a like influence in all of its tissues and organs. The reader has noticed, no doubt, that the present study is gradually leading in this direction. First the umbilical vesicle is most resistant, then the nervous system and now it is the skeleton. At first the blood-vessels possess the greatest power of growth before they were dependent upon the heart, but later when they are, they appear to suffer most, and the other structures are only affected in a secondary way, for they in turn receive their nutrition from the blood.

The changes in embryos of the sixth week can be followed with greater ease than those in earlier embryos, for they are less rapid and there are a larger number of known structures present to tell the story. In studying the pathological embryos, I naturally compare these changes with the normal in embryos of about the same age, and as a standard Nos. 109 and 144 are constantly employed (Plate IV, Fig. 10).

TABLE XII.

NORMAL EMBRYOS OF THE SIXTH WEEK.

Specimen.	Embryo.	Chorion.	Menstrual Age.
	mm.	mm.	days
His. (19)	12.8	40 x 32	
No. 35.....	13		37
No. 175.....	13	30 x 25 x 25	32
His (M2)	13		64
His (Br2).....	13.6	35 x 28	63
No. 144.....	14	40 x 30 x 30	60
No. 214.....	14	27 x 25 x 15	
No. 360	14		55
No. 167.....	14.5	30 x 30 x 30	65
No. 168.....	15		94
His (Dr1).....	15	45 x 40	
His (S2).....	15	35 x 28	
No. 256.....	16		60
No. 317	16	45 x 35 x 25	
His (Lhs)	17		51
No. 106.....	17		54
No. 216.....	17	35 x 35 x 25	

The first pathological specimen which I shall consider is No. 311, an unusually good one, for it is well preserved and there is every indication that the changes in it were produced gradually. Unfortunately, the menstrual age is not known, but I am of the opinion that it must be at least fifty days, that is, about two weeks more than normal embryos of the same size and degree of development. The chorion is covered with villi of unequal size, which show all degrees of activity, some being hypertrophic and others atrophic, fibrous and more or less invaded by leucocytes. The surrounding inflammatory process has gradually destroyed the villi. The condition of the vessels within the villi also indicates that the process of destruction has been gradual; the large villi contain fairly well developed capillaries, and the small ones are devoid of them altogether. The umbilical cord is enlarged in its center and very small at its attachment to the chorion. In general, it is fibrous and its blood-vessels are contracted and empty. The enlargement in the cord is due to the mucoid masses

TABLE XIII.

Arrested Development of the Embryo.

(Sixth Week.)

No.	Length of Embryo.	Dimensions of Chorion.	Menstrual Age.	Changes in the Chorion.
	mm.	mm.	days.	
311	12½	36 x 30 x 30		Fibrous.
69	13	70 x 40 x 20		Atrophic and fibrous
174	13	35 x 25 x 25	56	Atrophic. Invaded by syncytium.
182	13			
325	13	55 x 55 x 35	73	Hyaline.
346	13	50 (?)		Normal. Mucus and pus between villi.
375	13			Fibrous.
276	13½	70 x 35 x 35	80	Fibrous.
232	14	45 x 25 x 25		Fibrous (?).
262	14	80 x 15 x 15		Villi invaded by leucocytes.
270	14	40 x 30 x 20		Fibrous and atrophic.
365	14			
341	14	70 x 60 x 50		Fibrous. Some villi œdematous. (Twins in a single chorion.)
81	15	65 x 55 x 35	84	Atrophic, infiltrated with leucocytes.
132	15	42 x 30	89	Atrophic.
142	15	50 x 40 x 30	129	Fibrous. Invaded by syncytium.
200	15	35 x 25 x 15		Fibrous.
212	15	Very large	189 (?)	
364	16	90 x 50 x 40	99	Fibrous and atrophic.
137	16	60 x 50 x 30	86	Fibrous.
207	16	70 x 45 x 45		Chorion hyaline(?). Syncytium irregular. Decidua infiltrated with leucoytes. (Twins in a single chorion.)
339	16	50 x 30 x 30		Hyaline.
344	16	45 x 45 x 45		Fibrous and atrophic.
203*d*	15	27 x 27 x 27		Normal(?).
188	17	45 x 40 x 40	66	Very fibrous.
215	17	45 x 40 x 40	12 wks.	Fibrous.
357	17	90 x 40 x 40	13 wks	Fibrous. Invaded by syncytium.

within, seen so often in pathological specimens. Within the embryo the vascular system and heart are much dilated and filled with blood. The whole condition of affairs indicates that the circulation was interrupted shortly before the abortion took place.

The embryo is imbedded in an irregular mass of granular magma, and from its external form it seems to be nearly normal. However, its neck is kinked too much in front, and

sections show that there is an active growth of scar tissue at this point. In general, all the tissues are more or less dissociated, the cartilages and precartilages being most resistant. The walls of the heart and blood-vessels are not sharply defined and many blood cells spread from them into the surrounding tissues. The central canal of the spinal cord is distended and the peripheral nerves are well infiltrated with round cells. The dissociation of the fore-brain and mid-brain is pretty complete, and the walls of the medulla are spreading out into its ventricle. In general, the head is reduced in size,

The most marked secondary changes are seen in the mesenchyme of this specimen. At points there are fibrous thickenings in the skin, which frequently form papillomata, covered more or less with a single layer of epithelium. In front the face and chest have grown together, the point of union naturally closing the mouth, including the tip of the tongue in a mass of round cells.

I picture the whole process as follows: In general, the destruction of villi in the chorion is followed by fibrous atrophy of the umbilical cord and arrest of the heart beat. After the circulation has ceased the organs and tissues gradually dissociate and blood cells enter the tissues. Probably before the changed conditions had reached this extreme state the brain began to dissociate and became solid, and the face atrophied and united with the chest below. The changes in the rest of the embryo were not marked until the circulation ceased altogether. It is clear, however, that the brain dissociates before the rest of the embryo, for we constantly find in it more radical changes than in other portions of the embryo.

Practically the same pathological changes described for specimen No. 311 are found in Nos. 375, 69, 174, 182 and 325. No. 174 has horn-like processes and No. 182 has a straw-colored necrotic mass in front of the head. No. 325 shows still more advanced changes, the necrosed liver is disintegrating, this process having begun in 311. From all appearances this embryo has been dead for a long time, which is also indicated by its menstrual history.

Embryos 14 mm. long repeat the story given by those 13 mm. long. The least amount of change is found in No. 270, which is nearly identical with No. 311. However, the brain is not quite so solid, the dissociation of the tissues of the body is about of the same degree and the frontal process is united to the thorax below. Within the medulla there are papilliform sprouts of nerve tissue which extend into the ventricle, just as in No. 311. No. 346 may be a little older than No. 270, but the changes in it are not quite so advanced, nor has the frontal process united with the thorax below. The head and neck are also straight in Nos. 262 and 232, the changes in the tissues being very advanced. In No. 262 the cerebral hemispheres form a solid mass, which looks like an abscess, the medulla is much distended and its thin anterior wall protrudes through the mouth. Much the same condition is found in the cylindrical head of No. 232. In it the large fifth nerve may be seen running to the surface of the body, and acts as an index to tell how much of the head has become atrophied. The arms and legs are gorged with well stained round cells, indicating that secondary changes have taken place in them.

The marked changes which have taken place in the brain and head have met their end in embryo No. 276. Here we find advanced changes in the head, but the body is much like the other specimens. The medulla is greatly distended and fills entirely the rounded top of the body, the rest of the brain having been expelled through an opening which is still present. Around the edge of it the epidermis is piled upon itself, apparently attempting to heal the wound. The severe changes in the chorion and the long duration of the process have ended by destroying entirely the brain and the top of the head, leaving the body of the embryo capped only with a remnant of a head containing a dissociated medulla.

Most radical changes are found in specimen No. 365. The embryo is within a fibrous chorion. There is spina bifida, iniencephaly and anencephaly. The mouth is closed completely by the tongue becoming adherent on all sides. The tissues of

the body are necrotic, and most of them are infiltrated with round cells, and there is irregular growth of the mesodermal tissues, especially those of the tendons and perichondrium.

The embryos of the second portion of the sixth week, that is, embryos 15, 16 and 17 mm. long, may be brought together in three groups, according to the degree of change in their tissues.

In the first group there are three specimens, Nos. 263d, 132 and 188. In these the first changes are seen after the circulation has been cut off. The tissues and organs are sharply defined, the vascular system is distended with blood, and more or less round cells are found in them. The fore-brain is solid and the medulla and cord are somewhat dissociated. In No. 263d the brain has broken through the palate and a considerable amount of it has escaped into the mouth. However, this embryo is macerated somewhat and is slightly torn in the region of the back, and the brain capsule may have been torn open by mechanical means. In embryo No. 132 the extremities of the right side of the body are atrophic, while those of the left appear to be normal.

In the second group of specimens (Nos. 344, 137 and 357) the changes in the embryo are more marked. The blood-vessels are gorged, their walls are not sharply defined and the blood cells extend from them into the surrounding tissues. In No. 344 the brain is reduced in size, is solid and occupies but a small portion of the head. The medulla is dissociated and expanded and has been pushed forward, almost reaching to the front part of the head. Below this the jaw is kinked over the chest, with which it has formed a secondary union. Over the regions of the fore-brain and mid-brain there are spots in which all of the surrounding tissue is wanting entirely, thus exposing the brain freely at these points.

The last group includes the embryos in which the changes are extreme, and includes seven specimens, Nos. 81, 142, 200, 212, 215, 339 and 364. In them the tissues are well dissociated and more or less filled with round cells. The usual changes are seen in the central nervous system, the spinal

cord being dilated, while the brain and medulla are solid. In these embryos the dissociation is carried to an extreme degree, the extremities being atrophic, and in some of them the embryos are pretty well disintegrated. In Nos. 81, 200 and 212 the face and the top of the head are composed of a thickened mass of necrotic tissue, and the changes in the central nervous system are extreme. The embryo of specimen No. 215 is broken into a number of pieces which barely hold together.

Specimens Nos. 142 and 339 are quite typical ones of this stage, for in them the dissociation of the tissue is pretty complete, and the outlines of the organs are quite obscure. Most of the blood has left the blood-vessels and is in the surrounding tissues. The fore-brain is completely separated from the medulla in No. 339, and in general it is reduced in size: some of it may have escaped through the front of the head, which is broken off. The medulla is rounded at its free end, is distended and fills most of the head. Had this specimen lived it would probably have formed an anencephalic fœtus. But in order to have lived through gestation the change in the whole embryo could not have been as radical as it is in this specimen, and judging by the anatomy of anencephalic monsters the destruction of the brain does not, in all probability, begin until some time after the sixth week. In No. 142 the changes within the embryo are also extreme, but the remnants of the organs remain within the body. However, the external features of the embryo have vanished entirely, the arms and legs having atrophied completely.

No. 364, which belongs to this group, is a most remarkable specimen, for it forms a typical monster and is accompanied by an excellent history. The ovum, covered with a few ragged villi, is from a first conception in a woman who had been married four years. It was from a natural abortion, the woman being very anxious to have children. In general, the woman appears to be healthy, but she has suffered from a variety of troubles with her uterus and vagina, which are given at greater length in the history of the case. The usual

changes are found in the chorion, indicating faulty implantation and inflammation. The embryo, whose menstrual age is 99 days, corresponds in length with a normal embryo 40 days old, that is, having a menstrual age of 68 days. In other words, the pathological process in No. 364 has been under way for fully a month.

The large blood-vessels and heart are still filled with blood and there is a general infiltration of the tissues with round cells; the vessels of the umbilical cord do not reach to the chorion, showing that the nutrition of the embryo has been cut off entirely. There is a general destruction of the tissue due to, or causing, the irregular growth of the embryo. This is especially well marked in the brain and spinal cord, which are rudimentary, are converted into a mass of vascular connective tissue capped by a rudimentary shield of brain tissue, as is illustrated in the figures.

There is pronounced hare-lip, the ears are displaced, and there is exomphalos, spina bifida and pseudencephalus; the latter is no doubt the forerunner of anencephalus.

That the pathological conditions found in most of the specimens reported in this contribution are not of germinal origin, but rather due to the changes in the environment of the ovum, as may be brought about by endometritis, is illustrated beautifully by two sets of twins of the sixth week, which I have been fortunate enough to procure—one from Professor Brödel and the other from Professor Minot. To these may be added the twins of the fifth week (Nos. 330a and 330b), the two sets of specimens, each from the same woman (Nos. 110 and 141 and 308 and 325), kindly sent me by Drs. West and Ballard. These groups of specimens speak volumes against the germinal theory of merosomatous monsters. The facts of the case have been discussed under a special heading above, and they need not be repeated here. However, if the law of probability and the normal condition of the embryos in earlier pregnancies were not taken into consideration, they could be explained by the germinal, just as well as by the environmental theory. The conclusive evi-

dence in favor of monsters being due to a change in the environment, which causes faulty implantation of the ovum, thus impairing the nutrition of the embryo, is found in the study of the embryo in tubal pregnancy, where 96 per cent of them are monstrous. Were the primary trouble in the germ, no more pathological ova should be found in tubal than in uterine pregnancies. Furthermore, all this is vouched for by comparative experimental teratology.

To be sure, polysomatous, pansomatous and those merosomatous monsters that are due to an arrest of development at a very early stage (monopodia) and those variations of an hereditary nature (polydactyly, polymastia) and ordinary anatomical anomalies, cannot be due to changed environment at a stage so late as the fourth week of pregnancy, and some of them, like variations in the hands and feet especially, are markedly hereditary, and therefore germinal in nature. However, this digression is not altogether to the point; the merosomatous monsters, the subject of this report, are due to a direct experiment which is equivalent to the mechanical removal of most of the villi of the chorion.

The two sets of twins (Nos. 207 and 341) are alike in many respects, for each set is contained in a single chorion. The degree of development and degeneration is about the same for each set. In No. 207, the younger one, the process was severe but not of long duration, while in No. 341 the opposite must have been the case. In both sets the organs and tissues are well dissociated, showing the usual changes so often seen in the embryos studied. When I first took up the study of pathological embryos I was inclined to the idea that the changes in the chorion were often of a secondary nature, but as the specimens became more and more numerous and were preserved better and better, which enabled me to study them with greater care, this idea had to be abandoned. Now it is clear that we are dealing with a simple experiment which must bring about the changes in the ovum and embryo to make it pathological. The greater number of ordinary abortions in the first month consists of ordinary pathological

embryos. The changes in them are so radical that it is impossible for but few of them to develop into monsters had they not been aborted. However, it is not difficult to imagine specimens in which the changes are not so extreme, that is, they are due to minor changes in the chorion, which may retard the development of a part of the embryo, and afterwards become corrected, thus favoring the growth of a distorted embryo into a merosomatous monster. In nearly all of these embryos there is a tendency for the liquor amnii to increase in quantity, a condition which must also be viewed as a secondary process, and, therefore, cannot be of fundamental significance in the production of monsters.

It is evident from the study of pathological ova that in order to complete the chain of evidence it will be necessary to study anew and with much greater care the membranes of embryos which appear to be normal, for in them we shall no doubt find the very earliest stages of monsters which could have existed and grown throughout pregnancy. The recent publication of Fischel, as well as the more careful study of some of my "normal" embryos (Nos. 6, 10, 11, 12 and 80), indicates that embryos of this kind will probably serve to clear up entirely the subject under discussion.

Embryos of the Seventh Week.

In the seventh week, when some of the bones are ossified, the embryos have reached a stage in which interference with their nutrition does not shatter all of their tissues at once. The effect upon the central nervous system is still more pronounced than that upon the other tissues, and even at this late period the dissociated structures continued to grow in an irregular fashion.

The number of specimens at this time is also greatly diminished, as is naturally expected, for the younger ova are attacked early by the infected mucous membrane of the uterus and few of them survive until the seventh week. It follows, then, that as gestation continues pathological ova are

less and less likely to be found. To be sure, the diseased specimen may be retained in the uterus for a long time as a mole, with or without an embryo, but if the chorion is not affected during the first few months of pregnancy it is likely to go on to full term, or if it is aborted so late it rarely contains a dwarfed embryo. Furthermore, infections of the uterus are infrequent after pregnancy is well under way, and in case it does take place, the probability of its attacking the whole chorion is slight. The embryo would probably withstand the insult, since it is now more differentiated and more resistant.

There are in my collection ten pathological specimens of the seventh week and half of them may be normal, at least the changes in them are but slight. Furthermore, after the seventh week there is but one pathological specimen a week until the fourteenth week, and none from this time until the end of pregnancy.

A summary of the embryos brought together in the various tables is as follows:

Vesicular forms	19
Ova with neither embryo nor amnion	29
Ova with amnion but without embryo	15
Pathological embryos of the second week	4
Pathological embryos of the third week	18
Pathological embryos of the fourth week	21
Pathological embryos of the fifth week	13
Pathological embryos of the sixth week	27
Pathological embryos of the seventh week	10
Pathological embryos of the eighth week	2
Pathological embryos of the ninth week	1
Pathological embryos of the tenth week	0
Pathological embryos of the eleventh week	1
Pathological embryos of the twelfth week	0
Pathological embryos of the thirteenth week	1
Pathological embryos of the fourteenth week	1

This infrequency of pathological ova as pregnancy continues is not at all remarkable, for human monsters at term are not so very common, and while they are produced in the early months of pregnancy, at first the changes in them are slight, and consequently they are not aborted. The pathological ova in which the changes in the embryos are very severe are the kind that are aborted and the ones which I have been considering. However, the reactions in them give us a hint of what takes place in the early stages of monsters that continue to develop until the usual end of pregnancy.

Of the specimens of the seventh week, No. 128 is normal in every respect with the exception of the presence of a fairly marked magma reticulé in the amniotic cavity. This I have not found in other normal specimens, and, since it is the earliest and most constant sign of a diseased embryo, it is worthy of mention. No. 307 is also no doubt normal, although small clumps of leucocytes are found between the villi and at points they are found in the mesoderm of the chorion. Nos. 268 and 338a may be normal, although some tissue changes are seen. These may be due to maceration, for the specimens are not especially well preserved. Dissociation may have begun in No. 345, but it is more or less obscured by the extensive maceration which accompanies it.

Marked pathological changes are found in specimens Nos. 320 and 94. In them changes are found in the chorion, showing that the attack by leucocytes has been very severe. In one (No. 94) there is a great amount of granular magma within the amnion. The tissues of the embryos are dissociated, the brain and cord being nearly solid. In general, the boundaries of the organs are obscure, their tissues being more or less infiltrated with round cells. We have in these two embryos conditions found so frequently in younger specimens.

No. 293 is a specimen of unusual interest, for the sections show that most of the embryo is normal, only one portion of it being affected. The blister upon the back was recognized by Dr. Lamb when he opened the chorion. It is filled with a granular albumen, and at its edges burrows into

TABLE XIV.

NORMAL EMBRYOS OF THE SEVENTH WEEK.

Specimen.	Embryo.	Chorion.	Menstrual Age.
	mm.	mm.	days
No. 17.....	18	40 x 30 x 20	
No. 42.....	18	35	
No. 160.....	18		2 mos.
No. 5	18.5	40 x 30	
No. 28	19	50 x 30 x 20	47
No. 229.....	19		49
No. 128.....	20	50 x 43	76
No. 22.....	20	35 x 30 x 30	
No. 240.....	20	50 x 40 x 30	
No. 194.....	21	45 x 45 x 45	
Minot	22		53
His	22		56
No. 57.....	23	30	
No. 242.....	23	70 x 50 x 50	
No. 363.....	23		66
His. (Wt)	23	55 x 50	
No. 72.....	23	40 x 30	
No. 27.....	23	30	65
His (Lp)	23	55 x 50	
No. 6.....	24		77
No. 31.....	24	50 x 30 x 30	68
No 127.....	24	60 x 45 x 40	84

TABLE XV.

ARRESTED DEVELOPMENT OF THE EMBRYO.

(Seventh Week.)

No.	Length of Embryo.	Dimensions of Chorion.	Menstrual Age.	Changes in the Chorion.
320	18	70 x 50 x 40		Some fibrous. Ovum swollen and a few masses of leucocytes.
338a	18	45 x 45	6 to 8 wks.	Normal.
293	19		3 or 4 months	
345	19	60 x 50 x 50		
94	20	50 x 40 x 30		Atrophic and invaded by leucocytes.
128	20	50 x 43	76	Normal.
201	20			Fibrous and invaded by leucocytes and syncytium.
307	20	40 x 40 x 40		Invaded by leucocytes.
268	22			
226	24	60 x 60 x 30	87	

the mesoderm, which is well infiltrated with round cells. The "inflammatory" process extends along the middle of the back to the top of the head. Immediately over the cord, in the middle of the back, the infiltration of cells extends throughout the mesoderm and includes the meninges of the cord, showing a similarity with the changes in the case of spina bifida in the embryo described recently by Fischel (Plate I). It certainly would be very easy for a localized affair like this to prepare the way for the production of spina bifida. Possibly in the course of time, after my pathological collection contains thousands of specimens, intermediate stages will be found which will show that the changes found in this embryo favor the production of monsters with spina bifida.

Another embryo in which the spinal canal is broken open behind (No. 226) shows extensive alterations in its tissues. The changes in the chorion indicate that the circulation within the embryo had ceased some time before the abortion. Its mesoderm is fibrous and almost devoid of blood-vessels. Between the amnion and chorion there is a layer of organized blood from the mother, showing that there must have been a rupture some time before the abortion.

The external form of the embryo is interesting, the trunk, extremities and cord being normal in form, while there is a marked defect in the head. Here on the dorsal side the destructive process has also included the upper part of the spinal cord, producing complete spina bifida. The rest of the central nervous system is still intact, but the cerebral vesicles are reduced in size and are exposed to the exterior of the body. The free end of the upper part of the cord is broken quite abruptly, while that of the lower part of the medulla is rounded off, *i. e.*, it appears to have healed over. The larger portion of the cervical cord is missing; it may have escaped through the dorsal opening.

There are marked changes in the tissues of the body, which may be due to maceration rather than dissociation. The connective tissues, including the bone and the vascular system, are well preserved, with more or less round-celled infiltration and possibly some fibrous thickening.

A more advanced stage of the conditions found in No. 226 may be seen in No. 201, which in addition has cyclopia. In this specimen there are marked changes within the chorion to account for the degeneration of the embryo. The villi of the chorion are intermingled in irregular order with blood, fibrin and pus, and the mesoderm is fibrous and more or less infiltrated with leucocytes and syncytial cells.

The form of the specimen is that of a younger embryo, but the ossification centers of the maxilla, mandible, clavicle and humerus are present. The epidermis is nearly complete, thickened at points, but wanting over the top of the head. The mouth and anus are obliterated and the coils of intestine form a single mass, into which the entodermal cells ramify. In form the thoracic region, vascular system and liver are normal, although the cells of the latter are necrotic. Throughout the embryo there is an extreme growth of mesodermal tissue, apparently that of the precartilage being the most active. This newly-formed tissue seems to have invaded the embryonic muscles which are more or less destroyed.

The changes within the central nervous system are extreme, the brain being greatly deformed and separated by a growth of connective tissue from the spinal cord below. The cap-like process upon the head contains the medulla and mid-brain, which are well dissociated, partly necrotic and partly infiltrated with round cells. On either side of this there are two degenerated cerebral hemispheres which communicate in front of the medulla. The spinal cord begins quite abruptly in the upper cervical region, and ends in a marked fibrous tumor, one-half millimeter in diameter, in the upper lumbar region. In the lumbar and cervical regions the spinal canal is filled with mesodermal tissue rich in blood-vessels. Here, however, spinal nerves are present, showing that the destruction of the spinal cord is of recent date.

The two eyes form a single hour-glass-shaped body, with a double retina, two lenses, a single choroid and a single median optic nerve, which does not reach to the brain. Between this and the ear and tongue there are a variety of

structures—muscles and nerves—which are difficult to trace. Some nerves pass to the epidermis and may represent branches of the fifth, and one forms a commissure across the median line. At any rate, there has been a great deal of shifting, and it is natural to think that the eyes did likewise, as is the case in the experiments on Fundulus, mentioned above.

Embryos of the Eighth Week and Older.

There are but two specimens of the eighth week (Nos. 79 and 152), one about 56 and the other 57 days old. Both are strangulated embryos imbedded in a mass of granular magma with the chorion more or less infiltrated with leucocytes. No. 152 is from a woman suffering with endometritis, this being her third successive abortion, each of which took place during the third month of pregnancy. In this specimen the umbilical cord is thin and very much twisted, a condition which might also interfere with the nutrition of the embryo.

The bodies of the two embryos are more or less altered, the greatest change being found in the central nervous system, as found in so many of the younger specimens. The vascular system is dilated and there are clumps of blood cells in the surrounding tissues. In No. 152 the connective tissue appears to be more fibrous than is normal, the cutis being more or less hypertrophied.

The remaining specimens may be considered in two groups: (1) Those with a tendency towards club-foot, the most common malformation, and (2) those tending towards partial destruction of the central nervous system, also a very common malformation. At the present time, I cannot do better than to describe these specimens in regular order, for there are not enough specimens to allow following the changes from one to the other with any degree of certainty. Table XVII shows, however, that the placentæ are involved in all cases in which they were studied. The villi are more or less fibrous or hya-

TABLE XVI.

Normal Embryo of the Eighth Week and Older.

Specimen.	Embryo.	Chorion.	Menstrual Age.	Specimen.	Embryo.	Chorion.	Menstrual Age.
	mm.	mm.	days		mm.	mm.	days.
No. 118..	25		94	No. 315..	47		63
His (Dr2)	25	45 x 40		No. 105..	48		83
No. 99...	27	40	75	No. 184..	50	60 x 55 x 55	
No. 192..	27	70 x 60 x 50		No. 151..	52	80 x 60 x 60	
No. 403..	27		64	No. 169..	52		110
No. 45...	28	40 x 35 x 20		No. 139..	55	80 x 65 x 40	79
No. 203..	28	40 x 30 x 30		No. 326..	55		77
No. 26...	30		75	No. 267..	59		84
No. 155..	30	50 x 40		No. 30...	60		77
No. 202..	30		71	No. 171..	60	70 x 50 x 50	56
No. 227..	30	60 x 45 x 20		No. 92...	70		98
No. 274..	31		57	No. 23...	70		65
No. 373..	31	50 x 35	69	No. 300..	73		14 wks.
Minot....	32		68	No. 34...	80		104
No. 129..	32		66	No. 172..	80		103
No. 145..	33	60 x 50 x 40	78	No. 125..	83		84
No. 52...	33	40 x 30 x 15		No. 308..	84		101
No. 211..	33		9 wks.	No. 337..	90		64
No. 178..	35		61	No. 146..	95		115
No. 269..	35		77	No. 392..	95		101
No. 213..	37	70 x 60 x 50		No. 117..	100		111
No. 176..	38	70 x 70 x 70		No. 394..	105		125
No. 329..	39		82	No. 138..	112		127
No. 140..	40		72	No. 355..	113		14 wks.
No. 206..	40		89	No. 126..	125		125
No. 224..	40	60 x 50 x 40	78	No. 149..	130		126
No. 362..	40		44	No. 46...	135		140
No. 282..	42		36	No. 356..	150		130
No. 96...	44		84	No. 98...	160		125
No. 301..	44	60 x 40 x 30		No. 359..	160		156
No. 217..	45	80 x 60 x 60	78	No. 354..	190		178
No. 259..	45	65 x 50		No. 121..	210		190
No. 95...	46	68 x 50 x 50	83				

line, usually infiltrated with leucocytes, and sometimes attacked by syncytial masses. The umbilical cord is usually thin and much twisted. It appears as if the beginning of the trouble lay in the chorion or placenta, which was gradually poisoned by the products of inflammation in the uterus, and in the course of time this resulted in fibrous degeneration of its villi, main wall of the chorion and finally the cord. As a result of malnutrition, the tissues of the embryo grew in

TABLE XVII.

No.	Length of Embryo.	Dimensions of the Chorion.	Menstrual Age.	Chorion.
152	31	70 x 42 x 38	70	Fibrous and invaded by leucocytes and syncytium. Cord thin.
79	32	50 x 50 x 50	91	Invaded by leucocytes and syncytium.
124	35	90 x 75 x 50	126	Abscesses in the placenta Cord thin and twisted.
316	44			Cord thin and fibrous.
230	57	75 x 60 x 50	7 mos.	
286	60	100 x 50 x 40	225	Hyaline and infiltrated with leucocytes and syncytium. Cord thin and twisted.
308	84		101	Fibrous (?) Muco-purulent substance between villi. Cord twisted.
261	90	120 x 70 x 70		Very fibrous.

an irregular fashion, the central nervous system and extremities suffering most, as is the case in numerous younger specimens. Later the heart stopped, and then the most resistant cells of the body continued to grow for a time until everything came to a standstill.

These changes are beautifully illustrated in specimen No. 124, which is also described and well pictured in my first paper upon this subject. This embryo is of the nine weeks' stage, with a chorion twice too large and a menstrual history five weeks too long. This means that after the ninth week of pregnancy the embryo ceased to grow, but the chorion continued to expand at the same rate as the normal one grows, until the fourteenth week, when it aborted. What is also noteworthy is that the amnion did not keep pace with the growth of the chorion, leaving between them a large exocœlom. The placenta is more or less diseased, that is, infiltrated with leucocytes, which at points produce small abscesses. The embryo itself has atrophic ears, club-hands and club-feet. After the embryo had been in my possession for seven years it was cut into sagittal sections, which, unfortunately, did not stain well. However, they show that the skin is more fibrous than normal, being infiltrated with round

cells, especially in the deformed extremities, where all of the structures are involved, forming syndactyly.

Changes similar to the ones found in No. 124 are seen again in No. 316. Unfortunately, I failed to obtain the membranes or any history of this specimen, so its story must be told by its form and structure alone. The feet and one hand are club-shaped, and the other hand is spread out and is attached to the side of the head. The skin is thickened and much of the epidermis has fallen off. At points the epithelial cells form mounds without any tendency towards horny changes in them. The muscles, blood-vessels and nerves of the extremities are converted into one fibrous mass of spindle-shaped cells, giving much the appearance of myomatous tissue infiltrated with round cells. The cartilages are hyaline, and bone has formed in the center of the calcaneum. The hand has grown to the side of the head, the epithelial coverings having united. The true skin is composed of a mass of round cells.

No. 230 shows about the same changes, with additional "records" in the chorion, including the menstrual age. Together they show that the pathological process must have been under way for at least three months. There are some leucocytes in the chorion and the cord is thin and twisted. The tissues of the embryo appear normal, but they do not stain well, and the changes in the hands and feet appear to have been caused by mechanical twisting after the death of the embryo.

No. 286 is a similar specimen. The chorion is hyaline, infiltrated with leucocytes, and is attacked by syncytial cell masses. However, sections of different portions of the embryo show that its tissues are practically normal, which indicates that its death must have been sudden and not gradual.

The oldest specimen of this group is No. 261, which must also have been dead for a considerable time. The villi of the placenta have undergone fibrous degeneration and are devoid of syncytium. The cord is twisted, is of normal size, and at its attachment to the placenta is somewhat fibrous.

Its blood-vessels are filled with blood. The decidua is composed of large sinuses, which are well filled with round cells.

At this place it may be well to introduce the description of a specimen (No. 308) which may prove to be of unusual value. In every respect it appeared to be a normal one about 100 days old, but the amniotic cavity was found filled completely with a mass of granular magma which was easily brushed aside to expose the embryo. The umbilical cord was found wrapped around both of the arms like a pair of shoulder braces, as the figure shows. Sections from the middle of placenta, at the point of the attachment of the cord, show that there is a muco-purulent mass between its villi, which contain many fragmented nuclei.

It may be that the poisonous condition of the uterus stimulated the embryo unduly, which in its gyrations got well wrapped up in its own cord. This naturally affected its nutrition, the first sign of which is the presence of granular magma. Had it not been aborted it would probably have ended like some of the others just described.

In numerous younger embryos a destruction of the brain and cord were noticed, and in a few of them the brain was extruding from the head. Yet all these changes were by far too severe to end in anencephalic monsters at full term. In nearly all of these specimens the changes in the central nervous system followed the cessation of the heart beat, and naturally such embryos could not continue their development; at best they formed moles. At any rate, the changes in these embryos show in a most radical way the reactions of the various tissues when the circulation is gradually stopped.

However, development cannot continue long if the infection of the chorion is severe, and, therefore, we find but few older specimens of pathological embryos in a relatively large collection. Usually these appear to be uninteresting, are misplaced or are thrown away. This has not been the case with my collection, and in it there are but few older pathological embryos. If a variety of merosomatous monsters are due to endometritis, we should expect to find them in diminished

number as pregnancy proceeds, for it is likely that changes which arrest the circulation in the embryo, or fœtus, will soon end in abortion. Therefore it is to be expected that a small number of pathological embryos develop into well-formed monsters.

In specimen No. 293 we have, however, conditions which might end in a typical spina bifida, for the tissues over the spinal cord are infiltrated with embryo's blood and are being destroyed. The epidermis is intact. Had this embryo not been aborted the injury in it is sufficient to permit of an irregular growth of the cord to form spina bifida occulta.

In specimens Nos. 201 and 226 the changes in the central nervous system are very pronounced, and had they not been quite so severe it is easy to imagine their growth, at full term, into a cyclops fœtus in the first case and into anencephalus in the second case.

Specimen No. 295 has in it changes which, if extended, could affect the cerebrum, and I am inclined to the belief that most cases of anencephaly begin in these later stages rather than in very young ones, for if they did not how could a relatively normal base of the skull develop in them? In this fœtus correlated development has given form to all the bones of the skull, and the proportion of those of the base would not change very much in case the vault and brain were destroyed, as is found in typical anencephalic monsters at birth. Questions like these are open to investigation and will give us the key by which we may determine at what time in development anencephaly begins, or whether the time is at all constant.

PART II.

DESCRIPTION OF THE SPECIMENS AND THE FIGURES.

The description of each specimen is complete in itself, giving its main data, which were obtained from my note-books as well as from the specimens and their sections. Their numbers correspond with those in my catalogue.

The measurements of the embryo are as follows: *C.R.*, crown-rump or sitting height; *C.H.*, crown-heel or standing height; and *A.R.*, neck rump or length of the spinal column.

These specimens, with others which I am collecting, will be deposited in the Wistar Institute, where they may be studied by investigators interested in teratology.

DESCRIPTION OF THE SPECIMENS AND FIGURES.

No. 6.

Ovum, 40 mm. in diameter; embryo, C. R., 24 mm.

From Dr. C. O. Miller, Baltimore, October 27, 1892.

This specimen was obtained through the kindness of Dr. C. O. Miller, from whom the excellent specimen, No. 2, was secured some time before. (See JOURNAL OF MORPHOLOGY, Vol. 5, p. 459.) Both specimens were removed from the

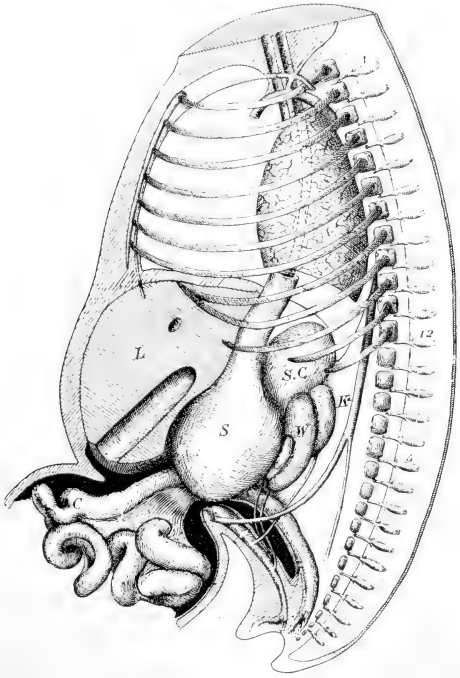


FIG. 6a.—Reconstruction of the body of the embryo. × 8 times. *1-12*, dorsal ganglia; *SC*, suprarenal body; *S*, stomach; *C*, cæcum; *W*, Wolffian body; *K*, kidney; *L*, liver.

uterus by self-inflicted mechanical abortions, which the woman was in the habit of performing upon herself and which finally caused her death. Dr. Miller informs me that when he was

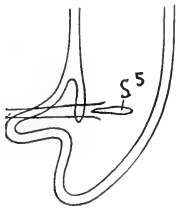


Fig. 6b.—Diagram of the lower part of the spinal cord through the vesicle protruding from its ventral side. S^5, fifth coccygeal nerve with its ganglion.

called to visit his patient she was bleeding profusely and he had considerable difficulty in removing this embryo and its membranes. In so doing the ovum was ruptured, but it still

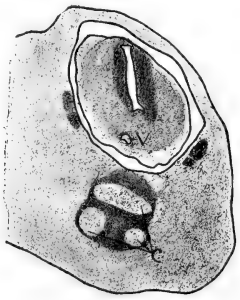


Fig. 6c.—Section through the upper part of the vesicle, shown in Fig. b. *V*, vesicle; *C*, cartilages at the tip of the spinal column, the last being double.

retained a sufficient quantity of fluid to protect the embryo. The time of the abortion was 77 days after the beginning of the last menstrual period. The entire membrane and embryo

were placed in 95 per cent alcohol one and one-half hours after the abortion.

The above history makes it highly probable that the embryo is normal, as does also its reconstruction. However, at the tip of the tail there is a small vesicle which cannot be considered normal. It is lined with a single layer of cylindrical cells, much like that of the central canal, is covered in part with round cells identical with those of the cord, and has on either side of it a spinal nerve, as shown in the diagram and the drawings.

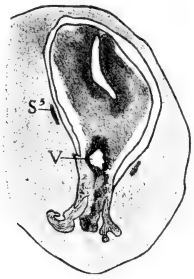


FIG. 6d.—Section through the vesicle, *V*, nearer the tail with a ventral, motor, nerve root on either side.

No. 10.

Embryo, C. R., 20 mm.

From Dr. W. S. Miller, Worcester, Mass.

At first I believed the embryo to be normal, but after it had been cut into sections 20 microns thick, and the entire body reconstructed from them, it was found that the abdominal viscera were clumsy in shape and that the liver protruded into the umbilical cord. A comparison of the picture of the reconstruction of the body of this embryo with those of both older and younger specimens will show that the body of the embryo is markedly distorted and that there is hernia of the liver.

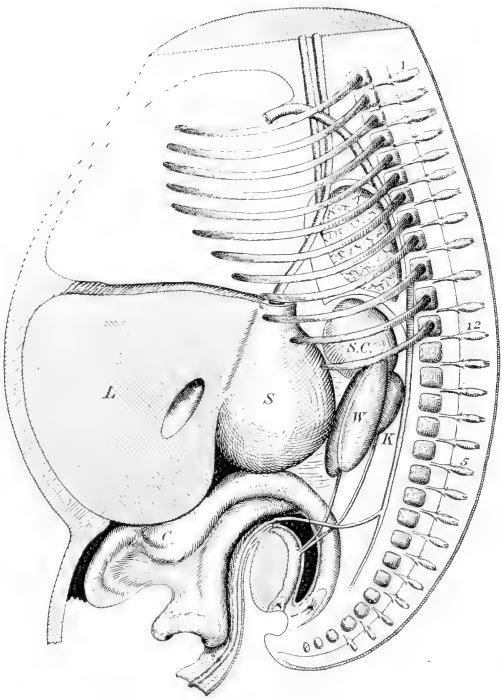


Fig. 10.—Reconstruction of the embryo. × 8 times. *L*, liver; *S*, stomach; *W*, Wolffian body; *SC*, suprarenal body.

No. 11.

Ovum, 10 x 7 mm.; umbilical vesicle, 1.5 x 1 mm.; "embryo," .8 mm.

From Dr. Kittridge, Nashua, N. H., March 16, 1893.

This specimen was sent me by mail in an ordinary 4-oz. bottle filled completely with alcohol. Dr. Kittridge has procured for me the following history: "The woman, from whom the specimen was obtained, is twenty-five years old, menstruates regularly every four weeks, the periods lasting from

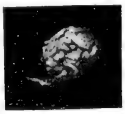


Fig. 11a.—Photograph of the entire ovum. Natural size.

four to five days. She gave birth to a child September 19, 1892, and had the first recurrence of menstruation December 19. The second period followed on January 25, and was very profuse; it lasted until February 1. The next period should have begun about February 22, but on account of its lapsing

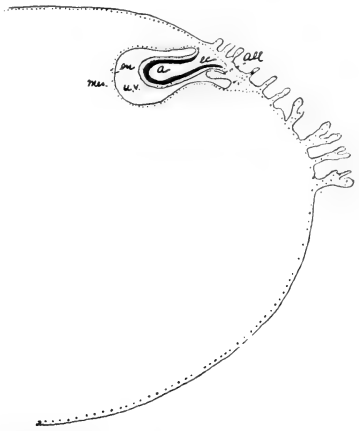


Fig. 11b.—Diagrammatic section of half the ovum with the embryonic mass attached. × 10 times. The villi are drawing only upon the upper quarter of the chorion. *Ec,* ectoderm; *en,* entoderm; *uv,* umbilical vesicle; *mes,* mesodern; *coe,* cœlom; *all,* allantois; *a,* amnion.

the patient concluded that she was pregnant, and called at my office a few days later. I did not examine her, but asked her to remain quiet and await developments, as I thought possibly that she might be pregnant. On the evening of March 1 she fell and sprained herself, and during the same night had a

scanty flow. The flow recurred each day, and on the 7th of March she passed the ovum. It was kept in a cool, moist cloth for twenty hours, and when it came into my hands was at once placed in a large quantity of 60 per cent alcohol."

The ovum is very large for its age, having a long diameter of 10 mm. and a short diameter of 7 mm. It is covered with villi only around its greatest circumference, having two spots without villi, as was the case with Reichert's ovum. The villi of the chorion are from 0.5 to 0.7 mm. long, are branched and are somewhat fibrous in structure.

Upon opening the chorion it was found that the embryonic vesicle is situated just opposite the edge of the zone of villi.

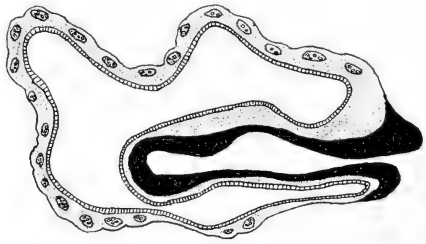


Fig. 11c.—Section through the umbilical vesicle and its invagination of specimen. × 50 times. The three layers correspond with the ectoderm, mesoderm and entoderm. The invagination marks the cavity of the amnion.

About it there is a considerable quantity of magma reticulé, which I did not remove, and therefore could not obtain good camera drawings. The portion of the chorion to which the vesicle is attached was cut out and stained with alum cochineal and cleared in oil, but even after this treatment it was impossible to obtain any clear picture. The specimen was next imbedded in paraffin and cut into sections 10 microns thick. The series proved to be perfect. From the sections a reconstruction was made in wax.

The dimensions of the different portions of the vesicle are as follows:

Diameter of stem	0.4 mm.
Length of stem	0.4 "
Length of vesicle	1.5 "
Width of vesicle	1.0 "
Length of invagination	0.8 "
Width of invagination	0.5 "
Diameter of opening of invagination	0.03 "

The sections and reconstruction show that the embryonic vesicle is attached to the chorion by means of a stem. The greater part of the vesicle itself is composed of two layers, ectoderm and mesoderm. In the neighborhood of the em-

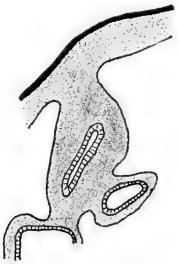


FIG. 11d.—Section through the stem uniting the umbilical vesicle with the chorion. × 50 times. The cavity within the stem lined with epithelium is the allantois.

bryonic stem there is a third outer layer which shows all of the characteristics of the ectoderm. Just beside the attachment of the vesicle to the stem there is a sharp, deep and narrow invagination of all three embryonic membranes. Within the stem there is a sharply defined allantois which communicates with the cavity of the vesicle just below the cavity of the ectoderm. The ectodermal plate of the invagination is very broad but not of equal thickness throughout its whole extent. It extends to the outside of the vesicle and ends quite abruptly in the neighborhood of the stem. The blood-vessels of the mesodermal layer extend to the stem but do not enter it, nor are there any blood-vessels in the chorion.

Since the first publication of this specimen, embryos both normal and pathological have been studied, all of which indicate more and more that this specimen must belong to the pathological class. The other pathological specimens in my collection as well as the perfect normal specimen described recently by Peters all speak for this conclusion. Yet the presence of all three blastodermic membranes in it, with blood islands in the mesoderm, and an allantois in the embryonic stem, indicate that this specimen cannot be far from the normal, but represents the earliest changes in the blastodermic membranes in a specimen of the Peters' stage under pathological conditions.

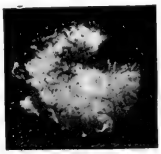


Fig. 12a.—Photograph of the entire ovum. Natural size.

No. 12.

Ovum, 20 x 20 mm.; embryo, C. R., 2.1 mm.

From Dr. Ellis, Elkton, Md.

"The patient from whom the ovum was obtained is twenty-three years old, menstruated first in her fourteenth and married in her twentieth year. Some time after her marriage she became pregnant and aborted July 6, 1893, having passed two periods at that time. The next time she became pregnant she aborted this specimen. She was last unwell November 7, the flow lasting five days, and she aborted on the 18th of December, that is, I found the ovum in the discharges of that day, although the waiting began the day before. The patient says it has always been her habit to go more than twenty-eight days, her periods recurring on the thirtieth day usually, but frequently the intervals are longer, thus: She was unwell on October 5 and on November 7 and in Sep-

tember she went a week over her time. The patient is an intelligent and truthful person and you can rely on her statements."

The ovum appears to be perfect and normal, being covered with the normal number of villi. The embryo within appeared normal and of the two weeks' stage. In reflecting over the specimen, I have concluded that its brain is too small,

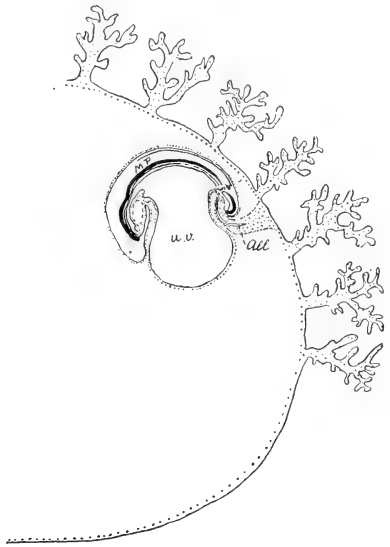


FIG. 12b.—Diagrammatic reconstruction of half the ovum with the embryo attached. × 10 times. The villi are drawn upon the upper half of the diagram only. *Coe,* cœlom; *uv,* umbilical vesicle; *all,* allantois; *mp,* medullary plate.

the central canal too wide open, and the optic vesicles too atrophic to be normal. The spinal cord is also too wide open behind. The embryo could be viewed as normal with the exception of the spina bifida in the lower part of the cord and the anencephaly in the anterior.

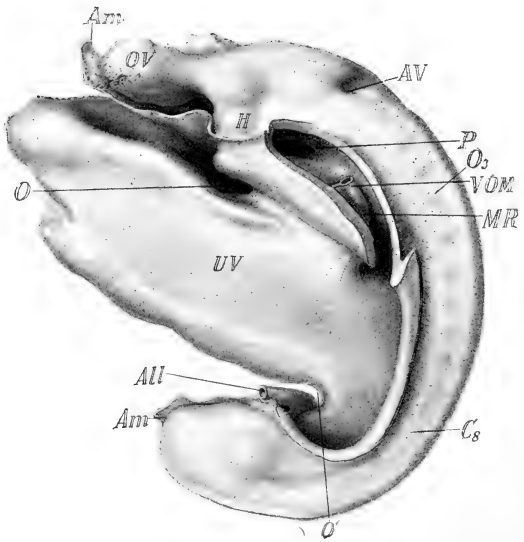


FIG. 12c.—From a reconstruction in wax. × 40 times. C_8, eighth cervical myotome; *OV*, optic vesicle; *AV*, auditory vesicle; *H*, heart; *VOM*, yolk vein; *P*, cœlom; *UV*, umbilical vesicle; O_3, third occipital myotome; *All*, allantois; *Am*, amnion.

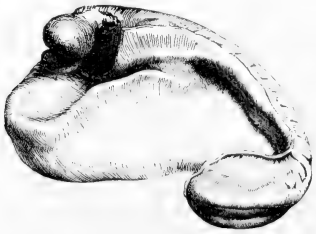


FIG. 12d.—View of the embryo to show the open spinal cord below and the atrophic head with large neuropore.

Sections of the chorion indicate also that it is practically normal with the exception of some fibrinous masses between the villi. Otherwise there is no indication of a change in the structures of the villi nor in the syncytium.

It is certainly possible that all of these slight changes took place during the twenty-four hours before the abortion, while the uterus was making ready to expel the ovum.

No. 13.

Ovum, 8 x 7 mm.; vesicle within, 1.4 x .85 mm.

From Professor His, Leipzig.

This embryo is the well-known specimen No. 44 of the His collection. (See Anatomie mensch. Embryonen, II, pp. 32 and 87.) The ovum is not completely covered with villi.

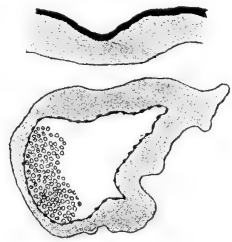


Fig. 13a.—Section through the umbilical vesicle and chorion of specimen No. 13, His's No. 44. Blood corpuscles are seen within the cavity of the vesicle. × 30 times.

Within there is a small double vesicle which appears to be the amnion lying upon the umbilical vesicle. Attached to the denser (umbilical) vesicle there are numerous fibrils which extend throughout the entire cœlom.

This specimen promised to be, at the time Professor His described it, the valuable early stage sought for by all embryologists, but unfortunately the sections prove it to be pathological. The great quantity of fibrils, magma reticulé, within the cœlom already indicated that the embryo is not normal.

FIG. 13b.—Section through the amnion, jugular veins, umbilical vesicle and chorion. × 30 times.

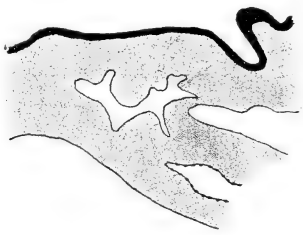


FIG. 13c.—Section through the umbilical vesicle as it joins the chorion. × 30 times. The large irregular space in the chorion is a blood space which communicates with the veins of the embryo.

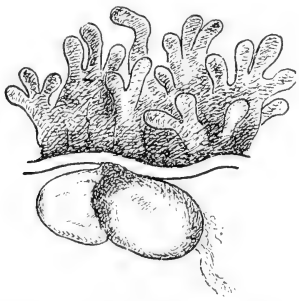


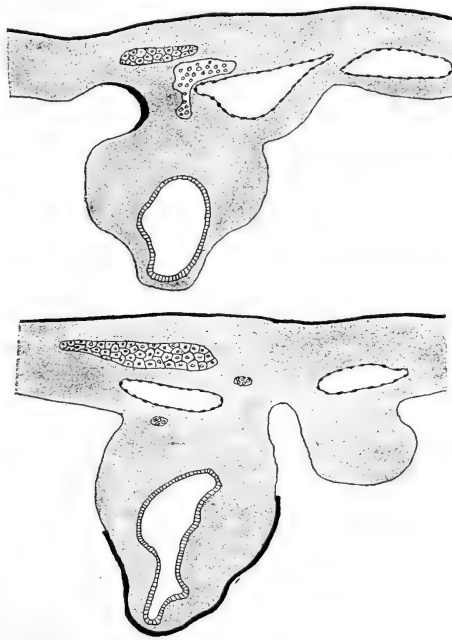
FIG. 13d.—Embryonic vesicle attached to the chorion. × 20 times. After His.

The sections show that there is a double embryonic vesicle composed of the amnion and umbilical vesicle, the walls of which are thickened and fibrous, with the embryonic layers but poorly defined. The tissues of the vesicle and its cavity are well filled with migrating cells. The chorion is also fibrous, with blood-vessels and migrating cells extending into the villi. The syncytial layer is not extensive.

No. 14.

Chorion, 30 mm. in diameter; within them is a small double vesicle with a short pedicle 1.5 mm. in diameter.

From Dr. Friedenwald, Baltimore, 1893.



FIGS. 14a and 14b.—Sections through the nodule (vesicle) of the specimen. × 25 times. A few blood islands as well as an enclosed mass of syncytium are within the stem.

The mesodermal layer of the chorion is thin and decidedly fibrous with but few cells scattered through it. There are no villi. There are groups of cells in the chorion, at the base of the embryonic vesicle, which are probably islands of syncytium inclosed within it.

The walls of the vesicle are thick and fibrous with no blood island within them. It is covered with a single layer of epithelial cells which have fallen off at points. Scattered throughout the mesoderm there are numerous migrating cells. At the base of the vesicles there are a few blood spaces with blood cells within them. The vesicle is lined with a single layer of epithelial cells.

At the base of the larger vesicle there is a large closed space lined with spindle cells. A similar space lies immediately below the smaller vesicle.

No. 20.

Chorion, 20 x 14.6 mm.

From Dr. J. W. Williams, Baltimore, February 14, 1894.

From the exterior, the ovum appears to be normal with well developed villi of the chorion. Within the cœlom, however, there is a great quantity of magma, within which were buried several nodules. These were removed and sectioned.

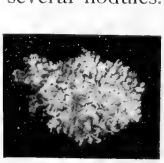


Fig. 20a.

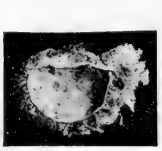


Fig. 20b.

Fig. 20a.—Exterior of ovum, showing long irregular villi. Natural size.
Fig. 20b.—Inside view of chorion, showing strands of magma reticulé.

Sections show that there is no amnion lining the chorion and that the small nodules are only small masses of magma which contain no cells. The villi are normal in form with the usual quantity of syncytium upon them. At isolated points between the villi there are small masses of a granular substance which look like coagulated albumin.

No. 21.

Chorion, 12 x 9 x 5 mm.; vesicle within, 5.5 x 3.5 mm.

From Dr. Cullen, London, Canada, January, 1896.

The fresh specimen, still inclosed within the decidua, was hardened in a large quantity of formalin and sent by express in the same fluid.

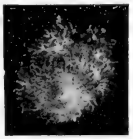


FIG. 21a.—Ovum covered with long irregular villi. Slightly enlarged.

From the external appearance, the ovum is apparently normal, with well-developed villi branching a number of times. Upon opening the specimen it was found that the cœlom is filled with a small quantity of magma reticulé, within which is embedded a very large transparent vesicle.

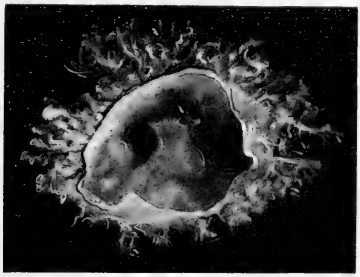


FIG. 21b.—Interior of chorion, showing magma and vesicle. × 5 times.

This, with its attachment to the chorion, was removed and cut into serial sections 20 microns thick.

The main vesicle is brought in contact with the chorion by means of a small secondary vesicle; both are inclosed with a layer of mesoderm within which are numerous blood islands

The smaller vesicle is lined with a layer of large spindle-shaped cells. Migrating cells are within the cavities of both vesicles, and are also scattered throughout the surrounding magma.

There are no blood-vessels in the chorion. The syncytial layer is diminished, but is well formed upon the tips of the villi. Here it often accumulates in layers, forming peculiar strata.

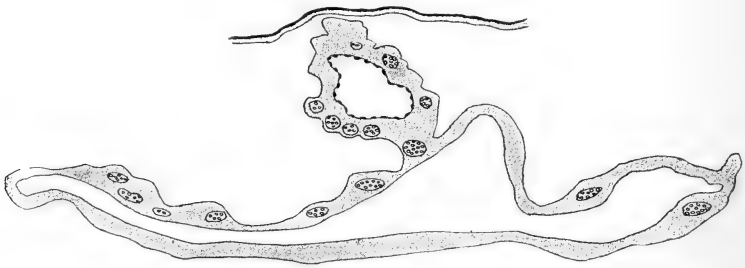


Fig. 21c.—Section through the vesicle and chorion. × 25 times. The second vesicle between the larger one and the chorion appears to be the stem with a dilated allantois, although it is not attached to the chorion.

No. 24.

Chorion, 21 x 16 x 5 mm.

From Dr. C. O. Miller, Baltimore.

The ovum was covered completely with villi which branch a number of times. Upon opening the specimen it was found that the cœlom was filled completely with magma reticulé of moderate density. No trace of an embryo could be found. From time to time I made renewed search and finally decided to cut the entire specimen into sections. After staining it with cochineal a small nodule became visible when the specimen was placed in direct sunlight. This, with a piece of chorion upon which it lay, was cut into serial sections 20 microns thick.

The walls of the vesicle are composed of three layers, the outer being greatly thickened at points, but retains sharp

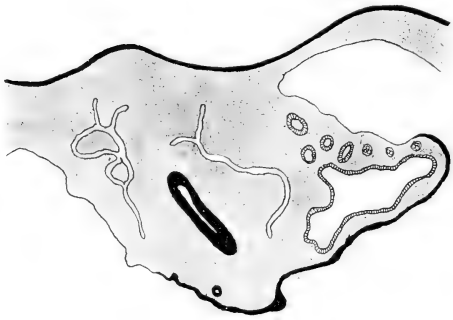


FIG. 24a.—Section through the vesicle attached to the chorion. × 25 times. There is a multiple allantois and multiple amnion with a thick layer of epithelium over the vesicle.

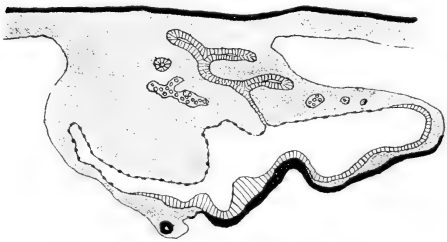


FIG. 24b.—Deeper section, showing the branching allantois.

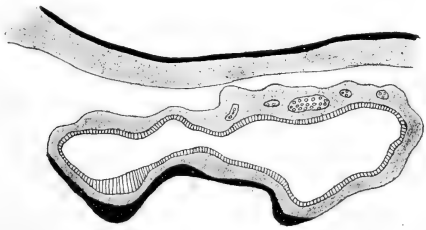


FIG. 24c.—A still deeper section of the vesicle, showing the irregular thickening of the ectoderm and entoderm.

borders. The mesoderm is hypertrophic. The inner layer is irregular, thick and thin, with a tubular branching process which extends to the stem of the vesicle. There are blood islands in the vesicle and stalk, and the vessels extend to the villi of the chorion. There are migrating cells in the tissue of the pedicle.

The syncytial layer is very extensive, forming large buds upon the chorion as well as upon the villi. At points these buds coalesce to form islands, the centers of which are composed of necrotic mass filled with fragmented nuclei.

No. 25.

Chorion, 25 mm. in diameter, with a pedicle within 6 mm. long and 2 mm. in diameter.

Dr. J. W. Lord, Baltimore.

The ovum is covered entirely with long villi, and has a hemorrhage on one side of it. The pedicle within has all of the characteristics of the umbilical cord of an embryo five weeks old. There is no trace of an embryo, but there are a

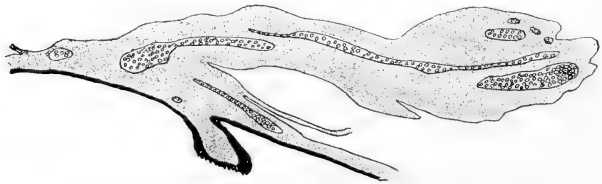


Fig. 25.—Section through the umbilical cord and amnion at their attachment to the chorion. × 10 times.

number of cells at the free end of the pedicle, which also has a ragged edge. The amnion lines the entire cœlom and is reflected over the pedicle just as it would be over the normal cord.

Sections show that the club-shaped cylindrical body is in fact the cord with its blood-vessels and amnion. The free end of the cord is rich in round cells, appearing much like the granulation tissue of healing wounds. At this point the end

of the cord is infiltrated with cells, in addition to the nucleated cells of the cord, and it has very ragged edges. It appears as if the embryo had gradually fallen off, piece by piece, leaving the ragged stump of a cord. The blood-vessels of the cord are but sparsely filled with blood. At the base of the cord there is a remnant of the umbilical duct. Apparently the chorion is normal.

No. 29.

Chorion, 30 mm. in diameter.

Dr. W. D. Booker, Baltimore.

The ovum is covered with but few atrophic villi, and within no trace of an embryo can be found. The cœlom is filled with a cheesy mass or granular magma, like that usually found within the amnion of pathological embryos. After the magma had been searched through most completely, the portions of the chorion which might have a remnant of an embryo attached were stained and cut into serial sections, but nothing whatever could be found.

Sections of the chorion show that its walls and villi are fibrous and thickened. There is no amnion present. The syncytial layer is very extensive over the villi and chorion, invading them at points. Immediately over the syncytium of the villi, and occasionally between them, there is a gelatinous envelope, which at times appears fibrinous. Within this envelope there are many leucocytes with fragmented nuclei.

No. 30.

Embryo, C. R., 60 mm.

From Dr. Snively, Waynesboro, Pa.

The woman from whom it was obtained is colored, and menstruated last from April 5 to 12. On June 19 she had her first pains, which continued until the 21st, when the abortion occurred, *i. e.*, 77 days after the beginning of the last period.

The specimen is apparently normal with the exception of a hernia of the liver into the umbilical cord. The communica-

tion between the cœlom of the cord and the peritoneal cavity is much too large for this stage and the liver protrudes into the cord fully 5 mm.

No. 32.

Ovum, 30 mm. in diameter, within a pedicle 9 x 2 mm.

From Dr. W. D. Booker, Baltimore.

"Mrs. N., colored. Last menstruation began December 26, 1893, and lasted 4 days, the usual duration being from 4 to 5 days. Cohabitation with husband December 12 and January

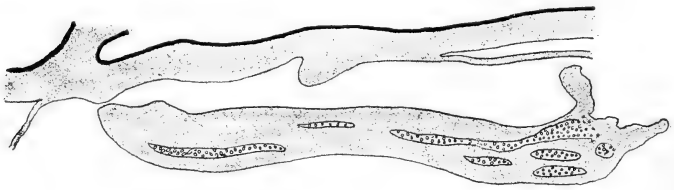


FIG. 32a.—Section through the cord and the amnion at its attachment to the chorion. × 10 times.

9. Hemorrhage began March 14 and continued until the 18th, when the abortion took place. The entire ovum was placed in 80 per cent alcohol one hour after the abortion." The time between the beginning of the last period and the abortion is 82 days.

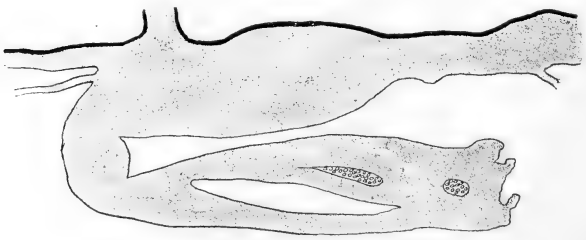


FIG. 32b.—Section through the attachment of the umbilical cord to the chorion. × 10 times.

Upon opening the ovum I found within it a large pedicle, 9 x 2 mm., which had every appearance of the normal umbilical cord of an embryo, 25 mm. long. The age of this ovum when estimated by the menstrual history calls for a cord of this size, but the chorion is undersized. At any rate, we have a cord without an embryo. At the point at which the cord should be attached to the body there is a mass of cells, making it appear as if the embryo ulcerated away. At this point the blood-vessels are greatly distended with embryo's blood, which also permeates the surrounding tissues. Within the cord there is a large space, the cœlom, as well as a reticular space, as is shown in Figs 32a and b. The mesoderm of the chorion and villi is fibrous.

No. 37.

Chorion, 25 x 18 x 15 mm., within a small nodule 2 mm. in diameter.

From Dr. G. M. Gould, Philadelphia.

The entire ovum is covered with villi which appear normal in form, both to the naked eye and under the microscope.

The specimen was macerated considerably, but the thick sections I made of it are extremely instructive. The embryonic mass within proved to be an atrophic cord, embryo

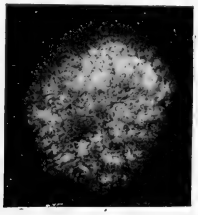


Fig. 37a.—Photograph of the entire ovum. Natural size.

and umbilical vesicle, as shown in Figs. 37b, c and d. The cord with its blood-vessels passes directly over into the head end of the embryo, which contains but a rudimentary nervous

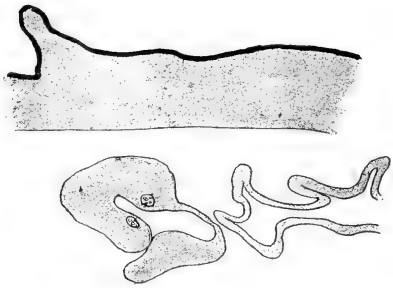


FIG. 37b.—Section through the head, umbilical vesicle and chorion. The pharynx and first aortic arches are cut across in the head. × 10 times.

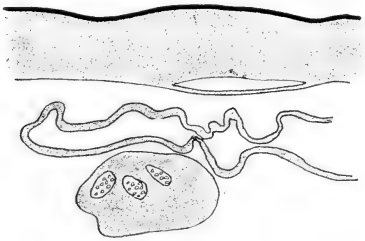


FIG. 37c.—Section through the umbilical cord, vesicle and the chorion. × 10 times.

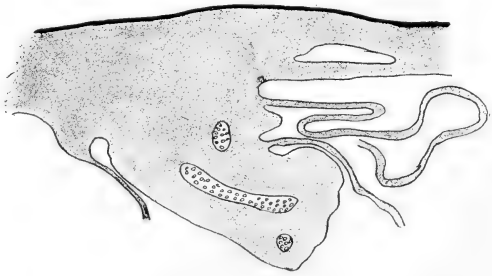


FIG. 37d.—Section through the attachment of the umbilical cord and vesicle to the chorion. × 10 times.

system. The mesodermal tissues are characteristic and the form of the pharynx and lower jaw is recognizable. From this region the two branchial arteries pass into the cord, as the figures show. A single vein, however, passes from the cord directly into the center of the body and ends just below the lower jaw. There is no heart, liver, myotomes, nor lower end of the body, these being replaced by the cord. The arteries are empty and the vein is distended with blood.

No. 54.

Embryo, C. R., 11 mm.

From Dr. McMorris, Belle Plaine, Iowa.

The embryo alone was given me. It shows an atrophic head, but otherwise appears like a normal embryo of 4½ weeks.

In the sections it is seen that the central nervous system is solid with the exception of the mid-brain, whose ventricle still communicates with the exterior of the body through an open neuropore. The head is atrophic. The vertebræ are well developed. The liver is large; the heart, other organs and cœlom are difficult to outline.

No. 55.

Ovum, 35 x 20 x 14 mm.

From Dr. Watson, Baltimore.

Last period January 18 to 22, abortion March 13, 1894. The specimen is a very solid fleshy mass, which contains a sharply defined spherical cavity, 15 mm. in diameter, with smooth walls. Absolutely no trace of an embryo found within this cavity.

Blocks of the tissue were imbedded in celloidin and some sections were cut. The sharply defined cavity proved to be the cœlom, as its walls were formed by the chorion. The thick fleshy mass proved to be villi of the chorion, syncytium, blood, fibrin and pus. The walls of the chorion contain remnants of blood-vessels, are partly invaded by leucocytes and are fibrous. The main bulk of the villi and syncytium stains poorly and

appears necrotic. The mesoderm is fibrous, more or less invaded by leucocytes and covered in part with a very active syncytium. The cavity of the cœlom is partly filled with a granular magma, in which are imbedded some cells.

No. 58.

Ovum, 20 x 18 x 12.

From Dr. Howard, Cleveland, Ohio.

"The specimen is from the first pregnancy of a woman who has been married for one year. The duration of the menstrual periods is usually from 3 to 4 days, the last one having ended July 25. The August and September periods were passed, and September 30 she had a hemorrhage which she believed to be the usual menstruation; this ended October 1

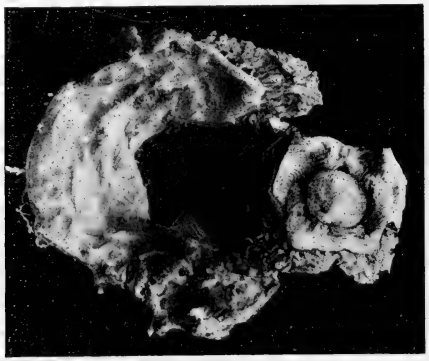


Fig. 58a.—Photograph of the ovum with the piece to which the vesicle is attached cut out and turned over.

with the abortion of the ovum. The time between the beginning of the last period and the abortion is 71 days. Cohabitation July 25 to August 5 and again on August 15, or several days before the first lapsed period."

The ovum is only partly covered with villi and is filled with a jelly-like mass of magma. Floating within this mass there

is a large vesicle, 6 mm. in diameter, with transparent walls. This vesicle in turn is partly filled with a granular deposit.

The syncytium is excessive. The mesoderm of chorion and inclosed vesicle is very fibrous. There are blood islands and

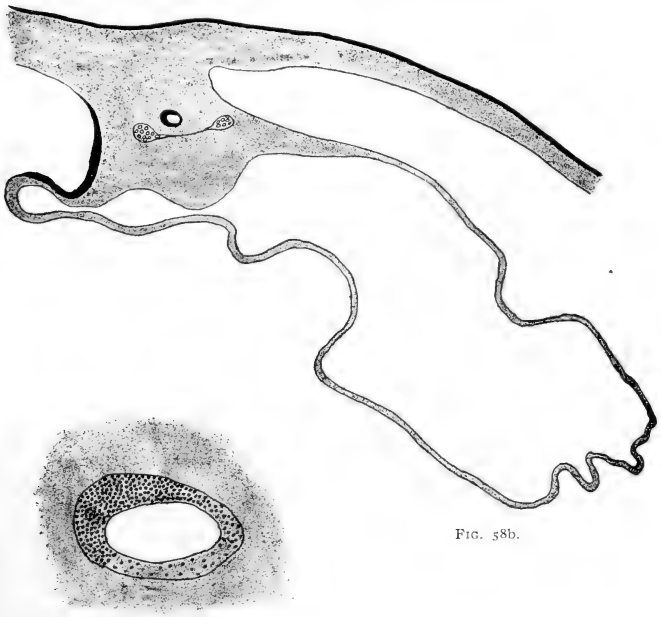


FIG. 58b.

FIG. 58c

FIG. 58b.—Sections through the vesicle at its attachment on the chorion. × 10 times. Within the stem there is a sharply defined cavity lined with epithelium and an hour-glass-like space filled with blood. On one side the stem is covered with epithelium.

FIG. 58c.—The cavity of the stem, shown in Fig. 58b, enlarged 50 times.

a cavity lined with epithelium in the stem of the vesicle. The main portion of the vesicle is composed of two layers, but near the stem there are three layers present. The mesoderm of the villi is hyaline and œdematous, and between them there is a stringy mass of mucus or fibrin rich in leucocytes.

No. 60.

Embryo, C. R., 8 mm.

Dr. Dobbin, Baltimore.

The body and extremities of the embryo appear normal in form. The tissues are considerably macerated and may be normal. The spinal cord is solid. There are large islands of blood cells in the liver.

No. 69.

Ovum, 70 x 40 x 30 mm.; embryo, C. R., 13.

Dr. Chabot, Baltimore.

The chorion is smooth, not being covered with villi. The head of the embryo is atrophic and club-shaped and the body is fairly plump. The arms are well developed, of the five-weeks stage, and appear normal.

The central nervous system is distended and the brain is macerated. The outline of the organs and of the peritoneal

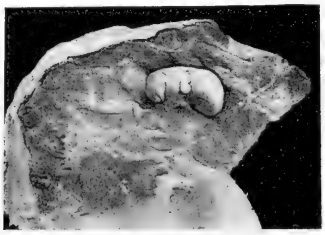


Fig. 69.—Photograph of the embryo attached to the chorion. Natural size.

cavity is not distinct, and the entire body is filled with migrating cells. The main bundles of nerves are filled with spindle-shaped cells, making them look like the nerves of amphibian embryos. The epidermis is hypertrophied and at many points forms papillæ. The embryo end of the umbilical cord is atrophic, invaded by migrating cells, and its blood-vessels are greatly distended. The whole chorion and part of the cord have undergone fibrous degeneration.

No. 70.

Mole, 45 x 30 x 28.

Dr. Ellis, Elkton, Md.

"The specimen is from a woman whose periods were regular until July 28, 1896, when she passed her period. In October she had a profuse hemorrhage, and on the 20th, aborted, the time between the beginning of the last period and the abortion being 113 days."

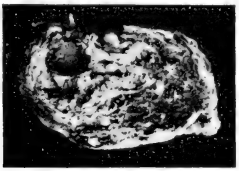


FIG. 70.—Photograph of the cut surface of the mole. Reduced one-half.

The specimen, as the figure shows, is very solid, and sections proved it to be composed of a mass of distended chorionic villi, forming an hydatidiform mole. Between the villi there is a large quantity of blood with an extensive syncytium which forms a large solid mass on one side of the specimen. Within the center of the specimen there is a small collapsed chorion with poorly defined walls. The specimen was not cut into serial sections, so it is impossible to state definitely whether or not the embryo has been destroyed entirely.

No. 71.

Ovum, 10 x 9 x 5 mm.

Dr. G. H. Whitcomb, Greenwich, N. Y.

Dr. Whitcomb writes me: "The specimen is from a woman twenty-three years old who had been married three months before the abortion occurred. She had been troubled with chronic cystitis and endometritis but menstruated regularly. After marriage she had two menstrual periods, but the third failing to appear she concluded that she was pregnant. Seven days after the lapsed period she slipped while descending the

stairs, and this was followed with some tenesmus. Four days later I examined her and found a free flow of unstained mucus from the uterus, with tenderness, hyperæmia of the pelvic organs and irregular pains. I requested a specimen of urine, which was given me on the following day. It was found loaded with pus and blood, and also contained the ovum. Two days later the decidua was discharged. The specimen was preserved in 50 per cent alcohol. Shortly after this the woman became pregnant again, which went on to full term."

The abortion from the above data took place 40 days after the beginning of the last menstrual period. When the ovum came into my hands, three years later, it was well preserved

Fig. 71.—Photograph of the ovum. Enlarged 2 diameters.

and had not been opened. The villi are well developed and even, but slightly deficient on one side. I cut the specimen in half around its greatest circumference and then stained the two halves. Within there was a small amount of magma reticulé and at the bottom of one of the shells of chorion there was found a very small nodule. Otherwise there is nothing within the ovum. The nodule was imbedded and cut into sections 20 microns thick.

The syncytium and the chorion appear normal. There are no blood-vessels. The nodule within the chorion is a solid mass which appears in structure like dried red blood corpuscles of the frog.

No. 77.

Ovum, 70 x 40 x 30 mm.

Dr. Horn, Baltimore.

The fresh specimen was sent to the laboratory and it was immediately preserved in strong alcohol. After it was hard-

FIG. 77a.—Photograph of the specimen cut open. One-half natural size.

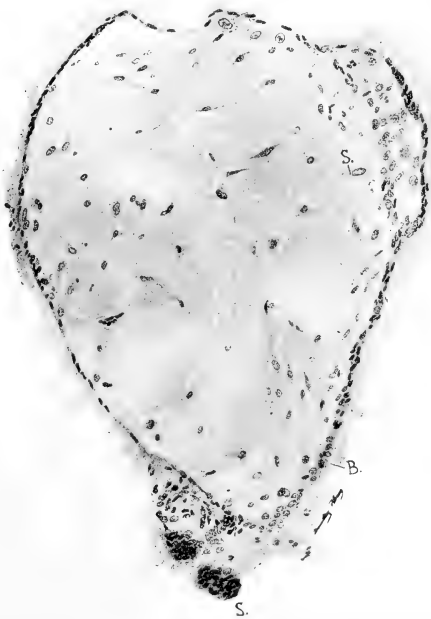


FIG. 77b.—Section of one of the villi. Enlarged 160 times. *B*, blood; *S*, syncytium, small cells of which are scattered throughout the mesoderm.

ened it was found to be of firm consistency and of a very red color, indicating that it must be pathological. Later, when it was cut in half, there was found within it a spherical cavity, 20 mm. in diameter, lined with a smooth, fibrous membrane and filled with a clear fluid which permitted of a careful inspection of its interior. On one side of the cavity there was a small elevation, one millimeter in diameter and one-fourth of a millimeter high.

Sections were made of the walls of the specimen through the elevation which proved to be a fibrous thickening of the amnion at its junction with the chorion. There are no blood-vessels in any portion of the chorion. Between the villi there is a great quantity of syncytium, fresh blood and fragmented leucocytes. At many points the syncytium and leucocytes invade the chorion and the villi with the apparent intention of destroying them. Where fresh blood and syncytium come in contact there are many fragmented leucocytes present.

No. 78.

Ovum, 36 x 33 x 13 mm.; nodule within, 14.6 mm.

Dr. A. P. Stoner, Harlan, Iowa.

"The woman from whom the specimen was obtained menstruated last on December 1, 1896, and the abortion took place on February 26, 1897. The sac was perfectly smooth when it was passed, and, without opening, it was placed in 50 per cent alcohol. After the abortion two or three pieces of

Fig. 78a.—Photograph of ovum with piece of chorion and nodule lying on top of it. Natural size.

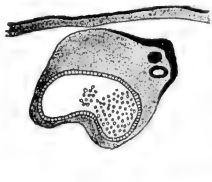


FIG. 78b.

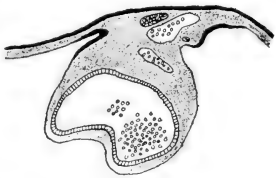


FIG. 78c.

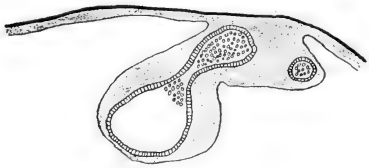


FIG. 78d.

FIGS. 78b, 78c, and 78d.—The sections through the vesicle and chorion. × 10 times. Blood is within the cavity of the vesicle. The stem is partly covered with epithelium and there is a double amnion, shown in Fig. 78b.

decidua and placenta were passed, weighing together about 30 grams, the right quantity, it seemed, for a ten-weeks ovum. The woman's husband has been absent for over ten weeks, thus making the specimen at least that old. It appears as if there had been an arrest of development of the embryo and that the membranes continued to grow."

When the specimen came into my hands the walls of the chorion were perfectly smooth without any villi whatever. It was filled with a clear fluid and within there is attached a small double vesicle, measuring 1 x .6 mm. This was imbedded and cut into serial sections.

The chorion is atrophic and has no villi upon it. The nodule within is covered with a single layer of epithelial cells which becomes thickened over the pedicle. At one point the thickening is greatly increased and immediately below it there are two small vesicles lined with epithelial cells. The main cavity of the vesicle is lined with a layer of cubical cells, and is filled with a considerable quantity of round cells. This cavity is hour-glass shaped and extends to the walls of the chorion, as the figures show. The mesoderm of the vesicle

is increased in quantity and is filled with wandering cells. At the base of the vesicle there are several blood spaces filled with blood.

No. 79.

Ovum, 50 x 50 x 50 mm.; embryo, C. R., 32 mm.

Dr. Briggs, Blackville, S. C.

Dr. Briggs writes that the abortion took place 91 days after the beginning of the last menstrual period.

The specimen came into my hands well hardened in strong alcohol with all of the membranes intact. When opened it was found that the inner walls of the amnion and the embryo were entirely covered with a layer of firmly coagulated granular substance or granular magma.

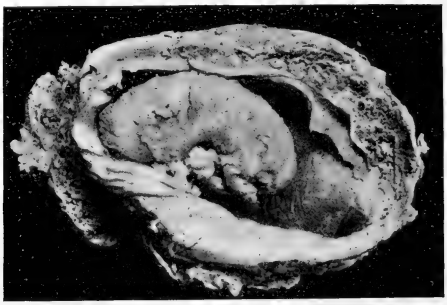


Fig. 79.—Photograph of ovum cut open, showing the embryo encrusted in granular magma. Natural size.

The chorion is very hemorrhagic and thick on one side, while on the other it is very thin. The sections show apparently normal structures in the thick portion, while in the thin portion there is an extensive leucocytic infiltration. At this latter point the walls of the chorion are markedly changed, being invaded by the syncytium as well as by leucocytes.

Serial sections of the embryo show that it must have been strangulated before the abortion took place. The central

nervous system is greatly macerated, the liver has disintegrated and the aorta is greatly distended. The rest of the embryo appears normal. The intestine is almost entirely within the peritoneal cavity; a single loop of it still remains in the opening communicating with the cœlom of the cord.

The embryo is completely covered with a layer of magma which contains but few cells in it. Below this the epidermis is wanting at many points, while at other points it appears normal. At the edge of the epidermis there is every appearance of an attempted regeneration, as its border is thickened and has rounded and not ragged edges.

No. 80.

Ovum, 24 x 18 x 18 mm.; embryo, C. R., 4 mm.

Dr. Branham, Baltimore.

Embryo and ovum are apparently normal, with the exception of a mass attached to the ovum, which proved to be diverticulum, its cavity communicating with the main cœlom. The lower part of the embryo is bent upon itself. The whole ovum had been preserved without opening it. Some magma reticulé is within the cœlom.

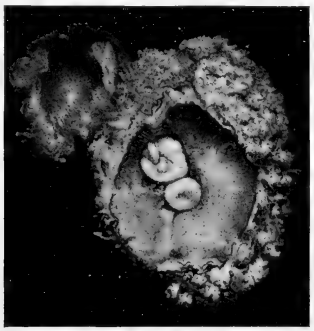


Fig. 80a.—Photograph of the ovum and embryo. Enlarged twice. The small additional mass forms a diverticulum of the ovum, the cavity of which communicates with the exocœlom through a narrow opening. The tail of the embryo is twisted.

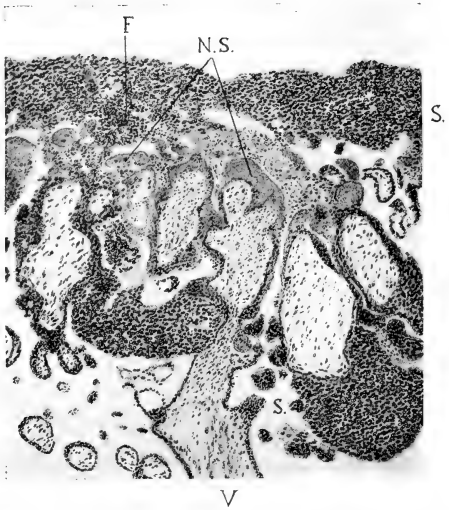


Fig. 80b.—Section through the tips of the villi, including the surrounding syncytium. *S*, syncytium; *NS*, necrotic syncytium; *V*, villus. Enlarged 62 times.

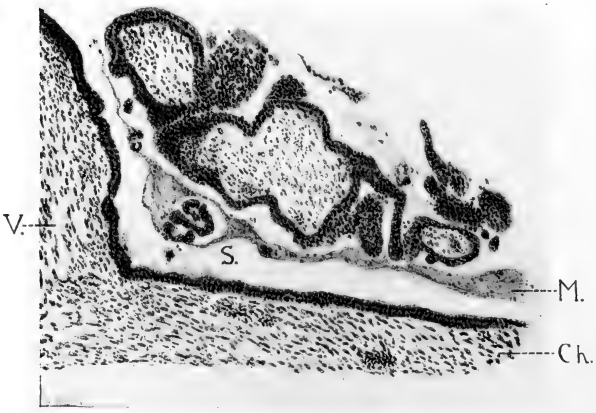


Fig. 80c.—Section showing the mucoid mass, *M*, rich in leucocytes and containing a nest of syncytium, *S*. *V*, villus; *Ch*, chorion.

As far as it is possible to determine, the sections indicate that the embryo, cord and yolk sac are normal. The villi of the chorion, however, which are well developed, have a considerable quantity of a fibrinous mass between them which is rich in leucocytes. The syncytium is well developed and at the tips of a number of villi it is decidedly necrotic. It may be that these changes are of sufficient importance to account for the abortion.

No. 81.

Ovum, 65 x 55 x 35 mm; embryo, C. R., 15 mm.

Dr. Branham, Baltimore.

"The abortion took place just three months after the beginning of the last menstrual period."

The unopened ovum had been placed in a large quantity of alcohol, and when it reached the laboratory I cut a window into it to allow the alcohol to enter its cavity. Within an embryo was found, which appears macerated and is broken

FIG. 81a.—Photograph of the whole ovum. Slightly reduced.

in its middle. The crest of necrotic tissue on the head of the embryo, the stumpy leg, the distended cord and atrophic chorion, all indicate that it is pathological.

Two parts of the embryo were cut into serial sections and different portions of the chorion were also examined.

Macroscopic as well as microscopic examination of the chorion shows that it has undergone extensive degeneration. Its walls are filled with large islands of blood and at points there is leucocytic infiltration, showing that an inflammatory process had also invaded it. Accompanying the inflammatory process the syncytial layer of cells has invaded the walls of the chorion, thus helping along its destruction. The mesoderm of the chorion has undergone fibrous degeneration and within its walls there are numerous cysts, some lined with flat epithelial cells and some with cylindrical. The amnion appears normal and lines the entire cœlom.

Fig. 81b.—Photograph of the embryo within the chorion.

The embryo is somewhat atrophic. Its central nervous system is macerated and there is a marked cyst-like dilatation at the tip end of the spinal cord. All the tissues, including the cartilages, show more or less dissociation. The necrotic crest covering the top of the head gives all the appearance of an ulcer; the ectoderm is destroyed and the mesoderm covering the brain is greatly thickened and pigmented with round cell infiltration of the surrounding tissue. The marked dilatation in the cord encloses double cavities filled with a mucoid reticulum, much as in embryo No. 32. This tissue is similar in appearance to the normal notochord in amphibian embryos.

No. 82.

Solid mole, 75 x 60 x 40 mm.

Dr. Cassidy, Baltimore.

"Last period began June 3, 1896, and this tumor was passed March 8, 1897, about 40 weeks later."

The specimen was brought to the laboratory fresh and was hardened in formalin. It is pear-shaped, ulcerated on the pointed end and the interior appears to be composed of fresh blood clots.

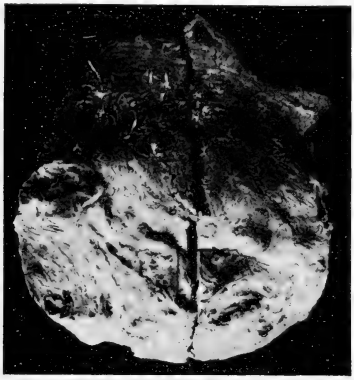


FIG. 82a.—External surface of the mole, slightly reduced.

Sections of the large solid mass show that within it there is a collapsed ovum with folds of the chorion extending throughout the specimen. On one side of the specimen there are long slender villi. Most of the layers of the collapsed chorion are composed of double walls, usually in apposition and occasionally completely blended. There is no amnion lining the chorion. Along the main central body of the collapsed chorion there are large quantities of fresh blood. The rest of the tumor is composed of old blood clots and nests of leucocytes and of syncytium. The syncytial nests are located in great part along the chorion, show active growth when

Fig. 82b.—Cut surface of the tumor, showing large masses of blood between the distorted chorion. Reduced one-third.

they come in contact with fresh blood and are necrotic elsewhere. At no point does the syncytium invade the walls of the chorion.

It is impossible to interpret this specimen without admitting that the chorion continued to grow after the ovum had collapsed.

No. 87.

Ovum, 24 x 16 x 9 mm.; embryo, C. R., 4 mm.

Dr. Cole, Peru, Ill.

"The last period took place April 15, 1896. On May 15 the woman had a slight flow which repeated itself every few days until the 27th, when the abortion took place. The day before the abortion the woman worked very hard."

The lower end of the embryo looks atrophic and on the opposite side of the ovum there is a vesicle 2.5 mm. in diameter.

Both the embryo and vesicle with pieces of the adjacent chorion were cut into serial sections.

The embryo proved to be a normal specimen about 21 days old, with normal umbilical vesicle and so on. The vesicle on the opposite side of the cœlom appears to be anything but an

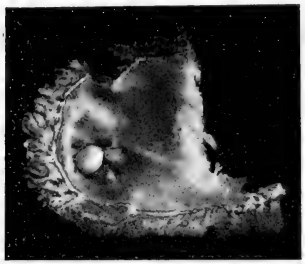


FIG. 87a.—Additional vesicle on the side of the ovum opposite the embryo. Enlarged 2 times.

umbilical vesicle. It lies free in the cavity of the cœlom imbedded within the magma, and is in no way torn. It is composed of three distinct layers: a thick middle layer, in which are numerous blood islands, an epithelial lining layer, and an outside layer, which does not completely cover the specimen. On one side of the vesicle there is a sharp invagination of all three layers, which projects well into the vesicle. The mesoderm of this invagination has also within it several blood islands.

The chorion is normal in appearance, with blood-vessels entering it from the normal embryo.

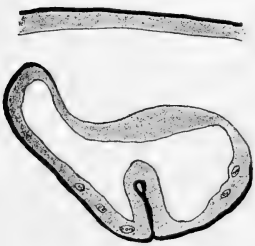


FIG. 87b.

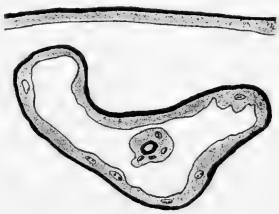


FIG. 87c.

FIGS. 87b and 87c.—Sections through the vesicle and chorion. × 25 times. The deeper portion of the invagination in Fig. 87b is shown cut in cross-section in Fig. 87c. Blood islands may be seen in the mesoderm.

No. 93.

Solid mass, 40 x 20 mm.

Dr. Cassidy, Baltimore.

The specimen was sent to the laboratory fresh and was hardened in formalin. Upon opening it, it was found that within there is a cavity into which projected a large tongue of fleshy tissue. Within this tongue there is a clot of blood as well as a sharply defined cavity.

Sections, through different portions of the specimen, showed that the outer sac is the decidua and that the tongue of tissue is the chorion. Within the central cavity of the tongue (cœlom) lies the amnion. I cannot state definitely whether or not the remnants of an embryo were present, for the specimen was not cut into serial sections. The walls of the chorion are thickened and irregular, and around it are packed hypertrophied villi with great quantities of syncytium and blood between them. Covering the villi and syncytium there is a layer of blood and fibrin separating them all from the decidua. Within the mesodermal tissue of the chorionic walls there are occasional islands of syncytium.

No. 94.

Ovum, 50 x 40 x 30 mm.; embryo, C. R., 20 mm.

Dr. Knill, Detroit, Mich.

Ovum is smooth with villi on one side of it only. The amnion does not fill the chorion completely; it measures 30 x 20 mm. Within the amnion there is much coagulated matter which envelops the embryo completely. This granular magma can be picked off easily in large flakes. The embryo thus exposed is bent upon itself more than usual and appears macerated, as if it had been dead for a number of days. The features are not clear, the tips of the hands and feet not being well defined. The lower part of the embryo is necrotic and the spinal cord is protruding. The entire ovum has been hardened in alcohol.

The sections show that the villi of the chorion are somewhat atrophied, with occasional nests of leucocytes within

FIG. 94.—Embryo partly imbedded in granular magma within the chorion. Natural size.

them. The mesoderm of the chorion and amnion show clearly marked fibrous degeneration. The embryo itself is normal in shape, but the brain is greatly dissociated and the liver is cloudy and projects into the cord.

All of the epidermis is exfoliated with great masses of migrating cells lying between the embryo and the envelope of magma.

No. 97.

Ovum, 33 x 30 x 15 mm.; embryo, C. R., 7; A. R., 9 mm. Dr. Goldman, Baltimore.

"Beginning of last menstrual period, March 8, 1897. Abortion, May 8. The entire ovum was hardened in 95 per cent alcohol."

The ovum appears normal with the villi distributed equally over it. Upon opening, it was found filled with dense magma reticulé, in which could be discerned the faint outline of a four-weeks embryo. A block of the chorion, including magma and embryo, was cut into serial sections.

The form of the embryo, amnion and umbilical vesicle is normal. On one side of the embryo the epidermis is wanting and the amnion is filled with cells. The umbilical vesicle is

filled with migrating cells, but its blood islands and its entoderm appear normal. The chorion is fibrous. The outer covering of the vesicle is composed of a short layer of columnar epithelial cells. The magma of the cœlom is filled with wandering cells.

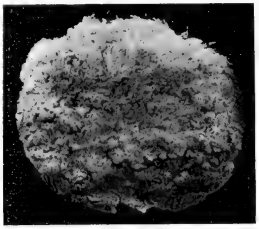


Fig. 97.—External surface of the chorion. Natural size.

The nervous system is greatly dilated and dissociated. The liver tissue is obscured and filled with migrating cells. The contour of the abdominal viscera is obliterated and they are likewise filled with migrating cells. Pharynx, heart, large veins and aorta are greatly dilated.

No. 104.

Ovum, 35 x 35 x 15 mm.; embryo elongated, 12 mm. long. If curled upon itself it would measure, C. R., about 7 mm.

Dr. J. P. West, Bellaire, Ohio.

"The beginning of the last menstrual period was on May 7, and the abortion took place on June 11, 1897. The entire ovum was preserved in strong alcohol."

The villi of the chorion appear atrophic, being wanting on one side of the ovum. After the ovum was carefully cut in half it was found filled with magma partly reticular and partly granular. On one side is a snake-like embryo with straightened head and atrophic extremities. The embryo, with a piece of chorion to which it is attached, was cut into serial sections.

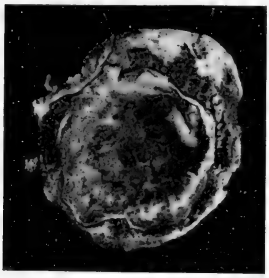


FIG. 104.—Photograph of the embryo within the chorion surrounded by magma. Natural size.

The main walls of the chorion are fibrous. The amnion is intact. The brain and spinal cord of the embryo are dilated and dissociated,—probably macerated also. The outlines of the organs and body cavity are obliterated. The boundaries of the liver can no longer be determined. The tissues of the body are generally dissociated and they, with the umbilical cord and magma, are infiltrated with migrating cells. The heart, large veins and aorta are greatly distended with blood. The head is atrophic.

No. 110.

Ovum, 46 x 30 x 30 mm.; embryo, C. R., 8 mm.

Dr. West, Bellaire, Ohio.

"The last period of the woman began September 22, 1897, and lasted five days. On December 8 there was a slight flow which continued until the 13th, when the abortion took place. Hardened in alcohol."

The shape of the ovum is oblong and its walls are fleshy, the villi having all disappeared. Within there is a clear fluid with a granular deposit covering the embryo. The embryo is greatly macerated and is but slightly attached to the chorion. At the point of attachment there is an elevated mound of necrotic tissue, to which the embryo is stuck. There is no distinct cord and the amnion is wanting. Evidently both chorion and embryo have been dead for a long time.

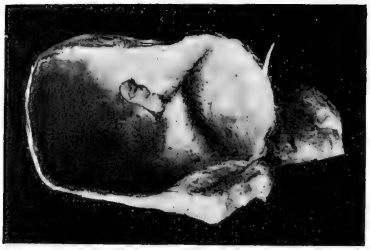


Fig. 110.—Photograph of the embryo within the chorion. Natural size.

The chorion is atrophic and the decidua is infiltrated with leucocytes. The amnion, umbilical vesicle and the attachment of the umbilical cord to the chorion are completely destroyed. The embryo is atrophic, the face not being developed at all. The central nervous system is swollen; the outlines of the viscera and body cavity obliterated and filled with migrating cells. The liver is small. The heart and large blood-vessels are greatly distended.

No. 115.

Ovum, 30 x 27 x 22 mm.; amnion, 10 x 5 x 5 mm.; embryo, C. R., 3 mm.

Dr. A. S. Atkinson, Baltimore.

The abortion took place two months after the beginning of the last period. During the second month of pregnancy there was continuous bleeding.

The ovum was brought to the laboratory fresh immediately after the abortion and placed in strong formalin. It was opened at once in formalin and found filled with a gelatinous, transparent mass, which became fibrous after the formalin had acted upon it. Later on alcohol made it opaque. The chorion is practically free of villi and looks necrotic. The embryo is well in the middle of the ovum and is apparently separated from the chorion. The head as well as the tail is atrophic.

Sections show that the villi of the chorion are atrophic, with but a small quantity of syncytium attached to them. The

entire chorion is surrounded with a mass of decidua filled with leucocytes.

The magma of the cœlom is very dense and has within it but few migrating cells. Within the greatly distended amnion lies the embryo, looking much like a chick of the third day. The peritoneal cavity communicates freely with the exocœlom, in which hangs an atrophic umbilical vesicle. The lumen of the umibilical vesicle is filled completely with entodermal cells, its

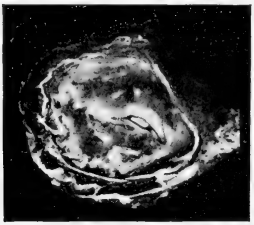


FIG. 115a.—Embryo imbedded within the magna reticulé of the cœlom. Natural size.

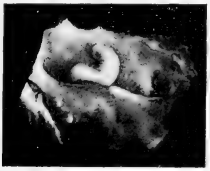


FIG. 115b.—Embryo attached to the chorion. × 3 times.

blood spaces are greatly distended but nearly empty, and its solid stem ends abruptly after it enters the body of the embryo. There is no trace of either alimentary canal or liver left. Rudimentary Wolffian bodies and ducts are present. The central nervous system is solid. The heart and large veins are simple in form and greatly distended with blood.

The mesodermal layer of the chorion and its villi appear normal, with the exception of the tip ends of the villi, which are enveloped in a mass of leucocytes.

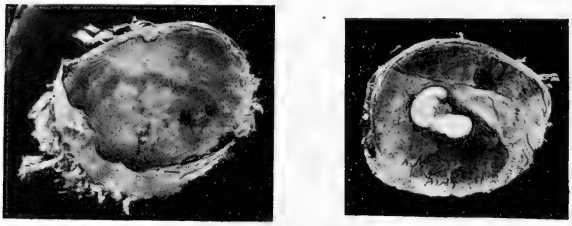
No. 122.

Ovum, 20 x 16 x 6 mm.; embryo, C. R., 5 mm.

Dr. J. W. Williams, Baltimore.

"Last period began April 19, 1898, and the abortion took place on June 23. Continuous bleeding for eight days before the abortion."

The transparent and fibrous chorion is covered with a few scattered villi of irregular length. The embryo is atrophic with a club head, large heart, stump tail and no limb buds.



Figs. 122a and b.—Two halves of the ovum, showing cœlom and embryo. × 2 times.

The nervous system is greatly distended and dissociated. The front of the head and the branchial arches are atrophic. The liver is small, the Wolffian body well marked and the body cavity sharply defined. The large veins of the body and of the liver are greatly distended with blood, the aorta being much enlarged and empty. The tissues of the entire embryo are partly filled with loose round cells. The chorion is thin and fibrous.

No. 123.

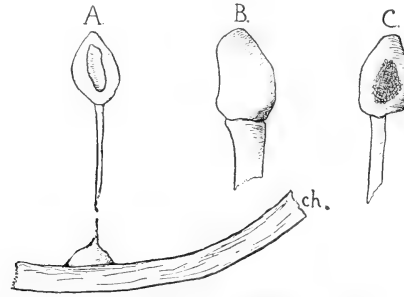
Ovum, 17 x 14 mm.; vesicle within, 1.8 x 1.5 x 1 mm.

Dr. H. J. Boldt, New York.

"The last menstrual period prior to the abortion occurred August 14 or 15, 1898. Abortion, September 10. The whole ovum was placed in 95 per cent alcohol within 10 minutes after the abortion."

The entire ovum was covered with villi, apparently normal, but surrounded by a layer of pus and blood. After opening it I found the cœlom filled with a mass of coagulated fibrinous albumin, the magma reticulé, within which no embryo could be seen. The two halves of the ovum were then stained, which brought out prominently a small vesicle imbedded in the magma. This vesicle had a rounded opening upon one side (Fig. a), with a long pedicle upon the other, which extended towards but was not attached to a small mound on the inside of the chorion. Vesicle and chorion were both cut into serial sections.

The sections of the vesicle appear as those of the normal umbilical vesicle. The opening on the side is undoubtedly due to a tear, judging by its broken edges.



FIGS. 123a, b and c.—Three views of the vesicle, enlarged about 10 times.

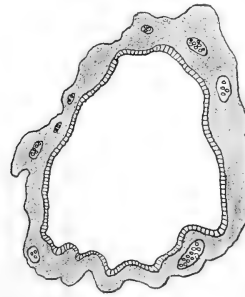


FIG. 123d.—Section through the vesicle. × 25 times. Entoderm, mesoderm and blood islands are shown.

No. 124.

Ovum, 90 x 75 x 50 mm.; embryo, C. R., 35 mm. Abortion 18 weeks after the beginning of the last menstrual period.

Dr. Cassidy, Baltimore.

The ovum was brought to the laboratory fresh and then hardened in a strong solution of formalin. It appears as a transparent cyst with a crescent-shaped placenta on one end, measuring 60 x 50 mm. Upon opening it I found within a second sac measuring 50 x 37 x 35 mm., which had tough

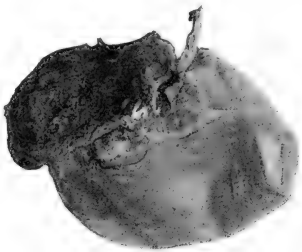


Fig. 124a.—Whole ovum with placenta attached to one side of it. Reduced one-half.

fibrous walls and proved to be the amnion. Within this was the embryo, with club hands and feet, pointed ears and a very thin, twisted, umbilical cord.

Sections of the placenta show that the villi are matted together and are covered with a thick layer of decidua cells. The entire thickness of villi is infiltrated with leucocytes, which at points are accumulated sufficiently to form small

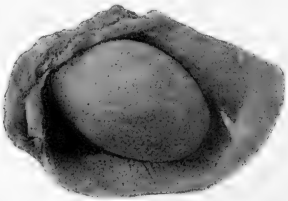
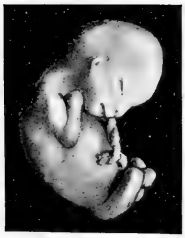
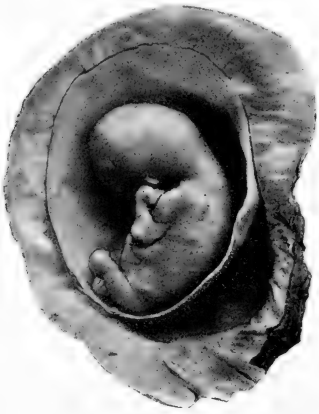


Fig. 124b.—Ovum cut open, showing amnion. Reduced one-half.





FIGS. 124c, d, e.—Three views of the embryo. Natural size.

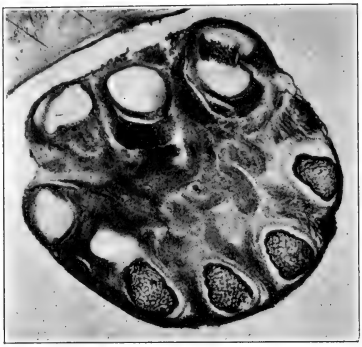


Fig. 124f.—Section through the hand showing well-formed bone and cellular infiltration of the surrounding structure. Enlarged 17 times.

abscesses. The walls of the chorion are considerably thickened immediately below the placenta and are fibrous in structure. Between the villi at their bases there is a quantity of fresh blood, and between their distal ends there is a great quantity of syncytium, which does not stain well and appears to be necrotic. Masses of fine granules are seen which stain intensely with hematoxylin, and on account of their uniform size they are probably bacteria.

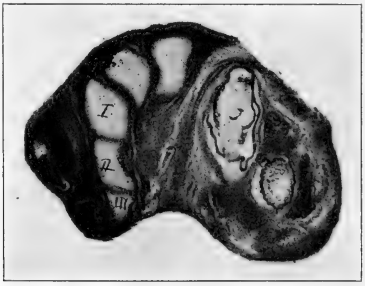


Fig. 124g.—Section through the foot with the phalanges numbered. Enlarged 17 diameters.

Sections of this interesting specimen do not reveal very much, for the tissues do not stain well. The form of the organs and skeleton, with the exception of that of the extremities, appears to be normal. However, the skin appears more fibrous than usual, being somewhat infiltrated with round cells. In the deformed extremities this infiltration is very pronounced and involves all of the structures of the hands and feet with the exception of the cartilages, forming syndactyly.

No. 128.

Ovum, 50 x 43 mm.; embryo, C. R., 20 mm.

Dr. Lupton, Baltimore.

The woman from whom this specimen was obtained is eighteen years old and has one child. The first recurring period after the birth of the child was on July 4, 1898; the second period, August 5; and the abortion on October 20.

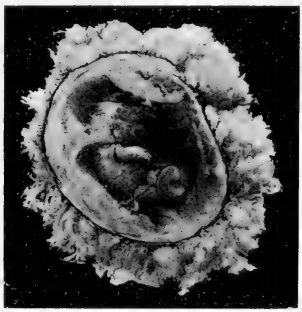


FIG. 128.—Embryo within the amnion and chorion covered with a delicate mass of fibrils and granules. Natural size.

After the abortion the entire ovum was placed in water, and 18 hours later was brought to the laboratory. It was a beautiful white specimen and I immediately placed it in formalin, in which it was opened at once. The water did not seem to have penetrated the ovum, as the embryo was not at all

swollen and appeared perfectly normal. The formalin, however, at once caused the coagulation of a delicate network of fibrils which enveloped most of the embryo. The sections show a delicate reticulum of fibrils within the amnion.

No. 130.

Ovum, 15 x 10 x 6 mm.; vesicle within, 4 x 3 x 1.5 mm.

Dr. De Saussure, Charleston, S. C.

"The specimen was passed by the patient while urinating, 14 days after the beginning of the last menstrual period. She had no idea that she was pregnant and thought that the specimen was a piece of mucous membrane from the bladder. It was hardened entirely in 50 per cent alcohol."

When the specimen came into my hands it was only half covered with villi, the other half apparently having had them stripped off. There was also a tear in the chorion through which a vesicle was protruding. Upon lifting the ovum this vesicle fell out. The ovum was then carefully cut open and

Fig. 130a.—Ovum with extruded vesicle. Natural size.

was found to contain a considerable quantity of magma reticulé. Within this there was a long pedicle, measuring 7 x 2 mm. There was also a space in the magma large enough to hold the vesicle which had escaped. Both ovum and vesicle were cut into serial sections.

The serial sections of the ovum show that the amnion is still unbroken, as shown in Figs. b, c, d. Its greatest meas-

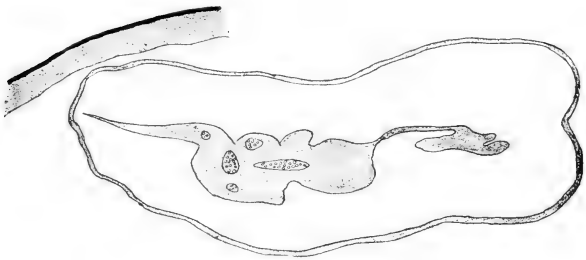


FIG. 130b.—Section through the amnion, cord and remnant of the embryo. × 10 times.

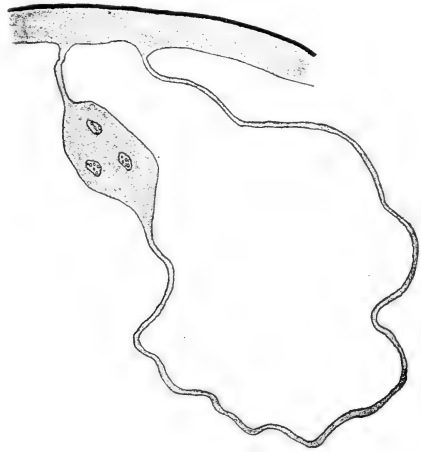


FIG. 130c.—Section through the amnion, cord and chorion. × 10 times.

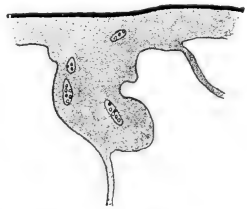


FIG. 130d.—Section through the attachment of the amnion and cord to the chorion. × 10 times.

urements are 10 x 4 mm., into which extends the umbilical cord. At the end of the cord there is a mass of tissue mostly broken down, the remains of the embryo. This mass is ragged, without any form corresponding to an embryo, and had the amnion been torn no doubt it would have fallen out. The blood-vessels of the cord are gorged with nucleated blood cells, but they do not extend into the embryo. The chorion is normal in appearance.

The umbilical vesicle (Fig. 130a) is pear-shaped and completely closed. At no place is there a break to show its attachment to the cord. Although considerably macerated, the sections showed the characteristic structure of an umbilical vesicle.

No. 132.

Ovum, 42 x 30 mm.; embryo, C. R., 15 mm.

Dr. Munson, Washington.

This specimen was kindly sent me by Dr. Lamb, who had obtained it from Dr. Munson. The woman from whom it was obtained menstruated last between August 15 and 20, and aborted November 12. The embryo was preserved in a 50 per cent mixture of commercial formalin. The chorion is

Fig. 132.—Photograph of the embryo. Natural size.

atrophic with but few villi. The embryo has a stub head and the extremities on the right side are atrophic, while those on the left appear to be normal.

The organs of the embryo are about normal in form and structure. The cord and brain are slightly dissociated. There is a small number of migrating cells in the tissues of the body as well as within the peritoneal cavity.

No. 133.

Ovum, 32 mm. in diameter.

Dr. J. M. Hundley, Baltimore.

"Last period began September 15, 1898, and continued eight days; bloody discharge began November 11th and abortion occurred on the 19th. Both parents perfectly healthy. Hardened in 75 per cent alcohol."

When the specimen came into my hands I believed it to be normal, but after cutting out a piece of chorion I found the cœlom completely filled with a dense mass of magma reticulé. In taking off the piece of chorion I cut the attachment of the umbilical cord and thus located the embryo. The mass of magma and a portion of the chorion encircling the embryo were removed and cut into serial sections.

FIG. 133.—Ovum with piece of chorion removed, showing dense magma within. Natural size.

The villi of the chorion are fibrous but normal in shape, with but litle syncytium at their tips. The syncytium immediately over the walls of the chorion is greatly increased in quantity. The cœlom is filled with magma and migrating cells. The amnion is complete. Umbilical vesicle is filled with desquamated entoderm cells. The embryo is distorted and cramped; epidermis is exfoliated at the points where the amnion contains masses of migrating cells; nervous system distended and dissociated; organs and peritoneal cavity fairly well outlined; liver filled with blood which forms large islands at points; front end of head greatly distorted, eye macerated and whole head gorged with round cells.

No. 134.

Ovum, 17 x 11 mm.; vesicle within, which is compressed, measures in the sections 9 x 3 mm.

Dr. G. N. Sommer, Trenton, N. J.

A number of the sections of this unique specimen were sent me by Dr. G. N. Sommer, of Trenton, N. J., who also informs me that the ovum had been passed with considerable pain and hemorrhage by a young multipara, due to the introduction of a bougie by the woman to produce abortion. The monthly period had been five days overdue when the abortion occurred. The bougie had been introduced several days earlier.

In stirring up the ovum the woman punctured it and it then became filled with mother's blood, which formed a clot around the embryo. The leucocytes invaded the walls of the ovum, the stem of the vesicle and even the blood-vessels of the embryo, and show all stages of fragmentation within the tissues of the embryo.

The vesicle itself is most interesting, as it shows the effect of an infraction upon a very young normal embryo. The

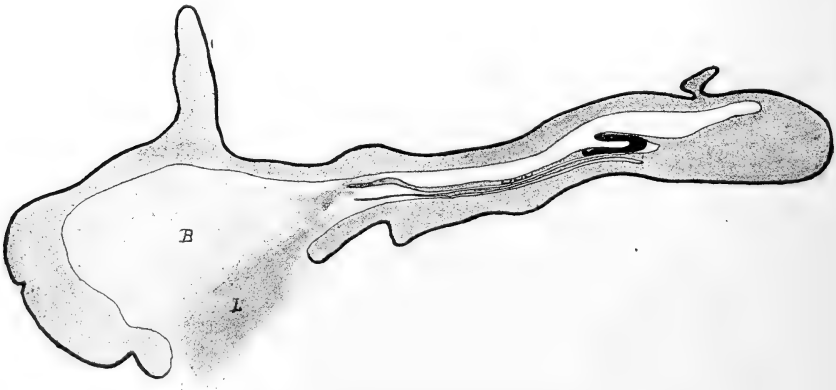


Fig. 134a.—Section through the ovum and embryonic vesicle. The umbilical vesicle is torn and collapsed. The invagination of its walls and the myotome-like bodies are shown in Figs. b and c. *B*, blood clot; *L*, leucocytes.

stem of the vesicle is quite extensive, in which are embryo blood-vessels filled with blood. Many of them extend into the chorion and some of them into villi. The walls of the vesicle are composed of three distinct layers. The inner is composed throughout of a single layer of sharply defined cubical epithelium, the entoderm. Immediately next to this is an extensive mesoderm, which continues into the mesodermal layer of the stem to the chorion. Near the attachment of the vesicle to the chorion there is a sharp invagination of the vesicle which is lined with a thick layer of epithelial cells, the ecto-

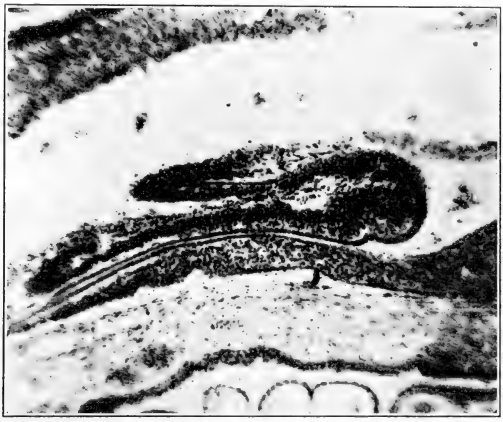


Fig. 134b.—Photograph of the invagination shown in dark in Fig. a.

derm. This layer lines only the invagination and does not extend over the rest of the vesicle. Beyond and on the distal side of the invagination the mesoderm is arranged in five groups of cells which suggest in every way myotomes. In this region there are embryonic blood-vessels filled with blood. The syncytium is very extensive.

The blood clot from the mother within the cœlom is recent, as is shown by the fact that there are present may red blood corpuscles. In the periphery of the clot next to the chorion

the red corpuscles are partly broken down and appear as an imperfect granular detritus, within which there is a network of fibrin. There are as yet no pigmentary changes in the tissues adjacent to the clot. The clot extends through a tear in the chorion into the cœlom, and as this portion is approached it is noticed that its characters change. The red blood corpuscles diminish in number, and the main coagulum consists of leucocytes which extend through the surrounding tissues. This mass of leucocytes also extends along the bor-

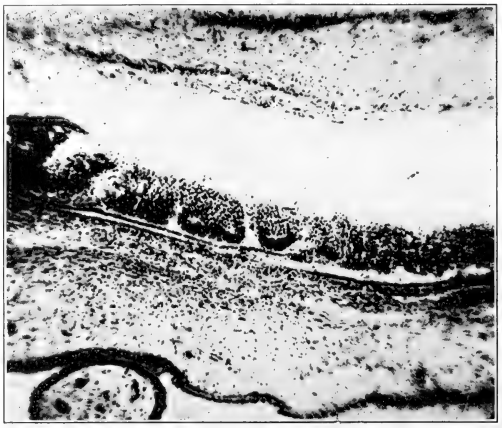


Fig. 134c.—Myotome-like bodies, three of which are shown in the collapsed vesicle shown in Fig. a.

der of the red clot into the cavity and walls of the vesicle. The blastoderm cells are intact on one side of the vesicle, whereas on the other they have suffered desquamation and have retracted from its walls. A part of the leucocytes composing this part of the clot are in a very imperfect state of preservation. They show great irregularities in the forms of their nuclei and are in a state of fragmentation. Fragmented leucocytes extend throughout the clot, a great portion of the chorion and through the walls of the embryonic vesicle.

The tissue elements of the embryo are for the most part well preserved. There is no evidence of extensive necrosis. Occasionally, where the clot of red and white corpuscles and fibrin becomes clearly intermingled with the villi of the chorion the syncytial cells stain imperfectly. The evidence of gross necrosis is entirely wanting.

The blood-vessels of the chorion contain numerous leucocytes, constituting in some instances what appears to be leucocytic thrombi. One section was stained for bacteria, but none were found.

The process as a whole is to be interpreted as an acute hemorrhagic inflammation of the embryonic structures. The large number of leucocytes undergoing fragmentation indicates that the inflammatory irritant was of a severe nature, and had acted with a considerable degree of intensity, as is not only shown by the rich immigration of leucocytes, but the severe retrogressive changes which they have undergone.

No. 135.

Ovum, 105 x 65 x 65 mm.; embryo, C. R., 9 mm.

Dr. Mosely, Baltimore.

The ovum was sent fresh to the laboratory and hardened in strong formalin. It is fairly smooth, its walls being thin and the villi are wanting. Upon opening it I found it filled completely with a gelatin-like mass which is neither fibrous nor granular. Within this mass there is an atrophic embryo standing upon a thin umbilical cord. The entire chorion is

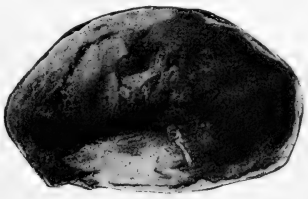


FIG. 135a.—Embryo upon a mass of magma within the cœlom. One-half natural size.

lined with the amnion. The head of the embryo is atrophic, the body being shaped like a grain of wheat. The extremities are more rudimentary on the right side than on the left.

The sections of the embryo show the cord distended, the brain almost completely destroyed and the mesoderm of the top of the head converted into a mass of mucoid tissue. The head end of the chorda is greatly hypertrophied, being converted into a mucoid tumor. On either side of this tumor there are two large cartilages, normal in structure. Farther headwards, buried deep in the mesoderm, there are two additional pearl-like bodies, which, on account of their appearance as well as by their being encircled by an oval zone of pigmented

Fig. 135b, c, d.—Three views of the embryo. Natural size.

cells, identifies them as the lenses of the eyes. These bodies have within them lens fibers, making them look much like the lenses of amphibians.

The front end of the head is necrotic. The heart is convoluted, its outline obscure and it is distended and filled with a mass of blood cells. The outline of all of the abdominal organs and of the peritoneal cavity can be determined, although the tissues are considerably obscured by the great quantity of round cells within them.

The entire wall of the chorion is very thin, and it is lined throughout with a delicate amnion. The villi have all disappeared and in their place there are islands of necrotic syncytium covered with a hyaline layer of fibrin. The whole chorion is infiltrated with leucocytes, which form small abscesses at points.

No. 136.

Ovum, 14 x 6 mm.; embryo, C. R., 5 mm.

Dr. Campbell, Halifax, N. S.

"Beginning of last period August 21, 1898. Abortion October 16. Entire ovum was hardened in 95 per cent alcohol."

The ovum is covered with rudimentary villi, and when opened was found to be completely filled with magma reticulé. Shining through this mass can be seen the embryo, curled up, with extremities, myotomes, heart and umbilical vesicle visible. This remarkable specimen is a four-weeks embryo within a two-weeks ovum. The entire ovum with the embryo was cut into serial sections.

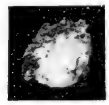


FIG. 136a.

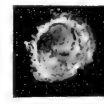


FIG. 136b.

FIG. 136a.—Photograph of the ovum. Natural size.
FIG. 136b.—Interior of the ovum, showing faint outline of the embryo buried in magma.

The villi of the chorion are atrophic and fibrous, with great buds of syncytium hanging to them as well as to the main wall of the chorion. Between the villi there is a small amount of mucus or fibrin, within which there are numerous leucocytes. Amnion, umbilical vesicle and embryo are apparently normal and of the four-weeks stage. The embryo is twisted on its long axis at about 90 degrees. The organs are normal. The peritoneal cavity is normal in shape and filled with blood, appearing as a fresh hemorrhage; the pericardial cavity is empty.

No. 137.

Ovum, 65 x 50 x 30 mm.; embryo, C. R., 16 mm.

Dr. Watson, Baltimore.

"Last period commenced September 26, 1898. Abortion, December 21."

The ovum is nearly covered with long and well-developed villi, having a bare pole on one side. The cœlom contains no magma. The embryo is broken from the cord and is macerated on its ventral end. The head is atrophic; arms and legs are normal. At the middle of the umbilical cord there is the marked swelling seen in other specimens of this kind.

Sections of the chorion show the villi to be normal in form but somewhat hyaline in structure and without blood-vessels. There is a considerable quantity of syncytium. The thickened umbilical cord has within it a cavity partly filled with a reticular substance, homogeneous in appearance, and more intensely stained than the surrounding tissues. Within the cord there are large blood-vessels, greatly distended with blood cells, which extend through the walls into the surrounding tissues.

Fig. 137.—Photograph of embryo. Natural size.

Ten millimeters from the attachment of the cord to the chorion is the umbilical vesicle. It measures 3 x 2 mm.; its walls are all degenerated and its cells, which are necrotic, fill its cavity. The stem of the umbilical vesicle reaches but half way to the umbilical cord.

The central nervous system of the embryo is distended and dissociated, the spinal cord being segmental to correspond with the vertebræ. The liver is necrotic and filled with blood. The heart is collapsed and dissociated. The large blood-vessels are collapsed and empty, while the small ones are filled with blood. The outlines of abdominal organs are pretty sharp, the tissues nearly normal in appearance and fairly free from migrating cells.

Most of the epidermis has fallen off the embryo, but where it remains intact it often shows irregular thickening.

No. 141.

Ovum, 40 x 30 x 20 mm.; embryo, 8 mm.

Dr. West, Bellaire, Ohio.

"The specimen is from a woman, a mother of nine children, who has always been healthy until about ten years ago. From this time her health gradually became worse and worse. She is extremely neurasthenic. Stomach is dilated, digestion poor. Bladder irritable and urine scanty. Uterus large, thick and retroverted; leucorrhœa. The uterus is about three times its normal size and has a number of cysts in the cervix. There were several earlier abortions, the one before this, which took

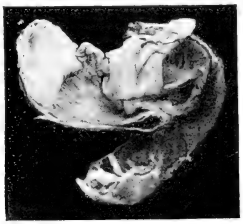


FIG. 141.—Piece of chorion with dense magma and misshapen embryo. Slightly reduced.

place December 13, 1897 (No. 110), having been sent to you. The last period began on October 27, 1898, and the abortion followed on January 13."

The chorion is fleshy, like No. 110, with but few villi, and within the cœlom there is a great quantity of magma reticulé and a dissociated embryo about four weeks old.

The sections show that the chorion and villi are matted together and contain but few blood-vessels. The syncytium is very extensive, and where it is in large masses the most central cells are necrotic. The mesoderm of the chorion is fibrous and hypertrophic. There is a considerable quantity of mucus or fibrin, rich in leucocytes, between the villi. This condition may have been more extensive elsewhere, as only the chorion in the neighborhood of the embryo was examined.

The great quantity of magma reticulé within the cœlom has numerous migrating cells scattered through it.

The amnion is partly in contact with the chorion and at the points of contact is normal in appearance. Where it is separated from the chorion by the excessive quantity of magma the walls of the amnion are greatly hypertrophied. The umbilical vesicle is collapsed and its walls have undergone hyaline degeneration completely.

The central nervous system of the embryo is greatly dilated and dissociated. The body cavity can barely be outlined. The large blood-vessels are faintly marked by the blood within them. The rest of the tissues are one homogeneous mass of tissue cells infiltrated with round cells, within which can still be recognized cartilages and nerve bundles. The boundaries of the heart and liver are wholly obliterated, due to their dissociation.

No. 142.

Ovum, 50 x 40 x 30 mm.; embryo, C. R., 15 mm.

Dr. Sommer, Trenton, N. J.

"Last period began September 28, 1898. On January 3 there were marked uterine pains; free hemorrhage February 1, and abortion February 4."

The chorion is fleshy, with some villi. Within there is a macerated embryo about five weeks old imbedded in a mass of fibrin-like magma. Between the magma and walls of the chorion there is a large space filled with clear fluid.

Serial sections of the embryo and chorion show most remarkable changes. The chorion and amnion are greatly thickened, are very fibrous and look in every respect like the membranes in fleshy moles (No. 82, for instance). The villi are matted together by a mixture of blood, fibrin and numerous necrotic as well as living cells. The fibrinous mass within the amnion is in all probability blood which has entered from the exterior. It has all the appearance of blood clots found elsewhere in the body, but in addition it has been invaded by wandering cells from the embryo. The cœlom is partly filled

with a granular magma into which project numerous slender villi arising from the walls of the thickened amnion.

The activity of the syncytial layer has been most pronounced. At all points it invades blood clots and the mesodermal tissue of the walls of the chorion and the villi. Occasionally it almost perforates the chorion to enter the cœlom. At one point syncytial cells are within the cœlom, but the serial sections do not extend far enough to show the point of communication. More marked is a great area of active syncytium within the amnion, surrounded with a clot of mother's blood. Not only does it spread as a double layer of cells to the attachment of the umbilical cord to the chorion, but at

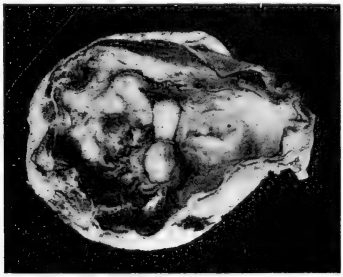


FIG. 142.—Ovum with embryo. Natural size.

numerous points the nests of syncytium have nearly perforated the walls of the thickened amnion to enter the cœlom. The whole picture reminds one much of cancer specimens. The presence of the large blood clots within the amnion indicates that the membrane must have been punctured, probably by the activity of the syncytium, long before the abortion took place. This, of course, would allow the syncytium to enter the cœlom and amnion to there make its further attack, which in turn may have caused the amnion to thicken and sprout so much.

The embryo itself has also undergone most marked changes. The dimensions of the ovum, the length and degree

of development of the embryo indicate that the pathological changes began not later than the sixth week of pregnancy, while the menstrual history of the mother indicates that at least 14 weeks have elapsed between the conception and the abortion. In other words, the pathological process has been under way for at least eight weeks. The extreme changes within the embryo also speak for this. The nervous system is markedly dissociated and macerated. Arms and legs, external features, as well as most of the internal organs, have vanished. The liver is still marked, but is necrotic. Wandering cells have invaded all of the tissues and are also beginning to attack the cartilaginous bodies of the vertebræ. Large nests of them are also imbedded in the clots of blood which surround the embryo. The main blood-vessels of the embryo can still be traced through the surrounding tissues. The cord is filled with embryo's blood, but this is also necrotic.

From all appearances had this ovum remained in the uterus much longer it would soon have become filled with mother's blood, which in turn would soon have solidified to make of the specimen a typical fleshy mole.

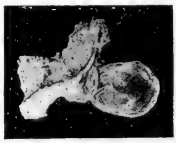


Fig. 143.—Photograph of the vesicle. Natural size.

No. 143.

Large double sac, 15 x 10 mm., attached to the wall of the chorion.

Dr. Stick, Glenville, Pa.

The chorion appears normal. The double cyst-like body has thin walls and is filled with a clear fluid. The specimen has been in strong alcohol for nearly twenty years.

Serial sections show a chorion, normal in appearance, to which is attached the double vesicle as shown in the photo-

graph. The structure of the walls of the two sacs is identical with that of the mesoderm of the chorion with all of the epithelial cells fallen off. The two sacs do not communicate; the larger has smooth walls; the smaller has numerous small vesicles, about 1 mm. in diameter, opening into it, and the cluster of "air cells" are directly blended with the mesoderm of the chorion. The specimen undoubtedly belongs to the vesicular forms, peculiar only on account of its size.

No. 147.

Ovum, 30 x 27 x 20 mm.; vesicle, 1 mm. in diameter.

Dr. Pole, Baltimore.

"Last period began January 1, 1899, and the specimen was discharged March 23."

The ovum is only in part covered with villi, the remaining portion of the chorion being clear and transparent. The cœlom is completely filled with magma which has turned very white in the alcohol in which this specimen was preserved.

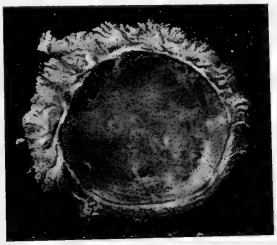


FIG. 147.—Interior of ovum. Slightly enlarged.

On one side of the cœlom, closely attached to the chorion, there is a small vesicle and an irregular mass which may represent the remnants of the embryo.

Sections of the chorion show that the mesoderm is very fibrous and rich in cells. The vesicle within is about one millimeter in diameter and is located two millimeters from the chorion, but not at all attached to it. Its walls are composed

of only one layer of cells on one side of the vesicle, while on the opposite side it has a second layer or mesoderm, .5 mm. thick, in which are imbedded numerous blood-vessels filled with blood. There are a few blood-vessels filled with blood in the chorion in the immediate neighborhood of the vesicle.

No. 150.

Ovum, 35 x 30 x 10 mm.; embryo, 5 mm.

Dr. Oertel, Augusta, Ga.

There are but few villi on the chorion. The embryo is distorted and the arm on one side is unusually large.

The sections of the embryo show an extreme degree of pathological change. The nervous system is swollen and solid, and the contour of the viscera is wholly obliterated. The large blood-vessels are greatly distended with blood. Round cells are distributed equally throughout the body of the embryo.

No. 152.

Ovum, 70 x 42 x 38 mm.; embryo, C. R., 31 mm.

Dr. H. J. Boldt, New York.

"The specimen is from a woman suffering with endometritis, this being her third successive abortion, which took place in each instance during the third month of pregnancy. The beginning of the last period preceding this abortion took place on April 16; conception April 20 (?); and abortion June 25, 1899."

When the specimen came into my hands the chorion was found to be smooth and apparently free from villi. The cavity of the amnion is filled with a mass of granular magma covering entirely an embryo over two months old. The umbilical cord is much twisted and thin, measuring .5 mm. in diameter. The embryo was cut into serial sections and different portions of the chorion were also examined.

Microscopic examination shows that the chorion and amnion are fibrous. The villi of the chorion are matted together with fibrin and a mass of cells, which have undergone hyaline

degeneration. The stroma of the villi is very fibrous, being invaded at many points by syncytial cells and leucocytes. At numerous points there are large nests of leucocytes forming abscesses. It is a plain case of the endometritis infecting the chorion.

The embryo is imbedded in a large quantity of magma giving every appearance of embryo No. 79 again. The organs of the embryo are dissociated and macerated and the tissues stain poorly, indicating that the embryo had died a considerable time before the abortion took place. Again the central nervous system is swollen and dissociated. Migrating cells are found in clumps or scattered in all of the tissues. In general, the connective tissues are more fibrous than normal, the true skin showing considerable hypertrophy. The epidermis is wanting.

No. 153.

Solid mass, 50 x 20 x 20 mm.

Dr. Stick, Glenville, Pa.

Last period began April 30; abortion, July 15, 1899.

The mass is pear-shaped and proves to be a ruptured chorion partly inverted and imbedded in an organized clot of blood and fibrin. The chorion is, of course, ruptured and at the point of rupture there is a mass of blood, which forms the large end of the pear-shaped mass. There is no amnion within the chorion, nor could the embryo be found. A portion of mucous membrane of the uterus is attached to the chorion. The villi of the chorion are normal in form, but the mesoderm of many of them have undergone a kind of coagulation necrosis. The syncytial cells are generally normal in appearance. There are many leucocytes throughout the tissues, especially within the mesoderm of the inverted chorion.

No. 154.

Ovum, 10 x 7 x 7 mm., found within a mass of blood within the uterine tube.

Dr. Boldt, New York.

The ovum was cut into serial sections, but no trace of an embryo could be found. The sections show, however, that the chorion had been torn, but the edges of the tear were rounded and infiltrated with mesodermal cells. The main wall of the mesoderm and the villi in the neighborhood of the tear are fibrous and artophic. The rest of the villi are normal in appearance.

No. 158.

Tubal pregnancy; vesicle, 2 mm. in diameter.

Professor W. T. Howard, Cleveland, Ohio.

The specimen came to me imbedded in celloidin and mounted on blocks ready to cut. From each block sections were cut, three of which proved to be through the chorion. In one of these sections there was the remnant of an embryo within the chorion; from this piece I removed the celloidin and reimbedded it in paraffin and cut it into serial sections 50 microns thick.

The microscopical examination of the sections shows that the chorion is denuded entirely of its villi, being in apposition and apparently continuous with the wall of the uterine tube. Occasionally the line of separation is marked by a row of irregular cells, probably the remnants of the epithelial covering of the chorion. The mesodermic portion of the chorion is somewhat fibrous, being smooth on its cœlom side and without an adhering amnion. The nodule within is shriveled and necrotic, only a few of its nuclei staining. It appears as a double sac, together measuring 2 mm. in diameter, with a clump of necrotic cells, appearing like those of the umbilical cord, between them. In none of the sections is the embryonic mass attached to the chorion. At one place, however, the cord-like structure runs into a long process toward the chorion with a blood-vessel (?) filled with blood in its center.

My interpretation of the embryonic mass is that it is composed of amnion and umbilical vesicle of about equal size, shriveled and partly torn into pieces, but still held together by the remnants of the embryo and umbilical cord.

No. 159.

Fragments of a chorion about as large as a walnut.

Dr. Golden, Elkins, W. Va.

"From a woman in good health who had aborted a year before during the third month of pregnancy. During the second month of the pregnancy, from which the present specimen was obtained, there was a slight flow of blood without any pain. It continued for two days. Ten days later it recurred and continued for 24 hours. Three days later it recurred again, became profuse and the abortion followed. The supposed duration of the pregnancy is ten weeks. No indication whatever of endometritis. Both father and mother are perfectly healthy and are very anxious to have children."

The specimen consists of portions of the mucous membrane of the uterus, large portions of the chorion, amnion, but no embryo is present. The mucous membrane is full of small abscesses, and leucocytes have invaded all portions of the chorion. The syncytium is very active, and at numerous points the syncytium and leucocytes have invaded the mesoderm of the chorion. The amnion is greatly curled up and thickened. Its walls have undergone hyaline degeneration. The cells covering the amnion on the side towards the cœlom are generally proliferated, often forming islands.

No. 161.

Chorion, 50 x 25 x 25 mm.; embryo, 10 mm.

Dr. Cassidy, Baltimore.

"Last period at the end of August. Abortion, November 17, 1899. After missing the next period patient took medicine and had a rubber tube introduced into the uterus. Purulent leucorrhœa during the past six months."

The entire ovum was given me hardened in alcohol. It was covered with hard clots of blood; on one side the villi appear to be normal. Upon opening the ovum a mass measuring 10 x 5 x 5 mm. was found attached to its walls, which, after sectioning, proved to be a strangulated embryo. It was imbedded and cut into serial sections.

The sections prove the mass to be an embryo of the fifth week, filled and covered with round cells. These cells have obliterated the structure of the head entirely, but as the tail end of the body is approached the outline of the organs can still be defined. The villi of the chorion are developed in a great mass of blood and pus; the syncytium is excessive. Within the stroma of the villi there are, at many points, many round cells which appear to be migrating cells from the embryo.

No. 162.

Mole, 70 x 30 x 30 mm.; embryo, 1 mm.

Dr. Wanstall, Baltimore.

The specimen came to me in formalin with the following note from Dr. Wanstall: "Last period from September 2 to 7, that is her usual time, five days. The woman began bleeding November 9, and passed the specimen on November 22. She is the mother of five children and says that this is the

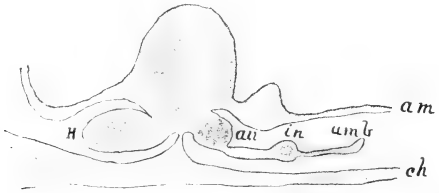


Fig. 162.—Section through the embryo. × 15 times. *Ch*, chorion; *am*, amnion; *h*, heart; *umb*, umbilical vesicle; *in*, intestine; *all*, allantois or possibly liver.

only time she has aborted. There is not the slightest indication of uterine disease."

Within the specimen there is a cavity measuring 35 x 12 x 12 mm., lined with a smooth wall and filled with a jelly-like substance, within which there is a very small embryo which was cut into serial sections 50 microns thick. The sections show a remarkable atrophy of the embryo and umbilical vesicle. The chorion is very thin and is composed of meso-

derm only. The villi and epithelial cells are wanting, but in their place there is a thick layer of mother's blood. The entire chorion is lined with an amnion and into its cavity the nodule-like embryo projects. Its tissues are not uniform, being thickened at some points, necrotic at others, and mucoid at others. Throughout the center of the nodule there are some capillaries filled with blood. At the point of juncture between the amnion and chorion there are three projections from the embryo into the cœlom—(1) the umbilical vesicle; (2) the allantois; and (3) the heart. That the second is the allantois is indicated by its cavity, which is multiple at points. The heart is within a pocket of the cœlom and has an irregular lumen which is well filled with blood. At the base of the nodule there is a short tube which communicates with the allantois, the intestine.

No. 166.

Ovum, 40 x 40 x 40 mm.; embryo, 2.5 mm.

Dr. Cassett, Baltimore.

Last period on October 18. On December 29 there was a discharge of blood which continued until the 31st, when the mole was expelled.

The mole is composed of very thick, fleshy walls within which there is a cavity with a smooth wall, measuring 30 x

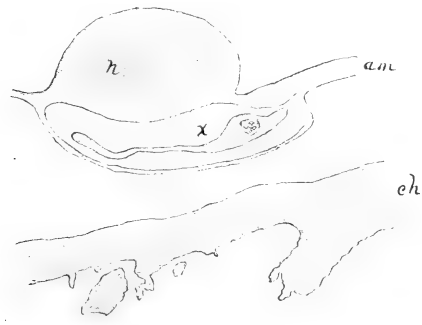


FIG. 166.—Section through the embryo. × 15 times. *Ch*, chorion; *am*, amnion; *n*, nervous system; *x*, heart or umbilical vesicle.

20 x 20 mm. On one side there is a small atrophic embryo 2.5 mm. long.

The sections of the chorion show that its villi are well formed and are imbedded in a mass of blood from the mother. Possibly the syncytial layer of epithelium is increased. The cœlom side of the chorion is smooth and is in contact with the amnion. Attached to the amnion there is the embryonic mass or remnant which does not reach to the chorion; there is no umbilical vesicle to be found. The amnion and embryo are completely separated from the chorion. There are no blood-vessels in the chorion.

The embryo is cylindrical in form, being attached throughout half of its length to the amnion and passing through it. In the center of the embryo there is a solid column of cells quite sharply defined—the remnants of the central nervous system. At the tail end of the embryo there is a blind tube, the allantois. The cœlom of the embryo, which is as a pocket on its ventral side, contains an irregular sac which may be either the heart or the umbilical vesicle; probably the former.

No. 174.

Ovum, 35 x 25 x 25 mm.; embryo, 13 mm. long.

Dr. Gibbs, Baltimore.

Last period January 11, 1900; bleeding five weeks later, which continued until the eighth week, when the abortion followed. The ovum is smooth, having but few villi, and is filled with a granular magma.

Sections of the chorion show a marked degeneration of its walls, nearly all of its villi having been destroyed. Those few

Fig. 174.—Embryo lying on piece of the chorion. Enlarged 2 diameters.

fragments of villi which remain are imbedded in blood and are riddled with the cells of the syncytial layer. The mesodermal layer of the chorion is no longer sharply defined and is more or less filled with cells with fragmented nuclei, the origin of which cannot be determined.

The embryo is of the stage of five or six weeks with pretty sharply defined organs and tissues which are more or less dissociated and infiltrated with wandering cells. Most of the epidermis has fallen off; in the region of the olfactory pit (which is almost obliterated) the epidermis forms two marked horn-like elevations. The central nervous system is swollen and dissociated more than the remaining tissues of the body, the change being greater in the brain than in the cord. The vascular system is gorged with blood which is beginning to invade the surrounding tissues. This increase is most marked in the umbilical cord, which appears œdematous.

No. 177.

Embryo, C. R., 12 mm.

Dr. Harrison, Baltimore.

The sections show well outlined all of the organs of an embryo of the end of the fifth week, but they are dissociated and swollen. So extensive is the dissociation in the head that the brain has become practically solid, the vesicles being nearly obliterated. The process is not so extensive in the spinal cord. Most of the epidermis has fallen off.

The vascular system is again greatly distended with blood which is infiltrating the tissues, especially those surrounding

FIG. 177.—The photograph shows the rounded head and stubby leg. Enlarged 2 diameters.

the larger arteries and veins. In general the tissues show the changes always seen in embryos which have been gradually strangulated before the abortion.

In this specimen there is one marked variation in the changes usually found. The precartilage outlines all of the vertebræ and ribs, but no true cartilage is as yet formed in them. Back of the eyes in the occipital region there are on either side of the head two cartilages well developed, much too advanced for embryos of this stage. A more advanced stage of this cartilage will be found in embryo No. 135. The head is also beginning to become stumpy; the frontal process is necrotic and is beginning to fall off.

No. 180.

Ovum, 20 x 15 x 10 mm.; vesicle, 2 mm.

Dr. C. W. Dodge, Rochester, N. Y.

"I have in my possession a human embryo which, if I may judge from some of your papers which I have seen, is likely to be more valuable to you than to me, and for this reason I have kept it intact, instead of sectioning it as I have been sorely tempted to do. Its history is as follows: The woman from whom it came is a patient of Dr. Edward Mott Moore, Jr., of this city. On March 28, last, her right ovary was removed. She left the hospital on April 15, and coitus occurred May 13. On June 19 menstruation appeared and this ovum was expelled, which was brought to me in a pill box (the membranes being broken in handling), and put at once into 4 per cent formalin, in which solution it still remains. As the dates given above are vouched for by the patient and the physician, it seems to me that we have here an unusually accurate and perfect history of the embryo, and, while it is not so very young, its history may give it additional interest."

Sections of the chorion show that its mesoderm is of normal thickness, but is fibrous and rich in nuclei. Throughout the main wall of the chorion, but not in its villi, there are numerous blood-vessels filled with blood, showing that at one time an embryo may have existed.

The villi are normal in form, with a very extensive syncytial layer of cells over them. At points the syncytium forms large islands, which can easily be seen with the naked eye. Immediately over the vesicle within, an island of this kind, a millimeter in diameter, arises from the main wall of the chorion and sends processes up between the villi. The mesoderm immediately below this island is thinner than the rest, making it appear as if the violent growth of the syncytium took everything before it, but that in the attempt to produce new villi the fibrous mesoderm of the chorion would not follow. At many points between the villi there is a slimy mass of albumen well infiltrated with leucocytes and numerous small islands of syncytium, some of which can be followed back to their origin from the villi.

The vesicle within is composed of but one layer of cells, those of the mesoderm with blood islands imbedded within it. No trace of an entoderm can be made out, although the lumen of the vesicle extends into a pedicle which, as a single strand of cells, attaches itself to the chorion.

No. 181.

Ovum, 18 x 18 x 10 mm.

Dr. D. S. Lamb, Washington.

The ovum is filled with reticular and granular magma and no remnants of an embryo could be found, although every particle which might contain it, with the adjoining chorion, was cut into serial sections. The mesoderm of the chorion and villi is œdematous; the epithelial covering is poorly developed, often being composed of but one layer of cells.

No. 182.

Head and upper end of the body of an embryo about five weeks old.

Dr. D. S. Lamb, Washington.

Sections of this embryo show an extreme degree of disintegration of the embryo. The brain is converted into a mass of cells filling the central canal entirely and extending into the surrounding tissues of the embryo, the line of de-

marcation being obliterated. The large veins of the body are gorged with blood which also extends into the surrounding mesoderm. On the frontal side of the head there is a straw-

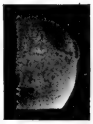


Fig. 182.—Piece of head showing necrotic mass over the mid-brain. Enlarged 2 diameters.

colored necrotic mass with some migrating cells within it. On the dorsal side of the head the mesoderm is thin and blistered, indicating the beginning of spina bifida. The cartilages alone are still well defined.

No. 185.

Ovum, 40 x 25 x 15 mm.

Dr. Sabin, Baltimore.

The abortion occurred seven weeks after the beginning of the last period. The specimen was brought to me in formalin, and upon opening it I found that the cœlom was stuffed with reticular and granular magma. No trace of an embryo could be found, although the entire ovum was cut into serial sections.

The main wall of the chorion is completely filled with leucocytes from the mother and show all stages of fragmentation of the nuclei. They form a fairly sharp border on the cœlom side, making the chorion appear as the wall of an abscess. The invasion of the chorion with leucocytes must have been merely from the cœlom side, as the villi are not invaded to any extent. Some of the villi are œdematous, others atrophied, being covered with a normal amount of syncytial cells.

No. 188.

Ovum, 45 x 40 x 40 mm.; embryo, C. R., 17 mm.

Dr. G. N. Sommer, Trenton, N. J.

"Last menstruation began January 6; bleeding began March 19, and ended in a few hours with the abortion. The

unopened ovum was immediately placed in ninety-five per cent alcohol."

The cœlom is filled with granular magma, the chorion is very fibrous, and the villi are mostly wanting. The tissue of the mesoderm is very rich in nuclei, none of which appear to belong to leucocytes from the mother. Three kinds can easily be recognized—(1) those which normally belong to the mesoderm; (2) blood cells from the embryo; and (3) an extensive invasion of the syncytial cells. This third group can be traced directly from large mounds of syncytium lying upon the chorion, from which they extend throughout the mesoderm, frequently entering the larger blood-vessels. Often large giant cells are seen, showing the usual characteristics of the syncytium after it has invaded the mesoderm of the chorion. The villi are affected less than the main walls of the chorion. No cells from the syncytial layer of the chorion were found in any of the blood-vessels of either the embryo or the umbilical cord.

The organs of this embryo are all normal in form and of the proper degree of development for an embryo of this size. The tissues are dissociated somewhat, the most marked being that of the brain. The veins of the body are all gorged with blood, with but little migration of blood cells into the surrounding tissues.

No. 189.

Ovum, 28 x 25 x 15 mm.; embryo, 4 mm.

Dr. T. E. Oertel, Augusta, Ga.

The ovum, filled with granular and reticular magma and contains a deformed embryo, lying within a distended amnion, 8 mm. in diameter.

The umbilical vesicle and amnion appear to be normal for an embryo of this size; the body, however, is greatly deformed, the central nervous system being open throughout its extent and encircles the dwarfed embryo like a broad hoop around a ball. A number of the motor roots of the spinal nerves are developed, more in the region of the tail than else-

where. There are no cranial nerves. The heart is a vesicle filled with blood, hanging into the cœlom and slightly attached to the body wall. Its vascular connection with the body is cut off entirely. The blood-vessels of the body are irregular in shape and entirely changed from the normal

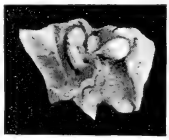


Fig. 189a.—Photograph of the embryo. Enlarged 2 times.

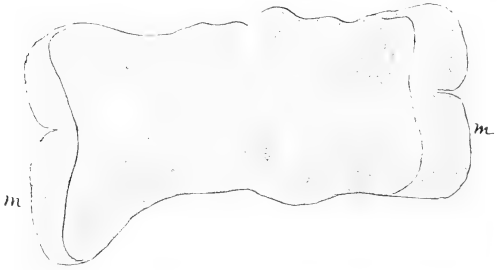


Fig. 189b.—Section through the head of the embryo. × 30 times. The medullary plate, *m*, is open throughout its whole extent.

type. They are filled with blood which extends through their walls into the surrounding tissues. The branchial arches correspond to an embryo of this size. There are still traces of optic vesicles, chorda and possibly allantois present, the liver and stomach and intestine having degenerated.

No. 190.

Ovum, 25 x 22 x 12 mm.

Dr. C. M. Ellis, Elkton, Md.

The ovum is filled with reticular magma within which no trace of an embryo can be found, although the entire specimen was stained and cut into serial sections. The chorion and villi are apparently normal, containing blood-vessels from the embryo.

No. 195.

Ovum, 30 x 30 x 30 mm.

Dr. D. S. Lamb, Washington.

No embryo could be found, although the entire ovum was cut into sections. The specimen is well covered with villi and contains some reticular magma. The mesoderm of the chorion and villi appears normal and is rich in blood-vessels filled with embryo's blood.

No. 196.

Tubal pregnancy; embryo, 2.5 mm. long.

Professor Brödel, Baltimore.

The specimen, hardened in formalin, contained two suspicious bodies which were both cut into serial sections. One of these proved to be the embryo greatly deformed, representing a stage about three weeks old. The tissues of the embryo are quite homogeneous, only the central nervous system being recognizable. One eye and a large blood-vessel can

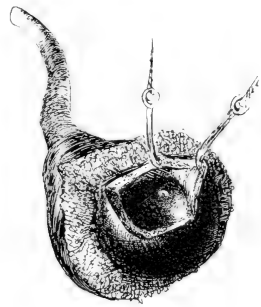


FIG. 196.—Tube cut open, showing the embryo. From a sketch by Professor Brödel.

still be faintly outlined. At points the amnion and umbilical vesicle are blended completely with the chorion.

The outside of the chorion has attached to it a few long and thick villi which do not branch. The chorion and these villi are covered with a layer of syncytium of unequal thick-

ness, which frequently invades the mesoderm. The whole chorion is embedded in a large mass of mother's blood.

The most remarkable part of this specimen is found within the blood-vessels of the chorion. They are gorged with nucleated blood corpuscles filled with a pigment of the same color as that of the surrounding mother's blood. It appears as if the syncytium, in destroying the mesoderm of the chorion and the mother's blood, at the same time made it possible for the blood of the embryo to take up the blood pigment thus liberated. At any rate, the blood of a human embryo three weeks old contains no pigment, and the sections of this specimen permit of this interpretation. There is also a considerable quantity of mother's blood within the ovum around the embryo, but as the specimen was opened before it was hardened and the corpuscles are all perfect, they need not be taken into consideration in the interpretation just given.

No. 198.

Ovum, 25 x 25 x 25 mm.

Dr. Larsen, Chicago.

The specimen came to me hardened in a mixture of bichromate of potash and formalin. The interior is filled with considerable reticular magma and large lumps of granular magma. Imbedded in this there is a large cylindrical pedicle 7 mm. long bent upon itself. Sections of this specimen show that pedicle to be the umbilical cord rounded off at its former juncture with the embryo.

Fig. 198.—Pedicle within chorion. Enlarged 2 diameters.

The mesoderm of the cord, chorion and villi is fibrous, having also an excess of spindle-shaped cells. The blood-vessels are all very large, those of the villi as well as most of those of the main wall being gorged with blood. The large blood-vessels of the cord are empty. Within the cavity of the amnion scattered throughout the magma there are numerous flakes of tissue of the embryo and a great many free cells.

No. 200.

Ovum, 35 x 25 x 20 mm.; embryo, C. R., 14 mm.

Professor Brödel, Baltimore.

The central nervous system is dissociated and macerated very much, the form of the brain and spinal cord being lost entirely. The organs are all deformed, the liver in addition being necrotic, as it does not stain at all. There is ulceration of the front of the head, but over the rest of it, in spite of the extensive internal change, the epidermis is intact.

The walls of the umbilical vesicle are broken down entirely and its lumen is filled with a mass of necrotic cells. The amnion, chorion, and villi are more fibrous than normal.

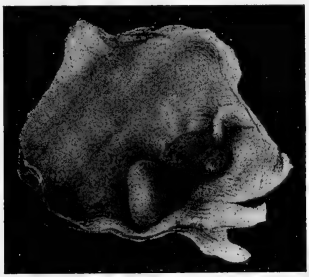


FIG. 200.—Broken embryo within piece of the chorion, showing stumpy arm. Natural size.

No. 201.

Ovum, 80 x 60 x 50 mm.; embryo, C. R., 20 mm.

Professor Brödel, Baltimore.

The ovum was received without villi and upon opening it it was found filled with a fluid which had hardened into a

jelly in formalin. The embryo is atrophic, with a necrotic mass on top of its head.

The fleshy chorion proved when sectioned to be a mixture of true chorion, villi, blood, fibrin, decidua, blood sinuses, pus and syncytium. The layers are not at all in regular order, and show all stages of disintegration. The mesoderm of the villi is fibrous and is often invaded by leucocytes and syncytium. At other points the syncytium invades the blood clot and frequently maternal blood sinuses are filled with leucocytes and syncytium.

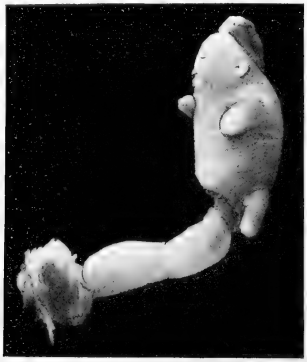


Fig. 201a.—Photograph of the embryo. Enlarged 2 diameters.

Within the embryo most extensive changes have taken place. The brain is greatly deformed and is severed, through a growth of tissue, from the spinal cord in the region of the medulla back of the deformed ear. In fact, the brain is included within the cap-like body on top of the head. The spinal cord begins quite abruptly in the upper cervical region and ends in the same way in the upper lumbar region. At its end there is a curious fibrous tumor measuring half the diameter of the cord. The cord, so far as it is developed, appears to be normal, but it is dissociated somewhat. Below the upper lumbar region the spinal cord is wholly wanting,

the spinal canal being filled up with mesodermal tissue rich in blood-vessels. Where the cord is missing most of the spinal nerves appear to remain, and many dorsal ganglia can be made out. This all indicates that the changes in the central nervous system took place after the spinal nerves were developed from it.

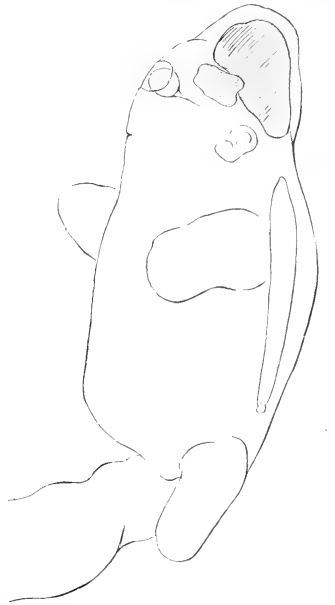


FIG. 201b.—Diagrammatic reconstruction of the embryo, showing the extent of the central nervous system. × 5 times.

The two eyes are united into a single one with a double retina, two lenses, a single choroid, and a single optic nerve; back of this it is double again. It certainly appears as if the two eyes have wandered together and have united in the middle line.

The epidermis is quite complete, being broken through at the back of the head. The extensive ulcer which is found

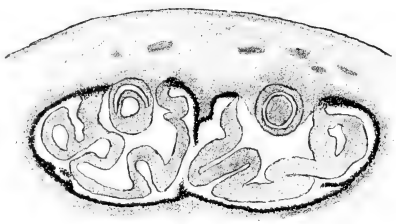


Fig. 201c.—Section through the top of the double eye of the embryo. × 30 times. The eyes are buried deep in the head, being covered with mesoderm and epidermis.

here is very rich in blood-vessels, involves the walls of the brain, but does not reach into its ventricle. At the highest point of the head the epidermis has developed into a papilliform body; below this there is a large necrotic area in which there is a great quantity of yellow pigment granules.

The mouth is closed, although the alimentary canal from there to the stomach is open and appears normal. The intestine is matted together, the cloaca and anus being obliterated. The epithelium of the upper portion of the intestine shows marked growths into this matted mass.

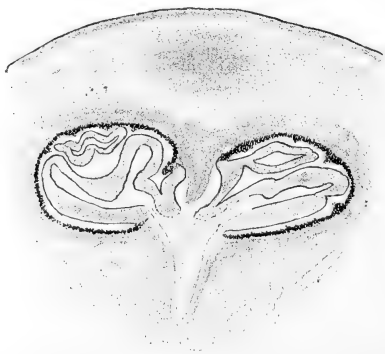


Fig. 201d.—Section through the optic nerve and double eye. × 30 times.

FIG. 201e.—Photograph through a section of the ear, showing the plug which closes the external meatus entirely.

The thoracic region, liver and vascular system have undergone practically no change. The extensive growth of mesodermal tissue throughout the embryo has caused an extensive destruction and arrest of further development of the muscular system. This is shown by all kinds of secondary changes in the connective tissue, especially that of the skin, which is markedly fibrous, as may be seen in Fig. 201e. Here the change is so great that it obliterates the external auditory canal entirely.

No. 204.

Ovum, 14 x 12 x 8 mm.

Dr. D. S. Lamb, Washington.

The specimen, said to be three weeks old, was found filled with a mass of granular magma. The whole ovum was stained and cut, but no trace of an embryo could be found. The chorion and villi appear normal.

No. 205.

Ovum, 40 x x30 x 30 mm.; embryo, C. R., 6 mm.

Dr. D. S. Lamb, Washington.

"The specimen is about four weeks old and is from a woman who had been married three months. Syphilis is suspected in the case."

The chorion is partly encircled with the decidua, which is more or less necrotic and well infiltrated with leucocytes, showing that an inflammatory process was present in the uterus. The chorion is fibrous at points and at others œdematous, with but few blood-vessels present. The villi are irregular and often very fibrous, being hypertrophied as well as atrophied. Their outlines are irregular and they are covered with a dense and very irregular mass of syncytial cells. But few of the villi have blood-vessels within them and they are all empty.

The amnion is completely adherent to the chorion throughout its extent, making these two membranes appear as one. On the amnion side there are numerous fibrous tuberosities

which appear much as small villi inverted. At other points the epithelial covering of the amnion builds by itself a double layer of cells, which often gives rise to papilliform processes much like the syncytium on the outside. Sometimes this layer of epithelium is raised, forming a blister with a fibrin-like substance, possibly magma reticulé, throughout which are scattered transparent round cells with very small nuclei.

The umbilical cord is quite fibrous, with large irregular openings scattered throughout it. These are filled with a mucoid substance in which a few nuclei are scattered. The blood-vessels are all obliterated with the exception of the point of the attachment of the cord to the embryo, where irregular vessels are filled with blood.

The external form of the embryo is well preserved and is covered entirely with epidermis which is much thickened. The brain and spinal cord are swollen, the former being practically solid in the region of the fore-brain. The heart and large vessels are gorged with blood which extends from them into the surrounding tissues, obliterating them almost entirely. Within this mass of migrating cells can be seen the outlines of some of the organs of an embryo about four weeks old. The liver, stomach, and lungs are riddled, and but the faintest mark of an endocœlom can be seen. It appears as if all the blood of this specimen accumulated within the embryo, the cord and the chorion being free, the extensive epidermis preventing the migration of the blood cells into the amniotic cavity.

No. 207.

Ovum, 70 x 45 x 45 mm.; twin embryos, 16 mm. long.

From Professor Brödel, Baltimore.

The specimen came to me unopened and hardened in a strong solution of formalin. Its exterior is smooth with small villi at one of its poles. Within there are two embryos, both macerated, with atrophic heads. The larger embryo measures C. R., 16 mm. The other is a little smaller, but as it is broken, an exact measurement could not be made. The cords of both embryos are atrophic.

There is some granular magma within the amniotic cavity with several large clumps in the cœlom, where the two amnions meet.

Sections of the membranes show that the chorion is denuded of most of its villi, with the exception of the point over the atachment of the cord of the broken embryo. The entire chorion is covered with its decidua, which is rich in blood sinuses and infiltrated with leucocytes. But few remnants of the syncytial layer of the chorion remain.

The whole embryo is still covered by epidermis excepting on top of the head, at the tail end of the body, and at the attachment of the umbilical cord. At these points there is a

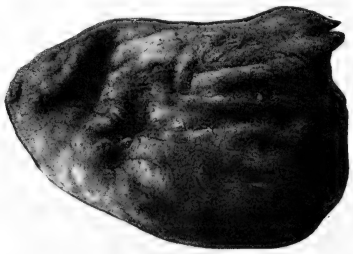


FIG. 207a.—A whole ovum. Reduced.

marked destruction of the tissues, which are beginning to disintegrate. The top of the head is ulcerated, in front it is necrotic and pigmented, as is frequently the case in other embryos. The nervous system shows the usual changes seen in strangulated embryos. The vascular system of the embryo is gorged with blood, but none is within the vessels of either the cord or the chorion. Within the body there is quite an extensive migration of blood cells into the tissues, obliterating them in part, but the process of destruction is not so far advanced as in No. 205. The majority of the organs can be still outlined. We have here a rapid infiltration with migrating cells of an embryo of forty days, with cytolysis rather than dissociation of the tissues.

The changes in the broken embryo are practically the same as in the unbroken one, although they are more advanced. Only the head, extremities and cord remain entire, and in

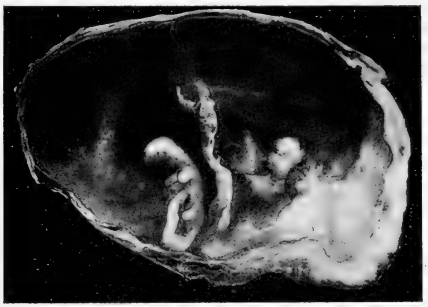


FIG. 207b.—Photograph of the interior of the ovum, showing both embryos. Natural size.

these the changes are more marked than in the corresponding parts of the unbroken embryo. In the former it is practically a mass of individual cells, while in the latter the brain is swollen and quite solid.

No. 209.

Ovum, 20 x 15 x 10 mm.; embryo normal in form, about two and one-half weeks old.

Dr. G. N. J. Sommer, Trenton, N. J.

"The woman from whom the specimen was obtained is thirty years old. Three years ago she had a miscarriage during the third month of pregnancy, and three months ago she was delivered of a monster at the end of gestation. The specimen was one of hydrocephalus and spina bifida with hydramnios, fully eight liters of fluid coming away at the time of delivery. She menstruated the first time yesterday since her confinement, bleeding profusely all day, and in the evening the ovum came away with a few blood clots. Within the sac I could see the embryo, about 5 mm. long, attached to the chorion by the cord."

The specimen came to me in 95 per cent alcohol, and upon opening it a large amount of magma was found within the cœlom. Not finding the embryo, the whole specimen was stained, imbedded in paraffin and cut into serial sections 50 microns thick. It happened that the embryo was cut into coronal sections, and those containing the embryo with the chorion attached to it were mounted.

The form of the structures of the embryo is normal, only the tissue did not stain well, indicating that it had been dead for some time before the abortion. Over the back and tail of the embryo the amnion is closely adherent, but it is wanting over the head. Here it ends abruptly, and this could not be due to rough handling, for the embryo is well packed with magma up against the chorion. Over the embryo the chorion is very thin and without villi, which explains why the embryo was seen in the fresh specimen. At some distance from the embryo the chorion appears to be normal in structure.

No. 212.

Embryo, C. R., 15 mm.

Dr. West, Bellaire, Ohio.

The macerated embryo is from a large ovum which was aborted October 9, 1902. Last menstrual period began on April 3, 189 days before the abortion.

The tissues of the embryo show that its development was arrested during the sixth week. The central nervous system is completely dissociated, being but a mass of cells. The other tissues of the body, except those of the head, have undergone no secondary changes. The face and the top of the head have been converted into a thickened mass of necrotic tissue, in which may be seen large veins filled with blood. The eyes are immediately below the skin, thoroughly dissociated, but the vesicular lenses can still be outlined.

No. 215.

Ovum, 45 x 40 x 40 mm.; embryo, C. R., 17 mm.

Dr. Unger, Mercersburg, Pa. Brödel Collection.

FIG. 215.—Photograph of macerated embryo in a piece of the chorion. Natural size.

The specimen is smooth and fleshy and filled with granular magma, in which was found the remnants of a macerated embryo. Sections of the chorion show that the decidua is attached and that the amnion lines the whole ovum. The chorion is well developed, but the villi are matted together; it corresponds with its history, which states that the specimen is about 12 weeks old. It was preserved in 10 per cent alcohol.

No. 223.

Mole, 40 x 18 x 15 mm.
Professor Brödel, Baltimore.
At the point of attachment to the uterus the "fibroid mass"

FIG. 223a.—Photograph of the mole. Natural size.

is very rich in villi, which at its rounded end is composed wholly of blood. The entire tumor is encapsulated with a layer of pus. Between the villi the meshes are filled with

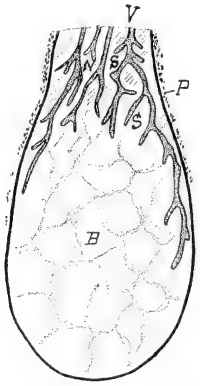


Fig. 223b.—Diagram of the structure of the mole. *B*, blood and fibrin; *P*, pus; *S*, syncytium; *NS*, necrotic syncytium; *V*, villi.

syncytium, which often give the picture of a cancer. Where the syncytial cells are far removed from the blood they are often necrotic.

No. 226.

Ovum, 60 x 60 x 30 mm.; embryo, C. R., 24 mm.

Dr. West, Bellaire, Ohio.

"The woman, mother of three children, menstruated last on March 3 and aborted on May 29." The ovum is covered with a few large villi two mm. in diameter at their base, and irregular clots of blood. Elsewhere it is smooth. The amnion is filled with a granular mass, which was swept out easily when opened. Between the amnion and chorion there is an irregular mass of mother's blood, which is partly organized, showing that the chorion had ruptured some time before the abortion took place. The tissues of the villi and the chorion are somewhat fibrous, with very few degenerated blood-ves-

sels within them, indicating that the circulation had ceased some time before the abortion; which is confirmed by a study of the embryo.

The external form of the embryo indicates that it was nearly 50 days old when it died, for, with the exception of the head, its form is practically normal. The menstrual history makes it 87 days, and if 28 is subtracted, ten days are still left, which is time enough in which to bring on the internal changes found within it.

In general the organs are sharply defined, but they do not stain well; the cells appear as in coagulation necrosis. The

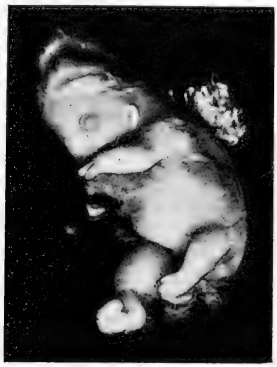


FIG. 226a.—Photograph of the embryo. Enlarged 2 diameters.

cartilages are also well formed, and the maxilla, mandible, clavicle, humerus, ulna, radius, femur and tibia have begun to ossify. All this indicates that this embryo died quite suddenly and that the changes within it are to be viewed as post-mortem changes.

The vascular system is well developed, the heart muscle being normal in shape but very fibrillar; it does not stain well. Most of the large vessels are empty and the blood cells are scattered throughout the tissues of the embryo and the cord. The muscle fibers are unusually well marked, and the connective tissue seems to be thickened.

The most marked changes are seen in the head. Much of the epidermis is still in place, but some of it has fallen off. At the back of the head the destructive process has included the back of the brain and the upper part of the spinal cord. The fore-brain, mid-brain and the spinal cord of the trunk are still intact and dissociated. The eyes are of normal shape and position, but much macerated. The nerves of the head can still be outlined, which shows quite conclusively that the destruction of the medulla is of recent date.

Fig. 226b.—Reconstruction of the central nervous system of the embryo.

No. 228.

Ovum, 60 x 25 x 25 mm.; embryo, 4 mm.

Dr. West, Bellaire, Ohio.

"The specimen is from the first pregnancy of a fairly healthy woman. Last period July 1 to 3, and the abortion took place on October 10, 1903."

FIG. 228a.—Photograph of the ovum. Natural size.

The solid blood-red specimen contains a regular cavity, 30 x 18 x 18 mm., which is filled with a granular magma, on one side of which is attached an embryo shaped like an hour-glass.

Sections of the mole show that it is composed of thick walls in which there is much blood, villi, a great deal of decidua and some pus, especially on its outside. The mesoderm of the villi and chorion is very fibrous and devoid of blood-vessels.

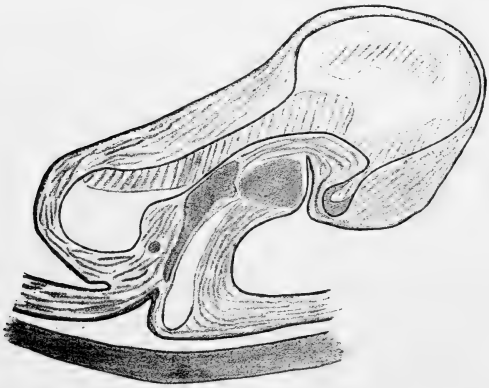


FIG. 228b.—Diagrammatic section of the embryo. × 20 times.

The cavity of chorion is lined with a very thick amnion and the remnant of an embryo indicates that its development was arrested towards the end of the third week. The deforming process must have been active for at least 50 days.

The vascular system is still represented by a mass of cells on the ventral side of the embryo, behind which there is a large vessel full of blood extending towards the remnant of the umbilical vesicle. No vessels extend to the chorion.

The central nervous system fills the main part of the embryo, being much dilated in the head and pretty well filled with round cells throughout. In front of the brain are two vesicles which communicate with it through two long tubes. These no doubt represent the eyes. In the neck there is a small gland, possibly the thyroid.

No. 230.

Ovum, 75 x 60 x 50 mm.; embryo, C. R., 57 mm.

Dr. West, Bellaire, Ohio.

"The mother has had three children and three miscarriages. She always menstruates regularly during her pregnancy, and she has been undecided during the past seven months whether or not she was pregnant."

Upon opening the ovum it was found that the fœtus is greatly cramped and imbedded in much granular magma. The cord is thin and knotted. The right leg has a club-foot and the left has a dislocated knee-joint. Evidently the embryo has been dead for a long time.

The tissues of the embryo and membranes appear normal; they barely stain at all. The outer zones of the chorion are slightly infiltrated with leucocytes.

The dislocated knee and the club-foot show that the cartilages are markedly deformed, but on account of the absence of tissue reactions it must be concluded that this change took place after the death of the embryo. The liver, brain, spinal cord and eye are macerated, converted into a pulpy mass and do not stain. All of the epidermis has fallen off. Apparently the embryo died suddenly, for there are practically no tissue reactions to suggest the contrary.

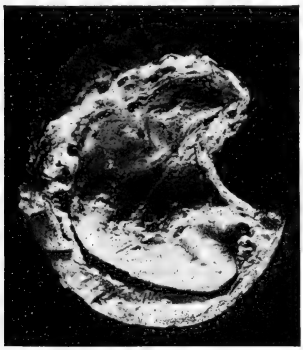
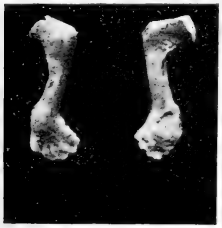


FIG. 230a.

FIG. 230b.

FIG. 230a.—Ovum cut open, showing embryo within imbedded in a mass of granular magma. Reduced.

FIG. 230b.—Embryo cleared of magma.



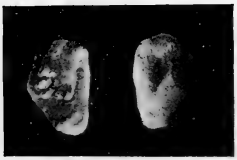


FIG. 230c.—Arms and legs of embryo. Two views of each are shown.

No. 232.

Ovum, 45 x 25 x 25 mm.; embryo, C. R., 14 mm.

Professor Brödel, Baltimore.

Most of the chorion is devoid of villi except that immediately over the attachment of the cord, which appears to be normal. The villi of the chorion are somewhat fibrous, with

FIG. 232a.—Entire ovum with villi on one end. Natural size.

blood-vessels less numerous than usual, and are covered with a rich layer of syncytial cells. The amnion reaches the chorion.

The embryo is atrophic and is imbedded in a mass of granular magma, in which there are numerous round cells.

FIG. 232b.—Embryo within the chorion.

Most of the epidermis has fallen off. The head is cylindrical in form, containing a solidified brain and dissociated eyes. The lenses are composed of broken cells surrounded by a very thick hyaline capsule. The organs of the body are not sharply defined, being filled with many round cells. The blood-vessels are mostly empty. Even the nerves and cartilages have lost their sharp borders. The extremities are stubby, being composed of densely packed round cells which show no differentiation.

No. 233.

Mole, 70 x 45 x 40 mm.

Dr. Miller, Hagerstown, Md. Brödel Collection.

The irregular mass appears as an ovum filled with blood. Sections show, however, that there is a mixture without rhyme or reason of all kinds of deformed villi, blood, syn-

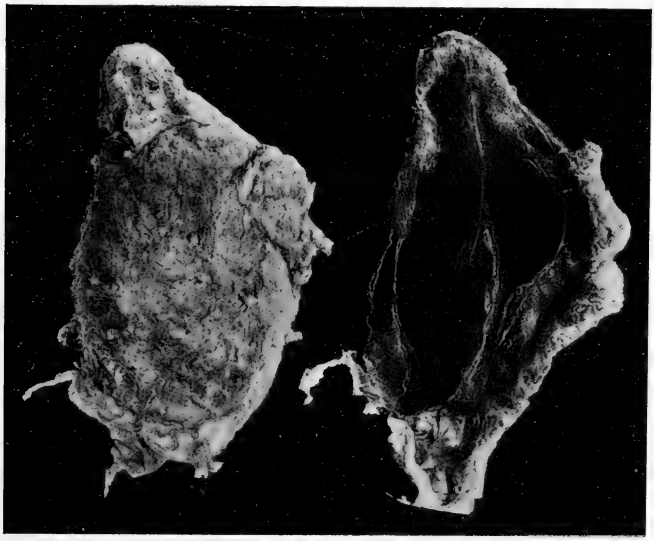


FIG. 233.—External and cut surfaces of the mole. Natural size.

cytium, decidua and pus. No doubt at its attachment to the uterus it received fresh blood into its center, while the leucocytes attacked it on its exterior. Most of the villi are encircled with fragmented leucocytes, which seem to have gained the upper hand.

No. 243.

Ovum, 30 x 20 x 10 mm.

Professor Brödel, Baltimore.

The specimen is pear-shaped with smooth thin walls, over which there are scattered a few thin villi. The whole specimen was cut into serial sections and no trace of an embryo could be found.

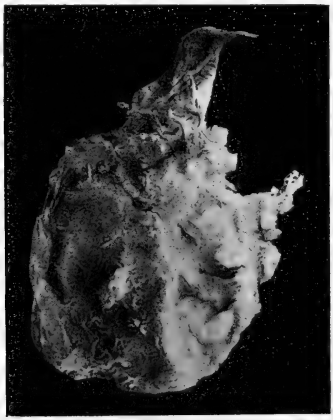


Fig. 243.—External view of ovum. Enlarged 2 diameters.

No. 244.

Embryo, 4 mm. long.

From Dr. Kelly's Sanatorium. Brödel Collection.

The specimen is enclosed in the amnion, which measures 25 x 15 x 15 mm. and is surrounded by a mass of granular magma.

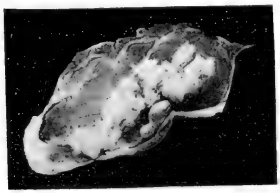


FIG. 244a.—Embryo, surrounded with granular magma, attached to the amnion. × 2 times.

FIG. 244b.—Section through the head of the embryo. × 20 times.

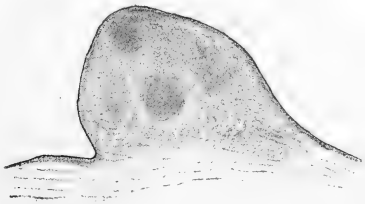


FIG. 244c.—Section through the body of the embryo. × 20 times.

The sections show that the amnion is attached along most of the ventral side of the embryo, somewhat as it is in the normal specimen at the end of the second week. The central nervous system is still quite sharply defined, being more characteristic in the head than in the trunk. The heart is composed of a solid mass of cells in front of the embryo, which extends as a horn-like process to the head. Between the heart and the body there is large group of epithelial cells, in which there are scattered some small round cells, probably the remnant of the liver. Otherwise the tissue of the embryo is of even structure with an occasional necrotic area. The epidermis is mostly wanting. There is neither umbilical cord nor umbilical vesicle present, the free embryo being attached to the amnion only.

No. 246.

Ovum, 30 x 21 x 14 mm.; embryo, 3 mm.

Dr. Wegefarth, Baltimore. Brödel Collection.

Dr. Wegefarth writes: "The woman from whom this specimen was obtained is the mother of two children, the youngest about seven years of age. Since then she has had five miscarriages, all of about the same age as this specimen. No history of syphilis, but have started to give her iodide of potash, with the hope that she may give birth to a child. I shall be glad to have you turn the specimen over to Professor Mall if it will be of any use to him. It would be interesting

Fig. 246a.—Ovum with window cut out of it, showing dense magma and embryo within.

if the great fire we had recently could have played any part in this trouble, as she felt well up to that time, and the fright due to the fear that the fire would burn out her neighborhood, too, kept her in a state of great excitement for about 24 hours."

The external surface of the ovum is normal in appearance, but when it was opened it was found to contain a deformed embryo lying beside a very large amnion. Sections of the chorion show that its structure is somewhat hyaline and the villi are devoid of blood-vessels. The embryo and membranes were cut together and the sections show that the amnion is greatly hypertrophied, folded and torn, and that the embryo

FIG. 246b.—Embryo covered with folds of the amnion. × 10 times.

is deformed and injured but lying outside of the amnion. The heart and great blood-vessels are empty, the brain is distended and partly filled with round cells; together they give the appearance of an embryo of the beginning of the third week. No liver can be found, but there are loops of intestine present, as during the fourth week. The otic vesicles are well defined, but the optic vesicles are wanting.

No umbilical vesicle can be found, but this may have been lost when the amnion was torn. The amnion, however, runs down in a thickened ridge which contains two large blood-

vessels and an epithelial tube, the allantois, between them. At no place is the amnion attached to the chorion, nor are there indications that they have been torn apart.

No. 247.

Ovum, 40 x 40 x 17 mm.; vesicle, 2½ mm.

Dr. Seymour, Trappe, Md. Brödel Collection.

The ovum was found filled with granular magma and in the center of this, far away from the chorion, a free umbilical vesicle was found. Sections of the chorion show that it is nearly normal in structure without any signs of an amnion on its inside. The villi are without capillaries. At points between the villi the syncytial cells form mounds below the epithelium, which have a tendency to penetrate the mesoderm of the chorion.

The pear-shaped body is probably the umbilical vesicle, with a cavity lined with epithelium and a considerable amount of mesoderm around it, in which there are numerous blood-vessels filled with blood. There are some accessory vesicles in this layer similar to those found in No. 78.

No. 250.

Ovum, 10 x 9 x 9 mm.; embryo, 2 mm.

Dr. Sampson, Baltimore.

The specimen came imbedded in a mass of decidua, which was obtained by scraping the uterus. When opened it was found filled with magma reticulé, in which could be seen, immediately beneath the chorion, a small embryo, and further away, towards the center of the cœlom, the umbilical vesicle. The whole ovum was cut into serial sections.

The chorion and villi are apparently normal in shape and structure, being also very rich in blood-vessels which are filled with embryo's blood. The villi are bathed in mother's blood and covered with an active syncytium. The decidua is somewhat infiltrated with leucocytes, but there are no abscesses.

The front end of the amnion is torn and its free edge and the embryo are imbedded in reticular magma, indicating that

the injury took place before the abortion. The general shape of the embryo and its degree of development are practically normal. The heart is well formed and it, with the blood-vessels, is filled with blood. The alimentary canal, brain, spinal cord, otic and eye vesicles, myotomes and branchial arches are much like embryo No. 12, which is practically a normal embryo of the beginning of the second week. The septum transversum is well marked and the thyroid gland is just beginning.

FIG. 250a.—Ovum, opened to show the embryonic mass, within the decidua. × about 2 diameters.

FIG. 250b.—Section of embryo, encircled with magma, within the chorion. The amnion is torn. × 17 diameters.

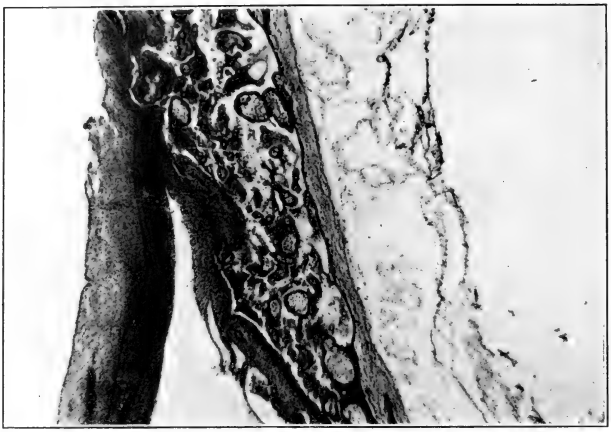


Fig. 250c.—Section of chorion, villi and decidua. There is a large quantity of mucoid mass between the villi. × 17 diameters.

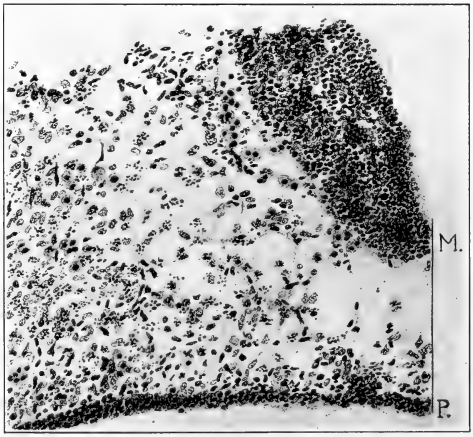


Fig. 250d.—Section through hind-brain, *M*, adjacent mesenchyme and epithelial lining of pharynx *P*, to show cytolysis and dissociation of the tissues. × 250 times.

The tissues of the embryo, however, and the cavity of the front end of the brain are filled with numerous small round cells wth fragmented nuclei. All stages of fragmentation are seen, just as may be seen in the leucocytes in small abscesses. Most of the red blood cells are within the blood-vessels, but those within the tissues appear perfectly normal. On account of the diminished number of mesoderm cells, in fact, they

FIG. 250c.—The dotted area in the section shows the portion which is enlarged in Fig. 250d.

diminish in proportion to the number of fragmented cells present, the conclusion must be drawn that the latter arise from the former. The epidermis covers the whole embryo.

The primary change in this specimen is no doubt in the mesoderm, for all the rest of the embryo appears normal. That the equilibrium was overthrown is indicated by the necrotic amnion and the great amount of reticular magma in the exocœlom.

No. 251.

Ovum, 30 x 25 x 25 mm.; embryo, C. R., 9 mm.

Dr. Ritter, Brooklyn.

Last period January 16, abortion April 3. Half of the chorion is covered with villi and the other half is bare, thickened and hemorrhagic. The amnion lines the entire chorion and the cord is very thin. Sections show that the mesoderm of the villi are rich in cells, fibrous and are devoid of blood-vessels. The main wall of the chorion is apparently normal, with a large number of vessels filled with blood scattered through it. The decidua is very extensive, is hemorrhagic and has a large number of abscesses in it. Apparently there was an extensive endometritis.

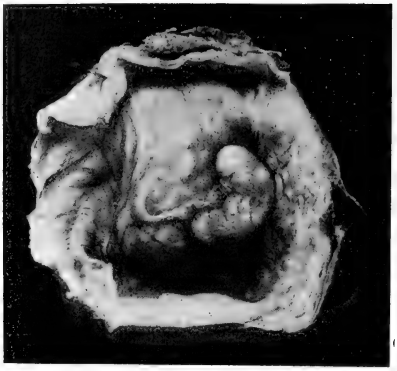


FIG. 251a.—Embryo attached to the chorion. Enlarged nearly 2 diameters.

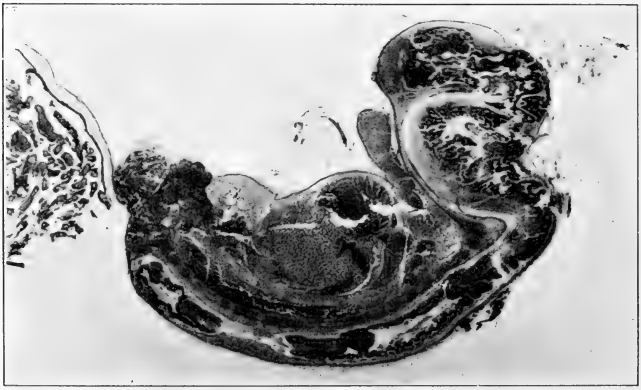


FIG. 251b.—Section of the embryo. × 8 times.

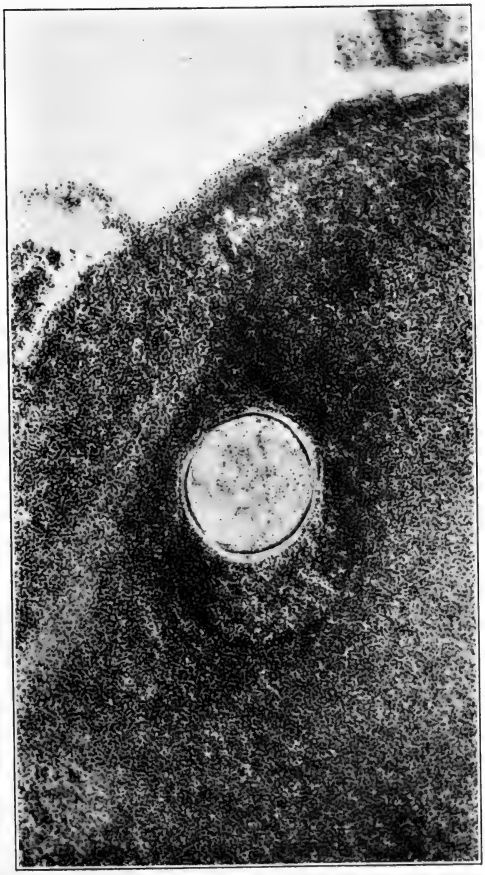


FIG. 251c.—Section through the lens and adjacent tissue.

The head of the embryo is atrophic and is nearly filled with a distended, dissociated and macerated brain. The eyes are solid and the lenses have become dissociated, but they are encircled with sharply defined and thickened hyaline capsules. The brain is protruding behind the head. The heart and blood-vessels are distended and filled with blood. The organs and tissues of the body are not well defined, and are filled with round cells. The epidermis is wanting. The extremities are stubby, without structure and filled with round cells. The cartilages are sharply defined, and the liver appears to be about normal.

No. 252.

Embryo, 5 mm. long.

Dr. Lamb, Washington.

"First pregnancy in an unmarried woman twenty-three years old. Patient missed a month, then had free hemor-

Fig. 252a.—Photograph of embryo, with amnion on one side and the thickened chorion on the other. Natural size.

Fig. 252b.—Section through the eye, small black spot in Fig. 252a. × 20 times. *E*, eye; *B*, brain.

rhage which continued for a month, when the embryo was expelled." This would make its age three months, counting from the last period.

This remarkable specimen shows to what extent an embryo may grow after its regular development has been arrested. The specimen came to me attached to a solid body, as the photograph shows, and it appears to be an embryo about three weeks old. The free end of the embryo is bent upon itself and runs to a point where two intensely black spots may be seen.

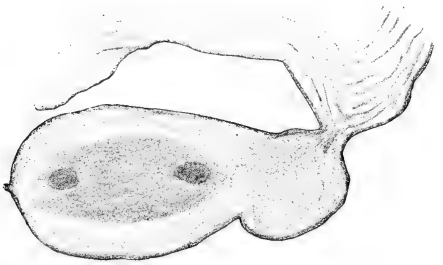


FIG. 252c.—Section through the embryo at its attachment to the chorion. × 20 times.

The membrane or body behind the embryo is undoubtedly the amnion curled up, for it is covered with epithelium on the side towards the embryo side, which continues over its body. On the other side the mesoderm, which is thickened and hyaline, is free, there being no border cells nor villi.

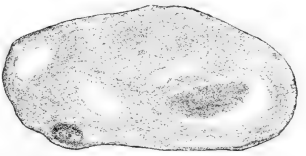


FIG. 252d.—Section through the embryo below its attachment to the chorion. The body immediately beneath the epidermis is a solid lentoid structure.

The skin is markedly thickened, the epidermis sometimes forming small papillæ, or are sometimes buried, forming pear-like bodies similar to those of epithelial cancer. Within

the body there is a large cavity filled with round cells. Near the attachment to the amnion there are several such "abscess-like" masses within the embryo.

The pigment dots, on account of their position, undoubtedly represent the eyes of the embryo. Each forms a small sac immediately below the skin filled with large free pigment cells. Deeper within the "head" of the embryo a band of pigment cells connects the two "eyes," as may be the case if we consider these cells as the connecting optic nerves.

No. 253.

Ovum, 35 x 30 x 15 mm.; embryo, 4 mm.

Professor Brödel, Baltimore.

Chorion and villi are somewhat hyaline, with indications of blood-vessels within them. Amnion, which measures 19 x

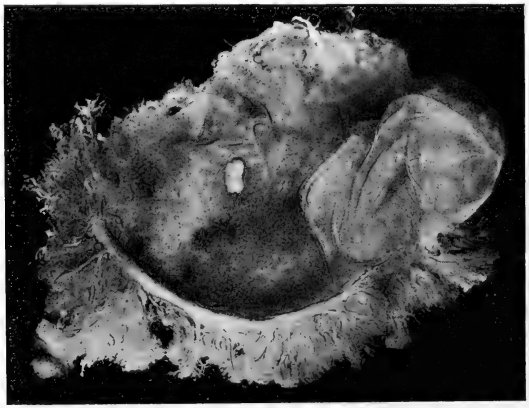


Fig. 253.—Embryo within the chorion. × 1.8 times. The collapsed bag behind the embryo is the amnion.

13 x 13 mm., is attached at one point, has hyaline walls and does not contain the embryo.

The embryo is a swollen infiltrated specimen of the third week, with no brain and little of its spinal cord left. The

rest of the structures (heart, cœlom and Wolffian body) are quite sharply defined, but are all infiltrated with round cells. Most of the epidermis is intact. The arm buds are well defined.

No. 255.

Ovum, 20 x 20 x 10 mm.

Professor Brödel, Baltimore.

The villi are atrophic and fibrous. At points the syncytial layer is well mixed with leucocytes, which also have invaded some of the villi as well as the mesoderm of the chorion. The whole chorion was cut into serial sections, but no trace of an embryo was found. There are no blood-vessels in the chorion, nor were any remnants of the amnion found.

No. 257.

Ovum, 55 x 40 x 40 mm., with a pedicle within, 14 x 2 mm., to which is attached a body 4 x 0.5 mm.

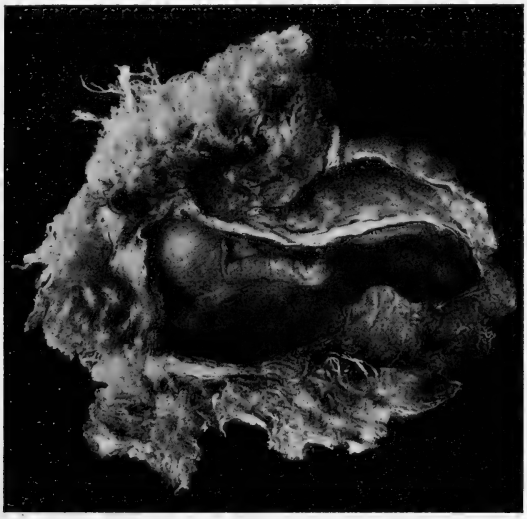


FIG. 257.—Photograph of the specimen. × 1.5 times.

From Mr. Lankford, Baltimore.

A large portion of the chorion is covered with well formed and apparently normal villi; a portion is hemorrhagic and another is fibrous, appearing as though it had protruded through the os. Sections through this portion show that the villi are atrophic and have undergone fibrous degeneration. The chorion is thickened and the decidua is infiltrated with leucocytes.

The inside of the chorion is lined with epithelial cells, which are continuous with those over the cord; it appears as if the amnion had become completely blended with the chorion.

The cord is also fibrous, with some spots which have undergone mucoid degeneration. It contains three large blood-vessels,—a vein and two arteries. The body at the end of the cord is simply its continuation, with the umbilical vein running throughout it lengthwise.

No. 261.

Chorion, 120 x 70 x 70 mm.; embryo, about 90 mm. long. Dr. W. M. Lewis, Baltimore.

Fig. 261a.—External view of specimen. Three-fifths natural size.

The ruptured and distorted fœtus, which no doubt had been dead for a long time, is imbedded in a mass of granular magma.

Sections of the placenta show that the villi and chorion are very fibrous and almost devoid of syncytium. The umbilical cord is somewhat fibrous, with blood-vessels within filled with

Fig. 261b.—Fœtus within its membranes. Reduced.

blood. The decidua contains large sinuses and is also well filled with round cells.

The tissues of the hand and skin are somewhat infiltrated with round cells, but other changes within them are not marked. It appears as if the embryo died quite suddenly, and therefore there are no marked tissue reactions.

No. 262.

Mole, 80 x 15 x 15 mm.

Dr. Giering, Baltimore.

The specimen was several days old when it came into my hands and was then hardened in formalin. The interior is

FIG. 262a,—Photograph of the mole. Natural size.

FIG. 262b.—Section of the embryo. Enlarged about 10 times.

filled with a large amount of granular magma, in which is imbedded a necrotic embryo, 14 mm. long.

The decidua is filled with small abscesses, the leucocytes invading the villi as well as the main walls of the chorion. The changes in the embryo are extreme, the nervous system being solid, filling up the stumpy head. The outlines of the organs are hazy, they being filled more or less with round cells. The embryo is falling into pieces; some of the epidermis is still intact.

No. 263d.

Ovum, 27 mm. in diameter; embryo, C. R., 15 mm.

Dr. Lyman, Baltimore.

The villi are apparently normal in form and in structure. Possibly the mesoderm is a little fibrous. The blood-vessels appear to be normal. The cord is dilated, showing the double enlargements, which are mucoid in structure.

The brain and spinal cord are dissociated, with the brain protruding into the mouth, but the other organs are fairly

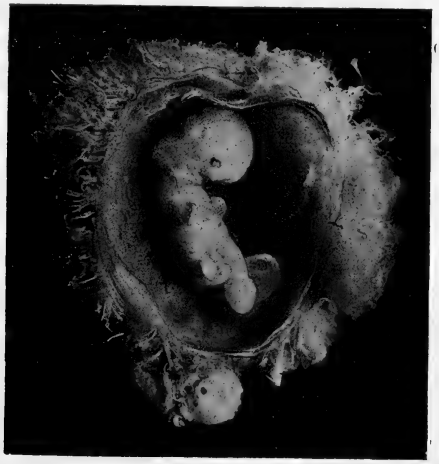


FIG. 263d a.—Embryo within the ovum. × 2 times.

well outlined. The heart and large blood-vessels are filled with blood and there is some infiltration of the surrounding tissues with round cells. The epidermis has fallen off. The changes within the embryo may be due to maceration, but on

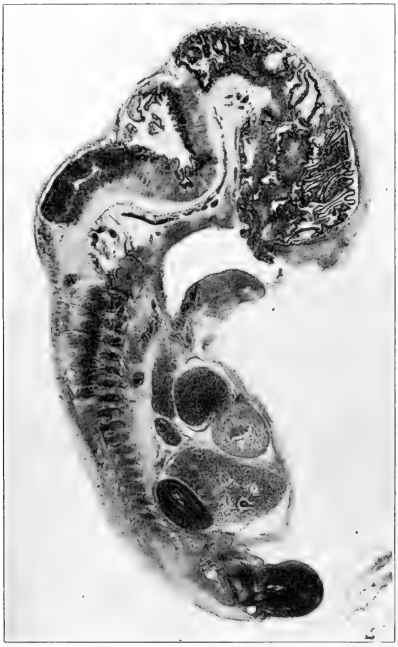


FIG. 263d b.—Sagittal section of the embryo. × 7 times.

account of the sharply defined tissues of the chorion and slight amount of fibrous changes in the villi and the mucoid dilatations in the cord with some wandering cells in the tissues, I am inclined to think that this specimen represents the earliest stage of a strangulated embryo of the sixth week.

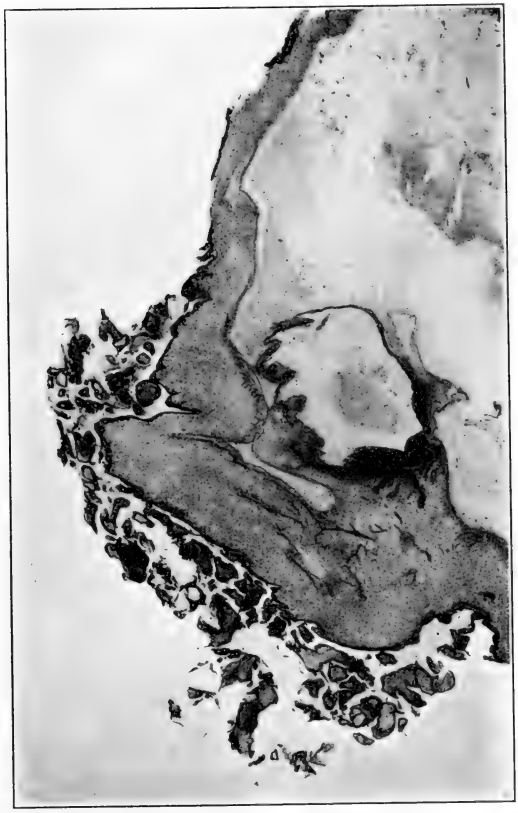


FIG. 264.—Section of the vesicle attached to the chorion. × 15 times.

No. 264.

Ovum, 25 x 20 x 15 mm., with a cavity within 10 mm. in diameter.

Dr. Gardner, Baltimore.

"Last period occurred on August 12; abortion October 9; but the menses had been irregular for three months before."

The cœlom is filled with hard hyaline magma, rich in round cells, in which is imbedded the umbilical vesicle measuring 2½ mm. in diameter. The chorion is thickened and fibrous and is covered with some villi, which are also fibrous. The vesicle shows all the characteristics of the umbilical vesicle and is attached to the chorion by a thick fibrous pedicle. At the point of juncture it is rich in large blood-vessels filled with blood. These radiate into the surrounding chorion, but do not reach into the villi.

No. 268.

Embryo, C. R., 22 mm.

Dr. Kammerer, New York.

The form of the embryo is normal, but its body is straighter than usual. It was hardened in formalin and some of the tissues are well preserved, but others, *e. g.*, brain, liver, lungs and muscles, are dissociated. The blood-vessels are filled with blood and there are no wandering cells in the tissues. Compare the form of this embryo with that of No. 256, Plate III, Fig. 8.

No. 270.

Ovum, 40 x 30 x 30 mm.; embryo, C. R., 14 mm.

Dr. Wilson, Baltimore.

The chorion is only partly covered with villi, which are atrophic and fibrous in structure, but contain some blood-vessels in them. The main wall of the chorion is also fibrous and of irregular thickness, with some blood-vessels in it. The amnion has reached the chorion and is filled with granular magma, which completely envelopes the embryo.

The central nervous system is distended, dissociated and macerated. The large blood-vessels and heart are distended

FIG. 268.—Photograph of the embryo. × 4 times.

FIG. 270a.

FIG. 270b.

FIG. 270a.—Photograph of the ovum. Natural size.

FIG. 270b.—Embryo within the chorion, containing granular magma. × 2 times.

with blood and the tissues of the body are somewhat infiltrated with round cells. The outlines of the organs are slightly obscured, but some of the tissues of the body are sharpened by the process of maceration, which does not seem to have been of long duration.

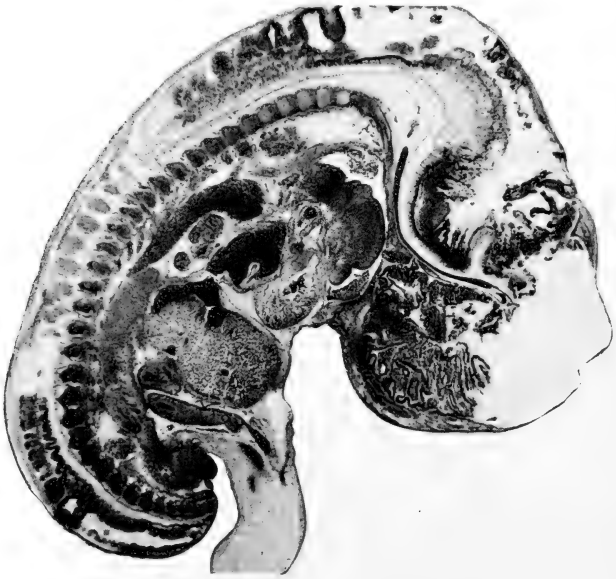


Fig. 270c.—Sagittal section of the embryo. × 7½ times.

No. 275.

Ovum, 40 x 30 x 25 mm.; embryo straightened and about three weeks old.

Dr. Tobie, Portland, Me.

The chorion of this specimen, thought to be two months old, is thin and covered with some villi which are imbedded in much blood. In structure it is fibrous, with a diminished amount of syncytium upon it, and contains no blood-vessels.

Within there is a cavity, the amniotic, filled with a clear fluid, into which the deformed embryo projects. The exo-

cœlom is from two to three millimeters wide, and is filled with typical magma reticulé.

The structures of the embryo form almost a continuous mass of tissue, in which the irregular central nervous system

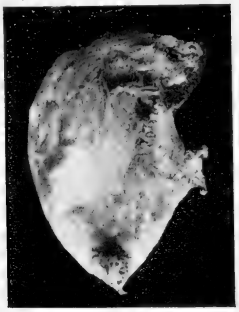


FIG. 275a.—Photograph of the ovum. Natural size.

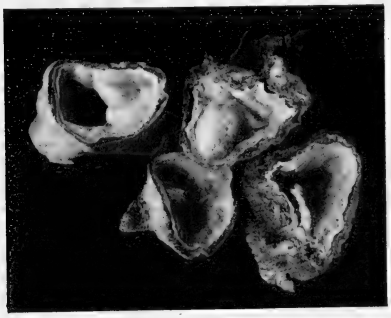


FIG. 275b.—Photograph of sections of the ovum, showing the embryo in one of them. Natural size.

can still be outlined. Enough is left to show that the specimen began to become infiltrated towards the end of the third week.

Most of the epidermis is still intact. The lenses of the eyes form small pearls enclosed in capsules lying beneath the

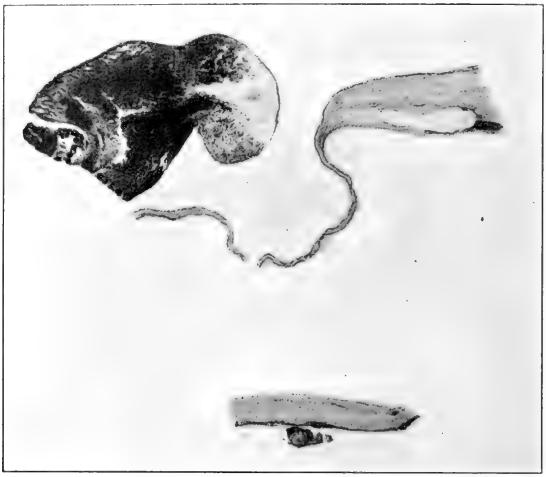


Fig. 275c.—Section of the head-end of the embryo, amnion and chorion. × 15 times.

skin. In front of them there are two small bodies connected with the epidermis, which might pass for lenses, but are probably changed olfactory pits. In a number of places the tissues are fibrous.

No. 276.

Ovum, 70 x 35 x 35 mm.; embryo, 13.5 mm.

Dr. Stanley, Portland, Me.

Dr. Stanley writes that the time between the last menstrual period and abortion is 80 days.

The walls of the chorion are partly infiltrated with blood and on one side is closely adherent to a fleshy mass—the decidua. Sections of these regions show that the decidua has large blood sinuses and numerous small abscesses in it. The villi of the chorion are imbedded in a mass of blood, are covered with a normal amount of syncytium, but in structure they are fibrous and devoid of blood-vessels. In addition,

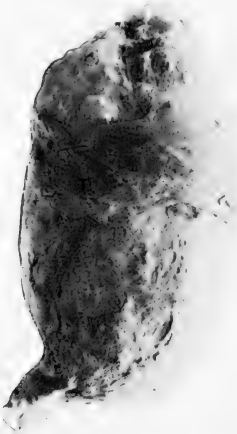


FIG. 276a.—Photograph of the ovum. Natural size.

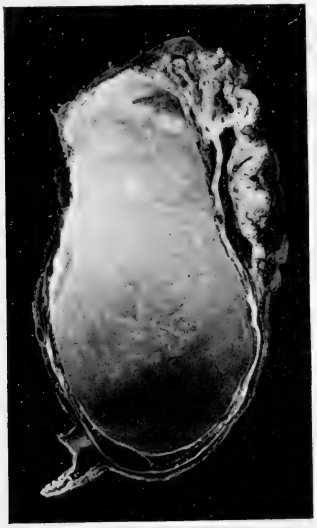


FIG. 276b.—Interior of the ovum, showing broken embryo on one side of it. Natural size.

they are invaded at numerous points by the syncytium, which forms in them small vesicles, lined with two layers of cells, and often filled with dense masses of small round cells. These vesicles are very numerous and usually communicate with the

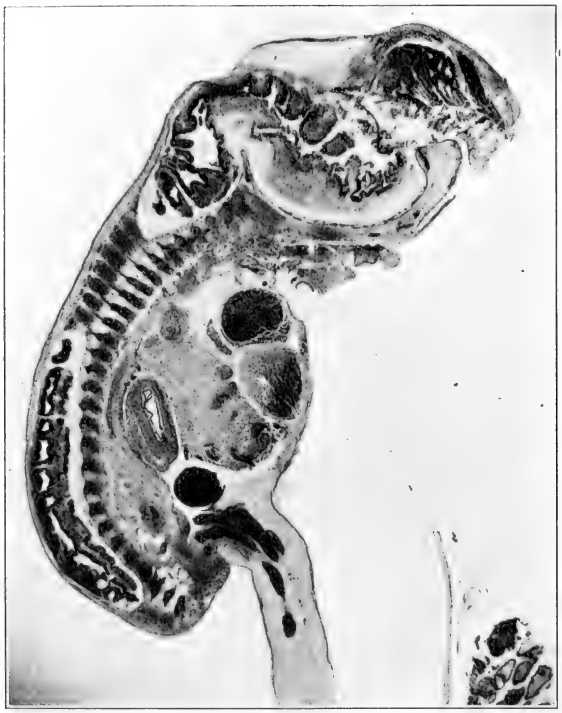


Fig. 276c.—Section of the embryo. × 8½ times.

surface of the villi by means of bands of epithelial cells. The walls of the chorion are in apposition to those of the amnion, but they are not invaded by syncytium.

The changes within the embryo are equally remarkable. The spinal cord is dilated and dissociated; the medulla is

solid, fills the entire head and protrudes from an opening formed by the destruction of the forepart of the head. In front of this opening the atrophic upper jaw may be seen, containing nerves, and behind the epidermis has grown into a small ridge, encircling the opening. What has taken place in this embryo took place mechanically in No. 256 (see Plate III): The outlines of the organs are not sharp, but those of the precartilages are very definite. The blood-vessels are greatly dilated and filled with blood cells, which make them look like abscesses. They are especially well marked along the line from the umbilical cord to the heart. In their immediate neighborhood there is more or less infiltration with round cells. The smaller veins and arteries are still filled with blood.

No. 278.

Ovum, 6 x 4 mm.

Dr. Stanton, Albany, N. Y.

"This specimen was found accidentally in curettings from a woman supposed to have chronic endometritis following pregnancy. There is nothing in the history by which the age of the specimen could be estimated." Part of the specimen had been cut into sections before the specimen was sent with the statement that no embryo had been found, it having fallen out.

I found that the half sent contained a cœlom, 3 x 2.5 mm. filled with magma, in which there was a cavity about 1.5 x 1 mm. Sections showed that the cavity was natural and not sharply defined, without anything to indicate that an embryo had been in it. On the contrary, it was found that the magma reticulé was filled with a loose net-work of mesoderm cells, which bound one side of the chorion with the other, as indicated in the diagram which is from a reconstruction. These cells are directly continuous with those of the mesoderm and resemble them in every particular. At one point there is a small group of epithelial cells, which may represent what was originally the embryo.

Otherwise the chorion and its villi are normal in appearance, being encapsulated in decidua which has in it some

Fig. 278a.—Photograph of a section of the ovum with syncytium and decidua. × 15 times.

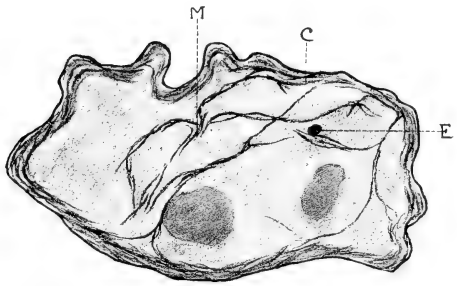


FIG. 278b.—Outline of the main wall of the chorion, *C*, showing the strands of mesoderm, *M*, that cross the cœlom, in which there is a small epithelial mass, *E*, possibly the remains of the embryo. × 18 times.

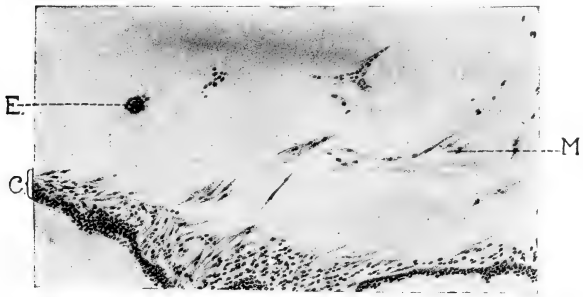


FIG. 278c.—High power drawing of the epithelial mass, strands of mesoderm and chorion. × 50 times.

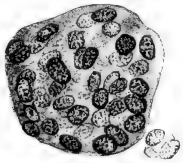


FIG. 278d.—The epithelial mass. × 500 times.

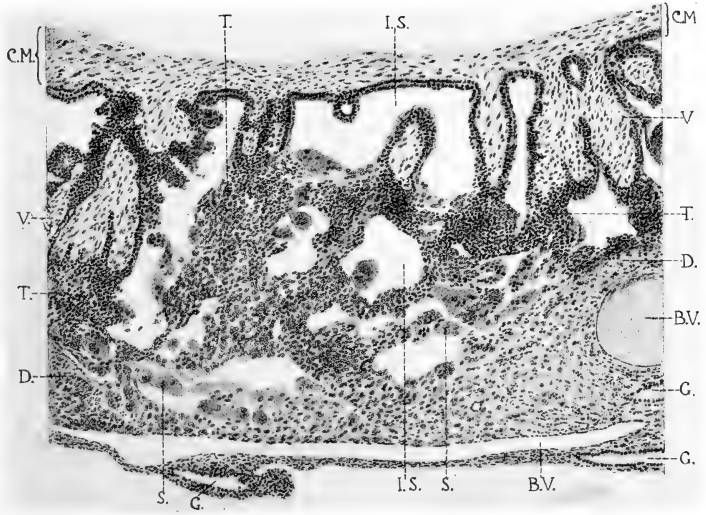


FIG. 278e.—Attachment of the chorion to the decidua. × 50 times. *CM*, chorion; *T*, trophoblast; *D*, decidua; *S*, syncytium; *BV*, blood-vessel; *G*, uterine gland; *I.S.*, intervillous space.

uterine glands. All in all, this specimen reminds one of Peters's ovum very much. There are some leucocytes in the decidua, but no accumulations of them, indicating inflammation of the uterus.

I consider this specimen one in which the embryo has been destroyed, leaving a normal chorion without an embryo.

No. 279.

Fleshy chorion, 100 x 60 x 60 mm. Into the cavity the umbilical cord, 30 x 5 mm., projects.

Dr. Kemp, Baltimore.

Part of the chorion is hemorrhagic; the rest appears normal. Sections show that the villi are nearly normal, with a deficient amount of syncytium over them, even where they are well imbedded in blood. Within there is an amnion, and the worm-like process which proves to be the umbilical cord, with its three blood-vessels. The vessels are well developed

FIG. 279.—Photograph of a section of the specimen showing the cavity and cord within. Slightly reduced.

and fully one millimeter in diameter; there are also numerous vessels in the villi of the chorion. The tissue of the chorion is hyaline, with a diminished number of nuclei in it.

Undoubtedly the fœtus escaped in some way shortly before the abortion, the membranes and cord remaining some time, long enough to undergo these changes. The blood-vessels of the cord and chorion are empty, but well developed.

No. 280.

Mole, 40 x 25 x 25 mm.

Dr. Magness, Baltimore.

Within the mole, which is said to be five or six weeks old,

FIG. 280.—Photograph of the mole. × 1½ times.

there is an irregular cavity with smooth walls, measuring 10 x 5 x 5 mm. Sections were cut of the thick hemorrhagic walls, which showed that the walls of the chorion are thin, with considerable reticular magma attached to them on the inside. No amnion was found. The villi are not very large, are well developed, contain remnants of blood-vessels and are covered with a mass of necrotic syncytium. The blood and mucus over the syncytium is filled with leucocytes, which invade the mesoderm of many of the villi. It is probable that the whole ovum has been dead for several weeks, the embryo and the amnion having been destroyed entirely.

No. 285.

Ovum, 45 x 35 x 35 mm.; embryo, 8 mm.

Dr. Keown, Baltimore.

"Last menstruation October 9 to 12; abortion December 20, 1904. The specimen came away unbroken, was washed in water and placed in alcohol. There is reason to believe that conception did not take place until the time for the period which lapsed. The mother insists that this is the case, and, inasmuch as all three of her children had diphtheria at that time it is probably true."

The chorion is mostly bare, with some hemorrhage in its walls. The villi which are left are very fibrous, with but few blood-vessels within them. The syncytium over them is very active, and at numerous points it is heaped up in small mounds, which form depressions, making it appear as if they

FIG. 285a.—Photograph of the embryo and chorion. Natural size.

are about to invade the mesoderm of the villi as well as that of the main wall of the chorion. The amnion fills the entire chorion.

Between the villi there is a reticular arrangement of blood and mucus, in which there are numerous leucocytes. The syncytial bodies enter this reticular mass at numerous points and make a very remarkable picture.

The embryo has an atrophic head and cord, showing, however, enough structures to fix its age at four weeks. The spinal cord is dilated and dissociated and the brain is solidified, filling the entire head. The eyes are destroyed. The blood-vessels are enormously distended with blood, which also fills the tissues of the body, obscuring them to a great extent. The epidermis is intact.

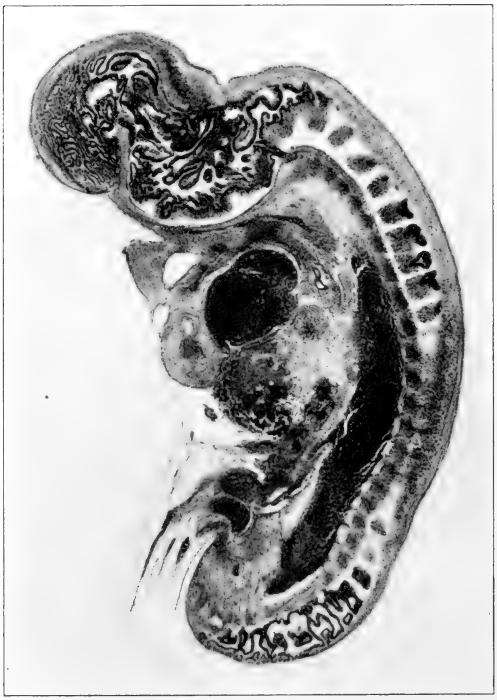


Fig. 285b.—Section of the embryo. × 13 times.

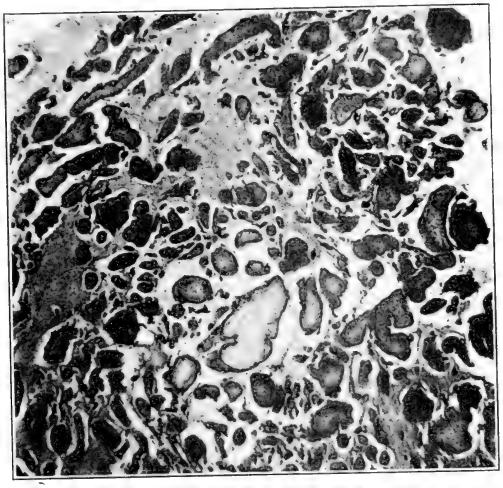


FIG. 285c.—Photograph of villi with mucus between them.

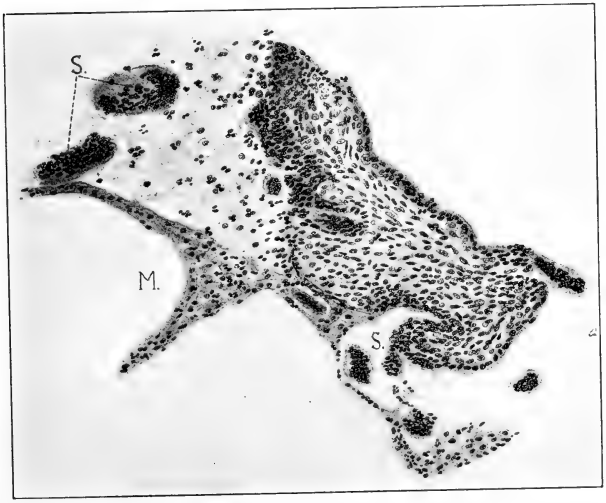


FIG. 285d.—Section of a fibrous villus which is invaded by leucocytes and adjacent syncytium, *S*, and mucus, *M*. × 250 times.

No. 286.

Chorion, 100 x 50 x 40 mm.

Dr. Girdwood, Baltimore.

This remarkable specimen must have been dead in the uterus for about five months, the last period having taken

Fig. 286a.—Photograph of the entire specimen. Natural size.

place during the latter part of May and the abortion on the 4th of the following January.

The chorion thickens as it passes into the large fleshy placenta on one side and is very thin on the other. The thin

twisted cord enters the chorion at the border of the placenta. The embryo is well imbedded in granular magma.

Sections from the placenta at the point the cord enters it show a most remarkable reaction. The amnion is folded upon itself and has undergone hyaline degeneration. The chorion is also hyaline and is infiltrated with leucocytes and syncytium. The villi are fibrous, with numerous spots of hyaline matter scattered through them. With them the lining cells of the large blood-vessels show remarkable growth, forming small

FIG. 286b.—Photograph of the embryo. Natural size.

pearls of endothelial cells. They are also invaded by syncytial cells at some points and at others by masses of leucocytes.

Between the villi there is a great mass of necrotic syncytium mixed more or less with fresh blood. Throughout this general mass numerous small islands of active syncytium may be seen; there are also a great number of scattered leucocytes

Sections of the cord, abdominal viscera and hand show that the embryo must have died quite suddenly, for there are no tissue reactions seen in them. However, the tissues do not stain well, the epidermis has fallen off and the large blood-vessels are filled with blood containing the proper number of leucocytes.

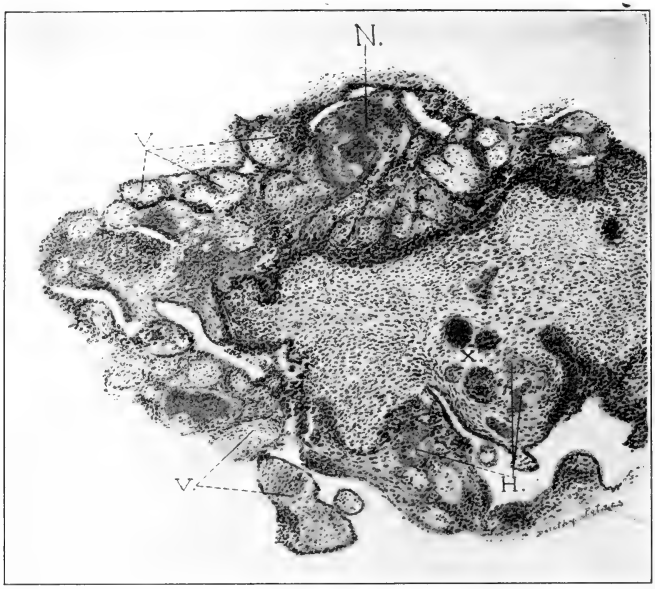


Fig. 286c.—Section of a villus. × 62 times. *V*, villus; *N*, necrotic villi and syncytium; *H*, hyaline degeneration of mesoderm and syncytium; *X*, peculiar masses of cells in the mesoderm, probably degenerated blood-vessels.

No. 288a.

Ovum, 85 x 35 x 35 mm.; embryo, C. R., 11 mm.

Dr. Brülle, Baltimore.

On one end of the chorion there is a space (30 x 30 x 5 mm.) filled with reticular magma. Within this, and pushed to one side, a collapsed amnion may be seen, containing the embryo.

The entire mole is surrounded by decidua and pus, in which there is the collapsed ovum. The intervening space is filled with blood through which ramify a few long slender villi. These are fibrous and devoid of blood-vessels. At points they are invaded by syncytium and leucocytes.

The amnion, which is also fibrous, is partly filled with magma reticulé and is very rich in degenerated migrating cells

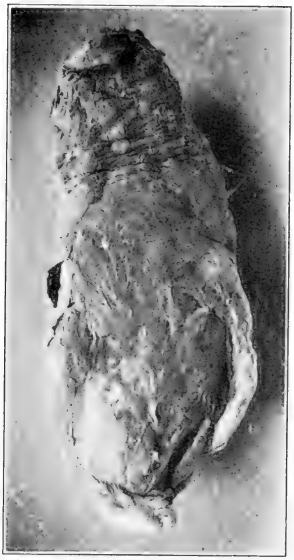


FIG. 288a a.—Photograph of the specimen. Natural size.

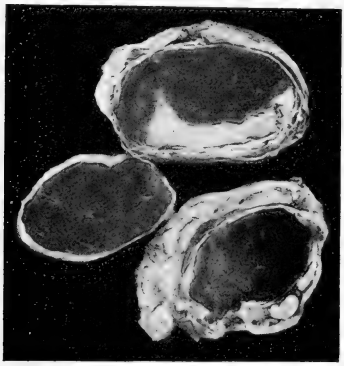


FIG. 288a b.—Photograph of sections of the mole, showing the embryo pushed to one side. Natural size.

from the embryo. The embryo is pushed to one side of the chorion and is pretty well dissociated, but the tissues are sharply enough defined to recognize that the embryo is not over six weeks old. They are well infiltrated with round cells which extend into the surrounding magma; there is no epidermis present.

No. 289.

Embryo of the fourth week, 8 mm. long.

Dr. Brülle, Baltimore.

The specimen is distorted and macerated and it is impossible to determine definitely whether or not it is normal.

No. 290.

Mole, 50 x 15 x 10 mm.

Dr. Warren, Portland, Me.

The specimen is said to be from a six weeks' gestation, and the abortion is believed to have been induced by some emmenagogue. Sections were cut from different portions of this

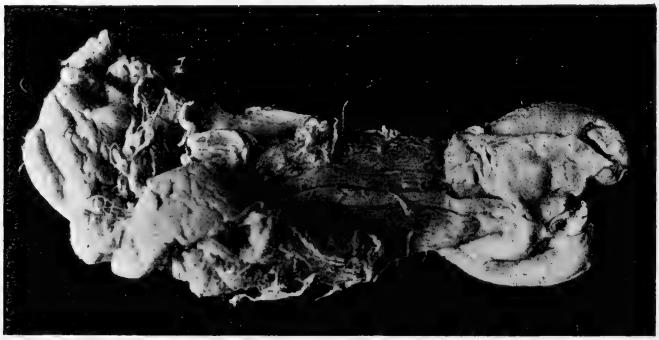


Fig. 290.—Photograph of the mole. × 2 times.

irregular mass and the remnants of a few villi were found, which were more or less infiltrated with leucocytes. The bulk of the mole is composed of decidua, mucous membrane of the uterus, blood, fibrin and pus.

No. 291.

Embryo, 5 mm.

Dr. Wegefarth, Baltimore. Brödel Collection.

The membranes are devoid of villi and very thin. The umbilical vesicle is necrotic and filled with an irregular mass.

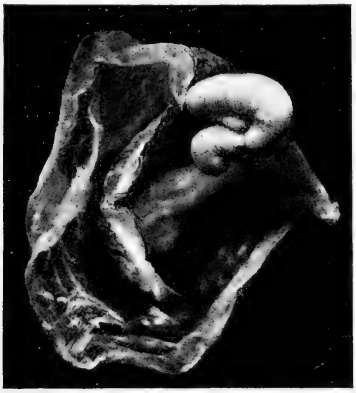


FIG. 291.—Embryo attached to the chorion. × 4 times.

Sagittal sections of the embryo show that the specimen is pathological, its head being rounded and the epidermis having fallen off. The spinal cord is distended and the brain is solid. Veins and arteries are greatly distended with blood. Eye vesicles are atrophic, and the lenses are dissociated, but encircled by a sharply defined capsule.

No. 292a.

Ovum, 50 x 30 x 30 mm.; embryo, 3½ mm.

Dr. West, Bellaire, Ohio.

"The ovum is from a woman thirty-one years old, who has been married for ten years, but never had been pregnant before. Last period November 10, and on December 24, after a hard day's work, she had a sudden gush of blood, and since then has been wasting at times. The ova was expelled February 4."

The chorion is partly covered with long villi, which are fibrous in some places and œdematous in others. The amnion within, which fills the entire ovum, is partly filled with granular magma, through which can be seen the outlines of an atrophic embryo. Sections of it show that the brain and

FIG. 292a a.—Photograph of ovum. Natural size.

FIG. 292a b.—Photograph of the embryo lying within the magma. × 7 times.

most of the spinal cord have been destroyed; at one point the cord ramifies through the embryo. In the middle of the embryo the aortae and cœlom are sharply defined, but elsewhere the tissues are entirely obscured by numerous round cells. The epidermis is intact.

No. 293.

Embryo, C. R., 19 mm.

Dr. Lamb, Washington.

Dr. Lamb writes: "Yesterday I sent you an embryo aborted at the third or fourth month of pregnancy. I trust that it may be of interest to you. It is from Dr. Munson, of this city.

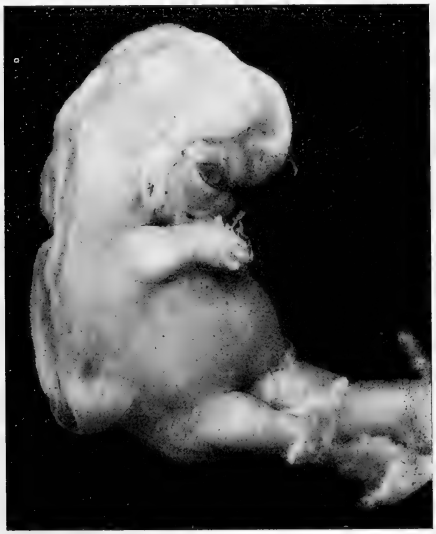


FIG. 293a.—A photograph of the embryo. × 4 times.

"I send it more particularly, however, to get some information. I myself cut it out of the ovum, so that I know that its

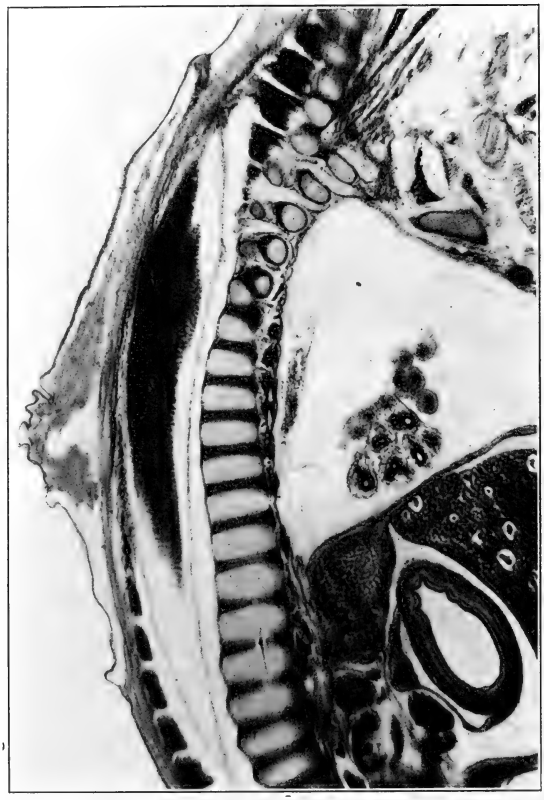


Fig. 293b.—Section through the swelling in the back of the embryo, showing the blister of the epidermis. × 12 times.

condition was not caused by any rough handling. The sac contained some fluid in which were many flocculi, which no doubt are the absent portions of the embryo. Along its spine the embryo is whitish, but for the remainder was dark. Now I do not understand that micro-organisms played any rôle in this case to bring about the condition of the embryo, but it is only a maceration produced by the surrounding fluid medium. Still, I feel some doubt about my being correct, because I have seen so many cases in which there was no evidence of such maceration. In fact, the condition of this embryo is rather exceptional in my observation. Apparently the soft visceral parts have first given way to whatever cause it was. Perhaps to you this is a trivial matter, but I would like to know what you think of it."

Sections of the embryo show that all of the tissues are normal in form and in structure, with the exception of a great excess of round cells within them. Especially is this true on the top of the head and along the back of the embryo. The œdematous mass on the back is as a blister with the epidermis lifted off. It is filled with a granular mass, within which there are but few cells. All of the blood-vessels of the embryo are distended with blood; there is also a great quantity of blood within the pericardial cavity and some within the ventricles of the brain. Possibly this condition accounts for the excess of round cells in all of the tissues, but it cannot very well account for the condition on top of the head and along the back. Here there is a most decided infiltration of cells.

No. 295.

Fœtus with pointed head.

Dr. Miller, Hagerstown, Md. Brödel Collection.

The vessels going to the vertex are much enlarged. The scalp of the protruding vertex is very hemorrhagic, the blood filling the subcutaneous tissue as well as that of the skin. The embryonic hair follicles appear to be normal, but the epidermis is also infiltrated with blood cells, and is crumbling off in flakes.

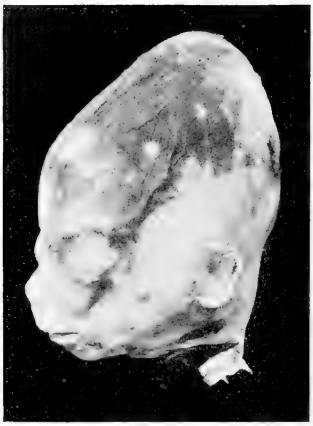


FIG. 295a.—A photograph of the head. Natural size.

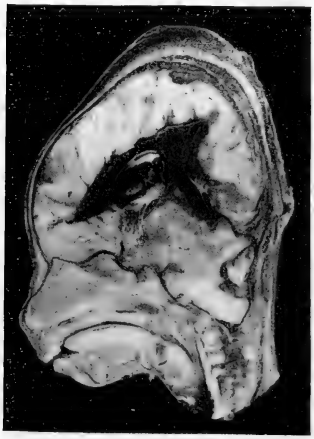


FIG. 295b.—Photograph of a section of the head. Natural size.

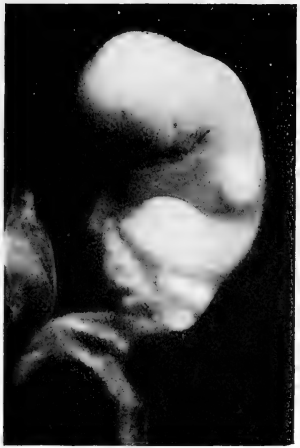


FIG. 297a.—Photograph of the embryo. × 8 times.

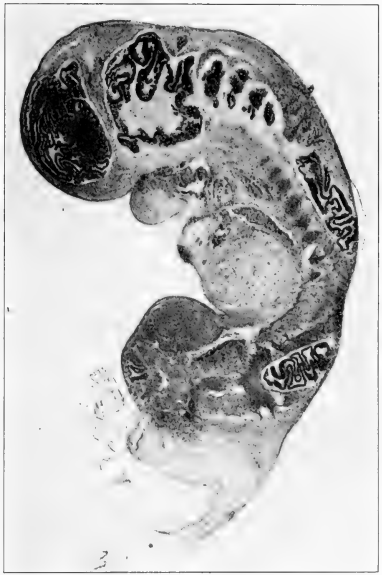


FIG. 297b.—Section of the embryo. × 15 times.

No. 297.

Embryo, 6 mm. long.

Dr. Lamb, Washington.

This specimen was removed from the uterus with a curette and is said to be nearly three months old. The distorted embryo is of the three-weeks' stage and shows extreme changes in its organs and tissues. The chorion is thin and atrophic. There is no trace of an umbilical cord, but instead the embryo sits upon the amnion. The spinal cord is dilated and the brain is fully dissociated, filling up the stumpy head entirely. The blood-vessels are much dilated with blood and all of the tissues are infiltrated with round cells which deform the organs and obscure their outlines. The mandible is necrotic and the distended medulla reaches almost to the mouth.

No. 298.

Tubal pregnancy.

Dr. Pearce, Albany, N. Y.

"I am sending you by this mail a Fallopian tube removed at an operation on March 13. The tube shows rupture over an hemorrhagic swelling. The clinical diagnosis is rupture of ectopic pregnancy. It is from a young woman, aged twenty-six, married, who states that the last menstruation was three weeks before the operation. The surgeon is positive that it is a case of ectopic pregnancy. I am not so sure of the diagnosis, but with the history given I thought it worth while to send it to you, without close examination, etc."

I found two nodules, each about 10 x 6 mm., one hemorrhagic and the other with hemorrhagic walls with villus-like bodies upon it. This second body has a lumen—the cœlom (?). Neither of them contained any trace of an ovum. Then the ends of the rupture were cut into serial sections, and in one of them the remnants of the ovum were found. It is about 4 mm. in diameter, composed of small fibrous villi surrounded by an irregular syncytium, decidua and blood. Some of the villi are invaded by leucocytes.

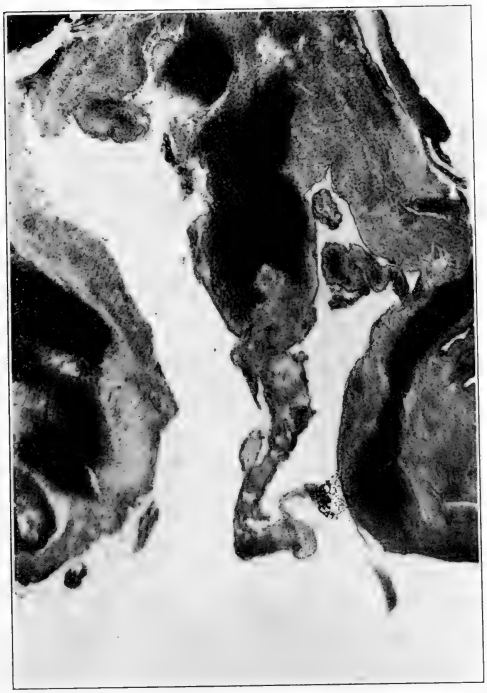


FIG. 298.—Section of the tube containing remnants of the chorion and villi.

No. 299.

Ovum, 16 x 12 x 10 mm.

Dr. Burns, Memphis, Tenn.

The specimen, apparently normal, is filled with a mass of dense magma reticulé. Serial sections failed to show even a remnant of an embryo. The structure of the chorion and villi is normal, possibly a little œdematous. No blood-vessels are present.

No. 302.

Ovum, 25 x 20 x 15 mm.; embryo, 4 mm.

Professor Brödel.

The ovum is apparently normal, being covered with irregular villi. Sections show, however, that the villi are fibrous, with remnants of blood-vessels within them. The syncytium is very active and is imbedded in a reticular mass of mucus rich in leucocytes and pus.

Fig. 302.—Section of the embryo. × 16 times.

Within the chorion there is a vesicle (amniotic) one centimeter in diameter imbedded in much magma reticulé. This in turn is filled with granular magma, in which there is an embryo about 3½ weeks old. The umbilical vesicle is degenerated and lies in the reticular magma.

The blood-vessels and tissues of the embryo are gorged with blood and the outlines of the organs are obliterated. The brain is solid and the spinal cord is distended and dissociated. The eye vesicle and lens are nearly destroyed. The umbilical cord is very short and wide, without marked blood-vessels, but it is infiltrated with round cells.

No. 304.

Ovum, 15 x 7 x 6 mm.

Dr. Hunner. Brödel Collection.

The specimen is surrounded by some of the decidua and much mucus, which is well infiltrated with leucocytes. The

FIG. 3042.—Photograph of half the ovum containing the embryo. × 4 times.

villi and chorion are apparently normal, with remnants of blood-vessels within them, and they are covered with an active syncytium. The decidua is encircled with pus and fragments

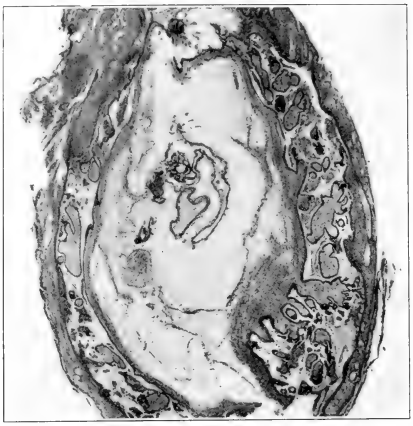


FIG. 304b.—Section of the whole ovum encircled by the decidua. × 10 times.

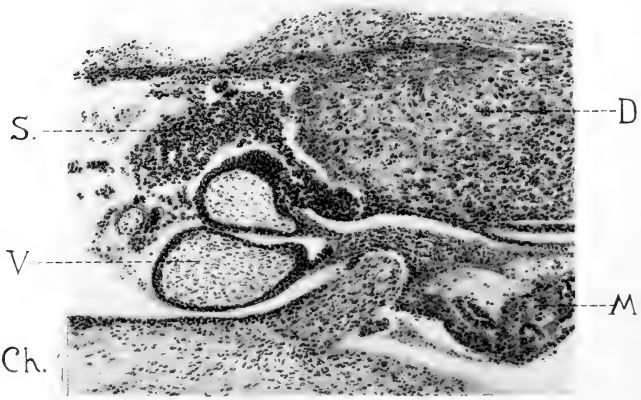


FIG. 304c.—Section of the villi and surrounding tissue. × 65 times. *D*, decidua; *S*, syncytium; *V*, villus; *Ch*, chorion; *M*, mucoid substance rich in leucocytes.

of uterine mucous membrane, showing that an extensive inflammatory deposit cuts off the normal nutrition of the ovum.

The ovum is partly filled with magma reticulé, in which there is imbedded an umbilical vesicle two millimeters in diameter attached to the remnants of an embryo, without myotomes. The neural canal is present and the body runs out into a stem, containing a tube (allantois), which does not attach itself to the chorion. There are also remnants of an amnion present. All in all, the embryo appears to be much like Graf Spee's specimen, which is 1.54 mm. long. There is no trace of a heart, but there are numerous blood islands in the umbilical vesicle and there are remnants of blood-vessels in the chorion, showing that the two were connected at an earlier date.

No. 307.

Ovum, 40 mm. in diameter; embryo, 20 mm. long.

Dr. Coe, New York.

The chorion and villi are imbedded in an hemorrhagic mass, and the latter do not appear normal; they are often surrounded by small clumps of leucocytes, which invade the mesoderm of the villi. The embryo was said to have been a beautiful normal one, but it had been harshly treated and practically ruined before it came to me. Sections of the embryo show that the tissues are macerated and distorted and probably normal.

No. 308.

Fœtus, C. R., 84 mm.

Dr. Ballard, Baltimore.

"Without any previous bleeding, on February 28, 1905, the fœtus as you have it was passed suddenly, accompanied by the usual amount of hemorrhage. Probably one-half of the placenta was retained, and was removed by curettement. Patient is regular in menstruation, and previous to miscarriage menstruated November 19, 1904. She has one boy who will be thirteen months old May 10, 1905, and another

FIG. 308.—Photograph of the fœtus, showing the cord wrapped around its arms, with a mass of granular magma in the amniotic cavity. Natural size.

son sixteen months older; no other children nor miscarriages."

[The woman aborted again on November 27, 1905 (specimen No. 325), and the ovum proved to be decidedly pathological. On December 31, 1906, after being pregnant for five months, she was taken with penumonia and aborted on January 4. The placenta was strongly adherent and was removed with difficulty. She died January 7, 1907. Apparently this fœtus was normal, but it was not sent to the laboratory.]

After the abortion Dr. Ballard found that the woman had an interstitial fibroid, somewhat diffuse in shape, in the anterior uterine wall. During some years she had some otorrhœa. There is no reason to suspect that her husband has ever had gonorrhœa or syphilis.

The specimen appears to be normal, but when I opened the amnion, which had not been torn, I found it filled with a mass of granular magma, some of which is shown in the illustration. The cord is well tied around the arms, indicating that the fœtus had been doing some lively jumping. Sections of the placenta, at the point the cord enters it, show that the villi are fibrous (?) and covered with an active syncytium, which is imbedded in bloody mucus containing large numbers of clumps of leucocytes with fragmented nuclei. The tissues of the cord appear to be normal; the blood-vessels contain but few blood cells.

No. 309.

Dr. Steensland, Syracuse, N. Y.

Ovum, 23 x 20 x 20 mm.; embryo, 4 mm.

The specimen, apparently normal, had been in alcohol for three or four years, but has been well preserved. The amnion filled the entire chorion, otherwise the interior also appeared normal. Section showed, however, that the dilated amnion was accompanied with marked changes in the embryo. All of the tissues of the embryo are infiltrated with round cells, obliterating, to a great extent, the organs and tissues. The

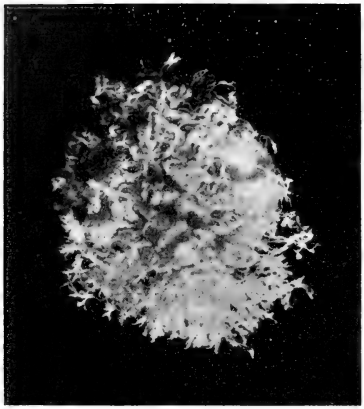


FIG. 309a.—Photograph of the ovum. × 2 times.

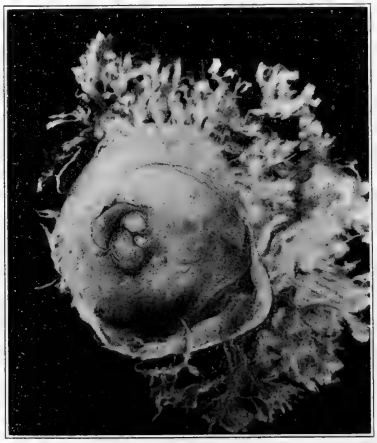


FIG. 309b.—Interior of the chorion, showing the embryo. × 4 times.

central nervous system is markedly dilated and filled with round cells. In front the walls are broken and the round cells are extended into the tissues of the front of the head. The eye and ear vesicles are also dilated and filled with round cells. No trace of a lens is seen, and the ear vesicle has two sprouts on its ventral side. The whole epidermis is intact.

No. 310.

Ovum, 18 x 14 x 14 mm.

Dr. Watson, Baltimore.

The specimen is covered with villi which in sections proved to be markedly changed. The mesoderm is hyaline, with vacuoles in which there are free nuclei. The epithelial layer is irregular and invades the wall of the chorion as well as

FIG. 310a.—Exterior of the ovum. × 2 times.

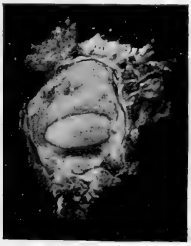


FIG. 310b.—Interior of a piece of the ovum, showing a large lump of magma. × 2 times.

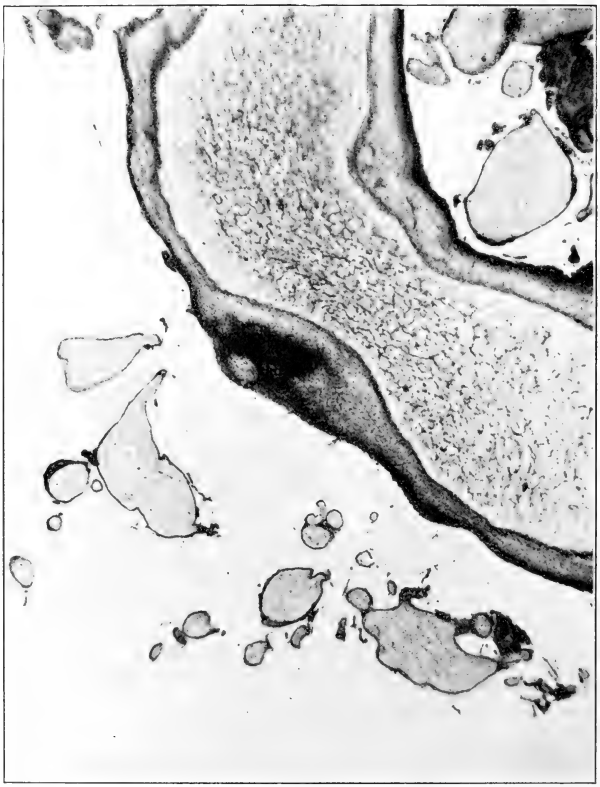


FIG. 310c.—Section of the ovum, showing a large mass of syncytium invading the main wall of the chorion.

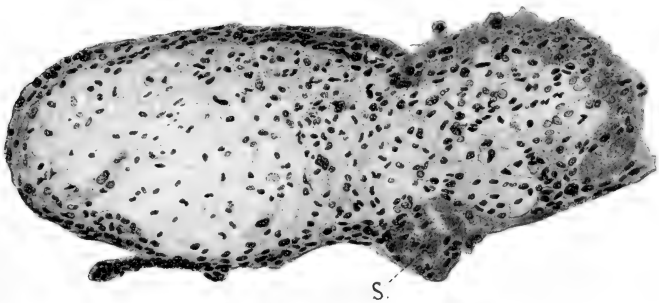


Fig. 310d.—Section of a villus. × 250 times. *S*, syncytium.

some of the villi. The villi are vacuolated, contain some blood-vessels, and are covered with a fairly active syncytium. Over this there is a mass of mucoid fibrin rich in leucocytes. The interior of the ovum is filled with magma reticulé, and contains no trace of an embryo nor amnion.

No. 311.

Ovum, 36 x 30 x 30 mm.; embryo, C. R., 12.5 mm.

Dr. Watson, Baltimore.

The walls of the chorion are thin and covered with a few scattered and irregular villi. Sections show them to be in all stages of degeneration, the large ones with blood-vessels and a rich syncytium, and the small ones, which are fibrous, devoid of syncytium and infiltrated with leucocytes. The spaces between the villi have a considerable amount of blood between them, and where this comes in contact with an active syncytium the nuclei of the leucocytes are fragmented; elsewhere they are not. Portions of the main wall of the chorion are very thin, fibrous and devoid of an epithelial covering. Throughout the amnion is in contact with the chorion and is often blended with it.

Within the amniotic cavity there is a mass of granular magma which could be seen through the thin walls of the chorion before it was opened.

FIG. 311a.—Ovum covered with ragged villi. × 1½ times.

FIG. 311b.—Interior of ovum with embryo imbedded in granular magma.

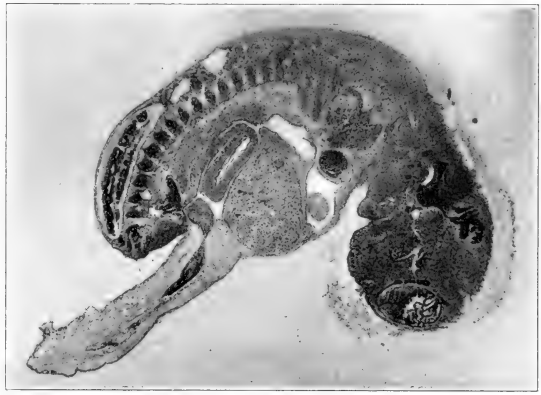


FIG. 311c.—Section of the embryo lateral to the middle line. × 6 times.

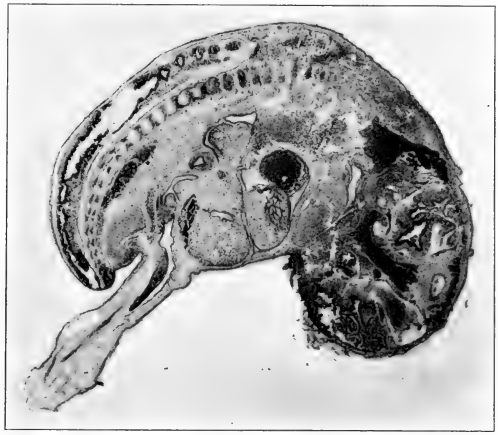


FIG. 311d.—Sagittal section through the middle line. × 6 times.

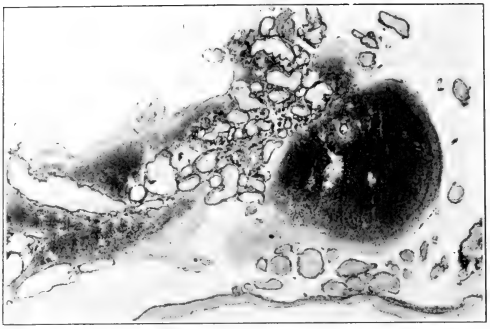


Fig. 311e.—Section of the chorion, showing blood clots between the villi. × 7½ times.

The umbilical cord is enlarged in its middle and is very thin at its attachment to the chorion, which is also atrophic at that point. Sections show that the center of the cord is fibrous and that the enlargement is due to the extreme mucoid degeneration of sides. Near its attachment to the body the cord is infiltrated with round cells and the intestine within the cœlom of the cord is irregular and gorged with them; the lumen of the intestine is destroyed entirely.

The embryo is imbedded in the granular magma, and is normal in form. Within, however, most radical changes have taken place. The blood-vessels and heart are distended enormously with blood, and the tissues are gorged with round

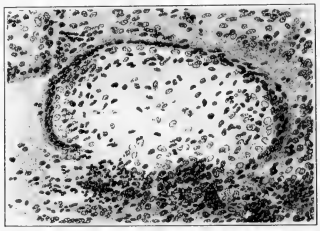


Fig. 311f.—Section of a villus which is invaded and partly destroyed by leucocytes.

cells. Liver, heart wall, intestine and mesenchyme are being destroyed. The precartilage is more sharply defined than in the normal embryo. The spinal cord is dilated, the brain and eye nearly solid and the ear vesicle is destroyed. The ganglia and nerves are disintegrating. The epidermis is partly wanting, and in the head region the skin is studded with numerous papillomata. The face is adherent to the thorax.

No. 312.

Ovum, 25 x 15 x 10 mm.; embryo, straightened and 8 mm. long.

Dr. Stanton, Albany, N. Y.

"Abortion followed a blow upon the abdomen." One side of the ovum is very hemorrhagic and the other side thin. The villi are few in number, fibrous, without a syncytial cov-

FIG. 312.—Solid ovum with embryo hanging from it. Enlarged nearly 2 diameters.

ering and possibly invaded by leucocytes. The main wall of the chorion appears to be necrotic.

The embryo is straight, showing three gill arches and some myotomes. Its tissues do not stain well, but the spinal cord can still be outlined. The tissues appear to be infiltrated with round cells.

No. 316.

Embryo, C. R., 44 mm.

Dr. Simms, Baltimore.

There are peculiar patches upon the skin, the cord is atrophic, feet and hands club-shaped and one hand is ad-

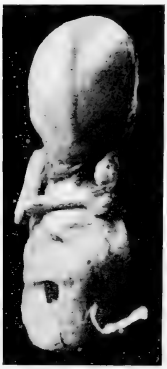


FIG. 316a.—Front view of the embryo. Slightly enlarged.

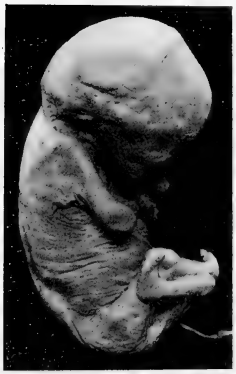


FIG. 316b.

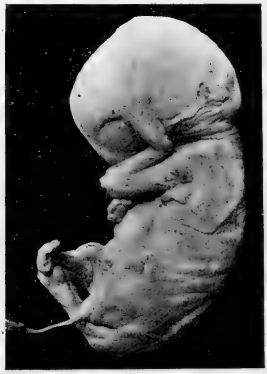


FIG. 316c.

FIG. 316b.—Right side. Notice smooth face with eye obliterated and fold of skin under the axilla.

FIG. 316c.—Left side. The eye is nearly closed and the hand has become adherent to the side of the head.

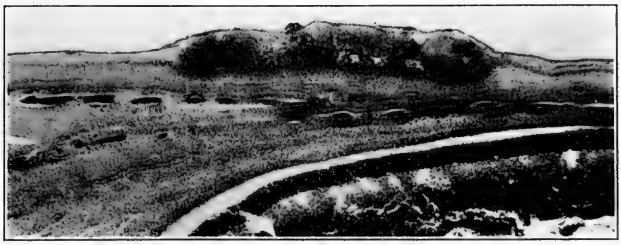


FIG. 316d.—Section of one of the elevations of the skin of the head shown in Fig. 316c.

herent to the side of the head. Sections of the cord show that it is fibrous and infiltrated with round cells along the course of the blood-vessels.

The skin is thickened and much of the epidermis has fallen off. At points the epithelial cells form mounds without any horny changes in them. The muscles, blood-vessels and nerves of the extremities are converted into one fibrous mass composed of spindle-shaped cells, giving much the appearance of myomatous tissue, infiltrated at points with round cells.

The cartilages are still hyaline, richer, however, in cells than is normal. The bone formation is very extensive, which at the border line between it and the cartilage shows peculiar changes in the latter. There is a mass of this changed cartilage in the os calcis without any surrounding bone formation. In general the cartilages are deformed, due no doubt in part to the distorted joints. Where the hand is adherent to the side of the head the epidermis of the two is continuous and blended. The skin and subcutaneous tissue are thickened, being composed of one mass of round cells.

The form of the brain and its structure are pretty well preserved, while the tissues of the liver and intestine are necrotic and macerated. It appears as if the growth of the embryo had been retarded with a continued growth and change in the connective tissues. Then, after its death, the embryo was retained in the uterus for some time. At points all over the body there are thickened spots in the skin which are epithelial in nature, but they are located below the epidermis.

No. 320.

Ovum, 70 x 50 x 40 mm.; embryo, 18 mm.

Dr. Gibbs, Baltimore.

The chorion is fleshy and thick, with irregular spots of villi covering its surface. Some of the villi are fibrous and others are swollen; all are deficient in syncytium. The decidua is not typical, being well filled with fibrin, with occasional masses of leucocytes. Within, the entire chorion is lined by the amnion, which contains no magma. The umbilical cord is

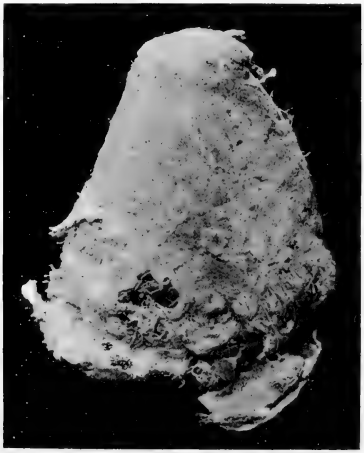


Fig. 320a.—Whole ovum. Natural size.

thin at its attachment to the chorion, but in its middle it is swollen, which, upon microscopic examination, proved to be a vesicle filled with a hyaline stringy mass tinged with carmine. Otherwise the cord is fibrous, and in its center are seen the remnants of its blood-vessels. They are practically obliterated.

The tissues of the embryo are pretty well dissociated, the cord and brain being nearly solid, with occasional irregular spaces representing the central canal. The outlines of the

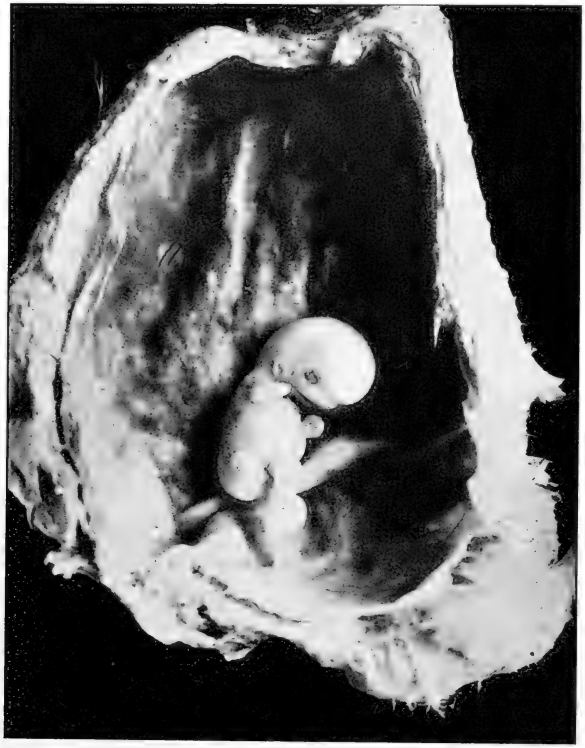


FIG. 320b.—Embryo within the ovum. × 2 times.

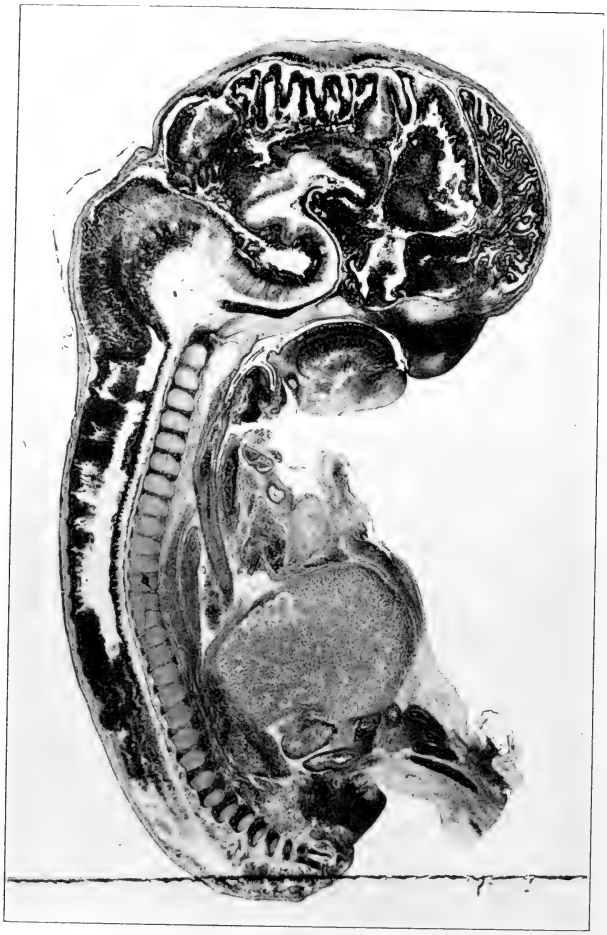


Fig. 320c.—Sagittal section of the embryo. × 8 times.

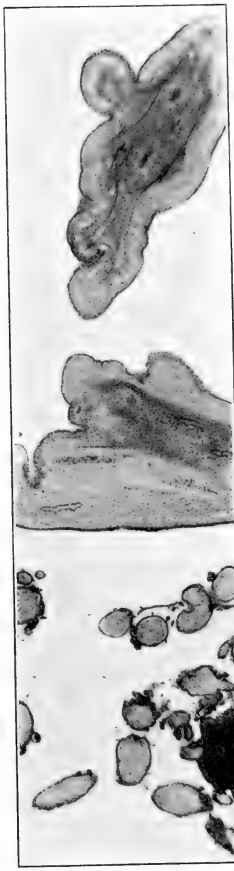


Fig. 320d.—Section of the chorion and part of the umbilical cord. × 10 times.

alimentary canal are obscure and its epithelial lining is nearly lost. The blood-vessels are distended with blood in an irregular fashion. The liver is necrotic and free from blood. The tissues of the body are all dissociated, which condition obscures the muscles and nerves and sharpens the outlines of the cartilages. The epidermis is intact.

No. 321.

Ovum, 40 x 40 x 20 mm.; embryo, 2 mm.

Dr. Wentz, Hanover, Penna.

The ovum is covered entirely with villi and contains some reticular and much granular magma. The whole chorion is lined by the amnion and the embryo is attached to it at its middle. Traces of the central nervous system can still be seen, and in front of it there is a structure which may represent the heart encircled by a large space, the cœlom, this ex-

FIG. 321a.—Photograph of the ovum. × 2 times.

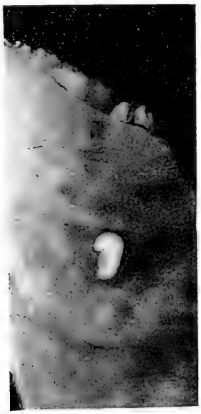


FIG. 321b.—Embryo attached to the chorion. × 4 times.

tending to the umbilical cord. The tail end of the embryo is nearly solid. A large share of the dissociation may be due to the dilute alchohol (50 per. cent) in which the embryo had been placed ten days before I got it. This, however, could not alter the general shape of the embryo and its attachment to the chorion.

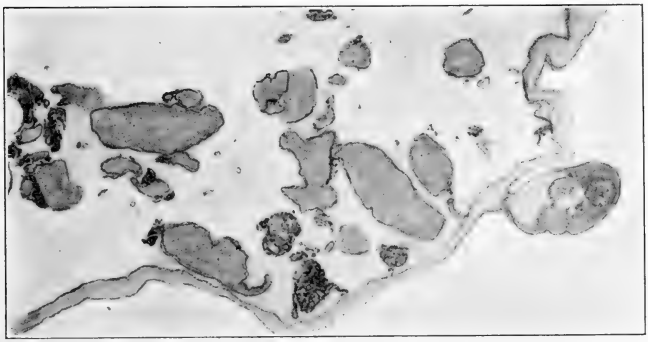


FIG. 321c.—Section of the embryo, main wall of the chorion and villi. × 9 times.

No. 323.

Pear-shaped hydatidiform mole. 120 x 90 x 65 mm.

Dr. Van Williams, Baltimore.

The fresh specimen was brought to the laboratory and was found to be composed of enlarged villi, most of which measure about 5 mm. and a few fully 20 mm. in diameter. On one end the specimen is fibrous, from which the villi extended into a bloody mass.

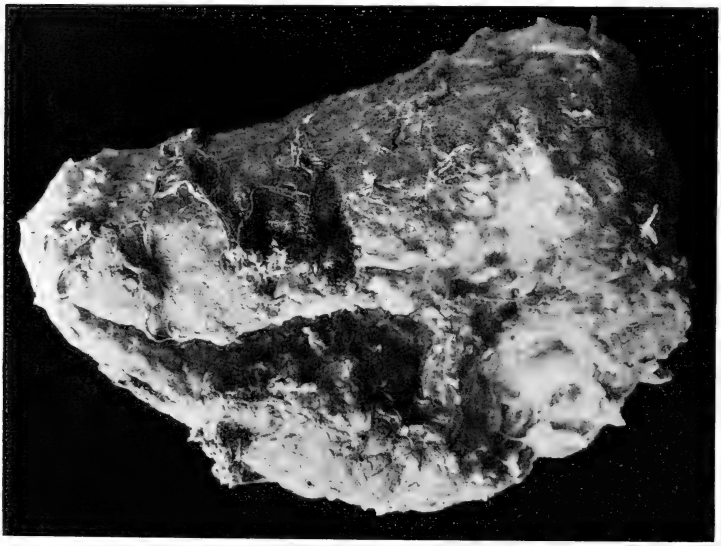


FIG. 323.—Photograph of the mole. Natural size.

The villi are very irregular in form, the mesoderm being hyaline, in which there are numerous spindle-shaped nuclei. Some of the "large villi" have in them a lumen which has all of the characteristics of the cœlom; in fact, it appears as if the main wall of the chorion ramified in all directions with the growth of the villi. One of these openings is 15 x 10 mm. and another just beside it is 7 x 2 mm. in diameter.

Between the villi there are great masses of necrotic syncytial cells. There is more or less blood between the villi and occasionally small masses of leucocytes may be seen. A few of the villi are being invaded by their epithelial coverings.

No. 324.

Ovum, hemorrhagic and fleshy, 45 x 45 x 22 mm.; embryo, rounded and 3½ mm. long.

Professor Brödel, Baltimore.

The walls of the chorion are thin and fibrous and are lined by the amnion. The villi are few in number, fibrous, devoid of syncytium and imbedded in a large quantity of blood. Unfortunately the embryo was lost while being imbedded, but the excellent drawing of it tells pretty well that its tissues and organs are markedly changed and deformed.

No. 325.

Ovum, 55 x 55 x 35 mm.; embryo, C. R., 13 mm.

Dr. Ballard, Baltimore.

"The specimen was obtained from the same woman that gave No. 308. Last menstrual period, September 15; abortion, November 27, 1905. Periods regular monthly." The specimen was clean, well covered with villi and well hardened in formalin. The amnion and cœlom are filled with magma reticulé, in which is embedded the trunk of an embryo attached to the chorion by a thin cord. On the opposite side of the ovum the head is located, also imbedded in magma. Over the body of the embryo there is a greenish-colored nodule 4 mm. in diameter, which proved to be the degenerated umbilical vesicle. The legs are poorly formed and stubby.

Sections of the chorion show that the mseoderm of the villi is hyaline, in which remnants of blood-vessels may be seen, with a normal number of round nuclei scattered through it. The syncytium also appears to be normal. Between the villi some mucus may be seen, in which there are leucocytes. No decidua is attached to the villi.

The cord is thin at its attachment to the chorion, and it is slightly enlarged midway between the chorion and the em-

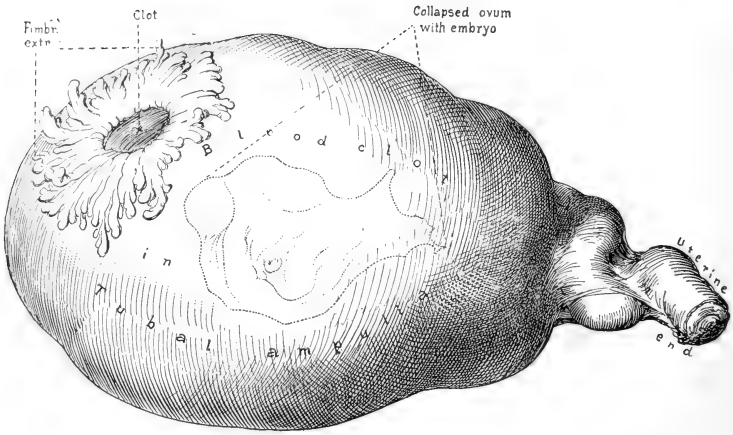


FIG. 324a.—Ovum within the distended uterine tube. Natural size. After Kelly.

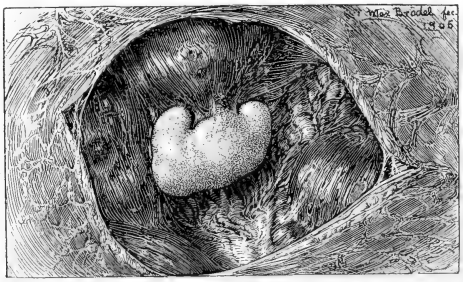


FIG. 324b.—The embryo attached to the chorion. × 7 times. After Kelly.

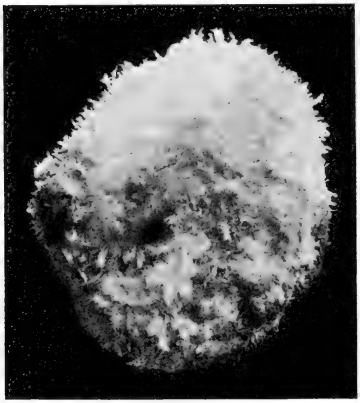


FIG. 325a.—Photograph of the whole ovum. Natural size.

bryo. Here it contains large mesodermal spaces, which at points are infiltrated with round cells. The umbilical vesicle is present only in outline. Its lumen is partly filled with debris. However, some beautiful multipolar mesoderm cells may be seen.

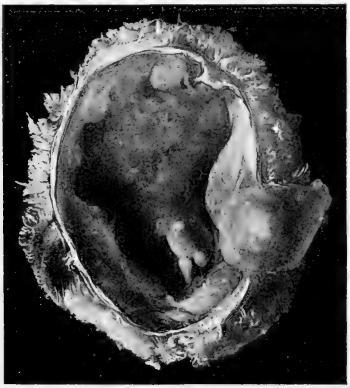


FIG. 325b.—Body of the embryo within the ovum. Natural size. There is a large amount of magma within the cœlom, and the lump of it overhanging the cut edge of the chorion contains the umbilical vesicle.

The epidermis covers the embryo only in part; a shell of granular magma covers the rest of the body. The tissues of the body are greatly dissociated and macerated, which has caused almost complete obliteration of the outlines of the epithelial lining of the alimentary canal. The central nervous system is nearly solid and the large blood-vessels are gorged with blood. The liver is necrotic. The mesodermal tissues are obscured, with the exception of the cartilages, whose outlines are sharpened.

No. 328.

Embryo, 4½ mm. long.

Dr. Pohlman, Bloomington, Ind.

The chorion extends into irregular fibrous villi, which are covered with a necrotic decidua infiltrated more or less with leucocytes. The main wall of the chorion is about normal in structure and contains numerous blood-vessels. Within the amnion nearly reaches the chorion; the degenerated umbilical cord is attached to the amnion, but not to the chorion. The umbilical vesicle is well imbedded in magma, is very rich in blood-vessels and on its outside has many papilliform

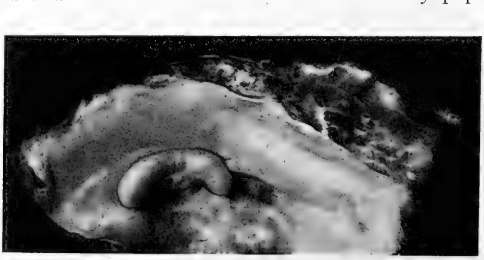


Fig. 328a.—Embryo attached to the chorion. × 4 times.

processes, some of which seem to blend with the chorion. In fact, it appears as if the blood-vessels of the umbilical vesicle passed directly over into those of the chorion.

The embryo is somewhat deformed, and it is difficult to follow the outlines of some of its viscera. The central

nervous system is dilated and is converted into a mass of round cells lying in the mesoderm without any epithelial lining; the otic and optic vesicles are likewise filled with round cells. The larger vessels are filled with blood, and the tissues are fairly well infiltrated with round cells. The epidermis is intact. Dissociation of the tissues has taken place to such a degree that it is difficult to outline all of the organs with certainty.

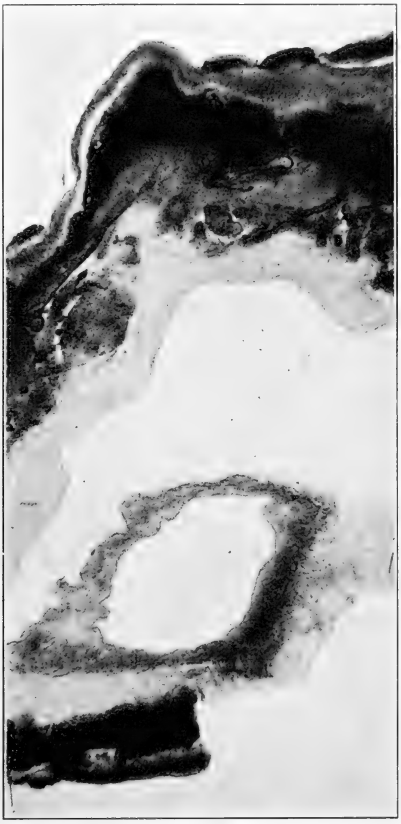


FIG. 328b.—Section of the chorion and adjacent umbilical vesicle. The chorion is hemorrhagic. The walls of the umbilical vesicle are rich in blood-vessels, which communicate directly with those of the chorion.

Fig. 330Aa.

Fig. 330Ab.

Fig. 330Aa.—Photograph of ovum. Natural size.
Fig. 330Ab.—The embryo. Nearly two diameters.

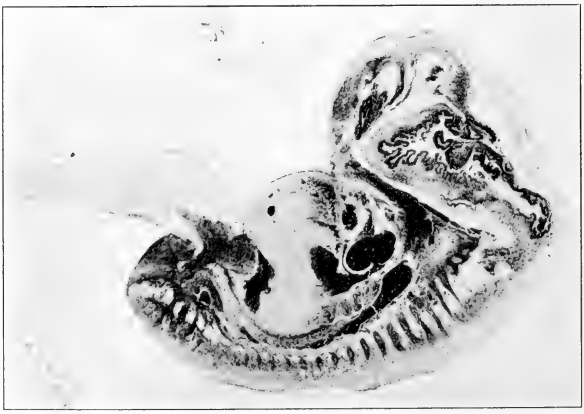


Fig. 330Ac.—Section of the embryo. × 6 times.

No. 330.

330A. Ovum, 60 x 55 x 50 mm.; embryo, C. R., 12 mm.
330B. Ovum, 55 x 50 x 45 mm.; embryo, C. R., 12 mm.
Dr. West, Bellaire, Ohio.

"The woman from whom these twin specimens were obtained is about 25 years of age. Fifteen months ago she gave birth to an eight-months child, which lived for two days. Her last regular menstrual period took place during the middle of September. The October and November periods were missed. About the middle of December, at her regular time, bleeding began, which continued until January 21, when these two ova were aborted. I am quite positive, but not certain, that woman has syphilis."

Both ova have smooth surfaces, being composed of thin walls, upon which there are occasional villi. In both specimens the villi are imbedded in a mass of pus, in which may be found irregular villi, much necrotic syncytium, fibrin and blood. Many leucocytes are found in the mesoderm of the villi. The main wall of the chorion and the amnion of both specimens are of irregular thickness and are well blended with each other.

The changes in the two embryos are very similar. In both the epidermis is intact and the dermis is thickened. In front of the head in the region of the deformed mouth there are peculiar thickenings of the epidermis. Both spinal cords are markedly dissociated. The dissociation of the brains is so extensive that in consequence the cerebral vesicles and midbrains are nearly destroyed and the hind-brains occupy spaces in the centers of the deformed heads.

The large vessels and heart are gorged with blood. In B the wall of the ventricle is well infiltrated and in A nearly destroyed by the migrating cells. The outlines of the organs and tissues are very obscure, the whole being more or less filled with round cells. Some of the liver tissue is necrotic.

No. 334.

Fleshy mole, 50 x 40 x 30 mm.; embryo, 5 mm.
Dr. Merrill, Stillwater, Minn.

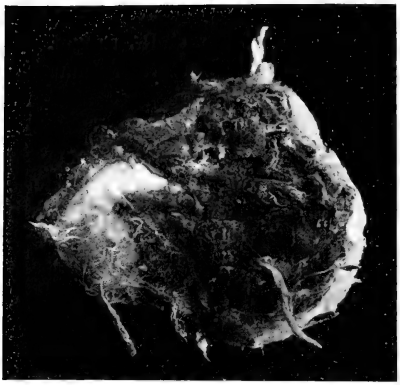


FIG. 330Ba.

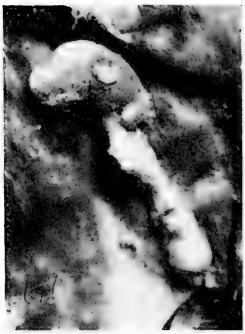


FIG. 330Bb.

FIG. 330Ba.—The ovum. Natural size.
FIG. 330Bb.—The embryo. Nearly two diameters.

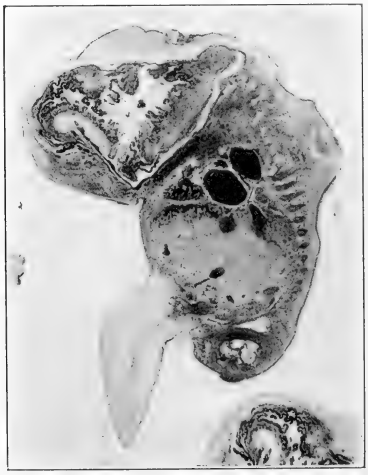


FIG. 330Bc.

FIG. 330Bc.—Sagittal section of the embryo. × 6 times.

"Last period four weeks ago. About ten days ago some bleeding, which repeated itself at intervals, and was finally followed by the abortion."

Examination of the mass proves that it is made up mostly of uterine mucous membrane, decidua, blood and pus, and contains a cavity 15 mm. in diameter. The chorion can still be made out as a fibrous band, infiltrated on the outside with leucocytes, and on the inside with small masses of syncytial cells. At points the chorion forms branches, which ramify partly through the mole. These are accompanied with syncytial cells and leucocytes.

The embryo is pretty well destroyed, of the five-weeks stage, and infiltrated with round cells. The head and back have fallen off, leaving only the viscera attached to the umbilical cord.

No. 336.

Ovum, 35 x 25 x 15 mm.; embryo, 8 mm.

Dr. West, Bellaire, Ohio.

The ovum is smooth, one end being covered with well-developed villi. Their mesoderm is hyaline, with scattered nuclei containing some remains of blood-vessels. The main wall of the chorion is fibrous and infiltrated with blood-cells from the embryo. Within there is a cavity (15 x 10 mm.) filled with granular magma and containing the umbilical vesicle and the embryo, which is closely encircled by the amnion.

The embryo is somewhat distorted, with large blood-vessels filled with blood and tissues infiltrated with rounds cells. The muscle wall of the ventricle of the heart is normal in appearance and there is every evidence that it kept beating until the last. What is especially noteworthy is that the circulation with the chorion has been cut off, the cord being atrophic and infiltrated, but instead the large omphelo-mesenteric vessels are filled with blood and spread over the yolk sac. The walls of this, however, are necrotic.

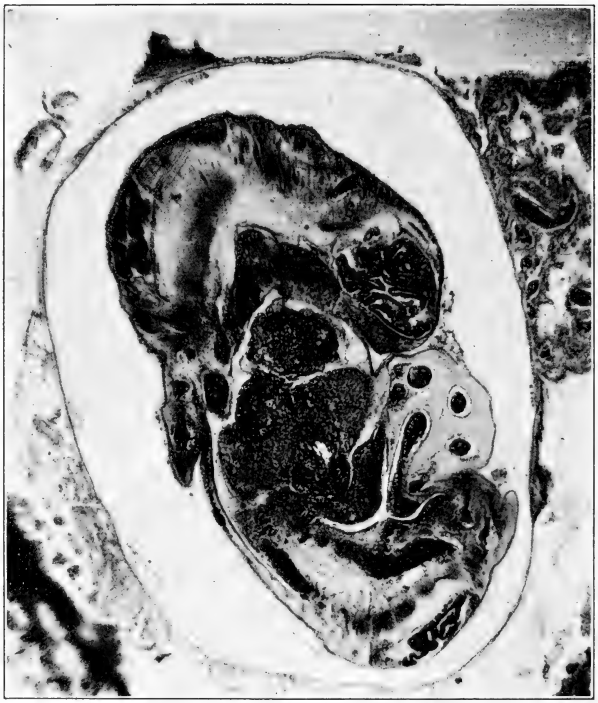


Fig. 336.—Section of the deformed embryo. × 12 times. The mass attached to the exterior of the amnion is a portion of the umbilical vesicle.

No. 338a.

Ovum, 45 x 45 mm.; embryo, C. R., 18 mm.

Professor Minot, Boston.

The specimen is from a patient suffering with arterio-sclerosis, who died of cerebral apoplexy in the Boston City Hospital. Pregnancy said to be of from six to eight weeks duration.

FIG. 338a.—Photograph of the specimen. × 2 times.

The chorion is normal in appearance and in structure. The cord of the embryo shows a marked constriction, which in sections appears to be fibrous. The embryo in general is normal in appearance, with the blood-vessels well distended with blood. The central nervous system is dissociated and somewhat macerated. The wall of the heart ventricle is also dissociated, that is, it is infiltrated with round cells.

Fig. 339.—Photograph of the embryo. × 2 times.

No. 339.

Chorion, 50 x 30 x 30 mm.; embryo, C. R., 16 mm.

Professor Minot, Boston.

The chorion is thin, is covered by but few villi and is hemorrhagic on one end. In structure it is somewhat hyaline at points and at others somewhat fibrous. The cord is thickened and also fibrous. The walls of its blood-vessels are dissociated and the blood from them is infiltrating the surrounding tissues. The embryo is somewhat distorted but normal in form. Within the tissues are dissociated and macerated. The large blood-vessels are distended with blood, and within the liver and heart the blood cells from them have extended into the surrounding tissues.

No. 340.

Stumpy embryo, 6 mm. long.

Professor Minot.

The embryo is well infiltrated with round cells, and the dissociation of the tissues is quite complete. Large blood-vessels can still be outlined, and the central nervous system is practically solid.

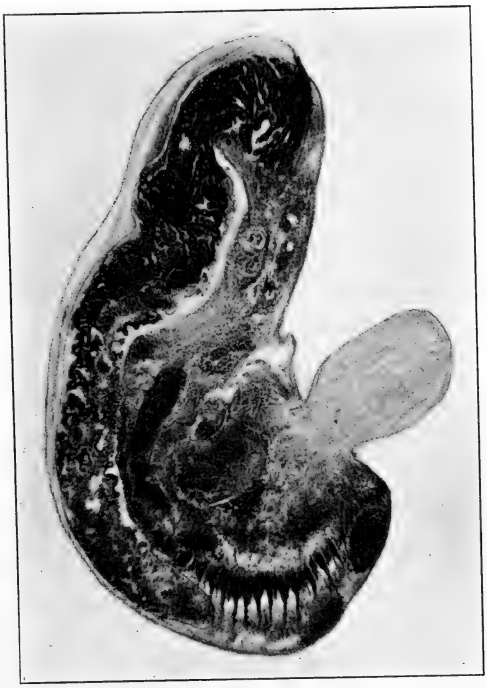


FIG. 340.—Sagittal section of the embryo. × 17 times.

No. 341.

Ovum, 70 x 60 x 50 mm.; embryo, 14 mm.

Professor Minot.

The ovum is pear-shaped and smooth, being covered with some decidua and at points with hemorrhagic masses. Its tissue does not stain well, but it appears as if some of the villi were fibrous and others œdematous. There is not much syncytium present. Possibly there are masses of leucocytes in the decidua.

Within there are two stumpy embryos, both of which have dilated cords which come to a point where they are attached

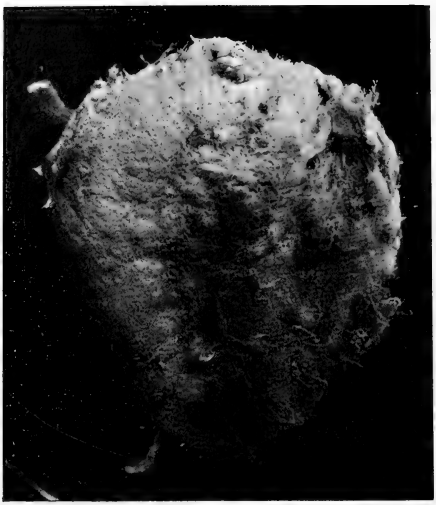


FIG. 341a.—Photograph of the ovum. Natural size.

FIG. 341c.—The twin embryos. Nearly two diameters.

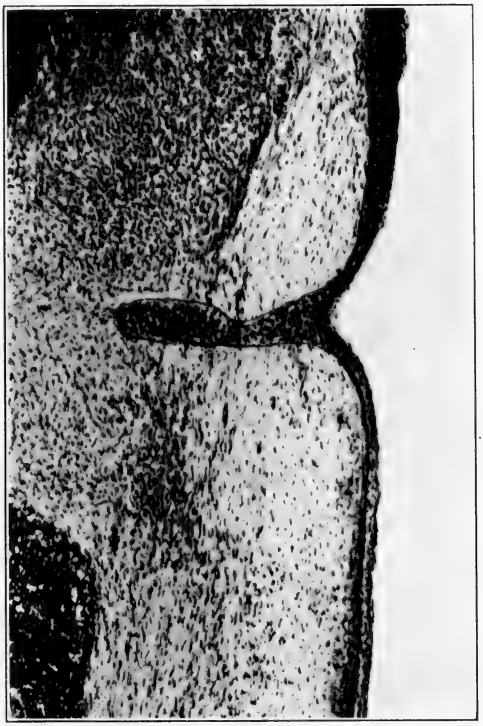


FIG. 341c.—Curious invagination of the epidermis on top of the head of one of the embryos.

to the chorion. These dilatations show the usual mucoid changes, with cavity formation. The embryos are dissociated and macerated. The large blood-vessels are filled with blood, and it appears as if the migrating cells had infiltrated much of the tissues.

No. 342.

Ovum, 30 x 20 x 20 mm.; pedicle within, 5 x 1 mm.

Professor Minot.

The specimen is from a tubal pregnancy and has a very thin fibrous chorion, with traces of blood-vessels, and is practically without villi. Within there is a thickened fibrous

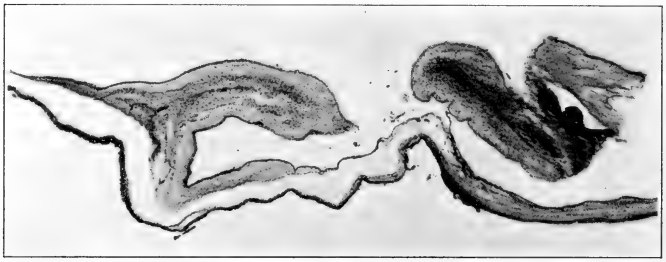


Fig. 342.—Chorion, amnion, cord and remnant of the embryo. × 15 times.

amnion, to which the process, the umbilical cord, is attached. The cord is also fibrous, contains remnants of its blood-vessels and has attached at its free end a curious group of round cells, which probably represents what remains of the embryo.

No. 343.

Ovum, 55 x 45 x 35 mm.; embryo, 11 mm.

Professor Minot.

The chorion is of unequal thickness and mostly smooth. Sections show that not only is the decidua attached to it, but also portions of the uterus. The decidua is necrotic and infiltrated with numerous leucocytes. Below the decidua there

FIG. 343a.—Photograph of the embryo attached to the chorion. × 2 times.

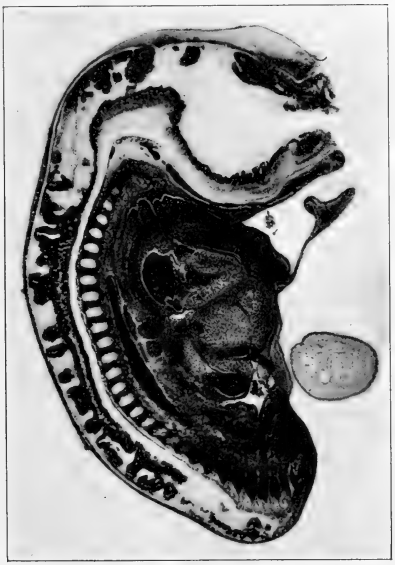


FIG. 343b.—Section of the embryo. × 8 times.

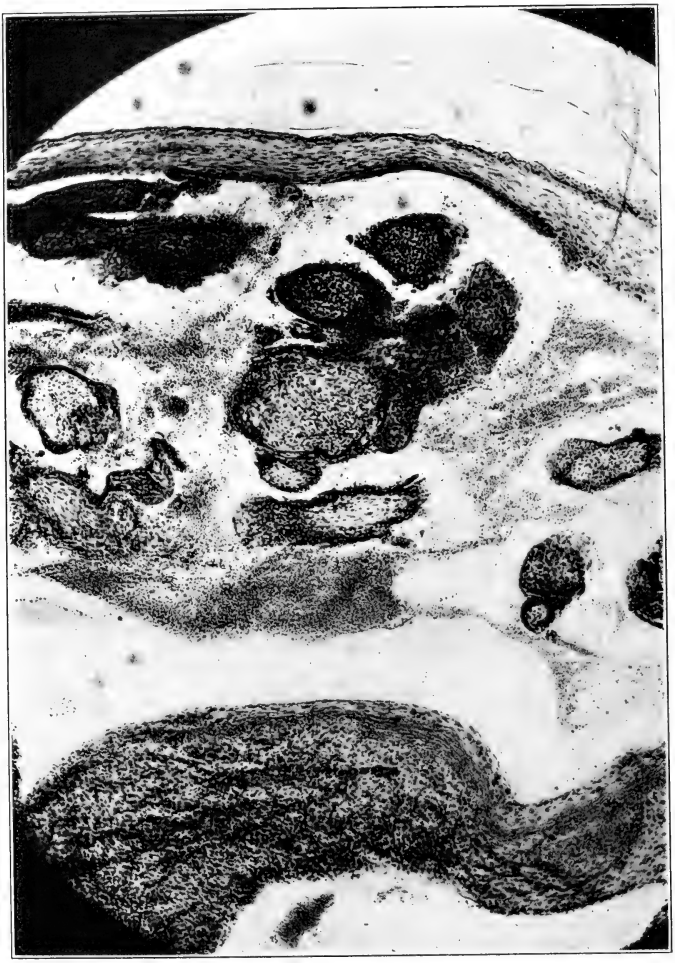


FIG. 343c.—Photograph of a section of the chorion, showing the mucoid mass infiltrated with leucocytes between the villi.

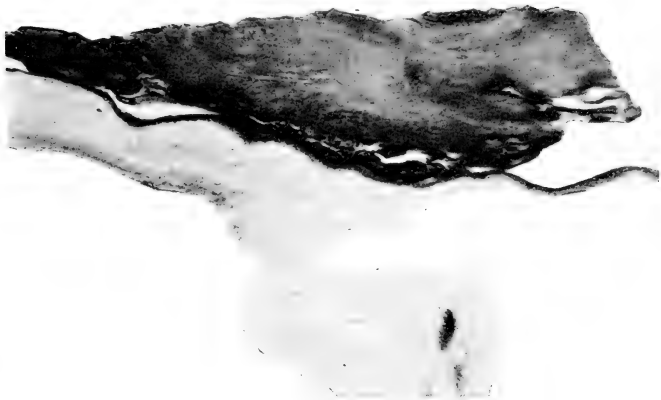


FIG. 343d.—Section of the point of juncture of cord, amnion, chorion, villi and decidua. × about 20 times.

are distorted villi with fibrous mesoderm. The amnion is in contact with the chorion. Between the villi there is a stringy mucoid mass rich in leucocytes.

The stumpy embryo is attached by means of a fibrous umbilical cord. Its tissues are dissociated and infiltrated with round cells; the blood-vessels and heart are greatly distended with blood. The liver is necrotic. In front of the head the tissue is broken away, leaving a pocket which contained the fore-brain. Above this the brain protrudes. The cord and fourth ventricle are distended and dissociated. The epidermis is intact.

No. 344.

Ovum, 45 x 45 x 45 mm.; embryo, C.R., 16 mm.

Professor Minot.

The wall of the chorion is very thin, with a few fibrous villi scattered over it. It contains no blood-vessels. The long thin umbilical cord is fibrous and shows remnants of blood-vessels.

The embryo has a rounded head and stumpy legs. Its tissues are dissociated, the brain being distended and macer-

ated, too. The medulla has expanded towards the mouth. Heart and blood-vessels are distended and in many places the walls are destroyed and the blood cells extend into the surrounding tissues. This is very marked in the liver. The legs are filled with an even mass of round cells, *i. e.*, the tissues are dissociated. Some of the epidermis has fallen off.

Fig. 344.—Photograph of the embryo attached to the chorion. × 2 times.

No. 345.

Ovum, 60 x 50 x 50 mm.; embryo, 19 mm.

Professor Minot.

The fleshy ovum is composed largely of decidua, in which are buried plugs of mucus, pus and necrotic villi of the chorion. The embryo is normal in shape. The tissues of the

embryo are macerated, but on account of the distended medulla which encroaches upon the mouth I think it likely that the tissues were dissociated before they became macerated.

No. 346.

Embryo, C. R., 13 mm.

Professor Minot.

A piece of hemorrhagic chorion, which may have been 50 mm. in diameter, is attached to the embryo. Its tissues are macerated, but they are well enough preserved to show that much mucus and pus are between some of the villi. The

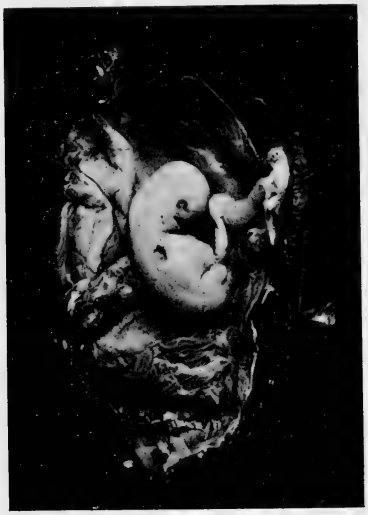


FIG. 346.—Embryo attached to the ovum. × 2 times.

structures of the chorion, amnion and cord appear normal, but the umbilical vesicle is filled with a necrotic mass.

The embryo is dissociated and macerated. The central nervous system is dilated and the heart is distended with blood, some of which infiltrates the surrounding tissues.

No. 347.

Ovum, 40 x 35 x 30 mm.; embryo, C. R., 11 mm. If the head is replaced the C. R. measurement will be less than 8 mm.

Professor Minot.

The decidua is hemorrhagic and necrotic at points and well infiltrated with leucocytes. The villi and main walls of the

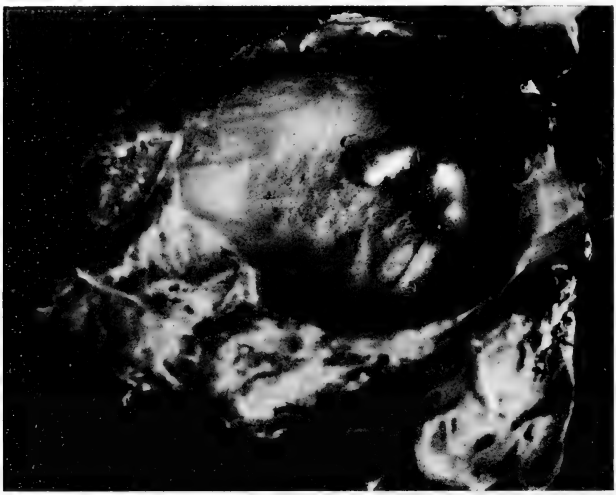


Fig. 347.—Embryo within the chorion. × 2 times.

chorion are fibrous and at points infiltrated with leucocytes. Very little syncytium is present, and but few traces of blood-vessels are found in the chorion.

The embryo is dissociated and macerated, with dilatation of the central nervous system and extension of the medulla. The blood-vessels are distended and the blood cells are continued through their walls into the surrounding tissues.

No. 348.

Ovum, 50 x 30 x 25 mm.; embryo, 12 mm.

Dr. Pearce, Albany, N. Y.

The specimen is smooth, being covered with numerous small hemorrhagic spots and irregular masses of small villi. Sections show that the decidua is infiltrated with leucocytes, with a consequent fibrous degeneration of the villi of the chorion. The villi, as well as the main wall of the chorion, are being invaded by leucocytes and frequently by syncytial cells.

The dissociation of the tissues of the embryo is extreme, the blood from the blood-vessels having passed through their walls to infiltrate the surrounding tissues. This is especially well marked in the heart and liver. The nervous system is pretty well broken up and the epidermis has fallen off.

No. 357.

Ovum, 90 x 40 x 40 mm.; embryo, C. R., 17 mm.

Dr. Russell, Baltimore.

"The specimen came from an unmarried woman twenty-two years old, who said that she was glad it had come away,

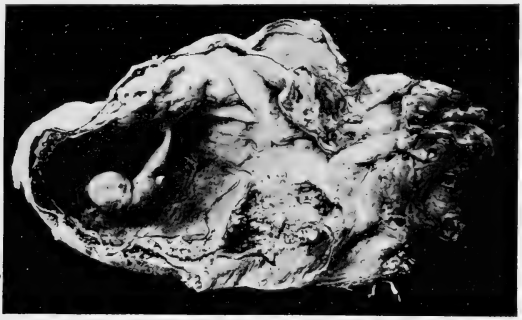


Fig. 357.—Embryo within the chorion. Natural size.

for it saved her the trouble of having an abortion induced. Her menstruation was irregular, sometimes every two weeks, sometimes every six weeks. The last period occurred about

the middle of January. On March 29 she began to bleed and aborted on April 19. Apparently her uterus is normal."

The unruptured specimen is inclosed in a layer of decidua and is covered with villi of unequal size, some being very large, as the photograph shows. Within there is a stumpy embryo without a neck and with atrophic leg buds. The cord is transparent and partly filled with granules, which indicates that the embryo had been dead for some time before the abortion.

The mesoderm of the chorion and amnion is thickened, of even structure, and contains no blood-vessels. In fact, its cœlomic cavity is entirely obliterated. The main wall of the chorion is very thin, often being composed of epithelial cells only. The mesoderm of the villi is unusually fibrous and contains no blood-vessels. The very large villi are degenerated, often hollow and do not stain. The syncytium is very deficient in quantity; at points it invades the mesoderm. Over the villi there is a mass of fibrin and disintegrated blood. Leucocytes are not numerous, even in the decidua, which appears to be normal.

The tissues of the embryo are not only dissociated, but also macerated, and they do not stain well. The sharp boundaries are lacking, showing that adjacent tissues have begun to coalesce. In fact, the whole head down to the thorax seems to have been converted into a bag in which fragments of cartilage and nerve tissue may be seen. The front of the head is adherent to the thorax immediately over the heart. The contours of the cartilages, liver, heart and adrenal can be made out, but those of the blood-vessels are obscure.

According to the menstrual history this embryo was in the seventh week when bleeding began, which was followed by the abortion three weeks later. However, the degree of the development of the cartilages and other structures places the embryo in the sixth week. The lack of inflammatory reaction, and the inactivity of the syncytium, suggests that the continued bleeding may have been the primary difficulty, which was followed by death and degeneration of the embryo.

No. 358.

Ovum, 30 x 16 x 10 mm.

Dr. Swett, Bangor, Me.

"Pregnancy of six weeks duration." The outer surface of the ovum is smooth and the specimen runs out into a pedicle which was undoubtedly attached to the uterus. Sections show that the villi are matted together, with much blood and syncytium between them. Around this there is a fibrous decidua in which there are many leucocytes. The mesoderm of the chorion is somewhat fibrous, the change being especially well marked in some of the villi. No blood-vessels are present in the villi.

The cavity within (cœlom) measures 8 x 6 x 6 mm., is lined by a layer of reticular magma, but contains no trace of the amnion nor embryo.

No. 361.

Ovum, 10 mm. in diameter.

Dr. Egbert, Washington.

"The ovum was found in a mass of blood within the abdominal cavity, due to a tubal abortion. The operation was performed just 41 days after the beginning of the last menstrual period."

The specimen came into my hands after it had been in water for 24 hours. It was well covered with villi and filled with a mass of dense reticular and granular magma. No embryo could be found by direct observation. The specimen was macerated too much to allow careful microscopic examination.

No. 364.

Ovum, 90 x 50 x 40 mm.; embryo, 16 mm.

Dr. Merrill, Stillwater, Minn.

The ovum is covered with a few ragged villi, over which there is some decidua which is more or less detached. Dr. Merrill had placed the specimen in formalin and sent it to me accompanied with the following letter, dated July 6, 1906:

"Yesterday I sent another specimen by express. It seemed to me that it would be a good specimen for you. April 7 was the date of the last menstruation; the abortion followed on July 5, 1906. The first flow and pain appeared on the night of July 4. The woman has been married four years; this was her first conception. Both she and her husband are very anxious to have a child, so the miscarriage could not have been aided. There was no incident, accident or otherwise to give cause for the abortion. The woman is unusually healthy and the miscarriage took place without chill or rise of tem-

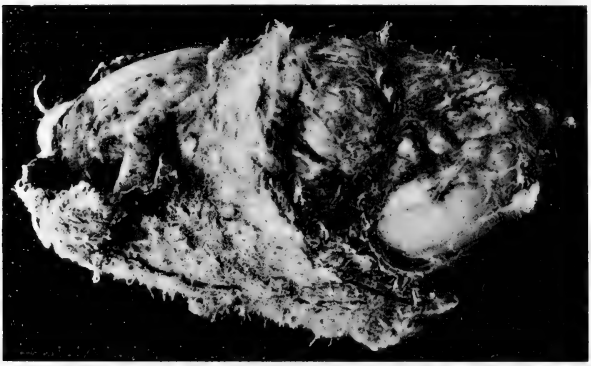


Fig. 364a.—The ovum. Natural size.

perature. The specimen was placed in formalin, 10 per cent, within two hours after its expulsion."

This history did not satisfy me, so I wrote Dr. Merrill asking a number of questions, for it is from specimens like this that we may hope to find the cause for such malformations. His second letter, dated October 24, 1906, reads as follows: "This specimen is from the first conception, after several years of married life. The woman had been operated upon several years ago for appendicitis. She has not been altogether regular with her menstrual periods, and there is some pain connected with them. She had been treated, some

FIG. 364b.—Front view. × 3 times.

time before I saw her, for vaginal discharge; there may have been endometritis. Prior to her conception I gave her some treatment for leucorrhœal discharge, also made some slight dilatation of the cervix. She had a long cervical os with a narrow canal. There was some vaginitis and, as I remember, some endocervicitis rather than endometritis; none of them

FIG. 364c.—Right side. × 3 times.

FIG. 364d.—Left side. × 3 times.

very marked. Probably there was enough uterine trouble to cause the delayed development of the embryo and the abortion. It was a natural abortion, as the woman was very anxious to have a child. She is what I call a perfectly healthy woman compared with the average woman of the day. The husband is ordinarily healthy, but about a year ago, his wife states, he had some trouble with his genital appa-

FIG. 364e.—Section to the left of the middle line.

FIG. 364f.—Section near the middle line.

ratus. He has night emissions and I judge took medicine for them. As far as I can ascertain, from her outline, he has not had a venereal disease. If so, he did not contaminate her. If he has, as she states, night emissions, perhaps the virility of his semen is below par."

These letters give the difficulties in obtaining histories in these cases, but they indicate that the cause of the change in

FIG. 364g.—Sagittal section.

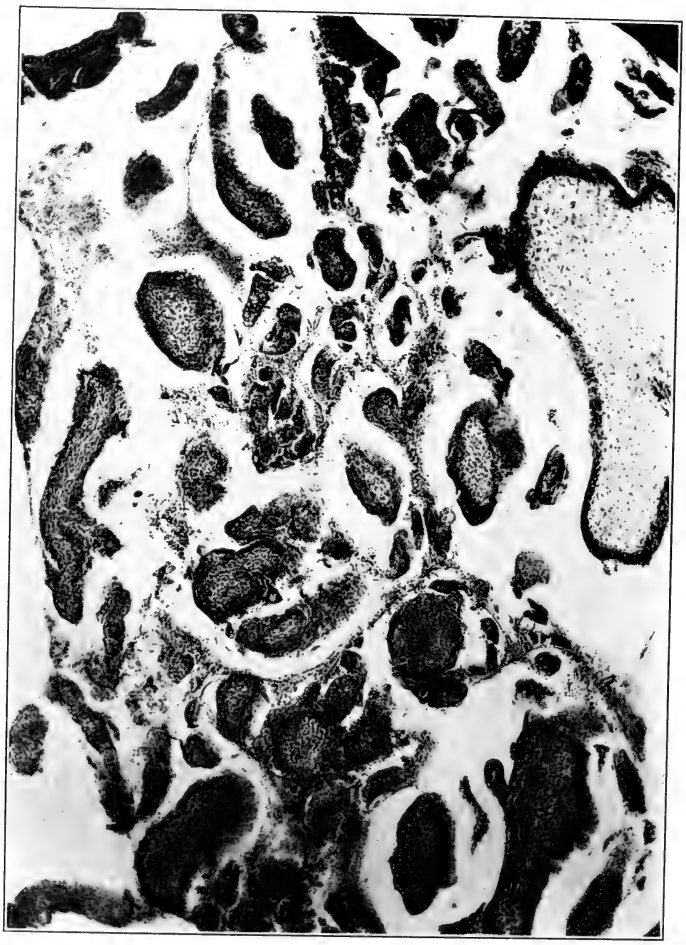


FIG. 364h.—Section through the villi, showing large amount of mucoid substance rich in leucocytes between them.

the embryo is to be sought in the chorion, which probably failed to attach itself well to the uterus.

Sections of the chorion show that the villi are far more numerous than was suspected from the simple inspection with the naked eye. The main wall of the chorion is thin and atrophic and is lined with the amnion, which is fully detached where it connects with the umbilical cord. However, it must have been attached at one time, for remnants of blood-vessels from the embryo are seen in the villi of the chorion. The mesoderm of the villi is very fibrous and the villi are matted together by a slimy mass rich in blood and leucocytes with fragmented nuclei. The syncytium is well developed and extends into the mass of blood and slime. The decidua over the chorion has large sinuses within its walls, is quite hemorrhagic and at points has large islands of leucocytes, usually situated along the course of the blood-vessels.

The photographs show the condition of the embryo. Hare-lip, displaced ears, protruding viscera in front and spina bifida behind. The large blood-vessels and heart are still filled with blood and there is quite a general infiltration of the tissues with round cells. The vessels of the embryo end in the cord and do not reach to the chorion. In general, there is mainly a destruction of the tissues due to the irregular growth of the embryo.

The central nervous system has been converted, in great part, into a mass of connective tissue, with remnants of the cord below and a rudimentary brain above, which forms a shield upon the protruding mass. A portion of this shield has grown into the connective tissue below, forming a gland-like structure.

The clavicle, mandible and maxilla have begun to ossify and some of the muscles are well developed.

No. 365.

Embryo, 14 mm.

Professor Pohlman, Bloomington, Ind.

This embryo, with spina bifida, iniencephaly and anencephalus, and extremities of normal form, has a straight body

Fig. 365a.—Front view of the embryo. × 2 times.

Fig. 365b.—Section through the middle line of the head. × 6 times.

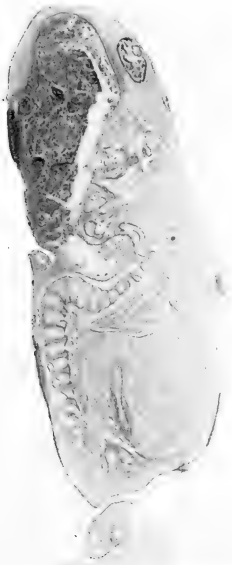


FIG. 365c.—Section through the middle line of the neck. × 6 times.

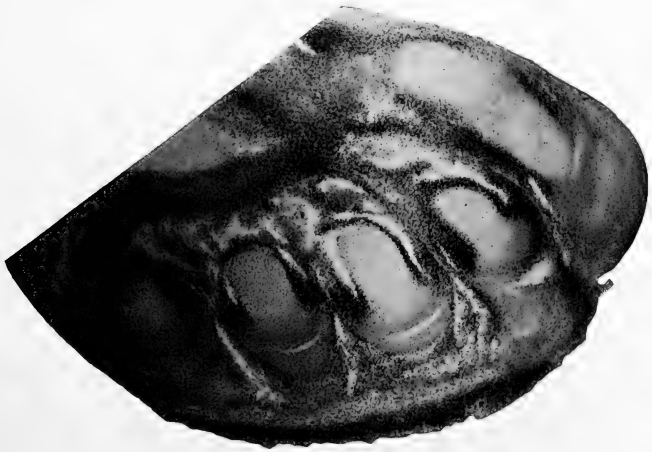


FIG. 365d.—Section through the hand.

and is attached to the end of a very large umbilical cord. Sections show that the spinal cord is absent, but there is a solidified brain which is more or less infiltrated with round cells at its periphery. The same is the case with the eyes. The mouth is closed by the tongue, which has become adherent to the lips. The nodules in front of the body are composed of necrotic epithelial cells.

Some of the tissues of the body are necrotic, but most of them are infiltrated with round cells, and those of the head are quite fibrous in character.

The walls of the alimentary canal and the lungs are also pretty well filled with irregular patches of round cells. Especially well marked is this change in the region of tendons and perichondrium, showing that there is an irregular growth of the mesodermal tissues. The clavicle, maxilla and mandible are well ossified, which should not be the case in so small an embryo.

No. 366.

Embryo, 9 mm.

Professor Pohlman, Bloomington, Ind.

Sections of the chorion, which is fleshy in appearance, show that its main wall is very thin and that it is lined with the amnion. The villi, few in number, are fibrous or hyaline, are covered with some syncytium, and the spaces between them are filled with blood. Some of the villi adhere by means of the syncytium to the decidua, which is fibrous and necrotic. There is no leucocytic infiltration of the chorion nor the decidua.

The embryo is pretty well infiltrated with round cells and the tissues are dissociated. The tissues are well preserved and appear to have been very much alive. There is a considerable quantity of blood within the cavity of the heart and in the blood-vessels. The central nervous system is dissociated. The lower jaw is large and is adherent to the head above and to the trunk below. The arms and legs are atrophic.

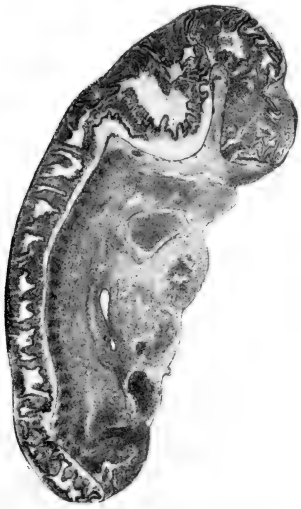


FIG. 366a.—Sagittal section of the embryo. × 10 times.

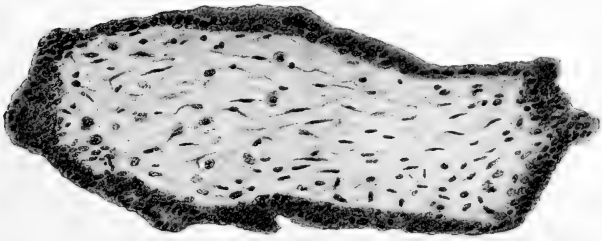


FIG. 366b.—Section of a villus. × 250 times. Notice large epithelial cells scattered in with the stroma.

No. 367.

Ovum, 10 x 7 x 5 mm.

Professor Brödel, Baltimore.

The ovum from a tubal pregnancy came to me unopened and with some adhering cells and blood clot it was cut into serial sections. The chorion was found to be torn on one side, but its interior is packed with a dense reticular magma. No trace of an embryo was found.

Fig. 367a.—Ovary and tube, clot within and ovum. Natural size.

The mesoderm of the main wall of the chorion is of normal thickness, but on the side towards the cœlom it is not sharply defined. Frequently strands of cells are found partly separated and running out into the magma. The tissue of the mesoderm of the villi is not as clearly defined as in normal

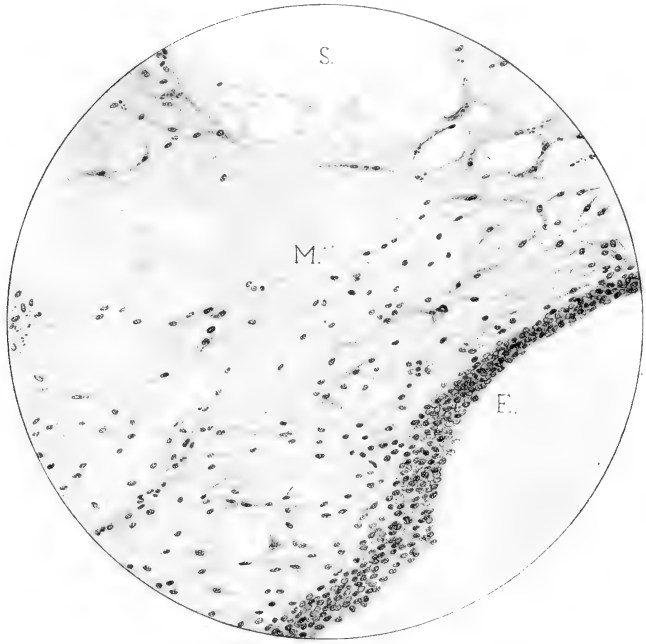


FIG. 367b.—Section of a portion of a villus as indicated by the adjoined outline, *V*. × 250 times. *E*, epithelial covering; *M*, mesoderm; *S*, space within formed by a destruction of tissues.

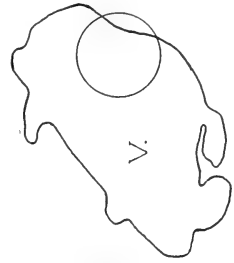


FIG. 367c.—Outline of a villus showing the portion from which Fig. 367b was drawn.

specimens, some of them having undergone marked degeneration. The villi are developed better on one side of the chorion than on the other, and here they contain structures which are undoubtedly blood-vessels.

The syncitium is not very marked and is held together by a slimy mass which contains some leucocytes. The surrounding tissue, the "decidua," is full of fibrin and contains numerous fragmented nuclei and some blood.

It is natural to read into this specimen the following history: The embryonic mass grew long enough to send its blood-vessels into the chorion and then the nutrition was cut off because the villi did not attach themselves properly. That this was the case is shown by the capsule of necrotic tissue which encircles the villi. As a result of impaired nutrition the embryo was destroyed, leaving only the isolated chorion filled with reticular magma.

No. 369.

Ovum, 7 x 3 x 3 mm.

Professor Brödel, Baltimore.

The specimen was removed by operation from a tubal pregnancy on October 9, 1906. The woman's last period began September 17. The distended tube measured 25 mm. in diameter and when cut open a small lump, 2 mm. in diameter, was seen on one side of its cavity. This was believed to be the embryo, but serial sections proved it to be a small mass of blood very rich in leucocytes.

The sections show the chorion pretty well folded upon itself, which is torn at several points. The torn edges are well rounded, that is, they are healed and are therefore not due to the operation. Few villi are left, and they, with the main walls of the chorion, are very fibrous in structure. There is but little syncytium present. The entire chorion is separated from the wall of the tube by a thick layer of blood, and the tube wall is well infiltrated with leucocytes. What is most remarkable in this specimen is that the amnion lines the chorion completely and all of the mesoderm of the chorion is

well filled with blood-vessels from the embryonic mass, which must have been present at one time.

No. 375.

Embryo, C. R., 13 mm.

Professor Gage, Ithaca, N. Y.

A piece of chorion accompanied the embryo, both of which appear quite normal. However, sections of the chorion show that the mesoderm of the villi is very fibrous, while that of its main wall appears normal. The syncytium seems to be deficient in quantity.

Sections of the embryo indicate that it is nearly normal, with some dissociation of the tissues. The larger blood-vessels are gorged with blood, and some of the tissues, especially those in front of the head, are infiltrated with round cells. The central nervous system is swollen and dissociated, as is so frequently the case in many of the other embryos.

No. 377a.

Ovum, 30 x 22 x 14 mm.

Dr. Crawford, Cedar Rapids, Iowa.

The specimen is well covered with villi, which appear quite normal to the naked eye, but upon microscopic examination it is found that they are very fibrous and tipped with syncytium; at points it forms islands with necrotic centers.

The interior of the ovum contains a considerable amount of reticular magma, within which there is embedded a large sac (5 mm. in diameter) containing a nodule (.5 mm. in diameter)—the embryo.

Sections show that the whole chorion is lined with the amnion except at the point of the "inclosed sac," which proves to be the exocœlom. The embryo is composed of an amorphous mass of cells which invade the mesoderm of the chorion. It may represent the last remnant of the umbilical vesicle. No traces of blood-vessels are seen in any portion of the embryonic mass, nor in the mesoderm of the chorion.

No. 378.

Ovum, 12 mm. in diameter.

Professor Brödel, Baltimore.

The specimen came from a tubal pregnancy, is dumb-bell-shaped, and had been opened by Professor Brödel, who found no trace of an embryo in it. It was hardened immediately and later cut into serial sections. At no point in the sections could any trace of an embryo be found, although it is possible, but improbable, that it was lost while the fresh specimen was being examined.

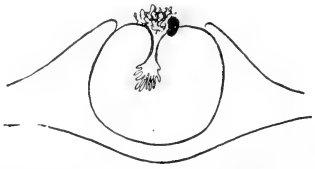


Fig. 378.—Outline of the tube, blood clot and ovum. Natural size.

The cœlom contains some granular magma. The mesoderm of the main wall of the chorion is apparently normal, but that of the villi is œdematous. There are no blood-vessels present. At many points the syncytium is necrotic, frequently rising from the villi, leaving small vesicles below. The necrotic masses are held together by a slimy mass, within which there are a great many small round cells, undoubtedly leucocytes.

No. 379.

Ovum, 35 x 25 x 15 mm.

Dr. Meyer, Baltimore.

"Last period early in August; abortion, October 20, 1906."

The specimen is well covered with villi and filled with a considerable amount of reticular magma. Within there is a sac, the amnion, measuring 10 mm. in diameter. It contained a granular mass, which, when floated from alcohol into water, took on the form of an embryo of the fourth week.

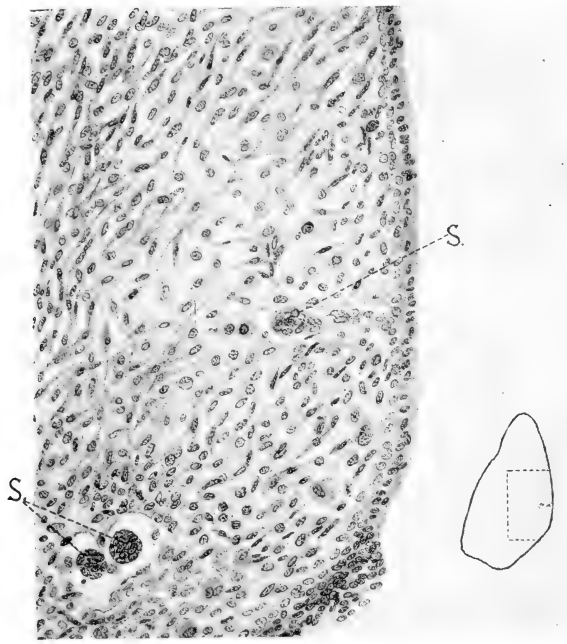


FIG. 379.—Section of a portion of a villus. × 250 times. The synctium, *S*, is invading the mesoderm of the villus.

No internal structures could be seen and in handling the embryo it fell into pieces. No doubt the embryo had been dead for some time.

Sections show that the mesoderm of the umbilical cord, main wall of the chorion and the villi are fibrous, with a curious growth of the blood-vessels in some places. Within them there are numerous fragmented cells, which may have come from the blood of the embryo. The syncytium is very extensive, necrotic at points and is not infiltrated with leucocytes. In many places it dips deep into the mesoderm of the villi and forms islands of epithelial nests. The wall of the amnion is composed of two layers of cells and appears to be normal.

No. 395.

Ovum and decidua, measuring 17 x 10 x 7 mm.

Dr. Pearce, Albany, N. Y.

Dr. Pearce writes: "I am sending you to-day a small encapsulated mass, found among curettage material, which appears to be a young ovum. I have refrained from attempting to determine definitely whether or not it contains an embryo, for fear of injuring a specimen which might be of value to you.

"The specimen was removed April 20, 1907, six weeks after the last menstruation. The uterus was emptied because the patient had eclampsia three years ago, and since then has had premature delivery of two dead children. The specimen is preserved in 10 per cent formalin."

The whole mass was stained in cochineal and cut into serial sections, but no embryo was found in it. The sections show it to be composed of numerous villi, decidua and inflammatory tissue. Most of the villi are also fibrous and degenerated, some few, however, contain blood-vessels filled with embryo's blood. The fragmentary walls of the chorion are very fibrous and the growth of the syncytium is very irregular. Undoubtedly the ovum "collapsed" some days before the uterus was scraped. The whole specimen is buried more or less in a slimy mass rich in leucocytes, which indicates that the uterine tissue was markedly inflamed.

No. 396.

Ovum, about 7 mm. in diameter, with the cœlom measuring 3 x 2 mm. Tubal pregnancy.

Dr. Castler, Baltimore.

"The tube was removed April 24, 1907, from a woman twenty-one years old. Last period, March 5, followed by a brownish discharge on April 11. Diagnosis of tubal pregnancy on April 23. The abdominal cavity was found well filled with blood and the tube was still bleeding through the internal ostium. The whole tube was removed and placed in a 10 per cent solution of formalin."

The hardened tube is 40 mm. in length and 20 mm. in diameter. It was cut into blocks 5 mm. thick and imbedded in colloidin. Two of the blocks were found to contain the

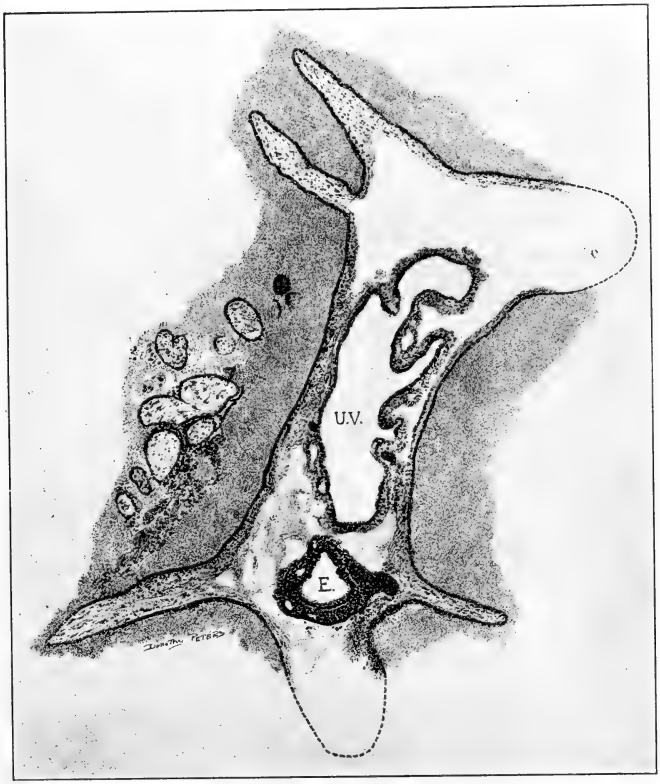


FIG. 396.—Section of the chorion containing the embryonic mass. × 35 times. *E*, remnant of the embryo; *UV*, umbilical vesicle.

ovum and these were cut out and reimbedded in paraffin and cut into serial sections. The sections show that the ovum has unusually long villi, fully 5 mm. long, which ramify

throughout the blood in the tube and in many instances are attached to the decidua. The syncytium is well developed. The walls of the tube are markedly distended, infiltrated with red corpuscles and leucocytes, many contain fragmented nuclei, which are also scattered throughout the decidua.

Within the cœlom of the chorion there is a double vesicle, the large one, 2 x 1 mm. in diameter, showing all the characteristics of the umbilical vesicle. Its layer of mesoderm appears to be thickened and at numerous points it has become adherent to the inner wall of the chorion. At these points the blood islands extend over to the mesoderm and from them blood-vessels ramify to all of the villi. These vessels are all filled with nucleated blood cells. The smaller vesicle is about a millimeter in diameter, is lined with cylindrical cells and is covered with quite an even layer of mesoderm, in which there are some quite large blood-vessels but no blood. Towards one of its ends it is covered with a marked layer of cylindrical cells. It may be that this second vesicle represents what is left of the embryo. Around these two vesicles, filling the whole cœlom, there is a dense reticular magma.

The main wall of the chorion and many of the villi are somewhat fibrous in structure. Some of the villi are being invaded by syncytial cells.

This specimen is especially valuable inasmuch as it shows the early changes which take place in an ovum after it became lodged in the uterine tube. No doubt owing to its faulty implantation the nutrition of the embryo was affected and it consequently grew in an irregular fashion. The umbilical vesicle became adherent to the chorion and its blood-vessels grew out into most of the villi.

No. 398.

Embryo, 5 mm. long.

Professor C. R. Bardeen, Madison, Wis.

The embryo is markedly changed and of the three-weeks' stage. Most of the organs can still be recognized and the embryonic cœlom is fairly definite. The front of the head is

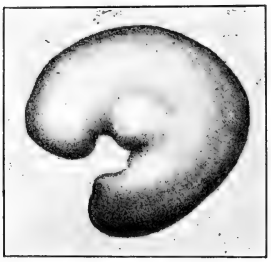


FIG. 398.—Outline of the embryo. × 8 times.

adherent to the thorax below and the face is pretty well atrophied. The central nervous system is dissociated and distended, as are also the heart, blood-vessels and the liver.

No. 399.

Embryo, 4 mm. long.

Dr. Thompson, Mt. Horeb, Wis. Bardeen Collection.

"The specimen is from a woman twenty years old who has been married ten months. She is a marked bleeder, other-

FIG. 399.—External form of the embryo. × 8 times.

wise strong and healthy. The pelvic organs are normal. The last period occurred during the first week in September and the abortion followed October 9, 1906."

The external form looks much like that of a chick. Sections show that the tissues are generally dissociated and also macerated.

No. 400.

Embryo, 3.5 mm. long.

Dr. Kaumheimer, Milwaukee, Wis. Bardeen Collection.

"Last menstruation October 21; abortion December 19. Placed in 10 per cent formalin an hour after the abortion."

The external form is that of a normal embryo, but the sections show that marked pathological changes have taken place. The central nervous system is distended and partly

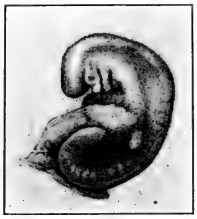


FIG. 400.—Drawing of the embryo. × 8 times.

filled with round cells. The walls of the brain of the embryo are dissociated and apparently are giving rise to the numerous round and fragmented cells which are present. The heart and large blood-vessels are distended and well filled with blood. The tissues of the mesoderm are generally filled with round cells as well as with numerous fragmented nuclei, the infiltration including the myotomes and the peritoneal cavity. The amnion and epidermis are intact.

No. 401.

Embryo, 5.5 mm. long.

Dr. Hay. Bardeen Collection.

Much of the chorion and many of the villi and the syncytium are necrotic and infiltrated with many leucocytes. The tissues of the embryo are dissociated, macerated and infiltrated

with round cells. However, all of the organs are recognizable. The umbilical vesicle is necrotic and filled with a mass of broken-down cells.

No. 402.

Ovum, 40 x 25 x 20 mm.; embryo, 4 mm. long.

Dr. O'Shaughnessy, New Canaan, Conn.

"The woman, age 30, from whom the specimen was obtained is well built, strong and healthy. Menstruated regularly, but was married 3½ years before she became pregnant. After the birth of this child she had a slight discharge and was attended by a physician, who stated that she had an ulcerated cervix, for which he made local applications. Shortly after this, two years after her first confinement, she became pregnant again. This confinement, which was attended by me, was rapid and normal in every respect. She remained in bed for 15 days, the uterus not reducing in size as it should normally.

"Since the second child was born she has had some discharge, but became pregnant again about six or eight weeks ago. This time, however, she aborted. She has never done anything to prevent pregnancy, and both she and her husband are anxious to have a large family. The patient is at present unwell and still has her chronic discharge."

The villi of the ovum are not well developed, being irregularly distributed over its surface. Within, the cœlom is well filled with reticular magma. The embryo is club-shaped, its head being much too large for the body, the external form being very much like that of No. 399. The umbilical vesicle is of normal size and shape, the heart is well outlined and the extremities are just beginning to develop.

PLATES.

The plates include a number of illustrations which were borrowed from the literature to illustrate various points in this article. There are also some sections of normal embryos with which to compare the numerous sections of pathological specimens.

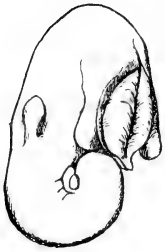


FIG. 1.

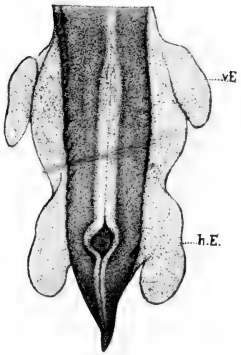


FIG. 2.

FIG. 1.—Human embryo 8 mm. long with spina bifida. After Torneau and Martin (Journal d'Anat. et Physiol., XVII, 1881).

FIG. 2.—Spina bifida in a human embryo 10 mm. long. After Fischel (Ziegler's Beiträge, XLI, 1907, Fig. 16).

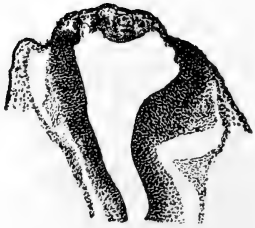


FIG. 3.

FIG. 4.

FIGS. 3 and 4.—Sections through the middle of the spina bifida shown in Fig. 2. After Fischel (Ziegler's Beiträge, 1907, Figs. 21 and 22).

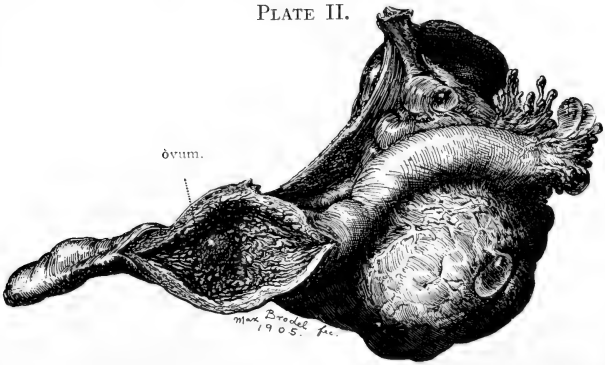


FIG. 5.—Ovum in tubal pregnancy. From a drawing by Professor Brödel. Natural size. After Kelly (Operative Gynecology, 2d edition, Vol. 2, Fig. 635.)

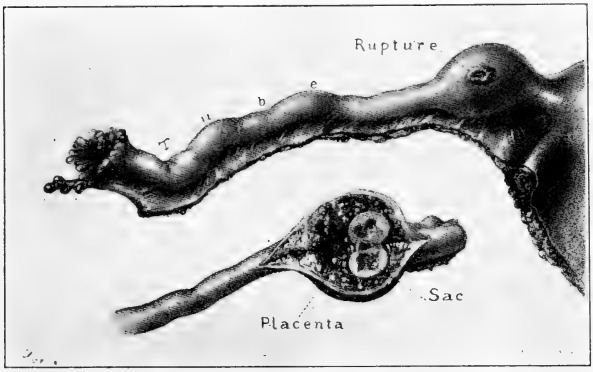


FIG. 6.—Ovum in tubal pregnancy. Reduced one-tenth. (After Kelly's Operative Gynecology, Fig. 640.)

FIG. 7.—Tubal abortion. Natural size. (After Kelly's Operative Gynecology, Fig. 643.)

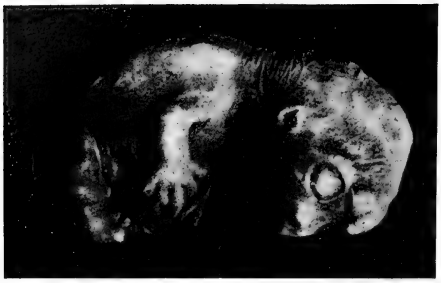


FIG. 8.—Normal human embryo 16 mm. long (No. 256). The head had been crushed in handling and the brain escaped through the two openings over the eyes.

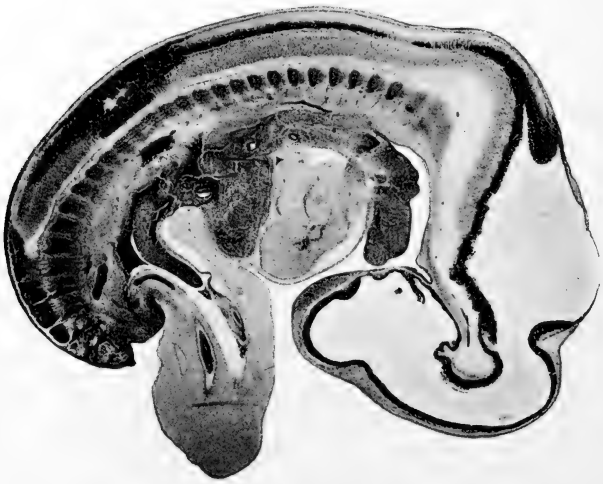


FIG. 9.—Sagittal section of a normal human embryo 7½ mm. long (No. 221).

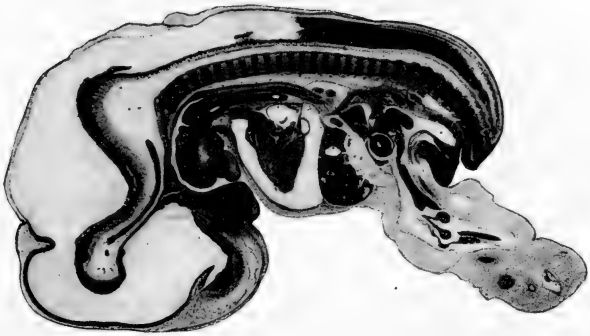


FIG. 10.—Sagittal section through a normal human embryo 14 mm. long (No. 144).

FIG. 11.—Sagittal section through a normal human embryo 35 mm. long (No. 199).

THE OÖGENESIS OF BUFO LENTIGINOSUS.

HELEN DEAN KING.

The present paper records the results of an investigation of the oögenesis of the American toad, Bufo lentiginosus, which was undertaken, primarily, in order to trace the history of the chromatin from the oögonia to the maturation period of the oöcytes and thus to complete my study of the chromatin behavior in the germ-cells of this amphibian. The work has necessarily involved a detailed study of the nucleoli, since these structures are closely associated with the chromatin at certain periods of development; and it has been extended to include an investigation of the yolk formation, as the material seemed especially favorable for this purpose.

This study was begun several years ago at Bryn Mawr College, but was laid aside for various reasons until this past year, when it was completed at the Biological Laboratory of the University of Pennsylvania, where I was holding a University Fellowship for Research in Zoölogy. I take this opportunity to express my obligations to Professor E. G. Conklin for many valuable suggestions during the course of my investigations.

I. MATERIAL AND METHODS.

Bufo lentiginosus is found very abundantly in the vicinity of Philadelphia; and, as the tadpoles are easily reared in the laboratory, several different series of preparations have been obtained consisting of larvæ killed at frequent intervals from the time of hatching until metamorphosis. These series give all stages in the development of the germ-cells up to the early growth period of the oöcyte. For the study of the later development of the ova, young toads with a body length of 1.5-5.5 cm. were collected at various times from June until

September. In order to compare the development of the ova in the young toad with that of the ova in the adult, portions of the ovaries of mature females were preserved at different times during the summer months. As the eggs appear to develop along similar lines in all toads, ovaries of young females were used principally for these investigations, since in them the ova are more nearly uniform in size than they are in the adult, and in a single section it is possible to find a large number of eggs in practically the same stage of development.

The very conflicting results that have been obtained by the investigators who have studied the development of the germ-cells in amphibians can doubtless be attributed, in part at least, to the great diversity of ways in which the material has been preserved. Carnoy and Lebrun, who have studied the germinal vesicle in the eggs of many different species of amphibians, unhesitatingly recommend Gilson's fluid as the best fixative for the amphibian egg. I have not found that this liquid gives a satisfactory fixation of the egg of Bufo, as it usually causes a decided shrinkage of the nucleus and, at certain stages, a distortion of the nuclear contents. A number of different fixing fluids have been tried during the course of these investigations, among which may be mentioned Zenker's fluid, corrosive-acetic (5 per cent acetic acid), corrosive-formalin (Bouin's method), picro-acetic, Flemming's solution, chromic-acetic, and Hermann's fluid. Flemming's solution (strong formula) is the best fixative for the oögonia and the early growth stages of the oöcytes, although Zenker's fluid and Hermann's fluid give very good results. After the yolk has formed Flemming's solution does not penetrate the egg sufficiently well to give a satisfactory fixation. For this later period I have found that the chromic-acetic solution recommended in a previous paper (King, 49) gives the best preparations. Corrosive-acetic acid is also a good fixative for the egg at this period of its development, but it is especially valuable for the maturation stages. Corrosive-formalin and picro-acetic do not give a satisfactory fixation of the egg of Bufo at any stage of its development.

Of the great variety of combination stains that have been experimented with at different times in the hope that it might be possible to differentiate the chromatin from the nucleoli, safranin and gentian violet, used in the manner recommended by Hermann (39), proved to be by far the best. This stain is rather difficult to use, but when a satisfactory preparation has been made, all of the plasmosomes are stained a vivid red and stand out in sharp contrast to the chromatin which is a deep blue, while the structures which I have called "compound-nucleoli" are stained purple. Much of the material was stained with iron hæmatoxylin and orange G. This combination does not differentiate the nucleoli from the chromatin; but it gives such clear, sharp outlines that it is of great value in studying the early stages in the development of the oöcytes when the chromatin stains but faintly and cell boundaries are difficult to determine. Borax carmine combined with Lyon's blue, safranin followed by Lichtgrün, and Delafield's hæmatoxylin with orange G. also give good preparations, particularly of the later growth stages of the oöcytes.

II. The Primordial Germ-Cells.

Owing to the large amount of yolk in the embryo and to the vagueness with which the cell boundaries are defined, it is impossible to trace the germ-cells in Bufo back to the segmentation stages of the egg, as has been done in other more favorable forms. Not until a tadpole is five or six days old and has attained a length of about 4 mm. can one point out definitely the group of cells that will develop into the genital ridge. At this stage of development the lateral plates of mesoderm (Fig. 1, L. M.) are well defined; the cells are small, with clear outlines, and they contain but comparatively little yolk. In the endoderm (Fig. 1, E.) on the contrary, the cells are very large; they are filled with yolk spherules which stain very deeply with iron hæmatoxylin, and their boundaries are irregular and difficult to determine. In the mid-dorsal region of the embryo there is usually found at this time a ridge of

cells (Fig. 1, G) which lies directly beneath the aorta (Ao.) and between the lateral mesodermal plates (L. M.). The cells of this ridge resemble the cells of endoderm in all respects and they are continuous with the endodermal cells which later form the lining of the digestive tract: there is no probability that these cells are of mesodermal origin, since they are always sharply marked off from the lateral mesodermal plates. When the tadpole is eight or nine days old this ridge of cells becomes separated from the endoderm, and it then forms a median cord of cells, the genital ridge, lying between the cardinal veins and supported by the mesentery (Fig. 2 G). The cells of the genital ridge are very conspicuous in sections at this time, as they still contain numerous large, deeply staining yolk spherules, although the neighboring cells have already absorbed the greater part of their yolk.

Many investigators who have studied the origin of the germ-cells in amphibians have asserted that these cells are derived from a germinal epithelium which is a modification of the peritoneal epithelium lining the body-cavity. This is the view advocated by Waldeyer (91), Semon (84), Hoffmann (44), Kolessnikow (55), Leydig (60), Spengel (86), Iwakawa (45), and more recently by Bouin (11). The last named investigator, however, admits the possibility that the first germ-cells in Rana are derived from endoderm, since in early stages of development they have all of the characteristics of the primitive endodermal cells. On the other hand, Nussbaum (73) maintains that in amphibians, and also in other vertebrates, the germ-cells are not derived from peritoneal epithelium, but that they are developed from undifferentiated embryonic cells which are set apart during early cleavage for this especial purpose. This theory has been supported by the researches of Woods (95) on Acanthias, and by Beard (7) on Raja batis. The latter investigator states: "The germ-cells may be regarded as unicellular organisms which pass one part of their life-history within a multicellular sterilized stock, the embryo or metazoön, formed by one of them at a definite period in the life-cycle."

In Bufo, as in the turtle and in the frog according to the investigations of Allen (1, 2), the germ-cells arise in connection with the endoderm. Allen's recent account of the origin of the sex-cells in Rana pipiens agrees essentially with what I have found in Bufo. I cannot be sure, however, that in Bufo the ridge of germ-cells is separated from the endoderm "by the approximation of the lateral plates of mesoderm," although many sections give this impression. During this early period of development, when so many organs are rapidly being differentiated from embryonic tissue, it is impossible to tell exactly what forces or combination of forces are at work shifting the materials from one place to another. It is possible, as Allen suggests, that the germ-cells themselves take an active part in the processes which separate them from the endoderm, since it is apparently only through their own activity that they reach their final position in the embryo. Since Hertwig (41), Boveri (12), and others have traced the germ-cells back to segmentation stages, and Conklin (21, 22) has found various organ-forming substances in definite areas in the unsegmented egg, it seems meaningless to speak of organs as arising from any definite "germ-layer," although the convenience of such a starting point for the study of the development of any structure is obvious. Owing to the character of the embryonic cells it is seemingly impossible to trace any organ in Bufo back to early cleavage stages, and the sex-cells are not clearly defined until the tadpole is about five days old. At this stage of development the germ-cells still retain their earlier embryonic character, and they are in contact with and closely resemble the endodermal cells. Instead of asserting that the germ-cells in Bufo are endodermal in origin, it seems to me more in keeping with the results of the investigations on other more favorable forms to assume that these cells in Bufo are of like generation with the primitive endodermal cells and that both kinds of cells arise from neighboring regions of the unsegmented egg. It may sometimes be possible to determine the organ-forming regions in the unsegmented egg of Bufo as Conklin has done in the egg of Cynthia.

When the genital ridge is first clearly marked off from the endoderm it occupies a median position between the cardinal veins and beneath the aorta (Fig. 2), as Bouin and Allen have stated is the case in Rana. If a section of the ridge in this stage of development is examined under high power one finds that it is composed of two distinct types of cells, one many times larger than the other (Fig. 3). The large cells, which are filled with yolk spherules and have vaguely defined boundaries, are the primordial germ-cells. The nuclei of these cells have the "mulberry" shape which La Valette St. George (78) discovered to be a characteristic of the nuclei in the spermatogonia of Salamandra, and they are usually crowded by the yolk spherules into one corner of the cell. The chromatin in these nuclei is in the form of minute, faintly staining granules which are distributed on linin threads or along the nuclear membrane. Each nucleus contains several rounded, deeply staining nucleoli of various sizes. Judging from their staining reactions most of these nucleoli are plasmosomes, and only one or two of the smaller ones are karyosomes. Scattered among these germ-cells, and frequently flattened against them, are numerous small cells which resemble in all respects the cells of the peritoneal epithelium from which they doubtless have been derived. These cells are very much smaller than the germ-cells; they contain no yolk and they have an elongated, deeply staining nucleus which is very large in proportion to the size of the cell. Doubtless these cells migrate into the genital ridge after the formation of the mesentery, since there are no cells of this type in the genital ridge at the stage of Fig. 1, and I have seen nothing that would indicate that they are derived from the germ-cells.

Bouin has stated that he finds in Rana temporaria transitional stages between peritoneal cells and primordial germ-cells, and he believes that before the metamorphosis of the tadpole new germ-cells are constantly arising from peritoneal cells. These observations have not been confirmed by Allen (2) in his study of the origin of the germ-cells in Rana pipiens, and in Bufo I can find no evidence that the germ-cells are

derived from peritoneal cells at any stage of development. The peritoneal cells in the genital ridge vary considerably in size and some may be nearly twice as large as others. In all cases, however, the cell contains comparatively little protoplasm and no yolk; while the nucleus maintains a characteristic appearance and stains very deeply, thus standing out in sharp contrast to the larger, more irregular, and more faintly staining nuclei of the germ-cells.

The development of the genital ridge proceeds from before backward. In a section of the anterior part of the ridge there are usually from 5-8 large germ-cells (Fig. 3), while in a more posterior section there are rarely more than three of these cells. In older tadpoles the difference in the rate of development of the different parts of the genital ridge is even more strongly marked, since the anterior portion of the ridge may have taken on its definite character as an ovary or a testis while the posterior portion remains in an apparently indifferent state.

When a tadpole is ten or eleven days old, the yolk spherules begin to disappear from the cells of the genital ridge and the structure of the germ-cells can then be more clearly seen (Fig. 4). At this time the germ-cells are more rounded than they were at an earlier period and, as they contain fewer and smaller yolk spherules, the polymorphic nucleus is usually found in the centre of the cell. With the exception of the large plasmosomes, the nuclear contents still show little capacity for staining either with plasma or with chromatin stains. By this time many peritoneal cells have become flattened against the germ-cells and have thus assumed the rôle of follicle cells. The boundaries of these follicle cells become very indistinct, and in many cases the cytoplasm seems to disappear entirely leaving the deeply staining nuclei in contact with the germ-cell.

In early stages of development the germ-cells are not always confined to the genital ridge. At the right, in Fig. 4, is a cell (Y) which lies considerably outside of the germinal area and directly under the Wolffian tubule; in Fig. 5, at the

left of the aorta are two germ-cells which lie above the level of the genital ridge. Such germ-cells must eventually come into the germinal area or degenerate, since cells of this character are never found outside of the genital ridge in later stages of development. I have never found cells with the characteristics of germ-cells in the mesoderm or in the ectoderm.

In a tadpole twelve to fourteen days old there is usually found the beginning of a separation of the median genital ridge into two ridges symmetrically placed one on each side of the middle line (Fig. 5). This division of the genital ridge is evidently brought about through the activity of the germ-cells, although I have never been able to find any evidence of amœboid movement in these cells. A longitudinal section through a tadpole thirteen days old (Fig. 6) shows that, at the time the genital ridge is dividing, the germinal area extends from about the level of the liver nearly to the posterior end of the body-cavity. When the division is completed the anterior portion of each genital ridge contains from two to five germ-cells (Fig. 7), while the middle and posterior portions rarely contain more than one or two germ-cells (Fig. 8). Sections through the posterior region of a genital ridge frequently contain only the peritoneal cells (Fig. 9) which seem to be crowding into the germinal area in increasing numbers at this time.

The primordial germ-cells in the sex-gland of a tadpole about to undergo metamorphosis are similar to those found in the genital ridge at the stage of Fig. 4, except that they contain only a small amount of yolk. After the greater part of the yolk has been absorbed there is found in the cytoplasm of these cells a small, round, deeply staining, apparently homogeneous body which is sometimes, though not invariably, surrounded by a clear area (Fig. 8, V). This body, which I shall call the vitelline body, divides previous to the cell mitosis (Fig. 7, V), and one of these bodies is to be found subsequently in each of the daughter cells.

In addition to the vitelline body, there is found in the

cytoplasm of the germ-cells, usually close to the nucleus, a small centrosome which is surrounded by a rounded, granular attraction-sphere (Fig. 8, C). This centrosome divides very early in preparation for the cell mitosis, and, as shown in Fig. 7, it is sometimes possible to find a section of a cell which contains two centrosomes as well as two vitelline bodies. Such a section shows conclusively that the vitelline body is not derived from the centrosome and that there is no relation between these bodies. I have, as yet, no clue to the origin of the vitelline body which, as will be shown later, is undoubtedly concerned in the formation of yolk nuclei. A structure similar to the vitelline body is found in the cytoplasm of the spermatogonia of Bufo, and it can be traced directly to the spermatids where it gives rise to the acrosome of the mature spermatozoön. Since Meves (69), McGregor (63), and Broman (13) have found that the acrosome of the amphibian spermatozoön is derived from the idiozome, I suggested in a previous paper (King, 52) that the body in Bufo which forms the acrosome might possibly be derived "from a condensation of a portion of the attraction-sphere at an early period in the history of the primary spermatogonia." My study of the primordial germ-cells has not given any support to this hypothesis since, although this body is usually found near the attraction-sphere (Fig. 7), the two structures are clearly distinct at all times and there is not the slightest evidence that the former is derived from the latter. In its size and general appearance the vitelline body closely resembles the small nucleoli in the nuclei of the primordial germ-cells, but I have seen nothing that would indicate that it is of nucleolar origin. The later history of this structure in the ova strongly suggests that it is a secretion product of the cytoplasm formed, possibly under the influence of the nucleus, but not from nuclear material.

According to the investigations of Bouin, the increase in the number of germ-cells in Rana is brought about through a continuous process of transformation of peritoneal and mesenchyme cells into sex-cells, not by mitosis nor by direct division

of the germ-cells already present in the germinal area. In Bufo I have found that the multiplication of the germ-cells is solely through mitotic division of the primordial cells evolved from embryonic issue. Although mitotic figures are comparatively rare during the early stages of development they are found very abundantly when the tadpole approaches metamorphosis, and in a single section of the ovary of a toad killed at this time one may find several cells that are preparing to divide (Fig. 17, P). Stages in the division of the primordial germ-cells are shown in Figs. 10-14. In the early prophase of mitosis the chromatin forms a thick spireme which is so much convoluted that it is impossible to determine whether it is continuous or not. This spireme is subsequently broken into segments of various lengths (Fig. 10). There are 24 of these segments, this being the number that is characteristic of the somatic cells of the species. Usually all of the nucleoli have disappeared before the segments are formed, but sometimes, as shown in Fig. 10, Nu., a nucleolus will persist until a much later period. This would seem to indicate that the nucleoli are not used in the formation of the chromosomes. The chromatin segments shorten gradually and form broad, V-shaped loops which can readily be arranged in pairs according to their lengths (Figs. 11, 12). In the metaphase the chromosomes are arranged in a circle with the angle of the V turned towards the centre of the spindle (Figs. 11, 13, 15); and, as they subsequently undergo a longitudinal division, much narrower V-shaped chromosomes are found at the spindle poles in the late anaphase (Fig. 14).

In sections of the ovary of a tadpole killed at the time of metamorphosis germ-cells are frequently found which appear to contain two or more separate nuclei (Figs. 15, 16, X). Judging from these figures alone one might feel justified in concluding that the germ-cells divide amitotically as well as by mitosis. I have never found a division of the cytoplasm in any of the cases in which sections of the germ-cells contain two or three nuclei, and in every instance the following or preceding sections invariably show a connection between the various nuclei in the cell. It is evidentfi therefore, that the

apparently multinucleated cells are not preparing to divide by amitosis. Their appearance is doubtless due to the fact that in sectioning the cells the polymorphic nuclei were cut in such a way as to completely separate two or more lobes. In my study of the germ-cells of Bufo I have never found a single instance where I could be sure that a cell was dividing amitotically; and I am convinced that this mode of division does not normally occur in any of the germ-cells of the ovary or of the testis.

By the time that a tadpole is sixteen to eighteen days old the anterior portion of each genital ridge has developed into a small rounded body, the so-called "Bidder's organ." The structure and development of this organ will form the subject of a separate paper and therefore no further mention of it will be made here, as it has seemingly nothing to do with the development of the ova.

Although sex is doubtless determined at a very early stage of development, the germ-cells of Bufo remain in an apparently indifferent condition for a long period, and it is not until the tadpole is about to undergo metamorphosis that its sex can be ascertained with any degree of certainty. Several investigators of amphibian oögenesis have stated that the presence of a central cavity in the genital ridge is the first characteristic by which the young ovary can be identified. In Bufo it is possible to distinguish the sexes at a somewhat earlier period of development by means of the arrangement of the cells in the more anterior portion of the sex-gland. In the young male the germ-cells are scattered evenly throughout the testis, each being surrounded by a number of follicle cells; in the young female the germ-cells have a definite arrangement around the outside of the ovary, while the centre is filled with peritoneal cells (Fig. 15). There is no central cavity in any part of the genital ridge at this time.

When the genital ridge has taken on the definite character of an ovary, some of the oögonia still contain a few small yolk spherules (Fig. 15), although all traces of yolk have long since disappeared from the other cells of the body. There is no ovarian wall at this time and the oögonia are surrounded

by follicle cells as in an earlier period. At a slightly later stage of development (Fig. 16), the central part of the ovary is no longer completely filled with peritoneal cells, but it contains a number of intercellular spaces which later unite to form one large cavity (Fig. 17). The central cavity in the ovary of Bufo is not, therefore, a portion of the general body-cavity which is brought into the ovary by a fold of peritoneal epithelium as Hoffman has claimed is the case in Triton and in various other amphibians, but it is the result of a fusion of the many intercellular spaces which are produced by the rapid increase in the size of the ovary. In Fig. 16 is shown the beginning of the formation of the outer ovarian wall. At the upper part of the ovary a number of peritoneal cells are found with their nuclei flattened against the outer surface of the germ-cells. The outlines of these cells become obliterated and their cytoplasm forms a continuous layer over the oögonia. At a slightly later stage (Fig. 17), many of the peritoneal cells in the interior of the ovary become arranged along the inner side of the oögonia to form the inner wall of the ovary. In the young female as well as in the adult, the ova develop between the two ovarian walls.

The small cells with deeply staining nuclei which are so conspicuous in the ovary at the stages of Figs. 15-17 have been called by various observers mesenchyme cells, peritoneal cells, and follicle cells; while Bouin considers them to be "petites cellules germinatives." In Bufo these cells are found with the primordial germ-cells when the latter are first separated from the endoderm at the stage of Fig. 3, and from their general characteristics they are doubtless to be classed as mesodermal cells. Occasionally these cells are found dividing mitotically (Fig. 15, R); but division figures in them are rare as compared with those that are found in the germ-cells. The number of these cells increases enormously as the ovary enlarges; and, since there is no evidence that they divide amitotically, it is probable that there is a continuous migration of cells from the mesentery through the ovary pedicle into the ovary. Many of these cells later become the follicle cells which are found around the egg as long as it remains in the

ovary; the others, as far as I can determine, are actively concerned in the formation of the ovarian walls, the cyst membranes and the zona pellucida.

According to the observations of Bouin there are fewer primordial germ-cells in a tadpole of Rana temporaria that is 33 mm. long than in one 20 mm. long. As the difference in numbers is considered too great to be attributed to individual variation, Bouin believes that a reduction in the number of primordial germ-cells is brought about at this stage of development through an expulsion of a large number of these cells from the ovary into the body-cavity. He calls this process "ponte d'ovules primordiaux," and he considers that it is analogous to that which occurs in the adult frog when the ripe eggs are expelled from the ovary. Bouin suggests that this process may take place so that "la glande, qui évolue dans le sens mâle, élimine les éléments qui seraient inutiles à son développement ultérieur." None of the other investigators who have worked on the development of the sex-glands in amphibians have described such a reduction in the number of primordial germ-cells and there is nothing similar to it to be found in Bufo. The expulsion of primordial germ-cells from the ovary is, therefore, either a process that is peculiar to Rana temporaria, or it is one which takes place so quickly in other species that it has escaped the attention of the investigators working in this field.

As the tadpole approaches metamorphosis, the ovary increases in size very rapidly and it usually appears lobed when examined in toto under a low power of the microscope. This lobed appearance of the young ovary furnishes a means by which it can be distinguished from the testis without making use of sections.

III. The Secondary Oögonia.

Soon after metamorphosis the primary oögonia give rise to a new generation of cells, the secondary oögonia, which are aggregated into cysts or "cell nests" that are arranged much

as are the primary oögonia shown in Fig. 17. The cells of a cyst are all descendants of one primary oögonium, and the cyst wall is formed evidently by the follicle cells which had previously surrounded the parent cell. The secondary oögonia are somewhat smaller than the primary oögonia, but they closely resemble them otherwise. They have a polymorphic nucleus containing a faintly staining reticulum and several plasmosomes. In the cytoplasm is a vitelline body (Fig. 18, V) and also a minute centrosome surrounded by a granular attraction-sphere (Fig. 18, C).

The cells of a cyst do not always divide simultaneously, and resting cells as well as cells in all stages of division may be found in the same cyst (Fig. 19). In the early prophase of mitosis a thick spireme is formed, as in the primary oögonia. This spireme breaks into segments (Fig. 19, S), presumably twenty-four, which condense into V-shaped chromosomes in the metaphase (Fig. 19, O). The spindle is the same shape as that found in the earlier generations of cells, and there are distinct centrosomes at the spindle poles which are devoid of any radiation (Fig. 19, O, R).

IV. The Development of the Oöcytes to the Synizesis Stage.

Considerable controversy has arisen among investigators regarding the origin of the oöcytes in the amphibian ovary since, at the period of the transformation of oögonia into oöcytes, cell and nuclear boundaries are frequently obscured and the cyst contents appear as a syncytium.

In his classic work on Bombinator igneus, Göette (35) states that in the young ovary the protoplasmic bodies of the central cells of a cyst fuse into a single mass which contains at first several separate nuclei; later the nuclei also fuse to form the mulberry shaped germinal vesicle of the egg. This view has been slightly modified by Bataillon (6), who concludes, from his observations on Rana and on Bufo, that after the fusion of the cytoplasmic bodies of the cells of a cyst one

of the nuclei wins the upper hand and subsequently absorbs all of the others.

Gemmil's (34) observations seem to indicate that "in der Regel geht aus einem Zellnest nur ein Ei hervor, und zwar durch directe Entwickelung aus einem der Elemente des Zellnestes. Von den übrigen Elementen bilden sich einige wieder zurück und betheiligen sich an der Bildung der Granulosa, der Rest aber geht zu Grunde." According to Gemmil, there appears to be a struggle among the cells as to which shall form the ovum; space being the chief factor which decides the contest. The cell which lies in the centre of a cyst has seemingly the most room for development and this is the one which usually wins. The fate of the other cells depends upon how far they have differentiated before the one cell becomes the ovum and so governs the rest. The cells which are least differentiated assume the rôle of granulosa cells. Those further developed cannot go backwards; they either have to become eggs or disintegrate. If the cyst happens to be larger than usual, as many as four of the cells may have room to develop into functional eggs. Since extra space is rarely obtained by the cyst, all of the cells which have passed a certain stage of development before the egg has formed are, as a rule, forced to disintegrate, and traces of the débris from these cells are to be found for some time in the protoplasm of the developing egg. Hoffmann's opinion regarding the origin of the egg in the Anura is similar to that of Gemmil, since he believes that one cell of a nest outstrips the others in development and forms the ovum while the others degenerate and become granulosa cells. Semon's observations lead to a similar conclusion.

Nussbaum and also Knappe (54) find a mulberry shaped nucleus in the primordial germ-cells, and they assert that this nucleus divides by amitosis into several small nuclei. One of these nuclei increases rapidly in size and becomes surrounded by the greater part of the cytoplasm of the cell, thus forming the egg; the other nuclei become arranged around the periphery of the egg to form the follicle epithelium. Ac-

cording to Eismond (27) an ovum may arise either from one of the cells of a nest which has outstripped the others in development, or from a fusion of all of the cells of a cyst. He also considers that "la formation des nids n'était pas un anneau indispensable dans le cycle de l'oögenèse, c'est-a-dire qu'en même temps que la formation des nids du sens strict, se faisait aussi la différenciation progressive des oöcytes directement aux dépens des produits de la dernière division des oögonies, comme cellules independantes." The conclusion that ova may arise directly from oögonia accords with the view advanced in 1870 by Waldeyer (91) and supported later by the researches of Balfour (4) on elasmobranchs.

Bouin has investigated the formation of the ova in much greater detail than have any of the other workers on amphibian oögenesis. He finds, as do other investigators, that secondary oögonia are enclosed in cysts, and he states that all of the oögonia in a cyst divide simultaneously. After several divisions, the number of which he does not determine, the character of the cells changes considerably and "oögonia of transition" are formed. The latter are clearly defined cells with rounded nuclei in which there are several chromatin nucleoli, but no traces of a chromatin reticulum. This stage is succeeded by one in which the nuclear membrane disappears and the karyoplasm is separated from the cytoplasm only by clear area. At a later stage of development granular threads appear in the nucleus which are formed, doubtless, of the minute chromatin granules scattered in the karyoplasm. These threads increase in number very rapidly and form a distinct chromatin reticulum, while a new nuclear membrane encloses the nuclear contents. All the cells of a cyst develop up to this stage, but later, owing to some unknown causes, only a part of the cells continue their development as oöcytes; the others degenerate and are either dissolved gradually or devoured by the phagocytes. Degenerating cells never form follicle cells but probably serve as nutriment for the victorious oöcytes. The results of Bouin's investigations agree essentially with those reached by Balfour in his study of

elasmobranchs. The latter investigator states that "some of the nuclei of each nest are converted into the nuclei of the permanent ova, others break down and are used as the pabulum at the expense of which the protoplasm of the young ovum grows."

Judging from the number of cells in a fully formed cyst, there are at most four or five generations of secondary oögonia in the ovary of Bufo. After the last oögonial division resting nuclei are formed, and the cyst is filled with small cells which appear much like that shown in Fig. 20. At this time cell and nuclear boundaries are very much more indistinct than in earlier stages, yet they can readily be made out in preparations fixed in Flemming's solution and stained with iron hæmatoxylin. If the material is properly preserved the cells never form a syncytium; nor is there any fusion of the nuclei, or any absorption by one nucleus of its less fortunate neighbors. Each cell in a cyst develops into an oöcyte, and, although I have examined a large number of cysts in this stage of development taken from many different individuals, I have yet to find a single instance in which there is a degeneration of any of the germ-cells in a cyst or any change of germ-cells into follicle cells. It seems very probable that the cells which several investigators have considered to be degenerating young oöcytes, were, in reality, cells in which the nuclei were in the condition shown in Fig. 25. This contracted state of the nuclear contents, to which McClung (62) has applied the term synizesis, is a definite constructive stage in the development of the young oöcyte of Bufo, and it is not due in any way to a degeneration of the nucleus or of the cell.

Owing to the crowded condition of the cells in a cyst the young oöcyte is more or less polygonal in outline. The nucleus is very large in proportion to the size of the cell, and it is invariably oval or slightly irregular, never possessing the polymorphic form characteristic of the nuclei in the earlier generations of cells. At this period the chromatin shows little capacity for staining and, as in the resting oögonia, it is in the form of minute granules which are either scattered along

the nuclear membrane or distributed on the linin fibres which form an irregular reticulum. The nucleus contains several deeply staining nucleoli of various sizes which are suspended in the meshwork of the reticulum or held against the nuclear membrane. In preparations stained with safranin and gentian violet the larger nucleoli invariably take the safranin while the rest of the nucleus is stained blue with the gentian violet, and these bodies must, therefore, be considered as plasmosomes; the very small nucleoli, which are found chiefly at the points of intersection of the linin threads, are karyosomes since they take the chromatin stain. In the cytoplasm, which stains very faintly and appears somewhat reticular, there is a vitelline body (Fig. 20, V); but I have not been able to find any traces of a centrosome or of an attraction-sphere in this or in any later period in the development of the oöcyte. As there are no centrosomes at the poles of the maturation spindle (King, 51), it seems probable that the egg centrosome disappears after the last oögonal division and that the attraction-spheres found at the poles of the segmentation spindle are formed in conjunction with the sperm-nucleus, probably under the influence of the centrosome imbedded in the substance of the sperm-head.

As the oöcyte enlarges its outline becomes more regular and much more distinct. The nucleus, which measures about 0.01 mm. in diameter at this time, soon assumes the rounded form which it retains up to the maturation period (Fig. 21), and its reticulum appears continuous and much more sharply defined than at an earlier period (Fig. 22). The number of nucleoli is not appreciably increased during the early growth period of the oöcyte.

V. Synizesis and Post-Synizesis Stages.

Although the stage in the development of the oöcyte shown in Fig. 22 is practically at the beginning of the growth period it corresponds, apparently, to the stage at the end of the growth period of the spermatocyte (King, 52; Fig. 15). In

both cases the nucleus contains a granular reticulum which appears to be continuous; and in both oöcyte and spermatocyte this stage is followed immediately by one in which there is a gradual condensation of the nuclear contents leading to synizesis (Fig. 25). The beginning of the process of condensation in the oöcyte is shown in Fig. 23, where the greater part of the chromatin reticulum is seen to be collected in the centre of the nucleus. In the following stage the contraction of the nuclear reticulum becomes more marked (Fig. 24), and eventually all of the nuclear contents forms a more or less rounded mass in the centre or at one side of the nucleus (Fig. 25). In favorable preparations the contraction figure is found to be composed of a tangled mass of exceedingly fine filaments in the meshes of which there are several round, apparently homogeneous bodies which are doubtless the plasmosomes: a number of the filaments run out from the central body and connect this structure to the nuclear membrane. At this stage it is impossible to follow in detail the changes that are taking place in the nucleus or to determine what relation the fine filaments bear to the nuclear reticulum of the earlier stage. The condensation of the nuclear contents in synizesis is not carried quite as far in the oöyctes of Bufo as it is in the spermatocytes where the contraction figure frequently appears as a rounded, apparently homogeneous mass connected by a few fine filaments to the nuclear wall (King, 52; Figs. 20-22).

In toads killed at the time of metamorphosis the ovaries contain large numbers of secondary oögonia and young oöcytes, although only a few of the latter have reached the synizesis stage at this time. Contraction figures are frequently met with in the ovaries of young toads killed about four weeks after their metamorphosis, and they are found very abundantly afterwards until the toad has attained a body length of about 1.5 cm. As I have already pointed out in the case of the spermatocytes of Bufo, I do not think it possible that the contraction figures are due to a bad preservation of the material as Janssens (47) has asserted is the case in Batracoseps atten-

uatus, since oöcytes with their nuclei in synizesis are found in all parts of the ovary and frequently lie adjacent to oöcytes in which the chromatin is in the form of a clearly defined continuous spireme (Fig. 22). Any method of fixation that would cause such a decided distortion of the nuclear contents in the one cell must of necessity have some effect on a neighboring cell which is in but a slightly different stage of development. In Bufo synizesis is not due to the degeneration of certain cells as Kingsbury (53) has claimed is the case in Desmagnathus fusca, since only in very rare instances are degenerating eggs to be found in the ovaries of young toads. Degenerating eggs, whether they are found in the ovaries of young toads or in those of adults, are usually deeply pigmented and they are invariably filled with phagocytes; they never resemble in any way the oöcytes shown in Figs. 24-25.

Synizesis, which is a well recognized stage in the development of the oöcytes and spermatocytes of many forms, has, for the most part, been ignored by the investigators who have worked on the germ-cells of amphibians, or its presence has been considered as evidence of a degeneration of the cell. Gemmil describes a stage in Pelobates fuscus in which the nucleus of the young oöcyte contains a star-shaped mass of chromatin which lies in the middle of a clear area and sends out processes to the nuclear membrane. It is evident, from the figures which Gemmil gives, that synizesis is the normal stage in the development of the ova of this amphibian. Nussbaum figures condensation stages in the young germ-cells of Rana fusca when they are enclosed in a cyst membrane. He has, however, mistaken the order of sequence in the development of the cells, as he considers that the contraction stage preceded one in which the cell contains a mulberry-shaped nucleus. Bataillon, Leydig, and Hoffman also mention the appearance of contracting figures in the course of the normal development of amphibian ova, although they venture no opinion as to the significance of these bodies.

Bouin has entirely overlooked in Rana temporaria the young oöcytes shown in my Figs. 20-22, and the earliest stage that he

figures as an oöcyte (Plate XII; Fig. 6), is about like that of my Fig. 39. He does not believe that synizesis is a normal stage in the development of the oöcytes of Rana, although he figures contraction stages of the nuclei in cells which he considers as oögonia that are not able to develop into oöcytes. His description of the nucleus of one of these "degenerating" cells is as follows: "On constate que les microsomes constitutifs du réticulum chromatique se gonfluent, se soudent les uns aux autres, forment des amas irréguliers qui se colorent comme les chromosomes des noyaux en mitose. Ces amas peuvent rester isolés dans l'aire nucléaire ou s'amalgamer en un bloc chromatique de faibles dimensions." The one figure which Bouin gives of such nucleus (Plate XI; Fig. 15), shows the synizesis stage in Rana which corresponds closely to that in Bufo shown in Fig. 25; and many of his other figures show post-synizesis stages comparable to those in Bufo (Plate XI; Figs. 10, 11: Plate XII; Figs. 2-5).

In a recent paper Lams (57) has given a description of the stages in the early development of the oöcytes of Rana temporaria which were overlooked by Bouin. According to this investigator the nuclear membrane does not disappear at any time during the transition of the oögonia into the oöcytes. In the young oöcytes the chromatin filaments gradually condense at one pole of the nucleus until they form a rounded, deeply staining mass which appears much like that shown in my Fig. 25. In post-synizesis stages this contracted mass resolves into a system of filaments which subsequently divide longitudinally and scatter throughout the nucleus. This work of Lams, with that of Bataillon and Leydig, furnishes conclusive evidence that synizesis is a normal stage in the development of the oöcytes of Rana.

It is unfortunate that the contracted condition of the nuclear contents during synizesis prevents a detailed study of the changes taking place in the chromatin at this time. It is evident that during synizesis the nuclear reticulum is no longer continuous, and that it becomes broken up into a large number of exceedingly fine filaments. Some of these filaments appear

to be composed of a series of minute granules; others of delicate linin threads. As the plasmosomes can still be found during synizesis it is probable that they play no part in the changes taking place in the chromatin.

From the contraction figure shown in Fig. 25 there is evolved a long, apparently continuous, much convoluted spireme which is made up of a series of deeply staining chromatin granules distributed on a linin thread (Fig. 26). In the meshes of this spireme there are several nucleoli of various sizes, and there are also from one to five irregularly shaped, apparently homogeneous nuclear masses which are distributed along the nuclear membrane. These masses all stain intensely black with iron hæmatoxylin as does also the spireme. If, however, preparations have been satisfactorily stained with safranin and gentian violet the spireme is deep blue, the very small nucleoli appear red, while the large nucleoli and the masses against the nuclear membrane are purple, thus indicating that they are composite structures although they usually appear homogeneous at this time.

From the stage shown in Fig. 21 to that of Fig. 26 the oöcytes do not grow to any appreciable extent and the nuclei measure from 0.011-0.013 mm. in diameter. After synizesis there is a rapid increase in the amount of cytoplasm and in the size of the nucleus (Fig. 27). The chromatin spireme becomes more evenly distributed throughout the nuclear space, and it is noticeably thicker than at the stage of Fig. 26. In the succeeding stage the spireme begins to split longitudinally (Fig. 28). As the sister portions of the spireme are only about one-half of the thickness of the spireme at the stage of Fig. 27 it is evident that there is a true longitudinal division of the spireme at this time and not a folding together of chromatin filaments similar to that which occurs in the young oöcytes of the rabbit according to the investigations of von Winiwarter (93). At the stage of Fig. 29 the greater part of the spireme has divided and many of the sister threads have separated a considerable distance. When the splitting of the spireme has been completed the sister threads lie parallel, for the most

part, although they are not connected in any way. The threads do not present the clear cut, granular appearance of the spireme shown in Fig. 26, as they have a jagged outline and send out fine projections on either side.

There is absolutely no uniformity in the arrangement of the chromatin threads after the splitting of the spireme. At times the sister threads seem to lie close together throughout their whole extent (Fig. 30); again the sister portions of the spireme lie parallel for a short distance and then become widely scattered throughout the nucleus (Fig. 31); in rare cases, as shown in Fig. 32, the chromatin threads are as evenly distributed throughout the nucleus as they are at the stage shown in Fig. 27, and there is nothing except the size of the nucleus and the character of the threads to indicate that there has been a splitting of the spireme. I am very sure that such a condition of the chromatin as that shown in Fig. 32 could not have been brought about by a gradual lengthening of the spireme, since the great majority of nuclei intermediate in size between that of Fig. 27 and that of Fig. 32 appear similar to those shown in Figs. 28-31.

Soon after the stage of Fig. 30 the spireme breaks transversely, forming, in most cases, long double segments which vary considerably in length (Figs. 33, 34, 36, 37). The sister portions of the segments may lie parallel or they may be intertwined in various ways; they may be united at one or at both ends, forming a figure 8 or an oval ring; in other cases both ends of the segments are free and the threads cross in the form of an X or a Y. The condition of the chromatin threads shown in Figs. 30-34 is found in nuclei having a diameter of 0.015-0.02 mm.

I have tried to reconstruct a nucleus in the stage of development shown in Figs. 33-34, by placing together a series of camera drawings of all of the sections of the nucleus, in the hope that I might be able to determine by this means the total number of chromatin segments. Owing to the fact that the segments are of different lengths and that they are united in a great variety of ways, it has been very difficult to arrive at any

exact conclusion regarding their number. I believe, however, that the nucleus at this stage contains only the somatic number of chromosomes (24) which are usually arranged in twelve pairs. The question at once arises as to the value of the sister segments which form a pair. Is the splitting of the spireme shown in Figs. 28-30 a longitudinal division of chromosomes united end to end in the spireme or is it a separation of univalent chromosomes which had conjugated side by side? This question is very difficult to answer since it is impossible to determine what changes the chromatin undergoes during synizesis. As the nucleus apparently contains but twenty-four chromatin segments which in later stages of development are scattered throughout the nucleus and only occasionally found in pairs, I am inclined to the opinion that each of the sister segments represents an oögonial chromosome. The paired arrangement of the chromosomes at the stage of Figs. 33-34 strongly suggests that in the oöcytes of Bufo synapsis is coincident with synizesis as it is apparently in the spermatocytes; yet for various reasons, which will be given later, I am inclined to consider that synapsis does not occur until the beginning of the maturation period.

At the stage of Figs. 20-21 all of the young oöcytes in a cyst are approximately of the same size and in practically the same stage of development. As the synizesis period approaches the oöcyte which lies nearest the cavity of the ovary grows very rapidly and soon becomes several times the size of its neighbors. A section of a cyst with the oöcytes in this condition is shown in Fig. 37. The large cell bordering the cavity of the ovary has a diameter of 0.043 mm., and its nucleus measures 0.023 mm. in diameter. This oöcyte is surrounded by a number of follicle cells and its nucleus contains paired chromatin threads. The other cells in the cyst are very nearly of the same size; each measuring about 0.015 mm. in diameter and containing a nucleus measuring 0.01 mm. in diameter. These smaller oöcytes are in early post-synizesis stages of development, and they are not degenerating, as several investigators who have found a similar condition of the

cells of a cyst have claimed. The development of these cells is slower than that of the one cell simply because the size of the cyst is limited and there is no space for a more rapid growth.

Soon after the stage shown in Fig. 37 the cyst wall is ruptured, owing doubtless to the pressure of the growing oöcytes, and the larger cell becomes separated from the rest of the cyst and surrounded by a membrane which attaches it to the wall of the ovary. Inside of this membrane there are always found a number of elongated follicle cells which are undoubtedly concerned in the formation of the zona pellucida which later develops around the egg (Figs. 36, 39). As the other cells of the cyst enlarge each in turn becomes similarly attached to the ovarian wall. The cysts do not all develop at the same rate. In the ovaries of toads with a body length of 1.5 cm. one may find some cysts containing oögonia, others filled with young oöcytes in various stages of development up to that shown in Fig. 37, while in many cases the cysts have become disorganized and the ova are separately attached to the ovarian wall.

VI. The Nucleoli and the Later Growth Stages of the Oöcytes.

The irregular shaped masses of nuclear substance found against the nuclear membrane or in the meshes of the chromatin reticulum at the stage of Fig. 26 seem to increase in size as the nucleus grows and one of them usually becomes much larger than any of the others. These bodies appear homogeneous and stain black with iron hæmatoxylin or purple when the preparation is stained with safranin and gentian violet. When the nucleus has attained a diameter of about 0.025 mm. and the splitting of the spireme has been completed, numerous fine granular fibres are seen to project from these masses which do not stain quite as intensely as before (Fig. 34). At the next stage (Fig. 35) one obtains the first clue to the structure of these bodies. With the use of iron

hæmatoxylin the masses now appear grayish, and they are found to be composed of a meshwork of exceedingly fine fibres inclosing several darker homogeneous bodies. In preparations stained with safranin and gentian violet a much better differentiation is obtained. The meshwork of fibres invariably takes the blue of the gentian violet, while the rounded bodies in the interior, which are of various sizes, react differently towards the stain; the larger of these bodies, which are usually slightly irregular in outline, stain a reddish purple; the medium-sized ones, which are rounded and have a smooth outline, stain uniformly red, while the very small granules take the gentian violet. From the staining reactions of these masses, therefore, it is evident that they contain two different substances; fine fibres which are doubtless composed of chromatin not used for the chromosomes, and rounded bodies which are nucleoli.

For convenience in description I shall apply the term compound-nucleoli to the complex masses shown in Figs. 26-35 and also to the irregular nucleolar bodies shown in Figs. 39, 40, 43, 45, etc., reserving the term nucleoli for the smaller rounded bodies found in the interior of the larger masses at the stage of Figs. 35-36. The nucleoli which stain uniformly red with safranin will be called plasmosomes, while those that stain like chromatin will be considered karyosomes. In order to distinguish the chromatin of the chromosomes from that of the meshwork which forms part of the compound-nucleoli I shall refer to the former as "basichromatin" and to the latter as "oxychromatin." I am aware that these terms are not being used strictly in the sense in which they were introduced by Heidenhain (37), since both kinds of chromatin show the same color reactions with all methods of staining employed. Their use has been considered advisable here, however, in order to avoid the introduction of new terms.

At the stage in the resolution of a large compound-nucleolus shown in Fig. 36, the oxychromatin meshwork is much more clearly defined than at an earlier period and the threads are thicker and more granular. The number of nucleoli found in

the nucleus at this time greatly exceeds that found at any previous stage in the development of the oöcyte, and it is evident that a new formation of these bodies must take place during or soon after the synizesis stage. The compound-nucleoli in a nucleus do not resolve simultaneously. The larger masses are always the first to break up, and one or two of the smaller bodies may remain unchanged until the nucleus is twice the size of that shown in Fig. 36. Soon after the stage of Fig. 36 the meshwork of fibres becomes very loose and frequently breaks into several parts, while the nucleolar bodies begin to leave the fibres and scatter about the nucleus (Fig. 38). At the stage of Fig. 39 the resolution of the largest compound-nucleolus has been completed and the nucleus contains a number of nucleolar bodies of various sizes as well as several masses of tangled oxychromatin threads which are entirely separated from the nucleoli and easily distinguished from the chromosomes.

In his Fig. 15, Bataillon shows a portion of the nuclear contents of an ovarian egg of Rana which is very similar to one of the larger resolving masses shown in my Fig. 38. Bataillon believes that his figure shows the beginning of a connection between the chromatin filaments and the nucleoli, and he states that later the filaments disappear entirely, all of their substance going into the uncleoli. These results do not accord with what I have found in Bufo, since in the oöcytes of this amphibian the nucleolar bodies are preparing to leave the chromatin meshwork at the stage of Fig. 38, and chromatin filaments are to be found in all of the later growth stages of the ova.

At the stage of Figs. 33-34, the chromosomes stain somewhat more faintly than at an earlier period, and they are composed of a series of minute granules from which numerous fine fibres extend out a short distance on either side. In later stages these side projections become much more numerous and somewhat longer, and the chromosomes then come to have the feathery appearance shown in Fig. 39. At a later period the chromosomes stain so very faintly that in many cases they are to be found only with the aid of an immersion lens, yet

they retain the characteristic structure shown in Figs. 39, 40, 43, etc., and are therefore always to be distinguished from the oxychromatin threads. The chromosomes are never united with the nucleoli, although sometimes, as shown in Figs. 36, 37 and 39, a nucleolus is in contact with a chromatin thread; neither is there any connection between the basichromatin filaments and the oxychromatin threads. The latter stain much more intensely than the former and always appear to be composed of a series of rounded granules, they never have the feathery structure of the chromosomes. After the stage of Figs. 33-36, the chromosomes become widely distributed throughout the nucleus, and only a very few of them are found paired in later stages of development.

In a preliminary paper on the oögenesis of Triton, Janssens (46) gives a brief account of the changes taking place in the young oöcytes which seems to show that the behavior of the chromatin in the eggs of this amphibian is somewhat different from that I have found in Bufo. Janssens finds that synizesis occurs during the early growth period of the oöcyte, but he states that the reduced number of chromatin filaments appears shortly after this stage and that these filaments subsequently split longitudinally, the sister threads always remaining together in later development.

Carnoy and Lebrun (15-18; Lebrun, 58, 59) have written an elaborate series of memoirs dealing with the germinal vesicle and the polar bodies in various species of Batrachians. They have not studied the primordial germ-cells or the early growth stages of the oöcytes, and in every case their investigations begin with the young ovum at a stage about like that of my Fig. 27. Although the details of the developmental processes in the ova differ somewhat in the various species, Carnoy and Lebrun invariably find that, in the earliest stage which they have studied, the nucleus contains a chromatin filament which seems to be continuous. Later this filament disintegrates and gives rise to "primitive nucleoli" which move to the centre of the nucleus and there resolve into chromatin threads of various types. These chromatin threads soon break up into minute

granules from which new nucleoli develop to undergo the same series of changes as their predecessors. When the germinal vesicle disintegrates at the beginning of the maturation period certain of the nucleoli escape dissolution to form the twelve chromosomes which undergo a double longitudinal division in preparation for the maturation mitoses. At certain periods during the development of the ova, therefore, the nucleus contains no chromatin except that found in the nucleoli, and there is no "individuality" of the chromosomes or any reduction in the Weismannian sense during the maturation divisions. For Carnoy and Lebrun (16) the most important structures in the nucleus are the nucleoli. "Les nucléoles sont le chef-d'œuvre du noyau: ils représent le degré le plus élevé de l'organisation nucléinienne." In another paper (15) the statement is made that "les nucléoles sont des noyaux en miniature. Il renferme toujours un appareil nucléinien filamenteux plongé dans un plasma et logé dans une coque mince."

Carnoy and Lebrun give a large number of figures which are supposed to furnish evidence in support of their conclusions. They have, however, seemingly overlooked the important stages which give the clue to the nature of the "primitive nucleoli" and of their relation to the chromosomes (Figs. 28-39). In many of their figures they show feathery chromosomes similar to those shown in my Figs. 39-41, etc.; yet they consider that these chromosomes are products of the resolution of the nucleoli, as are also the granular threads which correspond to my oxychromatin filaments. The feathery chromosomes are often figured in pairs, the sister threads lying parallel or intertwined in various ways. Carnoy and Lebrun state that these paired filaments are not formed by a longitudinal or by a transverse division of a pre-existent nuclear element, but that they are either produced by a single filament folding back on itself and the parts separating, or they are two filaments which have been resolved from two nucleoli lying close together. Sections of nuclei are given by Carnoy and Lebrun which contain numerous nucleoli and no chromatin threads. Such figures are considered to prove conclusively that there

has been no continuation of the primitive filament. It is not difficult to find sections of the nuclei of the young ova of Bufo, particularly at the stages of Figs. 40-44, in which no chromosomes can be found. Such sections are possible because the chromosomes, which stain very faintly, are sometimes collected together at one side of the nucleus and sections passing through the centre of the nucleus show only granular karyoplasm, nucleoli, and possibly some of the oxychromatin threads. After the yolk has formed, many fixing fluids do not seem to penetrate the egg sufficiently well to preserve the delicate structure of the chromosomes. I have examined, under an oil immersion lens, every section of the nucleus of an egg preserved in Gilson's or Flemming's solution without finding the slightest trace of chromosomes; while in the nuclei of eggs from the same ovary that were fixed with chromic acetic or corrosive acetic the feathery chromosomes show very distinctly with a comparatively low magnification. I have never found a nucleus in which it was impossible to find the chromosomes, provided the egg had been properly preserved and stained.

In his earlier work on Axolotl, Fick (28) states that the nucleoli are independent structures which probably represent "eine Art Reservestoffbehälter." In a later paper on the ripening of the egg of Rana (29) he confirms the work of Carnoy and Lebrun and states that during the growth period of the oöcyte there are several generations of nucleoli which alternate with chromatin figures, consequently the continuity of the chromosomes is not maintained during this time. Fick considers that the nucleoli in the egg of Rana represent "eine Ruheform des Nucleins im Gegensatz zu den Chromatin-Figuren und Chromosomen, Formen in denen das Nuclein offenbar eine active Rolle spielt." Carnoy, Lebrun, Fick, and also Bataillon agree, therefore, with the conclusion reached many years ago by Schultze (83) from his study of the ripening of the egg of Rana, that the chromosomes "nicht aus einem präformirten Kerngerüst entsteht, sondern sich direkt aus den winzigen Keimkörperchen herausbildet."

The observations of other investigators of amphibian

oögenesis stand in direct contradiction to those cited above. Born (9, 10) states that in the egg of Triton the chromatin skein "sich aus dem Chromatingerüst des Ureies direkt herleitet." Although at one period of development the chromatin threads stain faintly and the chromatin substance can only rarely be distinguished from the granular karyoplasm, Born does not believe that the chromatin disappears or leaves the nucleus at this time, but that "sich dasselbe nur äusserst fein in der umgebenden Kerngrundsubstanz vertheilt habe." Later the chromatin threads are formed again, and they appear in pairs, lying parallel or closely intertwined as in Bufo. Born does not find that the nucleoli ever give rise to chromosomes, and while he ventures no conjecture as tō the function of these bodies he believes that they "stehen in Beziehung zum individuellen Zellleben nicht zur Fortpflanzung."

According to the observations of Jordan (48) on the newt, the chromatin threads "are distinctly traceable through the whole history of the germinal vesicle," although large chromatin granules break loose from the threads at various times and pass over into true nucleoli. Janssens has also asserted that in the egg of Triton the chromosomes persist throughout the entire growth period; but in this egg the chromosomes are entirely independent of the nucleoli.

Lubosch (61) has recently studied the history of the nucleoli in the ovarian egg of Triton with the avowed purpose of testing the work of Carnoy and Lebrun. His material was preserved and stained in a great variety of ways, and he concludes that many of Carnoy and Lebrun's results are due to the methods of technique which they employed. As Lubosch did not study the very young oöcytes, he ventures no opinion as to the origin of the primitive nucleoli. He states that nucleoli are formed periodically at the nuclear periphery, and that they then wander towards the centre of the nucleus where they undergo one of three modes of dissolution: (1) through vacuolization and subsequent differentiation into karyoplasm; (2) through distintegration into granules; (3) through transition into various sorts of chromatin filaments, some of which

are indistinguishable from the chromosomes at the time that the latter are surrounded by a ring of nucleoli shortly before the maturation period. While Lubosch finds that the primitive chromatin network becomes extraordinarily fine at certain stages of development, he states that it never completely disappears and that it is morphologically present in the ripening egg, although it is in a finely divided form.

From the stage of Fig. 39 until that of Fig. 50, when the nucleus has reached its maximum size and the nucleoli have migrated to the centre preparatory to their final disintegration, the nuclei in the ova of Bufo contain an almost endless variety of nucleolar figures. In the nucleus shown in Fig. 39 the nucleolar bodies are of various sizes and they react very differently towards the gentian violet and safranin stain. The very small rounded nucleoli are karyosomes, since they stain like the chromatin; the larger, rounded nucleoli may be considered as plasmosomes, since they stain red and are not connected in any way with the chromatin; the irregular body marked X stains purple, and is a compound-nucleolus which has not yet begun its resolution; while the small, slightly irregular bodies are secondary compound-nucleoli which have been evolved from the resolution of a large mass similar to that shown in Fig. 35. At Y is shown a nucleolar body which is very similar to certain of the resolving nucleoli figured by Carnoy and Lebrun. This body is composed of a large, rounded central plasmosome (staining uniformly red) which seems to be giving off a number of small buds that also take the safranin. The outer surface of this plasmosome appears somewhat irregular and stains purple because a number of oxychromatin granules are attached to it. This structure has been produced, evidently, by the resolution of one of the compound-nucleoli which contained only a comparatively small amount of chromatin. The pinching off of small plasmosomes from a larger mass is a very common phenomenon in the ova of Bufo, and it is evidently one of the ways in which the number of these bodies is increased.

Several small nucleoli are shown in Fig. 39 which are composed of an outer ring of substance, evidently chromatin, since

it stains deep blue, surrounding a central portion which either stains very faintly or appears colorless; similar bodies are shown in Figs. 40, 41, 43, etc. At a later period the central portion of such nucleoli disappears, leaving only the chromatin ring. Subsequently the ring breaks at some point, thus becoming a crescent (Fig. 41), and it then disintegrates into small granules. Nucleoli of this character are probably derived from the oxychromatin of the larger compound-nucleoli, since they seem to be found most abundantly at the stages of Figs. 39-43. Similar nucleoli are figured by Carnoy and Lebrun and also by Lubosch.

The oxychromatin threads produced by the resolution of the large compound nucleoli are massed together at the stage of Fig. 39; but they soon become scattered throughout the nucleus and are bent and twisted in a great variety of ways (Figs. 40-43). Occasionally, as shown in Figs. 40 and 43, two of these filaments lie parallel or cross each other in the form of an X. Such an arrangement is purely accidental, since the filaments never have any definite arrangement in the nucleus. In some cases oxychromatin threads seem to be united with nucleoli (Fig. 43); but as the nucleoli stain differently from the filaments, it is readily seen that there is no true connection between them.

Many of the figures given by Carnoy and Lebrun show granular chromatin filaments strikingly like those shown in my Figs. 40-43. These investigators consider that such filaments are derived from the substance of the nucleoli, and the contact of a nucleolus with a chromatin thread, as shown in my Fig. 43, is considered to be proof that the chromatin thread is being formed at the expense of the nucleolus. The feathery chromosomes are considered by Carnoy and Lebrun to be merely a special form of the filaments and in no way different from the others in origin or in fate. My observations do not admit of such an interpretation, since in Bufo the feathery chromosomes can be traced back to the continuous filament formed after synizesis (Fig. 26), while the oxychromatin threads are undoubtedly derived from the chromatin

which did not go into the spireme and they are always produced by the resolution of compound-nucleoli. Oxychromatin filaments similar to those shown in Figs. 40-43 are figured in Bouin's work on the oögenesis of Rana. Bouin considers that these filaments are a part of the general chromatin of the egg, and he does not distinguish them from the true chromosomes.

By means of a series of camera drawings of all of the sections of nuclei in about the stage of development shown in Fig. 43, I have endeavored to ascertain the number of oxychromatin filaments and of chromosomes at this time. While the chromosomes appear to be twenty-four in every case, the number of oxychromatin threads seems to vary from 20-50 in different nuclei. This difference in the number of oxychromatin threads in various cases can doubtless be attributed to the fact that the compound-nucleoli from which the filaments are derived vary in number and in size in different nuclei and that these bodies do not all resolve at the same time.

Carnoy and Lebrun distinguish three distinct stages in the development of amphibian oöcytes, and they state that there are many generations of nucleoli which alternate with various kinds of chromatin figures; the nucleus frequently containing one kind of structure exclusive of the other. In Bufo I have never found an oöcyte in a stage of development between that shown in Fig. 38 and that of Fig. 50 in which the nucleus did not contain nucleoli, chromosomes, and oxychromatin filaments provided the egg had been satisfactorily preserved and stained. As the ova grow the number of nucleoli increases; but the number of chromosomes remains constant, and the maximum number of oxychromatin filaments is found at the stage of Figs. 40-43. After this time the oxychromatin threads stain more faintly; the granules of which they are composed gradually draw apart (Fig. 48), and finally become scattered throughout the nucleus. Many of these minute chromatin granules can still be found in the nucleus at the beginning of the maturation period.

Although in Bufo there is no periodic resolution of nucleoli into chromatin threads followed by the development of a

new generation of nucleoli from chromatin granules, there is a constant formation of new nucleoli and a gradual dissolution of the old ones during the growth stages of the ova. The disintegration of small nucleoli composed of a ring of chromatin enclosing a plasmosome body (Figs. 39-43) has already been described. The beginning of a dissolution of some of the larger nucleoli is shown in Fig. 42. In this nucleus many of the nucleoli are stained black with iron hæmatoxylin; others appear grayish, since they seem to have lost their capacity for staining intensely. The latter nucleoli are gradually dissolved in the karyoplasm; they are never resolved into chromatin threads. Although the process of dissolution usually involves the whole nucleolus, sometimes only a portion of it disappears leaving one or several small, rounded bodies (Fig. 40, X). It is possible that the small groups of nucleoli shown in Figs. 40 and 43 may have been formed in this manner.

As a rule the majority of the nucleolar masses which lie in the meshes of the chromatin spireme or against the nuclear membrane at the stage of Figs. 26-34, resolve into plasmosomes and oxychromatin filaments at about the time that the larger compound-nucleoli undergo their resolution (Figs. 35; 39, Y). These masses differ from the larger ones in that they contain a relatively greater quantity of plasmosome material and a much smaller amount of chromatin. One or two of these nucleolar bodies, rarely more, escape dissolution at the stage of Figs. 35-38 and appear in later stages as slightly irregular, round or oval structures which stain as uniformly, though in many cases not as intensely, as in an earlier period (Fig. 39, X). These bodies increase rapidly in size after the stage of Fig. 39, and they usually undergo a somewhat different mode of resolution from that of the other compound-nucleoli. At an early period in the resolution of these bodies the oxychromatin, which has been attached to the outer surface of the plasmosome substance (Fig. 40), breaks away and becomes scattered throughout the nucleus, being indistinguishable from the oxychromatin produced by the earlier resolutions of nucleolar masses. Sometimes all of the plasmosome

substance in these bodies forms one large rounded mass which contains either one large vacuole or a varying number of small ones (Fig. 40). Such a structure greatly resembles the large vacuolated nucleoli found by Carnoy and Lebrun and also by Leydig in the ova of various amphibians, and it is also very similar to the "principal nucleolus" described by Maréchal (67) in the selachian egg. In many cases the plasmosome substance in these bodies is divided, the greater part of it forming a large, rounded central mass which usually stains rather faintly and appears either homogeneous (Fig. 41, R) or vacuolated (Fig. 41, S), the remaining portion being broken up into a varying number of small, round, deeply staining bodies which are attached, for a time, to the outer surface of the larger mass and later separate from it to form small plasmosomes.

The large plasmosome bodies shown in Figs. 40, 41 and 51, disintegrate in various ways and at different times. In some cases they persist as rounded, vacuolated bodies until the germinal vesicle disintegrates at the beginning of the maturation period when they are slowly absorbed by the cytoplasm; in other cases they break open during the later growth stages of the ova (Fig. 51, b), and subsequently divide into several rounded portions which are gradually dissolved in the karyoplasm (Fig. 43). It is not uncommon to find these large plasmosome bodies budding off portions of their substance (Fig. 51, c, d); and it is probable that many of the nucleoli found at the stage of Figs. 48-50 have been formed in this way.

The central vacuole of these large plasmosomes frequently contains a number of nucleolini which may be separated or so joined together that they simulate a granular chromatin thread (Fig. 51, C, D). These nucleolini always stain like the plasmosome, and they are evidently granules which have broken away from the inner surface of the ground substance in a manner similar to that by which the small plasmosomes are budded off from the outer surface. It seems probable that Carnoy and Lebrun have in mind linear aggregations of

nucleolini when they state that chromatin filaments are often found in the interior of large nucleoli. As the result of an investigation of the structure of the nucleoli in many kinds of cells Montgomery (70) states: "I am forced to conclude that in all probability there are no skeins of chromatin lying in any metazoan nucleolus, since I have never found any evidence of chromatin in it in any metazoan cell." This statement may well be extended to include the nucleoli in the egg of Bufo, since in no case have I ever found chromatin filaments in the interior of rounded nucleoli, although they are often found wrapped around the exterior of a nucleolus (Figs. 38, 40, 43).

In preparations stained with safranin and gentian violet nucleolar bodies are sometimes found which are similar to the compound-nucleoli described above in size and general outline, although they have a very different structure as they are composed of a number of rounded plasmosomes imbedded in a mass of chromatin granules (Fig. 51, a). These bodies are compound-uncleoli containing a large amount of chromatin, which for some unknown reason did not undergo a resolution at the stage of Figs. 35-38.

Nuclei having a diameter of 0.04-0.08 mm. usually appear as in Figs. 39-43. They contain one or two large unresolved compound-nucleoli, a varying number of round plasmosomes and small karyosomes, together with numerous small compound-nucleoli which were set free from the large nucleolar masses at the stage of Figs. 35-38. These small compound-nucleoli, which I shall call secondary compound-nucleoli to distinguish them from the larger bodies, appear homogeneous and only slightly irregular at the stage of Figs. 39-43, and the greater number of them are masses at the side of the nucleus where the largest of the primary compound-nucleoli underwent a resolution at the stage of Figs. 35-36. In a slightly older egg metabolic processes occur which lead ultimately to the formation of yolk. These processes are accompanied by, if indeed they do not produce, a marked change in the appearance and in the behavior of the nucleolar bodies. At this stage of development (Fig. 44) there is a mass of very

irregular nucleolar bodies at one side of the nucleus which greatly resemble the structures figured by Carnoy and Lebrun as nucleoli resolving into their chromatin constituents. As the preparation from which Fig. 44 was drawn was stained with iron hæmatoxylin the nucleolar bodies appear homogeneous and stain very intensely. Their true character is shown only when preparations are stained with safranin and gentian violet. In such cases these fantastically shaped bodies are found to be composed of a number of rounded plasmosomes imbedded in a meshwork of oxychromatin granules (Fig. 45). The plasmosomes always appear homogeneous and they invariably take the safranin stain; the chromatin stains blue and it is always in the form of fine granules which may or may not be strung together in a filament. There is nothing to indicate that the chromatin in these structures is derived from the plasmosomes or vice versa. These peculiar bodies, which are found very abundantly in the oöcytes of toads with a body length of 4-5.5 cm., are unquestionably secondary compound-nucleoli which have increased considerably in size during the stages of Figs. 39-43 and are now resolving into their constituent parts, oxychromatin granules and plasmosomes. Soon after the stage shown in Fig. 44 these irregular masses break up, and for a short time the nucleus contains a number of plasmosomes surrounded by chromatin granules (Fig. 46). At a later period the oxychromatin granules separate from the plasmosomes and scatter thoughout the karyoplasm, and for the first time since before the synizesis stage the nucleus has all of its chromatin separated from the plasmosome substance.

As I was unable to obtain any young toads in the fall with a body length of over 5.5 cm., I have not been able to follow the later changes in the oöcytes in the ovaries of young females, and I have had to make use of the oöcytes developing in the ovaries of adults to complete my study of the oögenesis of Bufo. If adult females are killed soon after the breeding season in April or in May the ovaries are found to be filled with young ova, many of which contain nucleolar bodies simi-

lar to those shown in Fig. 44. A section of the nucleus of an egg taken from the ovary of an adult toad killed the latter part of April is shown in Fig. 48. The nucleus has nearly attained its maximum size, and it is slightly oval, measuring 0.19 mm. by 0.22 mm. All of the irregularly shaped nucleolar bodies found at the stage of Figs. 44-46 have disappeared and the nucleus contains a large number of round or oval nucleoli of various sizes which are entirely distinct from the chromatin threads. The smallest of the nucleoli, which stain like chromatin, have evidently been formed by a fusion of a number of the chromatin granules set free by the disintegration of the oxychromatin threads. Most of the larger nucleoli stain very intensely at this time and only a few of them show, by their lessened capacity for staining, that they have begun to dissolve. Since the majority of the nucleoli are derived from the resolution of the secondary compound-nucleoli the greater number of these bodies are massed together in one part of the nucleus. A few oxychromatin filaments in the process of dissolution are still to be found in the nucleus at this time.

During early growth stages the nucleus occupies the centre of the ovum, but at or soon after the stage of Fig. 44 it begins to move towards the future animal pole of the egg. As at this time the nuclear membrane is usually somewhat irregular in outline, several investigators have maintained that the change in the position of the nucleus is brought about through amœboid movement. This explanation does not seem to me entirely satisfactory since in some cases the nuclear outline is perfectly regular when the nucleus is moving towards the upper hemisphere, and when irregularities in the nuclear outline are found they are invariably distributed uniformly around the membrane, no matter by what means the egg has been preserved.

At the time that the nucleus is changing its position the greater number of the nucleoli are massed together in one part of the nucleus (Figs. 44-48). I have examined many eggs in this stage of development and I have always found

that the greater number of the nucleoli lie in the part of the nucleus that is nearest the periphery of the egg. It hardly seems probable that this arrangement would be found so constantly if it had no significance. It seems to me a possibility, at least, that the accumulation of most of the nucleoli in one part of the nucleus may have something to do with the movement of the nucleus towards the periphery of the egg. This arrangement of the nucleoli strongly suggests also that the polarity of the egg is determined at or soon after synizesis, since the location of the largest of the compound-nucleoli, from which the greater number of secondary compound-nucleoli are derived, indicates the part of the nucleus which will be nearest the animal pole in the later growth stages of the oöcytes.

After the nucleus has reached its final position at the periphery of the egg there is a rearrangement of the nuclear contents so that the nucleoli become distributed fairly evenly around the nuclear periphery, while the chromosomes and the remains of the oxychromatin filaments are found in the centre of the nucleus (Fig. 49). Whether this arrangement is due to the activity of the nucleoli themselves, I have not been able to determine. These bodies always appear round and they never show processes similar to those found by Leydig and also by Eimer (26) and considered by these investigators to be the means through which the nucleoli change their position in the nucleus. It is at this stage of development shown in Fig. 49 that the nuclear membrane is most irregular in outline, and it is very probable that this irregularity is due to the close proximity of the nucleoli. As a rule all of the oxychromatin filaments have disintegrated at this time; the chromosomes can always be found in favorable preparations, although they stain very faintly.

Several investigators, among whom may be mentioned Will (92), Fick, and Leydig, maintain that at or before the stage of Fig. 49 nucleoli pass out of the nucleus into the cytoplasm where they either dissolve or take part in the formation of the yolk. Although I have examined a large number of eggs in

which there were many hundreds of nucleoli lying close to the nuclear membrane I have never found a single case in which a nucleolus was passing through the membrane into the cytoplasm. At certain stages in the development of the ova there are a number of rounded bodies in the cytoplasm which greatly resemble nucleoli, and it is doubtless this similarity in appearance that has led to the assumption that the cytoplasmic bodies are of nucleolar origin.

Soon after the stage of Fig. 49 the nucleoli leave their peripheral position and move towards the centre of the nucleus. Their arrangement at first is somewhat irregular (Fig. 50); but later, as shown in a previous paper (King, 49; Fig. 3), they form a closed ring which surrounds the chromosomes. This arrangement of the nucleoli in the ovarian egg previous to maturation seems to be characteristic of all amphibian eggs, as it has been noted by all of the observers who have studied this period in the development of the ova. All of the nucleoli have begun to disintegrate by the time that the germinal vesicle breaks down at the beginning of the maturation period. They first lose their capacity for staining and many of them become vacuolated. Later they break into small fragments which are absorbed by the cytoplasm.

Although this study of the ovarian egg of Bufo has shown that investigators of amphibian oögenesis have classed together, under the general name of nucleoli, several different kinds of structures, it has not, unfortunately, disclosed the manner in which these bodies are formed or their function in the nucleus.

From the resting stage of the primary oögonium to the synizesis period in the oöcyte the nucleus of the germ-cells contains several rounded nucleoli which stain differently from the chromatin and there is not the slightest evidence that there is any genetic relation between them. During synizesis the nucleoli can still be distinguished from the chromatin (Fig. 25); but in early post-synizesis stages (Figs. 26-34) these bodies are contained in the amorphous masses of nuclear substance (compound-nucleoli) left over after the formation of

the spireme, and they cannot be followed since the large masses stain very intensely and uniformly at this time. When the large compound-nucleoli resolve (Figs. 35-38) they liberate, with the secondary compound-nucleoli, many more of the rounded nucleoli, which I have called plasmosomes, than were found in the nucleus previous to synizesis. It is evident, therefore, that plasmosomes are being formed in the nucleus during early post-synizesis stages (Figs. 26-34). Since these nucleoli are formed only in the midst of oxychromatin granules it seems probable that the oxychromatin is concerned in some way with their formation; but the number and size of these bodies and the fact that they invariably stain differently from the chromatin seems to preclude the possibility that they are derived from chromatin substance as Flemming (30), van Bambeke (5), Macallum (65), Hertwig (43), Obst (7), Schockaert (79), Carnoy and Lebrun, Fick (29), and many others have maintained. The part played by the oxychromatin in the formation of the plasmosomes is obscured. There is no apparent decrease in the amount of this substance associated with an increase in the number and size of the plasmosomes during the early development of the oöcyte, and at the time that the oxychromatin filaments disintegrate the nucleus apparently contains its maximum number of plasmosomes (Fig. 48).

The plasmosomes seem to be of a plastic, semi-fluid consistency; they appear homogeneous until they begin to disintegrate, and in some instances they seem to be capable of increasing in size and of budding off portions of their substance. Judging from the appearance and behavior of these bodies and from the fact that their formation is associated with the rapid growth of the cell and with the formation of vitelline bodies in the cytoplasm, it seems probable that they are products of nuclear metabolism which are possibly depositors of nutritive substance that are to be used at a later period in the history of the cell. This is substantially the view advocated by Korschelt (56) and by Rhumbler (75). Montgomery (70-71) is one of the few investigators who believes that the nucleoli are of extranuclear origin. He states that in the egg of

nemerteans the nucleoli are first found closely applied to the inner surface of the nuclear membrane. "It would seem that the yolk is at first present in the cytoplasm in the form of a diffused, unstainable fluid; that a portion of it, that remaining in the cell body, later becomes segregated as, or chemically changed into yolk globules; and that another portion of it is taken into the nucleus and, after passing the nuclear membrane, is changed into nucleolar substance." Such an origin for the plasmosomes in the ova of Bufo seems unlikely since these bodies are not found close to the nuclear membrane until a late period in the development of the ova.

The large nucleolar masses found in the oöcyte at the stage of Figs. 26-34 correspond evidently to the "primary nucleoli" of Carnoy and Lebrun. I have shown that these bodies are complex structures composed of plasmosome material and of the chromatin which did not go into the formation of the chromosomes and that they later resolve into their constituent parts; they are never formed entirely of chromatin, as Carnoy and Lebrun maintain. The fantastically shaped nucleolar bodies found at the stage of Fig. 44 are similar in structure to the large compound-nucleoli shown in Figs. 26-34, and they too resolve into chromatin threads and plasmosomes. In the egg of Bufo there is never any connection between the nucleoli and the chromosomes. Only oxychromatin goes into the formation of the compound-nucleoli, and the oxychromatin filaments which are formed by the resolution of these bodies do not at once disintegrate to form a new generation of nucleoli but they gradually break up into minute granules which seem to be absorbed by the achromatic substance of the nucleus. It is impossible to determine whether these granules take any part in the formation of the chromosomes which are found on the maturation spindle.

VII. The Chromosomes.

At no period in the development of the oöcyte does the basichromatin disappear nor does it become condensed in the form of nucleoli, and the chromosomes can be traced continuously

from the time that they are first formed by the breaking of the spireme (Fig. 33) up to the stage when the germinal vesicle disintegrates in preparation for the maturation mitosis. At the time that the spireme divides longitudinally (Figs. 28-29) the chromatin filaments are found to be composed of a series of rounded granules from which a few fine fibres project on either side. When the spireme breaks into chromosomes the number of fine projections increases, evidently at the expense of the chromatin granules (Figs. 33, 34, 36). By the time that the oöcyte has reached the stage of Fig. 39, the appearance of the chromosomes has changed considerably. The axial portion of the thread is now composed of minute, faintly staining granules, evidently formed by the breaking up of the larger ones, and the fine projections from the sides are longer and more numerous than at an earlier period. The chromosomes, of which there are undoubtedly twenty-four, thus come to have the feathery appearance that characterizes them from this time until the beginning of the maturation period, and they greatly resemble the filamentous chromosomes found by Rückert (76, 77) in the selachian egg and by Born in the egg of Triton. After the stage of Fig. 39 the chromosomes stain very faintly, since the greater part of their substance seems to be in the form of fine fibres as it was during the synizesis period; they can always be found, however, if the egg has been properly preserved and stained. When the germinal vesicle is about to disintegrate the chromosomes lose their filamentous structure and become greatly condensed, appearing as a single series of perfectly round granules (King, 49; Fig. 8).

The arrangement of the chromosomes in the young oöcyte depends, evidently, on the extent to which sister portions of the spireme have separated before the spireme breaks into segments. The transverse division of a spireme like that shown in Figs. 31 and 32 produces chromosomes that are scattered irregularly throughout the nucleus and only occasionally paired; while the division of a spireme similar to that shown in Fig. 30, gives a paired arrangement of all of the chromo-

somes (Fig. 33, 34). In the later growth stages of the oöcytes the chromosomes become widely distributed throughout the nucleus and they seem to have no definite arrangement, although it is not unusual to find two chromosomes paired as in Fig. 43, or two chromosomes crossed as in Fig. 40.

In a previous paper (King, 49) I have shown that, at the time the germinal vesicle is about to break down in preparation for the maturation mitoses, the twenty-four chromosomes come together forming twelve pairs. The chromosomes of a pair are of the same length, but there is considerable difference in the lengths of the chromosomes of the various pairs. At this time "two of the chromosomes may be united in the form of an X or Y, a single or double figure eight, or they may lie parallel for a part of their length and the ends intertwine in various ways." At a slightly later period the ends of each pair of chromosomes unite forming a closed ring. Immediately following this stage the chromosomes apparently break up into granules and, owing to the changes occurring in the nuclear substance preparatory to the formation of the first polar spindle, it is impossible to trace the chromatin for a short period. When the polar spindle forms a large number of rounded chromatin granules are found near it which soon fuse into several irregular clumps from which the reduced number of chromosomes (12) is formed.

It is unfortunate that the chromatin cannot be traced during the period of the formation of the first polar spindle since it thereby becomes impossible to identify the chromosomes of the first polar spindle with the chromosomes that are found in the oöcyte previous to the disintegration of the germinal vesicle. If, however, the chromosomes can maintain their individuality in the resting nuclei of the oögonia and of the young oöcytes when the chromatin is in the form of minute granules which are scattered irregularly along a linin meshwork or distributed on the nuclear membrane, I see no reason why they should be considered to lose that individuality when they break up into granules at the beginning of the maturation period. After all it may be through the linin that the morpho-

logical continuity of the chromosomes is maintained; and it is very probable that there is a linin connection between the chromatin granules during the early maturation stages which I overlooked in my previous work. When opportunity offers I shall collect new material showing the formation of the first polar spindle in the egg of Bufo, in the hope that with some new method of fixation or of staining I can follow the history of the chromatin granules and thus trace the chromatin continuously from the early growth stages of the oöcytes through to the maturation spindle.

As far as I am aware, no investigator of amphibian oögenesis has as yet traced the chromosomes from the ovarian egg directly into the maturation spindle. Schultze, Carnoy and Lebrun, and Fick believe that the chromosomes of the polar spindles are derived from chromatin nucleoli which escape dissolution at the time that the germinal vesicle disintegrates. Other investigators have tacitly assumed that the chromosomes of the ovarian egg pass over into the maturation spindle and they have not given any figures of the critical stages.

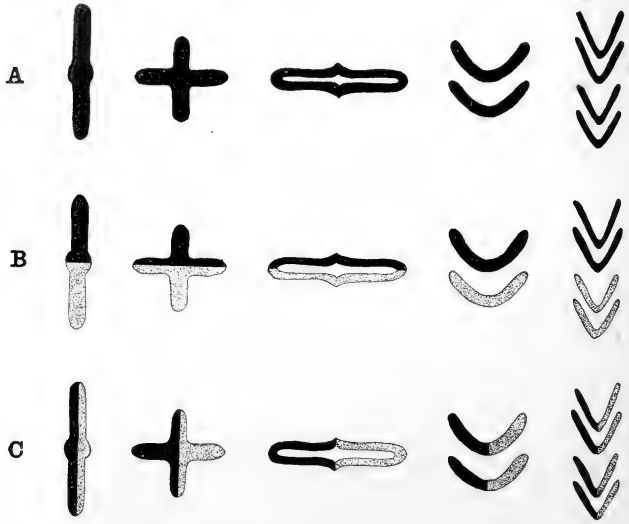
Since there are two periods in the development of the egg of Bufo when it is impossible to follow the changes which the chromatin undergoes, it is a difficult matter to decide when and how synapsis occurs. The first period when the history of the chromatin is obscured is during the synizesis stage in the young oöcyte when the nuclear contents become massed together as shown in Fig. 25 and the chromatin appears in the form of exceedingly fine granular threads. This stage is succeeded by one in which the chromatin, which is to form the chromosomes, is in the form of an apparently continous spireme. Later this spireme splits longitudinally and then divides transversely forming the somatic number of separate segments. Assuming that the chromosomes maintain their individuality during synizesis, it is obviously impossible to determine how they were joined together in the spireme which is formed after the synizesis stage. If the chromosomes were joined end to end, then the splitting of the spireme shown

in Figs. 28-30 is a longitudinal division of univalent chromosomes. On this assumption synapsis is coincident with synizesis and, at the stage of Figs. 33-34, the nucleus contains twelve bivalent chromosomes which are divided longitudinally. This is the interpretation which Janssens has given to the early post-synizesis stages which he finds in the egg of Triton. The fact that in later growth stages the great majority of the chromosomes are not arranged in pairs makes this interpretation improbable for the egg of Bufo, since in the eggs of other forms when bivalent chromosomes divide longitudinally the parts remain together or are connected in some way.

If we assume that two chromosomes united side by side in the spireme, then the subsequent longitudinal splitting of the spireme is merly a separation of the chromosomes that were previously paired, and the transverse division of the spireme is the means by which the chromosomes are completely separated from each other. Synapsis, on this assumption, does not necessarily occur during synizesis, since the somatic number of chromosomes is evolved from the spireme. I am inclined to believe that synizesis in the egg of Bufo is a process by which the chromatin which bears the hereditary qualities and is to be used for the chromosomes of the maturation spindle is separated from the chromatin which has other uses in the cell. This would seem to bear out Gardiner's (33) contention that "there are two kinds of chromatin stuff, the one insoluble and bearing the heredity which is to be transmitted to the daughter-cells, the other food for the cytoplasm." If this interpretation of synizesis is correct, the chromosomes must have been united in pairs in the spireme that was evolved from the synizesis stage, but synapsis does not take place until the beginning of the maturation period. This interpretation seems the more probable since Jordan failed to find a pairing of the chromosomes in the ovarian egg of the newt at any stage of development.

The second period when it is impossible to trace the history of the chromatin occurs just previous to the formation of the

first polar spindle when the twelve chromatin rings break into granules which cannot be distinguished from the granular achromatic substance of the nucleus. The twelve bivalent chromosomes that are finally formed from the mass of chromatin



A.—Diagrams showing the changes in the shape of the chromosomes of the first polar spindle in the egg of Bufo and the direction of the maturation mitoses. In both mitoses the chromosomes are divided longitudinally.

B.—Diagrams showing the character of the maturation mitoses if the chromosomes were united end to end in synapsis. The first division separates univalent chromosomes; the second is a longitudinal division.

C.—Diagrams showing the character of the maturation mitoses if the chromosomes conjugated side by side during synapsis. Both mitoses divide the bivalent chromatin segments longitudinally.

granules that surround the polar spindle vary somewhat in size and in shape. When they have become arranged on the spindle they undergo considerable modification in form, and the longitudinal axis of the chromosome at the time that the

first maturation mitosis occurs is the transverse axis of the chromosome at an earlier period (King, 51). In both maturation mitoses the bivalent chromosomes are divided longitudinally (Text-Figure I, A).

If synapsis occurs when the germinal vesicle disintegrates then the chromosomes must have united end to end, as shown in Text-Figure I, B, otherwise both of the maturation mitoses are equation divisions of bivalent chromatin segments and in neither division are univalent chromosomes separated (Text-Figure I, C). The investigations of Stevens (87, 88) have shown that in Sagitta synapsis takes place in the egg by a side by side conjugation of the chromosomes and in the spermatocytes by an end to end union. This is probably the plan that is followed in the germ-cells of Bufo if synapsis occurs in the egg during the synizesis period. I am inclined to the opinion that in the egg of Bufo, as in the spermatocyte, synapsis occurs shortly before the maturation mitoses and that the chromosomes are united end to end. On this assumption the first maturation division in the egg is a reduction division in the Weismannian sense since it separates univalent chromosomes, and the second division only is an equation division (Text-Figure I, B).

A. and K. E. Schreiner (80-82), who have recently examined the chromatin relations in the germ-cells of many different forms, conclude from their studies that in the germ-cells of *all* animals the chromosomes conjugate in pairs during synapsis, never end to end. This generalization finds an exception in the germ-cells of Bufo. In the spermatocytes of this amphibian synapsis occurs during the synizesis stage which immediately precedes the prophase of the first maturation mitosis, and in the prophase and metaphase of division the chromosomes behave in such a way that there seems no possibility of avoiding the conclusion that they were united end to end in synapsis. In the egg, as I have shown, it is not possible to determine when or how synapsis occurs; yet the evidence at my command seems to indicate that in synapsis the chromosomes are united end to end as they are in the spermatocytes.

VIII. The Formation of Yolk.

One of the most difficult problems met with in the study of amphibian oögenesis is that concerning the origin of the yolk. This problem includes not only a consideration of the origin and nature of the so-called "yolk-nuclei," but it also involves practically the whole theory of cell action since it cannot be supposed that the yolk formation takes place independently of nuclear activity. The anabolic processes taking place in the cell as a result of the interaction of the nucleus and the cytoplasm are as yet very imperfectly understood, and until we have obtained a clearer insight into the nuclear-cytoplasmic relations it will not be possible to solve the problem of yolk formation in an entirely satisfactory manner.

There is as great a diversity of opinion regarding the nature of the yolk-nucleus and the origin of the yolk in the amphibian egg as there is concerning the origin of the egg itself. The first observation regarding the presence of a yolk-nucleus in the amphibian egg were made by Cramer (23) in 1848. According to this investigator the cell body of the frog's egg contains a small granular ball which later spreads out in the form of a half-moon around the nucleus and gives off granules which develop into yolk spherules. In 1850 Carus (19) investigated the young egg of Rana temporaria and failed to find the granular ball described by Cramer. He states that the yolk first appears at the periphery of the egg in the form of single granules as it does in the egg of Alytes obstetricans according to the earlier observations of Vogt (90).

A few years later Thompson (89) wrote in regard to the presence of a yolk-nucleus in the frog's egg: "I have in general found it present, and think it more probable that it may be destined to form the external and larger corpuscles of the yolk, while the clearer part immediately surrounding the germinal vesicle may contribute to the production both of these and of the finer substance in which the germinal vesicle is found imbedded."

As Goette failed to find a yolk-nucleus either in the egg of Bombinator or of Bufo, Hertwig (40) is inclined to attach

but little morphological value to the rounded granular ball which he finds in the cytoplasm of the egg of Rana. "Mir scheint er einzig and allein mit der Bildung der Dottersubstanz in Beziehung zu stehen und eine eigenthümliche locale Ansammlung von Nährstoffen darzustellen." He suggests that the name "Dotterconcrement" would be more appropriate for this structure than "Dotterkern." Kolessnikow (55) mentions the presence of granular yolk-nuclei in the eggs of Rana temporaria, Rana esculenta, and Bufo variabilis, but he gives no opinion as to their origin or use.

Henneguy (38) finds a large granular mass, presumably a yolk-nucleus, in the egg of Rana, although he fails to find a similar body in the egg of Bufo vulgaris, Triton tæniatus, and Triton cristatus. Henneguy believes that wherever this body is found it is derived from the nucleolar substance of the nucleus and he ventures the interesting conjecture that "c'est un organe ancestral qui, avec les éléments nucléolaires de la vésicule germinative, correspond au macronucléus des Infusoires, le micronucléus étant représenté par le rôseau chromatique, prenant seul part aux phénomènes de fécondation."

Jordan finds a number of granular yolk-nuclei in the egg of the newt which he believes "arise from the cytoplasm and usually disintegrate in the cytoplasm." He is not sure whether these structures are of importance in the formation of yolk or not. Jordan's observations regarding the fate of these yolk-nuclei will be mentioned later.

The observations of several investigators seem to show that nuclear substance is used in the formation of yolk. In 1884, Will brought forward the view that in the ova of amphibians and of insects nucleoli leave the nucleus and migrate to the periphery of the egg where, as yolk-nuclei, they lose their sharp contour and break up into granules which become yolk spherules. Substantially the same view was advanced by Leydig in 1888 to account for the origin of the yolk in the egg of Triton. Leydig considers that the nucleoli arise in the nucleus "als Knotenpunkte in dem feinen Netz des Reticulums," and that they move to the periphery of the nucleus

where they "im losgelösten Zustande die Form und Natur kleiner Amöben zeigen." Subsequently they pass through the nuclear membrane into the cytoplasm and move to the periphery of the egg where they form groups of granules which develop into yolk.

Bataillon (6) also derives the yolk in the amphibian egg from the substance of the nucleus, but he believes that it is formed from the chromatin. "Des massules chromatiques issues de la vésicule germinative viennent donner dans le plasma ovulaire et à la périphérie d'abord, de véritables éléments cellulaires transitoires dont ils fournissent le noyau, et prendre part à la formation simultanié des tablettes vitellines et du pigment."

As a result of a study of the ovarian egg of Rana and of Necturus, Macallum (64) concludes that the peripheral chromatin nucleoli generate a substance which diffuses gradually through the nucleus into the cytoplasm. "I regard the yolk spherules as formed by the union of a derivative of the nuclear chromatin with a constituent of the cell protoplasm." Support for this view is furnished by the more recent cytological studies of Carnoy and Lebrun (15). These investigators state that the greater number of chromatin granules that are produced by the resolution of nucleoli are not used in the formation of a new generation of nucleoli, but that they are dissolved in the achromatin substance of the nucleus and transformed into nucleinic acid. This acid passes by osmosis through the nuclear membrane and is diffused through the cytoplasm. "Dans les plages formatrices, il rencontre les globulines de réserve imbibées d'eau, et se combine avec elles pour former la paranucléine d'abord, la vitelline en suite. . . . Nous considérons les plaques vitellines comme étant des produits de l'activité du noyau et du cytoplasme: celui-ci fournirait les globulines, le noyau, l'acide paranucléinique. Les vitellines sont, en effet, des paranucléo-albumines, c'est-a-dire des combinaisons de paranucléine avec un albumine qui est ici une globuline."

Jordan has observed in the newt appearances which might be interpreted as a migration of very minute granules from

the germinal vesicle into the cell body, and he also is inclined to the opinion that nucleus takes part in the formation of yolk. "One might suppose that granules from the germinal vesicle serve as starting points, centers of attraction or stimulation as it were, while the cytoplasm perhaps through the mediation of the yolk-nuclei, elaborates and supplies he requisite deutoplasmic material out of nutritive elements furnished it by the follicle cells."

Since it is seemingly impossible to harmonize these various observations regarding the yolk-nuclei and the yolk in the egg of amphibians, it can only be supposed, if these observations are correct, that the processes by which yolk is formed differ in various species. The details of these processes must, therefore, be worked out for each species separately, since there is no apparent similarity between them even in closely related forms.

In the egg of Bufo it is possible to trace the anlage of the yolk-nuclei back to the primordial germ-cells. As I have already stated, there is present in the cytoplasm of these cells a small, round, apparently homogeneous body which is sometimes, though not invariably, separated from the cytoplasm by a clear area (Fig. 8, V). This body colors very intensely with iron hæmatoxylin, and it always takes the safranin when sections are stained with safranin and gentian violet or with safranin and Lichtgrün. In preparations stained with Delafield's hæmatoxylin and orange G. this body is hardly discernible, since it takes the orange stain as does also the cytoplasm. Judging from its staining reactions this body is not chromatin; neither is it a centrosome, since the same section of the cell may show both of these structures (Fig. 7). I have not been able to determine the origin of this body owing to the fact that in very young tadpoles the large yolk plaques in the primodial germ-cells obscure the other cytoplasmic structures, while in older tadpoles, when the yolk is beginning to be absorbed, the small yolk granules show the same staining reactions as this body and therefore cannot be distinguished from it. Not until the tadpole is at least thir-

teen days old can this structure be distinguished with any degree of certainty. I shall apply the term "vitelline body" to this structure and also to other bodies of similar character which appear later in the cytoplasm, reserving the term, "yolk-nucleus" for the granular masses found in the cytoplasm at a much later period of development.

The vitelline body divides previous to each cell mitosis (Fig. 7, V). In sections of the ovaries of young toads this structure is found in the primary oögonia (Figs. 16-17), in the secondary oögonia (Figs. 18-19), in the young oöcytes at the critical period when the cell contents stain very faintly and cell boundaries and nuclear outlines are made out with difficulty (Fig. 20), and also during synizesis and early post-synizesis stages (Figs. 23-31).

In the early stages of development a cell rarely contains more than one vitelline body unless it is preparing to divide. During synizesis the vitelline body enlarges somewhat and at a slightly later period it becomes oval and then constricts through the middle so that it has the appearance of a dumb-bell (Fig. 47, a); subsequently it divides into two rounded parts (Fig. 47, b), which soon separate (Fig. 47, c). The vitelline bodies thus formed divide repeatedly, and by the time that the oöcyte has reached the stage of Figs. 36-39, its cytoplasm contains a considerable number of these bodies which vary greatly in size, although they all appear round and homogeneous. Sometimes at this stage a vitelline body is enclosed in a clear area which marks it off from the cytoplasm, but this is not a constant phenomenon. Occasionally a vitelline body does not divide in the manner described above, but it breaks into three small parts (Fig. 38, Y); in other cases division is unequal and one large and one small body are formed (Fig. 38, X). Since the vitelline bodies vary so greatly in size and are so widely scattered throughout the cytoplasm at the stage of Fig. 39, it seems very probable that some of them are newly formed secretion products of the cytoplasm which appear first as minute granules and gradually increase in size.

The vitelline bodies begin to increase in number before the resolution of the large compound-nucleoli and at a time when the nucleus contains but a very few plasmosomes: they are scattered throughout the cytoplasm, chiefly in the zone midway between the periphery of the egg and the nuclear membrane; and very few of them ever lie close to the nucleus. These facts would seem to preclude the possibility that the vitelline bodies are extruded nucleoli, although in their staining reactions and in their general appearance they are strikingly like these structures.

One of the reasons given by Will for considering the rounded bodies which he finds in the cytoplasm of the egg of Rana as extruded nucleoli is that he first finds these bodies in a light area close to the nucleus. Preparations of young ovarian eggs of Bufo that have been badly preserved frequently give the impression that nucleoli lie outside of the nucleus in a fluid space marked off from the cytoplasm. If such preparations are examined under an immersion lens, one finds that the light area which apparently surrounds the nucleus is, in reality, a portion of the nucleus itself, since the nuclear membrane is readily found where the clear area comes in contact with the cytoplasm. In such eggs, owing doubtless to the imperfect penetration of the fixing fluid, all of the more fluid portions of the nuclear substance seem to be collected at one side or around the periphery of the nucleus, while the granular achromatin and most of the nucleoli are massed together either in the middle of the nucleus or at one side of it. Projections from this mass sometimes extend across the fluid substance to the nuclear membrane and thus give the appearance of an amœboid nucleus without a nuclear wall. In nuclei of this character nucleoli are sometimes found stranded in the fluid substance and, under low magnification, they appear to lie in the cytoplasm. The clear area which separates the nucleus from the cytoplasm in many eggs is doubtless an artefact produced through the action of reagents: I do not think that it is present in the living egg.

The vitelline bodies are rarely found close to the periphery of the egg at the stages of Figs. 36-39, and I have seen noth-

ing that would indicate that follicle cells or their products enter the egg and produce these structures. As these bodies are not extruded nucleoli it is evident that they must be considered as secretion products of the cytoplasm itself. Since, as Bernard (8), Chittenden (20) and others have maintained, the nucleus is undoubtedly to be considered as an organ of constructive metabolism which "has controlling power over the metabolic processes in the cell, modifying and regulating the nutritive changes" (Chittenden), it is not to be supposed that the formation of the vitelline bodies in the cytoplasm takes place independently of nuclear activity. Although no substance can be seen to leave the nucleus at the time that the number of vitelline bodies is rapidly increasing, it is not improbable that a fluid, possibly an enzyme, passes from the nucleus into the cytoplasm and there causes the formation of these bodies. The same enzyme, acting in the nucleus itself, may be the cause of the formation of the plasmosomes; for these bodies are being produced in considerable numbers in the nucleus at the time that the formation of vitelline bodies is taking place most actively in the cytoplasm. On this assumption it is probable that the vitelline bodies "bear the same relation to the cytoplasm that the nucleoli do to the germinal vesicle," as Jordan has suggested. Whether the substance out of which the vitelline bodies are made is supplied entirely by the cytoplasm, or whether the follicle cells contribute material to the egg for their formation, I have not been able to determine. I have never found follicle cells inside of the egg, although they very frequently enter the cells of Bidder's organ. The function of the follicle cells seems to be to form the egg membranes during the early stages of development and, after the egg has left the ovary, to aid in the absorption of the follicle sacs (King, 50).

Several investigators of amphibian oögenesis, besides Will and Leydig, have found rounded bodies in the cytoplasm of the egg which are doubtless of the same nature as the vitelline bodies in the egg of Bufo. Hertwig (42) states that the small bodies which he finds in the cytoplasm of the egg of

Rana are composed of a hyaline substance and appear much like nucleoli, although they cannot be extruded nucleoli since nucleoli never wander into the cytoplasm. Born discovered small oval bodies near the nucleus in the cytoplasm of the egg of Triton which he hesitates to call yolk-nuclei since they never appear granular. Bataillon describes and figures the division of a small body lying in the cytoplasm of the egg of Rana which he considers to be a large nucleolus which has passed out of the germinal vesicle. He states that this body ordinarily disappears when the yolk is formed, and that he once saw it transformed into pigment.

Bodies similar to the vitelline bodies in the egg of Bufo have been found in the mammalian egg by von Winiwarter (94), Gurwitsch (36), and von Skrobansky (85). These bodies are present in the cytoplasm in addition to a granular yolk-nucleus. The latter structure, according to the researches of von Winiwarter and Gurwitsch, is homologous to the idiozome in the sperm-cells. The figures given by von Skrobansky of rounded bodies in the egg of the guinea pig are very similar to those shown in Figs. 36-38. Von Skrobansky states that these bodies increase in number as the egg develops and that they have a tendency to form in groups of two, three, or more which are often surrounded by a clear area. As their appearance is coincident with the disappearance of the yolk-nucleus, he suggests that the substance of the yolk-nucleus becomes differentiated into these rounded bodies, although it is not impossible that they are new differentiation products of the cytoplasm.

Small homogeneous bodies appearing like the vitelline bodies in the egg of Bufo have been found in the cytoplasm of the eggs of various arachnoids, myriapods, and vertebrates, and classed with large granular structures as yolk-nuclei. From the researches of Henneguy, Balbiani (3), and others, it is evident that the term yolk-nucleus has been used in a general way to cover a number of different structures in the cytoplasm, as the term nucleolus has been applied to a variety of structures in the nucleus.

When the toad has reached a body length of about 4 cm. and the egg has a diameter of from 0.18-0.2 mm., there appears simultaneously in different parts of the cytoplasm a number of irregular, granular masses which I shall call yolk-nuclei since they are similar in appearance to the structures described under this name by Foot (32), Henneguy, Jordan, Calkins (14), and Munson (72). These yolk-nuclei arise as rather small, irregular patches of granular substance that are not sharply marked off from the surrounding cytoplasm. There is at first no regular arrangement of these bodies; some lie near the nucleus; others are found near the periphery of the egg, while the majority lie in a zone midway between the nuclear membrane and the outer boundary of the egg. When sections are stained with any of the various combination stains previously mentioned, these yolk-nuclei take the plasma stain more deeply than does the cytoplasm and hence are easily seen. It seems strange that such a keen observer as Goette failed to find these bodies in the egg of Bombinator.

The manner in which these yolk-nuclei are formed is shown in Fig. 46. Around one of the larger vitelline bodies there appears a clear area, as if the vitelline body had in some way caused a liquefaction of the surrounding cytoplasm (Fig. 46, X). The substance of this area then becomes changed into an irregular mass of minute granules which at first stain but slightly darker than the cytoplasm. In some cases the vitelline body can be found in the centre of the yolk-nucleus (Fig. 46, Y), but as a rule it quickly loses its capacity for staining and then disappears, evidently being used up in the formation of the yolk-nucleus. As shown in Fig. 53, a yolk-nucleus sometimes contains several vitelline bodies of various sizes which stain as intensely as at the stages of Figs. 36-39. In cases of this kind it is impossible to determine whether the vitelline bodies in each yolk-nucleus are produced by the repeated division of the one vitelline body concerned in the formation of the yolk nucleus, or whether several vitelline bodies originally took part in the formation of a single yolk-nucleus. I am inclined to the former view since the vitelline

bodies grow rapidly and divide very readily both in earlier and in later stages of development and they are rarely found in groups of more than three before the formation of the yolk-nuclei.

In eggs with a diameter of 0.25-0.3 mm. the yolk-nuclei are very conspicuous since they stain more intensely than at the stage of Fig. 46, and their number is much less than at an earlier period as several small granular masses fuse to form larger ones. At this stage of development the yolk-nuclei come to have a definite arrangement in the cytoplasm, forming a more or less complete ring midway between the nucleus and the periphery of the egg (Fig. 44). This is not an accidental arrangement found in a few eggs, but it is a constant phenomenon in eggs of a given size taken from different individuals and preserved and stained in different ways. At this time the cytoplasm contains very few large vitelline bodies, most of these bodies having been used up in the formation of yolk-nuclei.

In the egg of the newt Jordan finds granular yolk-nuclei similar in appearance to those found in the egg of Bufo at the stage of Fig. 44. Jordan states that these bodies appear about the time that the yolk is beginning to form at the periphery of the egg "from points of independent origin," and that there are never more than nine of these structures. In the very young egg Jordan has found what appears to be "localized condensations of the cytoplasm and a consequent greater avidity for staining fluids," and he finds all gradations between these bodies and the granular yolk-nuclei. From his observations Jordan concludes that "in the newt the yolk-nuclei always arise first as condensations of the cytoplasm and subsequently increase in size and complexity with the growth of the egg." The figures given by Jordan do not show clearly the early development of the yolk-nuclei, although his Figs. 5, 10-12 are sufficiently detailed to indicate that the method by which the yolk-nuclei are formed in the egg of the newt is essentially the same as in the egg of Bufo. The subsequent history of these bodies in the egg of the newt is similar to that

of the yolk-nuclei that are first formed in the egg of Bufo since, for a time, they lie in a zone half way between the germinal vesicle and the periphery of the egg and later draw near to the germinal vesicle where they gradually disintegrate. The only conjecture Jordan makes as to the probable function of the yolk-nuclei is that "they have a real physiological significance probably related to the construction of yolk."

The granular yolk-nuclei found by Foot in the egg of Allolobophora fœtida bear a striking resemblance to those found in the egg of Bufo (Cf. Foot's Figs. 4-5 with my Figs. 44 and 46). According to Foot the yolk-nuclei arise in contact with the nucleus, but, judging from their staining reactions, they are not derived from the chromatin as Calkins (14) maintains is the case in Lumbricus. As the yolk-nuclei increase in size they become broken up, and they are either scattered in patches throughout the cytoplasm or aggregated at the egg periphery. Foot concludes that these yolk-nuclei are formed of "archoplasm," and she traces them into the attraction-sphere, the fertilization cone, and the polar rings. Munson finds granular yolk-nuclei in the egg of Clemmys marmorata which are similar to the granular masses shown in Figs. 44 and 46. Munson states that these structures are formed of "a kind of metaplasm (or archoplasm) arising in the neighborhood of the germinal vesicle through the combined influence of the nucleus and cytoplasm. From the place of its formation, it diffuses or flows throughout the cytoplasm where it serves as a culture medium of the living substance of the egg; in other words, it serves as food. The true yolk-bodies are a secretion of the living substance of the cytoplasm."

Soon after the yolk-nuclei become arranged in the form of a ring there appears near the periphery of the egg a number of small, round, homogeneous bodies which stain intensely (Fig. 45). These bodies, as their subsequent history shows, are a new generation of vitelline bodies which are directly concerned with the formation of the yolk. From their peripheral position one might, perhaps, be inclined to think that the follicle cells are concerned in some way with the formation

of these bodies. I have never found any evidence that would support such an assumption. Since these vitelline bodies are formed some distance from the yolk-nuclei and are at first nearly uniform in size, it would seem as if they must be new secretion products of the cytoplasm. There is the possibility, however, that they are derived either from the few vitelline bodies that were left over after the formation of the yolk-nuclei or from the granular substance of which the yolk-nuclei are composed. The vitelline bodies increase very rapidly in number and in size, and many of them give rise to small granular yolk-nuclei, similar to those shown in Fig. 46, which always remain at the outer surface of the egg (Fig. 54).

While the new formation of vitelline bodies is taking place at the egg periphery, the ring of yolk-nuclei is gradually moving towards the centre of the egg and at the stage of Fig. 54 it closely encircles the germinal vesicle. In many cases yolk-nuclei seem to come in actual contact with the nuclear membrane. Whether there is a fusion between these bodies and the substance of the germinal vesicle, as Jordan is inclined to believe, I am not able to state. There is no noticeable increase in the size of the nucleus or any unusual change in the nuclear structure as one might expect to be the case were a considerable quantity of substance taken at this time into the germinal vesicle. After reaching the germinal vesicle the circle of yolk-nuclei stains less intensely and gradually disappears (Fig. 56). I am inclined to the opinion that their substance is dissolved in situ to take part later in the formation of the yolk spherules in this region of the egg. This view agrees substantially with that advanced in 1859 by Thompson and since advocated by Jordan.

As a rule the yolk-nuclei that are first formed have moved close to the germinal vesicle by the time that new yolk-nuclei are to be found at the periphery of the egg, and there is a cytoplasmic zone between them which is free from vitelline bodies or yolk-nuclei (Fig. 54). In exceptional cases, as shown in Fig. 53, the formation of the peripheral vitelline bodies and yolk-nuclei takes place even before the older yolk-

nuclei have become arranged in the form of a ring, and the only portion of the cytoplasm which does not contain these structures is that surrounding the germinal vesicle. As these cases are found so infrequently I have not been able to determine whether the yolk-nuclei later become arranged as in Fig. 54, or whether all of them remain at the periphery of the egg to take part in the formation of the yolk there.

In Bufo, as in other amphibians according to the investigations of Vogt, Goette, Schultze, Born, Iwakawa, Jordan, and Dubnisson, yolk spherules first appear in the outer regions of the cytoplasm and usually simultaneously in different parts of the egg. There are apparently two methods by which the first yolk spherules may be formed in the egg of Bufo. Both of these methods sometimes take place in the same egg; whether this is true for all eggs I am unable to say. The yolk develops so rapidly that it is difficult to follow the processes of its formation in any detail.

Soon after the stage of development shown in Fig. 54 a varying number of small, oval bodies appear in the peripheral yolk-nuclei (Fig. 56). These bodies, which stain somewhat less intensely than the vitelline bodies, are yolk spherules which are being formed, evidently, from the substance of the yolk-nuclei. As the yolk spherules increase in number the granular yolk-nuclei fade away and they have completely disappeared by the time that the yolk forms a continuous layer around the periphery of the egg.

In some cases certain of the vitelline bodies at the periphery of the egg grow very large (Fig. 55). The outlines of these bodies become irregular (Fig. 52, a), and subsequently they break into from two to four rounded, homogeneous pieces (Fig. 52, b) which in turn divide into smaller bodies (Fig. 52, c-e). As a result of the repeated division of the one vitelline body there is formed a mass of small oval bodies (Fig. 52, f) which are of the same size and shape as the small yolk spherules, although at first they stain more deeply than the yolk spherules and can therefore readily be distinguished from them. Later these bodies stain less intensely and seem to pass

over into yolk spherules. It is very probable that the aggregations of small yolk spherules shown in Figs. 55-56 have had such an origin, although it is possible that they were derived from the substance of a yolk-nucleus. During the early stages of yolk formation the cytoplasm at the outer boundary of the egg frequently appears vacuolated. This does not seem to be a constant phenomenon, however, and it may be due in part, at least, to the action of reagents.

In the egg of Bufo there are two generations of yolk-nuclei, both formed from or under the influence of the vitelline bodies, and both evidently concerned in the formation of yolk. The first yolk-nuclei that are formed appear simultaneously in different regions of the cytoplasm and they later move close to the germinal vesicle where they gradually fade away. One can readily trace every step in their development from the stage of that shown in Fig. 46 to that of Fig. 56. The yolk-nuclei belonging to the second generation are formed at the periphery of the egg and they are transformed directly into yolk spherules. The various stages in the development of these bodies can also easily be followed. With these facts in mind the following statement by Crampton (24) is of interest: "The accounts of the origin of the yolk-spheres from cytoplasmic elements at places removed from the nucleus, or from several centres, or in all parts of the egg at once, fail to take into consideration an earlier stage marked by the origin from the nucleus of a true yolk-matrix which subsequently disintegrates and spreads throughout the whole cell-body as in Molgula." It would seem as if enough cytological work had been done to make it clear that one cannot deduce general rules applicable to all eggs from the study of one particular egg, no matter how carefully the work may have been done.

According to my investigations the formation of the yolk in the egg of Bufo is closely associated with the vitelline bodies and also with the granular masses produced by them, the yolk-nuclei. I have elsewhere stated that the vitelline bodies are probably secretion products of the cytoplasm formed, possibly, under the influence of an enzyme given off by the nucleus. The

granular yolk-nuclei are undoubtedly composed of nutritive material which is subsequently aggregated into yolk spherules. In some instances the substance of the vitelline bodies seems to be transformed directly into yolk spherules; the intermediate stage, that of the formation of yolk-nuclei, being omitted. This would seem to indicate that the vitelline bodies are themselves but aggregations of nutritive material which is in a semi-fluid condition rather than in the form of granules. It may be that during the early stages in the development of the ova "yolk is present in the cytoplasm in the form of a diffused unstainable fluid," as Montgomery has suggested, and that this fluid is first collected into the rounded vitelline bodies and later changed into yolk spherules.

The part taken by the nucleus in the formation of yolk in the egg of Bufo is as yet obscured. I have never seen any nucleoli or any minute granules leave the nucleus which might have an influence on the formation of the yolk. If, as seems probable, the nucleus directs and controls the nutritive processes in the cell, then in the formation of yolk it must act either through a fluid substance which it gives out into the cell-body, or it must exert its influence directly on the deutoplasmic substance of the cytoplasm. In many kinds of eggs, according to the investigations of Conklin, Crampton, Calkins, Foot and Floderus (31), the yolk is formed first around the nucleus and then produced progressively towards the periphery of the egg. In these cases it may be supposed that the cytoplasm surrounding the nucleus is directly stimulated by the nucleus to produce yolk. In amphibians and many other vertebrates the yolk first appears at the periphery of the egg. In these cases the nucleus has a less direct influence on the yolk formation, and this influence is probably exerted through the action of a fluid substance which passes by osmosis through the nuclear membrane into the cell-body. The investigations that have seemed to show that yolk is derived directly from nucleoli, or from chromatin, or from follicle cells, are all open to question, and until they have been confirmed by further research I shall be inclined to believe that yolk formation is

one of the anabolic processes in the cell which, although it is directly or indirectly controlled by the nucleus, does not depend upon the nucleus for its material substance.

Zoölogical Laboratory,
University of Pennsylvania,
March 14, 1908.

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EXPLANATION OF PLATES.

All figures were drawn with the aid of a camera lucida. They have been reduced one-third.

Abbreviations used in lettering the figures:

Al., alimentary tract.
Ao., aorta.
C., centrosome.
C. V., cardinal vein.
E., endoderm.
G., germ-cells.
H., heart.
L., liver.
L. M., lateral plates of mesoderm.
M., mesoderm.
N., neural tube.
No., notochord.
Nu., nucleolus.
P., early prophase of mitosis.
T., Wolffian tubule.
V., vitelline body.

Fig. 1.—Portion of a transverse section through the middle of a tadpole six days old. × 91.

Fig. 2.—Portion of a transverse section through the middle of a tadpole nine days old showing the location of the germinal ridge. × 91.

Fig. 3.—Section of the germinal ridge at the stage of Fig. 2. × 1,000.

Fig. 4.—Section of the germinal ridge in a tadpole eleven days old. × 1,000.

Fig. 5.—Transverse section of a divided germinal ridge in a tadpole thirteen days old. × 1,000.

Fig. 6.—Longitudinal section showing the extent of the germinal ridge in a tadpole thirteen days old. × 47.

Figs. 7-9.—Sections through the germinal ridge in a tadpole fifteen days old showing the character of the cells at different levels. × 1,334.

Fig. 10.—Early prophase of mitosis in a primordial germ-cell. × 1,334.

Figs. 11-12.—Equatorial plate in a primordial germ-cell. All 24 chromosomes are shown. × 1,334.

Fig. 13.—Longitudinal section of a spindle during the metaphase. Only 5 of the chromosomes are shown. × 1,334.

Fig. 14.—Late anaphase in a primordial germ-cell. × 1,334.

Fig. 15.—Section of the ovary at the time when sex is first apparent. Taken from a tadpole with very well-developed hind legs. × 1,000.

Fig. 16.—Section of a young ovary showing the beginning of the formation of a central cavity. Taken from a tadpole about to undergo metamorphosis. × 1,000.

Fig. 17.—Section of a young ovary containing a central cavity. Taken from a tadpole at the time of metamorphosis. × 1,000.

FIG. 18.—Cyst of secondary oögonia in the resting stage. × 1,334.

FIG. 19.—Cyst containing secondary oögonia in various stages of mitosis. × 1,334.

FIG. 20.—Young oöcyte with oval nucleus. × 1,334.

FIG. 21.—A slightly later stage than Fig. 20. The nucleus of the oöcyte has assumed a rounded form. × 1,334.

FIG. 22.—Early growth stage of the oöcyte. The nucleus contains a well defined, apparently continuous spireme. × 1,334.

FIGS. 23-24.—Stages showing the gradual condensation of the nuclear substance previous to synizesis. × 1,334.

FIG. 25.—Synizesis stage. × 1,334.

FIGS. 26-27.—Post-synizesis stages. Part of the chromatin has been evolved in the form of a continuous convoluted spireme: the nucleoli and the rest of the chromatin appear in the form of irregular masses lying against the nuclear wall or in the meshes of the spireme. × 1,334.

FIGS. 28-29.—Stages showing the longitudinal splitting of the spireme. × 1,334.

FIG. 30.—Slightly later stage. The sister portions of the spireme have begun to separate. × 1,334.

FIG. 31.—Young oöcyte surrounded by its zona pellucida. In the nucleus the sister portions of the spireme are almost entirely separated. × 1,334.

FIG. 32.—Section of an oöcyte in which there is a complete separation of the sister portions of the spireme. × 1,334.

FIGS. 33-34.—Nuclei of the young oöcytes showing the division of the spireme into double segments. × 1,334.

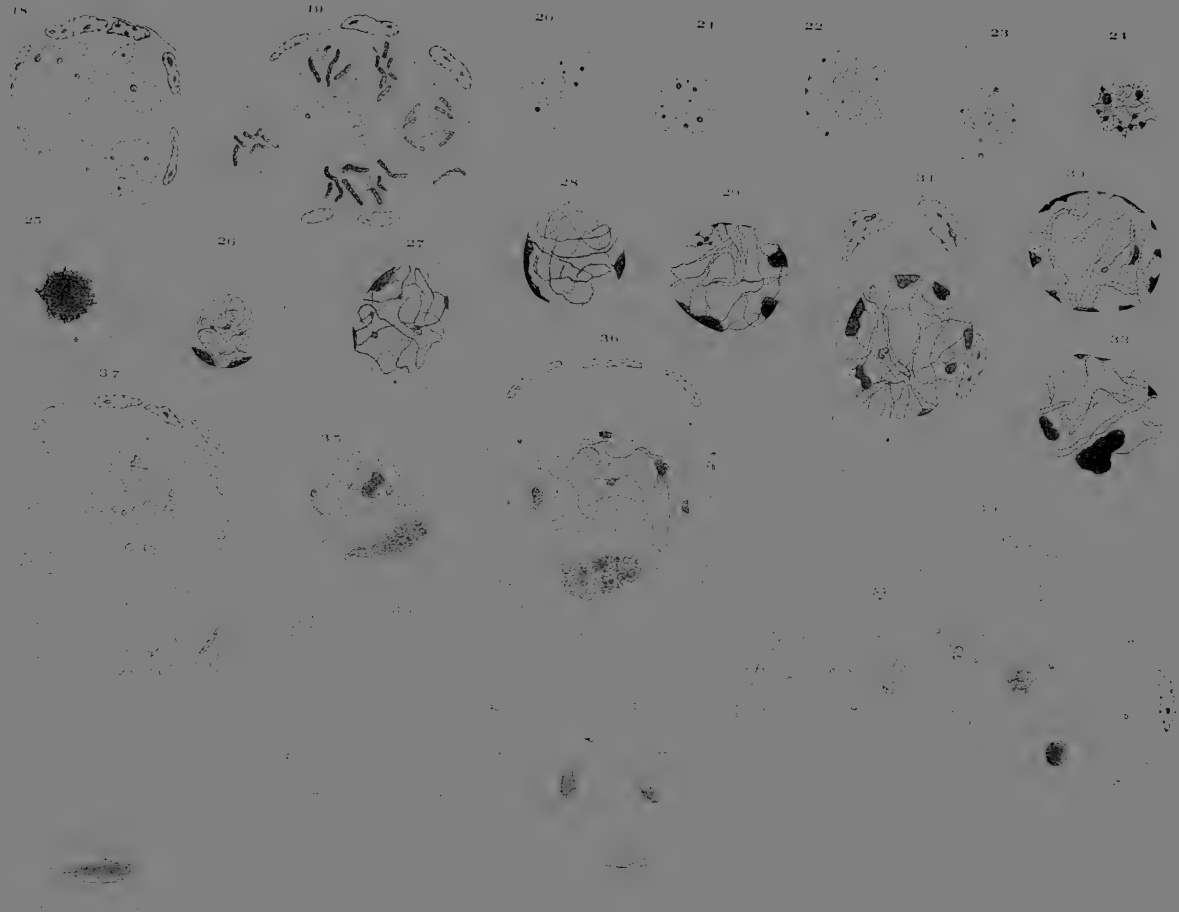
FIG. 35.—Section of the nucleus of a young oöcyte showing the beginning of the resolution of the amorphous masses shown in Figs. 26-34. × 1,334.

FIG. 36.—Section of a young oöcyte showing the differentiation of one of the amorphous masses into a meshwork of chromatin threads and rounded nucleoli. × 1,334.

FIG. 37.—Section of a cyst containing oöcytes in different stages of development. × 1,000.

FIG. 38.—Stage following that of Fig. 36, showing the relation of the nucleoli, the oxychromatin threads and the chromosomes. In the cytoplasm are numerous vitelline bodies. × 1,334.

FIG. 39.—Section of a young oöcyte. The chromosomes are scattered throughout the nucleus and they have assumed the feathery appearance which characterizes them throughout the rest of the growth period. The oxychromatin threads are entirely separated from the nucleoli and have become very granular. In the cytoplasm are numerous vitelline bodies of various sizes. × 1,334.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Figs. 40-41.—Sections of the nuclei in oöcytes of a young toad with a body length of 3.5 cm. A large nucleolar body, oxychromatin threads and feathery chromosomes are shown. × 1,000.

Fig. 42.—Section of the nucleus in the oöcyte of a toad with a body length of 3 cm. Some of the nucleoli stain faintly and are evidently in the process of dissolution. × 1,000.

Fig. 43.—Section of a nucleus in an oöcyte of a toad with a body length of 4 cm. showing the fragmentation of a large nucleolar body, scattered oxychromatin threads, and a pair of chromosomes. × 1,000.

Fig. 44.—Part of a section of an egg taken from a young toad with a body length of 5.5 cm. The yolk-nuclei are collected in a zone lying midway between the nucleus and the periphery of the egg. Diameter of the egg is 0.23 mm.; of the nucleus, 0.11 mm. × 1,000.

Fig. 45.—Drawn from an egg taken from the same ovary as that from which Fig. 44 was taken. Differentiation of the compound-nucleoli with the aid of safranin and gentian violet. × 1,000.

Fig. 46.—Part of a section of an egg taken from a toad with a body length of 5 cm. The yolk-nuclei are forming at the expense of the vitelline bodies. × 1,000.

Fig. 47.—Division stages of a vitelline body. × 1,334.

Fig. 48.—Section of the nucleus of an egg taken from the ovary of an adult toad killed the latter part of April. The plasmosomes are separated from the chromatin and most of them are massed at one side of the nucleus. Diameter of the nucleus, 0.2 mm. × 333.

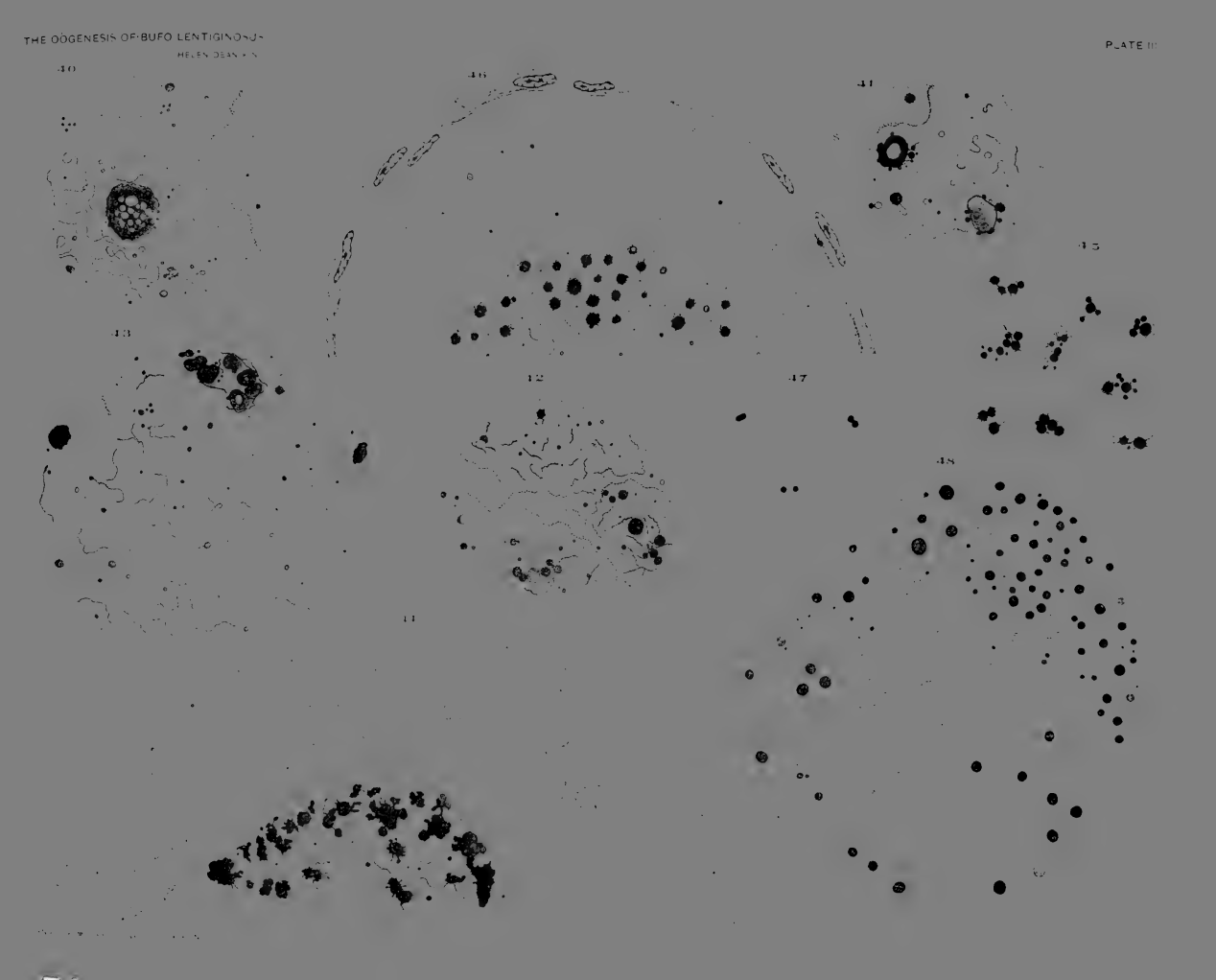
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Fig. 49.—Section of the nucleus of an egg taken from an adult toad killed the latter part of April. The chromosomes occupy the centre of the nucleus, while the plasmosomes are very evenly distributed about the nuclear periphery. × 333.

Fig. 50.—Section of the nucleus of an egg taken from an adult toad killed early in May. The plasmosomes have migrated to the interior of the nucleus and they enclose the chromosomes. × 333.

Fig. 51.—Peculiar types of compound-nucleoli found in many of the nuclei during the later development of the oöcytes. × 1,334.

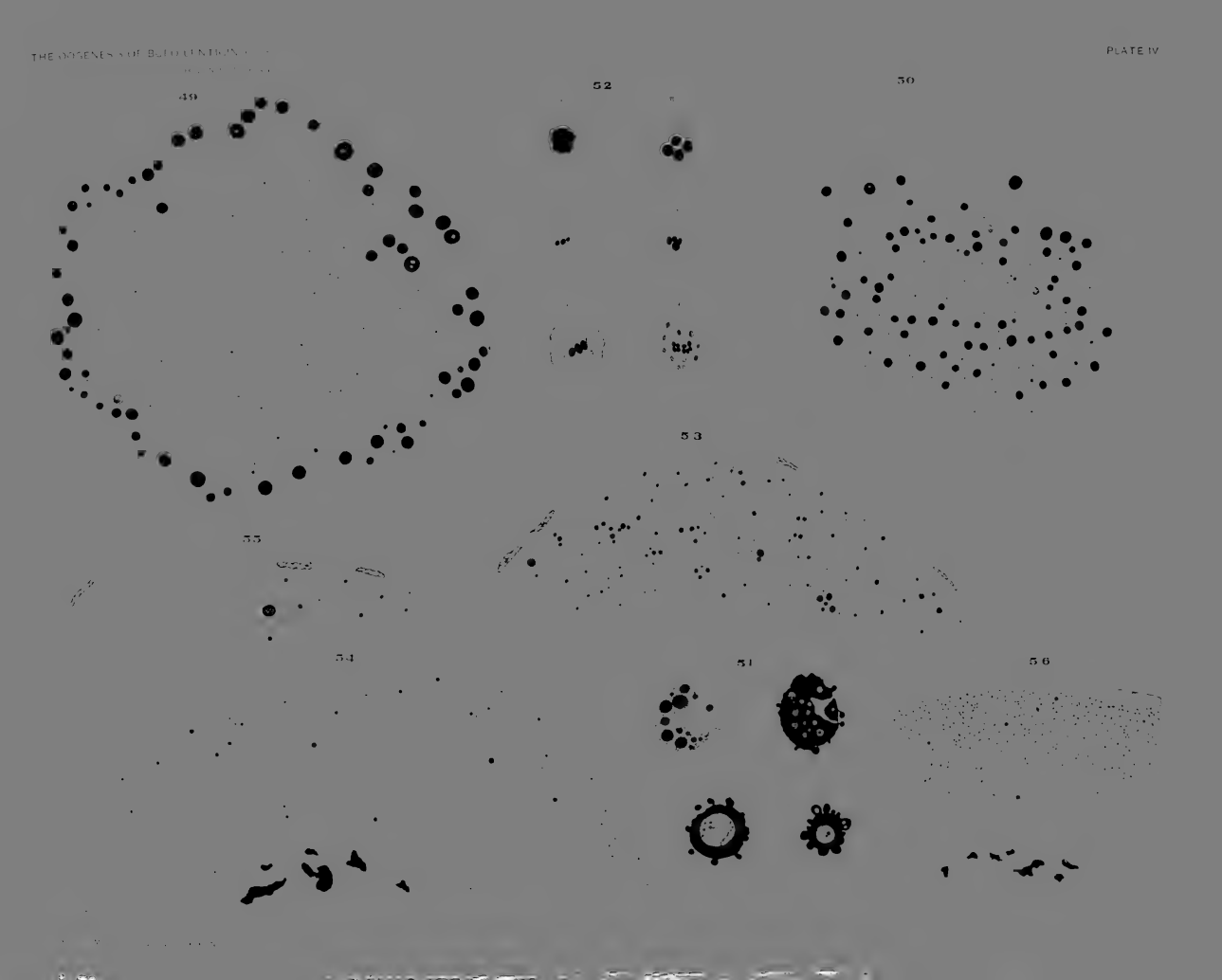
Fig. 52.—Stages showing the formation of yolk spherules from a vitelline body. × 1,334.

Fig. 53.—Yolk-nuclei and vitelline bodies in an egg taken from an adult toad killed the latter part of April. × 1,000.

Fig. 54.—Part of a section of an egg taken from a young toad with a body length of 5.5 cm. New yolk-nuclei are forming at the periphery of the egg, and the older ones closely surround the nucleus. × 1,000.

Fig. 55.—Formation of yolk spherules at the periphery of an egg taken from an adult toad killed in May. ×1,000.

Fig. 56.—Part of the section of an egg taken from an adult toad killed in May. At the periphery of the egg a layer of yolk spherules is forming at the expense of yolk-nuclei and vitelline bodies. The layer of yolk-nuclei around the nucleus is beginning to disappear. × 667.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THE STRUCTURE AND DEVELOPMENT OF BIDDER'S ORGAN IN BUFO LENTIGINOSUS.

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At the anterior end of the testis in all of the Bufonidae is the rounded body to which Spengel gave the name "Bidder's organ." Although many of the investigators who have worked on the germ-cells of the Anura have examined this organ and ventured a conjecture as to its nature and probable function, Knappe (18) is the only one who has studied its development in any detail. In his paper, which appeared over twenty years ago, Knappe gives but a brief account of the early development of this body and he pays but little attention to the nuclear changes in the cells. Those who have more recently worked on Bidder's organ believe, with Knappe, that this body is a rudimentary ovary, and they have been more interested in studying the maner in which the cells degenerate than in trying to determine the reasons for this degeneration. As an investigation of the nuclear and cytoplasmic changes occurring in the cells of Bidder's organ during its early development might possibly give some clue to the function of this body and to the causes for the degenerative processes which occur in it, I have studied the structure and the formation of this organ in Bufo lentiginosus in connection with my other work on the germ-cells of this amphibian. Especial attention has been given in this study to the behavior of the chromatin and to the differences between the germ-cells in Bidder's organ and those in the ovary which become functional eggs. For this investigation I have made use of material prepared for a study of the spermatogenesis and oögenesis of Bufo lentiginosus. Methods of fixation and of staining are given in detail in preceding papers.

The formation of Bidder's organ is first apparent when a tadpole is from fifteen to eighteen days old. Transverse sec-

tions through an embryo in this stage of development show that the anterior portion of each genital ridge has developed much more rapidly than the rest and that this region contains from five to eight large primordial germ-cells (Fig. 2), while the middle and posterior regions never contain more than three of these cells at this time. The anterior part of the genital ridge, which has begun to develop into Bidder's organ, is continuous with the part which later becomes the sex-gland, and the cells in one region appear exactly like those in any other (Cf. Fig. 2, Plate V and Fig. 5, Plate I). At the beginning of its development, therefore, Bidder's organ is composed of two kinds of cells: large rounded primordial germ-cells which have a faintly staining polymorphic nucleus; and small peritoneal cells in which the nucleus stains very deeply and is usually elongated. There are no intermediate stages between these two kinds of cells, and in Bidder's organ, as in the sex-gland proper, the primordial germ-cells must arise from undifferentiated embryonic tissue.

Bidder's organ develops much more rapidly than the sex-gland, and it has attained a considerable size long before it is possible to ascertain the sex of the individual. During the very early stages in the development of Bidder's organ the large germ-cells divide by mitosis; the stages in this process being similar to those taking place in the cells of the sex-gland. In the prophase of mitosis twenty-four deeply staining chromatin segments are formed which condense into V-shaped chromosomes (Fig. 3, X). The spindle has a small centrosome at each pole which is devoid of radiation (Fig. 3, Y).

It is only the early generations of germ-cells in Bidder's organ that are able to divide by mitosis; in later development the division of the cells is invariably by amitosis. In a series of papers dealing with the germ-cells of Moniezia, Child (8, 9) has maintained that amitosis is the usual method by which the germ-cells in this form increase in number, and he is inclined to believe that amitosis occurs much more frequently in normal development than most investigators admit. Amitosis is the normal method by which the germ-cells in Bidder's

organ increase in number after a certain period in the development of this organ, but the cells so dividing are not capable of becoming functional eggs. In Bufo, amitosis in the germ-cells is undoubtedly correlated with degeneration since the germ-cells which become functional always divide by mitosis (King, 16).

Hoffmann (14) is, I believe, the only investigator who has found the cells of Bidder's organ dividing by mitosis. Knappe states that from the beginning the increase in the number of cells in this organ is solely through amitosis. In tadpoles of Bufo lentiginosus it is possible to find amitosis and mitosis occurring simultaneously in Bidder's organ (Fig. 3, A and Y), but a cell dividing amitotically is always older than one dividing by mitosis, since the nucleus of a cell has to undergo a definite series of changes before amitosis occurs. After a cell has once divided directly it is not capable of again dividing by mitosis.

The resting germ-cells in Bidder's organ appear similar to the resting germ-cells in the sex-gland, as they are large round or oval cells with polymorphic nuclei which contain a faintly staining granular reticulum and several nucleoli (Cf. Fig. 4, Plate V and Fig. 18, Plate II). Owing doubtless to the rapid growth of Bidder's organ the yolk spherules disappear from its cells much sooner than from the cells of the sex-gland, and they are rarely to be found in the anterior part of the genital ridge after the tadpole has attained a length of 7-8 mm. When the yolk has disappeared the cytoplasm of the germ-cells appears granular, and it is found to contain one or two vitelline bodies (Fig. 4, V) and a centrosome (Fig. 3, C). At this stage of development there is nothing to indicate that these cells differ in any way from the germ-cells in the other portions of the genital ridge.

After the last mitotic division the cells take on the character of young oöcytes and they usually have several peritoneal cells flattened against their outer surface (Cf. Fig. 5, Plate V and Fig. 21, Plate II). The nucleus of the cell is no longer polymorphic but rounded in outline and it contains a faintly stain-

ing chromatin reticulum in the meshes of which there are several nucleoli. The nucleus maintains its rounded form during the later development of the oöcyte and it only becomes irregular when the cell degenerates. I cannot confirm Knappe's observations that the nucleus of the cells of Bidder's organ sometimes sends out amœboid processes by which it moves about to accelerate the taking up of nourishment. At the stage of Fig. 5 the cells are usually collected in cell nests, as are the young oöcytes in the ovary. All of the cells of a nest are in practically the same stage of development, and doubtless all have arisen by the repeated division of one primordial germ-cell. During the early stages of its development Bidder's organ has no outer membrane, but shortly before the tadpole undergoes its metamorphosis this body becomes surrounded by a capsule which is formed by the peritoneal cells in a manner similar to that by which the outer ovarian wall is formed.

The development of the young oöcytes in Bidder's organ parallels that of the ovarian oöcytes. After the stage of Fig. 5 the cell body and the nucleus enlarge very rapidly and the chromatin forms an apparently continuous spireme which stains rather faintly (Fig. 6). When the nucleus has attained a diameter of about 0.013 mm., the chromatin shows a marked increase in its capacity for staining and the spireme, which has become much thicker and somewhat jagged in outline, begins to condense (Fig. 7). This condensation continues until practically all the chromatin is collected in the centre of the nucleus where it forms a loose mass of fine fibres in which are imbedded several nucleoli (Fig. 8). This is the synizesis stage in the oöcytes of Bidder's organ. It corresponds approximately to the stage in the contraction of the nuclear contents in the ovarian oöcytes shown in Plate II, Fig. 24, since the meshwork of fibres is considerably looser and the fibres themselves are much coarser than those in the synizesis stage of the egg shown in Plate II, Fig. 25.

Slight as the differences appear between the synizesis stage in the ova of Bidder's organ and that of the ovarian oöcytes,

their effects on the latest development of the cells of Bidder's organ are far reaching since, after the stage of Fig. 8, the nuclei of these cells appear, as a rule, very different from the nuclei of the same size in the ovarian ova. The development of the ova in Bidder's organ is similar to that of the germ-cells in the ovary only until the synizesis stage. During the time that the chromatin forms a contracted mass in the centre of the nucleus changes occur which check normal development and eventually bring about a degeneration of the cells. Just what these changes are it is impossible to determine. A comparison of the early post-synizesis stages in the cells of Bidder's organ with similar stages in the ovarian ova indicates that during the contraction period in the cells of Bidder's organ the chromatin does not become arranged in a normal manner, since in later stages there is no separation of the chromatin that is normally used for the chromosomes from the chromatin that has other uses in the cell, as is the case in the ovarian ova. The turning point in the development of the ova in Bidder's organ is, therefore, the synizesis stage. Since normal development is not possible beyond this period, except in rare instances, it is evident that the causes which bring about the degenerative processes in the cells of Bidder's organ manifest themselves during synizesis and that they act in such a way as to prevent a normal arrangement of the chromatin granules. Since the amount of chromatin in the nucleus is apparently not affected by these changes it must be that it is the arrangement of the chromatin granules that is the important thing in synizesis. The fact that the cells of Bidder's organ show degenerative changes and divide by amitosis soon after synizesis strengthens my belief that synizesis in the egg of Bufo is a means by which the chromatin which bears the hereditary qualities is separated from the chromatin which has other uses in the cell. This separation is not effected during the synizesis stage in the ova of Bidder's organ, consequently these cells are not capable of developing into functional eggs.

Since Bidder's organ develops much more rapidly than the sex-gland, it is possible, perhaps, that the rapid growth of the

cells may in a measure be responsible for the deviations from the normal processes which occur during synizesis. On this assumption to determine the causes for the rapid growth of Bidder's organ would be to determine also the reason for the degeneration of its cells. These causes must be sought through experimental work; they cannot be determined from a morphological study of this body.

Occasionally, in later development, I have found nuclei in the cells of Bidder's organ which were very much like nuclei of ovarian eggs of the same size (Cf. Plate V, Fig. 20 and Plate III, Figs. 40-43). In such cases it can only be supposed that the nuclear changes which took place during synizesis were nearly like those occurring in the ovarian ova and, consequently, that the cells could continue for a longer time to develop in a normal manner.

Stages in the development of the young oöcytes of Bidder's organ through the synizesis stage shown in Fig. 8 are to be found in young tadpoles in which the sex-glands are still in an apparently indifferent state. Soon after the synizesis period the cell nests are broken up and the ova, which are surrounded by follicle cells, become separated by the connective tissue stroma which develops throughout the organ. The formation and development of the ova proceeds from the periphery centripetally; the oldest and largest cells lie towards the center of the organ, the youngest cells towards the periphery.

In tadpoles killed at the time of metamorphosis and also in young toads one frequently finds at the periphery of Bidder's organ nests of young ova in various stages of synizesis. It is doubtless such cell nests that Hoffmann and Cerruti (5) have considered as cysts containing sperm-cells, since the cells at this time bear but little resemblance to the later stages in the development of the ova and they appear somewhat like certain stages in the development of the spermatocytes. I have never found sperm-cells in the ova of Bidder's organ. Oblique sections passing through the posterior part of this organ may show the sperm-cells of the upper part of the testis

in close contact with the large ova of Bidder's organ, but as von Wittich (34) states, Bidder's organ is always clearly marked off from the testis; one never passes gradually into the other as Knappe and Ognew (24) maintain is the case in Bufo vulgaris. The sharp distinction between Bidder's organ and the sex-gland is not maintained in the female of Bufo lentiginosus after the first year, since at about this time the cavity of Bidder's organ becomes continuous with the central cavity of the ovary. Knappe asserts that he has found mature spermatozoa in the cells of Bidder's organ that have begun to degenerate, and he believes that these spermatozoa have developed from follicle cells that have entered the cytoplasm of the ova. In a recent paper (King, 17) I have shown that the structures considered by Knappe as spermatozoa are very probably parasites, since the figures which he gives of these bodies are very similar to certain stages in the life cycle of a sporozoan parasite which infects the cells of Bidder's organ in the American toad, Bufo lentiginosus.

As the nuclei in the cells of Bidder's organ emerge from synizesis the nuclear contents does not become arranged in a manner similar to that found in the nuclei of the ovarian ova in early post-synizesis stages. In the great majority of cases practically all of the chromatin goes into a continuous spireme (Fig. 9), while the greater portion of the plasmosome substance is collected into one or two rounded masses which lie in the meshes of the spireme or against the nuclear membrane (Figs. 10, 14). In some few nuclei the plasmosome masses have a smooth outline and they color uniformly red when preparations are stained with safranin and gentian violet (Fig. 14). In such cases it is evident that all of the chromatin, except that found in the few small karyosomes which are scattered about the nucleus, has gone into the spireme. In other nuclei a small amount of chromatin remains attached to the outer surface of the plasmosomes, giving these bodies a slightly irregular outline (Figs. 9, 10). Nucleolar masses of this kind correspond in structure to the compound-nucleoli found in the ovarian ova, and their subsequent fate is the

same since they undergo a resolution into plasmosomes and oxychromatin granules (Figs. 21-25).

The continuous spireme found at the stage of Fig. 9 appears granular and somewhat irregular in outline. It does not undergo a longitudinal splitting at any stage of development, but it divides transversely into a number of segments which are of various lengths (Figs. 10-14). These segments are scattered throughout the nucleus and they are never found in pairs. Camera drawings of all of the sections of a nucleus in this stage of development show that the number of chromatin segments is greater than the somatic number (24). All of the chromosomes appear granular, as a rule, and numerous fine projections extend out from either side (Fig. 16); only in rare instances (Fig. 20) do any of the chromosomes assume the feathery appearance which characterizes the chromosomes in the later growth stages of the ovarian ova. In later development all of the chromosomes break up into minute granules which are dissolved in the karyoplasm when the egg degenerates.

Usually the cells begin to divide by amitosis soon after the spireme has broken into segments. Nuclear divisions sometimes follow each other rapidly, and a cell may contain several rounded nuclei before the cytoplasm divides (Fig. 31). As a rule, the largest nucleolus divides once or twice before the nucleus itself divides. A constriction appears in the middle of the nucleolus (Fig. 11), and it subsequently breaks into two rounded portions which are nearly equal in size (Fig. 12). The two nucleoli thus formed usually move to opposite sides of the nucleus before the nucleus divides (Figs. 10, 13). The nucleus elongates considerably previous to amitosis (Fig. 15), and it is constricted into two nuclei of approximately equal size (Figs. 16, 17, 19). Each nucleus contains at least one large nucleolus and apparently half of the chromosomes (Fig. 19); and one or both of the nuclei may divide again before the cytoplasm of the cell shows any evidence of a division (Fig. 31). Amitosis is frequently seen in the cells of Bidder's organ in tadpoles killed at the time of metamorphosis, and it can be

found in practically every section of Bidder's organ taken from young toads or from adult males.

By the time that the nucleus has reached the stage of Figs. 10-13 the follicle cells have formed a membrane around each egg, the zona pellucida (Figs. 27, 28, 30). Not infrequently a blood corpuscle is to be found among the follicle cells which lie inside of the zona pellucida in contact with the outer surface of the cell (Fig. 19, B. C.).

In the early stages of the development of Bidder's organ the cytoplasm of the young oöcytes contains a single vitelline body which is sometimes surrounded by a clear area as it is in the ovarian ova (Fig. 7, V). About the time of synizesis this vitelline body divides repeatedly, and by the time that the egg has attained a diameter of 0.035 mm. there are a number of these bodies of various sizes scattered throughout the cytoplasm (Fig. 16). Will (33) and Leydig (20) maintain that the rounded bodies in the cytoplasm of the egg of Rana (which are similar to the vitelline bodies in the egg of Bufo) are nucleoli which have migrated from the nucleus into the cytoplasm in order to form the yolk. Such an origin for the vitelline bodies in the cells of Bidder's organ is impossible, since these bodies are increasing in number at the time that the nucleus rarely contains more than three or four small nucleoli. As is the case in the ovarian ova, the vitelline bodies bring about the formation of granular yolk-nuclei in the cells of Bidder's organ, and, at the stage of development shown in Fig. 19, the cytoplasm of the cells sometimes contains a large number of these structures. The arrangement of the yolk-nuclei in the cells of Bidder's organ differs from that found in the ovarian ova, since these bodies are always scattered irregularly throughout the cytoplasm and are never collected in a zone midway between the nucleus and the periphery of the egg. In many cases the yolk-nuclei form in a very abnormal manner, and a cell, instead of containing a large number of small yolk-nuclei, will contain only two or three of these structures which are very large (Fig. 18). In such ova one of the large yolk-nuclei almost invariably forms

a cap over one side of the nucleus, thus appearing very similar to the yolk-nuclei which, in many kinds of eggs, originate close to the nuclear membrane.

Knappe states that in Bufo vulgaris about one year old the cytoplasm of the cells of Bidder's organ contains a large, rounded, refractive body which is sharply marked off from the cytoplasm. He maintains, furthermore, that the nucleus puts out processes like pseudopodia which engulf this body; afterwards the pseudopodia are slowly withdrawn and the nucleus again becomes rounded, while the ball of substance is gradually dissolved in the karyoplasm. I have not observed this remarkable phenomenon in the cells of Bidder's organ in Bufo lentiginosus. In this species of Bufo, at certain stages in the development of Bidder's organ, the cytoplasm of the cells contains many granular yolk-nuclei which are more or less rounded in form and sharply defined (Fig. 19), but I have never seen anything that would indicate that these masses are ever taken into the nucleus.

The inability of the cells of Bidder's organ to develop into functional eggs has been ascribed by Knappe to the fact that these cells are not able to form yolk. A study of the early development of Bidder's organ in Bufo lentiginosus shows that in a great many of the cells the first stages in the formation of yolk take place, since yolk-nuclei are formed in a manner similar to that which takes place in the ovarian ova (Fig. 19). Except in the one case to be described later, I have never found the development of yolk in the cells of Bidder's organ progressing beyond the stages shown in Figs. 18-19. Were the processes leading to yolk formation independent of nuclear action it would seem as if they might continue beyond this point, since there is no evidence of cytoplasmic degeneration at the stage of Fig. 19. The degenerate condition of the nucleus at this time is shown by the arrangement of the nuclear contents and by the fact that the cells are dividing amitotically.

Fig. 26 shows a section of a cell taken from the Bidder's organ of a young male toad with a body length of 2 cm. In

this cell, and also in a few others lying near it, the cytoplasm contains a number of yolk spherules of various sizes. These spherules were not formed at the periphery of the egg, as is the case with the yolk spherules that first appear in the ovarian ova, but they were formed in, and from the substance of the yolk-nuclei scattered throughout the cytoplasm of the cell. The cytoplasm appears much vacuolated and the nucleus is in an advanced state of degeneration, while many of the large yolk spherules are disintegrating. The process of dissolution at first affects only a part of the yolk spherule, leaving a crescent shaped structure (Fig. 26, Y) which remains for a time and then disappears. This egg seems to me to furnish convincing evidence that yolk-spherules are formed from the substance of yolk-nuclei, and it also shows that the failure of the cells of Bidder's organ to develop into functional eggs cannot be due entirely to their inability to form yolk, as Knappe claims.

The compound-nucleoli which are found in some eggs during early post-synizesis stages (Figs. 9, 10) begin their resolution, as a rule, soon after the spireme divides into segments. These bodies first become very irregular in outline (Fig. 21), and subsequently several light areas appear in them (Fig. 22). In later stages a differential stain, such as safranin and gentian violet, shows that these structures are composed of a mass of rounded plasmosomes embedded in chromatin granules (Fig. 23). The component parts of these masses are soon separated (Figs. 24-25), and the plasmosomes become distributed throughout the nucleus. Very little chromatin is found in the compound-nucleoli in the cells of Bidder's organ compared with the amount that goes into the formation of the large nucleolar masses in the ovarian ova. Usually, after the resolution of the compound-nucleoli, the chromatin granules become scattered through the karyoplasm, only in exceptional cases (Fig. 20) is enough of the chromatin separated from the spireme after synizesis to form oxychromatin filaments. Nucleolar masses similar to those shown in Figs. 23-24 are evidently present in the cells of Bidder's organ in Bufo vulgaris since Ognew states that he sometimes

finds large nucleoli which have a very complicated structure, being composed of a number of deeply staining balls surrounded by a mass of granules.

No matter how many plasmosomes a nucleus may contain there is usually one of these bodies that is larger than the others (Fig. 29); and one of the first indications of the approaching dissolution of the nucleus is the formation of a fluid space around this large nucleolus (Fig. 17). The nucleolus itself at this time may appear homogeneous (Fig. 18), or it may contain one or many vacuoles (Fig. 17). In either case it stains less intensely than in earlier stages and it gradually decreases in size (Figs. 26-27), while the vacuole around it constantly grows larger (Figs. 18, 26, 29). As the fluid space becomes several times the size of the original nucleolus, its substance cannot be derived entirely from the nucleolus, but it must be obtained in part from the dissolution of the karyoplasm. The vacuole grows until it comes in contact with the nuclear membrane (Fig. 26). It then breaks at some point in its outer surface and the nuclear substance is in direct contact with the cytoplasm, as during the growth of the vacuole the nuclear membrane becomes very irregular in outline and it disappears entirely when the vacuole breaks (Fig. 27). While these changes are taking place the chromosomes gradually break up into granules that cannot be distinguished from the karyoplasm, and by the time the nuclear membrane has disintegrated most of the chromosomes have disappeared (Fig. 27). During the disintegration of the nucleus I have never found the chromatin in the form of irregular clumps as Ognew has found to be the case in the degenerating cells of Bidder's organ in Bufo vulgaris. The degenerative changes just described are not found in the cells of Bidder's organ until the young toad has attained a length of about 2 cm.

Degenerative changes usually appear in the cytoplasm soon after the stage of Fig. 19, since only in rare cases is a cell able to form yolk spherules. If a cell contains a large number of yolk-nuclei at the time that these degenerative processes begin the yolk-nuclei are dissolved in situ, leaving clear fluid spaces

in the cytoplasm which at first have the shape and size of the yolk-nuclei (Fig. 29). Later these spaces are united and the cytoplasm then contains several large vacuoles (Fig. 27). In cases in which the cytoplasm contains only a few large yolk-nuclei instead of a number of small ones (Fig. 18), these masses become sharply marked off from the cytoplasm when degenerative changes begin and they stain much more intensely than before. The appearance of these bodies thus becomes so very different from that of the yolk-nuclei shown in Fig. 19 that it might be thought that they were not yolk-nuclei but the products of a fatty degeneration of the cytoplasm. In order to determine this point definitely several cells appearing much like that shown in Fig. 18 were drawn with the aid of a camera lucida and the preparations containing them were then put in ether where they remained for about two weeks. At the end of this time the slides were remounted and the same cells were again drawn. The irregular granular masses had not been affected in any way by the ether and they were just as large and conspicuous as before. These bodies cannot, therefore, be products of a fatty degeneration of the cytoplasm or they would have been dissolved by the ether. Soon after the stage of Fig. 18 these masses dissolve in situ and several large vacuoles are formed in the cytoplasm which later become connected as in Fig. 27.

Knappe maintains that there are four ways by which the rudimentary eggs in Bidder's organ disintegrate: (1) through the penetration into the cytoplasm of follicle cells which absorb the egg substance; (2) through the development of pigment in the cytoplasm which seems to bring about a gradual collapse of the egg; (3) through the invasion of the cytoplasm by both follicle cells and blood capillaries; (4) through pigment formation combined with the penetration of blood capillaries into the egg. To these Ognew, from his study of Bidder's organ in Bufo vulgaris, adds a fifth method—a peculiar process in which the follicle membrane between two adjacent oöcytes disappears leaving a space which grows in breadth and finally becomes a large spherical vacuole which is

filled with a fluid derived from the disintegration of the oöcytes. Ognew also suggests as a special process of degeneration, the penetration of one cell of Bidder's organ into another.

From the very early stages in the development of Bidder's organ in Bufo lentiginosus the germ-cells are surrounded by follicle cells which are in direct contact with the outer surface of the cytoplasm, since the cells never seem to develop a yolk membrane as do the cells of Bidder's organ in Bufo vulgaris according to the investigations of Ognew. After the stage of Fig. 29 the egg shrinks away from its zona pellucida and its outline appears somewhat irregular (Fig. 27). At this time many of the follicle cells lie in slight depressions in the egg surface as if they were already beginning to enter the egg. It would seem as if the absence of a yolk membrane might make it possible for follicle cells to penetrate into the eggs at any stage of development, but I have never found these cells inside of the egg until the nucleus and the cytoplasm have begun to degenerate. Stages in the penetration of the follicle cells into the egg are shown in Fig. 30. The cells do not show any amœboid processes, but they appear to sink gradually into the substance of the cytoplasm. Fig. 32 shows a late stage in the absorption of the egg by means of the follicle cells; the nucleus has entirely disappeared and all that is left of the egg is a small amount of deeply staining, granular substance.

Sometimes, as shown in Fig. 30, B. C., blood corpuscles enter the cytoplasm with the follicle cells and evidently take part in the absorption of the egg. The zona pellucida becomes very irregular as the egg degenerates, and, owing to the pressure of the surrounding eggs, it collapses after the egg has become partially absorbed and evidently suffers the same fate as the egg itself.

The process described above is the usual method by which the eggs in Bidder's organ disintegrate in all toads under two years old. Sometimes in young toads, more often in adults, a blood capillary breaks through the zona pellucida and forces

its way into the egg, taking with it a number of follicle cells (Fig. 28). In such cases the egg disappears very rapidly, its substance being absorbed directly by the blood. I have never found the cells of Bidder's organ disintegrating as a result of the formation of a large amount of pigment in the cytoplasm. Pigment is rarely formed in the cells of Bidder's organ in Bufo lentiginosus, and then only in adult males. In all of the cases which I have found the pigment was confined to a narrow zone around the periphery of the egg; it did not develop throughout the entire egg as is usually the case in eggs which are degenerating in the ovary. There was nothing in any of these eggs to indicate that the pigment was concerned in any way with the degenerative processes taking place in the cell.

Although I have never found two adjacent oöcytes degenerating as a result of the development of a spherical vacuole between them, I have seen what Ognew considers as degeneration due to the penetration of one oöcyte into another. This phenomenon was first described by Cerruti (6) in 1905. Cerruti states that in Bufo vulgaris the cytoplasm of one oöcyte in Bidder's organ sometimes forces its way into the cytoplasm of another oöcyte. Later the nuclear substance of the entering cell flows towards the place of penetration and eventually one cell is engulfed by the other. Cerruti suggests that this process is analogous to the entrance of follicle cells into the egg, and that the entering cell may be considered as a parasite of the cell into which it penetrates. Ognew considers that this suggestion ventures too much since we would have to assume a subsequent struggle for existence between the two nuclei. Ognew does not think it possible that this phenomenon can be associated in any way with amitosis, and his only suggestion is that it is "a highly original process of degeneration" which requires further study. The figures given by Cerruti and by Ognew seem to me to show unmistakably that both of these investigators were dealing with cases of amitosis in degenerating ova which were greatly distorted in shape on account of the pressure of the surrounding cells. Bidder's

organ never grows beyond a certain size in adult males. During the summer months the cells of this body increase rapidly in number and also in size and they often become so crowded together that one cell forms a decided indentation in the surface of an adjacent cell. In preparing to divide the nuclei of such cells frequently become greatly elongated, much more so than shown in Fig. 17, and before the appearance of the division membrane it might readily seem as if the substance of the nucleus was flowing in a certain direction and that the one egg was trying to force its way into another. Very often, during the division of these cells, currents seem to be set up in the cytoplasm and a portion of the cytoplasm around one nucleus may appear sharply distinct from the remaining cytoplasm. On superficial examination such ova may give the impression that one cell has entered another since the egg contains two separate nuclei; one of them being surrounded by cytoplasm which appears differently from the other cytoplasm in the cell. The cell which has apparently engulfed one of its neighbors is never noticeably larger than the surrounding cells; and both of its nuclei are similar to the nuclei in the adjacent cells, while its zona pellucida appears perfectly intact in all places. These facts seem to me sufficient proof that the egg in question is dividing amitotically and that it has not been entered by another cell. I do not see how it would be possible for one egg to enter bodily into another egg of practically the same size without causing a break in its zona pellucida or without producing a marked increase in the size of the cell and a profound change in its structure.

Friedman (11) has observed that the cytoplasm of the degenerating eggs that are sometimes found in the testis of Rana viridis is often separated into two distinct portions which have no regular outline but dovetail into each other in various ways. One part of the cytoplasm has a granular structure and stains very intensely, while the other part is apparently homogeneous and stains very faintly. This appearance of the cytoplasm is probably due to an abortive attempt on the part of the cell to form yolk-nuclei similar to those shown in Fig.

18. There is the possibility, however, that it may be caused by currents in the cytoplasm of the degenerating eggs which separate the more fluid portion of the cytoplasm from the more granular, as is sometimes the case in the cells of Bidder's organ.

The degenerative changes taking place in the cells of Bidder's organ are very similar to those which occur in mature amphibian eggs which have remained in the ovary after the breeding season, according to the investigations of Bühler (4), Dubnisson (10), Ruge (28), and others. In such eggs the chromatin breaks up into granules and, after the disappearance of the nuclear membrane, the substance of the nucleus mingles with that of the cytoplasm, the egg being finally absorbed through the agency of follicle cells, leucocytes and blood capillaries which have penetrated into the cytoplasm. Eggs which are degenerating in the ovary are always heavily pigmented, however, while pigment is rarely developed in the cells of Bidder's organ and when it is present it never seems to be concerned in the degenerative processes.

Bidder's organ is a permanent structure in the males of all species of the Bufonidae so far investigated. In Bufo variabilis, Bufo cinereus, and Bufo calamita, this body disappears in the female at the end of the second year. In Bufo vulgaris, according to the observations of Knappe, Bidder's organ disappears in the adult female during the winter and a new organ is regenerated during the summer months. According to Ognew, Bidder's organ does not disappear in the adult female of Bufo vulgaris during the winter, but it persists as a small shrunken organ which lies near the fat bodies. Bufo vulgaris is, therefore, the only species so far studied in which Bidder's organ is a permanent structure in both male and female.

In Bufo lentiginosus Bidder's organ disappears in the female at the end of the second year and no traces of it are to be found in older females. During its early development this organ contains no central cavity, although there are a number of intercellular spaces between the rounded ova. After the

metamorphosis of the tadpole the cells of Bidder's organ increase in number very rapidly and, owing to pressure, they are often greatly distorted. The central cavity is formed when the cells in the interior of Bidder's organ degenerate; this occurs when a young toad has attained a body length of about 2 cm. In the female, after the first year, the cavity of Bidder's organ opens into the cavity of the ovary, as Knappe has stated is the case in Bufo vulgaris, and eventually the outer wall of this organ becomes continuous with the epithelial covering of the ovary. Bidder's organ then appears as a small lobe of the ovary which is easily distinguished from the other lobes as the cells never develop beyond a certain stage. Bidder's organ then gradually decreases in size and finally disappears. Although I have several times carefully examined entire ovaries of mature females, I have never been able to find any traces of this body.

In the male toad Bidder's organ varies greatly in size and in appearance at different seasons of the year. In the early spring this body appears shriveled and it is somewhat irregular in shape. Sections of Bidder's organ taken from toads killed at the height of the breeding season in April show that at this time the organ has a very large central cavity and that it contains a considerable number of degenerating ova and only a few young eggs. In the early summer large numbers of new eggs are formed at the periphery of Bidder's organ, and this body increases considerably in size and becomes more rounded. During the latter part of August and in September the large cells begin to degenerate in increasing numbers and only a very few young ova can be found.

Ognew states that the development of Bidder's organ is closely associated with the development of the sex-gland. When the sex-gland is resting, Bidder's organ grows and the number of cells increases, but from the time that the formation of the spermatozoa begins up to the period of sexual activity which occurs in April and May, this organ gradually decreases in size. I cannot agree with Ognew that the sex-gland is "resting" during the summer months when Bidder's

organ is increasing in size. My study of the spermatogenesis of Bufo lentiginosus (King, 16) has shown that early summer is the time when the sperm-cells are most actively dividing and that in August and September, when large numbers of the cells of Bidder's organ are beginning to degenerate, the testes are filled with spermatids and spermatozoa. The growth of Bidder's organ is therefore most rapid during the period when the cells of the testis are most actively developing into spermatozoa. The degeneration of numerous cells of Bidder's organ at the end of the summer is not due to the beginning of a period of sexual activity on the part of the testes, but to the fact that these cells have reached their maximum stage of development and, since they can go no further, they must of necessity degenerate. During the winter there is no active formation of new cells in Bidder's organ, and many of the cells already formed gradually reach their maximum development and then disintegrate. In the spring, therefore, Bidder's organ is only about one-half of its former size. New cells are formed in great numbers in Bidder's organ only at the time that new cells are developing in the testes.

In adult males the nuclei of the cells of Bidder's organ usually contain a number of small nucleoli rather than one or two large ones as is so often the case in the young animals. The cytoplasm of these cells usually appears uniformly granular until it is beginning to be absorbed by the follicle cells and by the leucocytes. Yolk-nuclei are very rarely developed in these cells, and when they are found they always appear like the granular masses shown in Fig. 18. Disintegration of the cells through the agency of blood capillaries which have penetrated into the cytoplasm occurs much more frequently in the Bidder's organ in the adult than in the young toad.

Investigations have shown that Bidder's organ is not found in the amphibians as a class, but that it is confined to the Bufonidae except in rare instances. In 1830, Müller (*22*) stated that a rounded body is present at the anterior end of the testes in tadpoles of Pelobates fusca and also in those of Rana. These observations have not been confirmed by other

workers and it is probable, as Knappe suggests, that Müller mistook tadpoles of Bufo for those of other species of amphibians. Knappe found a Bidder's organ in a young male Salamandra about two years old, but he gives no details of its structure. As far as I have been able to determine, these are the only recorded cases in which a Bidder's organ has been found in amphibians other than the Bufonidae. The numerous cases of hermaphroditism that have been reported in different species of Rana and the investigations of Pflüger (25) which show that large numbers of tadpoles of Rana temporaria are hermaphroditic would seem to indicate that a body somewhat of the nature of the Bidder's organ in Bufo is not infrequently formed in Rana. I am at present investigating the development of the germ-cells in a number of species of American amphibians, and I hope later to record my observations regarding the presence or absence of Bidder's organ in these forms.

Bidder's organ has been a subject of controversy ever since its discovery in 1758 by Rösel von Rosenhof (27), and a number of different theories have been advanced regarding its nature and probable function. The discoverer of this organ considered it a part of the fat body, while Ratke (28), who examined it in 1825, believed it to be a portion of the testis. Three years later Jacobson (15) came to the conclusion that all toads are hermaphrodites since the body at the anterior end of the testis is a rudimentary ovary. This view was adopted in 1853 by von Wittich (34), after a study of Bidder's organ in Bufo cinereus, and it has since been advocated by La Valette St. George (29), Nussbaum (23), Bourne (3), Cerruti (6) and Ognew. Hoffman (14) believes that Bidder's organ contains both ova and spermatozoa, and he therefore considers that this body is a "rudimentäre Zwitterdrüse." On the other hand, Bidder (1) maintains that the body at the anterior end of the testis is not a rudimentary ovary but an "Abtheilung des Hoden, und zwar eine auf einer niedrigen Entwickelungsstufe stehen gebliebene, welche die Bildung des Sperma und der Spermatozoen nun vorbereitet." Leydig (19)

and Spengel (31, 32) also consider that Bidder's organ is an accessory organ and not an ovary. The latter investigator thinks it highly probable that "dies Organ eine Rolle in den Leistungen der Geschlechtsdrüsen, spielt, etwa in irgend einer Beziehung steht zur Bildung des Materials von der die Entwickelung neuer Ureier ausgeht, im weiblichen wie im männlichen Geschlechte."

Spengel gives three reasons why he does not believe that Bidder's organ is a rudimentary ovary: (1) the anatomical differences between Bidder's organ and the true ovary consisting in a lack of a central cavity in Bidder's organ and the absence of pigment and yolk from the cells themselves; (2) the fact that Bidder's organ is found in the female as well as in the male; (3) the presence of Bidder's organ in hermaphroditic toads. Later researches have rendered the first of these reasons invalid since Bidder's organ has been found to contain a central cavity. Knappe has found pigment in the cells of Bidder's organ in Bufo vulgaris, and I have also found it in Bufo lentiginosus. The formation of yolk-nuclei is a common phenomenon in the cells of Bidder's organ in tadpoles of Bufo lentiginosus, and in one instance (Fig. 26) I have found the cells of Bidder's organ developing yolk spherules. Although Bidder's organ was found in the hermaphroditic toad examined by Spengel it is not present in a most interesting specimen of Bufo vulgaris recently described by Cerruti (7). In this individual there is a well developed testis in front of each kidney, and lying between each testis and the fat body is an ovary which appears to be intermediate in structure between a true ovary and Bidder's organ. In this individual Bidder's organ has been able to develop further than it normally does and it has thus become a part of the rudimentary ovary. This development would probably not have been possible if Bidder's organ were merely an accessory male organ.

The evidence brought forward by the investigators who have more recently studied the structure of Bidder's organ in adult toads has been unanimously in favor of the view that this body is a rudimentary ovary, and the results of my study

of the development of this structure point to the same conclusion. The germ-cells of Bidder's organ arise from primordial germ-cells which are similar in character to the cells which become functional spermatozoa or eggs, and the early development of these cells closely follows that of the ovarian ova up to the synizesis stage. During synizesis the cells of Bidder's organ appear similar to spermatocytes in which the nuclear contents are in a contracted condition, but at no other period in their development do they resemble in any way stages in the development of the spermatozoa; neither are they similar to any cells of the body except the ova. Even when the cells of Bidder's organ are degenerating their resemblance to the ovarian ova is very marked, and the degenerative processes occurring in them are similar to those taking place in the mature eggs which are not expelled from the ovary. There is, therefore, no probability that Bidder's organ is a portion of the testis which has been arrested in its development to serve as an accessory male organ as Bidder, Leydig, and Spengel maintain.

If Bidder's organ is a rudimentary ovary there are three possibilities that may be considered in a discussion of the origin of this body. It is possible, as Haeckel (13) suggests, "dass das älteste und ursprünglichste Geschlechtsverhältniss die Zwitterbildung war und dass aus dieser erst secundär (durch Arbeitstheilung) die Geschlechtstrennung hervorging." This primitive hermaphroditic condition has been lost by most of the vertebrates, although it still persists in many of the lower forms. If the amphibians were originally hermaphrodites then this primitive condition of the sex-glands still exists in the Bufonidae, being indicated by the presence of Bidder's organ. On this assumption Bidder's organ is a degenerate ovary in the male toad and a degenerate testis in the female.

In the female toad the cells of Bidder's organ have no resemblance whatever to the sperm-cells and in their structure and development they resemble the ovarian ova as closely as do the cells of the Bidder's organ in the male; neither is there

any tendency to the development of male organs by the female in any of the Bufonidae. These facts are considered by Marshall (21) to furnish sufficient reason to overthrow the view that amphibians were originally hermaphroditic. In spite of the objections that can be brought against it, this theory seems to me to offer the most satisfactory explanation of the presence of Bidder's organ in Bufo. It is conceded by most, if not by all, zoölogists that the spermatozoa are more highly differentiated than the ova. It is therefore only natural to suppose that in a degenerate sex-gland, such as Bidder's organ, the germ-cells would follow in the course of their development the type of the least specialized germ-cells, the ova, rather than that of the more highly specialized spermatozoa. Such an organ would, therefore, have the same structure in all animals regardless of sex. According to this view it is only necessary to assume that Bidder's organ has degenerated in the female more than it has in the male in order to have the conditions in this body what we find them at the present time. Since Bidder's organ usually disappears in the adult female, although it persists throughout the lifetime of the male, there is good reason to believe that this body is more degenerate in the female than in the male. That the Bufonidae, which are among the most highly differentiated of the amphibians, should retain a primitive hermaphroditic condition of the genital organs when such a condition is not found at the present time in other classes of amphibians, even among forms which are considered as primitive or degenerate types, is not an obstacle in the way of the theory outlined above. A somewhat similar condition is found among certain fishes, and many of the higher vertebrates have retained primitive organs which are at present in a degenerate condition and seemingly of no use to the individual.

Marshall has suggested that the formation of Bidder's organ "may be regarded as due to a further extension backward of that tendency to degeneration and atrophy which has caused the conversion of the most anterior part of the germinal ridge into the fat body." In accounting for the similarity in the

appearance and in the development of the cells of Bidder's organ in the two sexes, Marshall states that the "degeneration of the male genital gland may be regarded as taking the form of a reversion to the more primitive ovarian type." According to the investigations of Spengel (35), Semon (30), Goglio-Tos (12), and Bouin (2), the anterior portion of the genital ridge, which develops into the fat body, is composed entirely of small connective tissue cells. Sections of the young tadpoles of Bufo lentiginosus show that the germ-cells are never found anterior to Bidder's organ in this amphibian. In Bufo the germ-cells are not derived from peritoneal cells but from undifferentiated embryonic tissue. It hardly seems probable, therefore, that a mass of cells having a different origin from the germ-cells and totally unlike them in structure ever belonged to the sex-gland proper at any period in the history of the race. In very young tadpoles the cells which are to develop into the fat body form a forward extension of the genital ridge, but this does not necessarily indicate that they were primarily sex-cells. I am strongly inclined to believe that the peritoneal cells which form the fat body have secondarily come into connection with the anterior end of the genital ridge and that Bidder's organ marks the extreme anterior boundary of the sex-gland. If this be true, then Marshall's theory is untenable since the cells forming the fat body are not germ-cells, and even Marshall himself believes that the cells of Bidder's organ are degenerate ova.

There is a third possibility regarding the origin of Bidder's organ which may be suggested, although little can be said in its favor. Bidder's organ may, perhaps, be the remains of a primitive sex-gland which was functional when the Bufonidae were sexually mature in their larval state, as is the condition of the Axolotl at the present time. On this assumption the ovary and testis are structures secondarily acquired when the reproductive activity became manifested at a later period in the life of the individual. The similarity in the appearance of the cells of Bidder's organ in both sexes and their resemblance to ova can be accounted for on the supposition that, as the

organ is degenerate in both sexes, its cells have taken on the character of the least specialized germ-cells, the ova. The Axolotl is the only known amphibian that reproduces while in a larval condition, and its genital organs are similar to those of other salamanders. Since it is probable that neotenia in this form is a phenomenon of adaptation rather than a primitive condition, there is little ground for a belief that the amphibians as a class were ever sexually mature in a larval state.

The function of Bidder's organ is as yet undetermined. Since this structure is confined chiefly to the Bufonidae it hardly seems as if it could have an important rôle in the development of the sex-cells, as Spengel claims; neither can it be considered as a storehouse of reserve material that is to be used during the hibernation period, since measurements made of this body at different times of the year show that it loses not more than one-half of its volume during the winter months. As a rudimentary ovary Bidder's organ is apparently functionless, although further research, possibly by experimental means, may determine the part played by this body in the life history of the individual.

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EXPLANATION OF PLATES.

All figures were drawn with the aid of a camera lucida. They have been reduced one-third.

FIG. 1.—Outline drawing of the genital organs of a toad killed at the time of metamorphosis. C. A., corpus adiposum; B. O., Bidder's organ; O., ovary; R., kidneys.

FIG. 2.—Section of Bidder's organ taken from a tadpole 17 days old. × 1,000.

FIG. 3.—Section of Bidder's organ taken from a tadpole 35 days old. × 1,000.

FIG. 4.—Resting stage of a primordial germ-cell in Bidder's organ. V., vitelline bodies. × 1,334.

FIG. 5.—Young oöcyte in Bidder's organ. × 1,334.

FIG. 6.—Growth stage of a young oöcyte. ×1,334.

FIG. 7.—Beginning of the condensation of the chromatin spireme leading to synizesis. × 1,334.

FIG. 8.—Synizesis stage. × 1,334.

FIGS. 9-10.—Early post-synizesis stages. The chromatin is in the form of a continuous spireme. × 1,334.

FIGS. 11-14.—Post-synizesis stages showing the division of the spireme into segments. × 1,334.

FIG. 15.—The beginning of amitotic division in the nucleus of a cell in Bidder's organ. × 1,334.

FIGS. 16, 17.—Later stages in the amitotic division of a cell. × 1,000.

FIG. 18.—Section of an oöcyte which has begun to degenerate. A fluid space has formed around the large nucleolus and the yolk material is collected in several masses, one of which lies against the nucleus. × 1,000.

FIG. 19.—Section of an egg in Bidder's organ taken from a toad killed at the time of metamorphosis. Yolk-nuclei are forming in the cytoplasm. × 1,000.

FIG. 20.—Section of the nucleus of a cell in Bidder's organ which is very similar to nuclei of the same size found in the ovarian oöcytes. × 1,000.

FIGS. 21-25.—Stages in the resolution of compound-nucleoli in the cells of Bidder's organ. × 1,334.

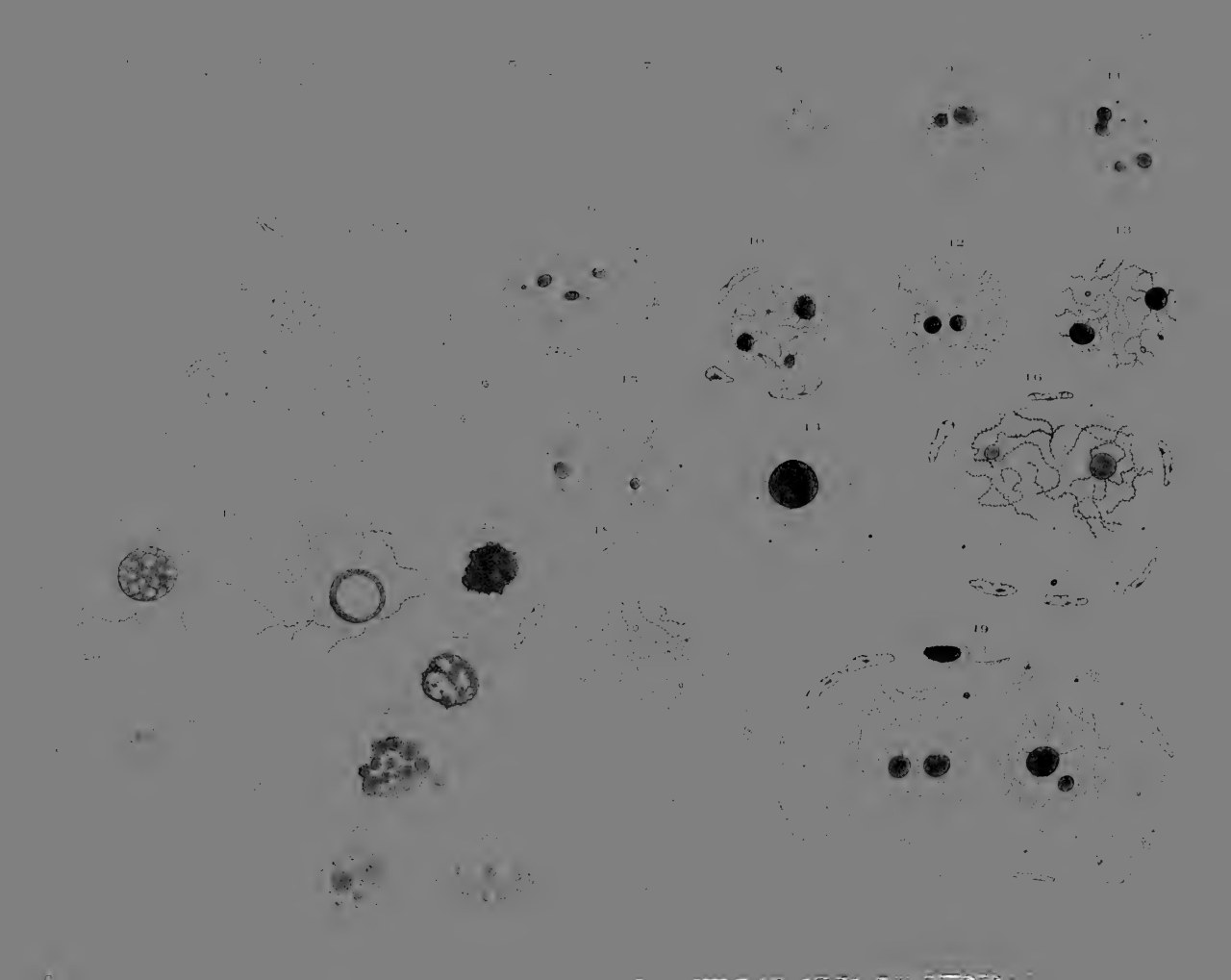


Fig. 26.—Section of an oöcyte in Bidder's organ in which yolk spherules were formed. Taken from a young male toad with a body length of 2 cm. × 1,000.

Fig. 27.—Section of a degenerating egg in Bidder's organ. Taken from a young male toad with a body length of 4.5 cm. × 1,000.

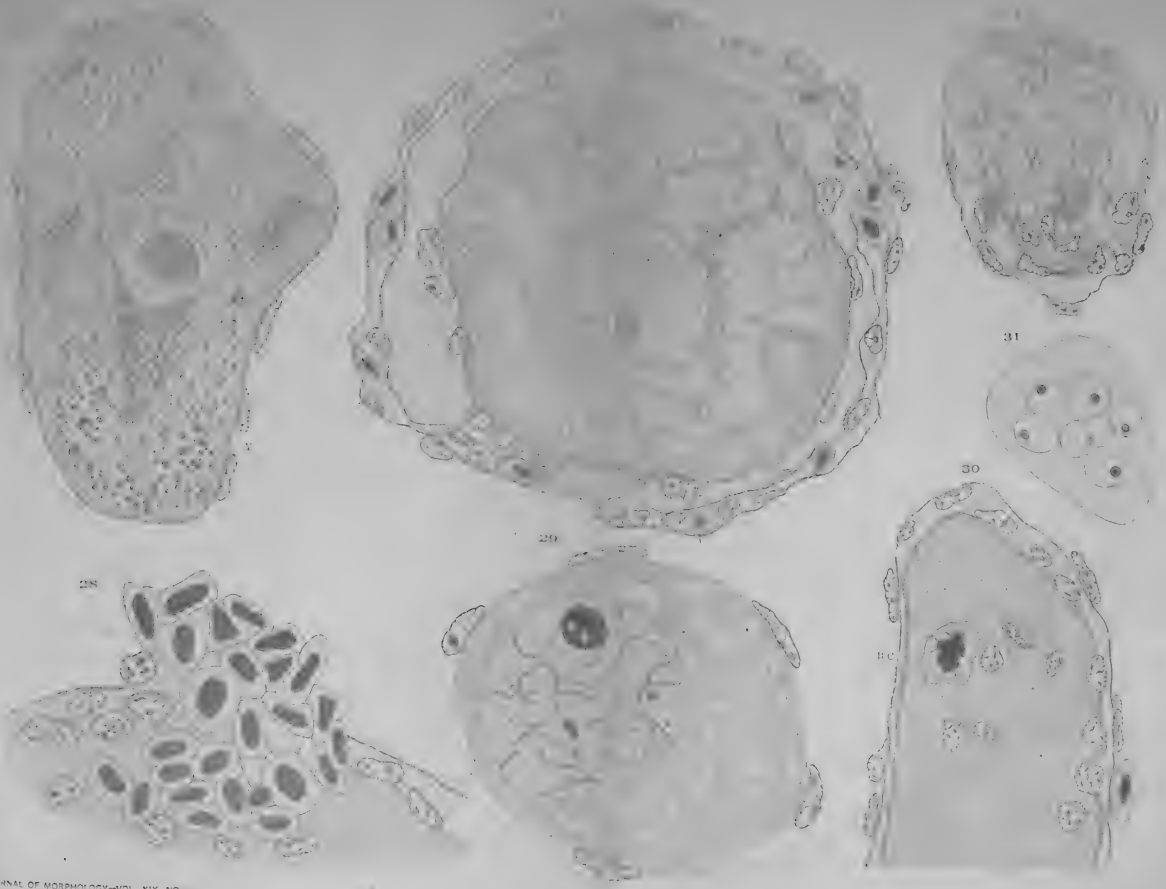
Fig. 28.—Part of a section showing the penetration of a capillary into a degenerating egg. Taken from an adult male toad killed in July. × 1,000.

Fig. 29.—Section of a degenerating egg taken from a young male toad with a body length of 5 cm. × 1,000.

Fig. 30.—Part of a section of a degenerating egg into which blood capillaries (B. C.) and follicle cells have entered. Taken from a young male toad with a body length of 5 cm. × 1,000.

Fig. 31.—Outline drawing of a section of a multinucleated egg in Bidder's organ.

Fig. 32.—Section of a degeneration egg containing numerous follicle cells. Taken from a young female with a body length of 5 cm. × 1,000.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THE DEVELOPMENT OF THE ADHESIVE ORGAN AND HEAD MESOBLAST OF AMIA.[1]

Jacob Reighard and Jessie Phelps.

In 1895[2] it was noticed by one of us that, at a certain stage in its development, the adhesive organ of Amia consists of a pair of diverticula whose walls and lumina are continuous with the corresponding parts of the foregut. Two years later we undertook a study of the development of this organ in order to determine whether it originates from the entoblast, as indicated by this observation, or from the ectoblast, as claimed by previous investigators of Amia, Aci-

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[2]In 1895 I noticed the connection referred to in this paragraph between the adhesive organ of Amia and the corresponding parts of the foregut. Two years later Miss Phelps undertook, under my direction, to trace the complete history of this organ. When Miss Phelps' work had been completed two brief preliminary notes were published (Phelps, 1899, 1900), but it seemed best, before final publication, to add the history of the head mesoblast in so far as this is related to the adhesive organ. For this part of the paper, as well as for the theoretical matter (see Reighard, 1902), I am responsible. The publication of the paper has been from time to time postponed in the hope that it might follow the "Normentafel of Amia" the preparation of which had been begun. The Normentafel has been greatly delayed by my inability to use my eyes for microscopic work, and I do not now know when it may be completed. The recent appearance of a paper on "The Adhesive Organ of Amia" by Eycleshymer and Wilson (1908) makes desirable the immediate publication of the present more detailed study. It is printed as it was completed in July, 1900, with no attempt to consider literature which may have appeared since that time. The only additional matter in the body of the paper is that in the foot notes, and concerns the publication of Eycleshymer and Wilson above referred to, whose results are in several respects at variance with our own. Some titles have been added to the literature list.—J. R.

penser, and Lepidosteus. This study has necessarily involved some consideration of the head mesoblast.

We shall distinguish two phases in the history of the adhesive organ: first a progressive phase which extends from the origin of the organ to a stage of the larva shortly after hatching, at which time the organ becomes functional, and second a retrogressive phase which extends from the close of the functional period to the time of the entire disappearance of the organ. In considering each of these phases we shall have occasion to refer to certain stages in the development of the embryo and larva, but shall describe these stages only in sufficient detail to render possible their subsequent identification.

The Progressive Phase.

The first stage.[3] *a. The Adhesive Organ.* The first external indication of the adhesive organ is found in embryos which still lie flat on the yolk (Plate, Fig. 1), with neither head nor tail protuberant. The brain at this stage shows externally two divisions only. The limits of the hindbrain are not distinguishable, but near its anterior end it appears

[3]This early stage of the adhesive organ has been found in surface views, only in embryos preserved after removal of the egg membranes or by a method which does not cause shrinkage of the membranes. If the eggs are preserved without such precaution shrinkage of the membranes brings them into contact with the surface of the embryo, obliterates many external features, changes topographic relations, and deforms certain internal structures. The sections figured by Eycleshymer and Wilson (1908, early stages) show the membranes still in situ and shrunk against the embryo. The use of such material probably accounts for their statement that the adhesive organ appears as a pair of diverticula. Shrinkage of the membranes obliterates the delicate "crescent" which is unpaired and includes the adhesive organ. This should appear in Fig. 13, Pl. I, of Eycleshymer and Wilson, 1906. They say that in embryos of ninety hours "the adhesive organs are not observable in surface views" (1908, p. 135), and they figure this stage without adhesive organ in Pl. I, Fig. 15, of their paper of 1908. On the contrary, we find the organ well developed and paired in surface views in a somewhat younger stage (*cf.* our plate, Fig. 2). Shrinkage of the membranes obliterates these delicate surface features in early stages, and gives rise to the impression that they appear only in later stages.

to be much broader. Opposite this broader part the auditory pit is found in sections. The broadening itself is due in part to the proliferation of cells from the ganglionic ridge. The future anterior end of the hindbrain lies just anterior to this broader portion. The pointed tip of the forebrain extends beyond the optic vesicles which lie close on either side of it and form the most conspicuous part of the head. On each side, behind the optic vesicles and at the side of the brain is an elevated, oval area, the branchial area, within which

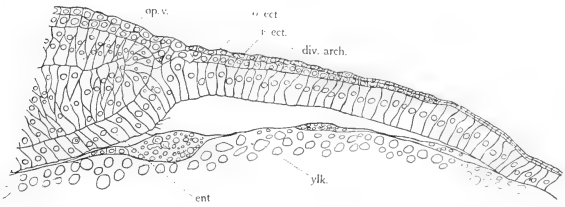

FIG. A.—Part of a parasagittal section of an embryo along the curved line a-b of the stage shown in Fig. 1. Camera outline drawing; details from a photograph. div. arch., diverticulum of the archenteron (crescent); ent., entoblast; i. ect., internal layer of ectoblast; o. ect., outer layer of ectoblast; op. v., optic vesicle; ylk., yolk. The section passes to one side of the median plane and does not include the hypophysis.

gill slits appear in a later stage. The axial mesoblast shows in sections at least twelve trunk somites. In front of the optic vesicles and lying against them and the anterior end of the brain is a crescent-shaped, elevated area which is shown by its subsequent history to include the fundament of the adhesive organ. It will be referred to hereafter as the crescent (anterior end of the foregut).

In a longitudinal section (Fig. A), the archenteron is seen to be enlarged slightly at its anterior extremity to form a broad, dorsally directed diverticulum which causes the cres-

cent-shaped elevated area seen externally. The dorsal wall of this diverticulum is made up of a single layer of columnar entoderm cells, which are in contact with the nervous layer of the ectoderm. Ventrally the cavity of the diverticulum is bounded by the yolk from the surface of which a few cells have been segregated. The cavity as seen in longitudinal section is crescent shaped.

b. The Mesoblast—In the trunk region, and as far forward as the midbrain region, the archenteric cavity is well developed and extends across the middle line. In the anterior head region, however, the increased depth of the brain causes the dorsal wall of the archenteron to be in contact with the yolk in the middle line, and thus obliterates the median portion of its lumen. The lateral portions of the archenteric cavity in the anterior head region are, on the other hand, well developed and connect the archenteric cavity of the trunk with the cavity of the crescent which is thus shown to be a part of the archenteron.

In an embryo a few hours younger than the one figured the crescent is not readily visible externally,[4] but may be easily found in sections. The columnar entoblast forming its dorsal wall is found to be much thickened and many layered at its anterior and lateral edges and to become there continuous with the yolk. The crescent is thus terminated in front and laterally by a germinal wall, much better developed laterally than in front. Posteriorly the crescent entoblast is continuous with the entoblast forming the dorsal wall of the archenteron in the head region. This entoblast of the head region is very thin in the middle line beneath the brain where it is in contact with the yolk. On either side of the middle line, however, it is many layered and very thick and is continuous laterally by means of a germinal wall with the yolk. Thus in the head region in front of the hindbrain there is as yet no mesoblast, but only the much thickened layer of entoblast continuous in front with the columnar entoblast of the crescent. About the borders of this entoblast sheet and

[4]Such a stage is figured by Beckwith, 1907, Plate I, Fig. 1.

about the lateral and anterior border of the crescent entoblast there is a continuous germinal wall by which the entoblast passes into the yolk.

In an embryo of the stage represented in Fig. 1 this entoblast of the anterior head region has separated into two layers. Of these the dorsal and thicker layer is composed of loosely aggregated mesoblast cells, while the ventral layer is a columnar entoblast, which forms the dorsal wall of the archenteron. The mesoblast begins at a point just in front of the optic vesicle, as a thick sheet of cells still connected with the entoblast near the median line, but free laterally. As the sheet is traced backward it becomes broader. It retains its connection with the entoblast near the median line and extends thence laterally to the limit of the archentron, where it becomes continuous with the germinal wall. It is without a cavity. The mesoblast of the anterior head region is thus clearly formed by delamination from the entoblast. No mesoblast is formed from the *columnar* entoblast of the crescent.

The second stage: a. The Adhesive Organ. The next stage of the adhesive organ to be described is found in embryos which show the following external features: The embryo (Plate, Fig. 2) is still flat on the yolk; the midbrain is now visible externally in addition to the forebrain and hindbrain; the tip of the forebrain extends beyond the optic vesicles for a short distance as in the preceding stage; the recessus laterales are distinguishable in the hindbrain, so that the cavity of the hindbrain may be described as triangular in outline, the anterior lateral angles of the triangle forming the cavities of the recessus laterales. Two oblique lines are seen on each side within the branchial area. These are gill slits. The first is the pre-hyoidean or spiracular slit, which subsequently disappears. The arch behind it is the hyoid arch. At its dorsal end the auditory vesicle is visible. The crescent of the preceding stage has given place to three hemispherical protuberances which are nearly as prominent a feature of the head as the optic vesicles in front of which they lie. The two lateral of these protuberances

form the fundaments of the adhesive organ. The central and smallest of them lies in the middle line between the other two and directly in front of the forebrain. It will be spoken of as the button. Bounded outside by a curved white line, and inside by the branchial area, are seen the halves of the body cavity extending backward over the yolk about the sides of the head. Between the three protuberances and the white line the stomodaeum appears as a long crescent-shaped depression.

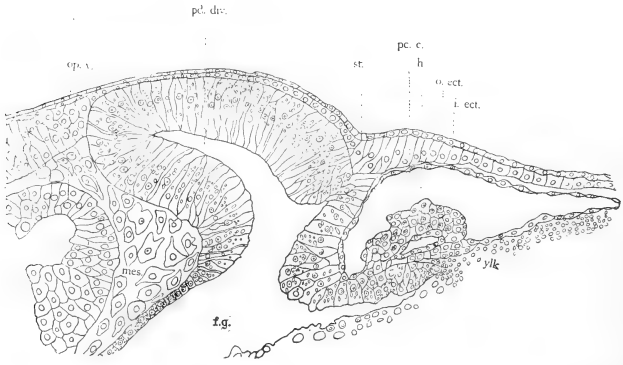

Fig. B.—Portion of a parasagittal section of an embryo of the stage shown in Plate, Fig. 2. The section passes to one side of the median plane through the optic vesicle and one-half of the adhesive organ. Camera outline; details from photograph. × about 150. f. g., foregut; h., heart; i. ect., inner layer of ectoblast; o. ect; outer layer of ectoblast; op. v., optic vesicle; pc. c., pericardial cavity; pd. div., one of the paired diverticula of the foregut (fundament of the adhesive organ); st., stomodaeum; ylk., yolk.

Sections of embryos of this stage (Fig. B) show that the entoderm in front of the crescent has been folded backward so as to form a small part of the ventral and side walls of the foregut at its anterior end. Ventral and anterior to the lower foregut wall thus formed and filling the entoderm fold, is the large pericardial cavity within which is seen the heart. Ventral to the pericardial cavity lies a portion of the yolk

sac which separates the entoblast beneath the pericardial cavity from the yolk. The broad, flat, anterior end of the foregut extends forward to the stomodaeum where its greatly thickened anterior wall is in contact with the ectoblast. From the dorsal wall of the short foregut rise three diverticula. The central one (Fig. C, med. div.) is thick-walled, with a very shallow cavity and forms the button. The lateral

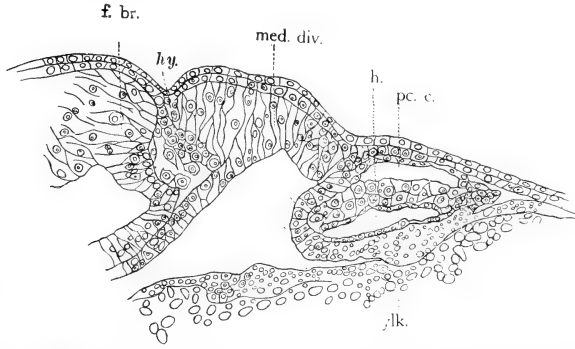

FIG. C.—Median longitudinal section of an embryo of the stage shown in Plate, Fig. 2. Camera outline, details from photograph, × about 150. med. div., median diverticulum which produces the "button" seen in front of the forebrain between the paired diverticula in Plate, Fig. 2. Other letters as in Figs. A and B. The hypophysis, hy., is not accurately represented.

diverticula are also thick-walled, but have long and narrow cavities. They leave the foregut at a point some distance from the middle line, ventral and anterior to the tip of the forebrain, and pass upward, backward and outward, so that their free ends touch the sides of the forebrain immediately in front of the optic vesicles (Fig. B). These paired diverticula produce the rounded protuberances referred to above as the fundaments of the adhesive organ. The button and the adhesive organ have evidently been formed from the crescent of the preceding stage. The button may be considered as a remnant of the central portion of the original crescent.

The single layer of cubical cells making up the posterior part of the dorsal wall of the foregut gives place to high columnar cells in the walls of the adhesive organ fundaments. The protoplasm of the peripheral end of each of these cells has embedded in it large conspicuous yolk granules and the nucleus with its prominent nucleoli also lies here, while the central end is largely composed of mucus (?) surrounded by a thin layer of protoplasm (Fig. B).

The planes passing through the long curved axis of the two adhesive organ fundaments are not coincident with each other, and if projected would intersect behind the optic vesicles at an acute angle. The space between these fundaments on the one hand and the forebrain and foregut on the other hand is wedge-shaped and filled with loose mesenchyme. The fundaments of the adhesive organ are in close contact with the ectoderm, without intervening mesenchyme.

The relations of the diverticula and foregut are shown in diagram in Fig. D.

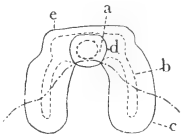

Fig. D.—Diagram of the foregut and its diverticula seen from the dorsal side in the stage shown in Plate, Fig. 2. The paired diverticula (adhesive organ) are shown displaced backward. The broken line indicates cavities: a, that of the button; b, that of the adhesive organ fundaments. The solid lines indicate outer surfaces; c, that of the adhesive organ; d, that of the button; e, the stomodaeum. The line composed of dots and dashes shows the posterior limit of the ventral wall of the foregut.

b. The Mesoblast. In the preceding stage the mesoblast sheets of the head, when traced forward, were found to become narrower and to end just in front of the optic vesicles. In the present stage the mesoblast extends forward at the sides of the adhesive organ and in front of the stomodaeum where it is continuous across the middle line.

The pericardial cavity has developed in this mesoblast sheet and may be seen in Plate, Fig. 2, extending backward between the outermost white line on the yolk and the branchial area to become continuous with the body cavity further back. Mesoblast also occupies the space between the adhesive organ and button on the one hand and the optic vesicles and brain on the other (Fig. B, mes.). Examination of intervening stages shows that the mesoblast of the region anterior and lateral to the adhesive organ has been produced, largely if not wholly, by progressive forward differentiation from the germinal wall. This germinal wall extends along the borders of the entoblast of the head and trunk region and forward along the lateral and anterior borders of the columnar entoblast of the crescent. The germinal wall of the lateral borders of the crescent is especially well developed and from it the mesoblast extends outward and also inward and backward toward the head, until it becomes finally continuous with the mesoblast previously formed in the head region.

The mesoblast at the anterior end of the brain and optic vesicles and between them and the adhesive organ and button owes its origin in part to proliferation from the button. The middle portion of the posterior surface of the button is in contact with the hypophysis (Fig. C) and no mesoblast is proliferated from this portion of its surface at this stage. At the sides of the hypophysis on the other hand the entoblast of the button may be seen to be in process of transformation into mesoblast. Cells here separate themselves from the button and at the same time assume the form of stellate mesenchyme. The whole button is thus finally affected so that by the next stage it is no longer recognizable. The crescent has given rise not only to the adhesive organ, but to a considerable amount of mesoblast which is proliferated from that portion of the germinal wall forming its borders as well as from the button. The mesoblast of the head is then seen to be formed principally by delamination from the primitive entoblast, but partly also by proliferation from the crescent in the manner just indicated.

c. The head cavity. In this stage the mesoblast of the head is mostly mesenchymatous, but extending forward from the anterior end of the recessus lateralis on each side, beneath the optic vesicle to its anterior border is a plate of more compact, delaminated mesoblast about 70 microns broad and 30 microns thick. It is connected across the middle line with the plate of the other side by a solid cord of cells which lies in the saddle cleft just behind the infundibulum and in contact with the front end of the notochord. These cell plates are surrounded by loose mesenchyme except at the ends. The anterior end of each continues into a slender cord of more compact mesoblast which connects it with the germinal wall at the sides of the adhesive organ. The posterior end of each is continuous with a similar cell cord which extends backward to the recessus lateralis and beyond it. Each of these plates contains a well defined cavity whose form is the same as that of the plate itself and whose extent is nearly as great. The cavities do not extend into the connecting cell cord. The dorsal wall of each cavity is formed of columnar cells which are highest toward its caudal end; the cells of the ventral wall are more flattened.

The third stage; a. The Adhesive Organ. In this stage the embryo, measured from the tip of the forebrain to the end of the tail (Plate, Fig. 3), encloses about two hundred and twenty degrees of the circumference of the yolk. The tail is protuberant, but so slightly that the re-entrant angle between its ventral surface and the surface of the yolk is obtuse. The midbrain is larger and the recessus laterales extend further forward than in the second stage. The lines indicating the gill pouches have changed their relative position so that they lie close to the recessus laterales, and the anterior pair is less marked than formerly.

As seen externally the adhesive organ has changed its form. In place of the two protuberances of the last stage we find two kidney-shaped ridges, the concavities of which face each other, and the median plane (Plate, Fig. 3). This gives the adhesive organ as a whole the form of a broad ring

interrupted dorsally and ventrally. Its form is therefore similar to that seen in the embryo at the time of hatching (Plate, Fig. 4), but its surface is not yet marked by the pits characteristic of the hatching stage. The median button-like protuberance is no longer visible.

In section the paired diverticula are seen to open wide as before into the foregut. They are longer and more sharply curved than in the preceding stage. We may distinguish for each diverticulum two surfaces, an anterior and a posterior and two borders, a convex outer and a concave inner (Plate, Fig. 3). The planes of the long curved axes of the diverticula, which before intersected, have rotated so as to make them coincident with each other. This common plane meets the frontal plane of the embryo at an obtuse angle; that is the dorsal ends of the diverticula are posterior to the ventral ends. The changed relations of the planes have resulted, apparently, in carrying the dorsal ends of the diverticula forward so that they no longer touch the forebrain. This rotation has also changed their relation to the ectoderm. In the preceding stage the convex border of each diverticulum was directed nearly forward and the diverticulum was in contact with the ectoderm over the middle portion of this border, so that there was produced a rounded protuberance of the external surface. In the present stage, owing to the rotation of the diverticula, the convexity of each is directed laterally, while the anterior surface, which before faced the median plane, is directed forward and pushes up the ectoderm with which it is in contact. There is thus produced, on each side, one of the low, rounded, U-shaped ridges referred to as visible externally (Plate, Fig. 3).

The nasal plates appear in sections of this stage. They are mere thickenings of the ectoderm which in Amia consists of an outer layer of flat cells and an inner layer of cubical cells. Only the inner, nervous layer is concerned in the formation of the nasal plates which lie near the median line just above the adhesive organ and in close contact with it. It is often hard to determine in longitudinal section the line of demarcation between the two structures.

b. The Mesoblast and the Head Cavities. The walls of the head cavities are but little altered. The cavities themselves are of about the same extent in a transverse direction as before, but have increased about three times in dorso-ventral extent. The dorsal wall of each is still composed of columnar cells, the ventral wall of flattened cells. In one specimen of this stage, although the cavity was continuous on the left side, it consisted on the right side of three isolated cavities contained in the common sheet of mesoblast. The posterior of these was the largest. It is probable that several small isolated cavities are formed on each side and that these subsequently fuse into the single head cavity.

The head cavities of the two sides now extend for some distance on each side into the connecting cell cord, but the middle part of the cord is still solid. The walls of the head cavities are separated from the structures anterior to them by loose mesoblast; that is the compact cell cord connecting them with the germinal wall in front is being transformed into mesenchyme. Posteriorly they are still continuous with the compact mesoblast which extends along the sides of the neutral tube backward to the auditory vesicle.

The fourth stage is one in which the embryos agree closely in external appearance with those of stage three (Plate, Fig. 3). The tail is more protuberant than in stage three, and the re-entrant angle beneath it acute. The only difference noted in the brain is in the greater length of the recessus laterales. The hyoid arch has become prominent and causes the second pair of gill pouch lines to be less marked. The pre-hyoidean pouches are not visible. The adhesive organ shows no external change, except that the plane of the two diverticula is nearly at right angles to the frontal plane instead of being at an obtuse angle as in stage three.

The further step in the development of the organ which has been taken since the last stage is one which is observable only in section (Fig. E). The lumen of each diverticulum is no longer of uniform diameter but shows a series of dilatations connected by narrower portions. These give to the

lumen a beaded appearance. The diameters of the narrower and wider portions are to each other as one to five or six. On the anterior face of the organ there are no grooves corresponding to the constrictions of the lumen, but on the posterior face a few such grooves are found. Each of the dilatations marks the fundament of a closed spherical vesicle, and each diverticulum is found to break up subsequently into as

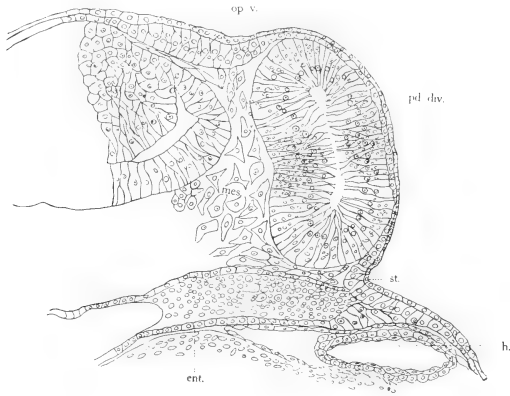

FIG. E.—Longitudinal section passing through one of the halves of the adhesive organ of an embryo of a stage a little later than that shown in Plate, Fig. 3. Lettering as in the preceding figures. The portion of the adhesive organ seen in the figure shows three dilatations of its cavity. The central one is not cut through its middle, and is therefore not well marked. Camera outline, details from photograph. × about 170.

many such vesicles as there are dilatations formed in its lumen at this stage. The head cavities are but little changed from the preceding stage.

The fifth stage; a. The Adhesive Organ. From the fourth stage to the fifth or hatching stage, the adhesive organ passes through a series of progressive changes which cannot well be described separately. There will be given first an account of the conditions at the time of hatching and then an analysis

of the processes which have led up to these conditions. At the time of hatching the embryo[5] is about seven millimeters long (Plate, Fig. 4). The most conspicuous change that has occurred in the brain is the great increase in the size of the midbrain and its division into two lobes. No gill slits are visible externally. The prehyoidean slits have disappeared and the hyoid arches now extend backward as narrow opercula so as to cover the second pair of gill slits and all others that have formed behind it. The pigment cells which are a conspicuous feature of this stage lie thickest in the region of the auditory vesicles. They extend for a short distance along the lateral lines posteriorly, and arch over the optic vesicles anteriorly. No pigment is observed on the adhesive organ or in its vicinity. The stomodaeum and pharynx are in communication.

The adhesive organ at this time consists of two sausage or U-shaped ridges which have the same position as in stage four (Plate, Fig. 3). Each ridge is made up of a series of six to ten cups whose cavities open to the surface so that they are seen from the exterior as pits. The dorsal ends of the ridges touch each other in the median plane while the ventral ends, still wide apart, abut on the roof of the stomodaeum, and thus help to form the dorsal border of the mouth opening. Indeed the ventral ends of the ridges run back along the roof of the mouth for a short distance. The planes of the ridges remain at right angles to the frontal plane as in stage four. Sections of the embryo at the hatching stage show that the lumen of the œsophagus is obliterated and that there is no longer any communication between the adhesive organ and the foregut. A longitudinal section taken just at one side of the median plane cuts through both limbs of one of the curved ridges of the adhesive organ. If the section falls through the center of one of the cups, a pit-like cavity appears which opens at the surface and is surrounded by thick walls made of a single layer of exceedingly high

[5]For a lateral view of an embryo in this stage, see Beckwith, 1907, Pl. III, Fig. 11.

columnar cells (Fig. F). The central ends of these cells next the cavity of the cup are distended, apparently with mucus. The peripheral third of each cell stains heavily on account of the larger amount of yolk it contains. The nucleus also lies in the peripheral portion of the cell. The walls of the cups are continuous at their edges with the adjacent ectoblast, so that the cups have the appearance of being thickenings of the ectoblast. Fig. F, which shows a film-like layer of ectoblast closing the mouth of the cup, belongs to a slightly earlier stage, but represents fairly the structure at the hatching stage.

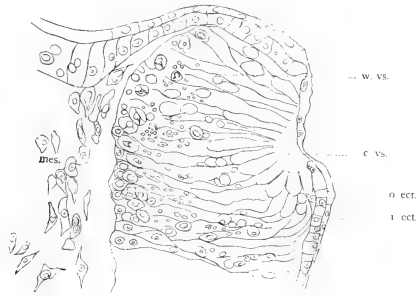

FIG. F.—Section through one of the vesicles of the adhesive organ of an embryo of Amia a little younger than that shown in Plate, Fig. 4. c. vs., cavity of the vesicles; w. vs., wall of the vesicle. Other letters as in preceding figures. The inner layer of ectoblast has disappeared over the cavity of the vesicle, but the outer layer still remains.

During the interval between the fourth and hatching stages important and rapid changes are thus seen to have taken place in the adhesive organ: *First*—Each fundament has been divided into as many vesicles as there were dilatations of its lumen in stage four. This division has resulted apparently from an extension and deepening of the grooves, present in stage four on the posterior faces of the diverticula, but the precise method of this process is unknown. The result is that each of the dilatations of stage four becomes the cavity of

one of the vesicles, while the cells composing the walls of the original diverticulum now form the walls of the vesicles. Six to ten such vesicles are formed on each side.

Second—Subsequently the cavities of the vesicles shift their position so that they come to lie excentric, close against the ectoderm of the snout. The vesicles are thus converted into cups whose mouths are still closed by the ectoderm (Fig. F).

Third—At the time of hatching, or just previous to it, the ectoderm stretched over the mouths of the cups disappears and their cavities are put into communication with the exterior. The edges of the cups become thus continuous with the adjacent ectoblast and the cups themselves have the appearance of being thickenings of the ectoblast. At first the cavities are ovoid or spherical, later they become shallower and their openings wider.

The processes described above as converting the beaded diverticula of stage four into the open cups of the hatching stage bring the organ to its functional condition, and the secretion of mucus enables the young Amia to adhere to objects with which it may come into contact.

b. The Mesoblast and Head Cavities. The head cavities are now greatly enlarged and have the form of large sacs which lie in the triangular space between the tweenbrain, optic vesicle and foregut and conform to it. The cord of cells connecting them is converted into a tube with a wide lumen. It lies in the saddle cleft dorsal to the infundibulum. The walls of the cavities are of cubical or slightly flattened cells. At one point they are greatly thickened and connected with the third nerve.

These head cavities from their position, their connection across the middle line by a tube which lies at the front end of the notochord and by their relation to the third nerve are clearly equivalent to the premandibular head cavities of elasmobranchs, reptiles and birds. Their further history has not been traced, but it is to be expected that they give rise to the oculomotor muscles as in other vertebrates. They are formed in the sheet of mesoblast which is delaminated from the

primitive entoblast of the head region. They are formed therefore in situ. We have traced their origin step by step and have found no evidence of their being produced by evagination from the entoblast as described by v. Kupffer (1893) in Acipenser. We consider such a method of origin impossible for Amia. No trace of a second head cavity has been found.

II. The Retrogressive Phase.

In larvæ 15 mm. long the adhesive organ as seen from the surface is in the condition figured by Allis (1889), Fig. 11. Its ring-shaped form still persists but it is not sharply outlined. It is much reduced in actual size and lies close above the mouth opening in the middle line, with the nasal openings on either side of it and slightly above it. The outlines of the individual cups composing the organ are still visible. The lateral line canals show distinctly.

Sections of such larvæ and of slightly younger ones show the epidermis over the end of the snout to be much thickened and filled with pigment cells and sensory buds. Beneath it are the regularly arranged lateral line organs. The cups of the adhesive organ are found to be still connected with the exterior, but the great thickening of the ectoderm has converted their openings into deep narrow tubes. The cells which make up the walls of the vesicles have the appearance of being greatly elongated. Their central ends are still filled with mucus, but the protoplasm of their peripheral ends contains a few vacuoles.

In larvæ varying in length from seventeen to eighteen millimeters the only external evidence of the adhesive organ is a small median opening lying at the apex of the upper jaw (Fig. G). This opening is at first slit-like and parallel to the edge of the jaw, later it becomes circular and resembles closely the opening of a lateral line organ. By means of sections one finds that the much thickened ectoderm entirely covers the adhesive organ except at this opening (Fig. G). The semicircular halves of the organ have the appearance, as seen in

section — they are not visible from the exterior — of having been flattened dorso-ventrally so that the two limbs of either curved row of cups lie parallel to one another and close together. The four ends of the two rows are thus brought together and the four median cups (lying at the ends of the rows) are in contact with one another. These four cups open

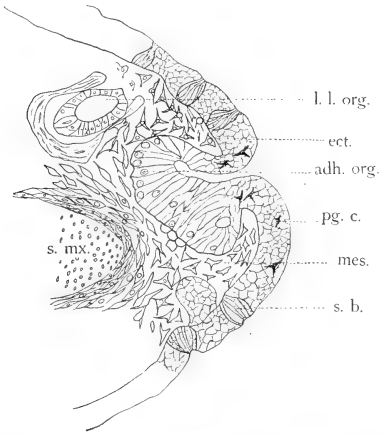

Fig. G.—Longitudinal (sagittal) section of the tip of the upper jaw of an 18 mm. larva of Amia, showing the adhesive organ in a late stage of degeneration. Camera outline drawing, × about 170. adh. org., adhesive organ; ect., ectoderm; l. l. org., lateral line canal; mes., mesenchyme; pg. c., pigment cell. s. b., sense bud; s. mx., cartilage of the upper jaw.

to the exterior through a common aperture, the median opening spoken of above, while the remaining cups no longer communicate with the exterior.

The cups have lost their original spherical shape and there is a tendency for them to open into each other. The degeneration of the organ appears to have begun at the sides and to have proceeded toward the median plane. The boundaries of the organ are still perfectly definite and the cells have the

same general arrangement that they had before the degeneration began. There is no yolk present and the entire contents of the cells are therefore much more transparent than formerly. The nuclei have the same peripheral position as before, but the central ends of the cells are no longer distended with mucus; the goblet character is not retained. Stellate mesenchyme cells crowd close about the organ on all sides, separating it from the superior maxillaries which lie immediately back of it, from the lateral line canals which lie deep below

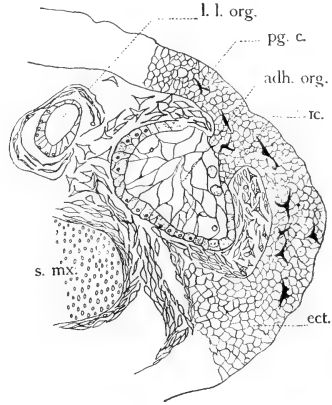

FIG. H.—Longitudinal section of the tip of the upper jaw of a 20 mm. larva of Amia with adhesive organ in final stage of degeneration. Camera outline, × about 150. lc., leucocytes. Other lettering as in Fig 10.

the surface, and from the epidermis except at the point where the epidermis dips down to form the tunnel through which the four median cups communicate with the exterior. Here, in the walls of the tunnel, the cells of the adhesive organ are continuous with the cells of the epidermis.

This tunnel is not a single, simple tube, but is divided just below the epidermis into two lateral branches, one of which goes to each half of the adhesive organ. Each of these branches is also bifurcated so that there are formed in all four

branches, which open one into each of the four median cups. The thickened epidermis is filled with pigment. Numerous sense buds also occur in it and through it the lateral line canals open to the exterior.

When the larva reaches 20 mm. in length (Fig. H), the median opening of the adhesive organ has disappeared and there is no external sign of the organ; but in sections (Fig. H) it is found to exist as a pair of irregular ovoid masses, one mass lying on either side of the median plane beneath the much thickened epidermis. The central portion of each of these is made up of an extremely vacuolated central mass, the remains of the central portions of the cells of the former cups. A few leucocytes occur among the meshes of the spongy core, surrounding which is a relatively thin sheet or layer which takes the stain heavily. It is evidently made up of the remnants of the peripheral ends of the cells of the former cups and contains the nuclei and all that is left of the protoplasm of these cells. The whole structure bears histologically a strong resemblance to the notochord in certain of its stages. This outer, deeply stained covering is wanting on the anterior faces of the ovoid masses where a median neck of ectoderm connects the two masses with the epidermis. This neck of ectoderm marks the position of the tunnel which led from the adhesive organ to the exterior in the 18 mm. larva. As the epidermis has thickened the tunnel has become entirely closed. A few days later no trace of the adhesive organ is to be found.

Summary of Observations.

1. The adhesive organ of Amia is an entoblastic structure which is developed from an unpaired fundament as a pair of curved cylindrical diverticula of the foregut. Each diverticulum subsequently loses its connection with the foregut and breaks up into six to ten closed vesicles. Each vesicle then acquires an opening at the surface and becomes thus converted into a cup continuous with the ectoblast.

2. The ends of the cells of the cups next their cavities form a secretion (mucous?) by which the organ is rendered adhesive.

3. In larvæ 18 or 20 mm. long the adhesive organ has been pushed beneath the surface by the thickening of the epidermis. Its cells subsequently become vacuolated, leucocytes appear among them and the organ finally wholly disappears.

4. There is at no time any genetic connection between the adhesive organ and the ectoderm, or any of the epidermal sense organs; nor is there more than a superficial resemblance between it and any of the epidermal sense organs.

5. The mesoblast of the head region is formed largely by delamination from the entoblast, but part of it is proliferated from the germinal wall bordering the crescent and part from the "button" (pre-oral gut).

6. A pair of head cavities is formed in the delaminated mesoblast and these later communicate with one another across the middle line. The communication lies in the saddle cleft at the front end of the notochord.

7. The head cavities are not formed by evagination from the archenteron, but appear in situ in the mesoblast. They are equivalent to the premandibular head cavities of other vertebrates and become later connected with the third nerve.

Conclusions.

An adhesive organ occurs in the larvæ of Amia, Lepidosteus and Acipenser, and in certain Amphibia. What we believe to be a homologous structure is found in Elasmobranchs and in Amphioxus. We shall not discuss the Amphibian organ at this time.

Amia. Dean, in his work on the larval development of this form (1896), says that in the four-day larva the two halves of the adhesive organ are crossed transversely by a row of pigment cells which "apparently demarcate the lines of the sensory tracts." This existence of pigment across the adhesive organ as well as along the sensory tract is regarded as evidence that the cups of the adhesive organ are homologous to

the sense organs of the tracts. Dean finds further evidence for this homology in the structural resemblance of the two organs. He says: "As far as histological evidence goes there is certainly no difference between the enlarged, thick-walled, cup-shaped organs which arise on the snout of the late embryos of Amia or of Lepidosteus and the typical pit organs or sense buds which later occur on other integumental regions. It is found, in fact, that a gradation in size exists which connects the huge sucking organs of the snout with the inconspicuous organs of the trunk." In a note he adds: "There is but little difference histologically between these (i. e. the cup-shaped organs of the adhesive disc) and the neighboring nasal pit."

Dean's inference that the existence of pigment across the adhesive organ indicates the homology of its constituents with the organs of the pigmented lateral line, need not detain us. There remains the histological resemblance of the two structures which he believes to indicate their homology.

Such similarity as exists is apparent only and depends wholly on the plane of section. Thus certain sections cut transversely through the peripheral ends of the cells of the adhesive organ cups, resemble sections of the nasal pits lying against the adhesive organ and cause the two to be easily confused. But when the whole series of sections is examined, and sections cut in other planes are compared, the differences are much more striking than the similarities. The goblet cells of the cups of the adhesive organ can hardly be long mistaken for the cells of pit organs. The presence of yolk in the entoderm cells aids in distinguishing ectodermal and entodermal tissue in this case as in others.

All histological differences aside, the development of the adhesive organ from entodermal pouches, and its entire disappearance make it impossible for any genetic connection to exist between the adhesive organ and the epidermal sense organs in Amia.

Lepidosteus. Alexander Agassiz (1879) first called attention to the adhesive organ in this form and Balfour (1881)

subsequently considered its constituent papillæ as "thickenings of the epiblast" and says: "These papillæ are probably sensitive structures, but I have not yet investigated their histological characters." Balfour and Parker (1882) subsequently considered the organ as composed of mucous cells, which were highly modified cells of the mucous layer of the epidermis. These cells they believed to pour out a sticky secretion. Investigations now in progress in this laboratory, which it is hoped shortly to publish, have shown that the adhesive organ of Lepidosteus has essentially the same developmental history as that of Amia. There is as little ground for considering its papillæ to be either ectoblastic or sensory as in the case of Amia.

Acipenser. Von Kupffer (1891) describes an organ which he calls the adhesive organ. It arises as a cushion-like thickening of the nervous layer of the ectoblast immediately dorsal to the stomodæum. The organ, as figured externally (v. Kupffer, 1891, Pl. 1), has somewhat the form of a crescent with enlarged ends which point dorsally. The convexity of the crescent lies just above the roof of the mouth. Three days after hatching the adhesive organ separates into two parts which have the form of hemispherical protuberances separated by the cleft-like space between the medial ends of the superior maxillary processes. Later each of the two parts of the adhesive organ thus formed again divides into two, so that there are four spherical projections which lie in a slightly curved row above the upper jaw. These become the four barbels of the adult.

In view of what we now know of the adhesive organ of Amia that of Acipenser should be re-investigated, especially since v. Kupffer did not pay especial attention to it. He points out that, although the external ectoderm and the ectoderm of the brain are free from yolk, yet the cells of the hypophysis and adhesive organ are filled with the same yolk materials as those of the entoderm, thus making it impossible to distinguish the tissues of these organs from the entoderm by means of stains. This presence of yolk indicates an entodermal

origin for the adhesive organ. A further suggestion concerning a possible homologue in Acipenser of the Amia adhesive organ will be found in a paper on the development of the hypophysis of Amia, by Reighard and Mast (1908).

Elasmobranchs. If we regard the adhesive organ of Amia as a pair of entoblastic pouches from the anterior end of the foregut it becomes an interesting question as to whether there exist in other vertebrates similar gut pouches with which they may be compared. The head cavities of Elasmobranchs at once suggest themselves. We have shown that in Amia the first head cavities (pre-mandibular) are formed in the mesoblast of the head behind and beneath the optic vesicles. If then the adhesive organ of Amia be comparable to a pair of head cavities it must be to that pair which lies in front of the pre-mandibular cavities, that is to the anterior head cavities of Platt.

The anterior head cavities were first described by van Wijhe (1882) in Galeus and later in much more detail by Platt (1891, 1891a), Hoffmann (1896) and Neal (1898) in Acanthias. According to the last three observers the foregut extends in early embryos nearly or quite to the anterior neuropore, (Hoffmann, Pl. III, Fig. 19, z, embryos with 15-20 somites). Hoffmann describes this 'pre-oral gut' as becoming subsequently divided by the down-growth of the infundibulum into a median and two lateral parts. The lateral portions lie beneath the optic vesicles and are for a time connected with one another by the median portion which extends from the infundibulum nearly to the neuropore. The median portion of the pre-oral gut breaks up finally into mesenchyme. The lateral portions develop cavities and become the anterior head cavities. After a time the proliferation of cells from the walls of these cavities renders them solid. Still later they break up into mesenchyme (embryos of 22 mm.) and are no longer distinguishable from surrounding mesenchyme.

The anterior end of the foregut of Amia forms a 'pre-oral gut' (cf. Fig. C). This becomes divided into three por-

tions. The central one of these, the "button," subsequently breaks up into mesenchyme, as does the middle portion of the pre-oral gut of Elasmobranchs. The lateral portions exist as diverticula of the 'pre-oral gut' (fundaments of the adhesive organ). Their relation to the foregut and to the optic vesicles and ectoblast is precisely the same as the relation of the anterior head cavities of Elasmobranchs to the same structures. This is at once evident upon comparing our figure B with Neal's Figs. 7 and 8, Pl. III. These lateral diverticula of the foregut of Amia differ from the anterior head cavities of Elasmobranchs in their larger size and in the fact that their lumina communicate with that of the foregut. These differences are referable to the fact that in Amia the diverticula are functional, becoming converted into an adhesive organ. In the Elasmobranchs the anterior head cavities are not functional as organs, but become converted into mesenchyme. They are potentially diverticula of the foregut. They are furthermore in process of reduction, as shown by the fact that, while they are still of considerable size in Acanthias (Platt, Neal, Hoffmann) and in Galeus (van Wijhe), they are much reduced in Scyllium (van Wijhe) and are apparently absent in other Elasmobranchs. In these other Elasmobranchs the pre-oral gut probably breaks up directly into mesenchyme, without first forming the anterior head cavities.

It seems to us that there can be but little doubt that these anterior head cavities were formerly much better developed in Elasmobranchs than they now are, and that they were then formed as diverticula of the foregut. According to Platt (1891) this method of formation still obtains, though this is emphatically denied by Neal. We believe these diverticula to be homologous with the adhesive organ of Amia. It is most probable that the anterior head cavities represent an adhesive organ that formerly existed on the end of the snout in developing Elasmobranchs. An adhesive organ of the character of that of Amia is of use only to larvæ of relatively small size like those of Ganoids and Amphibia. With increase in size of the larva the organ becomes useless owing to its inability

to support the increased weight. Assuming that such an increase in size has come about in Elasmobranchs through yolk accumulation, the adhesive organ of these forms has consequently become useless and has been reduced. It persists in but three genera in the form of the anterior head cavities.

Since an adhesive organ of this form is useless to a larva greatly burdened with food yolk, its retention in Ganoids indicates that the ova of these fishes have not at any time possessed more food yolk than they now have. The Ganoid ovum is not to be derived from that of the Elasmobranch through secondary reduction in the amount of food yolk. Beard's (1890) contention on this point is thus upheld. Hence we must conclude that the Ganoid and Elasmobranch lines separated before the great accumulation of yolk in the ova of Elasmobranchs had taken place.

Amphioxus. Here there is found a pair of diverticula at the anterior end of the foregut. Of these anterior gut pouches the right becomes the head cavity of Amphioxus, while the left acquires an opening to the exterior and is finally converted into the Räderorgan, a lobed tract of ciliated epithelium which surrounds the mouth. The left anterior gut pouch of Amphioxus thus agrees with the adhesive organ of Amia in opening to the exterior. Neal (1898) has already homologized these anterior gut pouches of Amphioxus with the anterior head cavities of Elasmobranchs. If this be conceded, they are in turn homologous to the adhesive organ of Amia. The evidence at hand does not seem to us to warrant any other conclusion, though possibly it does not warrant any conclusion.

Summary of Conclusions.

The adhesive organ of Amia (and Lepidosteus) is in no way homologous with epidermal sense organs as claimed by Balfour (1881) and Dean (1896). It is a pair of gut pouches comparable to the anterior head cavities of Elasmobranchs. These anterior head cavities may therefore be interpreted as adhesive organs which have been rendered useless by the in-

creased weight of the larva due to yolk accumulation and have been consequently reduced. They are, in their turn, probably homologous with the anterior gut pouches of Amphioxus.

We have in the adhesive organ of Ganoids an indication that their eggs have at no time contained more food yolk than at present, while the existence of a reduced adhesive organ in Elasmobranchs indicates that the Ganoid and Elasmobranch lines separated before the accumulation of yolk in the Elasmobranch ovum.

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EXPLANATION OF THE PLATE.

ad. org., adhesive organ.
a. p., auditory pit.
b. a., branchial area.
b. a. l., first branchial arch.
b. c., body cavity.
bt., button (prae-oral gut).
cr., crescent (fundament of prae-oral gut and adhesive organ).
h. a., hyoid arch.
m. a., maxillary arch.
st., stomadaeum.
th., thickening of neural crest.

FIG. 1.—Dorsal view of an early embryo of Amia, showing the first stage of the adhesive organ as part of a crescent-shaped elevated area. The line a-b indicates the plane of the section shown in text figure B. Description in text, p. 470. *Drawn from a photograph.* × 20.

FIG. 2.—Dorsal view of an embryo of Amia, showing the second stage of the adhesive organ, as a pair of protuberances with the button between them. Description in the text, p. 473. *Drawn from a photograph.* × 20.

FIG. 3.—Ventral view of an embryo of Amia with the adhesive organ in the third stage. Description in the text, p. 478. *Drawn from a photograph.* × 20.

FIG. 4.—Embryo of Amia at about the time of hatching. The embryo is between six and seven mm. long. Ventral view. Description in the text, p. 482. *Drawn from a photograph.* × 20.

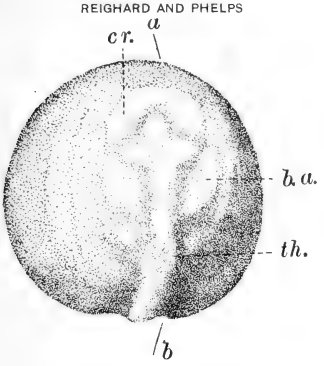

Fig. 1

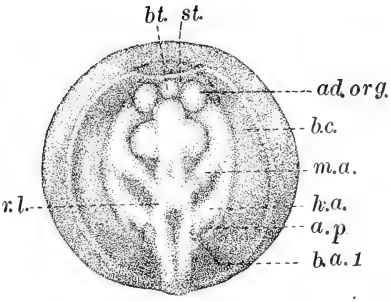

Fig. 2

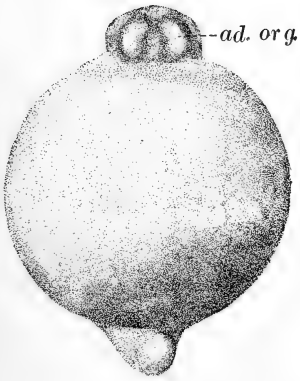

Fig. 3

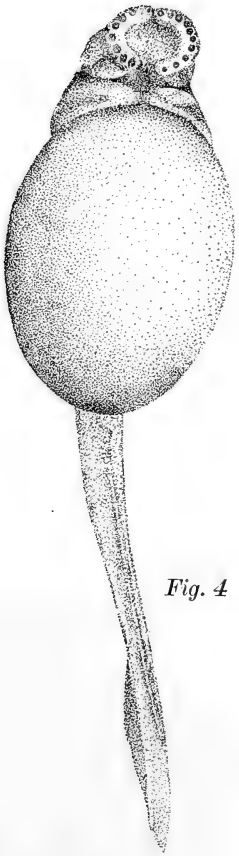

Fig. 4

STUDIES ON GANOID FISHES.[1]

II. The Development of the Hypophysis of Amia.

Jacob Reighard and S. O. Mast.

In describing the origin and early development of the hypophysis it is convenient to refer to definite stages in the development of Amia, as designated in the preceding article on "The Development of the Adhesive Organ and Head Mesoblast of Amia."

By referring to Fig. 1 accompanying the article just mentioned it will be noticed that there is a slight depression between the anterior end of the neural tube and the adhesive organ. This depression marks the position of the fundament of the hypophysis. A median longitudinal section (Fig. 1) through an embryo of this stage, shows the ectoblast to consist of two layers. The outer layer is thin and quite uniform in thickness. It is composed of a single tier of rounded opaque cells which are slightly flattened. The inner layer, also composed of a single tier of cells, approximately cubical in form, is about as thin as the outer except over a considerable area in the region dorsal to the anterior end of the neural tube, where it gradually becomes thicker from all sides, extends inward and becomes continuous with the walls of the neural tube which at this point extend outward forming a small funnel-shaped evagination in the dorso-anterior region of the neural tube, or forebrain. A similar evagination is designated lobus olfactorius impar by von Kupffer (1893) in Acipenser. In the following description we shall, following Haller (1897), speak of the tissues (Fig. 1, np.) between the

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above-mentioned evagination of the forebrain cavity and the exterior as neuropore (mediane Riechplatte and lobus olfactorius impar of von Kupffer).

Immediately in front of the neuropore the thickened portion of the inner layer of the ectoblast becomes still thicker, extending postero-ventrally in the form of the segment of a sphere. (Fig. 1, hy.)

This is the fundament of the hypophysis. The ectoblast of the neuropore and that of the hypophysial fundament are thus seen to form parts of the same thickening of ectoblast. The nervous or inner layer of the ectoblast anterior to the fundament of the hypophysis as well as that on both sides of it is composed of a single tier of columnar cells. In the ectoblast which forms the fundament of the hypophysis, the columnar cells, judging from the arrangement of the nuclei, appear to have divided transversely, thus forming approximately cubical cells. The cell walls could not be seen distinctly in the hypophysis in this stage, consequently it was impossible to determine the exact form of the cells and their arrangement. Some little distance anterior to the fundament of the hypophysis, there is a broad, low, thick-walled evagination (Fig. 1, ad.) of the dorsal wall of the alimentary canal near the anterior end. This is the crescent as described in the preceding paper. Immediately in front of the crescent there is a smaller but deeper evagination (Fig. 1, m.) marking the position of the mouth opening. In the space between the ventral surface of the hypophysis the dorsal wall of the alimentary canal and the anterior wall of the forebrain there is a mass of rather closely packed mesenchyme cells (Fig. 1, me).

A median longitudinal section (Fig. 2) of an embryo corresponding in age to stage three (Fig. 6) of the preceding article, shows that the fundament of the hypophysis which formed a part of the inner layer of ectoblast in the preceding stage, is now connected with other ectoblast tissue only at its posterior or dorsal end where it is continuous with the neuropore. It has become a solid rod of cells slightly flat-

tened. The original anterior end of the hypophysis is now directed postero-ventrally and projects into the slightly acute angle formed by the floor of the forebrain and the dorsal wall of the alimentary canal. The posterior surface (ventral surface of the preceding stage) comes in contact near its dorsal end with the wall of the forebrain in the region of the lobus olfactorius impar and near its ventral end with the wall of the forebrain in the infundibular region (Fig. 2, in.). The central portion of the posterior surface is separated from the forebrain by a mass of mesenchyme cells which in the preceding stage was situated between the anterior end of the forebrain, the dorsal wall of the alimentary canal, and the ventral surface of the hypophysis. The anterior surface of the hypophysis, which in the preceding stage was the dorsal surface and which was then in contact with the outer layer of ectoblast, is now in contact with the adhesive organ and button, which, developing from the crescent of the preceding stage, have grown up and separated the hypophysis from the ectoblast and forced it against the anterior wall of the forebrain. The cells composing the hypophysis are more or less columnar in form, arranged so that their longitudinal axes are approximately parallel with the longitudinal axis of the hypophysis, especially at its dorsal end, as if the entire organ had been forcibly elongated.

Mechanically, the change in the position and form of the hypophysis between stages 1 and 2, may be referred to the rapid enlargement of the cavity of the forebrain and to the development of the adhesive organ. The enlargement of the cavity of the forebrain causes the ectoblast in front of the neuropore, which in the preceding stage was nearly parallel with the dorsal wall of the alimentary canal to assume a position nearly at right angles to it. The upgrowth of the adhesive organ at the same time separates the hypophysis, anteriorly and laterally, from the ectoblast, and as already stated forces it against the anterior wall of the forebrain.

Sections of several embryos both older and younger than those of the stage just described were studied, but, contrary

to the observation of von Kupffer (1893) in Acipenser, no connection was found between the hypophysis and the entoblast, the cells of which may readily be distinguished from those of ectoblastic origin found in the hypophysis by the large round yolk granules they contain in contrast with the fine granules characteristic of ectoblast cells. Fig. 2, which is from a photograph, shows how distinct these two sorts of cells are and how sharply the ventral end of the hypophysis is separated from the entoblast.

Fig. 1a represents a median longitudinal section of an embryo somewhat younger than that represented in Fig. 6 of the preceding article, from which Fig. 2 is taken. The section is slightly oblique. It shows the hypophysis more broadly connected with the neuropore at its dorsal end than in Fig. 2, and lying against the posterior face of the button. Otherwise the condition of the hypophysis is not greatly different from that shown in Fig. 2.

In the fourth stage of the preceding article, the hypophysis (Fig. 3, hy.) has lost its connection with the ectoblast entirely and lies in a horizontal position in the somewhat enlarged space between the forebrain and the adhesive organ close to the dorsal wall of the alimentary canal with its posterior end projecting into the angle between the forebrain and the dorsal wall of the alimentary canal. The hypophysis considered as a solid has become somewhat tongue-shaped, and is now composed of a mass of more or less cubical cells. No definite arrangement of these cells could be determined. It contains three small inter-cellular cavities, none of which were found in younger stages. These cavities, some little distance apart, are situated on a median longitudinal line a little nearer the posterior than the anterior end of the mass of cells.

A stage between Figs. 2 and 3 is represented in Fig. 2a. The hypophysis is here more recently separated from the neuropore than in the stage of Fig. 3. The embryo from which this section was taken is externally scarcely distinguishable from that shown in Fig. 6 of the preceding article, except by the single character that its tail is free from the yolk for a distance equal to thirty degrees of the yolk's circumference.

Sections of embryos corresponding in age to the fifth or hatching stage in the preceding article (Fig. 8), show the hypophysis (Fig. 4, hy.)[2] to have changed considerably in position. It now lies between the ventral wall of the infundibulum and the dorsal wall of the alimentary canal with its posterior end extending to a point some little distance ahead of the anterior end of the notochord (Fig. 4, n.), which reaches the postero-ventral region of the infundibulum. At this point the infundibulum presents a slight evagination (Fig. 4, sv.), the fundament of the saccus vasculosus. The cavities first observed in the preceding stage are somewhat larger in this stage, and the cells, more columnar in form, are arranged apparently in a single layer around them, forming small vesicles (Fig. 4, c.). The ends of the cells next the cavities are clear, while their opposite ends contain numerous small granules among which the nuclei are found.

By comparing the position of the hypophysis in Figs. 3 and 4, it seems to have migrated backward between the dorsal wall of the alimentary canal and infundibulum independently of surrounding parts. Mechanically, this change in position may be explained by supposing that the entire forebrain moves forward in its courses of development, its ventral wall thus passing over the hypophysis, and that later its infundibular region moves backward, due to the formation of the cerebral flexure, carrying the hypophysis with it. The first supposition is supported by the fact that the large space between the anterior end of the forebrain and the ectoblast, seen in Fig. 3, disappears; the second by the fact that the saddle cleft is much narrower in Fig. 4 than in Fig. 3.

The hypophysis in embryos about 22 mm. long is found to be located in the same relative position as in the preceding stage. It has, however, increased rapidly in size and has become broader and flatter than it was in the earlier stages. The vesicles (Fig. 5, c.) have increased in number and be-

[2]The relative difference in size between Figs. 2, 3 and 4 is not due to a difference in magnification, as might be supposed, but to a difference in the actual size of the embryos, all being magnified 190 diameters.

come elongated to form canals, all of which end blindly at both ends, as far as could be determined. One exceptionally large vesicle (Fig. 6, c.) was found situated but a short distance from the anterior end of the hypophysis in the median plane, near its ventral surface. This vesicle is approximately cylindrical, its length being about ten times as great as its diameter, which was found to be about five times as great as the diameter of any of the other vesicles. It is formed by a layer of columnar cells whose inner ends are hyaline while their outer ends are granular. No opening could be found leading either into or from this cavity.

At this stage the hypophysis was found to be well supplied with blood by vessels (Figs. 5 and 6, b. v.) which enter both along the median dorsal and the median ventral surfaces from the posterior end.

On either side of the median line on the dorsal surface near the posterior end, there are depressions into which project anteriorly directed ingrowths (Fig. 5, hy.) from the ventral wall of the infundibulum. These ingrowths distinguish the nervous portion of the hypophysis, which is designated ramus hyoideus by Goronowitsch (1883), in Acipenser. They vary in number in different individuals, there being sometimes two on either side of the median line and sometimes one on one side and two on the other. They are composed of neuroglia (Haller) similar to that found in the walls of the brain tube at this stage. In the middle of each protuberance there is a rod-shaped core of nuclei which is surrounded by a layer of fibrous neuroglia tissue which is separated from the hypophysis by a limiting membrane.

We thus have here indicated a division of the hypophysis into two portions homologous with the two portions decribed by Haller (1897) and others in other vertebrates.

Summary of Observations.

1. The hypophysis of Amia originates at a much earlier stage than described by Dean (1896) as a solid mass of cells from the inner layer of the ectoblast between the funda-

ment of the adhesive organ and the neuropore. Its tissue is continuous with that of the neuropore and remote from the stomodæum.

2. It loses its connection with the ectoblast and the neuropore tissue and comes to lie between the infundibulum and the dorsal wall of the alimentary canal.

3. In changing its position it never unites with the entoblast.

4. Its cells contain smaller yolk granules than those found in entoblast cells and may consequently be readily distinguished from cells of entoblastic origin. This is true of the earliest stages.

5. In 22 mm. larvæ it shows a division into a number of elongated vesicles with well marked cavities.

6. By the penetration into it of nervous tissue (neuroglia, Haller, 1896) from the infundibulum, it becomes divided into an anterior and a posterior portion.

7. It is richly supplied with blood-vessels.

Discussion of Results.

From the foregoing account it appears that the hypophysis of Amia shows in its development a striking likeness in some features to the hypophysis of Acipenser as described by v. Kupffer (1893).

In both cases the hypophysis does not originate from the ectoblast of the stomodæum or near it, but from the ectoblast at the anterior end of the neural tube in connection with the anterior neuropore (mediane Riechplatte and lobus olfactorius impar of v. Kupffer). In both cases there is an adhesive organ intervening between the fundament of the hypophysis and the stomodæum. In both cases the hypophysis detaches itself from the ectoblast and neuropore and takes up a position posterior and dorsal to the stomodæum. In both cases its further history is not essentially different from that of other vertebrates (Haller, 1896).

Acipenser differs from Amia in that its hypophysis is, according to v. Kupffer, tubular in an early stage. This hypophy-

sis differs further from that of Amia in being continuous at its ventral end (in early stages) with the entoblast of the dorsal wall of the archenteron. The lumen of the tube opens at one end into the archenteron, while at the other end it is closed by the outer layer of ectoblast. In Amia we have not found the hypophysis at any time tubular, nor have we found any lumen until some time after the separation of the organ from the ectoblast. We have not at any time found a connection of the hypophysis with the entoblast.

The hypophysis of Amia is in close contact with the entoblastic button, which in an earlier stage is the middle of that part of the archenteric entoblast designated in the preceding article as the crescent (Fig. 1). The plane of demarcation between the ectoblast of the hypophysis and the entoblast of the crescent or button is not always easy to find. Its visibility depends on the plane of section. In suitable sections we have always found it as shown in Fig. 1, and we believe that it always exists. In respect to these two points, the existence in it from the beginning of a lumen and the continuity of its walls with the entoblast, the hypophysis of Amia may be in a less primitive condition than that of Acipenser.

It is not our purpose to discuss at length the theory advanced by v. Kupffer, on the basis of his work on Acipenser and Petromyzon, to the effect that the hypophysis is a palæostome. The development of the organ as we have described it in Amia certainly adds force to the array of facts adduced by v. Kupffer in support of his theory.

In this connection it should be noted that we know but three vertebrates in which the hypophysis originates in connection with or near the neuropore, Petromyzon, Acipenser and Amia. In Acipenser and Amia the adhesive organ intervenes between the hypophysis and the stomodæum. In Ammocœtes the upper lip occupies the same position as the adhesive organ of the Ganoids. This suggests the possible homology of the Ammocœtes upper lip with this adhesive organ.

Von Kupffer believes the position of the hypophysis as found in these forms to be primitive, the hypophysis to be a

palæostome and its connection with the stomodæum in other vertebrates to be a secondary condition correlated with the increased size of the forebrain and the loss of the adhesive organ. That such a condition as the connection of hypophysis and stomodæum could come about through the secondary displacement of the hypophysis he considers "schwer zu verstehen." Whether the position of the hypophysis in these three forms is primary or secondary, depends, it seems to us, on whether the adhesive organ is a primitive organ formerly possessed by all vertebrates, or an organ which has made its appearance in only a few groups and has in these groups caused the displacement of the hypophysis from a position in the stomodæum to a more dorsal position. This question can scarcely be answered until we know more of the adhesive organ itself and its homologues. Until our knowledge on this subject is increased one of these alternatives seems to us nearly as probable as the other; although, as v. Kupffer points out, the early appearance and large size of the Acipenser hypophysis is probable evidence of its primitive character.

Since the foregoing was written there has appeared a paper by Prather (1900) on the development of the hypophysis in Amia. The earliest embryo in which Prather has found the hypophysis is one a few hours before hatching, corresponding, therefore, very closely to Fig. 8 of the preceding article. In this stage he finds it in process of being differentiated out of the entoblast of the dorsal wall, of the foregut, ventral to the infundibulum, that is essentially in its adult position. His account thus differs from ours chiefly in two particulars: (1) He has not seen the early stages in the development of the hyphophysis as described by us. (2) He derives the hypophysis from the entoblast; whereas, according to our account, it is ectoblastic.

Through the kindness of Dr. Eycleshymer one of us has been able to examine the sections on which Prather's studies were made. They show what Prather has described. Having had an experience of many years in the preservation of Amia material and in the preparation of sections from it, we be-

lieve that we are justified in saying that the differences between Prather's results and ours are due to the insufficiency of the methods used in the preparation of his material. His Fig. 1 shows that in the material used by him, the egg membranes had not been removed from the eggs before preserving them. The figure shows also that in this case the membranes had shrunken down close against the embryo. The result of this is that the tissues of the embryo are pressed together and the limits between adjacent tissues are frequently obliterated. The hypophysis is not then visible in its early stages, and in the stages some time before hatching it is so pressed into the entoblast of the dorsal foregut wall as to be scarcely distinguishable. Such a condition is shown in Prather's Fig. 3. At the time of hatching or upon removal of the egg membranes the pressure from them is, of course, removed, and the hypophysis, being no longer pressed into the dorsal foregut wall, becomes visible and appears to be differentiated in situ out of the entoblast.

We have tried every feasible method of preserving these eggs without shrinkage of the membranes and have not found any method hitherto in use that accomplishes this. One of us has, however, devised a fluid, consisting of a five per cent formalin solution (i. e., containing two per cent of formaldehyde) to which is added two or three per cent of potassium bichromate and ten per cent of glacial acetic acid. This fluid used for eight hours or longer causes not more than a very insignificant shrinkage of the membranes or none at all. It is not stable and should be used fresh. Eggs are transferred from it to four per cent formalin which is changed until no longer discolored by the bichromate, and are then preserved permanently in the formalin. The histological results are similar to those obtained from Flemming's or Hermann's fluids and are excellent, as may be judged from the figures of cell divisions visible in Fig. 2. In spite of the results obtained from this fluid we have made it a rule, before preserving the eggs, to remove the membranes from them in the earliest stages practicable. This may be done in the stage represented by Fig.

6 of the preceding article, and (by using very great care) in somewhat earlier stages.

A comparison of our Fig. 4 with Prather's Fig. 3 of approximately the same stage will show the differences between eggs preserved with and without removal of the membranes.

From the foregoing we believe that Prather is in error in regarding the hypophysis as of entoblastic origin. Prather gives a brief summary of opinions that have been hitherto held as to the origin of the hypophysis. From this it appears that the view that the hypophysis is of entoblastic origin exclusively has not been maintained since 1882. Nearly all recent writers have maintained its ectoblastic origin (though a few have regarded it as of mixed origin). We believe that we have shown that Amia, also, must now be placed among those forms concerning the ectoblastic origin of whose hypophysis there is no question.

Prather believes that the structure which v. Kupffer (1893) has described and figured in early stages of Acipenser (Figs. 13, 14) as the hypophysis is in reality the adhesive organ of that form. This belief he bases on the two facts; that the hypophysis is described by v. Kupffer as laden with food yolk, and that it has the appearance of being a dorsal diverticulum of the entoblast. Both of these characteristics distinguish the adhesive organ of Amia, and may be expected to be characteristic of that of Acipenser also. An investigation in progress in this laboratory on the adhesive organ of Lepidosteus has given figures of median sections strikingly like Fig. 13 of v. Kupffer (1893). In these sections there is in front of the brain an entoblastic tube with its lumen and walls continuous below with those of the foregut. The tube is flattened and may be traced in series of longitudinal sections from one side of the brain to the other. It is therefore as wide as the brain. Its position and relation to surrounding structures are the same as that figured for the hypophysis of Acipenser by v. Kupffer, but its subsequent history shows it to be the adhesive organ. These observations on Lepidosteus certainly lend weight to Prather's suspicion. On the

other hand, the very circumstantial account which v. Kupffer gives of the transformation of the tubular fundament of the hypophysis of the embryo of Acipenser into what is undoubtedly the hypophysis of the larva, and his clear though very diagrammatic figures make it necessary to wait for a re-investigation of Acipenser before pronouncing judgment.

With our present knowledge it seems to us most likely that what v. Kupffer describes as the fundament of the hypophysis in Acipenser is really such. This view is strengthened by the likeness of its development to that described for Amia. The dorsal diverticulum of the archenteron shown in v. Kupffer's Fig. 13, between en. and vd., is the probable homologue of the Amia adhesive organ. V. Kupffer speaks of this structure as a "quer gelagerten Wulste des Entoderms, . . . der jederseits von der Mittellinie sich taschenartig gestaltet." Its form is thus not unlike that of the Amia adhesive organ in an early stage, and its position is the same. V. Kupffer himself suggests its possible homology with the anterior head cavities of Acanthias, structures which in the preceding paper are considered to be the homologues of the adhesive organ of Amia.

If it be true that this diverticulum of the foregut of Acipenser seen in v. Kupffer's Fig. 13 corresponds to the adhesive organ of Amia we should expect that the structure marked hf. (Haftscheibe) in the same figure is in reality a part of this entoblastic diverticulum and not, as stated by v. Kupffer, a thickening of the ectoblast. With this slight alteration v. Kupffer's account of Acipenser would be in substantial agreement with that given for Amia in this and the preceding papers.

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EXPLANATION OF PLATE.

All figures are photographs of sections and are magnified about one hundred fifty diameters. They have been slightly retouched with the pencil, for the purpose of adding to the distinctness of certain features. Nothing has been *added* with the pencil to that which was visible in the original photographs.

Reference Letters for all Figures.

ad—adhesive organ (crescent).
al—alimentary canal.
b—button.
bv—blood-vessel.
c—cavity in hypophysis.
h—heart.
hy—hypophysis.
hy′—posterior lobe of hypophysis.
in—infundibular region of brain.
m—position of future mouth opening.
me, me′—mesenchyme.
n—notochord.
np—neuropore.
st—stomodæum.
s. v.—saccus vasculosus.

Fig. 1.—A median longitudinal section (sagittal) of an Amia embryo of the stage of the one shown in Fig. 1 of the preceding article.

Fig. 1a.—An approximately longitudinal section of an embryo somewhat younger than the one represented in Fig. 6 of the preceding article.

Fig. 2a.—A median longitudinal section of an embryo a little older than of the preceding article.

Fig 2a.—A median longitudinal section of an embryo a little older than the one represented in Fig. 6 of the preceding article.

Fig. 3.—A median longitudinal section of an embryo still a little older than that shown in Fig. 6 of the preceding article, and older than the last.

Fig. 4.—A median longitudinal section of the hypophysis and surrounding structures of an embryo of the hatching stage (Fig. 8 of the preceding article).

Fig. 5.—A longitudinal section of the hypophysis and surrounding tissue of a larva of Amia, 22 mm. long, taken a little to one side of the median plane.

Fig. 6.—A median longitudinal section of the hypophysis and surrounding tissue of a larva of Amia, 22 mm. long.

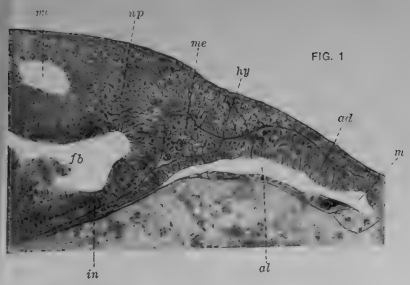


FIG. 1

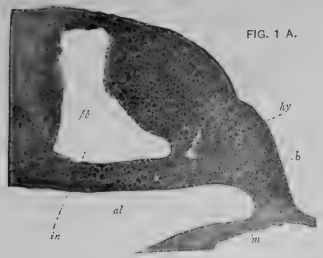


FIG. 1 A.

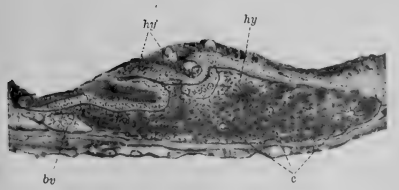
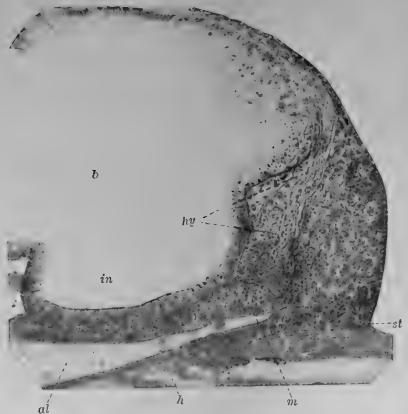


FIG. 5

FIG. 2



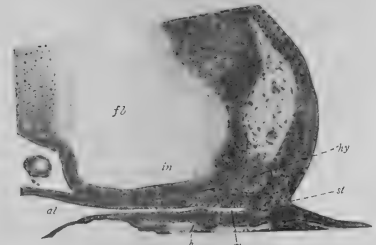
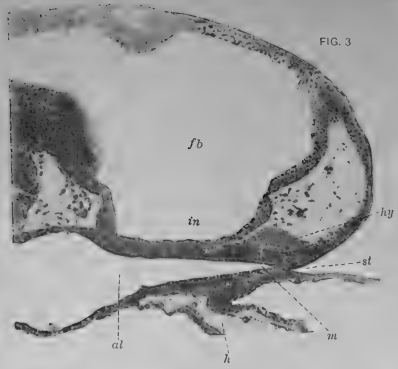


FIG. 2 A.

FIG. 3



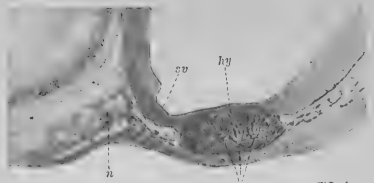


FIG. 4

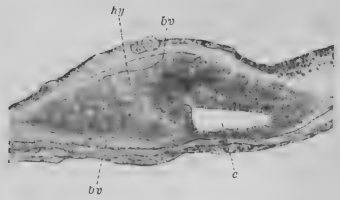


FIG. 6

THE LATERAL LINE SYSTEM IN EXTINCT AMPHIBIA.

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The study of the system of canals and sense organs in the lower vertebrates, known as the lateral line system, has interested many anatomists. The existence of the lateral line system was first observed, of course, in the fishes where it is clearly marked, more especially on the body of the fish. According to Collinge (1), the lateral line organs were first observed on a species of skate by Stenonis in 1664, and in 1669 on some of the sharks. The term canal was first applied to this set of structures under the preconceived idea that there was an actual canal under the skin, as indeed there seems to be in some cases, and M'Donnell (2) tells us of his attempts to inject the system of canals with a syringe. Various means were devised for studying the anatomy of this peculiar set of structures and many theories were propounded as to its possible functions. The general opinion was that the lateral line structures were secreting organs and the grooves on the crania of fishes and on the skulls of the ancient Amphibia are almost universally spoken of as *slime canals* or *mucous canals* or *grooves*.

Through the recent embryological and anatomical studies of Allis, Pollard, Platt, Collinge, Cole, Parker, Takahashi, Harrison, and others, the full development and functions of the lateral line system have been made out in a few forms.

It is now generally admitted, especially since the excellent experiments of Parker (3) on the function of these organs in fishes, that the lateral line system is a set of sense organs intermediate in function between the organs of touch and those of hearing, but more delicate than either in some respects.

Parker ascertained that the lateral line organs in some of the fishes seemed to respond to slow wave vibrations. It is thus through the action of wave vibrations on the external lateral line organs that the animal is notified of a disturbance in the water near it, the wave vibrations of which were too slow to affect the organs of touch or hearing.

It is not our purpose to discuss here the functions of these organs, but to describe the manner of their occurrence in the ancient Amphibia, on the crania of which the canals are often clearly marked. Unfortunately there are but comparatively few skulls in existence and they are scattered far and wide in the museums of the world. The remains of the early Amphibia are represented in great part by fragments. Occasionally, however, a good skull is discovered and almost always the lateral line canals are well shown on such skulls. But there is another source of grievance. When such good skulls are described, the describer of the specimen either pays but scanty attention to the subject of the lateral line system, or omits a discussion of it altogether or describes the canals inadequately. We are indebted to the paleontologists of the world for the knowledge we have of the lateral line canals of the ancient Amphibia and more especially to von Meyer and Fraas.

The lateral line system, as preserved in the extinct Amphibia, is of a very peculiar character and is unlike anything with which I am acquainted among the fishes, although some degree of correlation is possible. The entire set of structures on the skull of the Stegocephala is usually spoken of as the *lyre* or *lyra,* the former being the more correct term. The system of organs is represented by canals of various forms and with varying directions. They are usually in the form of gutters with more or less vertical sides, although sometimes, especially among the Microsauria, the canal is represented by a row of elongate pits. The bottoms of the well-formed canals may be smooth or roughened by pits such as commonly occur in the crania of the Stegocephala. The smoothness of the bottoms of the canals is, I think, an indication either of age

or of specialization, for I have observed that in the more generalized forms the line canals are roughened by the vascular pits, but in the highly specialized labyrinthodonts the bottoms of the canals are usually rather smooth, and this smoothness increases as the animal grows older. The canals of the lateral line organs are always open and never have the canals roofed over as is the case, according to Dean (4), in some of the Arthrognathid fish-like vertebrates.

Pollard (5), Baur (6), and Allis (7) have attempted some correlation of the cranial elements of the fishes and Stegocephala on the basis of the lateral line system. Baur's work had to deal with the stegocephalan side of the question and the others treated the question from the fish point of view. Allis' paper on the homologies of the squamosal and other elements is especially instructive. Pollard, according to Baur, did not fully understand the arrangement of the lateral line organs in the Polypterus which he was studying, having failed to comprehend the significance of the occipital cross-commissure. Baur did not complete the homologies of the crania of the fishes and stegocephalans, but carried the correlations as far as was then possible. In my studies on the Carboniferous Amphibia, I have been led to investigate the conditions of the lateral line system in the extinct forms to see if some definite idea might be formed as to the homology of the squamosal and supratemporal elements in the skull of the Stegocephala. The investigation was undertaken in the hopes of ascertaining just what the element which lies laterad to the parietal in most forms of the Stegocephala is, whether it is the squamosal, prosquamosal or supratemporal, all of which names have been applied to it by various authors.

An attempt has been made to correlate the lateral line canals in the Stegocephala with those of the fishes and recent Amphibia. In the accompanying diagram (Fig. 1), there are represented all of the canals which occur on the crania of the Stegocephala. They do not all occur in any one species nor indeed in any one group, but are all found in more or less well-developed form in some of the extinct Amphibia. The

nomenclature here proposed, it is hoped, does not depart widely from that given by Allis for Amia (8). The supraorbital (Fig. 1 "d") and infraorbital (Fig. 1 "c") canals are readily correlated with the canals of the same name in the fishes. The anterior commissure (Fig. 1 "a") is also homologous with that of fishes, as is also the canal which is here called the

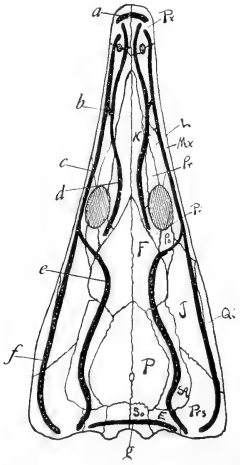

Fig. 1.—A diagram of the lateral line canals in the Stegocephala.

a, anterior commissure; b, antorbital commissure; c, infraorbital canal; d, supraorbital canal; e, temporal canal; f, jugal canal; g, occipital cross-commissure.

E, epiotic; F, frontal; J, jugal; L, lachrymal; Mx, maxilla; P, parietal; Pf, postfrontal; Po, postorbital; Pr, prefrontal; Prs, supratemporal; Px, premaxilla; Qj, quadrato-jugal; So, supraoccipital.

"antorbital commissure" (Fig. 1 "b"). The others are not so readily homologized. The upper canal in the posterior part of the cranium is here designated the "temporal canal" (Fig. 1 "e"). It is, however, clearly a part of the infraorbital canal of the fishes. Its relations in the Stegocephala are such that a new name is deemed necessary, for otherwise the canal would have to be referred to by the circumlocution: "the upper posterior portion of the infraorbital canal." For the

lower posterior portion of the infraorbital canal I propose the term "jugal canal" (Fig. 1 "f"), since it lies in great part on that element of the skull. The jugal canal is, I believe, a new formation in the Stegocephala. I am unable to homologize it with anything which I can find occurring in fishes. It may be composed of the infraorbital and a portion of the operculo-mandibular canal, but of this I am not sure. The most posterior canal of the stegocephalan skull is homologous with the

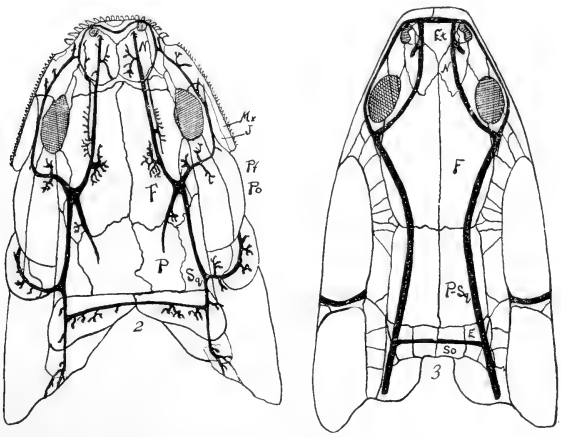

Fig. 2.—Outline of the lateral line canals on the skull of Amia calva. Lettering as in Fig. 1. After Allis.

Fig. 3.—Outline of the lateral line canals on the skull of Polypterus. After Baur and Traquair.

supratemporal cross-commissure of Amia as defined by Allis, and it is here designated the "occipital cross-commissure" (Fig. 1 "g"). Allis (1899) was doubtful as to whether this canal could be homologous with the same placed canal in Amia and Polypterus, and was inclined to correlate it with a canal which occurs in other fishes and which would distinctly change the correlations of the cranial elements. There can be no doubt, however, that the "occipital cross-commissure" is the

same as the supratemporal cross-commissure of Amia. It will be noticed that the main canal in the Stegocephala is the infraorbital canal as it is in Amia and Polyterus (Figs. 2, 3). In the Stegocephala likewise, the main canal always, or almost always, cuts the epiotic as it does that element in the fishes which Baur would correlate with the epiotic.

There are five suborders of the extinct Amphibia (9) in nearly all of which there have been detected evidences of the lateral line system. The extinct Amphibia of the pre-Jurassic are known as the Stegocephala and the five suborders of this group may be designated: the Branchiosauria, the Microsauria, the Aistopoda, the Temnospondylia and the Stereospondylia. In all of these suborders, with the exception of the Aistopoda, the lateral line system has been observed. The canals are more clearly preserved on the skulls of the Stereospondylia than in any of the other groups, the reasons for which we will consider later.

The Branchiosauria are known by numerous individuals of several species found abundantly in Europe in the Upper Carboniferous and Permian, and by a single species founded on a single well-preserved specimen from the Carboniferous of Illinois. The Branchiosauria are the best preserved of all of the extinct Amphibia and not only is the complete skeletal anatomy known, but something of the soft parts, color markings, covering and habits. Credner (10) has been able to write an interesting paleontological embryology based on the Branchiosauria preserved in the Permian rocks of Saxony and adjacent regions. The European students of the Branchiosauria have not, unfortunately, paid any special attention to the study of the lateral line system as it is preserved in the Branchiosauria. Thevenin (11) barely mentions the occurrence of the lateral line on the specimens he studied from the Upper Carboniferous rocks of France. It is to be hoped that more may be added to our knowledge of this portion of the anatomy of the Branchiosauria.

The form from Illinois, described elsewhere (9) as Micrerpeton caudatum gen. et. sp. nov. (Fig. 4), is a very small ani-

mal, apparently adult. It measures 49 millimeters in length or a little less than two inches. It is preserved, almost perfectly, in one of the nodules from Mazon Creek, Grundy County, Illinois; the collector in splitting the nodule lost the chips containing the hands and feet. From this specimen not a few new characters have been added to those already known for the Branchiosauria such as certain color markings on the body, the character of the dermal covering, and most important of all, the presence of a distinct type of lateral line preserved clearly and distinctly on the impression of the fleshy tail. This

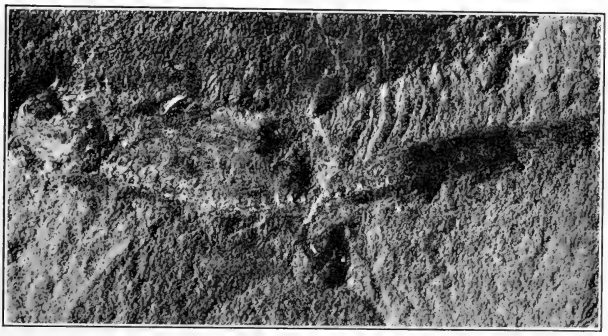

FIG. 4.—Impression of Micrerpeton caudatum Moodie, from the Carboniferous of Illinois. Twice natural size.

has been fully described elsewhere, but it is deemed of such importance that a redescription is here given.

"The most interesting and important single structure discovered on the specimen is the impression of the lateral line system which is clearly evident as two dark lines on the impression of the fleshy part of the tail. The sense organs are represented by two longitudinal rows of pigmented scales, one beginning at the tip of the tail, the other taking its origin from the median line somewhat further forward (Fig. 5). I am indebted to Dr. Takahashi for calling my attention to the similarity of this arrangement to that found in the modern

Necturus (Fig. 6). The arrangement and disposition of the lines containing the sense organs is practically the same in the two forms. The median lateral line takes its origin from the extreme tip of the tail or rather it ends there, and is continued to the base where the impression is broken. The dorsal lateral line has its origin rather abruptly from the median line at a distance of six millimeters from the tip of the tail. The sense organs were undoubtedly located beneath specialized pigmented scales on the surface of the animal's body as in

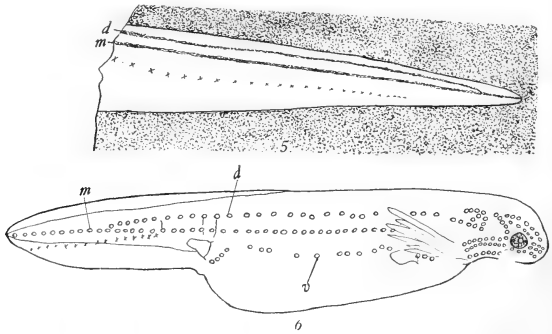

FIG. 5.—Detail of lateral line on impresion of the tail of Micrerpeton caudatum. d, dorsal lateral line; m, median lateral line; xxx, impressions of cartilaginous vertebræ. X 4.

FIG. 6.—Outline of the larva of Necturus maculosus Raf., showing arrangement of lateral line organs. d, dorsal lateral line; m, median lateral line; v, ventral lateral line. After Platt. Enlarged.

many of the modern fishes, e. g., the Holocephali, and to this pigment is due the preservation of the lines in a visible form.

"The fact that the arrangement of the lines of the sense organs of Micrerpeton corresponds so exactly to the condition found in Necturus is of considerable interest. Necturus alone among the modern tailed Amphibia has the arrangement of the lateral lines above described for Micrerpeton. All other forms of the Caudata as also the larval forms of the Salientia have an arrangement of the lateral line system which is per-

fectly distinct from that found in Necturus, although the same general plan is observable in all. The Necturus is, I believe, a more generalized type of amphibian so far as the lateral line is concerned than any of the other modern forms. This preservation of the lateral line system so perfect is due, without doubt, to the constant water habitat of the animal. Kingsbury (12) has expressed it as his opinion that Necturus is a primitive form and bases his conclusions on other grounds than that of the lateral line system. In very few of the modern Amphibia is the median line present as far back as the tip of the tail and in none, so far as I can learn, are the median and dorsal lines both present on the tail except in the Necturus. Other forms have lost the line from the tail, and this but gives expression to the general law that structures present over the entire body are lost first in the posterior region. This finds another expression in the loss of stripes by the zebras where in the quagga the hind part of the animal is destitute of stripes. In Ambystoma, for instance, the median lateral line is not present on the tail at all and the dorsal line is but imperfectly developed (13). The close similarity of the arrangement of the sense organs in the two forms, Necturus and Micrerpeton, may be of genetic significance with regard to the former group.

"The interval of time which has elapsed from the age in which Micrerpeton lived to the present is reckoned by many millions of years. But since the lateral line organs are of fundamental significance and since they are subject to comparatively little variation, this system of sense organs in the Amphibia may have persisted through the ages unchanged as we know it has done in some of the fishes (14). The ancestors of the modern Caudata and Salientia must have originated somewhere in the Carboniferous or earlier ages. Among all of the extinct Amphibia there are none which could have given rise to the modern forms save the Branchiosauria. The Microsauria are too highly specialized when we first know them in the Carboniferous, and they are already tending toward the reptilian type, to some groups of which they prob-

ably gave rise. The other groups of extinct Amphibia are, of course, out of the question so far as being ancestral to the modern Amphibia is concerned. The Aistopoda, when we first know them, are highly specialized, snake-like Amphibia and could not have given rise to animals with legs since it is well-known that they are descended from forms with well-developed limbs as is evidenced by the vestigial pectoral girdle in Ptyonius. The Temnospondylia and Stereospondylia, which are closely related, were also highly specialized along their own line, and like the Pterodactyles went out of existence completely and, so far as we know, left no descendants. There is certainly nothing in the structure of the Branchiosauria to prevent their being ancestral forms to the modern Amphibia. There is much in favor of it. The idea is not a new one but has been suggested by Baur and others. We have here, however, for the first time, something definite on which to base our conclusions." This matter is discussed more fully elsewhere. The cranium of the Branchiosauria, like the modern Amphibia, is never grooved by the lateral line canals, but the system was undoubtedly present on the skull in the skin, much as it is in the modern forms.

In the Microsauria, although there are numerous forms known, there have been but few observations made on the lateral line system. There are evidences of the canals on a skull and mandibles of Diplocaulus, Andrews (15) has observed rows of pits on the skull of Ceraterpeton galvani Huxley from the Carboniferous rocks of Staffordshire, England, and the writer has recently detected them on an excellent skull of Tuditanus tabulatus Cope from the Linton, Ohio, beds and traces of the supraorbital canal have been observed on the skull of Stegops divaricata Cope from the same locality.

In the Ceraterpeton specimen (Fig. 7) there are evidences of the lateral lines on the posterior and upper portions of the skull only. They consist in the occipital cross-commissure, the temporal canal and a portion of the supraorbital canal.

The infraorbital, the anterior commissure, and the jugal canals were not detected. The presence of a well-developed occipital cross-commissure in the skull of Ceraterpeton is of interest because this structure is rarely present in the more highly specialized labyrinthodonts. This is a generalized character as may be seen by referring to the figures of the Actinopterygian, Amia, and the Crossopterygian, Polypterus, in both of which the occipital cross-commissure is well-developed. The

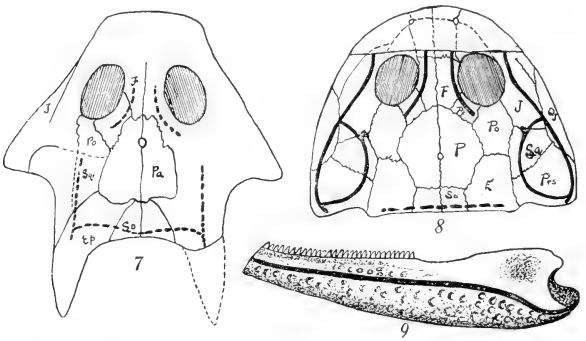

Fig. 7.—Outline of the skull of Ceraterpeton galvani Huxley from the Carboniferous of England, showing the distribution of the lateral line canals. After Andrews. Natural size.

Fig. 8.—Outline of the skull of Tuditanus tabulatus Cope, showing the arrangement of the lateral line canals in their relations to the cranial elements. One and one-third natural size.

Fig. 9.—The mandible of Diplocaulus magnicornis Cope, showing operculo-mandibular canal from the side. One-half natural size.

temporal canal, which is a portion of the infraorbital, begins its course on the epiotic and crosses most of the squamosal when it is lost. The supraorbital canal occurs on the frontal and postfrontal and has the usual relations for that canal.

The lateral line canals are but weakly developed in the cranial region of Diplocaulus. On a nearly perfect skull and mandibles of the species D. magnicornis Cope in the collection of the University of Chicago, there are traces of only three of

the canals. On the mandibles, the operculo-mandibular canal is well-marked (Figs. 9, 9a). The canal, apparently, has a course completely around the mandibles, although the anterior portion of each mandibular ramus has been broken and lost. However, at the point where the ramus is broken the canal is still strongly marked. The canal has its course, for the most part, near the middle of the rami, but as it approaches the posterior angle of the mandible it suddenly changes its course and drops down to the lower edge only to rise again and to come out strongly marked near the median plane on the posterior angle of the mandible. On the dorsal surface of the skull (Fig. 10) there are faint traces of the canals and on the

Fig. 9a.—The mandibles of Diplocaulus magnicornis Cope as seen from below. The operculo-mandibular canal at the arrow. One-half natural size.

edges the infraorbital is clearly marked. The arrows on the skull indicate the positions where the canals were detected. The long arrow points to a doubtful indication of the supraorbital canal. The infraorbital is clearly marked where it is preserved, but the premaxillary region of the skull is lost so the entire extent of the canal cannot be determined.

On the skull of Tuditanus tabulatus Cope (in the Zoological collection of Columbia University, New York City) there are evidences of a nearly complete system of canals (Fig. 8). The fore part of the skull anterior to the transverse line has been lost so that portion is unknown since the species is represented by a single specimen. The occipital cross-commissure is rep-

resented on the posterior border of the skull by elongate pits such as Andrews has described for Ceraterpeton. I fail to detect any pores in connection therewith such as Andrews describes for the lines in Ceraterpeton, and indeed I would be surprised if there were any since there is no evidence that the lateral line system in the Stegocephala was other than superficial. The temporal canal forms with the jugal canal a complete ring in this form much as in Trematosaurus, only in Tuditanus the temporal canal does not touch the epiotic. I think there are evidences of a former connection of the tem-

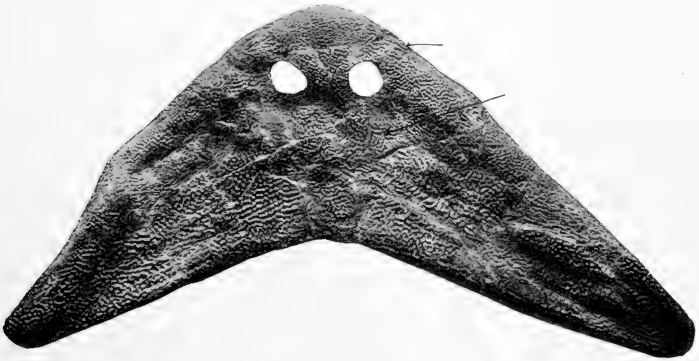

FIG. 10.—Photograph of the skull of Diplocaulus magnicornis Cope. The arrows indicate the regions where the canals occur. One-third natural size.

poral canal with the supraorbital, but am not sure of it. It is so represented tentatively in the diagram (Fig. 8). The temporal canal cuts the supratemporal (prosquamosal), the squamosal and the jugal. The jugal canal lies for the most part on the supratemporal and quadratojugal. It joins the infraorbital on the jugal. A portion only of the infraorbital is preserved and the remainder, i. e., its connection with the jugal canal, is restored. There is a portion of the supraorbital canal preserved. It seems not to be connected with the temporal canal, although there is a possible indication of this con-

nection as has been stated above. The supraorbital crosses the frontal, prefontal and a part of the nasal. The squamosal element in Tuditanus tabulatus Cope is peculiar in that it is excluded from the parietal by the extension of the epiotic and postorbital. This condition is found in several other species of the Microsauria. It will be noticed that with the changed condition of the position of the squamosal, the temporal canal has changed also and this is further proof of the close connection between the cranial elements and the lateral line canals.

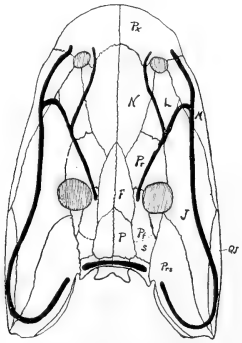

Fig. 11.—The cranium of Eryops megacephalus Cope with the cranial elements and the associated lateral line canals. Modified after Branson. One-ninth natural size.

Among the Temnospondylia the lateral line canals are well-developed on some of the skulls, such as Cricotus, Eryops, and Archegosaurus. I have had the opportunity of studying only one of these forms, that of Eryops megacephalus Cope (Fig. 11), from the Permian of Texas, represented by an almost perfect skull in the collection of the University of Chicago. This skull presents very striking characters as to the lateral line canals. The entire surface of the cranial elements in Eryops as in other of the Stegocephala is covered with coarse pits. The fossæ are present even in the bottoms of the grooves

which represent the lateral line system. This character is more marked in Eryops than in the Stereospondylia, a matter of considerable importance as we shall see.

The occipital cross-commissure is well-developed in Eryops. It is short and ends abruptly within the limits of the epiotics. Its ends are occupied by large pits. The cross-commissure, as in Amia, grooves the supraoccipital and epiotic elements. There is no evidence of an anterior commissure. I think there is an evidence of a temporal canal on the left side of the skull, but am not sure. This part of the skull is imperfect on the right side. The jugal and infraorbital canals are well-developed and strongly connected. The jugal canal starts far back on the supratemporal (prs), and after curving around over the quadratojugal joins the infraorbital somewhere on the jugal. There is nothing unusual in the characters of the infraorbital. The antorbital commissure is well developed in Eryops. It is longer and better developed in this form than in any other known to the writer. The supraorbital canal starts anterior to the nostril and makes a decided bend downwards to meet the antorbital commissure. It ends abruptly on the postfrontal bone. The squamosal is apparently not grooved in this form. The position of this element is a little doubtful as Branson has stated and the diagram of the cranial elements given is based on his studies of the skull. The antorbital commissure is primitively a branch of the supraorbital. It is such in the fishes and Dr. Takahashi informs me it is the same in Necturus. It would be an interesting matter if we could determine the points of origin of the lateral line canals in Eryops, and some day this may be known when we get material representing the youthful stages of the form. The peculiarly specialized condition of the lateral line canals bears witness to the specialization of the form. This is borne out by its osteology. Branson has shown that in its vertebral structure it is approaching the Stereospondylia, the most highly specialized of all of the Stegocephala.

There are faint traces of the lateral line canals on a poorly preserved mandible of Eryops in the collection of the Univer-

sity of Chicago. It does not differ greatly from that described below for the mandible of Anaschisma.

Although Archegosaurus (16) has been known for nearly sixty-five years, we have had as yet no adequate discussion of the manner of occurrence of the lateral line canals on the cranium of this form, where they assuredly occur. Burmeister, it is true, gave a figure of the canals as he thought they occurred on the cranium, but von Meyer states (16) that his representation is incorrect and seemed to be based in large part on the cranium of Trematosaurus. Although von Meyer criticised Burmeister's representation of the canals, yet he himself does not give any representation except in patches here and there on the skulls. Surely among the nearly two hundred specimens which von Meyer had at his disposal when he wrote on Archegosaurus there was sufficient information to have given such a restoration. There are two poorly preserved skulls of Archegosaurus decheni Goldfuss in the Field Museum. I have studied these specimens, which are preserved in the characteristic nodules much like those from the Mazon Creek region, but was unable to make out anything definite in regard to the lateral line canals on account of the poor state of preservation. The only other well-known form of the Temnospondylia with which I am acquainted, is that of Gondwanosaurus. This is represented by an almost entire cranium, but the cranial elements have almost all disappeared and have left only the sandstone cast.

The lateral line canals are well developed on the skulls of the Stereospondylia. Like the majority of the Stegocephala the cranial elements are strongly pitted and grooved. The sutures between the elements of the skull are usually clearly marked by smooth, narrow grooves. The lateral line canals can always be distinguished from the sutural grooves by the shape of the bottom, being U-shaped in the former, and V-shaped in the latter. The lateral line canals also at times have their bottoms roughened by pits occurring in them, the sutural grooves always have smooth bottoms. The lateral line canals are usually rather shallow and sometimes broad with the

edges of the grooves more or less perpendicular, but in Metoposaurus the canals are deep and the borders are sharply incised.

Why the lateral line canals are more deeply incised on the skulls of the Stereospondylia is not easy to determine. This suborder is the most highly specialized of all of the Stegocephala and it is a part of this specialization that the canals are deeply incised. The canals become more strongly marked as the individual grows older, and it is possible that the same will hold for the suborder. This suborder began, or at least we find the first evidences of it, in the Carboniferous at the same time that all of the other groups are represented by well-developed forms. All of the other groups, however, died out or became modified into other forms, so far as our present knowledge goes, before the Triassic or at least we know nothing of them after the close of the Permian. The Stereospondylia, however, did not become extinct until the latter part of the Trias or the early Jurassic. It is thus the longest lived of any of the groups of the Stegocephala as such, and for this reason we may consider that the lateral line canals are strongly developed. In Eryops, attention has already been called to the coarse pits occurring in the bottoms of the canals, and it must be stated that the canals in Eryops are not so well-developed as in the labyrinthodonts. It is thus evident, if we take Eryops to be a primitive form, that the primitive characters of the lateral line canals are the occurrence of deep coarse pits in the bottoms and their broad character and weakness of development. In other words the lateral line canals in the Stegocephala are, in their earliest development, rows of pits which later become developed into well-defined grooves. Whether the condition described for Eryops will hold for other of the Temnospondylia remains to be determined. In the Stereospondylia the canals, as stated, are usually clearly marked and often have smooth bottoms.

In the collection of the University of Chicago, there are two perfect skulls of labyrinthodonts, collected some years ago by Mr. N. H. Brown, of Lander, Wyoming, in the Triassic rocks

some eight miles southwest of Lander. These skulls were described in 1905, by Dr. Branson (17), as two species, Anaschisma browni for the larger specimen (Figs. 12, 14), and A. brachygnatha for the smaller skull. From my own study of the type specimens, I believe that the latter is but a youthful form of the former species. This is evidenced in several

Fig. 12.—The larger skull of Anaschisma browni Branson, showing distribution of canals. After Branson. One-fourth natural size.

particulars. The skull designated A. brachygnatha is narrower and shorter than the skull designated by A. browni. The broadening and lengthening of the skull would, of course, come with age. The vascular pits on the upper surface of the smaller skull are weaker and their borders are not so clearly defined as in the large skull. The position of the orbits is slightly different in the two skulls, but not enough to be of specific importance, and the difference is, in all probability, an

individual variation since it is very slight. But it is the lateral line canals that the chief points of resemblance lie. The general plan of the canals is identical in the two skulls. The only difference is this: the connection between the temporal and supraorbital canals is absent in the younger skull while in the adult it is well developed. The pits in the bottoms of the

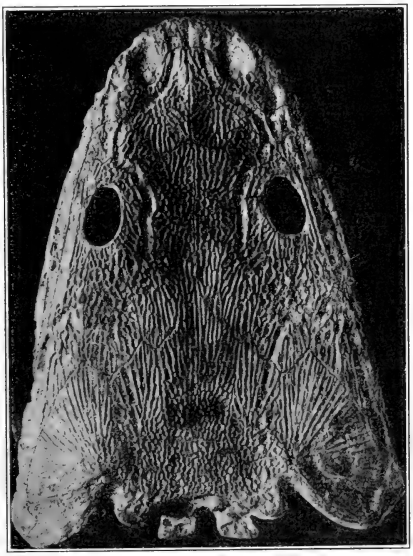

FIG. 13.—The skull of Metoposaurus diagnosticus von Meyer, showing the manner of distribution and occurrence of the canals. After Fraas. One-fifth natural size.

canals are more clearly marked in the younger than in the adult skull, and this is an indication of youth. There is another factor which tends to corroborate the statement as to the specific identity of the two skulls. They were found to be slightly overlapping each other in the rocks. This might

not be of importance were there remains of other species of labyrinthodonts known from these deposits. There are literally millions of fragments of labyrinthodont skeletons scattered along the exposures of the Popo Agie beds of Wyoming for a distance of more than thirty miles, but all of the fragments are of the same character and these two skulls represent the sum total of skulls, save fragments, from the Triassic deposits of the West. It is, I believe, therefore safe to assume that A. brachygnatha is a youthful form of A. browni, and that the lateral line canals really do become more deeply incised with age, lose the pits from the bottoms of the canals and become more clearly marked and more closely connected.

The temporal canal in Anaschisma browni (Fig. 14) is represented by broken furrows. The portions preserved exhibit the usual downward tendency of the canal to unite with the infraorbital on the postorbital element. In its course forward from the epiotic the temporal canal cuts the squamosal; a matter of considerable importance to be referred to later on. The supraorbital canal has an unusually deviating course in Anaschisma, but aside from the minor twists and curves it does not differ essentially from the same canal in other forms. It ends abruptly at the anterior end of the muzzle. In its course it gives off the vestige of an antorbital commissure which tends to join a vestige from the infraorbital canal. The jugal canal begins broadly at the very posterior edge of the skull as though it were continued, as it undoubtedly was, to the body of the animal. In its course forward it joins the infraorbital canal on the jugal. The course of the infraorbital is not unusual in any respect. There is no anterior commissure on the skull nor is the occipital cross-commissure developed in either of the specimens. There are distinct indications of the operculo-mandibular canal on the mandibles of Anaschisma. Each skull has a mandibular ramus preserved and the canals are identical on the two rami. The canal enters the mandible on the surangular and passes forward around the mandible as described for Diplocaulus.

Whether or not the canal completed the course around the mandibles is not to be determined from the specimens at hand.

The close resemblance between the arrangement of the lateral line canals of Metoposaurus (18), and that already described for Anaschisma is made evident by a glance at the figures (Figs. 12, 15). This is indicative of the affinity which the osteology of the two genera exhibits. The forms are, however, generically distinct as Branson has pointed out, by the absence of the characteristic ear slit in the latter genus. The arrangement of the lateral line canals would separate the forms generically if other characters were lacking. Since the canals have such a similar arrangement in the two forms only the differences need be pointed out and the detailed description given for Anaschisma will serve for Metoposaurus. There are no traces of an occipital cross-commissure in either form, nor indeed have I been able to detect traces of this canal in but one of the stereospondylous forms. The main difference between the two forms lies in the absence of connection between the temporal and supraorbital canals (Fig. 15), and in the more direct course of the latter canal in Metoposaurus. Other characters are almost identical in the two groups. The skull of Metoposaurus shows its specialized characters in the smoothness of the bottoms of the canals in the anterior part of the skull, and in the canals all over the skull, being more sharply defined than they are in Anaschisma.

The skull of Mastodonsaurus giganteus Jæger, fragments of which were first discovered by Dr. Jæger, in 1824, is the largest and first known of all of the extinct Amphibia. The skull reaches, at times, enormous proportions for an amphibian, often attaining a length of four feet with a posterior breadth of two and one-half feet. The lateral line canals are clearly marked on all of the skulls known or at least on all which have been figured (Fig. 16). There are no evidences of the occipital cross-commissure in an excellent photograph, published by Fraas (18, Pl.I), nor does that author indicate such a canal in the figure (18, p. 44), he gave of which the

accompanying cut is a copy. The skull of Mastodonsaurus differs greatly from the other skulls described in the large size and posterior position of the orbits. This has had an effect on the arrangement of the lateral line canals. The temporal canal is deflected strongly downwards and its union with the infraobital takes place to the side of the eye. The jugal canal according to Fraas's figure does not touch the supratemporal. In fact the canal is not represented in its most posterior portion. It may have had the course indicated in Fig. 16. The supraorbital is weakly connected with the jugal and with the temporal canal. It has the usual course forward and ends near the nares. The only character of interest in connection with the infraorbital canal is the distinct bend near the anterior termination as though it were a remnant of the former connection between the infra- and supraorbital canals through the intervention of the antorbital commissure. It is to be noticed that the temporal barely cuts the outer edge of the squamosal.

The skull of Trematosaurus brauni Burmeister (16) has already been figured and described in this connection by Baur and Miall, although the figures given by both of these authors are inaccurate in detail when compared with the original drawing of von Meyer. Fritsch in his translation of the report published by Miall also copies his figure and thus continues the inaccuracies. There is nothing unusual in the Trematosaurus (Fig. 17) except the strong development of canals. The occipital cross-commissure is well developed and is contained within the borders of the epiotics. The temporal has no connection with the supraorbital and the antorbital commissure is well developed. There is no anterior commissure. It is to be noticed that the squamosal element is clearly cut by the temporal canal which ends at the ear slit. It was stated above that there was rarely any evidence of an occipital cross-commissure in the Stereospondylia but Trematosaurus seems to be an exception to this rule. There may be others.

The skull of Anthracosaurus from the Carboniferous of

Great Britain has been figured and described by Atthey (19). In regard to the lateral line canals he says: "The mucous-grooves are two pairs. The anterior pair run backwards and inwards along the inner side of the naso-lachrymal suture as far as the posterior margin of the nasals; the posterior are deeper, and appear in two disconnected portions along the outer margins of the jugal and quadratojugal bones. The anterior pair of grooves are less deep and less distinct than those of Loxomma; the posterior are deeper, wider and rougher than those of that labyrinthodont." The skull studied by Atthey was in rather poor state of preservation, but it is evident that he detected the anterior portions of the supra-orbital canals and the jugal canal on one side, with possibly a portion of the infra-orbital. He does not indicate the "mucous-grooves" on his drawing of the skull.

The skull of Loxomma allmanni Huxley (20) is of peculiar interest in connection with the study of the lateral line system of the extinct Amphibia on account of the presence of a distinct anterior commissure which extends, clearly marked, according to the figure given by Embleton and Atthey (20), between the anterior extremities of the supraorbital canals. The antorbital commissure is also clearly preserved. The occipital cross-commissure is not represented in the drawing of Embleton and Atthey nor in that given by Miall (21). Miall's representation of the canals of Loxomma is manifestly inaccurate in regard to the antorbital commissure. In the skull of Gonioglyptus, Huxley (22) has figured the lateral line canals as more strongly developed than usual. Unfortunately only fragments of the skull remain. Huxley's restoration of the lateral line canals in this form is, so far as I can learn, conjectural. The manner in which the supraorbital canals curl around the nostril, if such is their normal course in Gonioglyptus, is without parallel among the other labyrinthodonts.

One of the main points to be brought out in this discussion of the lateral line canals of the extinct Amphibia is the correlation of the cranial elements with those of the fishes so

far as is possible. Professor Thyng (23), in his study of the squamosal bone in the tetrapodous Vertebrata, reached the conclusion that the element usually called squamosal in the Stegocephala is not that element, but is the supratemporal. Baur had made the same statement many years previously when he says: "Das sogenannte 'Squamosum' der Stegocephalen (Huxley, Miall, Fritsch, Credner, etc.) ist aber nicht dieses Element, sondern in Wirklichkeit das supratemporale, während das 'supratemporale' dieser Forscher das Squamosum repräsentirt" (Baur, Anat. Anz., I, p. 349). He afterward withdrew from this position (Anat. Anz., Bd. XI, p. 660) and concluded that the squamosal really was that element. Thyng now revives this idea and bases his conclusions on embryological studies of several forms. His major thesis is not, however, well taken since he assumes it to be an accepted fact that the quadrate of the lower vertebrates is represented in the mammals by an element of the ear, the incus. This is by no means so generally accepted as he would have us think. His conclusions are briefly these: since the incus of the mammals represents the quadrate of the lower vertebrates and since there is an intimate relation between the incus and the squamosal bone in certain embryos, ergo the so-called squamosal of the Stegocephala is the supratemporal. Whatever the element lying next to the parietal may be in other forms, I am fairly well convinced that in the Stegocephala it is the true squamosal and not the supratemporal (prosquamosal).

This position is sustained by the study of the lateral line canals on the crania of the extinct Amphibia and their correlation with those of the fishes. In the skull of Amia the temporal canal, in its course forward, cuts the squamosal as it does in all of the Stegocephala examined where the lateral line canals are preserved. I take the conclusion of Allis, Pollard and van Wijhe that this element in the fishes is really the squamosal and the reader is referred to them for their reasons. In Polypterus according to van Wijhe, when in his discussion of the lateral line system he says: "Bedenkt man, dass

ein Theil des Hauptastes, nämlich der, welcher bei andern Fischen dem Squamosum zukommt, durch das Parietale geht, dass dieser Knochen auch an der Begrenzung der Gelenkpfanne für das Hyomandibulare theilnimmt, was bei andern Fischen ebenfalls das Squamosum thut, und endlich dass das Schädeldach keinen besonderen Knochen besitzt, der auf den Namen Squamosum Anspruch erheben kann, so ist es kaum zu bezweifeln, dass der bis jetzt als Parietale bei Polypterus beschriebene Knochen ein Squamoso-parietale ist," the so-called parietal is the squamoso-parietal. He is followed in this by Pollard.

I do not find that the arrangement of the canals on the skull of Polypterus has ever been given in an accurate diagram. Tranquair gives a verbal description of their course (24). Van Wijhe remarks: "Ihren Verlauf (i. e. die Schleimkanäle in Polypterus) habe ich nicht verfolgt, er ist aber von Traquair beschrieben" (25). Pollard's representation is modified after Wiedersheim and Baur's figure is modified after Pollard. According to Traquair's description of the course of the canals, all of these figures are inaccurate in regard to the course of the main canal. Traquair says the infraorbital gives off the supraorbital canal on the postfrontal and I have represented it thus in the diagram (Fig. 3). In Polypterus according to Traquair the main canal is the infraorbital as it is in the Stegocephala.

The course taken by the lateral line canals is not subject to great change. This is suggested by the condition described for Micrerpeton and Necturus. If the temporal canal, which is the posterior part of the infraorbital canal, cuts a certain element in one form and cuts the same element with the same relations in another and related form, it is safe to assume that the element is identical in the two forms. We have thus a definite basis for conclusions regarding the homologies of the cranial elements in the two groups; fishes and stegocephalans. It is easy to understand the correlations of the premaxillaries, maxillaries, nasals, frontals, prefontals and parietals of the fishes with the same elements in the

Stegocephala but the correlation of the other elements is not so definite. Pollard is of the opinion that the post-suborital (postorbital) of fishes is homologous with that of the Stegocephala and it probably is. Baur was inclined to correlate the epiotics and supraoccipital of the Stegocephala with the supratemporal elements of the fishes and the arrangement of the lateral line canals substantiates his suggestion. He also would correlate the supratemporal (prosquamosal) with the preoperculum of the Amiadiæ and the quadratojugal with the suboperculum. The lateral line canals of the Stegocephala offer nothing which would contradict this view, so far as I am aware. The squamosal has been shown above to be the squamosal of the fishes, so that we have a nearly complete correlation of the elements of the Stegocephala with those of the fishes and more especially the Amia.

Maggi has offered some interesting suggestions with regard to the correlations of the elements of the stegocephalan skull with those of the higher forms and he would even go so far as to correlate the epiotics of the Stegocephala with the interparietal of man (26). While I have no reasons to doubt his conclusions in regard to some of the correlations yet I doubt his statements in regard to the cranial structure of Archegosaurus and Loxomma. If I understand Maggi correctly his homologies are, in large part, based on the assumption of the fusion of several of the cranial elements in the Stegocephala. I doubt very much if there has ever been a true case of the fusion of the cranial elements of the stegocephalans proved. Jaekel (27) thought he had a case of such a fusion in the skulls of Diceratosaurus punctolineatus Cope from the Carboniferous of Ohio, but in a perfect skull of a closely allied species of this genus I find the elements all clearly separated, as one would expect. Maggi is also inclined to doubt, according to Allis, the correlation of the occipital cross-commissure in the fishes and stegocephalans. It has been definitely shown above, I believe, that the canals are homologous in the two groups.

Summary.

1. There are present on the skulls of the extinct Amphibia seven distinct lateral line canals, all more or less connected.

2. These canals may be partly homologized with those of fishes. They may be termed: the anterior commissure, homologous with the same canal in fishes; the antorbital commissure, homologous with the similarly placed canal in fishes; the infraorbital canal, homologous with that of fishes; the supraorbital canal, homologous with that of fishes; the temporal canal, homologous with the posterior portion of the infraorbital canal of fishes; the jugal canal, homologous with the operculo-mandibular (?) and the posterior portion of the infraorbital (?) canal of fishes; and the occipital cross-commissure, homologous with the supra-temporal cross-commissure of fishes.

3. The lateral system has been discovered in four of the five suborders of the Stegocephala.

4. The Branchiosauria have a type of lateral line on the tail which is similar to that on the tail of the modern Necturus. The branchiosaurian skull is not grooved by the lateral line canals.

5. In the Microsauria, so far as known, the system of the lateral line canals is well developed. The occipital cross-commissure is present on the skulls of at least two genera.

6. In the Temnospondylia the lateral line canals are of a peculiar type, especially so in the form described, Eryops. The occipital cross-commissure is well developed.

7. The Stereospondylia always have the lateral line canals well developed. This character is an indication of age and specialization. The occipital cross-commissure is present in a single species of the Stereospondylia.

8. The so-called squamosal bone in the skull of the Stegocephala is really that element and not the supratemporal.

9. The following elements of the stegocephalan cranium are homologous with the same elements in fishes: the premaxillæ, maxillæ, nasals, frontals, prefrontals, parietals, squamosals, and postfrontals. The epiotics and supraocci-

pitals of the Stegocephala are homologous with the supratemporal elements of the fishes. The quadratojugal is homologous with the subopercular of fishes. The supratemporal (prosquamosal) is homologous with the preoperculum of fishes.

I am indebted to Dr. Katashi Takahashi for several interesting suggestions in regard to the lateral line system of the modern Amphibia and more especially Necturus. Dr. S. W. Williston has, with his usual kindness, read the manuscript and has offered interesting suggestions. I am indebted to him likewise for the use of the material on which the greater part of this discussion is based. To Dr. Bashford Dean I am grateful for the loan of the large part of the Newberry collection of Carboniferous Amphibia belonging to the American Museum.

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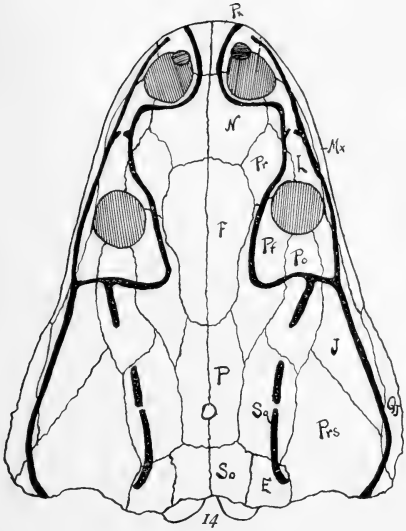
Fig. 14.—Outline of the larger skull of Anaschisma browni Branson, showing the distribution of the lateral line canals. Modified after Branson. A little less than one-fifth natural size.

Fig. 15.—Outline of the cranial elements and lateral line canals of the skull of Metoposaurus diagnosticus von Meyer. Modified after Fraas. One-fifth natural size.

Fig. 16.—Diagram of the cranial elements and the associated lateral line canals of Mastodonsaurus giganteus Jaeger. After Fraas. One-tenth natural size.

Fig. 17.—Outline of the arrangement of the lateral line canals and the cranial elements on the skull of Trematosaurus brauni Burmeister. After H. von Meyer. Paleontographica, Bd. VI, Pl. XXVII. Two-fifths natural size.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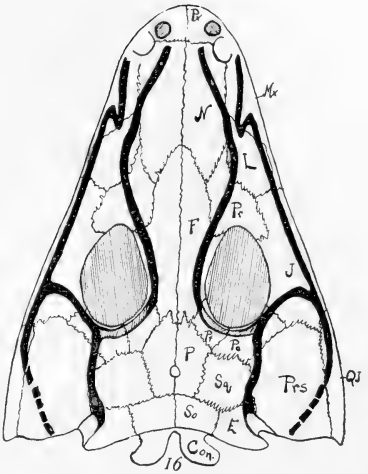
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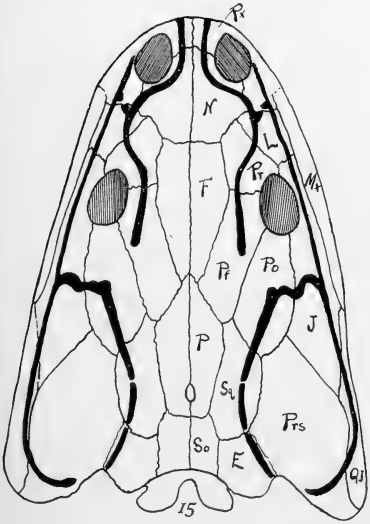
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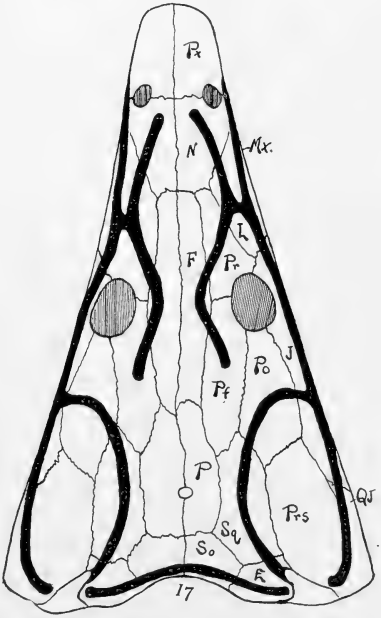
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THE LARVA OF CTENOPHORA ANGUSTIPENNIS LOEW.

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I. INTRODUCTION.

The great majority of the Dipterous insects are unfortunately known only in the adult state. This lack of knowledge in regard to the larval and pupal conditions of many forms leaves a gap which cannot be filled for some time. This gap, as pointed out by Kellogg, is especially noticeable in the case of the lower forms in their immature stages, which have hardly been studied at all. This lack is particularly serious inasmuch as these are the more generalized forms, and represent the ancestral types from which the highly specialized Tipulids have been evolved. It is to these lower forms that we must look for information regarding the genesis of the group.

The larva of Ctenophora angustipennis is peculiarly interesting, and as the main anatomical features can readily be worked out, it offers a most suitable subject for study in elementary entomology. The chief structural details can even be made out in observing the live specimen and the larvæ can usually be secured in sufficient abundance to furnish plenty of material.

II. Habitat and Mode of Life.

Ctenophora larvæ are somewhat gregarious in habit and are usually found massed in runways, as it were, in much decayed cottonwood or alder logs. They are rarely found in other logs and never in such great numbers. Where a single specimen is found a half hundred or more will commonly be found in the same log, though sometimes only a few will be secured. As the larvæ require moisture, they are usually found in the soft, punky wood along the lake shores. The numerous specimens used in this study were all secured from the small strip of the Lake Washington shore on the university campus. The larva probably obtains its food supply from the bacterial life found in this spongy wood; as when many of them are kept for some time in the same material they gradually absorb the plentiful adipose tissue, which apparently furnishes a reserve food supply. Numerous protozoan parasites are frequently found in between the cells of the alimentary canal, especially in the proventricular cæca.

III. Description of the Larva.

The Ctenophora larva (Fig. 1) is cylindrical, tapering somewhat towards the hinder end, and is bluntly rounded in front, especially when the head is much retracted. The larva is from three-quarters of an inch to a trifle over an inch in length. There are no external appendages or protuberances, but the animal is found covered with numerous fine hairs, extending backwards. When the larva is held to the light, these cause it to appear yellow on the edges. The larva moves quite freely by vermiform movements, and these are possibly facilitated by the presence of the hairs. The body proper consists of eleven segments, which, with the exception of the first and last, are not clearly marked off. Most of these segments are subdivided into annuli, but as the number of annuli varies in different specimens, and even in the dorsal and ventral portions of the same individual, their morpholog-

ical value must be slight. The prothoracic segment, the one just posterior to the head, appears at first sight double, as it is divided transversely by a distinctly-marked fold. This fold, however, is merely the result of the very frequent retractions of the head, which may be completely withdrawn within the first segment.

Indeed, it is generally so withdrawn, except when the animal is moving or eating. The head is thus surrounded by a fold of the body wall which greatly facilitates the retraction and protrusion of it. The top of the head (Fig. 2) is defended by a strong chitinous shield. The occipital region of this, as is the usual case in the retractile head of the Dipterous larvæ, is imperfectly chitinized and is posteriorly excavated by two deep notches. The antennæ (Fig. 3) are single jointed, projecting a little from the side of the head. No eyes are visible.

The extreme posterior segment (Figs. 4, 5 and 6) is modified as usual. The anus opens at the apex of this segment. Just above the anus and on each side of the median line are the two large oval stigmata (Fig. 7). The elliptical central core or plug looks coal black, while the surrounding ring of irregular chitinous threads is of a deep brown. The stigmata are of the primitive or generalized type, and are, therefore, without lips. The aperture can be closed by bringing down the surrounding lobes. The spiracles are surrounded by six backward projecting flexible lobes, four of these are in a line above the spiracles, while the others are just below and on each side of the anal opening. When these are contracted they serve to protect the stigmata, and are strengthened by the presence of small chitinous patches on the posterior tips.

As the larval skin is quite transparent, the main body systems can easily be seen through the skin. If the fat-bodies are well-developed, as they are just before pupation, they completely envelop the alimentary canal and therefore the larva appears white and opaque. When the live animal is observed the heart can be seen as a delicate pulsating tubule,

which lies in the median dorsal line, extending from the second to the extreme segment. The much darker alimentary canal shows on either side of the heart, while the large lobulated cæcum extends two-thirds of the way up the left side. The superficial tracheal system, which consists of the two main lateral trunks with their cross-connections, stands out as slender, shining white strands. On the ventral surface, the ventral ganglia, with their lateral branches, show very clearly. It is this readiness with which the main anatomical features can be made out that renders Ctenophora larvæ such remarkably fine subjects for students in entomology.

The mouthparts are very complex, and their exact homologies cannot be accurately determined save by a very extensive embryological study. The short single-jointed antennæ arise from the small lobe of the plate covering the top of the head. The antennæ bear at their extremity two groups of sensory papillæ. The mentum is triangular, with three serrations on each side and a larger apical tooth. The mandibles (Fig. 8) are strong and heavy. On the inner side of each mandible there is attached the serrated mandibular lacinia, which is evidently of great importance, as it is so very well developed. The structure of the maxillæ (Fig. 9) is extremely complicated and no definite homologies can be made out. The lacinia, a row of fine projections, lies next the mentum. On the outer side a very short palpus shows, and is carried on the short, curved palpiger. Back of this extends the head sclerites.

The entire body of the larva is covered with fine, close-set, simple hairs, pointing backwards. These are very much thicker on the anterior part of the body, and gradually decrease in number toward the posterior. This fact of distribution would tend to show that these hairs are somewhat sensory in function. Besides these there are larger hairs (Fig. 10), collected here and there in groups of from two to six and probably sensory in function. These large hairs are hollow with a central pore canal and are, in common with the shorter ones, somewhat useful in locomotion.

The skin is very inelastic and tough, due to the deposit of the thick chitinous layer. The skin consists primarily of an irregularly curved layer of columnar epithelial cells, the hypodermis. This layer secretes the chitin, which is laid down in irregularly waved laminæ.

The muscle system is very complex and there are a great number of muscle bands. There is a wide band of longitudinal muscles along the side of the dorsal and ventral median line of the body. Some of the fibers reach from the exterior to the posterior border of each segment, but other fibers reach from the middle to either end, and still others reach from the middle of one segment to the middle of another. There is also an inner set of lateral transverse muscles. Each muscle is a bundle of long fibers, each of which is enclosed in an outer elastic membrane, the sarcolemma. Each fiber is in turn made up of several fibrillæ. The muscle fibers of the insect present a beautiful striated appearance, which is due to the alternate light and dark bands of substance. In life the muscles are colorless and transparent. They are so soft that they are of a gelatinous consistency.

IV. Methods of Killing and Staining.

Hot Gilson's fluid was found to be the best reagent for killing the larvæ. With this the animals were killed very quickly and without contraction, while a slower reagent caused much distortion of the tissues. It was found impossible to make paraffin sections on account of the tearing of the rest of the section while cutting through the thick chitinous wall. Excellent preparations may be made by the celloidin method, although even here there is danger of tearing the inner delicate tissues. The best stain for use in making out the general differentiation of the tissues was found to be Bohmer's hæmatotoxylin. This stain was also used to good effect in working out the finer histological details, such as the cell structure of cæcum, the finer details of the muscle structures, and the details of the nervous system. Iron hæmatoxylin was found an especially good stain for working out the cell

structure of the salivary gland and the proventricular cæca. For the differentiation of the muscle plexus of the cæcum, Congo-red was the most satisfactory stain. In preparing whole mounts of the cæcum the best fixation was obtained by slitting the animal open for a short distance along the side and at once immersing it in a weak Flemming's solution. The best mounts of the mouth parts were made by removing the organs and cleaning them in strong carbol-xylol with a little safranin in the clearing solution.

V. The Digestive System.

The digestive system of Ctenophora is very like that of Holorusia, the allied giant Tipulid, as described by Kellogg. (Psyche, June, 1901.) The exceedingly large diverticulum is characteristic of the vegetable feeding larvæ of Tipula and Ctenophora. The alimentary canal extends as a straight tube from the anterior to the posterior extremity of the body, and is nearly wholly enclosed in the coiled perforated sheets of adipose tissue. (Fig. 11.) The tube consists first of the long slender œsophagus, which opens into the hypopharynx. At about the middle of this, the œsophagus is embraced by the circumœsophageal commissures and the brain lobes. At its posterior end, the œsophagus suddenly dilates and passes into the proventriculus, whose diameter is about ten times as great as that of the œsophagus.

The finer structure of the œsophagus, as seen in a cross-section, differs in the anterior and posterior part of the tube. (Figs. 12 and 13.) The outer œsophageal layer is composed of a band of circular muscles, beautifully striated and showing large oval nuclei. These nuclei extend over several striations. Within this is a doubtful layer composed of a few strands of longitudinal muscles, which lie in the cavities formed by the invagination of the œsophageal epithelium. At places this epithelium is contiguous with the circular muscles and show no trace of any longitudinal muscles. Within this muscle layer is the much convoluted epithelial layer. This is com-

posed of a single layer of columnar cells and is thrown into numerous deep, irregular, longitudinal folds, which at times cause the lumen to be almost closed. The inner margin of the epithelial layer, unlike that of the epithelium of the cæca, forms a straight line. This layer secretes the heavy, inner chitinous layer, depositing the chitin in irregular laminæ. In the anterior portion of the tube the lumen is nearly filled by the long, stiff hairs which project into the cavity. (Fig. 14.) These hairs are entirely lacking in the posterior portion, which is also distinguished by having no signs of the longitudinal muscle strands.

The proventriculus comprises the adjoining abruptly dilated portion. At the point of juncture the œsophagus is surrounded by the fine sphincter muscle, so that the entrance to the œsophagus may be completely closed and so keep food from passing back up the œsophagus. (Fig. 15.) There is also a large œsophageal invagination. This has the same cell structure as the posterior portion of the œsophagus, and extends for about the length of the proventriculus, when the line separating the ventriculus and the proventriculus is taken to be the beginning of the ventricular cæca. The wall of the proventriculus is composed of a single layer of secreting epithelium and is surrounded by a thin muscular membrane. This membrane is composed of a layer of circular muscles and an incomplete layer of longitudinal muscle fibers. The epithelial layer shows no signs of great activity as the inner cell margin is straight and no discharged globules are present.

There is no sharp line of division between the proventriculus and the vertriculus. The ventriculus proper bears at its anterior end four elongated pouches, the ventricular cæca. These are not alike in structure, two of them being nearly twice as long as the other two. They differ in this respect from the gastric cæca of Holorusia, where all four are of the same size. The longer cæca extend along the ventriculus for about one-fifth of its entire length. The transition of the epithelium of the ventriculus to that of the cæcum is very sudden. (Fig. 16.) The epithelium exhibits a very different appear-

ance according to the degree of secreting activity. The cells of the cæca are evidently very active in secretion and exhibit many granular protrusions. In the longer cæca there is but a single layer of cells, while in the shorter cæca there are two incomplete layers. (Figs. 17 and 18.) The cells of the shorter cæca are evidently much more active and show many narrow-necked protrusions. These protrusions increase in size till the cell projects into the lumen; the connection between the cell and the protrusions constricts, and there results a separation of the spherical globule. After the globule becomes free in the lumen it loses its definite outline. (Fig. 19.) The globule is finely granular, but nowhere contains any sign of a nucleus, such as described by Needham (Zoological Bulletin, 1897) in the digestive epithelium of the dragon-fly nymphs. The discharged portion of the cell represents but a small part of the whole and does not contain any trophic center. The secretion here differs from that of the larva of Ptychoptera as described by Gehucten (quoted by Packard, pp. 326-329) for the globule is constricted off and does not burst through the cell membrane. The process closely resembles that of Collembola, although there are no such marked changes in cell alveolation. (Folsom and Welles, in the Univ. of Illinois Bulletin, 1906.) Many protozoan parasites are found in the cæca, being often wedged in, as it were, between the cells. (Fig. 20.)

Near the posterior termination of the ventriculus are four very small protruding pockets, the gastric cæca. (Fig. 21.) These pockets have not been described as occurring in the nearly allied form, Holorusia. The epithelium here is composed of two layers of cells and is very much convoluted. The cells are evidently most active and show many protrusions and discharged globules.

The termination of the ventriculus is marked by a pale transverse line and the four coiled Malpighian tubules pass off at this point. Each tube passes forward to the base of the ventricular cæca and then turns backwards. In a cross-section the tubules show a ring composed of from two to six large

polygonal cells, which have very conspicuous nuclei. The excretory canal is clearly marked. (Fig. 22.)

Back of the ventriculus lies the small intestine, which is of smaller caliber than the ventriculus. The diameter of the small intestine is only about one-fourth of that of the ventriculus. This opens into the fifth division of the alimentary canal, the large intestine. The large intestine is distinguished by the very large intestinal cæcum or diverticulum, which is one of the distinguishing features of the herbivorous Tipulidæ. This cæcum lies along the ventriculus and extends as far as the proventriculus. A somewhat similar surface is present in Holorusia, but it is only about one-third as large. On a surface view the cæcum appears flabby, this being due to the pouch-like effect produced by the transverse and longitudinal muscles. The walls of the diverticulum are very thin. The wall is composed of two layers, an outer one made up of annular muscles and an inner one composed of very large cells. (Fig. 23.) (Fig. 24.) The muscles of the diverticulum form a most peculiar network or muscle plexus, as it might be called, which in its branching and anastomosing bears a superficial resemblance to a nerve plexus. (Fig. 25.) There are eleven or more large bands of striated muscles which extend around the diverticulum, and these are connected by numerous finer cross-branches, which are also striated. (Figs. 26 and 27.) In a few places the muscles seem to radiate from a central mass, but in general the ladder-like appearance is very marked. The striations run across both the large muscles and the finer connecting bands, so that the striations at the junctures meet at right angles. (Fig. 28.) There are many muscle nuclei, which, on a side view, seem to stand out from the fiber itself and to be surrounded by a transparent wall. By means of this peculiar muscle plexus, the diverticulum can be contracted in all directions at once. The gross structure of the plexus may best be demonstrated by mounting the entire wall of the cæcum after first removing the inner cell layer by means of a very fine brush. The cellular membrane is also remarkable. The individual cells are very large and

have very conspicuous nuclei. (Fig. 29.) There is a close resemblance in structure between the cells of the intestinal diverticulum and those of the Malpighian tubules. The cells are but loosely approximated to the muscle walls and in places the cells are separated by distinct canals. (Fig. 30.) The nuclei are very large, with deeply staining chromatic filaments. This large diverticulum, the presence of which characterizes the herbivorous Tipulids, is excretory in function, though it may also act as a sort of food reservoir. The similarity of cell structure with that of the urinary tubules would seem to point to this conclusion, but besides this there is the additional evidence of chemical tests. There is a strong uric reaction to the murexid test, which would seem to show conclusively that the diverticulum, as well as the Malpighian tubules, is excretory in function.

The large intestine dilates gradually till it forms the rectum. In cross-sections the structure appears very similar to that of the œsophagus. The epithelial surface is thrown up into numerous irregular longitudinal folds. The cells are uniform in size, and show no signs of any activity in secretion. Within this epithelial layer there is a layer of chitin, which is thrown into irregular folds and laminæ. Outside of the epithelial layer there is a series of striated annular muscles, which extends outwards for a distance equal to about one-fourth of the central cavity. (Fig. 31.) The structure of the rectum proper is very much like that of the colon, and the transition is imperceptible, but here the folds of the columnar epithelium extend much farther into the lumen and the chitinous layer is so thick that it almost obliterates the central cavity. The muscular ring is much thicker, extending out for a distance equal to the diameter of the lumen. (Fig. 32.) The heavy muscular walls serve to retain the food in the absorptive portions of the digestive tract till all possible nutriment has been extracted.

There is a much coiled salivary gland lying on each side of the œsophagus. (Fig. 33.) Each consists of a greatly coiled tubule, with a slender collecting duct extending from

the anterior portion. The two collecting ducts unite to form a common duct which lies just beneath the œsophagus and opens at the base of the hypopharynx. The glands are hollow, the walls being only one cell thick. The cell wall consists merely of an epithelial layer with its intima and basement membrane. As seen in a surface view the cells are very large and polygonal in shape. (Fig. 34.) They possess very large nuclei in which a distinct chromatic filament can easily be made out by proper staining. The breaking down of the salivary gland is accompanied apparently by simple cell degeneration, the "selbständige" degeneration of Karawaiew, and therefore without the recurrence of phagocytosis. The cell nuclei are at first regularly circular and sharply marked out by a nuclear membrane, but later on the nucleus loses this membrane. (Fig. 35.) The histolysis here follows closely the course described by Kellogg in regard to Holorusia. (Am. Nat., 1901.) Of course in the case of such a generalized larva as that of Ctenophora the breakdown of the larval organs would be accomplished with less change of structure than in the more specialized forms, which have been largely the forms studied in this connection, and so there would be less reason for the occurrence of phagocytosis.

VI. The Tracheal System.

There are two main lateral tracheæ, one passing along each side of the medio-dorsal line. These main divisions of the respiratory system are seen very clearly when looking at the live larvæ, and appear as two glistening bands when the animal is expanded, but having a sinuous course when the animal is contracted. These two tracheæ are connected by transverse and anastomosing branches, one main connecting branch in each segment. These branches, as well as the lateral tubes, give off numerous side branches. As these commissures are connected with the alimentary canal they are very slack, especially those near the middle of the body. During the vermiform movements of the larva, there is a great deal of sliding

of the body wall and the alimentary canal, and the commissures must be able to stand the strain.

From these two main lateral branches ramifications pass off into every part of the body. (Fig. 37.) These branches pass among the different organs of the body and seem to serve somewhat as strands to hold them in place. These branches have very minute ramifications, which become so attenuated that they pass among the fibers of the muscles. Each of the main tracheæ at the anterior extremity breaks up into a number of fine fibers, most of which are connected with the brain. In the last segment, just above the hinder part of the heart, there arises a number of small branches which are connected with the stigmata.

The lining membrane of the tracheæ consists of a layer of polygonal cells fitting closely together as a pavement epithelium. The chitinous wall or intima is thickened at intervals to form thread-like ridges, the tænidia. These tænidia serve to keep the tracheæ open without affecting their flexibility.

There is but one pair of spiracles so that the larva is metapneustic, as is the case with nearly all the Tipulidæ. The prominent oval spiracles are inserted at the apex of the last segment, and turn backwards and upwards. The structure of the spiracles is very similar to that of the larvæ of Dicranota and Phalacrocera, as described by Miall and Shelford. (Fig. 38.) The central portion of the spiracle consists of an inner cone or plug, which is surrounded by a thick and solid chitinous wall. Outside of this is a chamber with a colorless chitinous wall, the vestibule. The cavity of the vestibule is crossed by many radiating fibers, which are irregularly branched and start from the outer wall. Some of the fibers connect with the inner cone, but many do not reach it at all. The stigma forms the outer end of an air chamber whose inner surface is lined with a zone of large cells. This region is evidently of great importance in the respiratory mechanism of the larva, as there are great masses of blood corpuscles lying about the spiracle. There is a circular muscle with which to draw in the spiracle and help bring the

fleshy protuberances about it. The lateral tracheæ lost their tænidia just before they join the floor of the vestibule. The minute tracheal ramifications which extend out from the sides of the vestibule are not brought into any direct contact with the main vessel, but serve rather to aerate the numerous blood corpuscles. The large tracheæ lead out from the spiracle and give off numerous branches to the body wall and viscera. There is connected with the stigmatic region a very minute nerve plexus, which helps to show the great importance of this region in respiration and circulation.

VII. The Circulatory System.

The dorsal vessel or heart is a slender delicate membraneous tubule which lies along the medio-dorsal line, and extends from the brain to the last segment. In the live animal this may be seen to pulsate. The heart is cut off from the body cavity by the usual diaphragm. This diaphragm extends outward from the heart and, with the dorsal wall of the body, forms a pericardial chamber. The diaphragm is formed largely of paired fin-like muscles, the alary muscles, which extend past the lateral tracheæ and connect with the body wall. There are apparently no ostia, but at intervals the wall is thickened and the heart is partially divided into a series of chambers. This lack of ostia is not an aberrant condition, as there are no ostia present in the very young larva of Musca (Kolbe). The heart is attached to the body wall by the very minute suspensory muscles. These are attached to the upper surface of the heart and radiate till they come in contact with the body wall.

In a cross-section the heart is somewhat lozenge-shaped. (Fig. 39.) There are three distinct layers which compose the heart, as may be seen in a cross-section. There is (1) a very fine, transparent, and structureless intima, which is not marked except under a very high magnification, (2) a central layer of circular muscles, which effect the contraction of the heart, and (3) there is an extremely thin enveloping layer, the endocardium.

Along the heart on the basal side and along the alary muscles occur the so-called pericardial cells, which from their position would seem to have a close relation to the circulation of the blood. Some œnocytes are at times found collected along the body wall in the posterior region, as described by Bengtsonn. According to Kowalevsky the function of the pericardial cells is to remove the foreign or injurious matter mingled with the blood. The pericardial cells themselves are elliptical in shape and with no easily distinguished cell wall. They often have two nuclei lying side by side in the cytoplasm and sometimes as many as four without any sign of a separating cell wall. The cell-nuclei stain very heavily, but show no distinct chromatic filaments. The pericardial cells are congregated about the sides of the heart and lie along the alary muscles for some distance from the heart.

The blood proper is a thin whitish fluid which contains the very pale oval corpuscles. These corpuscles have a rounded nucleus and are covered with fat globules. The fresh blood has a slightly alkaline reaction. These leucocytes are seen in great numbers at the posterior end of the heart, just in front of the stigmata. At the stigmata the blood is passed over the tracheal ramifications, so that it tends to traverse the normal condition in insects in which the air is always brought to the blood, while here the blood seems to be brought to the air.

VIII. The Fat-bodies.

The alimentary canal is nearly enclosed in the thin sheet of adipose tissue, which is perforated by many small holes. This tissue fills in the space between the other organs and so occupies a large part of the body cavity. The cells are regularly polygonal in shape, and possess a central oval nucleus. (Figs. 40 and 41.) The fat-bodies are probably reserve food material to be used during the rapid pupal metamorphosis, as the sheets of adipose tissue are very large and conspicuous in larvæ which are about to pupate. When the larvæ have been kept for some time without any adequate food supply the fat-bodies are gradually absorbed.

IX. The Imaginal Buds.

The imaginal buds are small whitish bud-like bodies which lie between the muscles and the body wall of the thoracic segments. There are two pairs of these imaginal buds in each segment. There are several present on the ventral surface of the live animal when studied with a hand lens. The ventral invaginations give rise to the legs, while the dorsal invaginations develop into the pupal respiratory organ, the wing, and the halterer.

The imaginal buds or histoblasts, which Kellogg suggests as a better name for these structures, are composed of an invaginated portion of the hypodermis which has become folded and in which there has been a special increase of cells. (Fig. 42.) During this modification, the outer portion of these cells is separated from the rest and forms the very thick enveloping membrane, the peripodal membrane. The thickened part of the histoblast is the portion which later becomes functional as the developed organ. This portion forms two folds, each fold being composed of several layers of cells. The so-called tracheal veins lie between the two layers of the functional portion of the histoblast. The peripodal membrane and the function portion of the bud are both direct outgrowths of the hypodermis.

There are also other structures which, according to Villanes, are homologous with the histoblasts; these are the so-called optic imaginal buds. (Fig. 43.) The eyeless larva, as is common with the other eyeless Dipterous larvæ, avoids a too strong light, and this perception of light through the integument is probably due to the presence of these optic histoblasts. If a ray of light is concentrated on the region of these optic imaginal buds the response is much quicker than if the light be concentrated on another part of the body. The optic imaginal buds are rectangular in shape and are like other imaginal buds in possessing a peripodal membrane and a wider functional portion of the histoderm, which later develops into the optic ganglion. In the central cavity there are several tracheæ and some loose mesodermal tissue.

X. The Nervous Tissue.

The central nervous system extends along the median line of the ventral surface as a series of ganglia connected by nerve cords. The ganglia are more closely approximated in the anterior portion of the chain and some of the ganglia are united to form the brain. (Fig. 44.) Behind the brain, which comprises the supra-œsophageal ganglion, there is the sub-œsophageal ganglion and a chain of ten ganglia. Just back of the sub-œsophageal ganglion there are four closely approximated ganglia. These are so closely applied that there is no connecting nerve cord. Posterior to these, there are six ventral ganglia which are widely separated and are joined by the slender ventral nerve cord. The ganglia are roughly pyramidal in shape and give off four large nerve trunks; two nerve branches from each side and these soon divide and redivide into finer and finer branches. The terminal, or tenth ganglion (Fig. 45), is larger than the others and gives off four large nerve trunks from the base of the pyramid. The other ganglia also give off prominent nerve trunks; the one rising from the basal apex of the ganglion and going to the muscles of the body wall, while the other, which rises from the middle of the side of the ganglion, goes to the viscera. The muscular branch can easily be seen in looking at the live animal. The first ganglion after the thoracic group lies in the sixth segment, and posterior to this there is one ganglion in each segment.

The brain proper consists of the supra-œsophageal ganglion, which lies above the œsophagus and is connected with the sub-œsophageal ganglion by the œsophageal commissures, so that the brain completely embraces the œsophagus. (Fig. 46.) In front of the brain and extending across it is the frontal ganglion, which composes a part of the sympathetic system. Anterior to this and on either side are the optic imaginal buds. These are small oval swellings of the optic nerve. There are two other nerves given off from the supra-œsophageal ganglion, one going to the antenna, the antennal branch, and the other running to the labium. Going out from the sub-œsophageal are three branches which connect with the mouth parts.

These serve to control the mandible, maxilla, and the labium, and are known as the mandibular, maxillary, and the labial branch.

The brain in its finer structure is exceedingly complex. (Fig. 47.) There are several histological elements, the various cell elements and the fibrillar or Punktsubstanz. The cell elements form the cortical portion of the brain, while the central portion of the brain is composed of minute granules and interlacing fibers. These fibers appear as cut ends in the sections, so that the whole medullary portion appears finely granular. In the cellular portion of the brain there are three distinct kinds of cells. There is first an outer layer of cortical cells which differ somewhat in size, but are always larger than the cells which compose the cell mass lying just within. Then there is the mass of rather smaller and rounded cells which lie on either side of the central body and extend almost around the brain. Among this cell mass may be seen a few very large cells with very darkly staining nuclei. These evidently represent the large motor cells described by Kenyon in his work on the brain of the bee. The same elements may be found in the sub-œsophageal ganglion, although there is not so much definiteness in limiting the cell mass and there are fewer of the large cells. (Fig. 48.) There is here, in common with other Dipterous larvæ, no sign of the complicated mushroom bodies. In the sub-œsophageal ganglion there is a greater amount of the Punktsubstanz in proportion to the size than there is in the supra-œsophageal ganglion.

The thoracic and ventral ganglia show the same histological elements as the brain. (Figs. 49-50.) The arrangement of these elements is in general the same. There is an outer cortex layer of cells, the inner portion is composed of the cell mass with a few large cells interspersed, and a central portion which is composed of the Punktsubstanz. This arrangement is common to both the ventral and thoracic ganglia. Each nerve consists of an axis cylinder, which has a striated appearance in a longitudinal section which is due to the fine fibrillæ, and then the enveloping membrane or neurilemma (Fig. 51).

XI. The Pupa.

When the larva reaches its full size, it has stored up a great amount of reserve food material in the sheets of adipose tissue; it ceases to feed and becomes very sluggish until the last larval skin is shed and the pupa emerges. Pupation here usually takes place some time in April. The pupa is shorter than the larva and is proportionately wider. (Fig. 52.) When it first emerges it is very soft and very transparent, but as the parts becomes chitinized they turn a rich brown. The compound eyes, antennæ, the labial palps and other mouth parts can be seen through the pupal skin, though the head is not marked off from the rest of the body. The three pairs of thoracic legs are short and do not reach beyond the first abdominal segment. The abdominal portion consists of the usual six segments, pure white save for the dark golden band down the ventral and lateral surfaces. On each of the lateral lines there are two brown projections, a large one near the posterior portion of the segment and a smaller one midway. On each median surface there are others; one on each side in the first two abdominal segments and two in the next four. The posterior segment is, of course, sexually modified. The tracheal system of the pupa is closed, as there are no spiracles present.

Since the posterior extremity is changed to conform to the corresponding segments of the adult, the sexual modifications are indicated. The female may at once be recognized by the long triangular valves which are to constitute the ovipositor. The posterior segment of the male shows no such modification, but ends bluntly and shows the transverse anal opening. There are three large lateral spines upon each side of the posterior segment, while the other segments only have one large and one small projection. The spines all project backwards and serve to assist the animal in locomotion by giving it sufficient hold in the soft wood in which the pupa lies. It also enables it to creep to the surface before its final transformation. Such aids are found in very many Dipterous larvæ and Dipterous pupæ, for example in Dicranota, Tipula, and Bibio. If the pupa could not come to the surface before the

emergence of the fly much damage would inevitably result and a perfect adult would be rare indeed. If the pupa is extracted from its burrow it very shortly establishes itself in an equaly convenient position just beneath the surface.

XII. The Fly.

The fly emerges toward the end of April or the beginning of May, and is a very handsome fellow with his gay coloring of red, brown, or yellow. (Fig. 53.) The distinctive feature of the crane-flies, according to Comstock, is the presence of the transverse V-shaped suture, and this feature is very marked in this species. The wings are long and narrow, with a characteristic venation, the veins being partially fused at the proximal end. The ovipositor is composed of two long, horny pointed valves, well fitted for depositing the egg in firm substances. The power of flight is not well developed and the ability to walk is also poor. The long legs are so feebly attached to the body that they are easily broken off. This species of Ctenophora ranges from Vancouver Island to California. In conclusion I wish to express my indebtedness to Prof. Trevor Kincaid, of the University of Washington, without whose aid and encouragement this paper would have suffered a great deal.

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LIST OF ILLUSTRATIONS.

Fig. 1

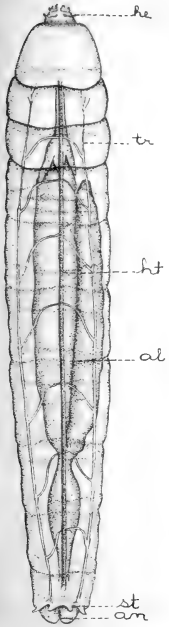
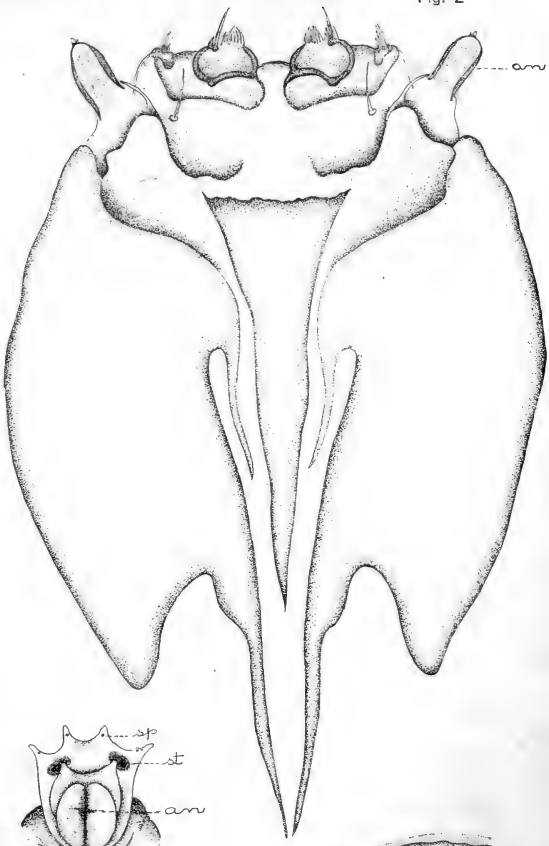


Fig. 2



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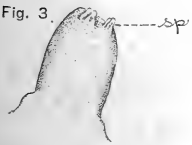


Fig. 6

Fig. 4

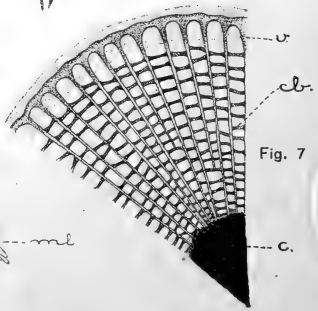
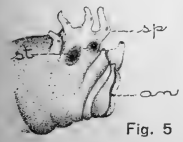


Fig. 7



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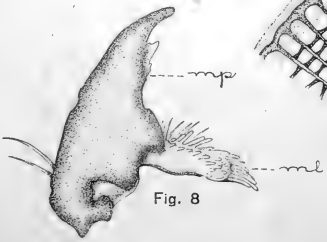


Fig. 8

Fig. 9.—Maxillæ. la., lacinia; ps., palpus; pr., palpiger; sc., head sclerites; me., mentum.

Fig. 10.—Large sensory hairs.

Fig. 11.—Surface view of alimentary canal. p., pharynx; o., œsophagus; pr., proventriculus; lc., long proventricular cæcum; sc.. short proventricular cæcum; g., gastric cæcum; v., ventriculus; m. Malpighian tubules; d., diverticulum; li., large intestine; r., rectum.

Fig. 12.—Cross-section of anterior œsophagus. mw., muscle wall; se., secretory epithelium; ch., chitinous layer; p., projecting hairs; im., inner muscle fibers.

Fig. 13.—Cross-section of posterior œsophagus. mn., muscle nucleus; mw., muscle wall; se., secreting epithelium; ch. chitinous layer.

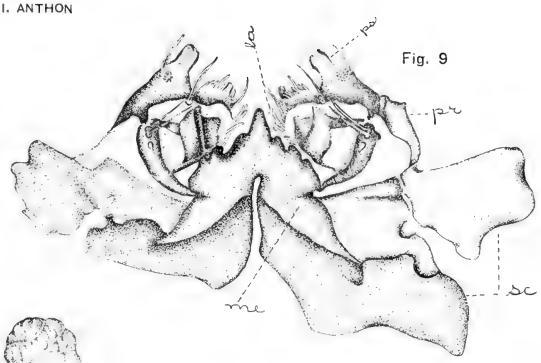


Fig. 9

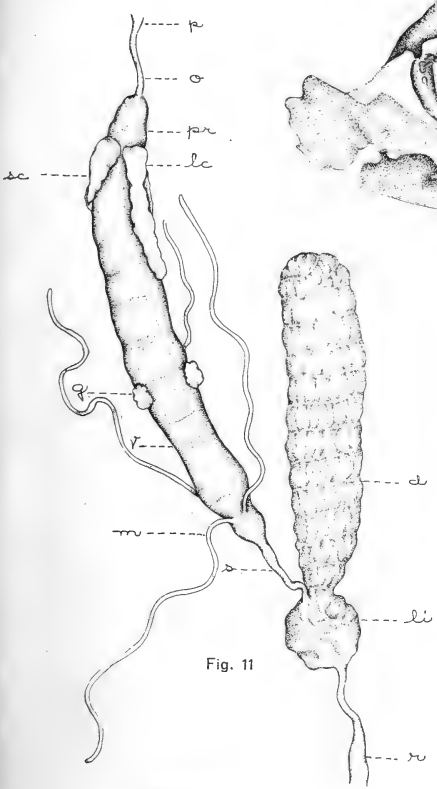


Fig. 11

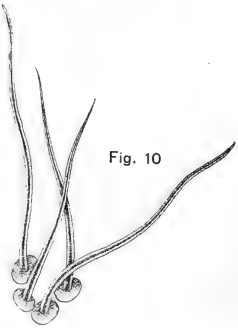


Fig. 10

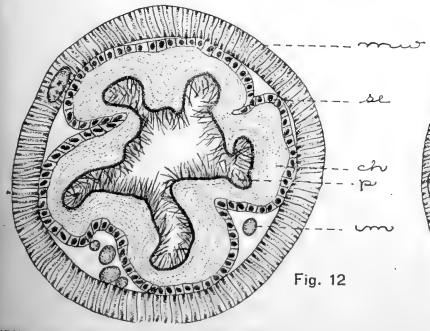


Fig. 12

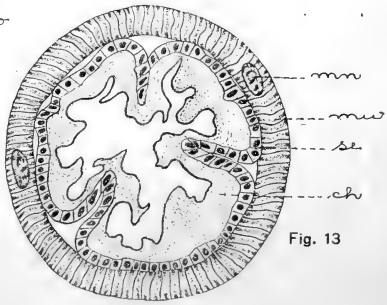


Fig. 13

Fig. 14.—Cross-section of anterior œsophagus to show the difference in the development of the chitin and hairs. se., secreting epithelium; ch., chitinous layer; p., projecting hairs.

Fig. 15.—Longitudinal section of œsophageal invagination. ch., chitin; e., epithelial layer; m., muscle layer; s., sphincter muscle; ov., œsophageal valve; me., mesoderm.

Fig. 16.—Longitudinal section of œsophagus and small cæcum. e., epithelium of stomach; as., active secreting epithelium; mw., muscle wall.

Fig. 17.—Cross-section of large ventricular cæcum. mw., muscle wall; se., secreting epithelium; sg., secreted globule.

Fig. 18.—Cross-section of small ventricular cæcum. mw., muscle wall; se., secreting epithelium; s., secretion thrown off.

Fig. 19.—Wall of ventricular cæcum to show active secretion.

Fig. 20.—Central cross-section. h., heart; ls., large ventricular cæcum; ss., small ventricular cæcum; a., alimentary canal; f., fat bodies.

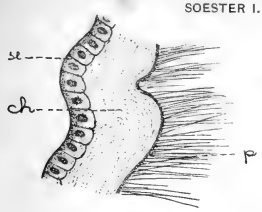
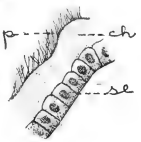


Fig. 14



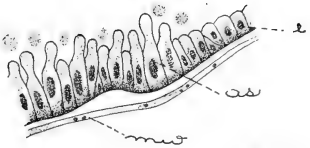
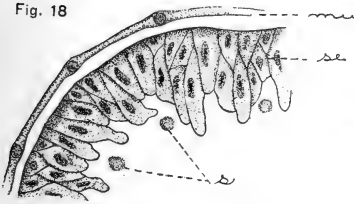


Fig. 16

Fig. 18



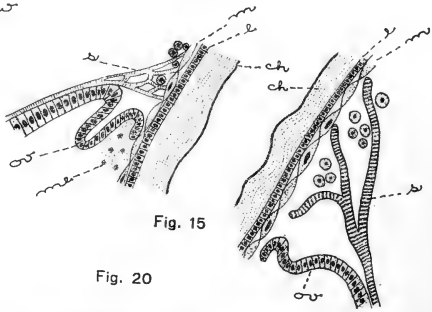


Fig. 15

Fig. 20

Fig. 17

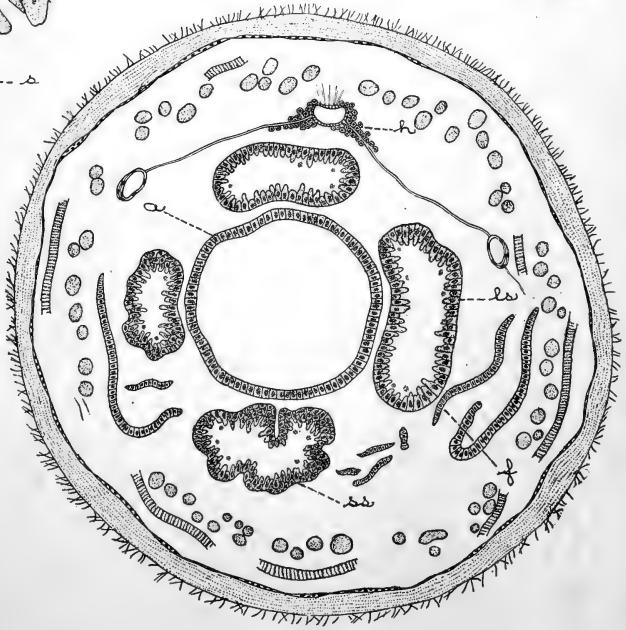


Fig. 19

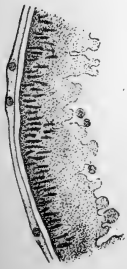


Fig. 21.—Posterior cross-section. h., heart; c., gastric cæcum; m., Malpighian tubule; t., trachea; f., fat bodies; d., intestinal diverticulum.

Fig. 22.—Longitudinal section of Malpighian tubule, to show the similarity of cell elements.

Fig. 23.—Cross-section of wall of diverticulum. m., muscle wall; c., cell layer.

Fig. 24.—Cross-section of cell of diverticulum.

Fig. 22

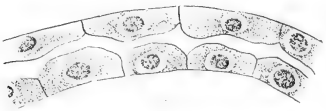
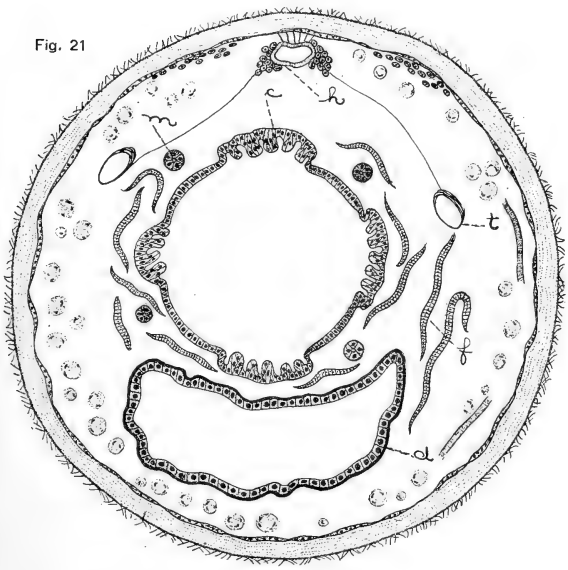


Fig. 21



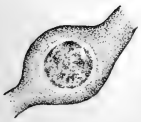


Fig. 24

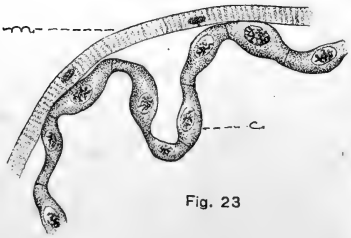


Fig. 23

FIG. 25.—Muscle plexus of the diverticulum.

Fig. 25

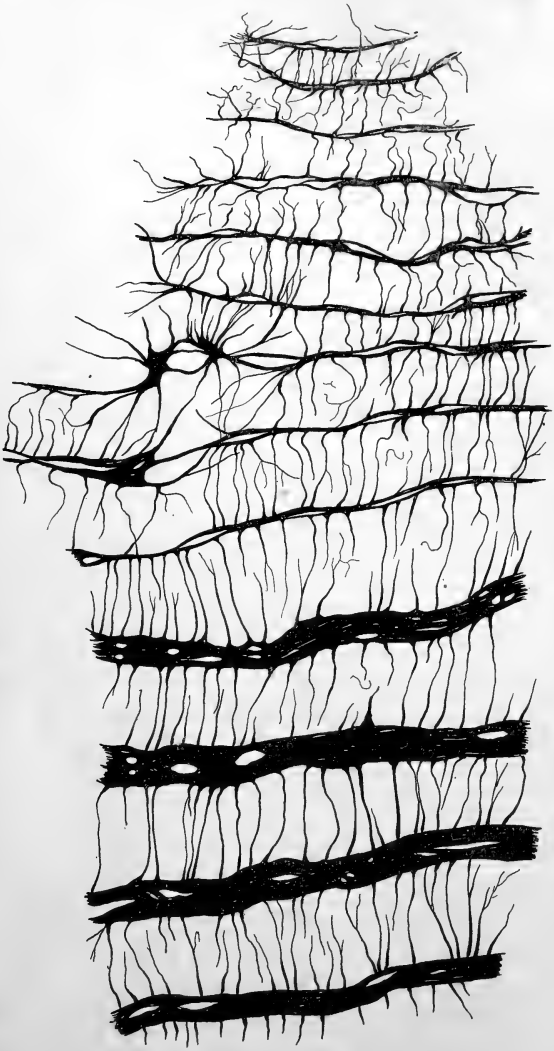


Fig. 26.—Muscle coat of diverticulum, H. P.

Fig. 27.—Same, L. P.

Fig. 28.—Muscle network from diverticulum.

Fig. 29.—Cell element from diverticulum.

Fig. 30.—Surface view of cell membrane of diverticulum.

Fig. 31.—Cross-section of colon. mw., muscle wall; se., epithelium; ch., chitinous wall.

Fig. 32.—Cross-section of rectum. Same as in Fig. 29.

Fig. 26

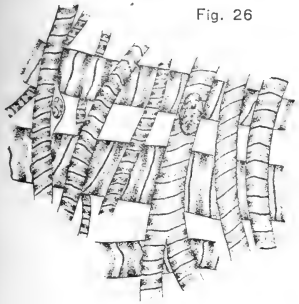


Fig. 28

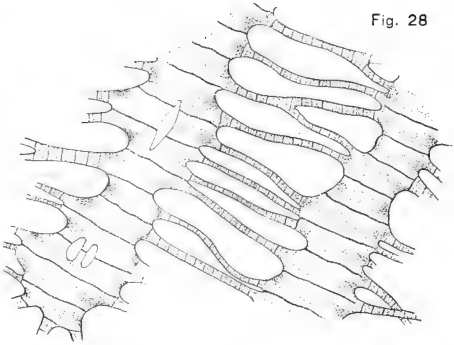


Fig. 27

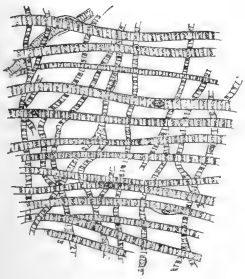


Fig. 31

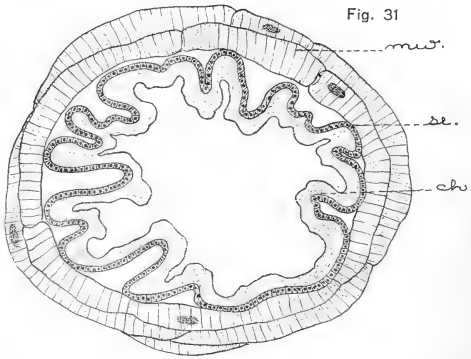


Fig. 32

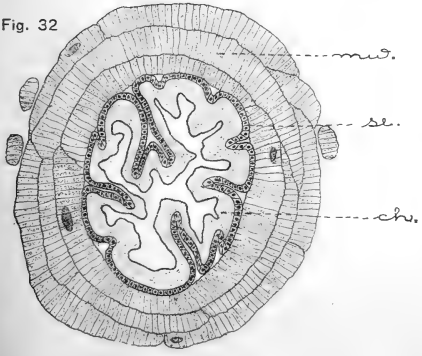
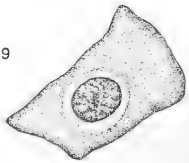


Fig. 29



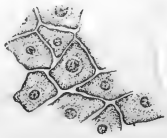


Fig. 30

Fig. 33.—Surface view of salivary gland. d., common duct; dc. collecting duct; g., gland proper.

Fig. 34.—Surface view of salivary gland. n., cell nucleus; cb., cell boundary; c., cells.

Fig. 35.—Cross-section of salivary gland (showing histolysis). n., cell nucleus.

Fig. 36.—Surface view of tracheal system. ad., anterior ramifications; mt., main lateral trunks; ct., cross branches; lb. lateral branches; pd., posterior ramifications.

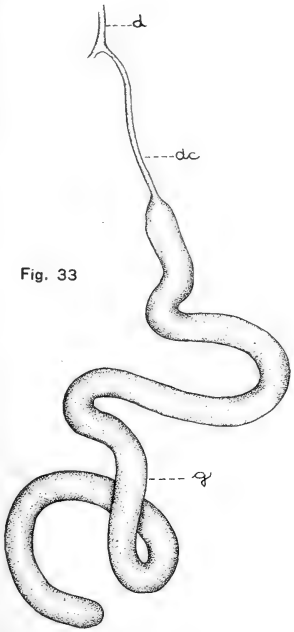


Fig. 33

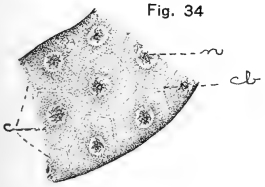


Fig. 34

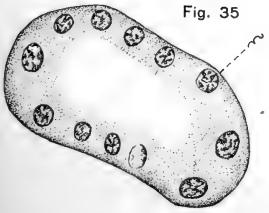


Fig. 35

Fig. 36

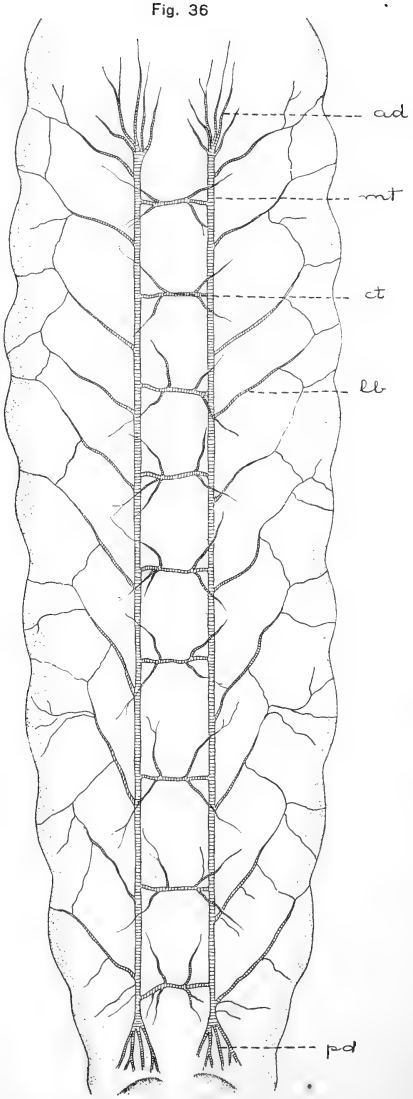


Fig. 37.—Right visceral tracheal system. ad., anterior divisions; l., main lateral trunk; b., branches to alimentary canal.

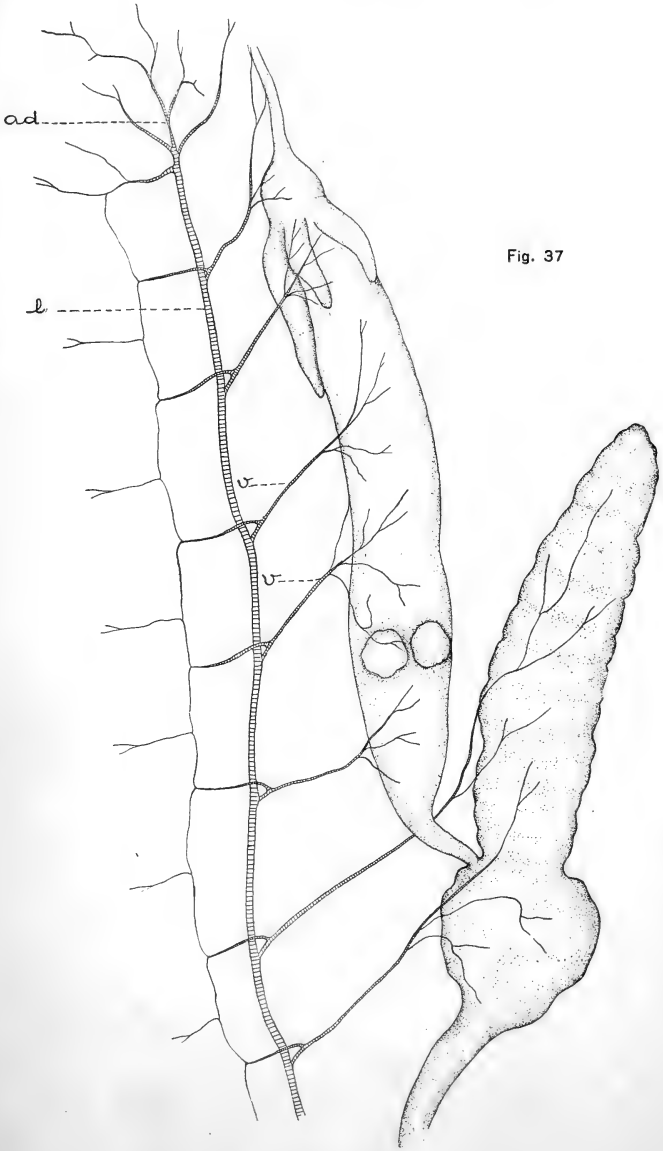


Fig. 37

Fig. 38.—Longitudinal section of spiracle. o., opening; cm., closing muscle; d., divisions of branches; bc., blood corpuscles.

Fig. 39.—Cross-section of heart. em., enveloping membrane; sm., suspensory muscles; l., leucocytes; am., alary muscles; i., intima; ml., muscular layer.

Fig. 40.—Closer adipose tissue.

Fig. 41.—Open adipose tissue.

Fig. 42.—Imaginal buds. c., cuticle; ch., chitin; t., trachea; pm., peripodal membrane; tv., tracheal vein; h., hypoderm; pv., peripodal vein; w., wing—forming part of histoblast.

Fig. 43.—Optic imaginal disc. e., endoderm; t., trachea; m., mesoderm.

Figs. 44 and 45.—Surface view of central nervous system. fg., frontal ganglion; og., optic ganglion; lan., nerve to labium; an., antennal nerve; br., brain; sym., branch of sympathetic nervous system; c., commissures; sg., subœsophageal ganglion; mn., nerve to mandible; mxn., nerve to maxilla; lbn., nerve to labium; tg., thoracic ganglion; vg., ventral ganglion.

Fig. 39

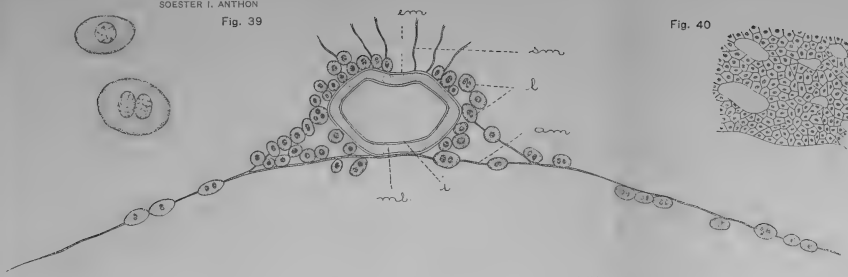


Fig. 40

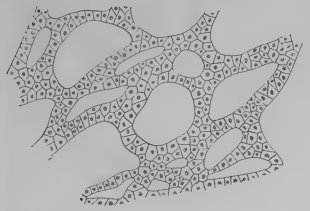
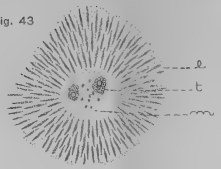


Fig. 41

Fig. 43



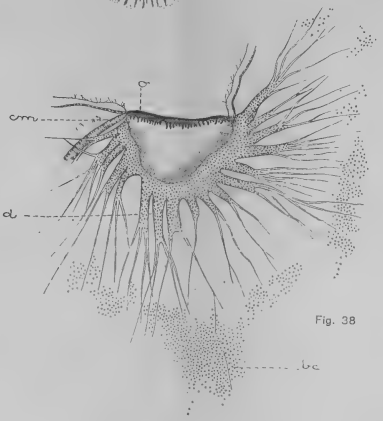


Fig. 38

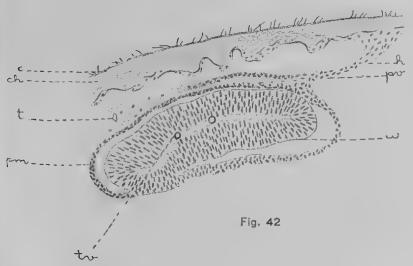


Fig. 42

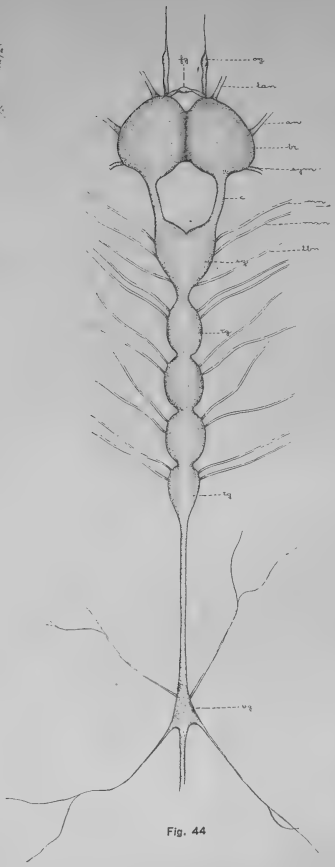


Fig. 44

Fig. 46.—Anterior cross-section of body. h., heart; f., fat-bodies; b., brain; o., œsophagus; s., salivary gland; sy., sympathetic nervous system; tm., transverse muscles; lm., longitudinal muscles; sk., skin.

Fig. 47.—Section of brain (transverse). mc., large cells; cm., cell mass; br., brain; ps., punktsubstanz; cc., cortical cells; œ., œsophagus; sym., sympathetic system; tr., trachea; sg., sub-œsophageal ganglion.

Fig. 48.—Cross-section of thoracic ganglion. Same notation as Fig. 50.

Fig. 49.—Cross-section of ventral ganglion. Same notation as Fig. 50.

Fig. 50.—Longitudinal section of chain ganglia. lc., large cells; ps., punktsubstanz; cm., cell mass; cc., cortical cells; ax., axis cylinder; nu., neurilemma.

Fig. 51.—Peripheral nerve plexus. ax., axis cylinder; nu., neurilemma; n., nucleus.

Fig. 52.—Pupa, female.

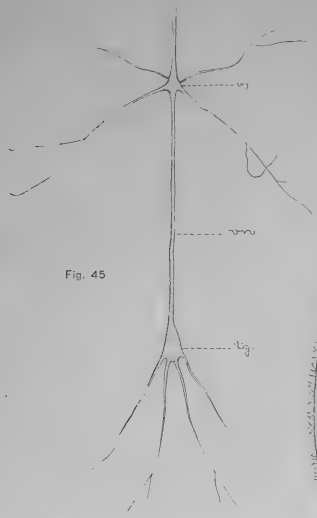


Fig. 45

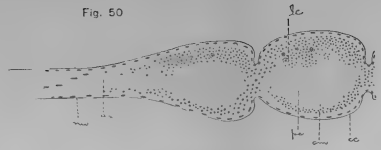


Fig. 50

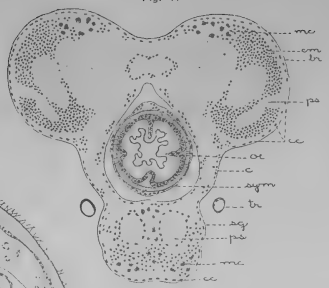


Fig. 47

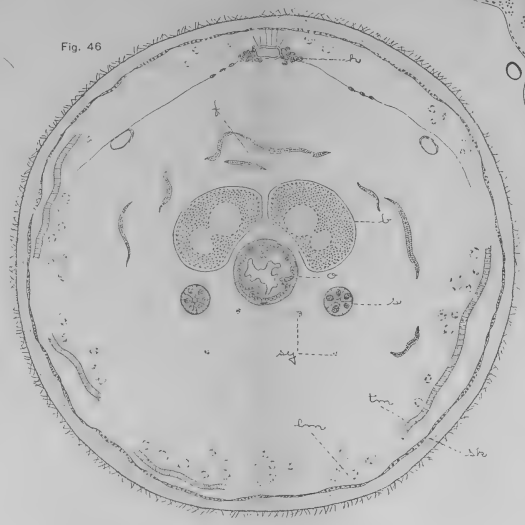


Fig. 46

Fig. 52

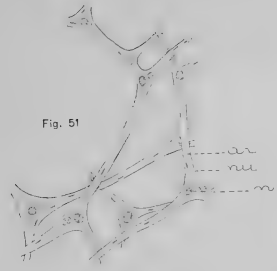


Fig. 51

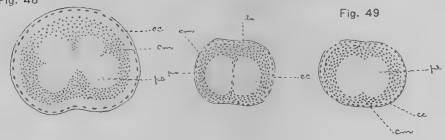
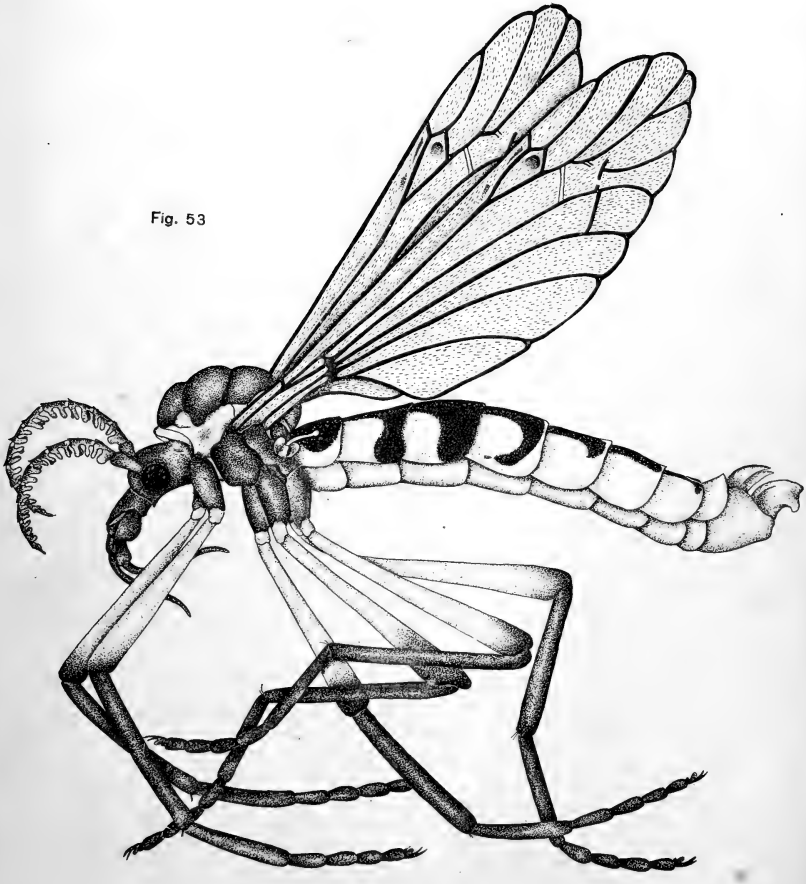


Fig. 48

Fig. 49

FIG. 53.—Adult fly, female.

Fig. 53



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THE VISUAL CELLS IN VERTEBRATES, CHIEFLY IN NECTURUS MACULOSUS.

Arthur Day Howard.

I. HISTORICAL INTRODUCTION.

From the earliest recognition of the layer of rods and cones as the receptive portion of the optic apparatus, this portion of the retina has naturally received considerable attention. The history of these researches has been quite fully dealt with by a number of writers. Of these I would mention Schultze ('72[a]), Hoffman ('75), Schwalbe ('87), Krause ('92), Greef (:00), and Hesse (:04).

Treviranus ('36), and after him Gottsche ('36) and Henle ('39), were among the earliest to declare the layer of rods and cones to be the expansion of the optic nerve. The error of believing that the "Sehpapillen" were turned toward the light, was corrected by Michaelis ('37), who first called attention to the fact that the rods formed the outermost layer of the retina. This discovery was confirmed by Bidder ('39) and Hannover ('40), but both believed that the rods could have no nervous function. Brücke ('44) agreed with Bidder and with Hannover in considering the rods to be refractive bodies for the transmission of light returning by reflection from the choroid.

The classic investigations of Heinrich Müller ('52; '56) and Kölliker ('52) helped to bring into general acceptance the belief that the rods and cones are the light-receptive organs of the retina. One of the grounds upon which Kölliker based his conclusion, was the similarity in structure, as he considered it, between nerves and rods. He says (p. 328): "Was die

Stäbchen selbst anlangt, so scheint mir aus ihrem Verhalten im frischen Zustande, ihrer leichten Veränderlichkeit und ihrer Reaction gegen Wasser und andere Substanzen unwiderleglich zu folgen, dass dieselben mit andern, blassen Nervenröhren, namentlich den Opticusfasern in der Retina, auf eine Stufe zu stellen sind und die Natur von zarten, mit einem zähflüssigen eiweissreichen und auch fettführenden Inhalt erfüllten Röhren besitzen." And he further adds (p. 329): "Eine wesentliche Differenz zwischen den Stäbchen und blassen Nervenröhren kenne ich nicht."

Kölliker by this declaration took the position, held for years by Henle, of identifying the rods with the axis cylinder ("Nervenröhren"). Leydig ('55; '64) also declared the rods and cones of vertebrates to be "eigenthümliche Umwandlungen der Nervensubstanz." The investigations of Max Schultze ('66; '71) cleared up many points in the structure of the rods and cones. Through these researches the contention of H. Müller and Kölliker was sustained.

The results of Schultze's researches and those of the workers up to his time, with regard to the structure of the visual cells, is well summarized in the following account, taken in the main from Hoffmann ('75). The description is based upon the amphibian rods and cones, which on account of their large size have yielded more results to investigation than those of any other animals.

The Rods consist of two parts, chemically and physically different, named the inner and outer segments or limbs. The outer segment is strongly refractive while the inner one consists of a homogeneous substance much less refractive. The segments are clearly distinguishable one from the other. What is evident in the fresh condition is especially marked after treatment with osmic acid. This reagent colors the outer segment black, while the inner one remains colorless or assumes a greyish shade. The reaction to (or behavior in) water, dilute acids, and alkalis is totally different for the two segments.

Valentine ('62) and Max Schultze ('67) found that the

outer segments are doubly refractive, while no trace of this was to be seen in the inner ones. Tests of outer segments or rods from the frog with a polarizing microscope, employing a gypsum interference plate, determined an optical axis in the length of the rod and that the rod has positive double refraction with respect to this axis. Preparations of frog retinas so disposed as to permit the light to pass through the length of the rod, show that in this axis no double refraction takes place.

The rods of amphibians are notable for their size, the dimensions of some in micra being as follows:

	Outer Segment.		Inner Segment.
	Length.	Breadth.	Length.
Frog	61	6	19
Triton	24-28	12	15-16
Toad	40-45	8	16-20
Bufo variabilis	76	8	17
Salamandra	44	12	18

Outer Segment.—In form the outer segment is cylindrical with a hemispherical slightly bulging distal end. Under high magnification its outer surface is marked by parallel striations, which deviate from a strictly longitudinal course only in that they are very slightly spiral. This appearance is due to superficial furrows alternating with ridges. The form of the outer segment may thus be well compared to that of a column with a slightly spiral fluting. The striations according to Schultze ('72[a]) have some relation with a deeper differentiation in structure, for in separated laminae, in addition to the grooving of the surface, there is a suggestion of a radial division of the substance.

Another character of the outer segment of the rod is the transverse banding, which appears at regular intervals. This is seen to best advantage by very oblique illumination of fresh preparations (Schultze, '69[b], p. 380) and is a surface indication of a plate-like structure. The thickness of the plates in micra according to the measurements of Schultze ('67) and Zenker ('67) is as follows: frog .5—.6, triton .55—.6,

salamander .6, dove .62, guinea pig .88, man .45—.6 (Greef :00). The thickness is constant for a given species and shows little variation even in the whole vertebrate series. Between the plates is a cementing substance, which becomes greenish instead of black in osmic acid. This cement is affected rapidly by certain reagents, and owing to its swelling it causes a characteristic disintegration of the outer segment into discs. This disintegration occurs earlier at the distal end of the segment than at the proximal, the difference being due to a protecting sheath over the latter.

The presence of an axial fibre in the outer limb, as maintained by Ritter ('59) and later called "Ritter's fibre," was questioned. Manz ('61; '66) and Hensen ('67), however, sided with Ritter; but Max Schultze concluded from Zenker's study of the refractive indices of these parts, that the appearance was due to a difference in refrangibility between the sheath and the substance of the rod. It was probably this opinion of Schultze which was responsible for the general discredit which "Ritter's fibre" eventually met with.

Inner Segment.—The inner segments of the rods in amphibians are usually short but as wide as the outer ones. In perfectly fresh condition the substance of the inner segment is homogeneous. Very soon after a preparation is made, however, a cloudiness appears, which seems to be due to coagulation granules. In the toad and frog there is observable near the edge of the outer segment a characteristic body in the form of a plano-parabolic lens. This body, which in fishes, other amphibians, and birds is much broader, was given the name "lens-shaped body" by M. Shultze, and later was called "ellipsoid" by Krause, and "outer-lens" by H. Virchow. The ellipsoid turns brown with osmic acid. It is more strongly differentiated by further treatment with fuchsin. With this stain the outer limb, as well as the ellipsoid, becomes a dark red while the remainder of the inner limb is light red. A more complicated condition is found in some forms (Triton, Salamandra), in which there are present two lens-like bodies having

the relation of the lenses in a compound achromatic objective, a proximal bi-convex body fitting into a distal plano-concave one. In osmic acid the plano-concave body is colored brown like the ellipsoid in other forms, and it also becomes dark red with fuchsin. On the other hand, the bi-convex body stains like the remainder of the inner segment. This body was called the "paraboloid" by Krause. In the fresh condition the plano-concave part, or ellipsoid, is finely granular, while the paraboloid is completely homogeneous. According to Max Schultze the paraboloid may be isolated.

The channeling of the outer surface of the inner segment, as reported by Merkel ('70), was not credited, and another question in dispute was the presence or absence of a membrane enveloping the segment. Landolt ('71) and Merkel reported its presence, while Max Schultze claimed that there was no distinct membrane, but that fibrous projections from Müller's fibres extended over the inner segment and the proximal third of the other segment. Schultze was at one time disposed to consider these as nerve fibrils, but was led finally to the view last given. He says ('72b, p. 823): "Although the fine fibres are with difficulty traced backwards into the external granule layer, I have ascertained this much with certainty, namely, that they are continuous with the tissue lying between the fibres of the rods and cones. But since this tissue can only be considered as connective substance, the fibrils in question represent a continuation of the delicate fibrillated connective substance of the external granule layer, and form supporting fibrillar framework for the bases of the rods and cones (Comp. Fig. 326)."

The Cones have parts corresponding to those in the rods, and these parts in general show similar reactions both to chemical reagents and to light. In amphibians the difference between the other segments of the rods and cones in these respects is less marked. The conical outer segments possess channeling of the outer surface, as in the rods, but with grooves nearer together. The outer segments of the cone are

exceedingly unstable and show the same plate-like structure as the rods, but owing to a sheath the disintegration is not as rapid. This fibrillar sheath in the cones, as in the rods, is easily demonstrated to be a prolongation from the inner segment. This inner segment shows the same complexity as that of the rods, possessing a distal ellipsoid (linsenförmigen Körper) and a proximal paraboloid ("blassere innere Hälfte"). In the frog and also in Sauropsida generally, there is present between the outer segment and the ellipsoid a highly refractive, colored, spherical body called the oil globule ("pigment Kugel"). Max Schultze ('72[a], p. 1003) found internal longitudinal fibres, in addition to peripheral fibrils, occupying the whole thickness of the inner segment of the cone in man. He was able to follow these from the beginning of the outer segment to a point short of the external membrane. Their further connections he was unable to make out.

Nuclei.—While in fishes and mammals the nuclei of the rods differ from those of the cones, in amphibians conspicuous differences are not apparent. The nuclei are arranged in two layers in both Rana and Bufo, but in a single layer in Triton and Salamandra. In frogs and toads the nuclei of the rods are commonly close to the external limiting membrane, while those of the cone cells are crowded into the second layer. Hoffmann ('75) described a thin granular cytoplasmic sheath about the nuclei and says there is a proximal extension (cone-foot) of the sheath into the outer granular layer. This extension possesses, according to somewhat inconstant appearances, a terminal spherule having a rough or ragged surface.

Double Cones, first described by Hanover ('40), are found in all vertebrates except mammals. They consist of two cones applied along a part of their lateral surfaces. In Amphibia as well as in Sauropsida there is a conspicuous difference between the cones of a pair. One of these is longer and egg-shaped, it is called by German authors the "Haupt-zapfen;" the other is shorter and retort-shaped, it is known as the "Neben-zapfen." In triton the Haupt-zapfen possess ellipsoids only,

the Neben-zapfen both ellipsoids and paraboloids. Hoffmann ('75) expressed doubt as to whether the double cones possess one or two nuclei, but inclines to the latter view. Schultze seemed to favor the former. He states (Schäfer '97, p. 49) that twin-cones possess two feet, one straight, the other oblique.

The investigators whose results are summarized in the foregoing account carried the analysis of the structure of rods and cones so far that for twenty-five years almost no noteworthy advance was recorded. Many of the structural features discovered by them seemed hardly to accord with the conception of the rods and cones as modified nerve fibres. The plate structure of the outer segments was generally considered as strong evidence against this view. M. Schultze ('71, p. 257), however, seemed disinclined to give up the older notion. He says: "Es ist das Wahrscheinlichste, dass Nervensubstanz auch mit den Aussengliedern in Contact oder Continuität stehe." However, he admits ('72[a], p. 1006) the possibility of a non-nervous function for the outer segment: "Somit könnte möglicher Weise die Nervensubstanz mit den Innengliedern abschliessen und das Aussenglied einen nicht nervösen physikalischen Hülfsapparat darstellen." He considered it probable that the outer segment, through its laminæ, serves as a reflecting apparatus and that the transformation of the luminous rays into nerve impulses is effected in this region. These views were strengthened by his studies on invertebrates, where the laminated visual rods in mollusks and arthropods were considered to be analogous to those in vertebrates.

Many writers on invertebrates, especially on arthropods, *e. g.*, Hensen ('65) and Watase ('90), believed that the visual cells were largely cuticular in structure. Thus Schultze's comparison apparently led other students to the assumption that the outer segment of the vertebrate visual cell was in the nature of an "Abscheidungsproduct" or cuticular substance, and this view was held to be supported by embryological evidence. Although Schultze seems to have given expression

to no such view, his results were so interpreted by others, *e. g.*, Schwalbe. This idea of the cuticular nature of the outer segments has met with general acceptance and has remained as authoritative almost up to the present day. Thus one finds frequently in text-books, where the outer segment alone is referred to, such expressions as: "entspricht einer Cuticularbildung" (Schwalbe, '87, p. 104), and "der Ausdruck einer kutikularen Auflagerung" (Rauber, :03, p. 810). Or the entire rod, or cone, is called a "Cuticularenbildung" (Wiedersheim, '86), an "Abscheideproduct" (Gegenbaur, '98, p. 935).

From time to time there have appeared in the literature on the retina results quite out of the ordinary, whose non-acceptance may be attributed to lack of confirmation by others, or to obvious insufficiency of evidence. The position, for instance, taken by Borysiekiewicz ('87), that rods and cones are non-nervous, seems to have received little support. Norris and Wallach ('94) describe "Distal connecting loops between visual cells." The photographs which they publish, to illustrate this condition, are certainly not convincing. Johnson ('95) finds a "branching central fibre" in the visual cell. Pes (:00) maintains that the ellipsoid acts as a nucleus. Bernard (:00-03) considers the visual cells to be vesicular projections from a syncytial retina.

The slow advance in the study of the retina since Schultze's time would seem at first sight rather strange in the light of recent progress in cytological technique. It might be partially accounted for, however, by the fact, that, owing to the extreme delicacy and instability of the rods and cones, many recently devised neurological methods are inapplicable.

In noticeable contrast with the condition of the problem in vertebrates has been the progress in the study of the terminal visual organs of invertebrates. In the arthropods, for instance, the rhabdomes, which were supposed by earlier writers (Hensen, '65; Grenacher, '79; Watase, '90) to have been formed by secretions, have been found by later workers to be living tissue of marvellous complexity. Schultze ('72[a]), who

found a fibrous structure in the molluscan visual cell, surmised a like structure in arthropods. Later, fibres were actually observed by Patten ('86) and others, and it was demonstrated by Parker ('95) that in the rhabdomes of the crayfish the fibrils composing them are neurofibrils and that the substance of the rhabdome is more correctly described as differentiated living material, comparable to the contractile substance of a muscle fibre, than as a secretion. This view, that the rhabdome is composed of neurofibrils, has been greatly extended for the invertebrates by the recent work of Hesse (:00, :01).

In the light of these discoveries in the lower animals it is not surprising that the attention of investigators should be drawn again to the more difficult problem of the finer structure of the rods and cones in vertebrates. That an interest exists, may be readily seen by reference to the literature of the last five years in comparison with that of the twenty years previous.

Another incentive to research in this direction is to be found, no doubt, in the interest aroused by the discoveries of Apáthy ('97) as to the minute structure of the nervous system. The further exploration of the nervous elements through the researches of Bethe ('98), Prentiss (:03), Schneider (:02), Parker ('95), Nissl (:01), Embden (:01) and others brings up, as a pertinent problem, the determination of neurofibrils in terminal sense-cells. Moreover, where these are found, is raised the question of their relation to other intra- and intercellular fibrils.

Schneider (:02) is, so far as I know, the first author since the appearance of Apáthy's paper to claim an identification of neurofibrils in the rods and cones of vertebrates. He used the frog in his studies; concerning the longitudinal markings, about which there has been so much controversy, he says (p. 789): "Das Sarc der Sehzellen ist zart längsfädig struiert; wir haben die leicht geschlängelt verlaufenden Fäden als Neurofibrillen aufzufassen." These neurofibrils he considers as continuous from the cell foot in the outer reticular layer

to the distal end of the outer segment, which is turned towards the pigment cells. In the red rods, the fibres are described as peripheral, starting from the rod foot and extending over the nucleus in the thin mantel sheath. Between the nucleus and external limiting membrane, they form a loose fibril group, but on reaching the membrane they become entirely peripheral, lying in the sheath of the inner and outer segments for the rest of their course. A granular cone proximal to the ellipsoid may be a new feature for the inner limb, but is probably identifiable with the paraboloid. The outer segment is stated to contain internally a homogeneous elastic mass, breaking easily into cross plates, but again, an appearance of cross striping is explained as due to a regular interruption of a "color mantel" on the peripheral neurofibrils. The club-shaped rods differ from the red rod in that the neurofibrils traverse the whole substance of their outer segments. As to the cone cells, Schneider says of the neurofibrils in their outer segments: "Diese Neurofibrillen dürften sich im Aussenglied in Windungen legen." Otherwise the condition of the fibrillæ is the same as in the rods.

Greef (:00), who depended chiefly upon osmic acid as a fixing agent, finds no fibrils. He describes, however, some details of interest in the structure of the rod, *e. g.,* the "Zwischenscheibe," a plate between the outer and inner segment; also the occasional appearance of two "Zwischenscheiben" between plates of the outer segment, and a sheath over the inner and outer segments. Levi (:00) holds much the same views as Greef as to the structure of the visual cells.

Hesse (:03), in a paper read before the Deutsche Zoologische Gesellschaft, reports the presence of spiral neurofibrils, and in a later paper he (:04) gives his results on teleosts, selachians, amphibians, and reptiles more fully. Hesse declares for two systems of fibrils, both of which he finds present in rod and cone cells. The first, which appear in general as parallel longitudinal lines, he believes to be thickenings of the sheath, confined to the periphery, where they have

a mechanical function. He does not trace them proximally beyond the external limiting membrane. The second is a system of parallel spiral fibres running from one end of the element to the other (including the nucleus) and always lying near the external surface. These he believes to be neurofibrils, the conducting elements of these optic organs. The spiral fibres described by Ritter ('91[a]; '91[b]) and Krause ('92) he mentions as possible records of the same feature. That these received so little credence (see criticism of Merkel, '92; Greef, :00; Ebner, :02) he thinks is due largely to the unconvincing appearance of the figures.

Very recently there have appeared publications from Kolmer (:04), Held (:04), and Retzius (:05), describing an appearance in the visual cells, not to my knowledge reported before, namely, a single relatively large peripheral fibre passing from diplosomes in the inner segment and over the whole length of the outer segment. The demonstration of this fibre was obtained by the use of silver-impregnation methods. Like results have been reported by Fürst (:04) using hæmatoxylin staining on embryonic tissue.

The investigations upon which the present paper is based were carried on chiefly in the Zoölogical laboratories of Harvard University at Cambridge, during the years 1902 to 1905. A part of the work was done in the laboratories of the United States Fish Commission at Woods Holl, during the summers of 1902 and 1903.

Before taking up this special problem I made studies under the direction of Professor William A. Locy, upon the development of the Vertebrate retina. In the fall of 1902, at the suggestion of Dr. G. H. Parker, I took up as a special problem the more limited field of the structure of the visual cells in the adult.

In this work I have received very considerable assistance from several persons, for this I owe especial acknowledgment. Dr. Parker has given the work his constant supervision and

rendered me every encouragement by helpful suggestions and valuable criticism.

I am indebted to Dr. E. L. Mark for the excellent opportunities of the Harvard Zoölogical laboratories and for help in the arrangement of plates, as well as kindly interest at all times.

Thanks are due Professors J. E. Wolff and Charles Palache for apparatus and assistance in the use of the same; also to Doctors F. B. Mallory and F. H. Verhoeff for suggestions in technique.

In addition to these, there are many to whom I am under obligation for kindly assistance. To these I wish here to express my thanks.

II. OBSERVATIONS.

A. General Methods and Technique.

I began my study of the visual cells in the frog, Rana pipiens Shreber, because of the ease with which this animal can be obtained at all seasons, and because of the large size of its elements. The importance of the latter consideration is obvious and led me finally to study the retinæ of the large salamander, Necturus maculosus Rafinesque (1819), the "Mud-puppy" of the St. Lawrence and Mississippi basins. The rods in this species proved to have a diameter two and a half times that of the rods in the frog, and larger than those of any other vertebrate known to me. This is in keeping with the well known fact that in Necturus, the histological elements are unusually large, the red blood corpuscle being the largest red corpuscle known. As this animal is commonly kept by dealers for supply to zoölogical laboratories, it can be obtained readily before ice appears and kept alive in laboratory tanks with running or standing water, of a depth just sufficient to cover it. The size of the visual elements in Necturus led me to use them in preference to those of the frog as a basis for my studies.

The advantage of size outweighed the two disadvantages of a small eye and a thick sclera. The small size of the eye renders manipulation difficult in the removal of the retina, and limits the amount of available eye fluids for study of the retina in the fresh condition, while the thickness of the sclera hinders the penetration of fixing fluids.

Two general methods of study were followed; permanent preparations were made according to the various devices of microscopical technique; and fresh material was studied while in the eye fluids, under as nearly normal conditions as possible.

In the technique of the rods and cones certain peculiarities of the tissue have to be taken into account. As they are such unstable and comparatively delicate bodies, special fixing fluids must be used. The finding of a suitable fluid was a matter of considerable experimentation, in which the mere preservation of a natural gross form of the bodies was the least perplexing part of the problem. It was more difficult to find a fluid which would permit staining, and still do little damage to the internal structure of the element. The tests for these requisites were dependent, in the main, on results obtained after differential staining, though tests with polarized light were also employed as a check on fixation. The latter furnished interesting data, which will be taken up in detail under observations upon the effects of different fixing fluids.

The difficulty of admitting fixing fluids to the retina without violent mechanical disturbance, is a problem which presents itself in the technique of small eyes, especially when they possess a heavy sclera. Immersion of the whole eye in the fluid does not guarantee immediate fixation, while cutting open the eye usually causes a wrinkling of the retina, even if no mechanical injury results. Wrinkling, and buckling of the retina away from the choroid, seems to be due to different degrees of contraction of the sclera and the retina, when treated with the fluids.

I found the following method most successful in preventing such effects, and in preserving the eye in an apparently natural

condition. The animals were anæsthetized, their hearts exposed, and the fixing fluid injected into the arteries as in ordinary injections for the demonstration of the arterial system.

Some of the fluids used were aqueous mercuric chloride containing 5 per cent. acetic acid, Perenyi's fluid, 1½ per cent. osmic acid and vom Rath's ('95) picro-platino-osmo-acetic mixture. The first two penetrated most successfully. The osmic preparations were only partially successful, for, owing apparently to the rapid constriction of the blood vessels, a smaller amount of the fluid reached the interior of the eye than by the other methods. After injection, the eyes were removed and placed in the fixing fluid, or else the whole head was immersed and the eye not opened until they were thoroughly hardened. Eyes thus prepared were imbedded in melted paraffine and cut for longitudinal or transverse sections of the rods and cones.

For a satisfactory study of the rods and cones it is usually necessary to free them from surrounding pigment. This may be done by bleaching on the slide, or, while the animal is alive, by keeping it in a dark box for a few hours, or conveniently over night, and then fixing the eye without exposure to the light. I got best results in Necturus by anæsthetizing with chloretone in the dark box. Chloretone was used to avoid the irritation and consequent advance of pigment which ether and chloroform seem to produce. The method described causes the retinal pigment to withdraw completely from the region between the rods, into the bodies of the retinal pigment cells.

Bleaching can be resorted to conveniently, if there is no reason for avoiding the necessary chemical treatment. This is obviously the most practical method of studying the rods and cones in their "light" phase. It also has the advantage that the rods and cones are protected, and prevented from breaking by the slipping of the retina over the pigment layer, when portions of the eye are removed for sectioning. Bleach-

ing was done on the slide by the potassium chlorate method (Lee, :05), or by the potassium permanganate and oxalic acid process (Mallory :05).

In the study of fresh material the cornea and lens were carefully removed, the eye divided, and the retina placed on a slide. In this process as much of the eye fluid as possible was removed with the retina, which was then teased in it and covered. The fluid at the edge of the coverglass coagulates and promptly checks further drying from exposure to air.

With such a preparation, a field containing detached rods can readily be found. These are usually broken, but occasionally a whole element was found intact, as for instance where a fortunate fold of the retina gives the visual cells in profile.

When more fluid than could be obtained from a single eye of Necturus was needed, humor from the eye of a frog or an artificial examination medium was used.

In tests for suitable media the most satisfactory that was found was the more fluid portion of white of a hen's egg. In the more artificial media that I used, the elements disintegrate very rapidly.

Fresh preparations put up as described were studied either under an ordinary microscope or a polarizing one.

The effects of reagents and stains were determined by introducing solutions of these (usually very dilute) at the edge of the cover-glass, while the object was under observation.

B. Permanent Preparations.

Observations of the visual elements begun on fresh unfixed material would be a logical introduction to their study, but, because of the difficulties in manipulation and interpretation, I found it hardly practicable. One must first gain some familiarity with the objects from a study of sections or, at least, from hardened material. On this account I have undertaken to describe first permanent preparations, selecting those which gave evidence of best preservation.

1. *Osmic Acid Material.*

Osmic acid, or osmium tetroxide, (OsO_4) has held probably as important a place as any fixing reagent in the investigation of the visual cells (Schultze, '66-72: Hoffmann, '75; Greef, :00).

I have used osmic acid without admixture of other reagents chiefly in the form of vapor. Eyes which were not opened were suspended over a 2 per cent. aqueous solution of osmic acid in a tightly closed bottle for three minutes. This method seems to have distinct advantages over the use of this reagent in fluid form. It avoids the danger of distortion and tearing of cells by osmotic pressure; as no opening of the eye is necessary, the danger of disturbance from other mechanical causes is also eliminated. Penetration is very rapid, two or three minutes' exposure being sufficient to fix the rods and cones. A retina thus fixed may be teased immediately upon a slide in glycerine, and isolated rods and cones found in the field of the microscope (Pl. 4, Fig. 33); or the whole eye, after it has been hardened in alcohol and its lens has been removed, may be cut into sections and mounted in balsam (Pl. 4, Fig. 35). Dyes do not readily stain tissues which have been treated with osmic acid; at least, I have been unable to get evidence of fine differentiation even after bleaching, and treatment with potassium bichromate as recommended by Lee (:05). If preparations fixed in this way were more susceptible to stains, the method would furnish, no doubt, a most valuable check on other methods of fixation.

In a retina from Necturus fixed as described by osmic acid fumes, the rods and cones have the following appearance. The cylindrical outer segments of the rods (Pl. 4, Fig. 33, *prs. dst.*) vary in tint from black to a translucent brown, depending upon the action of the fixing fluid, whether over a longer or shorter period. The other portions of the rod, as well as the whole of the cone, show varying shades of light brown or greenish grey, of much less striking character.

Between the outer and inner limbs is the intermediate plate ("Kittsubstanz," Schultze, '67, p. 218; "Zwischenscheibe." Greef, :00). This is unstained by the osmic acid and appears as a thin light band in marked contrast to the black or brown of the outer limb.

The inner limb shows a differentiation into a distal* ellipsoid and a proximal paraboloid (Krause). The ellipsoid, which has either the shape of the segment of a sphere, or is cylindrical, is distinctly granular. A sheath is not readily demonstrable in osmic-acid material, but is easily shown by other methods.

The paraboloid is more transparent than the ellipsoid and is without evidence of granulation. It has a well defined inner clear portion and an outer less transparent sheath. Its form varies from ellipsoidal or paraboloidal to plano concave or plano convex, with the plane surface always distal. The flattened and concave conditions are probably distortions due to fixation. The paraboloid takes a selective purple stain, different from any other retinal structure, in preparations subjected to the following treatment. Material fixed 3 minutes in osmic acid vapor is prepared for treatment on the slide in the usual way: slides are placed in a ¼ per cent. solution of permanganate of potash for 20 minutes; then in a 1 to 5 per cent. solution of oxalic acid for 30 minutes. After washing, they are stained for 1 to 24 hours in Boehmer's hæmatoxylin. The excess of stain is removed by 70 per cent. alcohol acidulated with hydrochloric acid and controlled by alcohol containing traces of ammonia.

The nuclei (Pl. 3, Fig. 20, *st. nl. ex.*), which in Necturus lie almost entirely proximal to the membrana limitans externa, are closely packed in a layer one deep in the functional part of the retina, but two deep toward the ora serrata. It is probable that extra-nuclear plasm from the inner segment is continued over the sides of the nucleus, but if so, it is

* *Proximal* and *distal* are used throughout this paper with reference to the center of the eye. In the figures on the plates, *proximal* is above and *distal* below, except in detached portions of cells.

so thin as not to be distinguishable from the nuclear membrane. This condition is evident in sections, but still more apparent in isolated elements (Pl. 4, Fig. 33). In such cases, ensheathing cytoplasmic material can be seen passing around the nucleus into the root-like process, the rod foot (Pl. 4, Fig. 33, *pd. bac.*) with dentritic extensions into the outer reticular layer.

The cone elements (Fig. 33) are of about the same length as the rods. The outer segments take a darker color than the other parts, but not as deep a shade as the rods. The osmic acid evidently (especially after longer fixation) has a destructive effect here, as the outer segment of the cone shows irregular fissures and has a broken appearance. The other parts, corresponding with those of the rod, agree with these in color and appearance, indicating similar chemical behavior as regards osmic acid. However, there are some differences in form beside those of the conical outer segment; thus the ellipsoid is longer and the paraboloid shorter, than the corresponding parts of the rod.

Cross sections of the outer segments of the rod appear roughly circular with corrugated outline (Pl. 4, Fig. 36). At a magnification of 1,450 diameters the dark circle appears to be bordered usually by a light line, outside of which is an intensely black corrugated edge, which evidently represents an enveloping sheath on the outer segment. In longitudinal thin superficial sections of the outer segment striations can be seen which correspond in number with the corrugations of the cross sections, and demonstrate that the outer segments of the rod are fluted (Pl. 4, Fig. 35).

At a much higher magnification (3,800 diameters) the contrast between center and rim is more marked, the latter being blacker while the white band between the two appears to be interrupted by radial extensions of the central substance (Pl. 4, Fig. 34) corresponding with the depressions of the corrugations. This appearance would indicate that the light band is not simply the result of the optical effect of the sheath,

but represents substance unblackened by osmic acid. After bleaching such material, and staining it with hæmatoxylin, I have obtained in cross sections evidence that the outward projection of the corrugated outline represents a fiber circular in cross section and stainable. The central mass included by these peripheral structures seems quite homogeneous, as in fresh material.

Frog rods treated by the same methods reveal a dark rim and a light line, but I have not been able to distinguish in them the corrugations. Such, however, have been reported by Hensen ('67, Fig. 7) and others.

Besides the ribbing of the surface and the underlying light spaces of the outer segments, I have seen no direct evidence of fibrillation in osmic material. That osmic acid fixation is in some ways very unfavorable for the demonstration of fine structural details, there can be no doubt. I have found no after-treatment by way of bleaching, etc., which satisfactorily restores the susceptibility to selective staining in the finer details. In other respects osmic fumes preserve very successfully the gross appearance of the fresh elements, in their almost optically homogeneous state. Whatever differentiation there is in the latter, is faithfully preserved in delicately fixed material.

2. *Vom Rath Material.*

Vom Rath's picro-platino-osmo-acetic mixture, often used in the study of finer details of nerve cells, gives good preservation for the nuclear portions of the visual cells, but not for the distal parts. The outer segment of the rods becomes an opaque black, and as a rule contracts to one-half its normal length. The outer segment of the cones becomes much broken. In certain cases the longitudinal striation of the outer segment of the rods is plainly brought out (Pl. 4, Fig. 32, *prs. dst.*). Cross sections seem to indicate that the peripheral lines are not always confined to the periphery, but extend as clefts into the central mass (Pl. 5, Fig. 40, *bac.*)

That these are clefts and due to the effect of the reagents, is indicated by the inequality of their depth and their absence in the osmic-acid fixation. The outer segments of the cones are as a rule poorly preserved, but show a cross striation with a tendency to an arrangement of the bands in pairs.

3. *Corrosive-Acetic Material.*

After trying a solution of mercuric chloride with different proportions of acetic acid, the best results were obtained from a saturated aqueous solution of mercuric chloride containing 5 per cent. of glacial acetic acid. The fluid was injected into the blood vessels, as already described, and the eye was immersed in the fluid for from 24 to 48 hours. I believe a short fixation with this fluid is not in general sufficient, notwithstanding theoretical considerations (Mann, :02). I have arrived at this conclusion empirically from the results of experiments by others, as well as by those I have made myself.

After the sections were cut they were stained on the slide by either the iron-alum-hæmatoxylin process of Heidenhain or by Mallory's (:00) triple stain. In the former case long staining of 40 hours in old hæmatoxylin was followed by decolorization so as to give on the same slide as great a range as possible between a strong stain and complete decolorization. Extremes are shown in Figures 1 (Pl. 1), 20, and 23 (Pl. 3).

I. NECTURUS.

Rod Cells.—The outer segments of the rod retain a slight stain, mostly in the form of reticulations apparently due to a very fine net-work. This is accentuated into stronger lines by a condensation due to the formation of vacuoles (Pl. 1, Fig. 1; Pl. 3, Fig. 20). In well preserved rods longitudinal striations are visible at upper and lower focus and usually more apparent in the latter (Pl. 1, Fig. 1; Pl. 2, Figs. 7, 8:

Pl. 3, Fig. 20). These lines have a parallel trend and ten to fourteen of them may be counted on one side of a rod. In cross sections of the outer segments (Pl. 2, Fig. 9) well defined circular dots are present in the periphery of the section. These correspond in number with the striations seen in lateral views, which, with other evidence, determines their character as definite fibers.

In most cross sections of the outer segment there are, just within the circumference, vesicles of somewhat regular arrangement but varying in size (Pl. 2, Fig. 9, *vac*). After fixation by mercuric chloride there is no differentiation of a definite sheath such as is seen in osmic acid preparations.

Now and then outer segments occur showing dark transverse lines at regular intervals, as well as dark plates separated by light lines. Fig. 24 (Pl. 3) is a drawing of such a rod. This was a detached outer segment and possibly had progressed more rapidly than the attached rods in the changes which bring about the plate-like cleavage of the outer segments.

The intermediate plate appears in this material as a very narrow light zone between the outer segment and the ellipsoid. It does not take the stain and seems more refractive than other portions (Pl. 1, Fig. 1, *dsc. i'm.*). The fibers, already described, pass over it to the ellipsoid.

The ellipsoid contains considerable stainable substance, being strongly stained by eosin, fuchsin, hæmatoxylin, etc. Where hæmatoxylin is used, the mass of the ellipsoid may be a homogeneous black, or this may be resolved into globules (Pl. 1, Fig. 1, *ell. bac.;* Pl. 3, Fig. 20). When the ellipsoid has been decolorized sufficiently to become translucent, superficial striations can be made out (Pl. 1, Figs. 1 and 6; Pl. 2, Fig. 7). In cross sections (Pl. 1, Fig. 6) these can be identified as fibrils, which have retained the stain and are undoubtedly continuations of those described for the outer segments. The ellipsoid body appears in some cases to be enclosed on all sides (Pl. 3, Fig. 20; Pl. 4, Fig. 33) by the light staining sheath substance. In most cases, however, a separation can

scarcely be distinguished distally between it and the intermediate plate and proximally between it and the paraboloid (Pl. 1, Fig. 1; Pl. 2, Figs. 7, 8). It seems probable, however, that there is in all cases a separation and that the ellipsoid is a distinct body entirely enclosed.

The paraboloid in this material often has an ellipsoidal form (normal), but is almost as frequently flattened by pressure. This seems to be due to a forcible contraction in fixation, of the "rod myoid," a name given by Greef (:00) to the contractile part of the inner segments of the rods and cones. In Necturus this myoid is often little more than a sheath to the paraboloid. As the ellipsoid and nucleus are hardened more quickly than the paraboloid, the latter is moulded to them.

The substance of the paraboloid is chemically unlike that of the ellipsoid, since most stains affect it but little. A close study of material stained in hæmatoxylin reveals a very close reticulum (Pl. 1, Figs. 1, 4, *pa'b. bac.;* Pl. 3, Fig. 20). The same material stained with Mallory's (:00) triple stain gives a coarser meshwork of a blue staining substance having nodal dots (Pl. 2, Fig. 8, *pa'b. bac.*).

In the periphery fibers are demonstrable in both lateral and sectional views, as in the parts of the rod previously described. Here, however, a clearly defined sheath of considerable thickness is present (the myoid described above), and in this the fibrils are deeply imbedded. Lying close to the paraboloid the fibrils (Pl. 1, Fig. 4, *B. fbrl.*) pass over the surface of the nucleus, as can be seen by cross sections of the latter at the distal end (Pl. 1, Fig. 5, *B., fbrl.*) or in lateral view of the nuclei in radial sections of the retina (Pl. 2, Figs. 7, 8, *fbrl.*). I have not succeeded in demonstrating with certainty fibrils in cross sections of nuclei, where the latter are closely packed.

Proximal to the nucleus the fibrils are sometimes convergent to the foot process and then divergent (Pl. 2, Fig. 8); in other cases there is no convergence (Pl. 2, Fig. 7, *fbrl.*), but the fibrils spread from the end of the nucleus directly out into the reticular layer.

The nuclei of the rods as well as of the other visual cells are larger than the other retinal nuclei and are characteristically elongated in a direction radial to the eye. Their chromation is either in the form of rather coarse or finer granules. The character of the nuclei no doubt changes at different stages of the metabolism of the cell; thus, for example, I have seen some evidence in experiments made by Mr. A. Forbes, that the distribution of chromatin varies with the degree of stimulation from light. I have seen nuclear division in embryonic material only. Such probably occurs in functional eyes of larvæ at the ora serrata but never in the fundus of the adult retina. Nucleoli have often been observed with appropriate stains (Pl. 2, Fig. 7, *nll.*).

Cone Cells.—The outer segments of the cones in Necturus are extremely unstable. In some respects corrosive-acetic fixation is as favorable as any other for them; but while this preserves the rods uniformly, the outer segments of the cones give evidence of considerable distortion. The conical form of these elements is quite constant, but they vary in length in consequence of varying degrees of contraction. The tip is often curled (Pl. 3, Figs. 20, 21), sometimes spirally. External parallel fibrils are present as in the rod (Pl. 3, Figs. 21, *fbrl.;* Fig. 25, *prs. dst.;* Pl. 1, Fig. 3; Pl. 5, Fig. 40). These are usually parallel to the long axis of the cone, but occasionally they have an oblique course, as is evident in Figure 23 (Pl. 3.). Here fine oblique superficial fibrils are to be seen at the distal end of the outer limb. In Figure 1 the cone shows the same condition both on the inner segment and over the paraboloid.

A central portion, which stains dark with Heidenhain's iron-alum hæmatoxylin, is generally present in the outer limb; it is of varying length, but as a rule it falls short of either end. Though it stains heavily, the dye is easily washed out in the mordant. This portion in certain cases shows a plate-like structure (Pl. 3, Figs. 21, 23). In these instances a spiral is suggested, but the appearance might be explained as due to

the irregular separation of plates by vacuoles. Three of the latter are shown in Figure 21 (Pl. 3) two on the left and one on the right. Sometimes the spiral appearance is much more striking, as if there were two fibers closely parallel, and suggests some of Hesse's (:04) figures. A common appearance presented by the outer segment of the cone is shown in Figure 20 (Pl. 3); here the tip only is dark and the proximal portion is marked by irregular lines, probably cleavage effects.

I have observed with certainty no intermediate plate in the cones of Necturus.

The ellipsoid of the cone differs from that of the rod in form, being of greater length and smaller diameter. The stain is retained longer and the resolution of the staining mass into globules is more apparent than in the rods (Pl. 1, Figs. 1, 6; Pl. 3, Fig. 21; Pl. 5, Fig. 39, *gran. ell.*).

The paraboloid is smaller than in the rod and more constantly ellipsoid or ovoid. The sheath is relatively thicker and fibrils are more easily seen than in the rods. In cross sections the fibrils appear in two layers, an inner and an outer (Pl. 1, Fig. 4*A*). No evidence of difference in these circles of fibrils could be detected with certainty.

The myoid, or contractile portion, between the nucleus and paraboloid is more distinct here than in the rods. The course of the fibrils over the nucleus corresponds to that in the rods. The nuclei themselves are commonly more pointed at the distal end than those of the rod are.

Double Cones.—As in other amphibians and in the other vertebrate groups except the mammals, there are in Necturus the remarkable elements called double cones. In fishes, so far as I have observed, the two cones of any pair are nearly alike, but in Rana and in Necturus they are in some respects quite different. The longer one (Pl. 1, Fig. 2, *A*) for convenience may be called the *far cone (con. dst.)* and the other (Fig. 2, *B*), which for the most part lies nearer the membrana limitans externa, the *near cone (con. prx.)*. These are

designated by German authors as Haupt-Zapfen and Neben-Zapfen respectively. The elements are united to each other along one side from the region of the membrana limitans externa to their ellipsoids.

I have been unable to distinguish any difference between the outer segments of the double cones and those of single ones.

As the ellipsoids of the pair do not stain alike, they differ chemically and this suggests a functional difference. That they are morphologically equivalent, or homologous, no one would question. In the far cone the ellipsoid takes the same color as it does in the single cone. That of the near cone shows much less affinity for stains, in this respect resembling the ellipsoid of the rod (Pl. 1, Fig. 2; Pl. 5, Fig. 38).

The paraboloids display the most conspicuous difference seen between the cell organs of the pair. That of the far cone is slightly smaller than the ellipsoid while the paraboloid of the near cone is three to four times as large as its own ellipsoid and looks like a large flask-shaped vesicle.

The myoid of the far cone (Pl. 1, Fig. 2, *my. con. dst*.) is very long, and reaches from the nucleus to the ellipsoid. In the myoid dark staining fibrils can be seen more plainly than in any other element. At the distal end, the fibrils pass around the paraboloid. Their farther course seems to be the same as in the single cones, where they have their origin on the outer limb. Seen in optical section the fibrils seem to end on the paraboloid in small bead-like enlargements, but by focussing, these are apparently resolved into a continuation of the fibrils (Pl. 1, Fig. 2, *cp*.). This appearance may be due to the fact that these fibrils are more distinct in cross section than when seen lengthwise. At the proximal end of the myoid the fibrils pass over the appropriate nucleus, as described for the other elements, and into the outer reticular layer.

The fibrils of the near cone lie embedded in the sheath of the large paraboloid, but otherwise their course is like the

course of those of the far cone. These relations are made clear in cross sections (Pl. 1, Fig. 5, *A.*) and different lateral views (Fig. 2, *A and B.*). In the preparation represented in Figure 2, *B,* the near cone, being out of focus, is shown by dotted outlines only.

The twin cones possess two nuclei, each element having its own, though the two lie in close contact. The nucleus of the far cone is like the other nuclei, but that of the near cone has a prolongation (Pl. 1, Fig. 2, *prc. nl.*), which reaches beyond the membrana limitans externa into the sheath of the paraboloid. Another difference between the two nuclei is usually discernible; the nucleus of the near cone (*nl. con. prx.*) contains a fine granulation giving a greyish cast, that of the far cone possesses larger, densely staining granules and is otherwise more transparent, because of the absence of the finer granulation.

A well defined foot in the double cone is sometimes distinguishable (Pl. 2, Fig. 7, *pd. con.*), the fibrils converging and then diverging into the middle of the outer retinular layer.

From estimates based on the fundus of the retina, the relative number of rods, cones, and double cones in Necturus are: rods, 4; cones, 1; double-cones, 1.

A comparison of double-cones with the other elements, when stained by Mallory's (:00) triple stain, brings out some interesting differentiations in corrosive-acetic material (Pl. 5, Fig. 38). The ellipsoids of the single cones take on a brilliant purplish-pink color, while the outer segments take a lighter but equally brilliant pink, with the exception of the fibrils, which are blue (Fig. 40). The rods also show the fuchsin-staining substance, but to a smaller degree, in both ellipsoids and outer segments. In the latter it is somewhat irregular in distribution, as if extending in the natural state throughout the segment, but confined in fixed preparations by condensation or contraction to its central portions. In the rods, the prevalence of a blue-staining reticulum masks the pink somewhat. The mixture of blue and pink in the ellip-

soids gives a purple effect. A glance at the double cones shows that this purple stain, characteristic of the ellipsoid in the rod, is also to be found in the ellipsoid of the near cone; while in the far cone the stain is like that of the single cones.

II. THE FROG.

Preparations of frog retina fixed by corrosive-acetic present a feature which I have not seen in Necturus when prepared by the same method. This is a central core *(med.)* in the outer segments of the rods (Pl. 4, Figs. 29, 30, 31) of both the long red rods and the short green rods. This core measures one-third to one-fourth the diameter of the whole rod. It takes none of the stains which I have tried, with possibly the exception of picric acid.

The rods otherwise seem to show the same general features as in Necturus. The intermediate plate, ellipsoid, and paraboloid show the same reactions to stains. The paraboloid in the frog is not as easily distinguished as in Necturus and has not usually been noticed by observers, but I believe it has been simply overlooked on account of its small size.

The fibrils I have not studied in this material, but, in rods of the frog preserved in Perenyi's fluid I have distinguished superficial longitudinal fibrils (Pl. 4, Fig. 27, *fbrl.*). The presence of visual-cell nuclei in a double layer, as contrasted with the single layer of Necturus, is a difference to be noted; also the presence of "green rods" and "cone color globules," which I have not observed in Necturus.

III. THE GOLDFISH.

In the goldfish (Carassius auratus) we find an outer nuclear layer of four or five strata of nuclei (Pl. 3, Fig. 19). There are present single cones, double cones, and rods; these are of the type of the "green rod" in frogs with long inner

segment. The rods (which may possibly be of two types) are extremely small in diameter and the staining effects so varied that I can not put much reliance on the appearances as an index of minute structure. Cross sections of the rods give evidence of a central core unstained by hæmatoxylin as in the frog.

The case of the large double cones is different. Here, in well preserved specimens, a constant appearance is presented of a system of peripheral spirals (Pl. 3, Fig. 18, *prs. dst.*) made up of four or five fibrils. On the inner limb, proximal to the ellipsoid, straight fibrils can be traced to, and over the nuclei. Their number does not correspond with that of the spiral fibrils. The nuclei of the double cones lie close together and each has a distinct cone foot.

C. Fresh Material.

In Ordinary Light.

A thorough familiarity with elements in their normal living condition, or as near as possible to this state, is of the greatest importance in histology. This is especially true of investigations into the nature of the optical properties of living bodies, since the process of fixing or "killing" is so liable to affect these properties. Though gross form and structure may not be as sensitive to chemical alterations as molecular structure, yet even here, as is well known, fixation may do the greatest violence. As a check upon such effects, a knowledge of fresh material is indispensable.

(a) rods.

The Outer Segments of the rods may be quite easily obtained by the method already described (pp. 574, 575), and a fortunate preparation may give complete rods and cones *in situ*. In Necturus, however, the cones, on account of their instability, are not easily found in teased fresh material.

The outer segments of the rods in Necturus are cylindrical, transparent, highly refractive bodies, measuring on the average 12 micra in diameter by 36-40 micra in length. The distal end is rounded, but not enough to make the end hemispherical (a form usually assumed in fixed material).

The most conspicuous finer details or markings in outer segments just removed from the eye, are visible under a magnification of 1,000 diameters at a low focus, but not at the lowest (Pl. 2, Fig. 13). These are slightly oblique lengthwise striations. They appear as dark lines with a lighter ground substance between them, and usually about eight to ten such lines are visible at once, evidently in focus on, or very close to, the lower surface. On the upper surface, usually, only a single line or possibly two or three are visible at one time (Pl. 2, Fig. 12). The difference is probably due to the optical effect of the cylinder converging the rays from the lower curved surface, so as to make them visible in one plane, thus bringing them into the same focus. At a focus about the lowest at which anything is visible, the dark lines appear bright, while the substance of the rod is dark (Pl. 2, Fig. 14). These lines evidently represent less refractive material separated by a more refractive substance.

The outer segments, soon after the preparation is made, show a slight bulging of the detached proximal end, later this is more pronounced, and lines of transverse cleavage become visible (Pl. 2, Fig. 15). The rod shown in Figure 15 had been under a cover-glass three hours. In this case the longitudinal lines were plainly visible from the upper surface. The lines were not noticeably spiral in this instance and illustrate an individual variation in this respect. In this rod (Pl. 2, Fig. 16) distinct plates could be seen separated from each other by small spaces. Occasional individuals in a field of detached outer segments, perfectly fresh, show dark transverse zones or bands, usually only one to three in a rod, as if some of the disks were darker than others. Transverse cleavage as thus described is not always found;

in fact, regularity in the breaking is very frequently not obvious.

In addition to the superficial striations visible during one to three hours after the removal of the rods from the eye, a central core is discernible; this is sometimes of slightly different color or shade from the rest, is regular in outline, and from one-fourth to one-third the diameter of the rod. It is very frequently seen at middle focus. If this appearance were the only evidence for the existence of such an internal structure, I should, on account of its inconstancy, look for its explanation in simple optical effects as many others have done. But additional evidence has been found in fresh material in the form of an occasional projection of a central portion beyond the broken proximal end of the outer segment. In one such case (Pl. 5, Fig. 42, *med.*) this axis took a differential stain in toluidin blue (see pp. 604, 605).

To aid in an interpretation of the appearance of the outer segments as seen lengthwise in the fresh condition, it is of advantage to have them in optical cross-section. This can be done by placing a fresh retina, proximal surface down, on a slide and employing a support for the cover-glass. The outer segments thus viewed (Pl. 2, Fig. 17) are generally circular, but frequently with considerable variation from a true circle. The outline appears crenate and in some instances nothing more is discernible. In others a distinct border can be made out, and this border in favorable light is distinctly resolved into small circles of highly refractive substance. It is evident, then, that these circles represent the highly refractive portions between the dark lines and are fibrils (Pl. 2, Fig. 13). The portion between the fibrils, of smaller diameter and less refractive, appears as dark lines when in focus (Pl. 2, Fig. 13) and as light lines on lower focus (Pl. 2, Fig. 14).

On first inspection one would think the dark lines of Figure 13 (Pl. 2) were identical with the staining fibril of Figure 1 (Pl. 1) because of relative sizes, but this seems not to be the

case, so far as can be judged from all the evidence obtainable. Cross sections from osmic material appear very much the same as the optical sections of fresh rods. There is no reason to doubt that the small refractive circles which in both cases produce the crenation are identical.

When the cross sections of osmic material are stained, after bleaching, a fibril is differentiated corresponding in position with the small circles or the ridges in the crenate outline and not to the grooves of this outline. The slight difference in size between the fibrils as seen in the fresh condition and when stained can be explained as due to the fact that a sharply outlined, stained fibril appears smaller than an unstained one, whose optical differentiation from the surrounding substance depends simply on a different index of refraction. Of course it is possible that the stained fibril might represent only the center of the small circles, analogous to the axis cylinder in the center of a medullated nerve. The minuteness of the objects, however, forbids the certain determination of this point with any apparatus one can command at present.

The intermediate plate in the fresh condition, when discernible, appears as a disk at the proximal end of the outer segment. It is apparently of a different refractive index from the latter, or at least is more transparent.

The ellipsoid usually has the form of a convex-concave segment of a cylinder lying within the substance of the inner limb; its diameter is only slightly less than the limb itself. The convex surface is distal. In the fresh condition the ellipsoid is more flexible than the paraboloid. From the condition presented by the fixed visual cells, however, the reverse would appear to be true. The probable explanation of this apparent contradiction is that the reagents used harden the ellipsoid more quickly than the paraboloid, the latter remaining for a longer time comparatively plastic. Immediately after death the ellipsoid is comparatively clear and highly refractive, perhaps possessing the latter characteristic to a greater

degree than the outer segment. In a brief time granulation sets in, followed, as soon as the ensheathing substance of the outer segment is broken, by complete dissolution. The degree of instability of the ellipsoid is next to that of the outer segments of the cone.

The paraboloid in the fresh rod of Necturus has, as its name appropriately signifies, a paraboloid form. As a rule its diameter is less than that of the ellipsoid, while the total diameter of the inner limb remains the same. From this fact it is seen that the ensheathing substance is thicker than that about the ellipsoid. This contractile sheath corresponds to the myoid, and the latter term is preferable to the word sheath, since the paraboloid is a separate body possibly possessing an independent sheath of its own. Contraction of the myoid undoubtedly flattens the paraboloid. A flattened condition is shown in Figures 10 and 11 (Pl. 2). The paraboloid has a lower degree of refraction than the ellipsoid, but greater than the ensheathing myoid. The paraboloid may be isolated, and when thus separated from its normal surrounding structures and suspended in the eye fluid, it assumes a spherical form. Here there is no granulation and clouding, such as we get so quickly in most protoplasmic substance after death. The appearance suggests the oil globules of the retinal elements in some amphibians and sauropsidans, but this resemblance is evidently only superficial.

The nucleus in the rod is often in contact at is distal end with the paraboloid. Its distal surface under these circumstances is concave, thus conforming to the convex surface of the paraboloid. Except for this occasional slight depression, the nucleus has an approximately ovoid form. A cytoplasmic sheath can be quite readily detected about the distal portion of the nucleus. I have not been able to distinguish it at the proximal end in fresh rods and cones. Granulation very rapidly sets in here, as is the case with most nuclei (Pl. 2, Figs. 10, 11, *nl.*).

(b) CONES.

The outer segments of the cones in Necturus are very difficult to observe in the fresh state, because of their distortion and dissolution immediately after death. Individual cases, however, may sometimes be found which preserve a constant form long enough to permit of observation and even of drawing with a camera lucida (Pl. 2, Fig. 10). In Necturus the marked conical form of the outer limb and differences in the ellipsoid make them readily distinguishable from the rods. The dissolution, so far as there is any regularity in it, usually results in an elongation of the cone (Pl. 3, Fig. 22) combined sometimes with a spiral twist. While still intact the appearance of the cone is like that of the outer segment of a rod, but less refractive. I have not observed an intermediate plate in the case of the cones.

The other portions of the cone appear like the corresponding parts of the rod, except that the ellipsoid of the cone is longer and the paraboloid is usually not in contact with the nucleus. Figures 1 (Pl. 1) and 20 (Pl. 3) give a fairer idea of the usual form than the fresh cone shown in Figure 10 (Pl. 2), as the latter is abnormal.

2. *In Polarized Light.*

During my studies on fresh material the question arose, whether there were not some means of making visible in the fresh elements structures which the inspection of fixed preparations led me to believe to be present in the living rod. Such a method, if found, must be in the nature of a variation in the light used. Polarized light seemed to offer the method desired, since, as is well known, some transparent bodies of similar appearance in ordinary light are brought out in strong contrast when seen under a polarizing microscope. Again, organic bodies of a fibrous structure give a definite reaction by this method and the direction of the fibrils seems to determine the result, since the color reaction is constant for a given direction.

Outer rod segments were first examined in a polarizing microscope with polarizer and analyzer alone. In this way no evidence of double refraction by extinction was found. However, certain features were brought out more plainly than with ordinary light, viz., the axial core and transverse banding. The visibility of the latter seems to depend upon the direction of the illumination with respect to the rod itself. The desired angle can be obtained for any given rod by turning the revolving stage of the microscope.

When a gypsum interference plate is introduced between the analyzer and polarizer (or Nicol prisms), and the prisms so placed as to give a sensitive red of the first order, the outer segments of the rod give a definite reaction. In a teased preparation of fresh retina a field may easily be found containing detached rods lying in various directions. Outer segments lying parallel to the *a* axis, or negative optical direction of the gypsum plate, were bright yellow while those at right angles to this were bright blue. The colors of an individual rod may be reversed by turning the preparation so as to bring the long axis of the rod into a line at right angles to its former position. (Pl. 5, Fig. 43.) The bright yellow or blue of the outer segments appears in marked contrast with the inner segments which retain the general red field color.

These observations were made on the rods of Necturus, but I have also tested the outer segments of rods in the frog, hen, guinea pig, mouse, rat, and ox, as well as in the outer segments of the cone-like elements of the turtle and snake, and I have obtained in all cases the same result. In the rods and cones of the goldfish I obtained no reaction, although the outer segments are fully as large as some which gave distinctly positive results. The smooth dogfish, Mustelus canis, gave results similar to those from Necturus.

The definite reaction obtained in the forms mentioned demonstrates that the outer segments (at least in their predominating substance) are doubly refractive or anisotropic, with the long axis in the positive optical direction; *i. e.*, as

regards their optical properties, they have axes of maximum elasticity at right angles to their lengths. As the red of the field color is lowered to a yellow when rod segments lie parallel to the *a* axis of the gypsum plate, it determines the long axis of the rod as optically the (*c* axis) positive direction.

To obtain an immediate basis of comparison, similar tests were made on other organic tissues which seemed, or were known, to be of a fibrous structure. Thus naked axis cylinders from the inner surface of the ox retina gave light reactions like those of the outer segments. The same was true of striped muscle fibers from the crayfish, frog and ox, connective-tissue fiber from the ligamentum nuchæ, vegetable fibers, longitudinal and radial fibers in hard wood, and central fibers of Spanish moss, etc. The rhabdomes from the compound eyes of crayfishes showed in general the long axis as the negative optical direction, but when it is remembered that the fibrous structure of these bodies is at right angles to their length instead of being parallel to it, it will be seen that they agree with the preceding examples in their optical reactions.

In the slug Limax maximus the distal portion of the visual cell, which corresponds with the outer segment in vertebrates, displays somewhat complex reactions. In Figure 46 (Pl. 5) I have indicated the character of these reactions as made out in a fresh preparation. In a diagrammatic way I have also shown the fibril relations, as figured by Dr. Grant Smith (:06) from fixed preparations. It will be seen from the diagram that the character of the reaction depends on the direction of the neurofibrils. Where the latter are parallel to the *a* axis of the gypsum plate the field color is lowered to a yellow, and where the fibrils are at right angles to this axis the field color is raised to a blue. Portions of the body in which the fibrils would be seen on end are neutral and so do not affect the reaction of fibrils in the plane perpendicular to the direction of the transmitted light ray.

From the above observations on fibrillar bodies, and especially on those that are nervous structures in which fibrils

have been demonstrated, the deduction would be natural, that the outer segments of rods are bodies possessing, not a transverse, but a longitudinal structure comparable to that of the axis cylinder of a single nerve fiber, in which the substance is differentiated for longitudinal conduction.

The question might arise as to whether polarization here were due to certain qualities inherent in individual fibers or whether it were due simply to the gross grouping together of fibers. It seems probable that the former is true and that a certain arrangement of substance, perhaps molecular and characteristic of organic fibers, accounts for the effect on light. A case of double refraction in a glass rod may help to illustrate the point. Such a rod ordinarily will not show double refraction; *i. e.*, the glass is isotropic, but if the rod be subjected to a stress (pressure or tension) the glass will cause polarization of light which passes through it. In the one case the rod (or fiber) is not doubly refractive, in the other the particles of glass are so disposed by the stress that they effect the light differently from what they did before. Tension in the direction of the axis will have the effect of determining the axis of maximum elasticity as transverse; while pressure in the longitudinal direction will change that (optical) axis to longitudinal. The mere cylindrical form of the glass rod will not produce polarization nor will polarization occur when several isotropic glass rods or fibers are placed together; the phenomenon depends upon a condition of the particles of glass in individual rods or fibers.

The view (based on comparison of reactions with fibrous bodies) that the outer segment of the visual rod is longitudinally fibrous may be opposed by another hypothesis, which is in accord with many observed facts. This view is, that the outer segments of the rods are made up of transverse plates of medullary substance and that between these plates are transverse neurofibrils which connect with longitudinal fibrils on the periphery of the rod. This hypothesis is suggested by a marked similarity in appearance, under polarized

light, between the rods and the sheath of a medullated nerve (Pl. 5, Figs. 43, 44). When seen in optical section the sheath reaction is opposite in character to that of the axis cylinder, being yellow when that is blue and *vice versa*. It will be seen from the figure (Pl. 5, Fig. 44) that the sheath substance is neutral where the light comes from a direction at right angles to a surface tangent. Thus a single nerve fiber with its highly refracting medullary sheath appears most conspicuously as two bright yellow or blue (depending on the angle of the microscope stage) parallel lines with a faintly colored axis cylinder between. The faintness of color in the latter is probably due to the predominance of the sheath reaction.

The rod outer segments are chemically similar to the medullary sheath according to researches of Kühne (see Schwalbe, '87, p. 104). The evidences for this are from such micro-chemical tests as the blackening with osmic acid, etc. If the rod were made up of plates of medullary substance with transverse nerve fibrils between, the relation of medullary substance would be as in the nerve trunks. These fine transverse nerve fibrils of the rod would not affect the light reaction perceptibly (in polarized light) unless in quantity great in proportion to the total mass of the rod. The longitudinal superficial fibrils of the rod are probably not sufficient by themselves to account for the very distinct anisotropic reaction of the rod, because their mass is much less than that of the comparatively faint axis cylinders of nerves. The quality of the rod reaction in its brilliancy is more like that of the medullary sheath.

The outer segments of the cones in Necturus show anisotropism, but not to so pronounced a dergee as in the rods. Outer segments from an animal kept in ordinary daylight and examined while in situ and in good condition gave the same reaction as rods; *i. e.*, positive with respect to the long axis. Cones from an animal kept in total darkness three days gave the long axis as the negative optical direction. This reaction is complicated in some way, for when the outer seg-

ments of the cone are at an angle of 45 degrees to the blue or yellow phase, in the upper field of the microscope, the half of the cone on the side of the yellow phase is blue and vice versa. By the theory that the reaction depends on the direction of the fibrils this would be accounted for by supposing that the cone was composed of fibrils whose direction was slightly ascending and oblique like the branches of a conical tree. In the yellow or blue position the fibrils on each side of the axis (or tree trunk) would not diverge enough from this axis to be at right angles to it and thus to give the same color to the whole, but between this position and that of parallelism with the axis there would be one where the color reactions would be opposite on the two sides of the axis.

This difference between cones exposed and unexposed to light is possibly due to a difference in tension between the elongated and contracted conditions. If different sets of fibrils are present one may be tense while another is relaxed. However, since such fibers have not as yet been seen and our knowledge of the outer segments of the cone is very limited, such considerations can be only speculative.

D. Effects of Reagents.

Early in this investigation I studied the effects of various fluids on the visual cells, particularly on the outer segments of rods, in order to obtain (1) neutral media in which intra-vitam stains could be used or other tests could be made; (2) suitable reagents for fixing and permanent preservation; and (3) means discovering the optical and other physical as well as chemical properties of these bodies. In judging of the state of preservation I compared the preserved material with living material in respect to its reactions to polarized light, its transparency, the form and behavior of its parts, and its subsequent staining, etc.

The use of polarized light as a basis of comparison seemed eminently suitable, for, assuming that minute structural con-

ditions determine the light reaction, it follows that if a fluid were found which preserved the same reaction as that of the fresh rod, one might infer that little violence had been done to that particular structural condition. Subsequent experiments showed that perhaps the test was too delicate for practical application to fixing fluids, since none were found which fulfilled that requirement. However, although none were found which preserved the reaction of normal rods, yet the rate of change and character of the final result furnished some index as to the relative merits of the fluids.

1. *Examination Media.*

For examination media the normal eye fluids would naturally be expected to be the best. In Necturus, however, the small volume of the fluid obtainable from an eye presents practical difficulties, there being scarcely enough in one eye after the necessary manipulation, to fill out one cover-glass. The eye fluids from the frog can be used for Necturus and make a satisfactory substitute. That the substitute is not perfect, however, is to be seen in the fact that the frog rods transferred with the fluid show better preservation after a given period, than do those of Necturus. Outer segments of rods of both species after twenty-four hours show in polarized light normal (positive) reactions but reduced brilliancy.

Glycerine (concentrated). Outer segments in one hour or less become granular and disintegrate somewhat, at the same time bending into horseshoe shapes. After seven days the reaction to polarized light is normal except for reduced brilliancy. The reaction of ligamentum nuchæ was normal even after it had been two years in glycerine.

Glycerine, 1 vol.; Water, 2 vols.; Alcohol, 1 vol. This mixture gave better preservation than glycerine and water alone, or concentrated glycerine, or in fact than any other purely artificial mixture tried. The reason for this, I suppose,

was that the fluid was probably isotonic with the cell fluids. When this solution was introduced at the edge of the cover-glass a remarkable reaction of the outer segments of the rods was observed. An individual rod was watched under an eye-piece micrometer, and the following changes in dimensions noted. Before admission of fluid the length of the rod was 6 divisions; at entrance of the fluid it contracted rapidly to 3 divisions; after which it slowly lengthened to 7 divisions. The experiment was repeated using another animal and the time noted. Before the admission of the fluid the length was 6 divisions; at entrance of the fluid contraction began and lasted for one minute, until a length of 4 divisions was reached; after this, the rod lengthened to 8 divisions, the period of lengthening being 7 minutes.

Physiological Salt Solution (¾%).—Outer segments of rods after remaining four hours in this solution showed normal positive reaction in polarized light. In this solution, however, a majority of the outer segments showed disintegration and distortion of form, with no refraction at all. After three days such outer segments as were still sufficiently intact to show any double refraction, gave reversed color reaction; *i. e.,* they were anisotropic and negative with respect to their long axes.

Amniotic Fluid.—This was obtained from pig embroys and was kept in sterilized bottles with a small piece of thymol as a preservative. Outer segments of rods rapidly disintegrated in this fluid, possibly because of the presence of thymol.

Egg Albumen.—As much of the fluid portion of this substance as would easily pass through a strainer of bolting silk proved as satisfactory an examination medium as the fluid from the eye. About 10 c.c. of liquid albumen can be obtained from a single egg.

2. *Fixing Agents.*

In giving the results of tests with fixing fluids I have included an account of the unsuccessful trials as well as the

more satisfactory ones, since the account of failures may at least save the time of other workers. However, in cases where a fluid has been unsuccessful in one animal it does not necessarily follow that it will be inapplicable to the same tissue in another animal, though the probabilities are against it. Again, slight modifications of a formula, or its method of application, might meet the requirements.

Osmic Acid Vapor.—Outer segments of rods show a reversal with polarized light after treatment with this reagent. The tints are more brilliant than normal and the blue phase has a decided greenish cast. The permanent preparations made by this method have already been described (Pl. 4, Figs. 33-37). The excessive blackening of the outer segments and their contraction are characteristic of long fixation. Short fixation gives appearances much like the fresh condition, but with a slightly brownish color.

Osmic Acid, ½% Aqueous Solution.—The outher segments were reversed in color when seen in polarized light; curling, breaking transversely, and lengthening occurred commonly in the outer segments; notwithstanding these results many segments were well preserved. Among the latter, many were observed which gave with oblique light alternating light and dark bands.

Vom Rath's Fluid.—As already stated on page 579, preparations fixed in this fluid show an exaggerated shrinkage in length (Pl. 4, Fig. 32).

Flemming's Fluid (strong formula).—The outer segments were poorly preserved by this method, but the superficial fibrils were brought out plainly.

Absolute Alcohol.—Double refraction is destroyed; *i. e.*, with light tests the outer segments become quite transparent and of the same color as the red field.

Formaldehyd.—In preparations made by exposure to the vapor of formaldehyd the "optical axes," as shown by the color reaction, were reversed as compared with those of

the fresh material. Treatment by this method did not yield well preserved outer segments. A 40 per cent. aqueous solution destroys the double refraction of outer segments of rods. A 4 per cent. aqueous solution gives reversal of reaction for outer segments. The form of the rod is not well preserved.

Nitric Acid (10%).—This reagent causes the outer segments of the rods to elongate to almost double their original length, but apparently they retain their former volume. This change is accompanied by a reversal of the colors in the light test and a loss of transparency by granulation.

Picric Acid seems to cause the loss of double refraction in the outer segments of rods; in polarized light with the gypsum interference plate they show the red "field color" at all angles.

Mercuric Chloride (saturated aqueous solution).—A reversed color reaction in polarized light occurs in outer segments when placed in mercuric chloride. Voluntary muscle seems to be unchanged in this optic character when subjected to the same treatment. However, after *long* fixation muscle shows the same results as the rods do.

A study of sections of the retina fixed by this method shows a very fair preservation of the general form of the elements, with slight contraction. The nucleoplasm is noticeably contracted, so as to be quite generally separated from the nuclear membrane. Staining seems to bring out less of the fine detail than is obtainable by some other fixatives. The membrana limitans externa of the retina is more distinct than with other fixatives.

Mercuric Chloride + 5% glacial acetic acid gave reversal with polarized light. A detached outer segment of a rod under observation when the fluid was admitted, showed suddenly a central axis, as if that portion were first affected, then the rod curled into a horse-shoe shape. Sections fixed in this fluid have been described above under "Permanent Preparations."

Zenker's Fluid.—With this reagent preservation is good

and fixation generally satisfactory, so far as I have observed. I have used it but little.

Petrunkevitsch's Fluid.—* This mixture causes a great deal of vacuolation in the outer segments of the rods.

Perenyi's Fluid causes outer segments to contract transversely and lengthen, probably preserving the same volume. I should attribute this effect to the contained nitric acid, which, when used alone, produces similar results. Vacuolation is greater than in mercuric chloride plus 5 per cent. acetic acid. The most marked effect in the whole visual cell is the vacuolation and consequent general expansion of the paraboloid. A similar effect is produced in the nuclei. The outer segments of cones seem to be as well preserved by this method as any I have seen, judging by external form, and from comparisons with fresh material.

Golgi's Rapid Process.—This method gave some indications of selective impregnation in the details of the elements. I was surprised and interested in this, but did not have the opportunity to carry on trials until complete impregnation was obtained. In other material treated by this process I have obtained elements so densely impregnated that there was little opportunity to make out finer cell details. The published results of other workers (Ramon y Cajal, Dogiel, et al.) who have used this method on the visual elements show the whole cell impregnated. On account of these results, I did not consider the method favorable for my special problem.

Drying.—Fixation by drying at a high temperature, in the manner of making "blood preparations," breaks up the outer segments into short pieces, but these appear in natural condition as to transparency and form. Light tests give normal reaction after twenty-four hours. Outer segments dried at

*Formula:

40% Alcohol	500 c. c.
Glacial Acetic	90 c. c.
Nitric Acid	10 c. c.
Mercuric Chloride	50 g.

room temperature disappear in a few hours, apparently by deliquescence, leaving a drop of liquid.

3. *Intra-Vitam Stains.*

Methods were considered for employing "intra-vitam" stains in the living animal, but as the proportion of successful impregnations is likely to be so small, a more practical method was sought for. The retina as a whole was placed fresh in examination media in which "non-poisonous stains" were dissolved in very small quantities, or the stain was added while the object was under observation to permit of watching the progress of the stain. Positive results were obtained with methylen blue, toluidin blue, and hæmatein. With Congo red and some other dyes I obtained no evidence of selective staining.

Methylen Blue.—A retina was placed fiber layer downward so as to leave the rods and cones vertical on the upper side and give an end view of these elements. When methylen blue is added the outer segments of the cones become immediately deeply stained, thus the field appears as a mosaic of larger white circles, the rods, and smaller blue ones, the cones. In teased retinæ where the rods are seen laterally, occasional individuals may be found with transverse bands stained deep blue alternating with light blue ones. In many cases the superficial fibrils stain blue, and there is some evidence of a central axis. The affinity of the stain for only occasional rods is probably due to some peculiar condition in the metabolism of the element. This selective character is in accordance with what has been observed for this method in other parts of the nervous system.

Toluidin Blue.—A retina was placed in fluid egg albumen in which was dissolved one three-thousandths by weight of ammonium molybdate; after ten to twenty minutes, toluidin blue, one three-thousandths in egg albumen, was added. In a few minutes the detached outer segments of the rods showed a marked differentiation of a central axis or core staining

purple. This color was noticeably different from the blue in other parts of the rod (Pl. 5, Figs. 41, 42) and in the field. In most cases this axis showed an irregular outline and discontinuity, as if broken by post-mortem changes, due perhaps to the reagents. In some outer segments this stained axis projected, at the proximal broken end, beyond the peripheral substance of the cylinder (Fig. 42).

Longitudinal superficial fibrils stain by the above method and also with toluidin blue alone. Whether the ammonium molybidate was an efficient factor or not, I did not ascertain.

Hæmatein.—From a retina immersed for twenty-four hours in fluid albumen containing a few crystals of hæmatein, outer segments of rods were obtained which showed alternating light and dark transverse bands. The bands appeared to be the edges of disks or rings and the darker ones projected slightly beyond the lighter ones, as do the successive segments in a muscle fibril when in a state of contraction.

III. DISCUSSION.

The various appearances presented by protoplasm or "cell plasm" after treatment with different fixing fluids is a matter of common knowledge. An interesting study of such effects on nerve cells is given by Floyd (:03). I have described some of the different appearances in the visual cells as prepared by various reagents, and I have shown that from this standpoint there is abundant opportunity for contradictory results where workers have depended on single preservatives.

A. Rod Cells.

The outer segments of the rods when prepared in osmic acid, have been shown to possess superficial longitudinal lines when viewed in toto. The interpretation of these lines from cross sections has been (Schultze, Greef, and others) in general that they "depend upon a grooving of the surface which has some rela-

tion with a deeper differentiation in structure" (Hensen, '67; Schultze, '72[a],). This differentiation was observed as regular rifts in the inner substance of the rods. But well preserved rods, I find, do not show these rifts. In such cases with comparatively low power (1,000 diameters) the sheath usually appears continuous, though sometimes thinner in the bottom of each furrow.

As I have shown (pp. 579, 590-591), the longitudinal ridges of the outer segment in osmic material correspond with the stained fibrils demonstrated by other methods of fixation and with the fibrils seen in optical sections of fresh rods. Hensen's ('67, Fig. 7) figure of the fresh rods of frogs corresponds with what I have seen in Necturus. It seems that the longitudinal cleavage lines occur between the fibrils and that the vacuoles of some material are due to penetration of fluid along these grooves, perhaps through a membrane. The appearance then of deeper radiating lines is probably due to artifacts determined by these peripheral structures.

Some preparations suggest the presence of a set of fibrils or tubes parallel to the stained fibrils and alternating with them, but I should put little reliance on such a conclusion without more evidence.

The fibrils of fresh and fixed material are usually slightly oblique, otherwise I have found no fibrils in the rods to compare with the spirals of Hesse (:04), or of Ritter ('91[a]; '91[b]). The evidence, such as I have obtained (Howard, :03), is all against W. Krause's ('92) opinion that the transverse cleavage is due to a system of closely wound spiral fibrils.

The fibrils which I have demonstrated in fixed material, most satisfactorily in Necturus, but also in the frog and the goldfish, are probably the same which Schneider (:02) has observed in the frog, though on this point I am not quite certain, for Schneider's description is accompanied by two small text-figures only. It is unfortunate that he has not published a more complete account of these "neurofibrils," as he terms them.

Schultze found no peripheral fibrils except those of the "fiber-basket," which he described as running over the inner end only of the rod and belonging to the system of retinal sustentative cells, "Müller's fibers." I have not identified any such structure on the rods of Necturus, though some might claim that the peripheral "stained fibrils," as I have termed them, are identical with the fibers of Schultze's "fiber-basket." The reasons against this view I will take up more in detail later, and I will merely add here that the intimate structural connection of the "stained fibrils" with the outer segment and the fact of their extension over the whole length of the segment argue against this opinion.

The large single peripheral fibril figured by Kolmer (:04) in the frog, can hardly be identified with any structure that I have described, unless in Kolmer's preparations only a single fibril was impregnated. Impregnation methods like Golgi's and Bielschowsky's, which Kolmer used, act selectively, as all know who have worked with them. If there is selection here in a single element, the fact would have considerable interest. That only a single fibril might be present, as Retzius (:05) described for the dogfish, is conceivable, in which case this would be a simpler condition than exists in amphibians and teleosts; but he figures some single rods, in which two fibrils have been impregnated. This, it seems to me, favors my contention that there may be selection of a single fibril, or at least of a smaller number, in certain elements. The same comment would apply to the fibrils described by Held (:04). The "diplosomes" mentioned by these authors imply a connection between fibrils and a couple of spherules in the region of the ellipsoid. I have seen nothing of such relations myself, but I am not prepared to deny their existence.

So far, then, as I have been able to demonstrate, the cylindrical outer segments possess peripheral fibrils running lengthwise and each fibril is raised slightly above the surface of the cylinder.

Another character of the outer segments which has been described is the transverse striation. This is demonstrated in

several ways. Its inconstancy of appearance in stained material is probably due to a uniformity in the laminæ or bands of an individual rod, which prevents differentiation under usual conditions. I have noted its appearance most frequently with methylen blue in fresh rods. Rarely transverse striation is to be seen in material stained by hæmatoxylin. Such a preparation is shown in Fig. 24 (Pl. 3). The cementing substance (Kittsubstanz, Greef, :00) is here evidently stained by the hæmatoxylin. This condition, in connection with other observed phenomena, has led me to suspect (see pp. 581, 596-597) that these excessively thin layers may be nerve conducting fibrils lying between thicker plates (catoptric in function or for isolation), and connecting on the outside of the rod with the longitudinal fibrils. Such a structure would seem to agree in general with that made out in invertebrate eyes (Schultze, Hensen, Patten, Parker, Hesse). I think we must agree that Schultze was right when he said: "However different the formation and development of the eyes of animals may be in general, still for the purpose of transferring the undulations of light to the domain of nervous conduction we may assume a conformity in the structure of the terminal organs" (Schultze, '72[a], p. 1006, '72[b], p. 826). The presence of transverse nerve fibrils has not been demonstrated, but such a condition would seem to be consistent with some polarized light reactions, and a few other observations, as I have already recorded (pp. 581, 596-597).

This hypothesis would, however, have to be supplemented to account for such further phenomena as the high degree of contractility and the alternating layers of optically different substances. These suggest an analogy in structure to muscle fibrils.

Direct observation of contraction in the outer segments of rods, such as I have reported, has not, I believe, been previously recorded. Several workers, however, have given the results of measurements on the visual elements examined in animals subjected to different degrees of light and darkness,

heat, etc., which showed elongation in darkness and contraction (as a rule) under the influence of light and heat (Hornbostel, '78; Angelucci, '85; Engelmann, '85; Gradenigo, '85; Krause, '92, p. 169; Herzog, :05).

In an attempt to explain the contraction of the visual cells we may employ a photo-contractile hypothesis (Cf. "Photo-musculaire theorie" of Dubois et Renaut, '89) and suppose that the final nervous impulse results from a mechanical stimulation (pressure or contact) produced by an intermediate process. The intermediate process would be the contraction of a structure (the free portion of a rod cell) extremely sensitive mechanically to differences of light or temperature; the contraction in response to heat or light might be conceived as comparable to that of a muscle from the effect (heat ?) of a motor-nerve discharge.

If to the photo-contractile hypothesis the objection were advanced that the introduction of mechanical stimulation is an unnecessary complication, it might be answered that it is consistent with observed parallel cases,—that the sensitiveness of a single cell to light might not be a type of stimulation transmissible over the long distances necessary to convey an impulse to the central nervous organs, in which case a transformation by secondary stimulation would be necessary.

To correlate the above hypothesis with the theory that rods are functional only for light of low intensities (see Rivers :00), we would suppose that the contraction was not due directly to light or heat but indirectly through the action of the visual purple, the chemical character of which was changed by light action.

That the laminæ of the outer segment in their dimensions come with the limits of the wave length of light and show a constant thickness has been taken, by some (Schultze, Greef, Zenker) to be of considerable significance (see p. 563). Schultze states that nocturnal animals and others living in diminished light have very long rods, thus increasing the number of plates. If stimulation depends on the relation of the

plates to wave lengths of light by some diffraction phenomenon, then expansion or contraction of the outer segment would necessarily affect the result by varying distances between plates.

Since I have not as yet systematically investigated the physiology of visual cells, my suggestions are purely tentative. Interesting as such considerations are, it must be granted that they can be little else than speculations until we have obtained a more complete knowledge of the structure of visual cells as well as further information as to their chemical and physical characters.

I wish now to discuss what I have called a "central core." The differentiation of this central core or axis in the outer segment occurs so frequently that I think we cannot but assume its existence in the living condition. When differentiated at all, this feature occurs in fixed and stained preparations as an unstained portion. On this account Greef (: 00) explained the appearance as a fixation effect and due to difference of consistency between the contents of the outer segment and its sheath. This hypothesis, however, does not explain the selective staining of the core by toluidin blue in fresh material, a fact which I consider of great weight. The irregular central staining mass in rods treated by Mallory's (: 00) triple stain is probably not identifiable with this body, since its distribution is inconstant and apparently limited to the center as a result of shrinkage only (Pl. 5, Fig. 38).

The more regular and constant appearance which I have mentioned as an axial core suggests the axial fiber, about which there has been so much controversy. This has been variously described, and, because of ambiguity in terms, it is scarcely possible to be certain, except from figures, to what the observations really referred. Probably the best known of these was the axial fiber described by Ritter, the presence of which was confirmed by Manz, Schiess, and Hensen, but not generally admitted by others. Schultze gave the question considerable attention, reporting ('72[a]; '72[b], p. 821) observations of his own on fresh mammalian material. He finally

concluded, however, that the appearance was due to optical effects alone. It is evident in some of the references to an axial structure that quite different objects were in the minds of the authors. Kuhnt (see Schwalbe, '87, p. 105) refers to the structure of the outer segment as, "Körnige axiale und eine streifige periphere Substanz." This description evidently, like that of Schneider (:02), who uses the term "Achsenstab," refers to all the mass of an outer segment inside the peripheral fibers, without any further differentiation within these. Dreser ('86) says, "Ich konnte eine axiale Substanz nachweisen," and goes on to describe it as an "Achsenkanal," which gives to a cross section of a rod the appearance of a ring. This differentiation he was able to bring out only by certain chemical treatments and stains. Bernard (:01, p. 465) in speaking of the conditions in a living rod says, that a reticulum is condenced into [*i. e.* to form] the axes of the rods, the reticulum being replaced by an inflow or absorption of substance from the pigment granules, through the walls of the outer segment. The fiber of Ritter as described and figured is too small in relation to the diameter of the rod to be identified with the axial core which I have observed. However, it seems probable from the figures of other investigators, that the same appearance gave rise to the various descriptions. For instance, an inspection of Hensen's ('67) figures shows that his cross section of the fiber are larger than they appear in the longitudinal view.

In connection with the double refraction of the outer segments I wish to call attention to some results which seem contradictory to my own. Valentine ('62) investigated with polarized light a large number of animal tissues including the rods of the retina and the axis cylinders of nerves, and as the following quotations show, he found that the reactions of these two bodies were not alike but opposite. "Die nähere Verfolgung des Gegenstandes zeigt, dass die optische Axe der Längsaxe der Nerven parallel geht; man also hier einen wahrhaft negativen Körper vor sich hat und die ganze Erscheinung nur von dem Marke herrührt" (Valentin, '62, p. 123). "Man

könnte theoretisch annehmen, das die Stäbchen an und für sich nicht anders, als die markigen Nervenfasern wirken" (p. 136). "Jene [Stäbchen] wären aber wahrhaft positiv und das Mark von diesen [Nerven] wahrhaft negativ" (p. 136).

It is thus evident that Valentin believed that the optical axes of the rods and of the nerve fibers were not in agreement, but were at right angles to each other, and this opinion was accepted by Max Schultze ('67), Krause ('92),* and Greef (:00).

It is not easy to account for Valentin's statement that the axis cylinders of nerves are negatively anistropic, unless we assume that in consequence of the imperfect knowledge of nerve structure at his time he has recorded the reaction of the *medullary sheath, which is negative,* instead of that of the *axis cylinder.* Valentin's work was done on Torpedo marmorata and shows that his observations were made almost entirely upon medullated nerves. It is quite evident that what he refers to as sheaths of the nerve must have been the positively reacting connective tissue of the peripheral nerves, for he makes no mention whatever of the brilliantly conspicuous *medullary sheath* as such. He does, however, speak of pressing out the retina of a frog with a cover-glass and finding fibers which he considers to be parts of the optic nerve. These, he states, also showed negative reactions, but there is no certainty that what he described were really optic nerve fibers.

In my tests of nerves I found medullated fibers unsatisfactory objects for clear demonstration of optical properties in the axis cylinder, because of the strong predominance of the reaction color of the medullary sheath. The non-medullated fibers from invertebrates (crayfish) were more satisfactory, but even here the presence of the positive Schwann's sheath, though comparatively thin, made conclusive observa-

*"Die Aussenglieder sind ferner positiv *doppelbrechend;* die optische Axe liegt in ihrer Längsrichtung und es ist bemerkenswert, dass sie sich entgegengesetzt wie das bekanntlich negativ Nervenmark verhalten." (Krause, '92, p. 159.)

tion out of the question, for the color of the sheath was projected on the less strongly reacting axis.

It was, therefore, necessary to use nerve fibers without protective coverings. The naked axis cylinders radiating from the entering optic nerve in the fiber layer of the retina, met this requirement. In order to get a clear demonstration of these, I made tests upon the retina from a perfectly fresh ox eye, where the large size of the eye made manipulation comparatively simple. In this case there was little difficulty in identifying the radiating bundles of nerve fibers, which were readily distinguishable from small blood vessels and other structures of a fibrous nature. The bundles of naked axis cylinders proved to be distinctly *positive,* thus agreeing with the rods, and I am consequently forced to conclude that in some way Valentin's observations were in this respect erroneous.

The agreement in reaction to polarized light between the outer segments of rods and the axis cylinders of nerve fibers, though not necessarily referable to identical structure, may be of significance. In the outer segment we would naturally look for some association between the polarization and transmission of light. According to the wave theory of light, the vibrations are at right angles to the direction of propagation. Since the axis of maximum elasticity is transverse in the rods we would seem to have in them a condition most favorable for the transmission of light rays in the direction of the longitudinal axis. This set of conditions may be simply incidental and not essential in the physiology of vision.

The reversal of polarization in the outer segments of rods with many reagents is a phenomenon the general occurrence of which is surprising, for one would naturally expect that killing fluids would destroy polarization (as some do) rather than that they would reverse it. The cause, which I only surmise, may be the contraction of certain structural elements producing a reversal of the conditions of stress in the body; *i. e.,* increasing the tension of one axis as compared with

that of another. Such an effect may be produced in a cylinder of glass which under ordinary conditions is isotropic; pressure at each end will produce polarization in the negative optical direction with respect to the cylinder axis, while tension at the same points will produce a positive reaction to the light. The latter would represent the normal condition in the outer segment of the rod; *i. e.*, the axis of maximum elasticity is at right angles to the cylinder axis.

From what has been shown as to the complex structure of outer segments, their delicacy and sensitiveness to slight stimulation, I think it will be unnecessary to try further to disprove the view that they are cuticular in nature or even secretions (pp. 567-568). Nor is there any good reason for considering the outer segment non-nervous, for, as I have shown, the fibrils run the whole length of the visual cell, and these fibrils satisfy well the conditions which Schultze ('72[a]; '72[b], p. 827) looked for when he wrote: "I at one time believed it possible to point out the way in which the outer segment might take a share in the act of perception, namely, by means of the fibers, discovered by me, which run over the surface of the inner segment and are continued upon the outer segment."

Having considered the characters of the outer segment, we may now take up the cell organs of the inner segment, treating of them in the order of their occurrence from the distal to the proximal end.

The *intermediate plate,* which has been observed in a number of forms, may be of more general occurrence than has been reported. Its small size would account for its having been overlooked, though it is usually differentiated clearly. The plate-like form and the fact that staining fibrils* pass over its edge only, and not through it, suggest an isolation function between inner and outer segments. Greef (:00) applied the name "Zwischenscheibe" to it, supposing that the structure had not been previously described; but from a glance

* Fibers which Schultze finally concluded were from sustentative tissue (Schultze, 72[b], p. 823).

at Schultze's ('67, p. 218, Taf. 13) description of what he calls the "Kittsubstanz," it is evident that he referred to the same object. The latter term has been since applied more generally to the substance between the plates of the outer segment. Herzog (: 05) figures an intermediate plate in both individuals of the double cones in Rana, but I am not certain of its presence in the cones of Necturus.

The *ellipsoid* or its homologue is found in most vertebrates. The name is hardly appropriate, for its usual form is not ellipsoidal; but such a term is better than "Aussenlinse," which assumes that its function is dioptric. Schultze's term, "outer lentiform body," has nothing objectionable except perhaps its rather prohibitive length. In our present state of knowledge concerning the ellipsoid, its significance, I think, can only be surmised. I can hardly agree with Pes (: 00) in his belief that it is a nucleus and that the visual cells extend as independent elements only to the membrana limitans externa. It is true that aside from what is usually taken to be the true nucleus, it is the distinctly chromophil portion of the element, and might have specialized nutritive functions, but I have not seen the slightest evidence that it ever exhibits karyokinesis. The visual cells, so far as I know, never divide normally in the adult animal, excepting possibly at the ora serrata. The highly refractive and clear appearance of the ellipsoid in the fresh condition, suggests a dioptric function, but again the frequent occurrence of globules within its substance is a fact not consistent with this view. That the globules are simply coagulation products is possible, yet they are perhaps too regular for that. In the cones of the goldfish the spherules are separated by equal intervals and are very uniform in shape and size (see also Hesse, : 04, Taf. 25, Fig. 4, 6; Chondrostoma, and Fürst, : 04, Taf. 3, Fig. 27, trout).

I have seen no connection between structures in the ellipsoid and the fibrils which pass over its surface. If, as has been supposed, the ellipsoid and paraboloid have each the function of a lens, we have here an interesting adaptation of

two bodies, in several ways quite dissimilar, to a like function. Contrasting them we find in the paraboloid little affinity for stains, comparative stability after death, persistent clearness and lower index of refraction, while the ellipsoid has a strong chromophilic character, marked instability, rapid clouding at death, and a higher index of refraction. Both are more refractive than the surrounding media and have such geometrical forms as to exert a marked effect on the light rays which traverse them.

The fibrils within the sheath of the *paraboloid* are probably what Hensen ('67) first described in the frog as the longitudinal striation of the inner segment. Schultze spoke of these as separate fibrils, saying that such were not demonstrable in the outer segment. If his observations were correct for internal and external fibrils in inner segments of the rods in man (Schultze, '72[b]; p. 824, top), then it would seem probable that the fibrils I have described as surrounding the paraboloid of Necturus would be the homologues of the internal fibrils.

The enveloping membrane reported by Merkel ('70) and Landolt ('71), I have not been able to find in Necturus; *i. e.*, there seems to be no conspicuous membrane distinct from the substance of the inner segment in which the longitudinal fibrils are embedded.

Observations upon the farther course of the stained fibrils over the proximal part of the visual cell are not numerous. Schneider (:02) describes these fibrils as passing over the nucleus. Hesse (:04) gives figures for the cones of Thalassochelys in which he shows spiral fibers proximal to the nuclei. I believe Bernard (:01) would have difficulty in upholding his contention that neurofibrils enter the nucleus. The fibrils which Schultze describes as forming the "fiber basket" (also Landolt, '71) surrounding the rods in the human retina, can hardly be identified with those I have described. The latter converge proximally in the rod foot (Pl. 2, Fig. 8) after leaving the nucleus. From the concentration in

the rod foot it is very evident that they diverge into the outer reticular layer. If the fibrils came from the "fiber basket" and belonged to the Müller's fibers, or sustentative cells, they would in general converge toward the nuclei of these cells located in the middle nuclear layer. Instead of this, the fibrils in question diverge on leaving the foot process of the visual cells.

Verhœff (:03) contends, with good reason, that the old idea that the membrana limitans externa is made up of the external end of the Müller's fibers, is incorrect. He finds a fenestrated membrane in the pigmented epithelium almost identical with the membrana externa, and argues that since this cannot be produced by Müller's fibers the probability is against that being the case in the membrana limitans externa. He finds these fenestrated, net-like membranes present generally in epithelia and believes they are produced by the epithelial cells themselves. Schneider (:02) describes in epithelia what are probably identical structures as "Schlussleisten" and "Desmochondren." I have examined Dr. Verhœff's excellent preparations of the human retina, in which this fibrillar membrane shows distinctly. I had previously seen the same in the pigmented epithelium of teleost retinas and puzzled over its meaning. Retzius (:05), however, shows in the pigment epithelium of the dogfish *sustentative* cells producing long intercellular fibrils. If such elements are generally present in retinal pigmented epithelia, Verhœff's opinion would be somewhat discredited. However, Verhœff states that such are not present in man.

B. Cone Cells.

Every indication points to a complex structure in the outer segments of cones and, because of their instability, to a more difficult problem than in the corresponding part of the rod. This peculiarity, making it difficult to obtain outer segments of cones in the fresh condition, accounts probably for the absence of reports as to their double refraction. That

the cones of some animals possess spiral fibers, seems fairly certain. Whether there is both an external straight system and an internal spiral one, as maintained by Hesse (:04), is open to question.

The external straight fibrils in Necturus have been sufficiently demonstrated. As to the inner dark staining structure, since it so frequently resembles a spiral, and since the latter is demonstrable in some animals, the balance of evidence would perhaps be in favour of the existence of that type of structure. That the external fibrils have a mechanical function (Hesse, :04), I think is no more likely than that that should be the office of a spiral. The high degree of contractility in the cone would not be inconsistent with a contractile spiral, such as is to be seen in the stalk of Vorticella. I have observed no sign of spiral fibrils in the inner segment of the cones of Necturus or the goldfish, except an occasional slightly oblique direction of the peripheral stained fibrils in Necturus. The double circle of fibrils, as seen in cross sections of inner segments of the cones, may, however, have some special significance, though they are, so far as I have been able to determine, all of the same character.

C. Double Cones.

The double cones of Necturus, showing the extreme of differentiation and evident specialization for particular functions, may offer a clue as to the functions of the various parts of the visual cells, and perhaps indicate the original line of differentiation between rods and cones. The far-cone, with its marked development of fibrils and other characteristics, practically the same as the single cones, evidently functions as these do, while the rear-cone, with its irregular and enormously enlarged paraboloid and ellipsoid of different form and staining qualities, must have a different office. On the theory that the nuclei of cells in general have a trophic function, it seems not unlikely that the near-cone

takes a large part in the nutrition of these joined cells. This is suggested by the relations of its nucleus. The process which the latter sends distally down over the side of the paraboloid must be of significance. The surface of the nucleus is increased thereby and noticeably that part of its surface which is in contact with the paraboloid and, through the paraboloid sheath, with the more distal cell organs. The nuclear sheath in this region seems to be very thin, or entirely absent, especially at the distal edge of the process. Here it is difficult to make out any distinction between nucleoplasm and cytoplasm, as there is no sharp differentiation in the staining of the two. The dark staining nucleoplasm, however, shades off gradually, as if there were some limit, though an indefinite one The constant difference in staining reactions between the two nuclei would seem a further indications of different functions. And again the loss of symmetry of the near-cone as to its nucleus and paraboloid must be associated with a loss of a light receiving office, as rays would not have the regular disposition that must result from the lens-shaped nuclei and paraboloid of the ordinary visual cells.

If we seek between double and single cones a distinct difference that might be of physiological importance in relation to visual function, it would seem to be the greater distance of the outer segments of double cones from the source of light. There is evidently a rather small range of contraction in the double cones, so that their outer segments are more constantly remote from their nuclei. If the increased distance were an advantage for a visual function, the distance from the nucleus might demand some new adaptation for more direct nuclear relations.

Levi's (:00) opinion that the double cones come from a single embryonic cell seems quite plausible. If that is the case, probably the nuclear division is complete while the cytoplasmic is less so. I have distinguished two nuclei in a great many cases and therefore do not agree with Schultze ('67) in the opinion that only one nucleus is present.

IV. SUMMARY.

1. The visual rods and cones of vertebrates represent distinct and separable elements of a sensory epithelium.

2. These elements are cells, usually much elongated, having a proximal fixed portion, containing the nucleus, and an extranuclear part ending free in the "ventricle of the primary optic bulb," or, more strictly, its morphological equivalent in the adult.

3. The fixed or nuclear portion is in close contact with other elements of the retina lying within the membrana limitans externa. It possesses in addition to the nucleus, a basal cytoplasmic extension, the rod-, or cone-foot.

4. The free portion (rod or cone) consists of two parts distinguished by chemical and optical properties, the inner and outer segments.

5. In Necturus there are present three distinctly differentiated types of visual elements called rod-cells, cone-cells, and double cone-cells. These have the following structure:

Rod Cells.

1. The outer segment has the form of a cylinder with a rounded distal end.

2. A sheath is demonstrable after fixation with osmic acid. On the inner side of the sheath are longitudinal, parallel, highly refractive fibrils, twenty to thirty in number, extending the whole length of the segment. Usually the fibrils vary from the strict longitudinal course so as to form a very open spiral. They project slightly on the surface, so as to produce a longitudinal ribbing.

3. With some stains and under certain light conditions the outer segment exhibits a banded appearance, as of alternating narrow and broad transverse stripes. As cleavage occurs on such lines, it seems probable that the inner substance of the rods is arranged in plates.

4. A further differentiation of the inner substance is an

axial portion, which is about one-quarter of the diameter of the rod and extends the whole length of the outer segment.

5. The outer segments contain a substance resembling the myelin of the medullated nerve sheaths. Evidence for this is the similarity in color produced by treatment with osmic acid and by other tests.

6. The outer segments are anisotropic with the positive optical direction parallel to their long axes. In this regard they agree with the axis cylinders of nerves and with muscle fibrils. In each of these bodies, then, the axis of maximum elasticity is transverse to the longitudinal axis of the fiber.

7. Outer segments in the fresh state when examined in eye fluids show a considerable range of contraction and elongation. If a very dilute foreign fluid is added, a rapid contraction occurs, like the reaction of a living body; this is followed by a gradual elongation to a length greater than the original. Elongation of a permanent character is produced by certain fluids of "fixing" strength. The contractility and transverse striation together with other characters suggest a possible structural analogy with muscle.

8. Between the inner and outer segments is the intermediate plate. This structure appears as a barrier between the two segments, in that the continuity seems to be interrupted, except for the fibrils, which pass over the edge of the plate.

9. The inner segment is in general form cylindrical, but less rigid in its outlines than the outer. No sheath comparable to that of the outer segment seems to be present. The proximal portion (myoid) shows considerable contractility. Two enclosures in the inner segment take up most of the space. These are the ellipsoid and the paraboloid. Outside of these are longitudinal fibrils, which are continuations of those on the outer segment. In the inner segment they lie so deep as not to affect the contour of the surface.

10. The ellipsoid varies in form from convex-concave to plano-concave, with the concave side proximal. It is composed

of a highly refractive substance strongly chromophilic. In fixed preparations this is often resolved into globules.

11. The paraboloid has the general form implied in its name. Distally it fits into the concave side of the ellipsoid; proximally it is often pressed against the surface of the nucleus, causing an involution of the latter. The paraboloid can be isloated and is more stable than the ellipsoid, but less refractive. Its substance does not stain readily. Paraboloid and ellipsoid must exert a definite action on the rays of light traversing them, because of their form and high refractive index, as compared with the surrounding substance.

12. The fixed portion of the rod cell contains the nucleus with its very thin cytoplasmic sheath, which is continued proximally in the rod-foot. The nucleus is an oblong to ovate spheroid in form. It is larger than the other nuclei of the retina and contains chromatic substance, usually in a finely divided form. Nucleoli are usually present. In the plasmic sheath are longitudinal fibrils, continuations of those seen in the outer and inner segments. These pass into the foot, whence they diverge into the outer reticular layer of the retina.

Single Cone Cells.

1. The outer segments have a conical form. In Necturus peripheral longitudinal fibrils are present, as in the rods.

2. The interior has a banded appearance, which is due either to a lamellar structure or represents the edge of a spiral. In the goldfish, four to five spiral fibrils are present having a peripheral position. This system only was observed in the outer segment, which suggests that it may be the homologue of the peripheral, stained fibrils in Necturus.

3. The composition of the outer segments of the cones differs in several respects from that of the rods. One of these is the presence of a substance having a marked affinity for methylen blue, another, the presence of a small proportion of "myeloid" substance.

4. The polarization is like that of the rod under ordinary conditions; *i. e.*, the cone axis lies in the positive optical direction, but the reaction is less decided. The evidence indicates that there are fibrillar elements present not responding to the predominating reaction.

5. No intermediate plate was observed in the cones of Necturus.

6. The ellipsoid differs slightly from that of the rod cell in its reaction to stains and in its form, which is more elongated in an axial direction.

7. The paraboloid seems essentially like that of the rods. The separation between it and the nucleus is greater in the cones than in the rods.

8. The nucleus of the cone differs from that of the rods in being an ovate spheroid with its small end distal. In other respects the nucleus of the cone cell seems to be like that of the rod cell.

Double Cone Cells.

1. The double cones consist of two complete cone cells united along their sides from their nuclei to their ellipsoids. The two individuals are evidently specialized for different functions, since homologous parts in each differ in form and staining properties, excepting perhaps the outer segments, which are not directly connected.

2. The far-cone retains the essential structural characters of the ordinary cones and evidently has the same function as these.

3. The near-cone is so unlike its fellow as to make it probable that its function is different. The nuclear relations suggest that the near-cone has a specialized trophic function for the pair, while the special development of fibrils in the far-cone and other considerations would indicate that its function was more highly developed along the line of that of the single cones.

General.

The fibrils which are seen in the three kinds of visual cells and pass from the outer limbs into the outer reticular layer seem to be intercellular neurofibrils. Their function is presumably that of conducting nerve impulses from the end-organ to the more central elements of the retina, ultimately to the optic centers. That these fibrils may arise from more central retinal elements and penetrate the more peripheral ones is conceivable, but in my opinion they have more probably taken their origin in the visual cells themselves.

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* TREVIRANUS, G. R., '36. Beiträge zur Aufklärung der Erscheinungen und Gesetze des organischen Lebens. Bd. 1, Heft 2, p. 42. Papillen der Netzhaut des Auges. Bd. 1, Heft 3, p. 91, Nachträge, Taf. 3-6. (Arch. f. Anat., Physiol. u. wiss. Med., Jahrg. 1837, Jahresbericht; Greeff, :00.)

* VALENTIN, G., '61. Die Untersuchung der Pflanzen- und der Thiergewebe in polarisirtem Lichte. Leipzig, 8°, vi + 312 pp., 3 Taf. (Valentin, '62.)

VALENTIN, G., '62. Histologische und physiologische Studien. Zeit. f. rationelle Med., Reihe 3, Bd. 14, pp. 122-181.

VERHOEFF, F. H., :03. A hitherto undescribed Membrane of the Eye and its Significance. Boston Med. Surg. Jour., Vol. 149, pp. 456-458.

WATASE, S., '90. On the Morphology of the Compound Eyes of Arthropods. Studies Biol. Lab. Johns Hopkins Univ., Vol. 4, No. 6, pp. 287-334, pls. 29-35.

WIEDERSHEIM, R., '86. Lehrbuch der vergleichenden Anatomie der Wirbelthiere. Jena, Auflage 2, xiv + 890 pp., 614 Fig.

ZENKER, W., '67. Versuch einer Theorie der Farben-Perception. Arch. f. mikr. Anat., Bd. 3, pp. 249-262.

VI. EXPLANATION OF PLATES.

All figures are from preparations of Necturus, except where otherwise stated. All, with the exception of Figures 22 and 46, were drawn with the aid of the camera lucida.

The magnifications are indicated in the descriptions of each plate, and the means of obtaining them are shown in the following table:

OBJECTIVE	MAKER	OCULAR	MAKER	TUBE LENGTH	MAGNIFICATION IN DIAMETERS
E	Zeiss	No. 3	Zeiss	136 mm.	830
E	Zeiss	" 4	Zeiss	136 "	900
$\frac{1}{18}$	Zeiss	" 4	Zeiss	136 "	1300
"	Zeiss	" 12	Zeiss	136 "	3800
$\frac{1}{12}$	Leitz	" 4	Zeiss	136 "	1450
"	Leitz	" 4	Zeiss	136 "	1000
"	Leitz	" 8	Zeiss	136 "	1450
"	Leitz	" 8	Zeiss	158 "	1850
"	Fuess	" 1	Fuess		1000
No. 9	Fuess	" 1	Fuess		450
No. 5	Fuess	" 1	Fuess		300

ABBREVIATIONS.

ax. cyl., axis cylinder.
bac. vir., green rod.
cl. pig. rtn., retinal pigment cell.
con., cone.
con'., double cone.
con. dst., far cone.
con. prx., near cone.
cp., corpuscle of fibril.
dsc., plate.
dsc. i'm., intermediate plate.
ell., ellipsoid.
ell. bac., ellipsoid of rod.
ell. con., ellipsoid of cone.
ell. con. dst., ellipsoid of far cone.
ell. con. prx., ellipsoid of near cone
fbrl., fibril.
gran. ell., granules of ellipsoid.
gtt. ol., oil globule.
ivlr., sheath.
ivlr. nl., nuclear sheath.
ivlr. pa'b., sheath of paraboloid.
ivlr. prs. dst., sheath of outer segment.
med., axial core.
mb. lim. ex., membrana limitans externa.
my., myoid.
my. bac., myoid of rod.
my. con., myoid of cone.
my. con. dst., myoid of far cone.
my. con. prx., myoid of near cone.
nl., nucleus.
nl. bac., nucleus of rod.
nl. con., nucleus of cone.
nl. con'., nucleus of double cone.
nl. con. dst., nucleus of far cone.
nl. con. prx., nucleus of near cone.
nll., nucleolus.
pa'b., paraboloid.
pa'b. bac., paraboloid of rod.
pa'b. con., paraboloid of cone.
pa'b. con. dst., paraboloid of far cone.
pa'b. con. prx., paraboloid of near cone.
pd. bac., foot of rod.
pd. con. prx., foot of near cone.
pd. con., foot of cone.
pd. con'., foot of double cone.
pd. con. dst., foot of far cone.
prc. cl. pig., process of pigment cell.

prc. nl., nuclear process.
prs. dst., outer segment.
prs. dst. bac., outer segment of rod.
prs. dst. con., outer segment of cone.
prs. dst. con. dst., outer segment of far cone.
prs. dst. con. prx., outer segment of near cone.
prs. prx., inner segment.
prs. prx. bac., inner segment of rod.
prs. prx. con., inner segment of cone.
st. nl. ex., outer nuclear layer.
st. nl. m., middle nuclear layer.
st. pig., pigment layer.
st. ret. ex., outer reticular layer.
vac., vacuole.
vsl., vesicle.

PLATE 1.

All figures are from Necturus and are magnified 1,450 diameters. All preparations were fixed in corrosive-acetic mixture and stained in Heidenhain's iron-alum-haematoxylin.

FIG. 1.—Two rods and one cone as seen in a radial section of the retina. The small rod at the right shows the peripheral system of stained fibrils. These are seen most distinctly at low focus; *i. e.,* on the lower surface of the rod. The sides are out of focus. Small portions of the rods are broken away at the distal end. (Cf. other figures.)

The paraboloid *(pa'b. bac.)* of the large rod, drawn in optical section, is clearly eccentric, a condition not very rare. The nucleus and other parts are shown from a focus at the near surface.

The cone (on the left) has an appearance typical of this material. The outer segment is much vacuolated and with only a slight general stain. Superficial fibrils show faintly and are slightly oblique. The fibrils over the paraboloid are more distinct. The paraboloid itself is out of focus.

The heavily stained granules are characteristic of the ellipsoid *(ell.)* of the cones.

FIG. 2.—The double cones showing the marked differentiation between the individual elements of the couplet. In *A* the couplet is seen laterally; *i. e.,* both cones are in the plane of section. In *B* the couplet lies in a plane at right angles to that of the section, and the far cone, only, is in focus. A comparison of the different views of the myoids *(my. con. dst.)* in the far cones in *A* and in *B* shows the flattening of the myoid.

FIG. 3.—Cross section of an outer segment of a cone; the surrounding elements are in outline only. The peripheral, stained fibrils are seen in section and also obliquely lengthwise; they are separated by some little space from the dark stained center.

FIG. 4, A.—Cross section of a cone through the paraboloid. The stained fibrils occur in two concentric circles, neither of which is on the surface.

FIG. 4, B.—Cross section of a rod through the paraboloid; the fibrils are closer to the paraboloid than to the surface.

FIG. 5, A.—Cross section of a double cone through the paraboloid of the near cone, and the myoid of the far cone.

FIG. 5, B.—Cross section of the distal end of a nucleus of a rod showing fibrils in the surrounding cytoplasm.

FIG. 6.—Cross section of the ellipsoid of a rod. The stained fibrils are superficial. The ellipsoid granules are distributed through the central portion.

Fig. 1

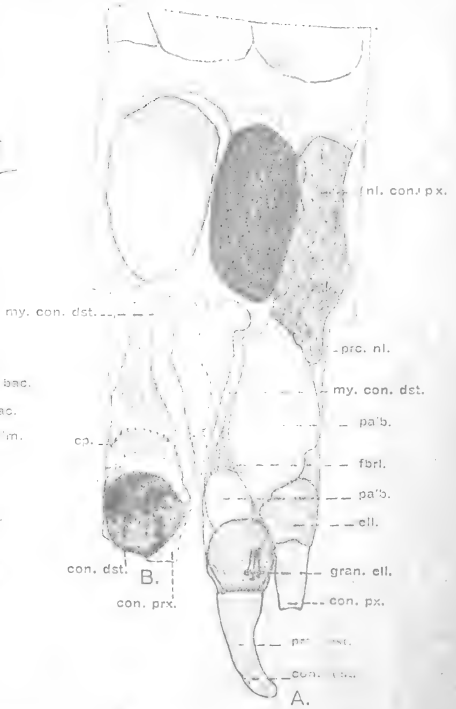

Fig. 2

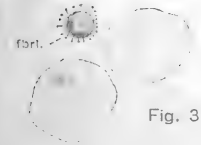

Fig. 3

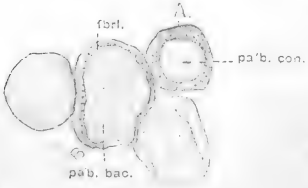

Fig. 4

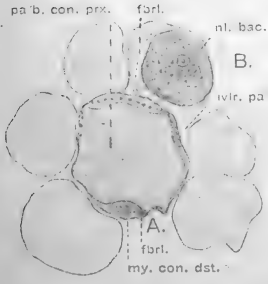

Fig. 5

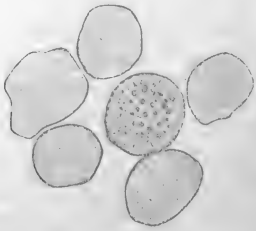

Fig. 6

PLATE 2.

All figures are from Necturus. Figures 7 to 11 are magnified 1,450 diameters; Figures 12 to 16 are magnified 1,000 diameters.

FIG. 7.—A large rod, slightly separated from its nucleus, and a double cone, the near cone in focus. In the latter, superficial fibrils are visible at the base of the outer segment, passing over the ellipsoid, paraboloid, and nucleus, and converging proximal to the nucleus in the cone foot. The fibrils of the rod in this particular preparation are more diffuse in the region of the foot than those in the foot of the neighboring cone. The material was fixed in corrosive-acetic mixture and stained in Mallory's triple stain (Mallory, :00).

FIG. 8.—A single rod showing the relation of the stained fibrils to the nucleus and the rod-foot. The fibrils of adjacent elements are distinct until they diverge at the outer reticular layer of the retina. The nucleus of the rod in the center has shrunken away from its sheath, at its proximal end. The parabaloid in optical section gives the appearance of a coarse blue reticulum. The fixation and stain were the same as in Fig. 7.

FIG. 9.—A cross section of the outer segment of a rod. The fibrils are distinct when seen in section. As a rule, vacuoles alternate with the fibrils.

FIGS. 10-11.—A cone and a rod, fresh, in normal fluids of the eye, and drawn in situ a few minutes after the removal of the retina from the living animal. Only a small portion of the nucleus of the rod is shown. The changes which so rapidly set in upon the death of the animal have already begun here. The outer segment of the cone, most unstable of all, shows evidence of disintegration; granulation has commenced in the ellipsoids and is more pronounced in the nuclei. The parabaloid remains comparatively clear for some time. The latter is more refractive than the sheath, but less refractive than the ellipsoid. In the cone (Fig. 10) the paraboloid is a great deal more flattened than usual. A common relation of paraboloid and nucleus for fresh cones is shown in Pl. 3, Fig. 20.

FIGS. 12 to 16.—Outer segments of fresh rods in fluid from the eye, showing progressive disintegration. Figures 12 to 14 were drawn within half an hour after the rods were removed from the eye. In Figure 12 the upper surface is in focus and only one diagonal is visible, while in Figure 13, where the lower surface is in focus, several of the diagonals (fibrils) are visible. The difference in the number of lines visible is apparently due to the optical effect of the cylinder. Figure 14 represents the appearance of a rod at the very lowest focus at which lines are visible. The more highly refracting fibrils are dark in low focus, while the less refractive intermediate substance appears light.

FIG. 17.—An outer segment of a fresh rod seen in optical section. This view was obtained by laying a portion of a retina flat on a slide and focusing upon the rods in situ. In a retina thus examined the crenated edge of rod outer segments is very apparent, and in many instances where disintegration has commenced, radial fissures of variable length are to be seen extending inward from the sinuses. The rods that are still intact show at first sight a homogeneous center with a crenated sheath; by close inspection this sheath proves to be made up of separate bodies (the fibrils in section). These are brought out more distinctly where the rods are illuminated by a direct light from the side.

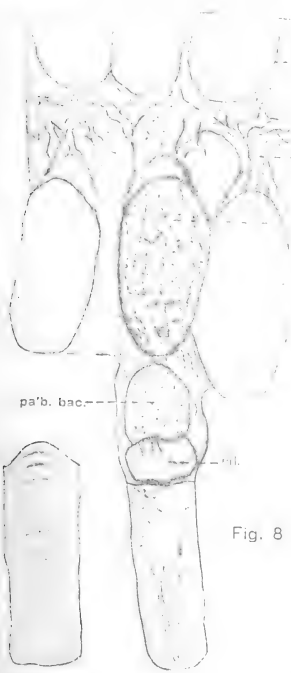

Fig. 16

Fig. 8

Fig. 9

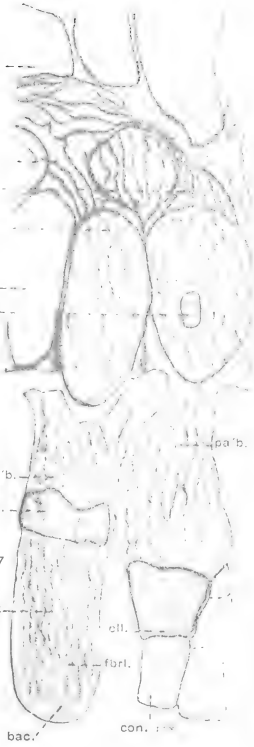

Fig. 7

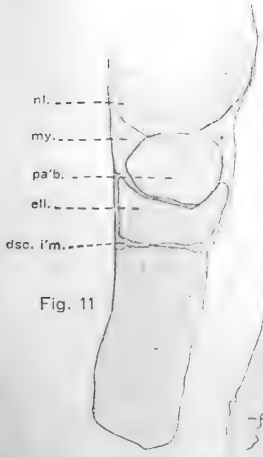

Fig. 11

Fig. 10

Fig. 17

Fig. 12 Fig. 13 Fig. 14

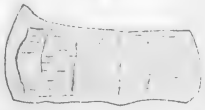

Fig. 15

PLATE 3.

Figures 18, 19, 21, 23 and 24 are magnified 1,450 diameters; Figure 20 is magnified 1,000 diameters.

All the preparations, except that shown in Figure 22, were fixed in corrosive-acetic mixture and stained with Heidenhain's iron-alum-hæmatoxylin; all are from Necturus, except Figures 18, 19.

FIGS. 18, 19.—Visual elements of goldfish seen in radial sections of the retina. In Figure 19 the three different types of elements, rods, cones and double cones are shown as they occurred in actual section. The rods with long, thread-like inner limbs occur at varying distances from the membrana limitans externa. The double cones with long inner limbs are more distal than the single cones. The outer nuclear layer consists of four or five strata of large cone and smaller rod nuclei.

In Figure 18 a double cone is shown in detail, only one of the couplet, however, being in focus. In the outer limb two to four spiral bands are seen. These do not correspond in number or in size with the straight parallel fibrils of the inner limb, seen just distal to the nucleus. The light area proximal to the ellipsoid is probably the homologue of the paraboloid in amphibians.

FIG. 20.—A portion of retina embracing the pigment layer and part of the outer nuclear layer. A rod and single cone are shown in detail and a double cone in outline. The end of the outer segment of the rod is surrounded by processes from the pigment cells. The nuclei and inner segments are in optical section; the outer segments in superficial focus.

FIG. 21.—The near cone of a double cone showing plate-like structure. Vacuolation on opposite sides between alternate "plates" gives a spiral appearance. Superficial fibrils are visible at the Proximal end of the outer segment.

FIG. 22.—A fresh cone in fluids from the eye and shortly after removal from the eye. The animal from which this was taken had been kept in the dark three days. This treatment induced elongation of the cone, but probably not to the extent seen here. The extreme elongation is a characteristic form assumed by the outer segments of cones upon the death of the organism. This outer segment gave the *negative* reaction with polarized light, being yellow when at right angles to the *a* axis of the gypsum plate.

FIG. 23.—A cone typical of heavily stained material. In the center of the outer segment are structures which do not persistently retain the hæmatoxylin stain. The appearance is that of plates connected by a lightly stained axis. Their oblique relation to the latter suggests a spiral, made up of a continuous band or broad fiber. However, an entirely satisfactory demonstration of such a spiral has not been secured in Necturus. At the distal end of the outer segment superficial fibrils are visible, which take a direction slightly oblique to the long axis of the cone. Between the nucleus and ellipsoid the superficial fibrils are straight. The paraboloid is drawn a little more distinctly than it would appear if the fibrils had been in focus.

FIG. 24.—Outer segment of a rod showing evidence of "plate" structure of great regularity.

FIG. 25.—A portion of a cone. Distinctly staining, superficial fibrils are visible on the fragment of the outer segment. The ellipsoid is densely stained with hæmatoxylin.

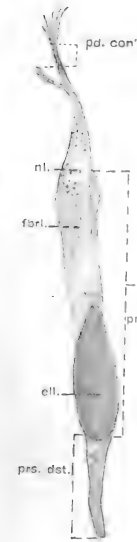

Fig. 18

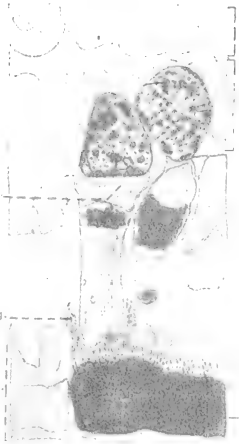

Fig. 20

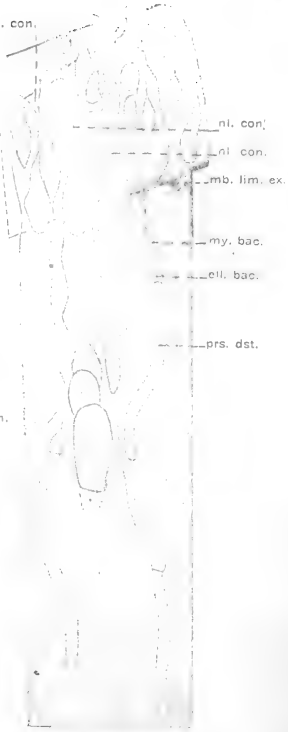

Fig. 19

Fig. 22

Fig. 23

Fig. 21

Fig. 24

Fig. 25

PLATE 4.

Figures 26-31, 37, from the *frog;* Figures 32-36, from *Necturus.* Figures 26 to 31 are magnified 1,300 diameters; Figure 32, 1,450 diameters; Figure 33, 900 diameters; Figure 34, 3,800 diameters; and Figures 35-37, 1,000 diameters.

FIG. 26.—Outline drawing of visual cells from the *frog* retina in situ. The outer nuclear layer is two nuclei deep. In the red rods the inner segments are short; in the green rods the myoid portion of the inner segments is long. The cones are much smaller than the rods and contain an oil globule between the outer segment and the ellipsoid.

The material was fixed in Perenyi's fluid and stained with Heidenhain's iron-alum-hæmotoxylin.

FIG. 27.—A single rod from the group shown in Figure 26. Detail is shown at the distal end of the outer segment and over the whole of the inner segment. Longitudinal peripheral fibrils are visible on the outer segment over the intermediate plate and on the myoid of the inner segment.

FIG. 28. Cross sections of outer segments of the same material as that shown in Figures 26 and 27. A central axial portion is differentiated from the peripheral part. The latter is subdivided by lines which correspond in number with the longitudinal fibrils on the surface.

FIG. 29.—A red rod from a radial section of the retina of a frog. An axial core is visible in the whole outer segment. In the inner limb is a body corresponding to the paraboloid in Necturus. The material was fixed in corrosive-acetic mixture and stained in Heidenhain's iron-alum-hæmatoxylin and orange G.

FIG. 30.—Cross section of the outer segment of a rod from the same material as that shown in Figure 29.

FIG. 31.—A green or short rod from the frog as seen in a radial section of the retina including the pigment layer. The fixation is the same as for the preparation shown in Figure 29; the preparation was stained with fuchsin.

FIG. 32.—Visual cell of *Necturus* fixed in vom Rath's fluid.

The outer segment is very much blackened and contracted. In a thin section the longitudinal grooving appears as light lines in the dark substance of the outer limb.

FIG. 33.—Rod and cone cells of *Necturus* fixed five minutes in osmic acid fumes and isolated in glycerine.

The individuality of the cells is demonstrated. The osmic acid has induced rather forcible contraction of the inner segments and the cleavage in the outer segments of the cone is excessive.

FIG. 34.—Cross section of the outer segment of a *Necturus* rod, fixed in osmic acid fumes and seen under high magnification. The

(Continued on next page.)

PLATE 4.

(Continued from preceding page.)

sheath, which has stained very black, is shown thicker in the drawing than is commonly seen. The light spaces beneath the sheath seem to correspond to the fibrils of material stained in hæmatoxylin.

FIGS. 35, 36.—Longitudinal and cross sections of a rod of *Necturus* fixed in osmic acid fumes. The grooving of the outer segment appears as alternating light and dark lines in the thin longitudinal section (Fig. 35). In the cross section (Fig. 36) of the outer segment the grooving appears as crenations of the boarder. Inside of the black sheath the light zone appears continuous at this magnification.

FIG. 37.—Cross sections of the outer segments of the rods from a *frog*. The fixation and magnification are similar to those of Figures 35 and 36. No crenation of the edge is distinguishable, but an outer black sheath and an inner light band can be seen.

Fig. 26

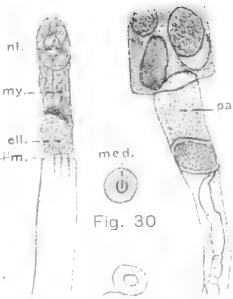

Fig. 27

Fig. 30

Fig. 28

Fig. 32

Fig. 29

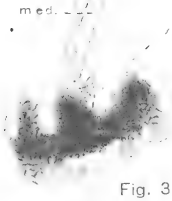

Fig. 31

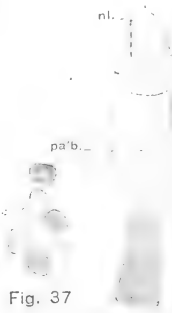

Fig. 37

Fig. 35

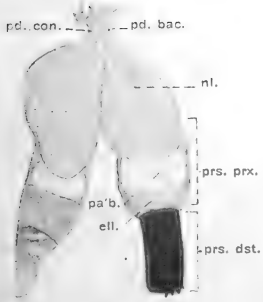

Fig. 33

Fig. 34

Fig. 36

PLATE 5.

Figures 38 and 45 are magnified about 1,000 diameters; figures 39 and 40, 1,450 diameters; figures 41 and 42 about 1,100 diameters.

FIG. 38.—Visual elements as seen in a radial section of the retina of *Necturus*, showing different staining qualities. The ellipsoids of single cones and of far cones stain alike. Those of the near cone and of rods also stain alike. All outer segments of cones stain alike The outer segments of rods contain a blue staining reticulum in addition to the substance that stains with fuchsin. The material was fixed in corrosive-acetic mixture and stained with aniline blue, fuchsin, orange G., phospho-molybdic acid, etc. (Mallory : 00).

FIG. 39.—Thin section of a cone from the same material as that shown in Figure 38. The substance of the ellipsoid, staining with fuchsin, is in the form of globules, the arrangement of which gives some suggestion of rows. Superficial fibrils staining blue are visible between the ellipsoid and the nucleus.

FIG. 40.—A field of the outer segments of visual cells from the same material as that shown in Figure 38. The superficial blue fibrils are seen as dots in both rods and cones. One rod is drawn in detail. In this the peripheral portiors are much vacuolated and devoid of substance staining in fuchsin.

FIGS. 41, 42.—Isolated outer segments of rods in natural fluid from the eye, but containing a trace of ammonium-molybdate followed by toluidin blue. A central portion has taken up the blue stain in a somewhat irregular war. The irregularity is probably due to a post-mortem disintegration. In Figure 42 the central staining core projects beyond the peripheral substance.

FIG. 43.—A field containing fresh isolated rods and a cone in eye-fluids as seen with a polarizing microscope having a gypsum interference plate. The latter when inserted between the Nicol prisms gives as a field color, red of the first order, while bodies in the field possessing polarity are yellow or blue according as their axes of maximum elasticity lie at right angles or parallel, to the *a* axis of the gypsum plate. This test shows that the long axes of the outer segments of both rods and cones are in the positive optical direction, indicating the short axis as that of maximum elasticity. The outer segment of the cone shows a much less decided reaction than that of the rod, while the inner segments of both show the neutral field color.

FIG. 44.—A single medullated nerve fiber from the sciatic nerve of the *frog* as seen by the same color test as that described under Figure 43. The medullary sheath shows a very decided double refraction with the axes of minimum elasticity radial with respect to the axis-cylinder. The reaction of the axis cylinder, though not decided in this instance, is discernible as opposite in character to that of the sheath.

(Continued on next page.)

PLATE 5.

(Continued from preceding page.)

When the sheath is absent the reaction of the axis cylinder is seen to be unmistakably the same as that of the outer segments of the rods; *i. e.*, the longitudinal axis is the positive optical direction.

FIG. 45.—Outer segments of a rod and cones from a *Necturus* that had been kept in the dark three days. The outer segment of the cone gives the long axis as the negative optical direction instead of the positive as usual.

FIG. 46.—Diagram showing the polarization color reaction observed in the visual rod of the slug Limax maximus. The fibrillar structure is shown in black as figured by Dr. Grant Smith (:06) from a preparation fixed in vom Rath's platino-osmo-acetic mixture.

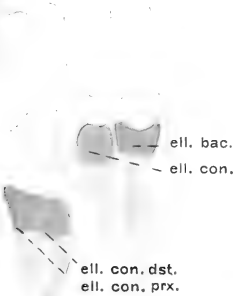

Fig. 38

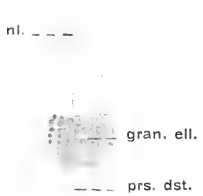

Fig. 39

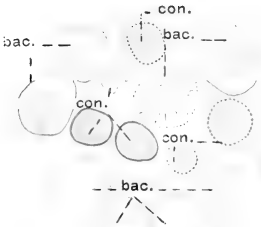

Fig. 40

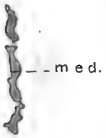

Fig. 41

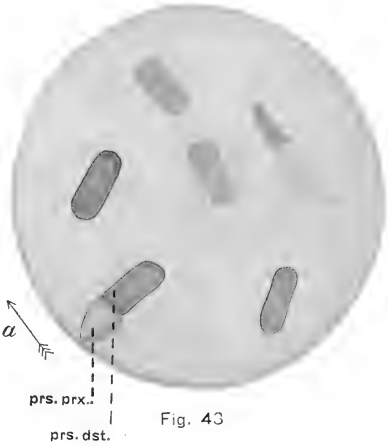

Fig. 43

Fig. 42

Fig. 44

Fig. 46

Fig. 45