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Historia de las células germinales de la lamprea de arroyo, Entosphenus wilderi (Gage) hasta el periodo de diferenciación.

Las células germinativas se segregan de las restantes en una época muy temprana de la vida del animal, aun antes de haberse formado definitivamente las hojas germinales. Pueden reconocerse por primera vez cuando el mesodermo se separa del endodermo (embrión de unas 191 horas de edad). Las células masculinas y femeninas definitivas no reconocen otro orígen que el de las células germinales primordiales; las células germinales tampoco toman parte en la formación de estructuras somáticas. Muchas de las células germinales degeneran y desaparecen en cada individuo.

Durante el periodo de diferenciación sexual las células germinales de cada glándula germinal son claramente de dos tipos: Unas que presentan marcada tendencia hacia una división contínua (células catabólicas) y otras que tienden a crecer (células anabólicas). El autor considera a las primeras como posesoras de una potencialidad masculina, y a las segundas como femeninas. La proporción relativa de células anabólicas y catabólicas determina si la larva ha de ser un macho o una hembra. Las observaciones del autor parecen justificar la conclusión de que cada larva de esta especie lleva la potencialidad para producir los dos sexos, y que el sexo, por consiguiente, no se fija de modo irrevocable en el momento de la fecundación. En el trabajo se describen las diversas estructuras nucleares y citoplásmicas y los cambios que sufren durante las diversas fases del desarrollo.

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THE EARLY HISTORY OF THE GERM CELLS IN THE BROOK LAMPREY, ENTOSPHENUS WILDERI (GAGE), UP TO AND INCLUDING THE PERIOD OF SEX DIFFERENTIATION

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FOUR TEXT FIGURES AND TWELVE PLATES (SEVENTY-EIGHT FIGURES)

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INTRODUCTION

Owing to their bearing on current theoretical questions, certain phases of the germ-cell cycle in vertebrates have received more attention than others. Maturation has been fully studied because it appears to involve a redistribution of parental hereditary factors. The small size and the brief maturation period of the spermatocytes make it possible to obtain all stages very easily on a single slide, whereas much time and material are needed to obtain the stages of maturation in the much larger oocytes. Since it usually is assumed that the maturation phenomena are practically identical in the two sexes, the male has been commonly selected for the study of this period because it presents lesser technical difficulties. A second phase on which attention has been focused in recent years, because of its bearing on the question of germ-plasm continuity, is that of the origin and early development of the germ cells. The period of differentiation of the male and female sex cells from the primordial germ cells has been relatively neglected, apparently because of the belief that in all animals sex is irrevocably determined at or before fertilization through the agency of sex chromosomes. The result is that we have much literature on isolated periods in the germ-cell history of vertebrates, chiefly in the male sex, but few comprehensive accounts.

The work of Brock ('81), Schreiner ('04), R. Hertwig ('05, '06, '07), and others has shown that in both sexes of certain species of vertebrates there is a tendency toward hermaphroditism at the period of differentiation of the primordial germ cells—an indication that sex is not irrevocably determined at the time of fertilization. This fact and the lack of a complete account of the germ-cell cycle in any vertebrate led me to undertake a study of the whole history of the germ cells in both sexes in the American brook lamprey, Entosphenus wilderi (Gage), in which species there is a decided tendency toward a condition of juvenile hermaphroditism. This seemed the more worth while because the lampreys and the hag-fishes are now elevated to a separate vertebrate class, and in this class very little work has been done on the germ-cell cycle.

I wish to express my sincere appreciation of the help and encouragement received from Prof. Jacob Reighard during the progress of the work.

SPAWNING HABITS AND LIFE-CYCLE OF ENTOSPHENUS WILDERI

A. Spawning habits

Entosphenus wilderi is abundant about Ann Arbor in several streams tributary to the Huron River. All the material used in the present study was collected from Honey Creek, a small stream about four miles west of the city.

On the average, the first lampreys appear on the spawning grounds at Ann Arbor about the 10th of April. Dean and Sumner ('97) report them spawning on the 16th of April in New York City. According to Gage ('93), they spawn between the 8th and 20th of May at Cayuga Lake, New York. The time of spawning is undoubtedly dependent upon temperature as determined by the progress of the season. The temperature of the water in Honey Creek, when the lampreys first appear in the spring, ranges from 13°C. to 14½°C. The water is warmer down stream than farther up, and it is usually down stream that the lampreys are first observed. This, of course, may be due to the fact that the temperature is higher in this part of the stream, but it may also be correlated with the fact that many larvae have been carried down stream during successive years of their life, so that the number ready to transform into adults is greater in the lower part of the stream. Evidence for this is the fact that older larvae are usually obtained from the lower part of the stream, while it is very seldom that any large or full-grown larvae are found in the upper part.

I have found that the males appear on the spawning grounds before the females. Usually also only males are found in the nests early in the morning and late at night. That the males appear earlier in the season than the females was observed also by Young and Cole ('00), and, according to Surface ('97), the same is true of the lake lamprey. It is therefore necessary to collect the animals when they are spawning under optimum conditions in order to obtain reliable data concerning sex ratio. Dean and Sumner ('97) report more males than females in the proportion of five to one. It is easy to see how one might get such results from collections early in the season or at certain times of the day. Loman ('12) found no such disproportion of sexes in the European brook lamprey and neither have I found in the American brook lamprey an excess of one sex over the other when spawning conditions were at their optimum.

It is claimed by Loman ('12) that in the European brook lamprey internal fertilization takes place. The same idea has been advanced by Ferry ('83) in regard to the marine lamprey. To test whether or not spawning females of Entosphenus wilderi contained spermatozoa, the urogenital sinus, as well as the posterior portion of the body cavity, of a large number of living specimens was examined. In no case could any spermatozoa be found, nor did the eggs from the posterior part of the body cavity develop without the addition of sperm after being stripped. This shows clearly that, if internal fertilization occurs in this species, it must be only as a rare exception. The spermatozoa of the brook lamprey are motile for less than a minute after being shed into the water, but they are extremely active and are extruded simultaneously with the eggs. It is, therefore, inherently probable that fertilization of nearly all the eggs is insured. In fact, all eggs collected from the stream after deposition are found to be developing.

B. Life-cycle

The life-cycle of the lamprey may be divided into three main periods: the embryonic, the larval, and the adult (table 2). There is no sharp structural change between the first and second periods, but the first period may arbitrarily be considered as ending at the time of hatching and the larval period at the time of metamorphosis. The duration of the embryonic period depends on the temperature of the water during development. Balfour ('81) estimated the length of the period to be from thirteen to twenty-one days in Petromyzon planeri. A. Müller ('56) says that in the European brook lamprey (kleines Neunauge) hatching takes place about eighteen days after fertilization. I have found that when the temperature of the water ranges from 17 to 20°C., the artificially fertilized eggs of Entosphenus wilderi hatch in the laboratory in about ten to twelve days. There may, however, be a great deal of variation in the rate of development, even in the same brood of eggs when kept in the same dish.

The length of the larval period is not known from direct observation. It would be difficult in the laboratory, if not impossible, to raise the larvae to the time of metamorphosis, and if one were successful in doing so, he could not be certain that the larval period was of the same length as in the natural habitat. Various estimates have been given. A. Müller ('56) states that metamorphosis probably does not take place before the fourth year in the case of the European brook lamprey. Loman ('12) found larvae of four sizes, which he took to represent four successive generations, or four years of development before metamorphosis. Lubosch ('03) estimates the adult age to be four years, the estimate being based on the size of the larvae found. According to him, they attain an average size of 5 cm. during the first year; 10, during the second year, and 15 to 18 cm. during the third year, or just before metamorphosis.

Schaffner ('02) makes the following statement concerning the age of Entosphenus wilderi at sexual maturity: "The larvae taken a few days preceding the breeding season can be grouped under three divisions as regards size, and we took this to indicate that it required three years for the complete development of the larvae." The sizes ranged as follows: Those almost fully developed, 17 to 20 cm.; a second group, 9 to 11 cm.; and a third group, 3 to 6 cm.

The variation in size of the larvae of each year (figs. 1 to 4) is so great that from any one catch a continuous series may be selected. The measurements of seventy-seven adult males taken at random show a range in total length from 13.5 cm. to 19 cm., and an average length of 16.12 cm. Similar measurements of sixty-five females give a range from 13.3 cm. to 18.5 cm., with an average length of 15.61 cm. It thus appears that size is so variable in the adults and larvae of this species that it cannot be regarded as a reliable index of the age of the individual.

I have sought, by means of average curves, to determine the length of the life-cycle in Entosphenus wilderi. Such curves were obtained from measurements of the lengths of larvae collected during single months of the year. When the number of specimens was small, the sizes graded into each other so that no suggestive curves were obtained. The greatest number of specimens was collected in the month of August, and the curve represented in figure 1 is the result of measurements of 167 larvae from this period. The first hump of the curve, with its peak at 2 cm., represents larvae hatched in the preceding April, and, therefore, about four months old. Most of these larvae are about 2 cm. long. The second hump has its peak at 5 cm., and presumably represents larvae which are about one year and four months old. The third, fourth, and fifth humps with their peaks at 8, 12, and 18 cm. presumably represent larvae 2⅓, 3⅓, and 4⅓ years old, respectively. The larvae of the latter age would undoubtedly transform into adults during the fall, as they have already attained their full larval size.

Another series of larvae from the month of February was measured and the results are represented in the curve shown in figure 2. The number of specimens measured in this case is 130. It will be seen that this curve has four distinct humps. The larvae, which in the first curve were represented by the fifth hump, have supposedly metamorphosed and are at this time of the year in the adult form. The other humps of the curve represent larvae about one, two, three, and four years old.

Two other curves are shown, each of which is constructed from combined collections of three successive months. The curve for April, May, and June (fig. 3) has the peaks of the humps at 3, 9, 12, and about 14 cm., respectively. The humps represent larvae one, two, three, and four years old. At this time of the year adults are found and also larvae that have just hatched, but these are not included in the graphs. The other curve from the months of July, August, and September shows practically the same thing. It appears from these curves that Entosphenus wilderi probably attains an age of five years before it is sexually mature. This does not exclude the possibility that individuals may reach their full size in four years.

The rate of growth varies greatly, as shown in figures 1 to 4, where individuals grouped in each hump of the curves are presumably of the same age. The twenty-six larvae represented by the first hump in figure 1 range in length from 15 mm. to 32.5 mm. and have an average length of about 24 mm. As shown by the curves, the same variation in the rate of growth exists among older larvae. This probably is due largely to differences in the food supply in different parts of the stream and to the inactivity of the larvae, which keeps them from seeking out the optimum environmental conditions. It was found, for instance, when the larvae were kept under observation in the laboratory, that they remained in their burrows for days at a time, even though they were kept in a small dish without running water. It was only when the water became warm and stale that they came out. In the stream the water is cool and well aerated, so that it is doubtful if the larvae ever come out unless disturbed by torrents after heavy rains and during spring thaws.

The time of metamorphosis probably varies somewhat in different species and also in different localities. Balfour ('81) says that it takes place from August to January in Petromyzon planeri. Gage ('93) believes it occurs about from August to September or October in Petromyzon branchialis, and estimates the time required for metamorphosis as probab'y ten to twenty-six days. Reese ('00) found that larvae of the same species, which were kept in the laboratory, metamorphosed about the 20th of October. Schneider ('79) found metamorphosing larvae of Petromyzon planeri as early as the middle of August. I have found larvae of Entosphenus wilderi metamorphosing in the brook during the months of August and September, but only four specimens have been obtained; two on August 16th, one on August 23d, and one on September 13th. No direct observations have been made on the length of time required for metamorphosis, but since adults have not been found during May, June, and July, the three months following the spawning season; since metamorphosing individuals occur in late August and early September while adults are obtained during the other months of the year, it is likely that the usual time for metamorphosis in this species is in August and September, and that it requires only a short time.

Entosphenus wilderi reaches its full length in the larval state. After metamorphosis the germ glands grow very rapidly and soon fill up the whole body cavity. The intestine atrophies and becomes so small that it can be distinguished only with difficulty in cross-sections of the body. After transformation no food is taken by the animal. The whole metabolism of the body seems to be changed and all the resources possessed by the individual are used toward maturing the sexual products. After the spawning season the adults die within a very short time. Dead lampreys may sometimes be found along the stream, and crayfish have been found feeding on the dead bodies lying on the bottom of the stream.

It is remarkable that during the period of its adult life, extending from August or September to the following April, the lamprey takes no food. During this time the germ gland increases greatly in size and most of the material for its growth must be furnished by the fat body (corpus adiposum) and by the other tissues of the body. Metamorphosis seems to be the beginning of the death process, since at this time the intestine and the portal vein degenerate, thus making it impossible for the animal to feed again. The metabolic processes taking place in the adult must be concerned largely with material already present in the body at the time of metamorphosis. It is not unlikely, however, that water and some nutritive substances may be absorbed during adult life through the external surface of the body. Pütter ('09) found that sea-water and probably also fresh water contains amino-acids, oils, and carbohydrates, and that many aquatic animals absorb nutrition from solution, thus rendering them only in part dependent upon plankton. Alcock ('99) found that the larvae of the lamprey secrete an enzyme through the skin which has some digestive action on bacteria that might attack them in their burrows. This renders it probable that some food may be absorbed through the skin of the larvae and perhaps of the adult.

MATERIALS AND METHODS

Adults have been obtained at all seasons of the year except during the three months following the spawning period, but only those taken during the spawning season contained fully matured sex cells. In the laboratory the adults must be kept in running water, but the larvae live for months in standing tap-water if it be kept cool. Early larval stages are best obtained from artificially fertilized eggs. The largest percentage of fertilization is had from eggs of females taken at the height of the spawning season and used at once. The fertilized eggs are placed in tap-water in covered bacteria dishes and require no more care than an occasional change of water, which should be made without considerable change of temperature. Larvae from such eggs have been kept without much difficulty in the same dishes for as long as forty days, i.e., until most of the yolk is absorbed. Older larvae were obtained from the brook at all seasons and for a period of about four years.

By recording the moment of fertilization and by watching the progress of development, it was possible to obtain any desired stage. The older larvae collected from the stream were usually anaesthetized and put into the fixing solution as soon as they reached the laboratory. Precaution was taken to insure a rapid fixation of the germ gland.

Various fixing reagents were used, but the best results were obtained with Flemming's, Meves', and Bouin's solutions. For embryos in which there was a great deal of yolk, Bouin's solution gave very satisfactory results; it also gave uniformly good results for all other stages. For certain nuclear and cytoplasmic structures, Flemming's and Meves' solutions were more satisfactory. After fixation the material was left in alcohol for a few days and then imbedded. The results were not as good if the material had remained in alcohol for a long time.

Haemalum with a counterstain of orange G gave the best results for early stages in which a great deal of yolk was present. This combination gave the yolk a yellowish or brownish tint, while the cytoplasm was stained more or less bluish. For later stages iron haematoxylin was used almost exclusively, either alone or with a counterstain of eosin or Licht-grün.

HISTORY OF THE GERM CELLS

A. *General outline of the whole germ-cell history in animals; special outline for Entosphenus wilderi*

In table 1 an outline is given of the different periods, as defined by various writers, in the development of the germ cells of vertebrates. The scheme recognizes, in the column headed 'periods in the germ-cell cycle,' an early segregation of the germ cells and the development of all the definitive germ cells from the early segregated cells. The table also admits the possibility of two alternatives as to the origin of sex in the young animal. There may be distinct male and female individuals from the beginning of embryonic development, as shown in columns I and II of the table, in which case sex is dependent on an hereditary factor or on other factors that influence the germ cells at or before fertilization. On the other hand, the young animal may be indifferent as to sex, as shown in column III

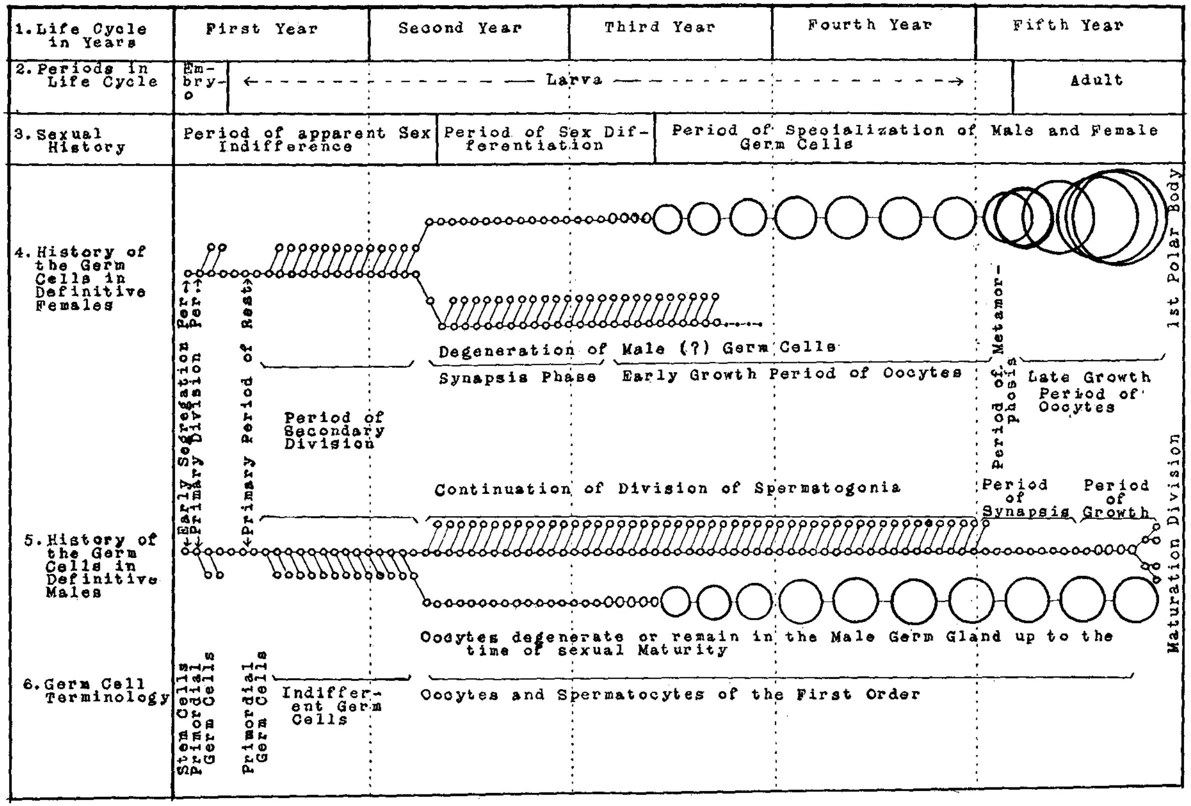
TABLE 1

A scheme representing in outline the different periods in the development of the germ cells in vertebrates, and the terminology that might be employed when the sexes are distinct from the beginning of development (columns I and II) and when the young animal appears indifferent as to sex (column III). See explanation in text

PERIODS IN THE GERM-CELL CYCLE	GERM-CELL TERMINOLOGY			
	Sex determined at or before fertilization		Sex determined after fertilization	
	I	II	III	
	Embryo and larva male	Embryo and larva female	Embryo and larva indifferent	
1. Period of early segregation	Primary spermatogonia	Primary oogonia	Stem cell	
2. Period of primary division	Primary spermatogonia	Primary oogonia	Primordial germ cells	
3. First period of rest	Primary spermatogonia	Primary oogonia	Indifferent germ cells	
4. Period of secondary division	Secondary spermatogonia	Secondary oogonia	Indifferent germ-cells	
			Male	Female
5. Period of maturation and growth *a.* Synapsis phase 1. Leptotene 2. Synaptene 3. Pachytene 4. Diplotene *b.* Growth phase 1. Dictyate 2. 1st maturation division	Primary spermatocytes	Primary oocytes	Primary spermatocytes	Primary oocytes
3. 2d maturation division	Spermatids	Ova	Spermatids	Ova
6. Period of cell metamorphosis	Spermatozoa		Spermatozoa	

TABLE 2

Outline of the history of the germ cells of the brook lamprey in relation to the life cycle of the animal. The germ cells are represented by circles



of the table, in which case factors influencing the animal during development may be responsible for the resulting sex. The possibility is not excluded of sex being the result of the joint action of hereditary and external factors.

Whether the germ cells of the larval lamprey eventually give rise to ova or to spermatozoa, their early history appears to be the same. Sooner or later some of the primordial germ cells transform into oocytes in practically all the larvae, irrespective of whether the larvae which bear them eventually become males or females. In the present work the history of the germ cells has been studied up to a period when males and females can be distinguished by an examination of the germ glands.

The scheme in table 2 presents in a graphic form the history of the germ cells in the lamprey in both males and females in relation to the development of the body. As here shown, the life of the animal extends over a period of five years, and only a small part of the life-cycle is spent in the adult stage. The scheme also forms a basis for the terminology employed in the subsequent pages.

B. Origin and early history of the germ cells up to the beginning of sex differentiation

1. Observations on Entosphenus wilderi. During cleavage and gastrulation in the lamprey all cells are more or less laden with yolk and, if the germ cells are segregated at this time, no character has been discovered by means of which they may be distinguished from other cells. But after the mesoderm begins to separate from the entoderm certain large cells occur which may be identified as the primordial germ cells.

a. Embryo 191 hours old (fig. 5). A camera-lucida drawing of an embryo 191 hours old (two or three days before hatching) is shown in figure 5, and figure 11 shows a section along the line *ab* of figure 5. In this embryo the mesoderm has already separated from the entoderm cranially, but at the caudal end, i.e., in the region from which the section (fig. 11) was taken, there seems as yet to be no division line between the two layers. The mesentoderm in this region extends dorsad as two ridges, one on either side of the nerve cord and notochord, and these are bounded externally by the ectoderm. The cells of the mesentoderm vary in size, are irregular in shape, and all of them are heavily laden with yolk. Several larger rounded cells stand out rather clearly among the more irregular surrounding cells. One of the most cranial of these is shown in figure 11. It lies directly under the ectoderm, in that part of the mesentoderm which later forms the mesoderm. Many other cells similar to this one are found farther caudad. That these are germ cells is shown by their later history, and the posterior region of the mesentoderm may therefore be considered a region for the proliferation of these cells. The shaded area in figure 5 shows in a diagrammatic way the relative position of the germ cells at this stage.

b. Embryo 238 hours old (fig. 6). A sketch of an embryo 238 hours old, about one day before hatching, is shown in figure 6, and a section along the line *ab* is shown in figure 12 In the latter figure three spherical germ cells, structurally identical with those of the preceding stage, are seen in the lateral portions of the mesoderm which have recently separated from the entoderm. Other germ cells may be recognized caudad of this section and also considerably farther craniad. The relative position of the cells in the whole embryo is shown by the shaded area in figure 6, and it will be seen that they extend much farther craniad than in the preceding stage. They are not arranged in regular groups in relation to the body somites, but form irregular bands, one on each side of the body. At this stage the dorsal part of the mesoderm has separated from the entoderm along its whole length, but its lateral plates are still continuous ventrally with the entoderm except along the cranial part of the germ-cell region, and craniad of it where a complete separation seems to have taken place, but even here one cannot be absolutely certain that the two germ layers are not continuous ventrally. Each lateral plate becomes thinner along the side of the body and terminates ventrally in a sharp edge, but the separation of this edge from the entoderm is not distinct. This makes it very difficult to determine whether the mesoderm extends ventrad at the expense of the entoderm or by independent growth.

In the caudal region of the body the germ cells are large and spherical and stand out clearly among the apparently smaller and more irregular-shaped cells of the yolk entoderm, as shown in figure 13, a section from the region *cd* of figure 6. Farther craniad the germ cells are more irregular in shape due to pressure from surrounding cells. No structural difference could be found between the germ cells and the large yolk-bearing cells of the entoderm, except that the former have more definite outlines. It is probable that even this difference is the result of location rather than of any inherent difference in structure. This suggests the possibility that any of the yolk-bearing cells of the mesentoderm which are so situated that they have a chance to get into the mesoderm at the time it separates from the entoderm may become germ cells. Another probability is that the germ cells are segregated in an earlier stage and that many more are produced in the early development of the embryo than can be included in the mesoderm when it separates. In this case all of the germ cells which remain in the entoderm probably degenerate in situ or are thrown off bodily into the lumen of the intestine. Later some evidence for this will be presented.

c. Larva 274 hours old (fig. 7). The larva of this stage has just broken out of the egg membrane and the anterior portion of the body has straightened out, as shown in figure 7. The caudal region, however, which includes most of the yolk, still forms a right angle with the cranial region. The position of the germ cells from three different regions is shown in figures 14, 15, and 16, taken from the parts of the larvae indicated by the lines *ab*, *cd*, and *ef*, respectively. At this stage the mesoderm extends farther ventrad than in the preceding stage. The germ cells lie in the nephrotome region, either ventrad or latero-ventrad of the newly formed pronephric ducts. They are much more numerous than in the preceding stage, and sometimes they lie so close together that in every section two or more cells are found. The absence of mitotic figures and the uniform size of the germ cells indicate that the increase in number is not due to any division of the cells, but to the fact that more and more germ cells are being included in the mesoderm as its separation from the entoderm extends caudad. There is no indication at this or at any other stage that the germ cells are segmentally arranged. They form two bands which are separated caudally, but converge cranially. The most cranial cells, although older in the sense that they were first to be included in the mesoderm as it became separated from the entoderm, are apparently not different from the posterior cells which were included much later.

d. Larva 286½ hours. This stage, shown in section in figure 18, is somewhat more advanced than the preceding. The mesodermic somites in the anterior region have become differentiated into a muscle plate, a dermal plate, and a sclerotome. The pronephric ducts lie in the regions laterad of the muscle plates. Between them and the yolk entoderm, and sometimes indenting the latter, are the large yolk-laden germ cells. They are of the same size and structure as the cells of the preceding stage. Occasionally the cells are found in groups, but no mitosis has ever been observed in the germ cells of this period, and this makes it probable that the cell aggregations are the result of a slight amount of migration or of several cells being separated from the entoderm at the same place. Due to a pressure from surrounding cells, many of the germ cells have lost their rounded appearance at this stage. The bands of germ cells of the two sides approach each other more closely cranially than in the preceding stage, but caudally they still lie far apart.

e. Larva 299½ hours (fig. 8). The larvae of this stage have increased considerably in length, but the caudal region is still loaded with yolk and remains perpendicular to the rest of the body (fig. 8). Figure 17 represents a section through the posterior part of the body, from the region indicated by the line *ab* in figure 8. In this section four germ cells are scattered along the lateral plates of the mesoderm. A section near the cranial end of the germ-cell area is shown in figure 19. This contains three germ cells which now have reached a position mediad of the pronephric ducts. Not only do the entoderm and mesoderm lie so close against each other that it is difficult in some places to see the line of separation, but the germ cells often lie in little depressions in the entoderm formed by the pressure of the germ cells against it. The result is that in certain sections the germ cells appear to be still in the entoderm. Such sections of this late stage examined without knowledge of the previous history of the cells might lead one to believe that they were migrating from the entoderm into the mesoderm. But there is no reason for believing that such a belated migration takes place in the lamprey, for other sections show that practically all the mesoderm has separated from the entoderm at this stage and that all the germ cells which are destined to become functional now lie in the mesoderm. Their position is about the same as in the preceding stage. They have not yet reached the midline cranially, while caudally they are scattered along the lateral plates of the mesoderm so that at the very extreme caudal end they are still found near the midventral line of the gut entoderm.

In later stages germ cells which lie in the lateral plates, far removed from their final destination, are often found in various stages of disintegration. It is also likely that many prospective germ cells never reach the mesoderm, but remain in the gut entoderm either to degenerate in situ or to be thrown off.

f. Larva 320 hours old. A larva of this stage is considerably longer than that of the preceding stage. The caudal part of the body is still slightly curved. Cranially the germ cells lie between the dorsal aorta and the pronephric ducts; caudally they lie ventrad or laterad of the ducts. The germ cells are in all respects similar to those of the preceding stage.

g. Larva 359½ hours old (fig. 9). The body of a larva of this stage is almost straight (fig. 9). The germ cells, two of which are shown in figure 20 are nearer the middorsal line than before, have lost their rounded contours, and are flattened between the gut entoderm and the pronephric ducts, dorsal aorta, and intervening mesenchyme. The germ cells are still filled with yolk globules and there is no indication that mitosis is taking place. The nucleus is usually eccentric and contains two deeply staining nucleoli, besides scattered chromatin granules. Each germ cell is surrounded by a number of flattened mesoderm cells. Figure 20 shows one germ cell cut through the nucleus and another cut along one side. The two germ-gland anlagen have not yet fused at any place along the midline, although they approach each other very closely at their cranial ends.

h. Larva $373\frac{1}{2}$ hours old. The germ-gland anlagen extend farther forward than in the preceding stages. Since the large, inert, yolk-bearing cells are very poorly adapted for independent migration, it is probable that their movement craniad as well as mediad is due, at least in part, to the mechanical shifting of the parts surrounding them. A coelom had formed in the cranial portion of the mesoderm and is also forming in the caudal region in front of the anal opening. In the middle portion, however, no body cavity is yet formed. The gut is loaded with yolk and is surrounded by mesoderm. In this mesoderm, on the doral side of the intestine, the yolk-laden germ cells occur, sometimes singly and sometimes in groups. Cranially, the two lateral germ-gland anlagen are well defined and practically come together. The cells are greatly flattened dorsoventrally by the pressure of the surrounding tissues. Caudally the germ cells are scattered along the whole lateral plate. In a cross-section from the caudal region slightly craniad of the anal opening, a large germ cell (fig. 21) was found lying in what may be considered the ventral mesentery. It is highly probable that a germ cell so situated will never become functional.

i. Larva $429\frac{1}{2}$ hours old. The posterior cardinal veins have appeared at this stage and the germ cells lie ventrad of these cranially (fig. 22). As the coelomic cavity is being formed by a splitting of the mesoderm, the germ cells become included in the somatic portion (fig. 23).

j. Larva $478\frac{1}{2}$ hours old ($4\frac{7}{13}$ mm. long). At this stage the germ-gland anlagen have fused cranially (fig. 24); caudally they still remain apart. The cardinal veins have increased in size and now lie dorsomediad of the pronephric ducts. Cranially the mesenchymal tissue has increased greatly in amount in the region in which the germ cells are found. It fills a considerable space between the germ cells, the dorsal aorta, and the cardinal veins. The germ cells are still flattened against the gut ventrally. They still retain their embryonic structure and are not dividing.

k. Larva $538\frac{1}{2}$ hours ($5\frac{5}{13}$ mm. long) (fig. 26). In the caudal region of the body the germ cells still retain their embryonic form. They are apparently not yet able to reach their final median position on account of the large amount of yolk in the entoderm of this region. Cranially, on the other hand, the yolk in the entoderm is being absorbed so that more space is left for the germ cells, and in consequence they shift their position toward the midline. As a result, the two bands of germ cells are now arranged in the form of a V with the apex pointing craniad. With the release of pressure and with the assumption of a median position, the anterior cells begin to show signs of activity. The yolk globules in many cells have lost their sharp contours and often appear fragmented (fig. 25). Sometimes they are absent from certain parts of the cells. The cytoplasm, hitherto clear, now has a granular appearance. The chromatin material in the nucleus now stains more deeply, and often chromatin-like granules are found in the cytoplasm surrounding the nucleus. It appears probable that there is at this time an active interexchange of material between the nucleus and the cytoplasm. No mitoses were observed, however, for a long time subsequent to this stage.

l. Larva 647 hours. At this stage the coelomic cavities have formed by a splitting of the lateral plates throughout their length, and the cavities of the two sides have fused and nearly the whole of the dorsal mesentery has disappeared. The germ cells are included in the somatic layer of the mesoderm. Most of them now lie along the dorsal midline directly below the dorsal aorta, but are spread out over a considerable area on each side of it. All have disappeared from the caudal region. No germinal fold is yet present. Some of the germ cells have lost the greater part of their yolk. None of the cells were found in mitosis.

m. Larva $902\frac{1}{2}$ hours. A few germ cells along the posterior portion of the body cavity still retain some yolk, but the great majority of them are now free from it. As compared with the mesodermal cells of the same region, they may be described as large spherical cells with large spherical nuclei each with two large nucleoli. No cells were found in mitosis. Sometimes two or more cells are so grouped as to suggest that they are derived from one cell by division; but since no mitoses are observed, the grouping is probably the result of a migration of the cells. The larvae of this stage have begun to feed. An examination of larvae between this and the former stage shows that they begin to feed when they are about 7 mm. long, although a great deal of yolk is still present in the intestinal wall.

n. Larva 10 mm. long (June 22). This larva was obtained from the creek on June 22nd and is in the neighborhood of seventy days old. The yolk is now all absorbed from the intestinal wall and the lumen of the digestive tract is full of diatoms and other organisms upon which the larvae feed. The germ-gland anlagen are in the middle two-thirds of the coelom, but are absent from its cranial and caudal parts. In later stages, when the cells begin to increase in number by division, their range is extended both craniad and caudad. The germ cells are irregularly distributed along the anlagen with no indication of a segmental arrangement. They lie in the mesenchyme on the ventral side of the dorsal aorta and close against the peritoneum, which consists of very flat epithelial cells (fig. 29). Some of the germ cells may project slightly into the coelom, but these projecting cells do not yet form a continuous germ fold. Although the germ cells may lie against the peritoneum, they never form a part of it. They may be distinguished from the epithelial cells and other cells of the same region by their larger size and spherical shape; by their large spherical nuclei, each containing two large nucleoli, and by their clear transparent cytoplasm (fig. 30). Each is surrounded by flat epithelial cells which are similar to those forming the peritoneum. The germ cells in this stage are absolutely distinct from the cells of the soma, as they appear to be from the time when they are first recognized as germ cells. They have lost all their yolk, but no signs of mitosis could be found.

At this stage the lumen of the intestine is very much enlarged and many of the cells from the walls of the intestine have been set free into the intestinal cavity (fig. 31). This is the case also in much earlier stages (larvae about 7 mm. long), and suggests that the extruded cells are germ cells which have failed to reach the germ-gland anlage.

o. Larva 20 mm. long. This larva is about four months old. The germ fold has now formed (fig. 27) and extends along the dorsal wall of the coelom as a low longitudinal ridge. Some of the germ cells have migrated into the fold, but others are still in the mesenchyme above it. Their position is such that they are practically surrounded by blood-vessels—the posterior cardinal veins laterally and the dorsal aorta above. Besides these vessels, a large number of smaller vessels permeate the tissues around the germ cells. The germ cells occur in groups and in some places long distances intervene between them so that the various groups do not form a continuous band. The germ cells are more numerous than in the preceding stage, and in some cases two are found which apparently are surrounded by a single follicular membrane—an indication of recent division. In most of the cells at this stage, a distinct attraction sphere occurs. It consists of a mass of closely set granules and lies against one side of the nucleus. It may cover as much as one-third of the circumference of the nucleus in each section through the middle of the cell (fig. 28). No distinct centrosome could be found. Besides the attraction sphere, there is in the cytoplasm a spindle-shaped body, the 'vitelline body' of King ('08), which is much smaller and is made up of coarser granules than the attraction sphere. In longitudinal section it appears oval, in cross-section, round. Judging from sections through various planes, it is shaped like a spindle which tapers abruptly at both ends. It may occur almost anywhere in the cytoplasm, sometimes near the nucleus but sometimes close under the cell membrane. The origin of this body could not be ascertained. The presence of a centrosphere indicates the beginning of mitotic activity. The cells may now be considered as having passed out of the primary period of rest and entered the period of secondary division. There is yet no indication of sexual differentiation and, in the absence of any characters which distinguish the secondary spermatogonia from the secondary oogonia, the germ cells may be regarded as still indifferent as to sex (table 2).

The germ cells lie against the peritoneum which covers the gland (fig. 30), but they are always separated from the coelom by peritoneal cells and they have never been found to form a part of the peritoneal epithelium. Epithelial cells may be seen, still in part included in the peritoneum, but with processes extending to the germ cells and forming a part of their follicles. These cells occur in all stages of detachment from the peritoneal epithelium and in all stages of inclusion in the follicular membranes of the germ cells. There is no evidence at this stage that the follicular cells are derived from any other source. Both in the peritoneum and in the follicles these cells are distinguishable from the germ cells and mesenchyme cells by their ovoid nuclei, each with one large plasmosome and several smaller chromatin nucleoli, and by their flattened form and indefinite contours. The mesenchyme cells are recognizable by their nearly spherical nuclei, and the germ cells by their large size and spherical nuclei, each with two large plasmosomes. It seems clear from their early history, from the fact that they are at no time seen to be included in the peritoneal epithelium, and from their distinguishing structural characters, that the germ cells are not derivatives of the peritoneal epithelium. It seems equally clear that the follicle cells are derived from this source.

2. Historical and critical. *a.* Invertebrates. A study of the early history of the germ cells in various species of invertebrates has disclosed the fact that they often are segregated during early cleavage. These primordial germ cells are at first distinguished either by the behavior of their chromosomes or by the presence of certain cytoplasmic inclusions. In vertebrates the primordial germ cells are usually not recognizable until the three germ layers are formed, although most investigators of the subject (Beard, Allen, Dodds, Nussbaum, King, Witschi, Rubaschkin, Swift, Tschaschkin, and others) believe they must have been segregated at a much earlier stage. The stanchest adherents of the theory of early segregation (Beard, Allen, Rubaschkin, Witschi Swift, and others) hold that all the definitive germ cells are derived from the primordial germ cells, a conclusion that the theory of the continuity of germ plasm naturally demands.

Others (Abramowicz, Bouin, Kuschakewitsch, Dustin, Firket), while admitting the presence of the primordial germ cells in the early embryo and the possibility that they give rise to definitive reproductive products, still think it probable, and even supported by very strong evidence in some cases, that many of the definitive germ cells are derived from other elements which, strictly speaking, have formed a part of the soma. Opinions vary as to whether these other cells should be considered true somatic elements or simply another type of undifferentiated cells. Child ('06) is convinced that in the cestode, Moniezia expansa, the germ cells develop from cells of the parenchymal syncytium which must be regarded as differentiated tissue cells. In his development of the 'theory of dedifferentiation' ('15) he makes the following statement:

> In the tapeworm Moniezia, for example, the sex cells arise from the parenchyma, and apparently any parenchymal cells which lie within the region involved in the production of sex cells may undergo dedifferentiation and take part in the process. Even the large muscle cells may give rise to testes. . . . . In such cases the muscle fiber undergoes degeneration, the vacuoles disappear, and the nucleus begins to divide, apparently at first amitotically (pp. 331–332).

C. W. Hargitt ('06) thinks that the germ cells in Clava leptostyla arise in the entoderm, and that it is unlikely, though possible, that these cells may be undifferentiated. In Campanularia flexuosa, George T. Hargitt ('13) has found that the egg cell arises in the entoderm by the transformation of single epithelial cells, or from the basal half of divided cells. He concludes: "Therefore the egg cells have come from differentiated body cells (so-called) and there is no differentiation of the germ plasm in the sense that the germ cells are early differentiated and set aside and do not participate in the body functions" (p. 111).

Max Jörgensen ('10) comes to the same conclusion for Sycon. He says: "Indessen zeigen mir meine Präparate dass auch eine Entstehung von Oogonien aus Mesodermzellen denkbar und morphologisch nachweisbar ist" (p. 169).

b. Vertebrates. The earliest theory of the origin of germ cells in vertebrates is the 'germinal epithelium theory,' advanced by

Waldeyer in 1870. He found some large spherical cells in the coelomic epithelium on each side of the dorsal mesentery in the early chick embryo, and supposing that they were young stages of eggs he called them 'Ureier'; the epithelium in which they were found he called 'Keimepithel' (germinal epithelium). In 1875 Semper found that both ova and spermatozoa were derived from the so-called Ureier. Waldeyer and his followers believed that these cells were derived directly from the cells of the germinal epithelium, and this idea is held by a few investigators at the present time.

Since the development of the theory of 'early segregation' by Nussbaum ('80) many investigators have worked on the origin of the germ cells in vertebrates. It has been found in most cases in which the early history of the cells has been traced that they do not originate in the coelomic epithelium, among the cells of which they are later found, but that they attain this position after a migration from other parts of the embryo (Woods, Allen, Dodds, King, Witschi, and others). These same investigators have found that the germ cells in very early stages are usually located in the entoderm, from which they migrate to their definitive position in the coelomic epithelium. When first found, they are large yolk-bearing cells of the entoderm and distinguished from the entoderm cells principally by their location.

From numerous investigations on the subject there seems to be no doubt about the existence of the so-called primordial germ cells which are segregated very early in the development of the embryo, but whether or not all, some, or any of the definitive germ cells are derived from these is a question about which there is very little agreement. Rubaschkin ('09, '12) and others hold that the definitive reproductive cells in mammals are derived exclusively from primordial germ cells. Firket ('14) thinks it is possible that a few of the oogonia may be derived from the primordial germ cells, but that most of them are derived from certain cells in the germinal epithelium, which he calls 'gonocytes secondaire.' Kuschakewitsch ('10) believes that in Rana esculenta the oogonia are derived from cells in the germinal epithelium which are descendants of the primordial germ cells; while the spermatogonia are developed from 'Paragonien,' or secondary germ cells, which take their origin in the axial mesenchyme. Von Winiwarter and Sainmont ('09) regard the primordial germ cells in mammals as only temporary structures, which later degenerate. The same conclusion has been reached by Kingery ('17). Von Berenberg-Gossler ('14), from his work on Lacerta agilis, comes to the conclusion that the migration of the so-called primordial germ cells from the entoderm is nothing but, "eine späte, sich nach längere Zeit hinziehende Mesodermbildung aus dem Entoderm." He thinks that these cells as well as other mesoderm cells, such as those of the coelomic epithelium, may give rise to the stem cells of the ova and spermatozoa. According to Gatenby ('16), there is in the frog (Rana temporaria) and other amphibians an annual transformation of peritoneal cells into germ cells, so that in these forms there can be no talk of a continuity of the definitive germ cells and the primordial germ cells.

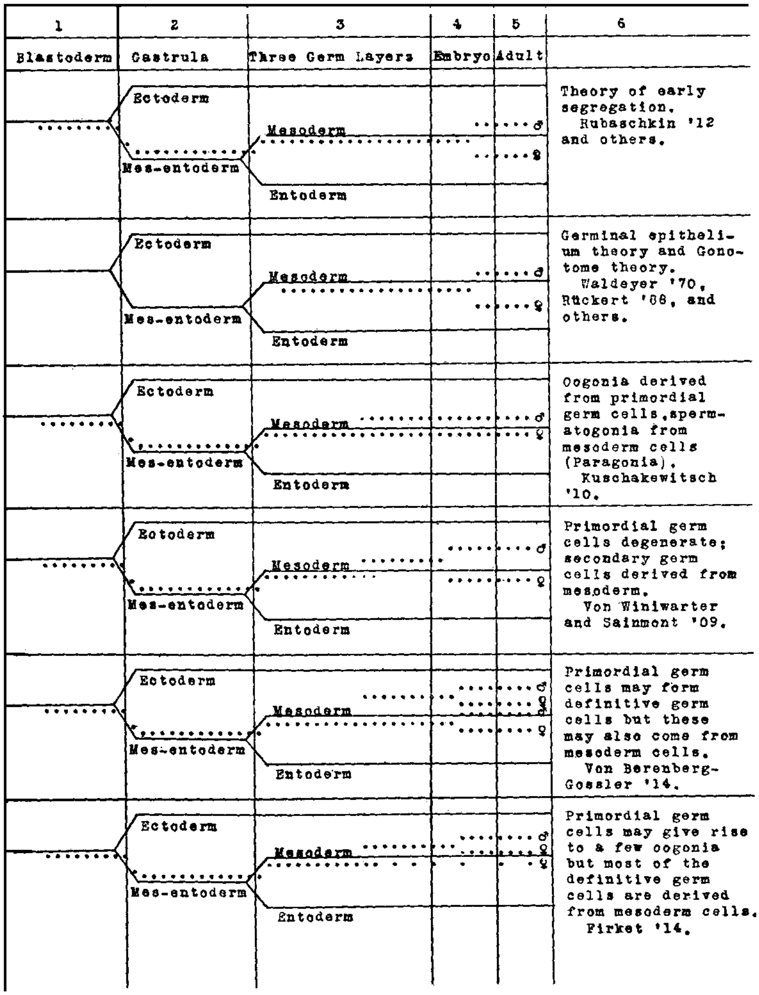
Some investigators believe that they have found evidence that the primordial germ cells are segmentally arranged, being derived from segmental portions of the mesoderm (Rückert, Van Wijhe, Dustin, and others). This has been termed the 'gonotome theory.' It is an attempt to homologize the condition found in vertebrates with that found in Amphioxus in which the gonads are segmentally arranged from the beginning of their development. According to this theory, the germ cells are derived from mesodermal cells.

Only a few investigators have followed the later history of the primordial germ cells and found that they actually give rise to definitive germ cells. Among these are Witschi ('14) for the frog, King ('08) for the toad, and Swift ('14, '16) for the chick. Many supporters of the theory of early segregation have studied only the early embryonic stages and have assumed that definitive germ cells originate from no other source than the primordial germ cells.

From the above it is clear that there exists at the present time a great diversity of opinion concerning the origin of the

TABLE 3

Outline of theories concerning the origin of germ cells in vertebrates. The various stages in the development of the individual are represented by five vertical columns. The dotted lines indicate the germ paths as conceived by the various theories. The different germ layers are represented by horizontal lines. The proximity of the germ paths to each of these lines indicates where the germ cells are supposed to take their origin and where they may be found during the different stages of development. In the sixth vertical column a brief summary of each theory is given



Theory of early segregation.
Rubaschkin '12 and others.

Germinal epithelium theory and Gonotome theory.
Waldeyer '70, Rückert '88, and others.

Oogonia derived from primordial germ cells, spermatogonia from mesoderm cells (Paragonia).
Kuschakewitsch '10.

Primordial germ cells degenerate; secondary germ cells derived from mesoderm.
Von Winiwarter and Sainmont '09.

Primordial germ cells may form definitive germ cells but these may also come from mesoderm cells.
Von Berenberg-Gossler '14.

Primordial germ cells may give rise to a few oogonia but most of the definitive germ cells are derived from mesoderm cells.
Firket '14.

reproductive cells in animals. Table 3 presents a summary of the various theories relating to the origin of the germ cells in vertebrates.

For one who adheres closely to the germ-plasm theory it is hard to conceive of the germ cells as coming from any other cells than the early segregated embryonic cells which have had no part in the building up of the body. Some who believe in the theory of early segregation maintain that, even when the germ cells appear to arise from so-called somatic elements, these are in the strict sense not somatic cells, but cells that have maintained their embryonic structure and have not specialized in any given direction. There is, of course, no direct evidence for this. In many forms, it is true, the germ cells seem to be segregated very late in the life of the individual. This is apparently true of annelids and flatworms among animals, and it seems to be true of all plants. There are no investigators of germ cells in vertebrates who maintain that they come from highly differentiated somatic cells, such as muscle cells, as observed by Child ('06) in Moniezia.

The various theories concerning the origin of germ cells in vertebrates have now been stated. Below is a partial list of the most important contributions on the subject, each followed by a brief statement of the conclusions which the various investigators have reached. The references have been arranged chronologically for the various groups of vertebrates.

AUTHOR	YEAR	SPECIES	RESULTS AND CONCLUSIONS
		Cyclostomes	
Goette	1890	Petromyzon fluviatilis	Germ cells derived from the mesoderm
Wheeler	1899	Petromyzon planeri	Germ cells derived from blastoderm cells
Beard	1902	Petromyzon planeri	Germ cells are early segmentation cells. Their number is 2^5–1
		Elasmobranchs	
Semper	1875	Plagiostomes	Germ cells are derived from the coelomic epithelium
Balfour	1876 1877	Scyllium, Pristiurus	Germ cells are probably derived from the mesoderm. They may have been introduced from elsewhere
Rückert	1888	Pristiurus	Germ cells are derived from segmental mesoderm cells. Gonotome theory
Van Wijhe	1889	Scyllium, Pristiurus	Germ cells are derived from segmental mesoderm cells. Gonotome theory
Beard	1900 1902	Raja batis, Pristiurus	Germ cells are derived from early segmentation cells
Woods	1902	Squalus acanthias	Early segregated germ cells are first found in the entoderm
		Ganoids	
Allen	1909	Amia Lepidosteus	Germ cells are segregated early and are first found in the entoderm
		Teleosts	
Nussbaum	1880	Trout	Germ cells are segregated early and are first seen in the region of the germ gland, but they are not derived from the mesoderm
MacLeod	1881	Hippocampus, Belone	Germ cells are derived from the germinal epithelium
Hoffmann	1886	Salmon	Germ cells are derived from peritoneal cells
Eigenmann	1891 1896	Micrometrus aggregatus	Germ cells are segmentation cells from about the fifth generation
Böhi	1904	Trout, salmon	Germ cells are derived from cells of the coelomic epithelium

AUTHOR	YEAR	SPECIES	RESULTS AND CONCLUSIONS
		Teleosts—Continued	
Federow	1907	Salmo fario	Germ cells are first found in the somatopleure and splanchnopleure
Dodds	1910	Lophius piscatorius	Germ cells are first found in the primary entoderm. They are early segmentation cells
Bachmann	1914	Amiurus nebulosus	Germ cells are first found in the lateral plate of the mesoderm. They are early segregated cells
		Urodeles	
Dustin	1907	Triton alpestris	Germ cells are derived from mesoderm cells (gonotome)
Spehl and Polus	1912	Axolotl	The germ cells are derived from mesoderm cells
Schapitz	1912	Amblystoma	The germ cells are derived from mesoderm cells (gonotome)
Abramowicz	1913	Triton	Primary germ cells are derived from the entoderm (early segregation cells). Secondary germ cells are derived from the mesoderm
		Anura	
Nussbaum	1880	Rana fusca	Germ cells are first found in the mesoderm, but are early segregated cells
Bouin	1901	Rana temporaria	Germ cells are derived from early segregated cells, and from peritoneal and mesenchyme cells
Allen	1907	Rana pipiens	Germ cells are derived from early segregated cells. They are first found in the entoderm
Dustin	1907	Rana fusca	Germ cells are derived from the lateral plate of the mesoderm (gonotome) and from peritoneal cells
Dustin	1907	Bufo vulgaris	Germ cells are derived from the lateral plates of the mesoderm (gonotome) and from peritoneal cells
King	1908	Bufo lentiginosus	Germ cells are early segregated cells and are first found in the entoderm
Kuschakewitsch	1910	Rana esculenta	Primary germ cells are derived from early segregated cells. Secondary germ cells are derived from the peritoneal epithelium and axial mesenchyme

AUTHOR	YEAR	SPECIES	RESULTS AND CONCLUSIONS
		Anura—Continued	
Champy	1913	Rana temporaria	Germ cells are derived from segmental mesoderm cells (gonotome)
Witschi	1914	Rana temporaria	Germ cells are derived from early segregated cells and are first found in the entoderm
Gatenby	1916	Rana temporaria	Germ cells originate periodically from peritoneal cells in adult frogs
		Reptiles	
Allen	1906 1907 1911	Chrysemys marginata	Germ cells are first found in the entoderm and are derived from early segregated cells
Jarvis	1908	Phrynosoma cornutum	Germ cells are first found in the entoderm and are derived from early segregated cells
Dustin	1910	Chrysemys	Primitive germ cells are derived from entoderm cells. Secondary germ cells come from peritoneal cells
Von Berenberg-Gossler	1914	Lacerta agilis	So-called primordial germ cells are formed in the entoderm. They are probably not germ cells, but give rise to mesoderm cells. Germ cells are derived from mesoderm
		Aves	
Waldeyer	1870	Chick	Ova are formed from cells of the germinal epithelium. Spermatogonia come from cells of the wolffian duct epithelium
Hoffmann	1892	Twelve species of birds	Germ cells are early segregation cells
Nussbaum	1901	Chick	Germ cells are first found in the splanchnopleure, but they are early segregated cells
Rubaschkin	1907	Chick, duck	Germ cells are early segregation cells first found in the splanchnopleure
Tschaschin	1910	Chick	Germ cells are first found in the splanchnopleure. They are early segregated cells
Von Berenberg-Gossler	1912	Chick	The so-called germ cells first found in the splanchnopleure may not be germ cells

AUTHOR	YEAR	SPECIES	RESULTS AND CONCLUSIONS
		Aves—Continued	
Firket	1914	Chick	Primordial germ cells may give rise to definitive germ cells, but most of these are derived from the germinal epithelium
Swift	1914 1916	Chick	Germ cells are derived from cells in the germ wall entoderm. They are early segregated cells
		Mammals	
Allen	1904	Pig, rabbit	All functional germ cells are derived from the peritoneum
Sainmont	1906	Cat	Primitive ova are present, but are not functional. Definitive germ cells are derived from epithelial cells
Winiwarter and Sainmont	1909	Cat	Primitive ova are present, but are not functional. Definitive germ cells are derived from the germinal epithelium
Rubaschkin	1908 1909 1912	Cat, rabbit Mole, porpoise Guinea pig	Germ cells are derived from early segregated cells and are first found in the entoderm. There is no secondary origin of germ cells

c. Review of work on the early history of the germ cells in lampreys. W. Müller ('75) described the germ glands of young lamprey larvae as median, unpaired thickenings of the peritoneum situated between the bases of the mesonephric bodies and extending along the whole length of the body cavity. At this stage groups of germ cells were found, but sex could not be distinguished.

Goette ('90) found the reproductive cells in larvae of a much earlier stage, corresponding approximately to that represented by my figure 7. He observed that, while most of the cells in the mesodermal plates soon lost their yolk and began to divide, some of the cells retained their yolk and remained undivided. These cells were found in the mesodermal plates on both sides along their thickened median portions directly under or outside of the pronephric ducts and sometimes against the yolk entoderm, so that it appeared as though they might belong to it. He says, however, "Eine genaue Untersuchung hat mich aber überzeugt dass es ursprüngliche Mesoderm-elemente und nicht etwa vom Darmblatt her eingewanderte Zellen sind" (p. 53).

Wheeler ('99) has given an excellent account of the early development of the germ cells in the lamprey (Petromyzon planeri). He recognized the germ cells in the posterior region of embryos as early as my figure 5, the stage in which they were first observed by me in Entosphenus wilderi. Wheeler found: "Just laterad to the myotomes a few very large rounded masses of yolk." He described each mass as containing a nucleus and "more or less distinctly marked off from the adjacent entoderm elements." He says further, "These large masses are the primitive reproductive or sex cells. They can hardly be assigned to the mesoderm because their appearance and position are those of entoderm cells in this stage. Still they lie in a portion of the entoderm which becomes mesoderm with the more lateral extension of the latter layer."

Beard ('02), in an attempt to work out a numerical law for the primordial germ cells in animals, says that the number of cells should in each case be 2^n-1. His theory is that the blastoderm in animals corresponds to the sporophyte in plants, and to it he applies the term 'phorozoon.' After a time one of its cells divides a definite number of times and forms the primordial germ cells. The number of divisions varies according to the species. One of these primordial germ cells is sacrificed to form the embryo so that the actual number of germ cells remaining is in each case 2^n-1. In the case of the lamprey (Petromyzon planeri) Beard finds that $2^n = 32$ and that therefore in this species the number of primordial germ cells is thirty-one.

Interesting in connection with the description of the early history of the germ cells in the lamprey is an observation made by Kupffer ('90). In the early gastrula of Petromyzon planeri, he found, between the ectoderm and the entoderm in the region of the blastopore, certain cells which he called 'teloblast cells.' They were easily distinguished from the yolk cells adjoining them, but their origin was not observed. Kupffer thinks that the mesoderm in this region develops at the expense of these cells. He says:

> Wenn aber das dorsale Mesoderm entstanden ist und bis zum Teloblast reicht, tritt es in dieselbe einige Verbindung damit wie der Neuralstrang und die Chorda, und nachdem die Segmentierung des Mesoderm bis zum Schwanzende fortgeschritten ist, ergänzt sich der jeweilig hinterste Abschnitt des Mesoderm durch Zellen die aus dem Teloblast stammen.

Hatta ('92, '07) describes certain cells as budding off between the ectoderm and the entoderm in the region of the blastopore. These cells he calls the 'peristomal mesoblast.' He could not find any cells that corresponded to Kupffer's teloblast cells. It is possible that Kupffer's teloblast cells and Hatta's peristomal mesoblast cells are identical and that they correspond to the large yolk-laden cells which later become included in the mesoderm and form the germ cells.

3. Discussion. *a.* Early segregation. We have seen that the germ cells in Entosphenus wilderi may be traced to the large yolk-bearing cells which at first are located in the mesentoderm. This is in agreement with the observations of Wheeler. The history of these cells, previous to their inclusion in the mesoderm is not known. They apparently lie among similar yolk-bearing cells belonging to the entoderm, and it is a question whether or not they are essentially different from these. The germ-plasm theory, as expressed by Weismann, demands a segregation of the germ cells at a very early stage, or their origin, at least, from cells that have never taken any part in the formation of body tissues. In one sense all the yolk-bearing cells of the entoderm may be considered as undifferentiated cells, but only some of these cells which are included in the mesoderm become germ cells. Most of the mesoderm cells, however soon begin to divide, become smaller, lose their yolk, and form various tissues, while the large cells that become germ cells do not change in the least for a very long time. This indicates that they are endowed with certain qualities which distinguish them from the cells that become somatic.

My observations and those of Wheeler ('99) show that the germ cells appear first in the posterior region of the body, probably in a small area around the blastopore. None have been seen to separate from the entoderm very far craniad of this region, so that those found later in the region further craniad gain this position by some form of migration. In Entosphenus wilderi the peristomal and the paraxial regions of the mesoderm are continuous and do not differ in structure or in origin as maintained by Hatta ('92, '07). The only distinction found between them is that the mesoblast of the paraxial region is delaminated earlier than that of the peristomal region, but only the peristomal mesoderm carries the germ cells in early stages. In Entosphenus wilderi no cells corresponding to Kupffer's teloblast cells were found in this region. These may have been either germ cells or, as he supposed, mesoderm cells.

The condition in the lamprey is in favor of the theory of early segregation. The large yolk-laden cells that are at first found among the other yolk-bearing cells of the entoderm in the caudal region of the body become included in the peristomal mesoderm when it separates from the entoderm by a process of delamination. These cells retain their embryonic structure for a long time after the other elements of the mesoderm have become differentiated. Even after all their yolk has been used up, they remain as large conspicuous cells among the smaller somatic cells of the germ gland. Up to this point the history of these cells has been traced and the later history to a time when they begin to divide. Later it will be seen that there is in Entosphenus wilderi no evidence that any other cells take part in the formation of definitive germ cells. What evidence there is in other forms will now be considered.

Two lines of evidence have been advanced for the secondary origin of germ cells from mesodermal elements. The first is that transitional stages have been found between mesodermal cells and true germ cells (Abramowicz, Dustin, Firket, Gatenby, and others), but in most cases the figures which purport to represent this transition are not convincing. The second line of evidence is that advanced by Kuschakewitsch ('10). He found that in the frog (Rana esculenta) all the embryos produced by eggs in which fertilization had been delayed for a certain number of hours were males. He believes that normally oogonia are derived from primordial germ cells which are situated in the germinal epithelium, while the spermatogonia are derived from the axial mesenchyme. When fertilization is delayed no primordial germ cells are produced and all the definitive germ cells come from mesodermal cells which he calls 'Paragonien.' These give rise only to spermatogonia, and the larvae are therefore males. Witschi ('14), who more recently has worked on the development of the germ cells in Rana temporaria, finds that in this form there is no secondary origin of germ cells. He says:

> So scheinen alle Tatsachen dafür zu sprechen, dass von ihrem frühesten Erscheinen an, die Keimzellen als Gebilde spezifischer Natur zu betrachten sind, welche, wenigstens unter Bedingungen die von normalen nicht sehr abweichen, weder sich in somatische Elemente umwandeln, noch aus solchen durch Unwandlung entstehen können.

Among others who have followed the history of the germ cells and have found no evidence of secondary origin, may be mentioned King ('08) for Bufo and Swift ('14, '16) for the chick.

b. Method of migration of the germ cells. Three opinions have been advanced concerning the method by which germ cells reach the germ-gland anlage in vertebrates: 1. Most investigators are inclined to the belief that, by some sort of ameboid movement, there is an active migration of the germ cells from the entoderm to the splanchnic mesoderm, and then through the mesentery into the germ-gland anlage. That such migration exists seems certain in forms in which the germ cells are so late in arriving at their final destination that they lose their yolk before leaving the entoderm.

2. A second theory is, that in some of the lower forms the migration of the germ cells may be accounted for partly by a shift in the position of the somatic tissues around them. In this case the germ cells are considered passive elements which take little or no active part in the migration.

It seem likely that in some forms migration is partly active and partly passive. This is probably true in the lamprey. No ameboid forms have been observed in the yolk-laden germ cells of the early embryo, and it is not likely that they are capable of independent movement, but it is probable that they are transported to the germ-gland region by a shifting of the tissues during somatic differentiation. In later stages, after all the yolk has disappeared, the germ cells extend farther and farther craniad. In sections of these stages the individual germ cells always have a spherical shape and no pseudopodial processes have been seen to indicate that they migrate independently; yet it seems probable that in life there may be a slight amount of migration in this way. Individual germ cells and cysts are usually not in contact, so that it is a little difficult to conceive of these shifting to a more cranial position as a result of the pressure of the cells or cysts against one another as they increase in number. Since the follicle cells show ameboid processes, it may be that the germ cells are carried along by a movement of these.

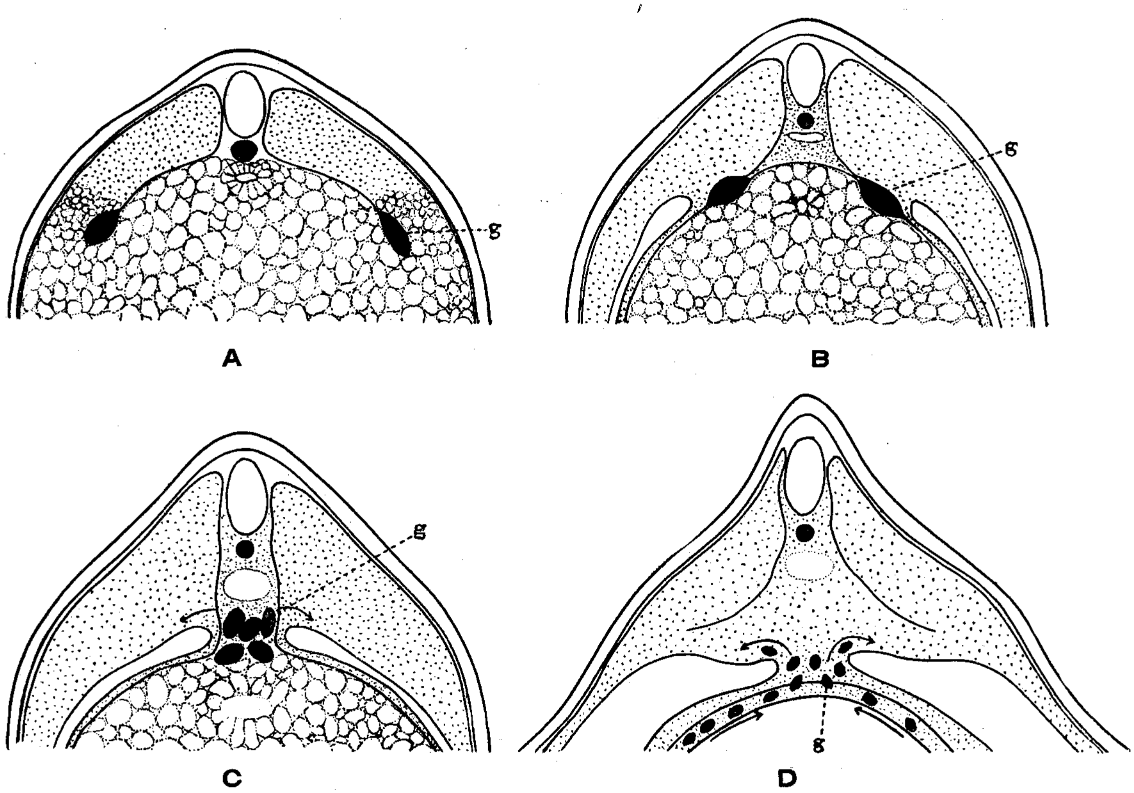
Some of the germ cells in the lamprey, as well as in other forms, never reach the germ gland, but remain in the entoderm or some other part of the body. Some of these cells may divide and form cell nests in other organs. Such cell nests have been found in the lamprey, both in the fat body and in the median and lateral portions of the mesonephros (figs. 32, 33). The fate of these cell nests is not known, but they probably degenerate.

3. A third method of migration appears to have been observed by Swift ('14) in the chick embryo. He says that the large yolk-laden germ cells, which are first found in the germ-wall entoderm, are taken up by the blood-vessels and carried by the blood-stream to the germ-gland region. In fact, the germ cells may be carried to any part of the body, but it is only in the germ-gland region that they migrate out of the blood-vessels. Swift ('16) has followed the later history of these cells and has come to the conclusion that they give rise to the definitive germ cells. Von Berenberg-Gossler ('14), who worked on the early germ cells in duck embryos, also found cells similar to those described by Swift, but he expresses a doubt as to whether or not they are germ cells. He says: "Alles in allem bin ich der Ansicht, dass das ganze Verhalten dieser Zellen in höherem Grade davor warnt sie für Keimbahnzellen zu halten" (p. 261).

A study of the methods by means of which germ cells migrate from the entoderm into the mesoderm in the various groups of vertebrates, and of the time at which this migration takes place, shows that these groups may be arranged in an interesting phylogenetic series as represented in the following diagrams (text figures A, B, C, D).

In the lamprey (text figure A) the germ cells are shown as being included in the mesoderm at the time when it becomes separated from the entoderm. In Triton (text figure B), according to Abramowicz ('13), the germ cells migrate into the mesoderm before the dorsal mesentery is formed, but much later than in the case of the lamprey. In the frog, according to Allen and others, the germ cells are separated from the yolk entoderm at the time the dorsal mesentery is formed, as shown in text figure C. In each of the first two forms, therefore, the original germ-cell anlage is double, while in the latter it appears to be unpaired at first and to lie along the middorsal line above the gut entoderm. In the lamprey, although the original anlage is paired, the germ gland later becomes single by a fusion of the two parts on the ventral side of the dorsal aorta. In Triton the paired anlagen fuse, but later they become paired again, and in the frog the original unpaired anlage becomes paired. In reptiles and mammals the germ cells usually separate from the entoderm much later than in the other three forms, often after they have lost all their yolk. Then migration follows: first into the splanchnic mesoderm, then through the dorsal mesentery, and from there to the germ-gland anlage on each side (text figure D).

c. Relation of germ cells to body cells. In the later embryonic stages of many forms, when the germ cells have reached their final destination in the region of the germ gland, they lie among the peritoneal cells covering the definitive germ-gland anlage and apparently form a part of the peritoneal membrane. This fact gave rise to the idea that the cells were derived from the peritoneal cells, so that this portion of the peritoneum became known as the germinal epithelium. In the lamprey there is, strictly speaking, no germinal epithelium since the germ cells never form a part of the peritoneal membrane, but are independent elements



Text figures A, B, C, and D A phylogenetic series showing by what method the germ cells of various vertebrate embryos attain their position in the mesoderm. *A*, lamprey; *B*, triton; *C*, frog; *D*, reptiles and mammals. (For description see text.)

situated in the mesenchyme dorsal to the peritoneum. Sometimes the germ cells may lie so pressed against the peritoneum that its cells become greatly flattened, but the continuity of the peritoneal epithelium is apparently never broken. I have found no evidence that any germ cells are formed out of peritoneal cells at any stage.

d. Number of germ cells. No evidence was found that division takes place in the germ cells of the lamprey before the larva is about 20 mm. long. The germ cells are few in number, and, as the young larva continues to grow, they become more and more scattered along the whole length of the body cavity, so that in following a series of sections they are often found far apart. In a larva about 8 mm. long, in which the germ cells stood out with great clearness, thirty-six were counted. These were distributed through 153 10-μ sections or along 1.53 mm. of the body length, as shown in figure 10. The distribution of the cells by sections was as follows (table, p. 40):

It will be seen that at this time no germ cells are found in the caudal region of the body for about 0.58 mm. in front of the anal opening, and cranially they do not extend any farther than to about the middle of the coelomic cavity.

The number of germ cells found in this larva comes very near that found by Beard ('02) in larvae of Petromyzon planeri, but it can hardly be cited as supporting his theory, especially since it is known that many of the germ cells never reach the germ-gland region. It is highly doubtful if the number of germ cells segregated in the development of the embryo is constant, and it certainly seems probable that the number of germ cells that reach the germ gland must vary greatly in different individuals. It would be futile, therefore, to form any conclusions concerning the number of primordial germ cells from a count of those that reach the germ gland.

4. Conclusions and summary. Evidence presented supports the theory that germ cells are segregated very early in the development of Entosphenus wilderi. These primordial germ cells are first found in the yolk entoderm and become included in the mesoderm when it separates from the entoderm. The germ cells occur at first only in the posterior region of the body, but they gain a more cranial position with the growth and development of the larvae. The movement of the germ cells craniad is probably a passive one in early stages and results from a shifting of the tissues during development. In later stages, after the cells have lost their yolk, migration is probably due partly to an active

SECTION CRANIAD OF ANUS	NUMBER OF GERM CELLS
58	1
61	1
68	1
76	1
80	1
84	1
86	1
92	1
98	1
102	1
104	1
105	1
106	1
108	1
111	1
120	1
122	1
129	2
135	1
144	1
148	1
155	1
156	1
157	1
158	1
159	1
165	1
166	1
174	1
177	1
182	2
197	1
207	1
211	1
Total.............................	36

movement of the cells. The original double anlage of the germ gland is reduced to a single median gland in later stages, due to a fusion of the two parts along the midline. This fusion begins at the cranial end of the gland. The germ cells which do not reach the germ gland probably degenerate. Some of them form extraregional cell nests, the fate of which is not known. There is no division of the germ cells before the larvae have reached a length of about 2 cm. Long before this all the yolk has been absorbed. Granules staining like chromatin are found in the cytoplasm around the nucleus when the yolk begins to disappear in the cells. When the yolk has been absorbed, two cytoplasmic bodies are found, one a spindle-shaped, granular vitelline body, the other an astrosphere (yolk nucleus). The origin of the vitelline body was not ascertained. The number of the primordial germ cells is small. In one count thirty-six were found. There is no indication during the early stages that the germ cells are derived from any other source than the early segregated cells.

C. Period of secondary division

After the primary period of rest, already described, during which the germ cells do not divide, they enter upon a period of division (table 2). In the following pages the changes are described which take place in the germ gland during this period, which begins when the larva is about 20 mm. long.

1. Observations on Entosphenus wilderi. a. Larva 21.5 mm. long, August 23 (fig. 34). In this larva, somewhat more than four months old, the germ-gland fold is still interrupted. While many single germ cells are found and many cysts of less than eight cells, there are a few of from eight to sixteen cells, and the latter number is rarely exceeded in this stage. Most of the germ cells are in the resting stage, but a few are in various phases of mitosis.

The gland is 2.5 mm. long and extends from a point near the middle of the mesonephros, about 2.5 mm. back of the cranial end of the coelom, to a point about 2 mm. in front of the anal opening. Its position, therefore, coincides about with the middle third of the coelomic cavity and not with its whole length as in older larvae.

Where the coelomic epithelium covers the germ gland it is thicker than elsewhere and its nuclei are much more numerous. These nuclei are ovoid in shape and are usually so oriented that their long axes are parallel to the long axis of the body and to one another. Each contains, besides a chromatin network, a large nucleolus, usually near its center (fig. 35). Many of the larger chromatin granules lie against the inner surface of the nuclear membrane. No membranes are visible between the cells of the peritoneum, but cell limits are indicated by the lessened thickness of the cytoplasm between the nuclei. All the cells of the germinal peritoneum are similar and in no case was the peritoneal membrane found to be interrupted so that the germ cells might be said to form a part of it. Evidence has already been presented to show that these two kinds of cells are of different origin and that there is no genetic relationship between them. That the follicle cells are derived from peritoneal cells, as in earlier stages, is indicated by the similarity in structure of their nuclei and by the fact that in various places peritoneal cells are migrating inward from the peritoneal covering of the gland to take part in the formation of follicles around the cysts and individual germ cells. When a germ cell or cyst lies against the peritoneum, the cells of the latter are usually heaped up around it in such a way as to suggest that they will eventually enclose it altogether. Some of the peritoneal cells also migrate into the interior of the gland without at first coming into relation with germ cells. This usually happens where the peritoneal membrane is indented, in which case the cells separate from the apex of the indented portion (fig. 35). Whether or not all these cells finally attach themselves to germ cells and form follicles for them is not known, although in some cases they seem to become associated with the more deeply situated germ cells. For a time at least, they make up most of the stroma of the gland, although part of the latter enters the gland from the mesenchyme dorsal to it. The nuclei of these few mesenchymal stroma cells may be distinguished from the peritoneal and follicle cells by their smaller size, structure, and rounded form. The cytoplasm ramifies in all directions so as to form a loose parenchymatous structure (fig. 36). Blood-vessels enter the gland from above with the mesenchyme and fill practically the whole of some sections. The endothelium of these blood-vessels consists of the usual flattened cells containing much flattened nuclei.

The germ cells remain as distinct elements with more or less clear cytoplasm, usually without observable limiting membranes, and with large rounded nuclei. In each nucleus there are, as before, two deeply staining nucleoli and a distinct chromatin network. One has no difficulty in distinguishing the germ cells from the somatic elements of the gland by their nuclear structure and the much greater size of both nuclei and cell bodies. No transitional stages were found between the germ cells and the somatic cells of the gland, and no indication of the transformation of somatic elements into germ cells or germ cells into somatic cells. The number of the germ cells is increased rapidly during this stage, but so many of them are in mitosis that the increase in their number may easily be accounted for without supposing that new germ cells are being formed out of surrounding tissue cells.

b. Larva 25 mm. long (fig. 37). The structure of the germ gland in a larva of this stage is similar to that of the preceding stage, except that, owing to the enlargement of the gland without corresponding increase in the number of germ cells and cysts, these are more scattered than in the earlier stages. In many sections not a single germ cell occurs. In some sections both germ cells and blood-vessels are absent and the peritoneum encloses only a loose stroma with greatly scattered nuclei.

The gland is now suspended from the middorsal line of the coelom by a broad mesentery, in which germ cells are present, while some are still found even in the mesenchyme above it. The germ cells and cysts often lie against the peritoneum and new follicle cells are being formed continually by division and migration of peritoneal cells.

c. Larva 27.5 mm. long. Although the histological features of the gland have not changed from those of the preceding stage, this particular larva was selected for study because certain cytological structures in the germ cells are more clearly shown. One of these is the centrosphere, which differs from that of the 20-mm. larva, in which it was last referred to, in that it shows a distinct centrosome. The vitelline body, as variable in position as in the 20-mm. larva, is visible. It is now more compact and stains black in sections treated with iron haematoxylin. In favorable sections the outline of each individual germ cell may be faintly seen in the cysts (fig. 38). The germ cells are in various phases of mitosis, but most of them are in the resting phase, as in previous stages. Those of a cyst do not divide simultaneously, but several cells within the same cyst may be in different phases of division, while others are in a resting condition (figs. 39 and 67). The cysts range from two to over thirty cells and there are a great number of isolated cells.

d. Larva 30 mm. long. In most of the larvae of this stage the germ glands are similar to those of the preceding stage. In one (larva no. 622) a large number of germ cells were in mitosis. The products of the division of an isolated germ cell may become enclosed, each in its own follicle, or they may remain together as a part of a nest of cells enclosed in a common follicle. The follicle cells of such a nest often stretch into the spaces between the germ cells, while other follicle cells, recognizable by the form and structure of their nuclei, are detached from the follicular membrane and lie free among the enclosed germ cells. Follicle cells occur in all stages of the process of detaching themselves from the follicles and also from the peritoneum, and of penetrating between the germ cells of the nests (fig. 40). In this way, as the germ cells increase in number, old cell nests are broken up and new ones formed, so that in this larva very few nests remain which have more than eight cells. The many follicle cells necessary to enclose the increasing number of nests are probably produced by proliferation of cells already in the follicles as well as of those in the peritoneal epithelium. If this be so, the mitoses must take place very rapidly, for dividing nuclei in either follicle or peritoneal cells are rare. There is no indication that follicle cells are derived from germ cells. If this were the case, transitional stages should be found which might be detected by nuclei of intermediate form and structure, but such have never been found.

e. Larvae 34 to 35 mm. long. In the gonad of this stage many single germ cells, as well as many cysts, occur ranging in size from two cells to one hundred or over. The cysts are surrounded, as before, with follicular cells. In many of them the germ cells are in a resting condition, but in some they are in mitosis. Sometimes cell nests, identical with those of the germ gland, occur in abnormal positions. One of these from the fat body is shown in figure 32. Some of the germ cells at this period have entered the synapsis phase, while others have transformed into growing oocytes, the first visible sex-distinguishing character.

2. Historical review and theoretical discussion. Wilhelm Müller ('75) studied the early stages of development in Petromyzon fluviatilis and P. planeri. He gives an account of the reproductive system in a larva 35 mm. long, but the earlier stages were apparently not studied by him. At this stage the sexes could not be distinguished. Oocytes were first observed in larvae 50 mm. long, and in larvae 65 mm. long the ovary and testis were fully differentiated.

Lubosch ('03) found the first anlage of the sex gland in larvae of Petromyzon planeri 18 mm. long. He found two types of cells in the gland, the follicle cells and the germ cells, and he believed that the peritoneum was the source from which both were derived. In a slightly later stage he describes a germ ridge as being formed, into which connective tissue and blood-vessels descend from the region dorsal to the gonad. He observed that peritoneal cells migrate inward to form the follicles surrounding the germ cells. He found that the glands remained undifferentiated until the larvae are about 4 cm. long when the ovaries could be distinguished from the male glands.

No further literature exists on this stage in the development of the germ gland in the lamprey.

In the lamprey, according to evidence presented above, most of the somatic part of the germ gland comes from the peritoneum. This is certainly true of the follicular epithelium and a considerable portion of the interfollicular tissue. On the other hand, some mesenchyme is included in the gland fold as it is formed and some is carried in later by the blood-vessels.

Whether or not the follicular cells take any active part in nourishing the germ cells in the early stages is not known. The only case of a true nurse cell observed in the germ gland of lower vertebrates in early stages is that described by Kuschakewitsch ('10). He found what he considered to be nurse cells in frog larvae which developed from his so-called 'Spätbefruchteten' eggs. They were distinguished by greatly concentrated nuclei and by cytoplasm filled with granules which he thinks are chromidia. These nurse cells were not, according to him, themselves used as food for the neighboring germ cells, but served as a source of certain ferments which were useful in preparing nourishment for the cells. He says concerning them: "Es handelt sich aber um eine Sezernierung von Fermenten, welche es bewirken dass die in der Keimanlage zirkulierenden Nährstoffe von den Ampullenelementen besser ausgenützt werden können."

A proliferation of peritoneal cells to form follicle cells has been observed by other investigators in various groups of vertebrates other than the cyclostomes. In the frog it has been observed by Bouin ('01), Dustin ('07), Kuschakewitsch ('10), and others. Bouin believes that these cells take part in the formation of germ cells as well as follicle cells. Kuschakewitsch does not believe that they take part to any great extent in the formation of germ cells. Dustin states that he observed the transformation of ordinary peritoneal cells into germ cells in the turtle (Chrysemys). Allen ('06), however, working on the same form, found no evidence of such transformation. In Triton, Abramowicz ('13) observed the proliferation of peritoneal cells in the germ gland, and believes that these cells give rise to follicle cells and interfollicular tissue as well as to germ cells, and to the cells of the sex cords. King ('08) thinks that in Bufo lentiginosus the peritoneal cells give rise to all the elements of the sex gland with the exception of the germ cells.

The germ cells of the lamprey have not been seen to divide except by mitosis. The nuclei of the cells are always spherical and never have the lobulated appearance which seems to be characteristic of the nuclei of the germ cells of amphibians. Several investigators (Vom Rath, '91; Meves, '91; McGregor, '99) have recorded amitosis as taking place in the germ cells of the latter group, but it is possible that the appearance of amitosis comes from sections through lobulated nuclei. Recently Macklin ('16) has made a study of apparent amitotic phenomena in heart cells of chick embryos growing in vitro, and has found that nuclei divide by bilateral and unilateral constriction, but that afterward the parts of the nuclei recombine and divide by normal mitosis. It may be that in some animals similar processes take place in the germ cells.

In the lamprey the cells in the same cyst do not always divide simultaneously, though generally, if the cells in a cyst are dividing, most of them are in one phase or another of mitosis. At the same time the cells in neighboring cysts may all be in a resting stage. Since the germ cells do not multiply very rapidly, there is a considerable period of rest between successive simultaneous divisions in a cyst. The synchronous division of the cells of a cyst may be due to their close relationship, all being derived from one cell and having been subjected to similar environmental influences, or to some stimuli from outside sources, the effect of which is limited to a single cyst. King ('08) found that in Bufo the cells of a cyst did not divide simultaneously. According to Jörgensen ('10), this is also true in Proteus. Bouin ('01), however, describes the cells of a cyst as dividing simultaneously in the frog. It seems likely that there may be considerable variation in this respect in different forms and even in the same species.

Since, in the lamprey, most of the cysts are broken up from time to time by the inward migration of follicular cells, it is impossible to say whether or not there is a constant number of divisions of the indifferent germ cells. In some cases the cysts become very large and contain hundreds of cells, but it is not certain that only these came from one primordial germ cell. In most, if not in all cases, each primordial germ cell gives rise to many cysts, probably to an indefinite number. In Bufo it appears that the cysts do not break up, for, according to King ('08), all the cells of a cyst are descendants of one primary oogonium in the female, and the cyst wall is formed out of the original follicle cells which surrounded the primary oogonium. Witschi ('14) states that the number of cells formed from the primary oogonium in Rana is at least thirty-two in some cases, but often fewer, while in the testis the number is greater. In the turtle Clemmys, Munson ('04) found that the number of divisions was three, each cell thus giving rise to eight cells.

It is impossible to distinguish oogonia and spermatogonia in the lamprey until the larva is about 35 mm. long. Before that time the cells in all larvae appear structurally alike and divide in a similar manner. The centrosphere with its centrosome corresponds to the yolk nucleus described by Lubosch ('04) in the larvae of lampreys about 4 cm. long. He describes it as an oval, clearly defined body of the same structure as the surrounding plasma, but not staining so deeply. Surrounding it, like a membrane, he found deeply staining granules. Lubosch believes that the yolk nucleus introduces the process of yolk-building; but this cannot be its function in very early stages (larvae up to 35 mm. in length) while the cells are still dividing and long before there is any formation of yolk. In these stages it probably functions in cell division in the germ cells as it does in other cells. During the subsequent growth period it may play some part in the process of yolk-building.

The vitelline body is probably present in all indifferent germ cells. It seems to be a permanent element in the germ cells of the lamprey, but it was not possible to determine its origin. King ('08) believes that in Bufo it must be considered as a secretion product of the cytoplasm itself, but she thinks it not improbable that a fluid, possibly an enzyme, may pass from the nucleus into the cytoplasm and there cause the formation of the body, and that this enzyme, while in the nucleus, may be in the form of plasmosomes. Dodds ('10) has described a body in the cytoplasm of the early germ cells of Lophius and believes that it is a mass of plasmosome material that has been separated and cast out of the nucleus. The act of extrusion was not observed. The body found by Dodds, however, has only a transitory existence in the cytoplasm, so it cannot correspond to the vitelline body. Since the vitelline body is found to be a very prominent structure in the oocyte of the lamprey, a full discussion of it will be reserved for a future paper which will describe the growth period of the egg.

Witschi ('04) found that sex could be distinguished in frog larvae before there was any differentiation of the germ cells, by the fact that in the larvae destined to become females the germ cells remained along the periphery of the gland, while in those destined to become males the cells migrated into the interior of the gland in very early stages. No differences of this kind have been found in the larvae of the lamprey during the indifferent period. Neither are any genital cavities formed in the gland by means of which the sexes may be distinguished previous to germ-cell differentiation, as is the case in amphibians.

3. Summary of the period of secondary division. During the period of secondary division (larvae 20 mm. to 35 mm. long) the germ cells multiply by frequent mitoses. The resulting cells may remain together after division, enclosed by a common follicle, or they may become separated by the migration of follicle or peritoneal cells between them. The result is that in all glands both cysts and isolated germ cells are found. The germ cells are distinguished from the somatic elements of the gland by their size, the structure of their cytoplasm, and the form and structure of their nuclei. There is no indication that germ cells are derived from somatic cells or somatic from germ cells. Usually most of the cells of a cyst are found in one or another phase of mitosis at the same time, but some of the cells of the cyst may be in a resting stage while others are dividing. In the cytoplasm of the germ cells a centrosphere with a centrosome is often visible; also a vitelline body of unknown function and origin. Spermatogonia and oogonia cannot be distinguished during this period. Neither are there any other characters by means of which future males and females may be distinguished.

D. Period of sex differentiation

1. General statement. The period following that of secondary multiplication of the germ cells is characterized in dioecious animals by the differentiation of male and female individuals. The latter may be distinguished from the former by the appearance in the germ gland of yolk-filled oocytes. This phenomenon is accompanied or preceded by somatic changes in the germ gland and elsewhere. The somatic differentiation may take the form of changes in the structure of the germ gland, the appearance of accessory reproductive organs peculiar to one sex or the other, or the development of other secondary sexual characters.

In the lamprey there are no secondary sex characters developed until after metamorphosis, before which the sexes cannot be distinguished except by an examination of the germ gland, and in early stages even the germ gland does not form a criterion of the future sex of the animal.

2. Sex characters in the adult brook lamprey. The reproductive gland in the adult lamprey is unpaired and is suspended by a mesentery from the middorsal line. Previous to spawning, it fills practically the whole body cavity in both sexes. The surfaces of both the male and female glands are thrown into more or less oblique folds, more easily seen in the testis than in the ovary. The testis is made up of numerous cysts filled with spermatozoa and enclosed by follicle cells. It is supported by the mesorchium, from which connective tissue cords radiate into the body of the gland. In the ovary each ovum is enclosed by follicle cells as are the cysts in the testis. Connective-tissue cords, similar to those of the testis, radiate into the body of the gland from the mesovarium above. The number of ova varies in different females as also does their size. In the same animal, however, the ova do not vary greatly in size.

The cysts in the testis and the ova in the ovary may be considered homologous structures. In the one case the germ cells have continued to divide, while in the other case they have stopped dividing early in the life of the animal and have entered upon a period of growth. The greatest amount of growth takes place in the female after metamorphosis, while in the male metamorphosis is followed by a period of very rapid multiplication of the germ cells. In both sexes the germ cells reach the outside through abdominal pores. These open in each sex into a common urogenital sinus which terminates externally in a urogenital papilla.

There are only a few external sex-distinguishing characters. The urogenital papilla is short and wide in the female, with a large opening at the end, while in the male it is long and slender with a small opening at the tip (fig. 66). Both sexes have a lateral fold of skin on each side of the papilla. In the female the papilla is hidden by these folds, but in the male it extends beyond the folds as a prominent structure. In the female a small anal fin, connected with the caudal fin by a low ridge of skin, lies directly behind the urogenital papilla, while in the male the anal fin is absent or rudimentary. Loman ('12) states that in the European brook lamprey the anterior dorsal fin is always lower in the female than in the male, and Gage ('93) found the cranial end of the second dorsal fin always swollen in the female of the American brook lamprey.

From the above description it will be seen that the reproductive organs in the lamprey are reduced to extreme simplicity. The sex glands and the accessory structures, by means of which the reproductive elements are extruded, are morphologically similar in the two sexes. This point is emphasized because of its bearing on the tendency of the animal toward hermaphroditism.

3. Changes in the germ gland during the period of sex differentiation. In larvae less than 35 mm. in length the majority of the germ glands are in an indifferent condition as regards sex. During these stages the germ cells are scattered irregularly through the germ gland, either singly or in cysts; either in a resting condition or in various phases of mitosis, but they are all alike. This indifferent period is followed by one in which distinct sex characters appear in the germ glands through the development of oocytes found both in the synaptic and growth phases (tables 1 and 2). Whether the germ glands eventually become ovaries or testes, they all develop this female character, and the animals may therefore during this stage be considered intermediate as to sex or hermaphroditic.

During this period (larvae 35 mm. to 70 mm. in length), therefore, one may find in any germ gland, germ cells and cysts which are in all respects like those of the indifferent period, as well as germ cells, located singly or in cysts, which are in the various stages of the synaptic phase or which have entered the growth period.

To avoid confusing the germ cells which are in the various phases of mitosis with the cells that had already entered the prophases of maiosis, it seemed necessary to follow as carefully as possible the various steps in the two processes. On account of the small size of the cells and the large number of chromosomes present, it was very difficult to get a complete history, but the main features were worked out.

a. Changes during mitotic division. The resting germ cell. The resting cell (fig. 41) has a spherical nucleus in which the chromatin material is in small and large granules or masses, united by fine achromatic threads. Two rather large plasmosomes, at some distance from each other, are present in the nucleus. In cells stained in iron haematoxylin and afterward destained so long that the chromatin masses can no longer be seen, the plasmosomes retain the stain and stand out as two very distinct elements. The formed elements of the nucleus are surrounded by a clear homogeneous nuclear sap, and the whole nucleus is enclosed by a nuclear membrane. The amount of cytoplasm is rather small. An astrosphere with a centrosome lies along one side of the nucleus, although the centrosome is not always visible. A vitelline body sometimes occurs. The cytoplasm is granular and, in cells, fixed in Meves' solution and afterward stained in iron haematoxylin, mitochondria, in addition to finer protoplasmic granules occur.

Prophase. During the first phases of mitosis (fig. 42), the amount of chromatin increases greatly. The original chromatin granules of the resting cell grow in size but remain united by linin threads, so that the whole gives the appearance of a network with conspicuous masses of chromatin at the crossings of the threads. The two nucleoli remain distinct as before.

A little later (fig. 43) the chromatin masses become still more conspicuous, but they continue to be held together by the achromatic threads. The nucleoli are still large, but generally one is considerably smaller than the other. They are closer together than in the preceding stage.

The chromatin granules or masses soon become separated from one another by the breaking of the achromatic threads which up to this time have been holding them together (fig. 44). Each mass is now clearly a short globular chromosome. It was impossible, however, to count the chromosomes with any degree of accuracy, either at this or any other stage. At this time only one nucleolus is visible. The nuclear membrane is still intact. There is some indication that the chromosomes are arranged in pairs.

In the next stage (fig. 45) the nuclear membrane disappears and the chromosomes come together in a mass at about the equator of the cell. The two centrosomes occur at opposite poles, and achromatic spindle fibers extend from the centrosomes to the chromatin mass. The nucleolus is no longer visible and its fate is not known. A polar view of this stage is shown in figure 46. The chromosomes are so closely massed that it is difficult to distinguish each individual chromosome.

Metaphase. Although numerous dividing cells were examined, none was found in which the splitting of the chromosomes could be observed. It seems likely that this process takes place so rapidly that the chances of finding a cell in this stage are slight. Furthermore, the chromosomes are so closely massed together on the equatorial plate that their division would be difficult to observe.

Anaphase. Figures 47 and 48 represent cells in early anaphase. The daughter chromosomes have already begun their migration to the opposite poles. Occasionally bodies having the appearance of chromosomes are found outside of the spindle in the cytoplasm of the cell, or they may be scattered along the spindle threads.

Telophase. In this stage (figs. 49 and 50) the daughter chromosomes have separated from each other and form irregular groups at opposite poles. The spindle is still distinct. The cell is constricted in the middle and the two daughter cells are about to separate. Sometimes a deeply staining strand or thread remains between the two chromatin masses after they are some distance apart (fig. 49). In a slightly later stage a distinct midbody is seen along the line of separation (fig. 50). Soon after this the two daughter cells separate, a new nuclear membrane forms around each daughter nucleus, and the two nuclei are in a stage of reconstruction. The two daughter cells are smaller than the mother cell (fig. 51). Small chromatin masses reappear and are united by connecting achromatic threads. At least one nucleolus occurs in each cell. After division the cells enter a period of growth until they have assumed the structure and size of the mother cell.

Discussion. There is nothing strikingly peculiar in common mitosis of the germ cells of the lamprey and only a few observations need further comment. First, the process of division is probably not very rapid. This statement is based on the fact that the number of germ cells does not at any time increase very fast. From the number of germ cells in the mitotic phase at any one time, it is evident also that the karyokinetic period must be rather long, probably occupying several days.

The chromosomes are more or less rounded and stand out most clearly in the middle prophase (fig. 44). The number of chromosomes is very large, and they often give the impression of being in pairs. This may mean one of two things: either that the chromosomes have already divided during the prophase stage before they have reached the equatorial plate or that the maternal and paternal chromosomes remain associated during the prereduction stages. The latter was found by Chubb ('06) to be the case during the multiplication period in the germ cells of Antedon. Stevens ('07, '08) found that in Diptera a pairing of the chromosomes took place in germ cells far removed from the reduction stages and occurred in connection with each oogonial and spermatogonial division. Metz ('16) has reinvestigated the problem in Diptera, and from a study of about eighty species has come to the conclusion that in somatic, as well as in germ cells, the chromosomes are associated in pairs. The paired condition persists throughout the various phases of cell division. An association of maternal and paternal chromosomes is apparently effected during early cleavages, and probably before the first cleavage, thus continuing from the fertilized egg to the adult stage. In Diptera the pairing is side by side (parasyndesis) and similar to synaptic pairing. The pairing, according to Metz, comes about through a physicochemical similarity of the homologous maternal and paternal chromosomes.

In the lamprey the granules on the achromatic network of the resting nucleus form the centers for the reconstruction of the chromosomes. There is little doubt that these granules actually represent chromosomes in all the various phases through which the cells pass; for this reason one may speak of a visible continuity of chromosomes from one cell generation to the next. Thus the individual chromosomes in the germ cells of the lamprey never lose their identity during mitosis.

The origin, function, and fate of the plasmosomes remain obscure in the cells of the lamprey. In the resting cell there are usually two plasmosomes which appear approximately of the same size and which lie some distance apart. As the phenomena of prophase advance, they approach each other, and somewhat later only one is present. Whether a fusion of the two takes place or one dissolves and disappears at this time is not known. During late prophase the single plasmosome also disappears or at least is no longer distinguishable among the chromosomes. During the telophase a new plasmosome soon appears in each daughter cell and, as the growth of the cell progresses, a second plasmosome also appears. It is likely that the plasmosomes dissolve during cell division to be formed de novo in the daughter cells.

b. Synapsis phase of the oocytes. After an indefinite number of divisions, some of the primordial germ cells (oogonia), which lie singly or in cysts, undergo a series of changes preliminary to the stage of actual growth. When the cells have entered this stage they are termed oocytes of the first order (table 1).

Very little was known concerning the processes that take place in the oocyte of any animal preliminary to its growth period, until von Winiwarter ('01) published his extensive observations of this period in the cat and in man. Based upon changes which take place in the nucleus, he divided the transition period from the oogonium to the oocyte into the following periods: 1) Noyaux protobroques; 2) noyaux deutobroques; 3) noyaux leptotènes; 4) noyaux synaptènes; 5) noyaux pachytènes; 6) noyaux diplotènes, and, 7) noyaux dictyes. In a later publication by von Winiwarter and Sainmont ('09) another stage has been added between the first two, namely, noyaux poussiéroides. Other investigators have divided the period differently and have applied other terms to the different phases, but on the whole the processes taking place in all animals in which the synapsis phase has been studied seem to follow the general course outlined by von Winiwarter. In the lamprey the changes correspond in the main with those of other forms studied. An abbreviated list of the above stages has already been given (table 1).

Nuclear changes. Early leptotene. After the last oogonial division the germ cell enters a period of rest, during which the chromatin of the nucleus becomes broken up into small particles (fig. 52). These are scattered throughout the whole substance of the nucleus so as to make its contents appear almost homogeneous. There are no very distinct chromatin bodies in the nucleus at this stage and the only stainable parts are a very fine network and two very distinct plasmosomes. This stage corresponds to the stage in the germ cells of the cat described by von Winiwarter and Sainmont ('09) as 'noyaux poussieroides,' and to the stage in the germ-cell history in Proteus described by Jörgensen ('10) as 'erste Zerstäubung.'

Sonnenbrodt ('08) studying this period in the germ cells of the chick, found that the chromatin at this stage was very small in amount, and he believes that the period succeeding the last oogonial division is devoted largely to the formation of new chromatin. He says it "besteht in der Hauptsache in der chromatin Aufnahme oder richtiger Chromatinbildung." In the lamprey, too, the chromatin network begins to reappear at a somewhat more advanced stage, but for a long time the chromatin remains in a finely divided condition. The nucleus appears to grow larger as the period progresses. The two nucleoli persist throughout the period.

Bouin ('01) studied this period in the oocyte of the frog, and called the cells of this stage 'ovogonies de transition.' He described the nucleus as losing its membrane at this time so that there was a free communication between the nuclear and cytoplasmic substances. This has not been confirmed for other forms and it is certainly not the case in the lamprey.

Late leptotene stage. During this stage (figs. 53 and 54) the chromatin network becomes much more distinct. The whole nucleus is now filled with chromatin threads which cross one another in various ways. Irregular thickenings are found on the chromatin threads not only at their intersections, but in other parts as well.

Synaptene stage. During this stage (fig. 55) the chromatin becomes massed together along one side of the nucleus in the form of an irregular tangle of rather thick, deeply staining threads, and forms what has been termed a 'contraction figure.' This is the stage described by von Winiwarter as the synaptene stage and by Maréchal ('04) as the 'bouquet stage.' On the side of the nucleus on which the chromatin is concentrated, the individual threads can no longer be distinguished; but in the clearer parts of the nucleus many of the ends of the chromatin threads extend out from the concentrated mass, sometimes as far as the nuclear membrane of the opposite side. Occasionally a nucleolus, which later appears to be lost, occurs during the early phases of this stage. It is possible that later, when it is not visible, it is hidden among the chromatin threads of the contraction figure. If in reality this be the case, it indicates a tendency of the chromatin to concentrate around the nucleolus during this stage; for otherwise, if the nucleolus be present, it should be found occasionally in the clearer portions of the nucleus. Some investigators have termed this period the synapsis stage, because in many forms the chromatin threads come together in pairs at this time. McClung ('05) has called it the 'synizesis stage,' and this is a more appropriate term, since apparently synapsis does not always occur during this period. In the case of the lamprey it was not possible to find any pairing of the chromatin threads at this time, although hundreds of cells were examined.

Pachytene stage. In the pachytene stage (fig. 56), the chromatin material again becomes uniformly distributed throughout the nucleus. It is now in the form of thick threads which appear more or less continuous in some places, but are generally broken up into segments. One large nucleolus appears. There is no indication that the chromatin threads are paired.

Diplotene-dictyate stage (diakinese) (figs. 57, 58, 59, 60, and 62). In an oocyte somewhat farther advanced than the above, the whole chromatin network has become broken up into definite chromosomes, and the paired structure of every chromosome is very apparent, a condition which persists throughout the early part of the growth period and probably up to the time of maturation. It is very difficult, however, to follow the history of the chromosomes during the later growth period, since the nucleus becomes very large and the chromatin material may be scattered throughout its whole extent. The nucleolus at this time is large and almost spherical. It has not been possible to observe any relation between the nucleolus and the chromosomes at this period of development. Many of the paired chromosomes lie against the nuclear membrane, but they may occur also in various other parts of the nucleus. Very often they are arranged in the form of tetrads which are best seen along the nuclear membrane (fig. 61). There appears to be no regularity in the arrangement of the chromosomes. The dictyate stage (diakinese) continues during the growth period of the oocyte.

Cytoplasmic changes. The nuclei of the oogonia are surrounded by a small amount of granular cytoplasm. Often no visible cell boundaries are present, although favorable sections show that the cells do not form a syncytium, but are morphologically independent of one another, in spite of the fact that no true cell membrane is found. In cells, fixed in Meves' solution or in other solutions that fix mitochondria, these occur in great numbers. They are usually granular and appear to be more or less grouped. There is no evidence that they are derived from the nucleus, so they may be considered as true cytoplasmic bodies. The mitochondria in the early oocytes of the lamprey are not essentially different from those found in the indifferent germ cells (figs. 41 and 52).

During the progress of the early nuclear changes, up to the time of the synaptene stage, there is a gradual decrease in the amount of cytoplasm and a gradual disappearance of the mitochondria. After the synaptene stage the amount of cytoplasm increases again, but no study was made of the mitochondria subsequent to the synapsis phases.

In the undifferentiated germ cells there is an astrosphere near the nucleus, even in the resting cells. Sometimes a minute centrosome has been distinguished in the middle of the astrosphere. The astrosphere may be distinguished from the surrounding cytoplasm by its more granular appearance. During the middle synapsis phases it seems to disappear with the decrease in the amount of cytoplasm, but it reappears in the early growth period and remains through this whole period as a very prominent structure (fig. 60).

The vitelline body may be traced through the various phases of the synaptic period, and during the growth period it becomes a very conspicuous structure (figs. 60, 62).

Discussion. The general history of the period has been outlined above, but certain features require further discussion. The changes taking place in the cells during this period initiate the period of heterotypic or reduction division. This is a period through which all germ cells apparently must pass before they can become functional ova or spermatozoa. In the female cell these changes take place at a very early stage in the development of the animal, and in the case of the lamprey they precede the maturation period proper by at least two or three years. In the male lamprey these changes occur much later in the life of the individual and usually precede the maturation divisions by only a very short time, probably not more than three or four months.

I have made a study of the synaptic phases of the male germ cells in the lamprey and found the process of development to be much like those of the female. In both sexes the germ cells presumably come out of the synaptic phase with the number of chromosomes reduced to one-half the number found in the presynaptic cells. The subsequent history of the cells, however, differs in the two sexes. In the female the cells grow to an enormous size by the accumulation of yolk, while in the male there is very little growth.

Aside from the apparent pairing of the chromosomes during the period of multiplication, no other instance of chromosome pairing was observed in the cells before they have reached the diplotene stage; but it cannot be said with certainty that a doubling does not take place before this stage during the synaptic phase. In some forms which have been investigated, synapsis seems to take place during the synaptene stage, or during the period of transition from the leptotene to the synaptene. Von Winiwarter ('01) figures a pairing of the chromatin threads in the germ cells of the rabbit during the early synaptene. Von Winiwarter and Sainmont ('09) describe a similar condition in the cat. In Proteus also, according to Jörgensen ('10) the leptotene stage is followed by a stage which shows a double nature of the chromatin threads. In Bufo, King ('08) figures double chromatin threads for the first time after the synaptene stage. Maréchal ('04) observed the double structure of the threads during the synaptene stage in Pristiurus and Scyllium and later ('07), in Ciona and Amphioxus. Janssens ('04) found that in Triton the reduced number of chromosomes, or chromatin filaments, appears shortly after synizesis and that these filaments subsequently split longitudinally forming two sister threads which remain together. d'Hollander ('04) found a massing of chromatin (synizesis) in the oocyte of the hen before synapsis.

The phenomenon of synizesis (McClung, '05) has been found by various investigators to occur in the oocyte of invertebrates as well as in vertebrates; it seems to be a universal phenomenon of the early heterotypic prophase. Chubb ('06) thinks that synapsis takes place in Antedon during the oogonial divisions, and that it is followed by still one more division. In Sycon Jörgensen ('10) thinks that the reduced number is present in the oogonia. According to these investigators, synapsis may take place previous to synizesis, and the two phenomena probably have nothing in common. Most investigators, however, agree that the double nature of the chromosomes is first visible during a late stage of the heterotypic prophases, but their interpretations of the doubling vary. Some consider it a suppressed mitosis (Hertwig, '08; Matscheck, '10, and others), while the majority of workers on germ cells look upon it as a pairing of parental chromosomes similar to that which takes place in the male germ cells previous to the maturation division. Very little light can be thrown upon this subject by the lamprey, since it was found impossible to count the chromosomes before or after synapsis. To all appearances, however, the chromosomes enter synizesis in the univalent condition. The bivalent nature of the chromosomes is not observable before the diplotene stage.

The meaning of the 'contraction figure' has been variously interpreted. Some investigators consider it simply an artifact due to poor preservation (Janssens, '05; Jörgensen, '10, and others). Maréchal and Saedeler ('10) insist that it is not an artifact in Raja clavata. King ('08) has shown that it is a perfectly normal condition in the toad. In the lamprey it appears to be a normal phenomenon, and forms a stage in the morphological changes which take place in the oocyte at this time. In the same gland were cells in the contraction phases, other cells in the various stages of the synapsis phase, normal resting cells, and cells in the different phases of mitosis. Degenerating cells also occurred in most glands, but no evidence was found to indicate that the contraction figure is a phase in the process of degeneration. Even in the same cyst, there are contraction figures side by side with resting cells and cells in other stages of the synapsis phase. It must be concluded, therefore, that the phenomena connected with synizesis in the lamprey are perfectly normal and due to some peculiar condition of the cells at this time—a condition the nature of which is not yet understood.

Whether the contraction figure is normally formed around the nucleolus or on the side next to the centrosome could not be determined. A body sometimes occurs in the cytoplasm near the nucleus on the side toward which the contraction figure is formed, but this was taken to be the vitelline body since it may often be found in other parts of the cells. No distinct centrosome or attraction sphere could be found in the cells at this time. Jörgensen ('10) figures a very distinct astrosphere during the bouquet stage in Proteus, but he was not certain of the presence of a centrosome. He also found that during this period there was an extrusion of chromatin material from the nucleus into the cytoplasm on the side next to the astrosphere. This was not found to be the case in the lamprey, in which the nuclear membrane appears to be intact throughout the period of transformation of the germ cells into growing oocytes. No centrosome was found by King ('08) in the germ cells of the toad during the synapsis period, and she concludes that probably the egg centrosome disappears after the last oogonial division. Lams ('07), on the other hand, observed a centrosome in the germ cells of the frog during the bouquet stage.

Although no centrosomes or astrospheres were found during the synapsis phase in the oöcytes of the lamprey, they are not permanently lost, for they reappear somewhat later in the growing oocyte. It is probable that special technique might make them visible also during the synapsis stages. Jörgensen holds that the centrosome is functional in connection with the convergence of the chromosomes along one side of the nucleus during the bouquet stage, with the radiations in the cytoplasm through the orientation of plasma inclusions, and with the formation of a permeable region in the nuclear membrane where chromatin bodies may be extruded from the nucleus. In the lamprey no extrusion of visible chromatin material from the nucleus at this stage has been observed, but there is ample evidence that such extrusion takes place in the dictyate stage, although there appears to be no special area of the nuclear membrane over which it occurs.

It has been shown that two nucleoli are present in the germ cells of the lamprey during the multiplication period. During mitosis these are reduced to one, which also later disappears. In the resting cells after mitosis a single nucleolus appears and shortly afterward a second. When the cells enter the synapsis phase, the nucleoli again disappear, apparently during synizesis. When the cells enter the growth phase, only one nucleolus appears in each cell. This remains during the whole period as a very prominent spherical structure. Whatever may be the function of the nucleoli, they are, as shown by their reaction to stains, true plasmosomes, and not composed of chromatin material. This is contrary to the idea of Lubosch ('03), who believes that in the lamprey and in other forms the chromatin material is stored in the nucleolus during the growth period of the egg. This view is based largely on the fact that the chromosomes seemingly disappear during the later stages of growth. Lubosch also thinks he has evidence that the maturation chromosomes are derived from the nucleolus. I have found some evidence which indicates that the maturation chromosomes appear in the clearer portions of the nucleus and do not come from the nucleolus.

Von Winiwarter and Sainmont ('09) found in the nucleus of the oogonia of the cat, at the time when the cells were preparing for mitosis, an elongated body which stained like chromatin. Often it had a horseshoe shape, and it was larger than the other bodies of the nucleus. During mitosis it divided, but much more slowly than the chromosomes. In the oocyte it was often attached to the plasmosome, but sometimes it was free. When dividing, it split longitudinally, and during the growth period it disappeared. The body was supposed by the authors to be a sex chromosome (monosome). Gutherz ('12) found a similar body in the spermatocytes of the cat, but came to the conclusion, on account of its staining reaction, that it was a true plasmosome "der einen Gestalt ein Heterochromosome in Herteropyknose vertauscht." Gutherz doubts that the body observed by von Winiwarter and Sainmont was a true sex chromosome, since no differential stain was used by them. Furthermore, there should be two sex chromosomes present in the oocyte of the cat, if such bodies are present at all, since in this form the male is apparently heterozygotic with respect to sex.

There is considerable danger of misinterpreting nuclear bodies. Many of the structures described in the germ cells of vertebrates as sex chromosomes may be plasmosomes and have nothing to do with the determination of sex. Wilson ('13) found a body in the spermatocytes of Pentatoma that simulated an accessory chromosome, and which he called a 'chromatoid body.' A similar body has been found by Wodsedalek ('14) in the spermatocytes of the horse and by Bachhuber ('16) in the spermatocytes of the rabbit. More recently, Swingle ('17) describes what he considers to be the same kind of body in the spermatogonia of the frog (Rana pipiens and R. catesbiana). The body was in all these cases of cytoplasmic origin and was found with the chromosomes only during mitotic division.

An examination of the various figures given of a so-called sex chromosome in vertebrates reveals a striking resemblance to plasmosomes similar to those that are found in the early oocyte of the lamprey. Stevens ('11) describes such bodies in the spermatocytes of the guinea-pig; two such bodies were found by Wodsedalek in the spermatocyte of the pig; similar bodies were found by Levy ('15) in the spermatocyte of the frog. Guyer ('09, '16) has described such bodies in the spermatocytes, in the oocytes, as well as in the body cells of the fowl, and ascribes to them a sex-determining function. Finally, Jordan ('14) has found such a body in the germ cells of various mammals. Bohring and Pearl ('14) have studied the body found by Guyer in the domestic fowl, and have come to the conclusion that it is not a sex chromosome.

At the present time the status of the sex chromosome in the germ cells of vertebrates is very uncertain. It is unfortunate that the subject has been studied almost exclusively in the male germ cells. Von Winiwarter's account of such a body in the oocyte of the cat and Guyer's description of the body in the female germ cells of the fowl seem to be about the only accounts dealing with the sex chromosomes in the female germ cells of vertebrates.

It is generally assumed that when the spermatozoa in a species are dimorphic, the female of the same species produces eggs only of one kind. These correspond in their chromosome make-up to the male cell possessing the sex chromosome. All the eggs, therefore, in such species possess accessory chromosomes. On the other hand, it is assumed that if the female produces two kinds of eggs which differ in their chromosomal structure by the presence or absence of a sex chromosome or chromosome complex, the male must produce only one kind of spermatozoa. In this case one-half of the ova should correspond to the spermatozoa in their number of chromosomes. Guyer ('16) says that in the fowl, where it has been shown experimentally that the female is heterozygous for sex, there are also two kinds of spermatozoa. He believes it is probable that only the spermatozoa containing the odd element become functional.

In the lamprey no evidence has been found of the presence of an accessory chromosome in the oocyte during the synaptic phase, the growth period, or the maturation division stage. A search has also been made for this body in the spermatocytes during the various stages of maturation, but without success. If, as seems to be the case in some invertebrates and in Necturus among vertebrates (King, '12), the sex chromosomes might be united with other chromosomes, it would be extremely difficult to find it in forms like the lamprey where the chromosomes are very small and numerous. Observations on the lamprey can neither affirm nor deny the existence of sex chromosomes which might be responsible for sex. It can only be said that such a body has not been found. Whether or not the assumption of the presence of such a body is necessary to account for sex in forms like the lamprey where the sex potentialities are so equally balanced, is a question which will be discussed later.

I have not found in the lamprey a transfer of visible material between the nucleus and the cytoplasm of the oocytes during the synaptic period, but have found that there is an intimate relation between the two parts of the cells. The absolute amount of cytoplasm decreases greatly during this stage, and it is not until the nucleus enters the diplotene phase that the cytoplasm begins to grow again. All the energy of the cell seems to be devoted to nuclear changes in the early oocyte and to cytoplasmic changes during the growth period which follows synapsis. The mitochondria, which are abundant in the oogonia, disappear during the synaptic phases or, at least, can no longer be seen. In this respect they seem to behave like zymogen granules in gland cells. In the resting gland cells the zymogen granules are very abundant, but in a cell which is secreting they decrease in number and size, and may disappear entirely if activity continue.

Most of the mitochondria in the germ cells of the lamprey are spherical, but occasionally rod-shaped ones may also occur. The theory that the early germ cells may be distinguished from the somatic cells by the shape of the mitochondria has been developed by Rubaschkin ('10) for mammals, Tschaschkin ('10) for birds, and Aunap ('13) for fishes. These investigators think that the mitochondria of the germ cells are spherical, and that during the process of development of the embryo they become chain-like and finally rod-shaped in the differentiated tissue cells. The primitive character of the germ cells is, therefore, according to these investigators, indicated by the fact that they possess granular mitochondria after the other cells of the embryo show rod-shaped ones. That this is a universal character of the early germ cells has been denied by von Berenberg-Gossler ('12) and others. Von Berenberg-Gossler found that in the individual germ cells of the duck and the chick, the shape of the mitochondria may vary from granular and chain-shaped to rod-shaped. Firket ('14) also found that in the germ cells of the chick, the shape of the mitochondria is not constant.

In the oocyte of the lamprey the mitochondria may be found again after the beginning of the growth period. They are cytoplasmic structures and not related to the chromidia which are so abundant in the growing oocytes of the various stages. This is in agreement with Schaxel ('10, '11) and others who consider the chromidia to be of nuclear origin and the mitochodria to be of cytoplasmic derivation.

Meves ('08), the first to study the mitochondria in embryonic cells, upheld the theory which had previously been advanced by Benda ('03) and others, that the mitochondria are bearers of cytoplasmic heredity. This theory has since been advocated by Duesberg ('08, '10) and others. Those who adhere strictly to the chromosome theory of inheritance are opposed to it. According to Cowdry ('16), the chemical nature of the mitochondria seems to oppose the idea that they are individual constant bodies in the same sense that the chromosomes are considered to be so. It is more likely that they play an active rôle in the metabolic activities of the cell and that they may vary in number, size, and shape as the activity of the cell varies.

c. History and fate of the germ cells during the period of sex differentiation. This period (table 2) includes larvae from about 35 mm. to about 70 mm. long. Figure 68 is a cross-section through the germ gland of a larva 54 mm. long, from the middle of this period. It shows many large, growing oocytes, as well as many cysts. A comparison of sections from various larvae shows that the oocytes are formed from germ cells which have entered synapsis, either while isolated or while constituent elements of small cysts, usually of less than eight cells. In the latter case the cysts become broken up by the penetration into them of follicular cells, so that each of their contained cells, while still in the synaptic phase or in the early growth phase, becomes isolated within its own follicle. Thus, as shown in figure 68, nearly all of the growing oocytes are sooner or later isolated cells. In addition to these cells the section contains numerous large cysts, and of these there are two kinds. In one kind the germ cells are still indifferent, as shown by the fact that they have not entered synapsis, but are dividing by typical mitosis. Such a cyst containing two cells in mitosis is shown at the left of the figure. In the second kind of large cyst the cells have entered synapsis. Such cysts are shown to the right of the figure and are recognizable by the different behavior of the chromatin. In large cysts the cells which enter synapsis rarely become growing oocytes, but sooner or later degenerate, until finally they and their enclosing follicles break up and disappear. A longitudinal section of a gland from a larva 59 mm. long, in which two such degenerating cysts are found, is shown in figure 69. The cyst at *deg.cy.2* is in a later stage of degeneration than the one at *deg.cy.1*. A detailed drawing of such a cyst from another section is given in figure 65. The cells of such a cyst rarely get beyond the synizesis stage before degeneration sets in. Degeneration begins with a condensation of the chromatin into solid masses, as shown at *deg.g.* in the figure. The cytoplasm disintegrates and finally the chromatin masses break up and the fragments are scattered through the whole cyst. Such fragments are shown at *g.f.* Other stages occur showing the various steps up to the final dissolution of the cysts. No large cysts containing cells in synapsis have been found to break up by the inward migration of follicle cells. The final fate of all of them and of their enclosed cells is degeneration. Rarely, however, a single cell of such a cyst may enter the growth phase. A cyst containing such a cell is shown in figure 64. This contains a large oocyte among the smaller cells of the cyst. Whether such oocytes continue to grow and form functional ova is not known, but it is certain that nearly all of the growing oocytes are derived from isolated germ cells or from the breaking up of small cysts. Other sections, showing both cysts and oocytes in about equal numbers, are given in figures 70 and 71 from larvae 55 mm. and 62 mm. long, respectively.

In the middle of the period of sex differentiation, therefore, there are in the same gland undifferentiated germ cells that are still dividing; germ cells in the various prophases of heterotypic division; cells that have entered the period of growth, forming recognizable oocytes, and cells undergoing degenerative changes.

The gland described above represents only one type of germ gland during this period—a type in which the number of growing oocytes and cysts is approximately the same. There are other glands with very few growing oocytes, and still others in which there are very few cysts. Figure 72 is a section through the germ gland of a larva 50 mm. long in which there are very few growing oocytes, and none of these are shown in the section. The gland is filled with cysts, some of which contain undifferentiated germ cells, while some contain cells in the synaptic phases. It is often found that if a gland seems free from growing oocytes when examined superficially, more careful search usually reveals at least a few of them scattered about through the gland. In one such gland only one growing oocyte could be found.

Figure 73 is a section of the germ gland of a larva 71 mm. long. In this larva there are only a few growing oocytes, only one of which is shown in the section. Numerous cysts are present, some with cells in synapsis, others with degenerating cells, and still others with undifferentiated cells.

Figure 74 shows a cross-section of a gland from a larve 65 mm. long. In this larva the gland contains practically only growing oocytes, although a few individual cysts are scattered throughout the gland. Such cysts may contain only actively dividing germ cells or only cells in the various stages of degeneration. Larvae with such glands quite certainly become females. Another gland of this type from a larva 63 mm. long, is shown in figure 75.

Figures 76 and 77 are two sections from larvae 50 mm. and 60 mm. long, respectively. In these the number of oocytes in the gland is greater than the number of cysts.

Germ cells of the various types mentioned above are distributed throughout the germ glands of this period, apparently without any regularity and without any relation to one another or to the somatic parts of the gland. All of the types may occur along the periphery of the gland or in the deeper portions. Cells may enter the synaptic phases whether they lie singly or in cysts, and irrespective of the position they occupy in the gland. Furthermore, no difference has been observed between the follicular cells surrounding those germ cells which have entered the synaptic phases and those enclosing the resting and dividing cells. There is, then, no indication that the somatic environment has anything to do with the initiation of the synaptic phases. The fact, however, that the cells of large cysts which have entered the synaptic phase usually degenerate indicates that the environment of the cells at this time may determine whether or not they shall form growing oocytes.

In none of the germ glands do all the cells that are destined to form growing oocytes enter the growth phase simultaneously, and therefore, in the same gland one may find oocytes of all sizes, as well as cells in the various stages of synapsis. Figure 78 is a section of the germ gland of a larva 72.5 mm. long, in which are oocytes of various sizes. There is no special limited period during the course of early development when the germ cells show a greater tendency than at other times to change into oocytes.

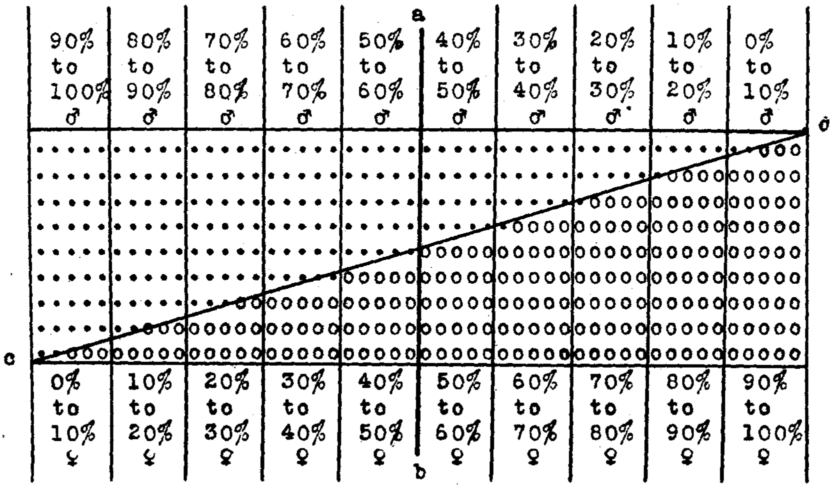
The change may take place in some cells when the larvae are less than 40 mm. long; while in other larvae no oocytes are found until a much later stage, in some cases not until the larvae are 70 mm. long. Few or many growing oocytes may form in the germ gland in very early stages. In most cases they are formed before the larvae are 70 mm. long, at which stage the sex glands are either predominantly male or predominantly female. A few oocytes may enter the growth phase after the larva is 70 mm. long, especially in glands that are predominantly male. After that time the cysts and undifferentiated germ cells gradually disappear in larvae destined to become females, while in those that become males the oocytes which have reached a considerable size or which may form in the gland subsequent to this time remain in the gland up to the adult stage. One such cell from an adult testis is shown in figure 63. It has been found that in the majority of adult testes such undeveloped oocytes occur.

Although the caudal portion of the germ gland remains smaller and less developed than the cranial portion, yet no difference has been found in the structure of the glands in the two regions. The tendency to form oocytes seems to be equally strong in the cranial and caudal portions of the gland.

So, whether the germ glands eventually become ovaries or testes, they all develop oocytes, and this is an undoubted female character. Sometimes only a few oocytes are present, and again, with the exception of a few indifferent germ cells scattered through the gland (singly or in cysts), oocytes may make up the whole of it. Hundreds of glands from young larvae have been examined to ascertain whether or not there are, in addition to the oocytes, any other sex characters which might indicate that the larvae are predetermined to form one or the other kind of sexual individuals, but none have been found. The germ glands vary somewhat in different regions, the result of the presence of blood-vessels and the irregular distribution of the various somatic elements, but the limits of such variation of the somatic parts of one gland are not appreciably different from those of any other gland. The only basis, therefore, for considering a larva male or female is the relative number of cysts and oocytes present in the gland. If in older larvae of this period, the number of cysts is greater than the number of oocytes, the larva probably becomes a male; if the number of growing oocytes is greater than the number of cysts, it probably becomes a female. In younger larvae of the period this cannot be a reliable criterion, for in some cases oocytes do not apparently begin to form in great numbers until the larvae are over 5 cm. long. Other larvae of the same size show evidence of having formed a large number of oocytes in their early stages, but appear later to have formed cysts only. Other larvae show about equal tendencies to form oocytes and cysts during the whole intermediate period.

TABLE 4

Diagram showing the relative number of cysts and oocytes in various lamprey larvae during the period of sex differentiation



The relative number of cysts and oocytes found in the various larvae of this period may be represented in percentages from one to a hundred as shown in the above table (table 4), which should be compared with table 2 (page 12).

The circles in the table represent ova, the small dots cysts. The spaces between the vertical lines may represent individual larvae, and the relative number of cysts and ova in the glands of each of these is indicated by the dots and open circles above and below the oblique line *cd*. The whole diagram may represent ten larvae of any size between 35 mm. and 70 mm. in length. If a large number of larvae of all sizes between 35 mm. and 70 mm. in length were sorted according to the number of cysts and oocytes present, approximately the same number of them would fall within each of the vertical spaces. Those to the left of the line *ab* would be predominantly male and those to the right predominantly female, as judged by the number of cysts and oocytes present in the germ gland. Those on the extreme left would be more strongly male and those on the extreme right more strongly female than those which would fall in the groups nearer the line *ab*.

Although the future sex of the larvae cannot be determined for a long time after oocytes have appeared in the germ gland, there is apparently developed out of these indeterminate larvae an approximately equal number of males and females. As previously stated, it is very difficult to obtain exact data on the sex ratio in the adult lamprey, since the habits of the two sexes are so different; but it has been found that out of all the adults collected from year to year and at various times of the day as well as at various times during the breeding season, there is only a slight excess of males over females, the ratio being about 118 males to 100 females. Many of the larvae, therefore, which bear the unmistakable female character of possessing oocytes must later develop into males.

d. Literature on the period of sex differentiation in the lamprey. W. Müller ('75) found that in larvae of Petromyzon planeri 35 mm. long the germ cells were not yet differentiated, while in larvae 50 mm. long oocytes were found. In larvae 65 mm. long he found the ovary and the testis to be fully differentiated. Lubosch ('03) found in the same species that sex differentiation takes place at the end of the first year. The youngest identified ovary was from a larva 40 mm. long.

Lubosch was the first to call attention to the fact that hermaphroditism is of common occurrence in the larvae of lampreys. He examined forty-nine germ glands from larvae of Petromyzon planeri. Among these 10.3 per cent were undifferentiated, 16.3 per cent older undifferentiated (probably young testes), 48.9 per cent true ovaries, and 24.5 per cent mixed glands. He came to the conclusion that the greater part of the hermaphrodite or mixed glands ". . . wird als männliche Anlage gelten können, in der eine kolosale, gleichsam atavistische Anlage von Eiern stattfindet." He states further: "Es ist anzunehmen, dass diese Eier später während oder nach Metamorphose der Rückbildung anheimfallen werden."

I published ('14) a summary of some work on the sex glands of the American brook lamprey, Entosphenus wilderi. Fifty larvae ranging in length from 20 to 75 mm. were examined for sex. Out of these 46 per cent were regarded as female because the germ glands contained practically nothing but growing oocytes, 10 per cent were taken to be true males on account of the absence of any growing oocytes in the germ gland, and 44 per cent were considered intermediates, for the reason that both cysts and growing oocytes were found in the glands. Since in the adult stage males and females occur in nearly equal numbers and since undeveloped oocytes were found in the adult testes, the conclusion seemed warranted that the intermediates became males.

Two other instances are recorded in which oocytes were found in the mature testis of the lamprey. Beard ('93) found in the testis of a specimen of Petromyzon planeri one well-marked oocyte in an individual follicle for every forty sections of 10 μ thickness. Ward ('97) describes the occurrence of a single microscopic oocyte in the testis of an adult Petromyzon.

Further discussion of juvenile hermaphroditism in lampreys will be found at the close of the next section following the discussion of like conditions in other vertebrates.

e. Other cases of juvenile hermaphroditism among vertebrates.

Cyclostomes. Cunningham ('86) found that in all specimens of Myxine glutinosa with very immature eggs, the caudal portion of the sex gland had the structure of a testis. In one specimen this testicular portion showed spermatogenesis and a number of spermatozoa. In all the sex glands of specimens with well-developed ovarian eggs there was, with one exception, no testicular portion. He concluded that in the young state nearly all females were hermaphroditic and that the testicular portion of the sex gland normally disappeared as the eggs became more mature. He believed that fertilization was normally effected by these hermaphrodites, since true males were so rare. Out of hundreds of specimens examined he succeeded in identifying only eight males.

Nansen ('87) also worked on Myxine, and came to the conclusion that all the animals are males up to a length of 32 to 33 cm., after which they change sex and become females. He regarded this as a case of protandric hermaphroditism among vertebrates. Dean ('97), as a result of his study of Bdellostoma stouti, doubted the conclusion reached by Nansen, as also did Price ('96). It was not, however, until 1904 that Schreiner proved by the examination of hundreds of specimens of Myxine that hermaphroditism in this form is of a juvenile character and that each animal matures only one kind of germ cells. Schreiner divided the animals examined into three groups, namely, males, females, and sterile. In the males the testes occupy the posterior portion of the gonads, while the anterior portion may not develop at all or may contain ova arrested in their development and showing signs of degeneration. In the female the ovary occupies the anterior portion of the gonad and is well developed, while the posterior or testicular portion is sterile and only slightly developed. The sterile individuals were of two kinds: those that showed neither follicles nor eggs and those in which both ova and follicles were found. Ova were usually found in the testes of the males, ranging from only one in some specimens to a large number in others. Out of hundreds of specimens examined only nineteen males were found without ova. The evidence obtained by Schreiner and later confirmed by Cole ('05) shows that Myxine is not a protandric hermaphrodite, but a juvenile hermaphrodite like the brook lamprey. This same conclusion has been reached by Conel ('17) for Bdellostoma as well as for Myxine. Conel believes that in the males the sex gland may degenerate with age, and that this accounts for the sterile individuals found by Schreiner.

Teleostei. Certain species of teleosts belonging to the families Sparidae and Serranidae are said to be normally hermaphroditic, and those of the latter family are even said to be self-fertilizing (Brock, '81; Howes, '91). Brock found in certain species of Sparidae that some of the young were hermaphroditic and others unisexual. In the former the ovarian elements did not mature and they became males, while the latter became females. I have shown that in the young lamprey the male part of the predominantly female gonad soon disappears, or is at least in many cases not very conspicuous, so that the impression may be gained by superficial examination of certain individuals that they are pure females. In the male, however, the female character, namely, the presence of oocytes, persists in many cases even up to the adult stage. For this reason one might conclude from the examination of older larvae that the males alone are hermaphroditic or heterozygotic as to sex. This was the conclusion that I first reported ('14). From a more careful examination of earlier stages I have now found that there is essentially no difference between the males and the females with regard to the juvenile hermaphroditic condition and that the apparent purity of the older larval females is due to the fact that the male character in the form of cell nests does not persist for any length of time after the female character has become predominant. Out of the hermaphroditic larvae, therefore, both males and females develop, and not males only as was at first supposed. In bony fishes there are no recent researches on sex differentiation, and the problem of juvenile hermaphroditism in this group needs to be reinvestigated. It may be expected to occur in both sexes.

Amphibia. It has long been known that in toads anterior to the true sex gland there is an organ (Bidder's organ) which has the structure of a rudimentary ovary. In males it persists throughout life, but in females it disappears after the second year in all forms that have been studied with the exception of Bufo vulgaris, in which, according to Ognew ('06), it is retained throughout life. Certain cells in this organ are, in structure and development similar to true oocytes. Ognew states that the boundary between Bidder's organ and the germ gland is often quite indefinite, so that one merges more or less into the other. In many cases oocytes occur even in the center of the testes, and Cerruti ('07) also found follicles which contained spermatozoa in Bidder's organ. With age, however, the boundary between Bidder's organ and the testis becomes more and more definite.

Pflüger ('82), one of the early workers on the sex problem in frogs, came to the conclusion that in recently metamorphosed frogs there are three kinds of individuals, namely, males, females, and hermaphrodites. During development the hermaphrodites become definitive males and females, so that in the adult condition the number of males and females is about equal. Pflüger also found that in certain races of frogs there is a greater tendency toward juvenile hermaphroditism than in others. An examination of recently metamorphosed frogs collected in nature from three different geographical regions gave the following results:

	PER CENT MALES	PER CENT FEMALES	NUMBER EXAMINED
Bonn	35.0	65.0	228
Utrecht	13.2	86.8	459
Königsberg	47.2	52.8	500

Collections of adult frogs from the three regions showed that the number of males and females was approximately equal. The conclusion was reached that the young are often hermaphroditic and do not reveal the final sex condition of the animal.

R. Hertwig ('05, '06, '07) also found that young frogs showed a tendency toward juvenile hermaphroditism. In two laboratory cultures, in which all the larvae were brought beyond metamorphosis, he found forty-three females and eighteen males in one and forty-seven females and eight males in the other. He believed that the females were pure and that only the males were hermaphroditic, and this form of hermaphroditism he termed rudimentary protogynic. Schmitt-Marcel ('08) made microscopic examinations of the sex glands of a large number of Rana temporaria in different stages after metamorphosis and concluded that all the intermediates or hermaphrodites became males. Kuschakewitsch ('10) worked on Rana esculenta and came to the conclusion that, if intermediates were found in a culture, all the individuals were intermediates and consequently some developed into males and some into females.

Witschi ('14) found that individuals of Rana temporaria that had just metamorphosed showed all the intermediate conditions between pure males and pure females. He believed that the testis developed out of an ovary and not from an undifferentiated gland. No evidence was found that an ovary developed out of a testis; for, usually, when a germ gland had begun to develop in the male direction, the whole or at least a part of it became male.

Witschi has advanced the idea that a germ cell in a frog becomes a male or a female cell according to the length of time it remains in the germinal epithelium. He found that when eggs developed under optimum temperatures (21°C.), no hermaphrodites appeared in the cultures; but when the eggs developed under low temperatures (10° to 15°C.) and under high temperatures (27°C.), intermediates were formed. Witschi concludes that heat and cold increase the chances of an early migration of some of the germ cells out of the germinal epithelium into the sex cords, and that when a part of the germ gland has thus once differentiated in the male direction, the whole gland usually changes into a testis, although the germ cells which remain in the germinal epithelium transform into oocytes. Under this assumption there would naturally be more males formed at either extreme of temperature. In the same way Witschi assumes that overripeness of the egg at fertilization so alters the trophic condition of the whole organism that only males are produced.

Abnormal temperatures and overripeness Witchi speaks of as 'Ausenfaktoren,' and claims that they may influence the sex of the organism. Under normal conditions they are absent and sex is then determined by 'Erbfaktoren' and 'Innenfaktoren.' The latter he speaks of as female determining: "Wenigstens spielen männchenbestimmende Innenfaktoren keine auffällige Rolle." These factors are local conditions in the germ gland which retard the migration of the germ cells into the sex cords. The 'Erbfaktoren,' however, are supposed to be the chief sex determining factors under normal conditions. In attempting to explain how these factor operate, Witschi has adopted the interpretation of Goldschmidt ('12) that the female is homogametic with respect to sex (MFMF), while the male is heterogametic (MFMF′). In these formulae the letters are ranked in value as follows: F>M, F>F′, and M>F′. Goldschmidt speaks of a variation in values of the factors as 'Potenzgraden,' which may be represented by figures; for example, M = 40, F = 60, etc. It is assumed that these potencies may vary for different fertilized eggs and that, in order that the resulting offspring may become a male or a female which is free from the characters of the opposite sex, the dominance of one sex tendency over the other must reach a certain epistatic minimum. If it falls below this minimum, intermediates are formed.

Witschi found also, as had Pflüger, that different races of frogs varied as to the number of intermediates produced. Races of Rana temporaria from northern Germany (Königsberg) and from the Alps (Ursprungtal) differentiated early and only a few or no intermediates were produced. In middle Europe (Utrecht, Munich), however, intermediates were commonly formed. Witschi designates these races as differentiated races and undifferentiated races.

Considerable space has been devoted to the results obtained by Witschi, for it is about the only experimental evidence we have that an external factor may influence the resulting sex of the individual. There are other cases in which the sex of an individual appears to be reversed by factors influencing the egg before fertilization, such as overripeness of the eggs, overwork in reproduction, desiccation of the eggs before fertilization, etc. Some of these cases wil' be discussed later.

f. Discussion. It has not been found practicable to test sex determination in the lamprey by experimental means, similar to those employed in the case of the frog, because of the length of the larval period and the difficulty, under laboratory conditions, of rearing the larvae through the period of sex determination. Witschi thinks that in the early phylogenetic history of the frogs the germ cells were probably all of the same value as to sex and that sex was determined wholly by external factors. In the lamprey it appears that the two sex potencies are almost balanced and that under normal conditions it is a matter of chance which sex develops, so that slight changes in the environment might suffice to throw the balance in favor of one or the other sex. It cannot be denied, however, that the eggs, from the time of fertilization, may show a greater tendency in favor of one sex than the other, as has been supposed by Witschi to be the case in normally developing eggs of the frog; but this inherited tendency may not be strong enough to prevent the formation of a series of intermediate individuals with glands ranging from those with no oocytes to those with no cysts. Under such conditions, it is not difficult to understand how a sex reversal might take place as a result of extraordinary external conditions. The more equally balanced the sex potencies are, the more easily a sex reversal might be effected.

Granting that sex potencies may be inherited factors, it does not seem necessary to assume that one sex is homozygous for sex and the other heterozygous, as Witschi and others have assumed, who felt themselves obliged to bring the phenomena of the inheritance of sex in line with those of the inheritance of mendelian characters. Before entering upon this question further, it will be necessary to summarize briefly the morphological evidences obtained from the present study in favor of a possibility of sex reversal in the lamprey.

Following an earlier indifferent period, when no sex characters are present, there is an indeterminate period in the early larval life of the lamprey during which the future sex of the individual cannot be determined, in spite of the fact that the sex characters are present. During this indeterminate period, all of the germ glands develop oocytes in greater or less number, with the exception of possibly a few in which no oocytes are found. At the same time many germ cells in all the glands remain in an indifferent condition and are found either as individual cells or in smaller or larger cysts. Since the secondary sexual characters do not appear until later in the life of the animal, there is during this period no other sex distinguishing character than the presence or absence of oocytes. The presence of large cysts of indifferent germ cells has been taken to be a male character, the whole cyst being homologous to an oocyte with its follicle; but to a certain extent the male character remains obscure, since it is not until the animal approaches the sexually mature condition that the male germ cells can be identified as such. The presumably male germ cells of the larger cysts continue to divide until after metamorphosis, but the cells resulting from each division are, for a long time, not essentially different from the primordial germ cells. The only secondary characters that distinguish the adult male are the long, slender urogenital papilla and the absence of an anal fin, but these do not appear until after metamorphosis. Since this study does not involve the stages in which secondary sex characters are present, we are concerned only with the primary ones, namely, the presence of male or female sex cell. Only the latter are structurally recognizable in the stages studied, and they give us the only definite clue to the sex condition of the young larvae.

Oocytes appear in practically all lamprey larvae; in normally dioecious species oocytes appear only in about one-half of the young, while in the other half only male cells appear. Whether or not male germ cells occur in all lamprey larvae cannot now be stated for reasons already presented. When a germ cell, however, shows no tendency to transform into an oocyte, but continues to divide and form cysts, it has been assumed that it is potentially more strongly male than female in constitution. The activity of the germ cells may be along either of two lines. In the one case their tendency is toward growth, and in the other toward rapid multiplication. This difference in the activity of the cells may be due to an inherent tendency in the cells themselves or to factors operating in the cell environment. If due to the former it must be admitted that all the germ cells of the same gland do not have the same make-up and that during the process of development each cell inherits a different constitution after each division. Some of the cells are endowed with a tendency toward rapid division and others toward an early cessation of division and entrance upon a period of growth. It may be due to an unequal partition of chromatin material during cell mitoses or to an unequal distribution of cytoplasmic material during the early divisions of the cells. Just as the germ cells are set aside from the somatic cells in early stages of development by some differences in their make-up, so the germ cells may also differ among themselves in their inherited structure after each division. There is direct proof that there is an unequal distribution of material among the cells in early stages of some animals, and that certain cells are destined to form certain parts of the body. These differences appear to be cytoplasmic in most cases; for example, in the case of Cynthia, in which (Conklin, '05) there are several kinds of organ-forming substances which are unequally distributed among the cleavage cells. In later stages, however, there is no direct proof of an unequal division of the cells; so in the case of the germ cells of the lamprey it must remain an assumption that unequal division does take place; but this would explain the two types of behavior of the germ cells.

Again the germ cells might all be assumed to have the same inherited structure, and yet they may develop along different lines on account of their different local environment in the gonad. In this case the behavior of the cells would be the result of factors or circumstances acting from without. These factors may be supposed to be differences in nutrition, the presence of various enzymes and toxins, differences in pressure, and various other factors operating in the germ gland of the animal. In this case it must be assumed that the germ cells are so constituted that they can respond to environmental factors in two different ways. Under certain conditions the cells will continue to divide, under others they will stop dividing and enter upon a period of growth.

As yet we know too little about the physiology of the cell to be able to decide between the two possibilities. We know that in a form like the lamprey, whether it eventually becomes a male or a female, the two kinds of cells make their appearance, some with a tendency toward rapid division and some with a tendency for growth. Now, since the latter is an undoubted female quality and the former is supposedly a male quality, practically every individual must possess both male and female potencies. That these potencies are practically in a balanced condition is seen from the fact that both male and female sex cells appear in the majority of larvae. Sometimes a larva is inclined more strongly toward the female side and at other times it leans toward the male side. In some cases it appears that a larva may fluctuate back and forth between the two extremes until finally one or the other sex condition takes the lead and sex reversal becomes more difficult. This is indicated by the fact that the sex glands of older larvae from the period of sex differentiation (larvae 50 mm. to 70 mm. long) often show that an earlier sex condition has been replaced by that of the opposite sex. After sex has become definitely established, one or the other sex potency becomes so strong that only unusual circumstances are able to reverse the condition. The elements in the body or in the germ gland, which have specialized in the opposite direction, stop developing and either degenerate or remain in an undeveloped condition during the whole lifetime of the animal. The cysts in the developing ovary contain small cells which soon degenerate and disappear so that the larva soon becomes apparently a pure female. In the developing male gland the undeveloped oocytes remain, in many cases, even up to the adult stage; but often they degenerate in early stages, so that fragments of oocytes occur in the developing testes. Out of the juvenile hermaphroditic condition, therefore, both males and females eventually emerge.

The condition in the lamprey is not essentially different from that in Myxine, except that in the latter the two kinds of germ cells develop in different parts of the gonad, while in the lamprey there is no segregation of the two kinds of cells. The whole gland is in fact hermaphroditic in the lamprey while in Myxine the anterior portion of the gonad is ovarian and the posterior portion testicular. In some individuals of Myxine there is a tendency for the two kinds of cells to be mixed. This is especially true on the border-line between the testicular and ovarian portions. There is also probably no essential difference between the condition in the frog and that in the lamprey, and an explanation of the phenomena in one case should hold for the other as well. Whether or not different races of lampreys show a greater or less tendency toward juvenile hermaphroditism is not known, and no opportunity has yet been offered for an investigation of this question.

What it is that keeps a larva with hermaphroditic tendencies from developing into a functional hermaphrodite is not known. It appears that when one of the sex tendencies takes the lead, it prevents the development of the structures characteristic of the other sex; or it may be that when one set of sex elements begins to degenerate, there are removed certain influences that have previously inhibited the development of the other set. Something similar to this is seen in most true hermaphrodites, where only one set of sex cells develops at a time, so that the animal is either protandrous or protogenous. In this case, too, the development of one set of germ cells is antagonistic to the development of the other, but a reversal always takes place when one group is exhausted. This may be due to the fact that certain hormones are eliminated from the germ cells which have taken the lead in development, and that these are unfavorable to the development of the other set. As soon as the first set of cells has been eliminated, a reversal takes place and the opposite set develops. It may be looked upon as alternate periods of vigor and depression as far as the particular germ cells go. In bisexual animals with juvenile hermaphroditic tendencies, it may be supposed that the animals never recover from the state of depression relative to the opposite sex.

Reviewing the case of the lamprey, the evidence seems to warrant the conclusion that sex is not irrevocably fixed at the time of fertilization; that the future sex of the animal is not definitely determined until the larva has reached a considerable size, and that sex is not the result of any unchangeable sex quality present in the egg at the time of fertilization, but is rather the outcome of a balanced sex potency which results in one or the other sex being formed, largely as a matter of chance under normal environmental conditions. It is possible that one sex potency may be stronger than the other from the beginning of development, and that even the germ cells themselves at the time of fertilization may be inclined in one or the other direction; but such an admission is not necessary for an explanation of what actually takes place.

Practically the same conclusion has been reached by Shull ('11) in the case of plants. He says:

> May not maleness and femaleness be thought of as alternative states which can be crudely analogized with the acidity and alkalinity of chemical solutions. . . . . In some species the sexes appear to represent a much more strongly polarized (?) condition than in other species, and a transition from the characters of the one sex to those of the other is attained only with the greatest rarity, if at all; while in other species the sex conditions may be so nearly balanced or neutral that individuals are not absolutely determined in their sex relations by their genotypic nature. . . . With such a conception of sex, it also appears probable that sex may be influenced sometimes by external factors as well as by internal ones, and in this case the preponderance of one sex over the other, which has been observed in many animals and plants, need not be attributed alone to selective disorganization of germ cells, a selective fertilization or a selective death rate, but might conceivably be controlled to a certain extent by environmental conditions, acting at some particular 'sensitive' period in the ontogeny of the organism in question (pp. 363-364).

4. Present status of the sex problem. We may now ask whether or not the view expressed above can be brought into harmony with current opinions concerning sex determination. The generally accepted view is that sex is established at the time of fertilization as a result of the presence or absence of so-called sex chromosomes in the fertilized egg. This view was first expressed by McClung in 1902. During the progress of his work on the maturation of the germ cells in insects, he found a certain body in the spermatocytes which was interpreted as being a sex-determining element. This body had been seen before by Henking ('91), Montgomery ('98), and Paulmier ('99), but it had not been suspected that it might be a sex-determining factor. McClung's statement concerning the function of the accessory chromosome as it was called, was as follows: ". . . . it is the bearer of those qualities which pertain to the male organism, primary among which is the faculty of producing sex cells that have the form of spermatozoa." This interpretation was quite generally accepted. Previous to this time numerous theories had been advanced concerning the cause of the appearance of two kinds of sexual individuals, each theory to be replaced by others, which further research found equally untenable. Investigators now began to search for this odd element in the sex cells of various species of animals. More and more forms, especially among insects were found in which the odd element was present in the germ cells and in which it became distributed to half of the mature cells.

It is natural that this discovery should have led to a qualitative explanation of sex. There apparently was something present in half of the male germ cells which, after fertilization, was responsible for the development of a male. This was McClung's interpretation and this explanation was accepted by the majority of his immediate followers.

The early work was done on the accessory chromosome of the male germ cells alone. When cytologists began investigations upon the chromosomal structure of the female germ cells (Wilson, '05; Stevens, '05, and others) it was found that this odd element was present there also, not singly but in duplicate. These two accessories were so distributed during maturation that every egg retained one, and consequently all the eggs were alike in their chromosomal structure. Theoretically, therefore, an egg which happened to be fertilized by a spermatozoon containing the accessory chromosome would give rise to a female, and not to a male as had been supposed to be the case. It became clear that the accessory chromosome could not be sex determining by virtue of any qualities it might possess, but rather that sex was due to a quantitative difference in the amount of the odd chromosomal material present in the fertilized egg.

Certain studies in heredity have shown that some characters are sex-linked. The interpretation of this fact is that the factors for such characters are carried by the sex chromosome. It was discovered that the inheritance of sex-linked characters in forms like moths, butterflies, and birds was such as to necessitate the assumption that the ova in these forms rather than the spermatozoa were dimorphic in regard to the sex chromosome. Later it was discovered by Seiler ('14) that there are actually two kinds of eggs in the moth Phragmatobia fuliginosa. In the case of birds the problem has not yet been cleared up. Guyer thinks he has evidence that the spermatozoa are dimorphic in this form, while the inheritance of sex-linked characters in birds points to the egg as being dimorphic. In his last paper on the subject, Guyer ('16) again emphasizes the fact of the presence of two kinds of spermatozoa in the common fowl, but admits the possibility of only one kind being functional. If it be admitted that the eggs also are dimorphic, it would be difficult to explain why two kinds of cells should be produced in both sexes of the offspring.

The assumption of a dimorphism of both spermatozoa and ova of the same species has been made before. Castle ('03) proposed a theory of this sort. Such a theory necessitates the further assumption of selective fertilization, for which there is apparently no direct evidence.

In a recent paper by Stockard and Papanicolaou ('16), dealing with the hereditary transmission of degeneracy and deformities in alcoholized guinea-pigs, a statement is made that the junior author is in possession of data which indicates that the female guinea-pig, as well as the male, shares in the determination of sex, and that in this species both ova and spermatozoa may be dimorphic. Previous to this, Papanicolaou published some of his results in Science ('15), where he states that the sex of the guinea-pig is determined, sometimes by two and sometimes by three factors, depending upon whether or not the mother had previously given birth to young. The three factors are: 1) The sex tendency of the father; 2) the sex tendency of the mother; 3) the change of sex tendencies in the female from litter to litter. If these observations prove to be correct, the sex potency of the fertilized egg is not determined by a sex chromosome, unless there be a selective fertilization that is subject to variation according to the physiological condition of the parent.

An accessory chromosome has not been found in all forms studied. This does not, however, exclude the possibility of its being present, since it appears that it may often be united with some other chromosome. This seems to be the case in Ascaris megalocephala among invertebrates and Necturus maculosus among vertebrates. If it occupy such a position, it might very easily escape observation. In plants no accessory chromosome has been found, except in Salamonia biflora in which Cardiff ('06) describes one, but his interpretation has been doubted by Strasburger and others. It is in insects that the accessory chromosome has been studied with most care, and in this group it has also been found that the secondary sexual characters apparently develop independently of the sex glands. It has been shown by Kellogg ('04), Meisenheimer ('09), Kopec ('11), and Steche ('12) that in moths, at least, the presence of a particular germ gland in the animal is not responsible for the development of the secondary sexual characters. In this case the primary and secondary sexual characters seem to develop in consequence of the presence in the developing embryo of a common factor or set of factors which may be located in sex chromosomes.

Many of the bodies that have been described as accessory or sex chromosomes are probably something else. Our knowledge concerning many of the cytoplasmic bodies in the cell is very limited, but it is known that some of them may occur among the chromosomes during mitosis. It will be recalled that Wilson ('13) in his work on Pentatoma warned against mistaking a so-called chromatoid body in certain cells for a sex chromosome. Wodsadelek ('14) found a similar body in sex cells of the horse, and Bachhuber ('16) found it in the rabbit. There also seems to be a certain relation between the nucleolus of the cell and the accessory chromosome in certain cases. Goldsmith ('16) thinks he has found evidence in Pselliodes cinctus that the nucleolus is composed of both chromatic and achromatic material. The achromatic material he thinks is linin, or closely related to it in composition, while the chromatic part constitutes the sex chromosome.

It must be admitted, however, that an accessory chromosome is undoubtedly present in the cells of a great number of forms, and that it may function as a sex determiner, at least in the absence of other factors. There is, however, good reason for believing that it forms only one link in a series of events that precede the development of sex. This conclusion has also been reached by Doncaster ('14) who says: "It seems evident that sex cannot depend on a chromosome alone for the chromosome must act by its relation with the cell-protoplasm and it is on this relation that sex determination depends." This same proposition is admitted by Morgan ('15). He says: "It is quite conceivable that one or more of these other factors might so change that the sex differentiation would become inoperative or even change so that these other factors themselves become the diferentiators that determine sex" (p. 95). He admits that the environment is one of the important factors that enters into the development of every individual and that it is quite possible that it may turn the scale and determine sex. Loeb ('16) accepts the cytological evidences for sex determination by sex chromosomes, but speaks also of a physiological basis of sex determination by specific substances or internal secretions. He thinks it possible that the sex chromosomes may favor the formation of specific internal secretions which are responsible for the formation of sex characters in the animal and that if it should be found "possible to modify secretions by outside conditions or to feed the body with certain as yet unknown specific substances the influence of the sex chromosome upon the determination of sex may be overcome" (p. 228).

From these statements it will be seen that the possibility is admitted by some of the foremost investigators of the sex problem that all germ cells carry the potentialities of both male and female, and that after fertilization the egg may be inclined in one or the other direction, but not so strongly that it excludes the possibility of a reversal in the other direction. There seems to be at the present time a decided tendency away from the idea that the sex chromosomes carry absolute sex determiners. We are, therefore, no longer antagonistic to the idea that other sex factors may exist, either in the cell itself, in the developing organism which comes from the germ cell, or in the environment of the cell or organism.

5. Discussion of the hermaphroditic condition found in the lamprey in connection with other sex phenomena, not easily explained by current theories. a. Normal hermaphroditism. The sex-chromosome hypothesis does not offer a satisfactory explanation of normal hermaphroditism in animals and plants. Hermaphrodites normally produce both male and female sex cells in the same individual, but in most cases the two kinds of cells are not matured at the same time. Usually the male germ cells are ripened first, and in such cases the species is known as protandric. When the eggs are matured first, the species is known as protogenic, and when the male and female germ cells are produced at the same time, the condition is known as simultaneous hermaphroditism. The latter condition is usually found in species with more or less widely separated male and female sex glands, but it may also appear in species in which an ovotestis is found, as, for example, in certain pulmonates. When the germ cells are ripened during successive seasons of the life-cycle of the animal, the condition may be called polycyclic. On the other hand, if the animal produces only one kind of germ cells during the early period of its life and the other kind of germ cells during the later period, the condition may be termed monocyclic hermaphroditism. The latter condition exists in Crepidula fornicata. Orton ('09) has made a study of this form and has found that the individuals associated in chains offer transitional series from maleness to femaleness both in primary and secondary sexual characters, beginning with a male in the young stage and ending with a female in the older stages. Three hundred and fifty chains were examined, and it was found that the individuals could be arranged as follows: 1) male; 2) male with rudimentary uterus; 3) hermaphrodite with small uterus; 4) hermaphrodite; 5) hermaphrodite with small penis; 6) female with rudimentary penis; 7) female.

In monocyclic hermaphroditism it appears that with the aging of the animal its metabolism becomes antagonistic to the development of one or the other of the two kinds of sex cells. In the case of Crepidula, the metabolism of the young animal is favorable to the development of the male sex cells, while the metabolism of the older animal is more favorable to the development of the female sex cells. In the case of polycyclic hermaphrodites, when the two kinds of germ cells are ripened in close succession, the development of the second kind of germ cells may be the result of a changed metabolism.

In some true hermaphrodites sex conditions seem to be disturbed at times so that true males and females appear. According to Maupas ('00), the number of males per thousand females in various nematode worms may vary from 0.13 to 45.

b. Alternation of the hermaphroditic and the dioecious condition. A more complex case of hermaphroditism than those mentioned above is that found in the nematode worm Rhabdites (Rhabdonema) nigrovenosum, which is parasitic in the lungs of frogs. While in the lungs, the worms are hermaphroditic, but in the free-living state, which alternates with the parasitic, two sexes occur. The free-living worms again give rise to hermaphroditic parasitic offspring. This has been explained by Boveri ('11) and Schleip ('11) as being due to the disappearance of one kind of spermatozoa in the free-living males, so that upon fertilization only one kind of sexual individual is produced, namely, a female which again becomes parasitic. This female is capable of giving rise to both spermatozoa and eggs, both of which should have the same chromosomal make-up. During maturation three kinds of spermatids are produced, with five, six, and seven chromosomes, respectively. It is supposed that the last kind degenerates.

Some evidence has been advanced for a chromosomal explanation of true hermaphroditism. Zarnik ('11) thinks that in certain hermaphroditic Pteropods, the female cells are of one kind only (homogametic); while the male cells are of two kinds (heterogametic), but that only one kind of male cells is functional, namely, the one corresponding in chromosomal make-up to that of the female cells. The offspring from such union should result in a female, but instead it develops into a hermaphrodite in which again half of the male germ cells degenerate.

Krüger ('12) found what she thinks is an accessory chromosome in the hermaphrodite Rhabdites aberrans. During spermatogenesis it becomes distributed equally among the spermatozoa, with the exception of a very few cases when it lags behind and is retained in one cell. Apparently in this species the spermatozoon simply initiates development in the egg and the sperm nucleus degenerates. The parthenogenetically developing egg forms a hermaphrodite. Only in one case was a fusion of the male and the female nucleus observed, and Miss Krüger assumed that the male nucleus in this case was one in which the accessory chromosome was lacking. This would give rise to a male of which there were a few formed.

Demoll ('12) thinks that in Helix pomatia, which is hermaphroditic, two kinds of spermatozoa result from the unequal distribution of the sex chromosome and that only one kind, that with the accessory, becomes functional.

It appears from the above cases, namely, that of the gastropods, which are true hermaphrodites, and that of Rhabdites, in which the hermaphroditic condition alternates with the dioecious, that sex cannot be the result of the action of the sex chromosome alone, but that the activities and behavior of the sex chromosomes which results in their peculiar distribution must be due to some physiological activity in the cell which antedates the sex chromosomes, so that the latter are simply the final link in a series of processes which determine the sex potentiality of the cell. This possibility has been admitted by Schleip in the case of Rhabdites. He says: "Es scheint, dass die Entwicklung mancher Keimzellen zu Spermatozyten statt zu Ovozyten zum Teil auf Ursachen beruht, die ausserhalb dieser Keimzellen liegen." . . . "Diese äusseren Ursachen brauchen nicht ausserhalb desselben befinden; man kann sogar vielleicht daran denken, dass innere Secretion dabei eine Rolle spielt" (p. 128). Further on Schleip adds that external conditions may influence the development of the sex cells. He says: "Wie bei manchen Tieren äussere Bedingungen einen Einfluss auf das Geschlecht der sich entwickelnden Tiere auszuüben imstande zu sein schienen, so beeinflussen also äussere Bedingungen bei der zwittrigen Generationen die Entwicklungsrichtung der Keimzellen." He says further: "Daher wird die Frage erlaubt sein, ob die verschiedene Chromosomenzahl überhaubt einen Einfluss auf die Geschlechtsbestimmung hat, und ob die Spermien nicht aus anderen Ursachen und in anderer Weise in männliche und weibliche differenziert sind und die verschiedenen Chromosomenzahl, die sie erhalten, nur die Folge davon ist."

Another line of investigation on hermaphroditism is the study of the segregation of the germ cells in the sex gland. In the case of Sagitta, Elpatewsky ('09, '10) finds a body in the cytoplasm of the cells during early cleavages, which he calls the 'besondere Körper.' This is retained by only one cell after each cleavage up to the sixth, when it divides during mitosis and part of it passes to each of the resulting cells. These two cells become the germ cells, and Elpatewsky believes that one becomes the forerunner of the spermatozoa and the other of ova, and that the former gets a larger portion of the 'besondere Körper.' Ancel ('03) has worked on the early development of the germ cells in Helix pomatia and thinks that three kinds of cells appear in the germ gland, spermatozoa, oocytes, and nurse cells. He thinks that the primordial germ cells become transformed into female and male elements according to whether or not the nurse cells are present at the time of transformation. Buresch ('11) thinks that in Helix arbustorum, also, the fate of the indifferent germ cell depends on its proximity to a nurse cell. The cases which have been cited, show that the differentiation of the germ cells into male and female cells has been interpreted as being due to nuclear differences in some cases, cytoplasmic differences in others, and to differences in the environment of the cells in still other cases. If we conceive of sex as a metabolic state rather than the result of definite sex factors, it is easy to see how any one of the above factors might result in a metabolic change which would throw the balance in favor of one or the other sex.

It is unfortunate that so little work has been done on the history of the germ cells in hermaphroditic animals; for it is in these forms that one undoubtedly must look for valuable clues to the problem of sex determination.

c. The effect of delayed fertilization on sex. An interesting case in which sex metabolism seems to be disturbed by outside factors is that of the frog in which delayed fertilization results in the development of the eggs into male individuals exclusively. It was found both by R. Hertwig ('05. '06, '07), and by Kuschakewitsch ('10) that the percentage of males increased with the length of time that fertilization was delayed. Kuschakewitsch found that when fertilization was delayed as much as eighty-nine hours all of the eggs developed into males. The mortality among all the eggs in the culture was about 4 per cent. In the case of the frog, an accessory chromosome has been described, both by Levy ('15) and by Swingle ('17). Levy found twenty-five chromosomes in the male germ cells of Rana esculenta. During maturation division these were so distributed that half of the cells received twelve and the other half thirteen chromosomes. The odd chromosome of the thirteen is the sex chromosome. Levy believes that the accessory chromosome undoubtedly has something to do with sex, but he thinks that it is not the only sex-determining factor. He says: "Man darf aber die Geschlechtschromosomen nicht als den geschlechtsbestimmenden Faktor bezeichnen, den sie sind nur die zuerst morphologisch erkennbaren Zeugen einer stattgefundenen sexuellen Differenzierung."

Swingle found the spermatogonial number of chromosomes to be twenty-five in Rana pipiens. He found some cases in which the sex chromosome divided during the second spermatocyte division instead of during the first, and one case in which the two parts of the X-body were unequal in size. He thinks that there may be some connection between the abnormality of chromatin distribution which results, presumably, in the production of three kinds of spermatozoa, and the fact that in certain strains of the species, males, females, and individuals possessing marked hermaphroditic tendencies occur.

In the case of the frog it seems evident, both from the experiments of Hertwig and Kuschakewitsch on delayed fertilization and from those of Witschi ('14) on the effects of temperature on the sex of the animal, that the accessory chromosomes, known to be present, are not the sole sex determiners. Such a conclusion is not a condemnation of the sex-chromosome theory. If other factors also affect the sex of an individual, it shows that the sex chromosome is but one of many such factors which may bring about the same result. Temperature, for instance, may result in reactions in the protoplasm of the cells or may so change the whole metabolism of the organism that the visible results might be quite different. An analogy may be drawn between the phenomena of sex and those of the red-flowered Primula which, according to Klebs ('03) becomes white when grown at high temperatures. In this case the two color potencies are present in the organism, and which one shall appear depends upon an external factor, namely, temperature. Similarly, sex potencies may assert themselves differently under various conditions, so that a reversal may take place, or intermediates be formed, such as are found in cyclostomes and amphibians, and possibly in many fishes as well.

d. Hermaphroditism and sex reversal due to external conditions. Another interesting case showing the double sex potentiality of early larvae is recorded by Baltzer ('14). He found in Bonellia viridis, the males of which live parasitic upon the females, that if the larvae have a chance to attach themselves to a female they become males, and if they do not succeed in becoming attached they form females. If they are allowed to attach themselves and are later removed, they become hermaphrodites. In the attached larvae the sex determining substances are undoubtedly taken up from the host, since the female-determining substance seems to be stronger in the free-living state. Baltzer concludes that sex is partly predetermined and partly epigenetic, and that both sex tendencies are inherited but in different degrees. He believes that the male tendency is stronger than the female. If this be so, we have here a case of sex reversal, providing the larva remains unattached. A case somewhat similar to that of Bonellia is that of the protandric hermaphrodite Crepidula plana, in which Gould ('17) finds that the development of the male phase is dependent upon the presence of a larger individual of the same species, but not necessarily a female. In the absence of a larger individual, the larva develops into a female, but the process of transformation in the female direction may be halted at any time, up to the period of formation of growing oocytes, by bringing the animal into proximity with an older individual. Gould does not offer any explanation as to the nature of the stimulus exercised by the older individual over the sex of the larva.

Among plants there seem to be many cases which indicate that every individual possesses a double sex potentiality. It appears that in some of the lower types of plants which are normally dioecious, the organs of the opposite sex can be made to appear on all the individuals under proper culture conditions; that is, the male plant will produce female organs and vice versa.

Bordage ('98) cut back the apex of young male plants of Carica papaya just before the appearance of the first male flowers. Lateral branches arose below the cut, and these produced female flowers and fruit. Strasburger ('00) found that the smut Ustilago violacea caused the dioecious plant Melandryum album to produce the opposite sex organs; that is, the male organs appeared on the female. The pistils remained undeveloped, while the normally rudimentary anthers grew large and produced pollen mother cells. Later, Strasburger ('09) came to the conclusion, from a consideration of many evidences, that sex determination in plants cannot be the result of mendelian segregation. He says: "Ich bin nach alledem der Ansicht dass alle Versuche, die Geschlechtsbestimmung getrenntgeschlechtlicher Organismen auf Mendelische Spaltungsregeln zuruckzuführen, erfolglos bleiben werden" (p. 17). Strasburger also did not consider the so-called sex chromosomes as true chromosomes. He says: "Denn nicht nur zeigen sie ein eingeartigen Verhalten, sondern auch ihre Beseitigung aus den Geschlechtszellen ist möglich, was für Träger von Erbeinheiten nicht zulässig wäre." Should they be proved to stand in some relation to sex, they might yet be individual linin bodies "die aber nicht Pangene führen, sondern der Aufnahme des über das Geschlecht bestimmenden Stoffes dienen" (p. 22).

It may be suggested in this connection that other bodies are present in the cell which may become unequally divided during mitosis. This is true of plasmosomes, which may not always dissolve and become diffused throughout the cell before division. It may be equally true of mitochondria and other cytoplasmic bodies. Such an hypothesis has been advanced by Schaudin

('05) in the case of Protozoa. A normally functioning cell is regarded by him as a hermaphrodite which has the male and female qualities equally balanced. The differentiation which leads to the formation of gametes is due to inequalities of cell division which result in a more or less imperfect distribution of the qualities of the parent cell between the daughter cells, so that some cells may receive more male and others more female properties. The male cells show greater kinetic energy; the female cells greater trophic energy. The opposite tendencies accumulate in different cells which thus become one-sided in their vital activities. The want of balance may reach a stage in which syngamy must take place or the cell dies.

A similar idea was advanced above, in my discussion of the appearance of two kinds of germ cells in the sex glands of the lamprey. In this case, too, the development of the two kinds of cells in the same gland may be due to a disturbance in the metabolism of the cell during mitosis, which results in the development of a cell along either one or the other of two potential lines. It is conceivable also that there may be various grades of male and female potentialities in the germ cells thus formed, and that even in their mature condition some cells may be more strongly sexed than others. After fertilization, the same differences of sex potentialities may exist, and, in so far as no other factors are introduced to disturb the relative sex potentialities, the sex of the resultant animal may be said to be determined at the time of fertilization.

Whether or not these differences in sex potentiality are the result of a variation in the chromosomal make-up of the cells is not certain. This suggestion appears contrary to certain known facts of sex-linked inheritance, which seem to require for their interpretation that the sex characters reside in the same chromosome as the sex-linked character. It might be assumed equally well, however, that certain characters appear, only when associated with a certain kind of cell metabolism which may be peculiar to one or the other sex. This conception might also account for the exceptions to the inheritance of sex-linked characters which are difficult to explain by the chromosomal theory.

Some further examples of mixed sex among plants may be given. Among the flowering plants some species are hermaphroditic, others dioecious, and still others produce three kinds of individuals, namely, males, females, and hermaphrodites, as, for example, the sweet pea. In the strawberry three kinds of flowers are produced, staminate, pistillate, and perfect. Valleau ('16) has investigated the inheritance of sex in grapes, and his results are as follows: The wild grape develops two kinds of individuals, staminate and pistillate, and both possess flowers of the opposite sex in a suppressed condition. The grape, therefore, occupies an intermediate position between purely dioecious plants, like the willow, and purely monoecious plants, like the apple. On individual plants of the grape all gradations are sometimes found, from staminate to functionally hermaphroditic flowers, and sometimes only hermaphroditic flowers are produced. Certain clusters of the vine may be entirely staminate, while other clusters on the same vine contain all gradations from staminate to functionally perfect flowers. In the grape, therefore, both staminate and pistillate vines carry the determiners for femaleness and maleness, respectively, but with one or the other partially suppressed. Valleau draws the conclusion that, if the chromosomes carry the determiners for sex, then in hermaphroditic plants the determiners for maleness and femaleness must be carried in the same chromosome. There are two possibilities, therefore, for the origin of functional hermaphrodites. The maleness may express itself fully in one of the chromosomes bearing the determiners for femaleness in a pistillate plant and femaleness may express itself similarly in staminate plants.

Pritchard ('16) discusses the change of sex in hemp. Hemp is dioecious, and the female plant is distinguished by its dense foliage as well as by the production of female flowers. The male plants have very scanty foliage. The sex ratio is normally 1:1. Hermaphroditic individuals appear in small numbers, but they are of the female type and predominantly female in flower development. Disturbances in the plant's physiological equilibrium were induced by the removal of flowers and of vegetative parts, as well as by the injection of various chemicals into the stem.

It was found that sex was alterable by removal of flowers. Removal of female flowers caused staminate flowers to appear, and the removal of staminate flowers resulted in the development of female flowers. Pritchard believes that the change is probably due to disturbances in nutrition. He concludes that maleness and femaleness are not always fixed characters, but frequently appear more like responses of the developing organism to external stimuli. He thinks that facts do not support the theory that sex is wholly a matter of zygotic constitution, but indicate that both males and females are partially hermaphroditic.

Certain plants which, under normal conditions are true hermaphrodites, will, under other conditions, produce two kinds of sexual individuals. This is true of certain mosses and ferns which normally produce antheridia and archegonia on the same plant, but which, by being supplied with a certain kind of nourishment will produce only one or the other of the two kinds of germ cells. Again, it has been found that under certain conditions some dioecious plants may become monoecious. Wuist ('13) found that Onoclea struthiopteris, which is normally dioecious, could be induced to become monoecious under proper culture conditions, so that the male plant produced female organs and the female plant produced male organs. Here, again, the appearance of the organs of the opposite sex is apparently due to the nutritional environment.

e. Hermaphroditism as a result of hybridization. During the last few years some interesting facts have been brought out in connection with hybridization in animals and these seem to throw some light upon the sex problem. Goldschmidt ('16, '17) found that by crossing European and Japanese races of the gypsy-moth many so-called gynandromorphs were produced. Different results were obtained if the material had a different race origin. The explanation of this seemed to be that the potency of the sex factors differed in different races. It will be seen that this case is somewhat similar to that of the frog, in which Hertwig and Witschi found a racial difference as regards the tendency toward juvenile hermaphroditism; but in the latter case the hermaphroditic condition was not retained up to the adult stage.

It was supposed by Goldschmidt that the sex potency varied with the geographical distribution of the moth, and for this reason it was decided to study the behavior of different local forms of the Japanese moths crossed inter se and with European moths. The result was that a great number of individuals were obtained, which, for the various crosses, showed all intermediate conditions between true males and true females; consequently, if maleness and femaleness are represented as the end points of a series, say one as zero and the other as one hundred, a given moth might be represented by twelve, thirty-five, forty-two, etc. These animals do not represent a mixture of the primary and secondary characters of the two sexes, but a definite point between the two extremes, maleness and femaleness. Since the term gynandromorphism applies only to individuals showing a mosaic of characters of both sexes, Goldschmidt discards this term; for in the moths the entire individual represents a definite quantitatively fixed point intermediate between the two sexes, and not a mixture of the characters of both sexes. Such sex intermediates he calls intersexes—female intersexes, if they are genetically female, but transferred to some stage toward maleness, and male intersexes, if they are genetically male, but transferred to some point in the opposite direction. Goldschmidt has succeeded in breeding every step from a normal female through the different intersexes to a normal male; also the steps starting with the normal males and passing through the male intersexes toward the female up to three-fourths of the way. Every single step can be produced by the right combination of races. The change in any given direction is through the secondary characters first and the primary characters last.

The explanation of the above condition appears to be that each sex possesses the potentiality of the other. In both sexes, irrespective of the zygotic constitution, both anlagen may become patent; which one shall appear depends entirely upon the quantitative relation between the two potentialities. Applying symbols and recognizing that the female is heterozygous for sex in moths, Goldschmidt makes use of the following formulae: FFMm = Female, FFMM = Male. The value of the sex factors he speaks of as a potency or valency. Now, it may be assumed that in a certain case the female factorial set, FF, has a value of 80 units, and the male factor, M, a value of 60 units. The formulae would then read as follows: $\underset{80}{\text{FF}}\ \underset{60}{\text{Mm}} = \text{Female}$; $\underset{80}{\text{FF}}\ \underset{60+60}{\text{MM}}$ = Male. In the first formula the female set overpowers the male set by twenty units, and in the second formula the male set overpowers the female set by forty. According to Goldschmidt, two possibilities are open. Either the slightest preponderance of one over the other, say only one unit, is sufficient to determine the male or the female sex, or there is a necessary minimum of preponderance beyond which only one or the other sex appears. This minimum he speaks of as the epistatic minimum. If the epistatic minimum be twenty; then when $\text{FF}-\text{M}$ is greater than twenty a female is produced, while if $\text{MM}-\text{FF}$ is greater than twenty then a male is the result. The intermediate points represent the intersexes and, if they are heterozygous for M, they are intersexual females, but if they are homozygous for M, they are intersexual males. Definite races possess special potencies for the male sex factors. A cross of races of similar potencies gives normal offspring. Races of different potencies of the male factors give female intersexes in the F_1 generation if the mother belongs to a race of lower potency. The degree of intersexuality depends upon the differences in the potencies.

Another interesting case which seems to show that sex may be disturbed by hydridization is that of the Norway rat when hybridized with the albino rat. King and Stotsenburg ('15) found a great excess of males among hybrid rats and came to the conclusion ". . . that hybridization alters the sex ratio by producing a marked increase in the relative proportion of males" (p. 110). Detlefson ('14) on the other hand, found a marked preponderance of females, especially in the early hybrid generations of the wild Brazilian guinea-pig and the common domestic guinea-pig.

Riddle ('16, and others) has conducted an important series of experiments on sex behavior in crosses between the various races of domestic pigeons. This work was begun by Professor

Whitman and, since his death, the experiments have been continued by Doctor Riddle. Whitman found that, if certain distantly related pigeons were mated, for example, individuals of different families, only male offspring resulted. If matings were made of individuals not so distinctly related, as, for example, between different genera, and to this was added the element of overwork in reproduction, males only were produced in the early part of the season and females only in the later part of the season. He also observed that at the transition period during the summer some pairs of eggs produced males and females, the first usually male and the second female. It was noticed, further that toward the end of the season the eggs were not quite able to hatch, and produced embryos of fewer and fewer days' development. This led Whitman to conclude that the developmental energy is greatest in the male-producing season.

Riddle, in a long series of experiments, has been able to verify the results obtained by Professor Whitman. He has also discovered many more facts which tend to show that in pigeons there is a reversal of sex, and that under certain conditions male offspring are hatched from normally female-producing germ cells, and vice versa.

In birds there should be, according to evidence obtained from experimental breeding, two kinds of eggs; one maleproducing the other femaleproducing. These two kinds should normally be produced in equal numbers. Riddle does not deny the existence of a chromosomal difference in the eggs of birds. He admits that it has been definitely shown that in some species, at least, when bred under stable conditions, certain chromosomes are associated with sex; but he denies that the sole cause of sex lies in the sex chromosome and that sex is definitely fixed and non-reversible from the very beginning of development. Data collected, he says, "strongly indicates that the basis of sex is a fluid, reversible process; that the basis of adult sexual difference is a *quantitative* rather than a *qualitative* thing."

In pigeons, therefore, it has been shown that eggs which normally develop into males or into females can have their developmental energy so changed by the introduction of spermatozoa from another species, or through overwork of the parent in reproduction, that they produce individuals of the opposite sex. Sometimes this sex reversal is not absolutely complete, for many of the females showed different grades of masculinity in their sex behavior. Females hatched from eggs laid earlier in the season were more masculine in their behavior than those of their own full sisters hatched later in the season; and a female hatched from the first egg of a clutch was more masculine than her sister hatched from the second egg of the clutch. Here there is, therefore, a second form of intersexualism which does not show in the primary sex characters, but in the sex behavior.

f. Sporadic hermaphroditism. Banta ('16) has published some observations on the appearance of sex intergrades in the parthenogenetic Phyllopod, Simocephalus vetulus. The culture was started from material collected in an outdoor pond and the propagation was continued in the laboratory. During the 131st generation of parthenogenetic offspring, one of the strains suddenly produced a large percentage of males, together with some normal females, and a large number of sex intergrades. These intergrades were either males, with one or more female secondary sex characters, or females, with one to several secondary male characters, together with some individuals which had hermaphroditic sex glands and showed various combinations of male and female secondary sex characters. The sex intergrades are of all possible sorts of combinations of secondary and primary sex characters. The highly male-like female intergrades produced few or no young, and males with one or more female secondary sex characters in nearly every case had incompletely developed reproductive organs.

Banta succeeded in propagating female intergrades for sixteen generations with no apparent change in the ratio of the various forms and with no apparent tendency of the stock to lose vigor or become less prolific. An attempt has been made to classify the intergrades on the basis of sex characters, and no less than twenty classes are distinguished. At the ends of the scale are the normal males and females.

Banta draws the conclusion from his observations that sex depends on environmental factors which influence the general physiological whole of the organism. In the intergrades the sexual balance has in some way been disturbed and the origin of this disturbance he considers a mutation.

g. Hermaphroditism as a result of hormone action. Another interesting case of disturbed sexual condition is found in the so-called free-martin in cattle. Frank Lillie ('16, '17) has made a study of this problem. Forty-one cases of twins were examined in utero and a classification made of them without a possibility of error. In fourteen cases both members were males, in six cases both were females, and in twenty-one cases the two were of opposite sex; 97.5 per cent were monochorial, but, in spite of this, nearly all were dizygotic as determined by the number of corpora lutea present. It was found that twins in cattle are nearly always the result of fertilization of an ovum from each ovary. As development proceeds, the developing embryos sink down into the median portion of the uterus and the blood-vessels anastomose in the chorion, so that it is possible to inject the blood-vessels of either foetus from the other. If both of the embryos are of the same sex, no harm results from the continuity of their circulations; but if of different sex, the reproductive system of the female is largely suppressed and certain male organs are developed. This is interpreted as a case of hormone action which may be due to a more precocious development of the male hormone or to its natural dominance. In this case, therefore, a distinction can be made between the effects of the sex-determining factors that are zygotic and those due to hormones. But the sex reversal is not complete and the result is the development of an intersexual individual.

h. Hermaphroditism as the result of parasitism. That a partial reversal of sex may be induced by parasitism has been observed by Geoffrey Smith ('10) in the case of the spider-crab, Inachus, when infected with the rhizocephalan, Sacculina. The males, under the influence of the parasite, are capable of assuming all the female secondary sex characters, and often even develop ova in the testes. In this case, however, the females do not seem to develop toward the male line when infected with the parasite. The explanation offered by Smith is that the parasite causes the host to elaborate a yolk substance similar to that which is elaborated in the ovaries during growth of the eggs. The apparent change of sex, therefore, is due to a change in the metabolism of the organism. It is clear that such a change could take place without the assumption that one sex is heterozygous for sex and the other homozygous, as Smith has assumed. If the change in the sexual condition be due to a change of metabolism in the direction that he has suggested, it follows that only the males should take on the characters of the opposite sex.

i. Sex in parthenogenetic animals. The determination of sex in parthenogenetic animals has been studied by various investigators. Woltereck ('11), who worked on Daphnia, came to the conclusion that there are, in each egg, competing sex substances, one kind becoming active at the maturation of the egg, while the other remains latent. In summing up his results he says:

> Die resultate meiner Versuche lassen sich nur verstehen, wenn wir in jedem Ei verschiedene konkurrierende Geschlechtssubstanzen annehmen, von denen jedesmal die eine aktiviert wird, während die andere latent bleibt . . . . die Geschlechtssubstanzen selbst können wir uns unter dem Bilde von (latenten) Profermenten und (aktivierten) Fermenten vorstellen.

In the rotifer Hydatina senta, A. F. Shull ('12) found that it is decided, in the growth period of the parthenogenetic egg from which the female hatches, whether it is to be a female-producer or a male-producer, or, in other words, that sex is determined a generation in advance. In some later experiments upon this form it has been found by Shull and Ladoff ('16) that an important factor involved in the production of male-producers is the amount of oxygen present in the culture, and that this probably acts by increasing the rate of the physiological processes taking place in the body. This conclusion is analogous to that arrived at by Riddle in connection with his experiments on sex in pigeons. Riddle says: ". . . the low-storage capacity of the male-producing eggs as compared with the high storage capacity of the female-producing eggs is therefore an index of higher oxidizing capacity of the male-producing eggs as compared with the female-producing eggs."

More recently Whitney ('19) has reinvestigated the problem relative to the effect of oxygen as male-producer in the rotifer, and has come to conclusions opposite to those of Shull, namely, that oxygen does not act as a factor in the production of male-producers. The question cannot be considered fully settled.

j. Variations in sex ratio. A variation in sex ratio might indicate that sex is not irrevocably established at the time of fertilization. It has been found that, in certain dioecious plants, females are more commonly derived from seeds of one region and males from those of another region. This may be due to differences in the metabolic activity of the two kinds of seeds, brought about, possibly, by differences in the conditions of the environment under which they were raised.

Montgomery ('08) found that there were 8.19 males for every female in a count of 41,749 spiderlings. Out of the total of 127 cocoons, only eight showed a male ratio of less than one. Out of the total number of eggs in the cocoons only 2871 failed to hatch, and even though all of these should be assumed to be female eggs, the ratio would not be appreciably altered and the results cannot, therefore, be due to selective survival. Examples of this sort might be given by the score, and they are not easy to explain on the hypothesis that the chromosomes are the only and absolute sex determiners; for this hypothesis demands that there should be an equal number of males and females produced.

Pearl and Parshley ('13) have found that, in cattle, the sex of the offspring is somewhat dependent upon the time of coitus. Early in the heat the number of males to one hundred females was 98.4; in the middle of heat the ratio was 115.5, and late in heat it was 154.8. The conclusion is drawn that, granting the presence of an X-chromosome, the results may be interpreted by assuming that it is not a positive cause of sex differentiation, but rather an inhibitor of the development of male characters—two doses inhibits maleness, while one dose is insufficient. On this hypothesis it is assumed that the general conditions of metabolism in the germ cells might modify sex. The case is similar to that of delayed fertilization in the frog which results in the formation of an increased number of males.

In fish cultures it is not rare to find an excess of males or of females. Woltereck ('08) has reported various records made by Thumm upon the sex ratio in fishes. In Jenynsia lineata broods were obtained of 68, 92, and 116 individuals without a single female. A female of Cichlasoma nigrofasciatum three years old, bred to a male one year old, gave a progeny of 800 individuals, of which not fifty were females. A female of the same species one year old, bred to a male two years old, (the same male as in the first case), gave 400 young, of which over 300 were females. To summarize: "aeltere starke Weibchen, verpaart mit jüngeren, daher schwächeren Männchen, brachten in Nachzucht vorwiegend Männchen und umgekehrt." It was also found that in viviparous 'Körpflingen' the percentages of males were higher in the spring than later in the season, and that in the fall it was often very low. This apparently corresponds with the results of Riddle on pigeons, where also the percentage of males is greater in the early part of the season. It does not seem possible that the results obtained by Thumm in the cases above are due to selective survival.

6. General conclusions in regard to the problem of sex determination. In the case of the lamprey it has been seen that, in young stages, a series of individuals may be arranged exhibiting all the intermediate forms between apparently pure females and apparently pure males. Pure in this sense is used to designate the individuals which possess no visible characters which normally distinguish the opposite sex. Out of the sex intermediates both males and females develop, so that in the adult condition only two kinds of individuals are found, functional males and females. The designation of sex intermediates among the young is based on the appearance in the germ gland of primary sex characters, namely, oocytes and cell nests. The presence of oocytes in the germ gland is unquestionably a female character, while the presence of cysts may indicate a juvenile condition or a male character. The oocytes, in one case, and the well-developed cysts, in the other, may be considered homologous characters of the opposite sexes.

Judging from the quantitative appearance of the characters in the germ glands of hundreds of larvae which have been carefully studied, the conclusion is reached that every individual is a potential hermaphrodite possessing the sex qualities pertaining to either sex. It appears, however, that some individuals are more strongly inclined toward the male side, others more strongly toward the female side. Some, on the other hand, are apparently in a balanced condition as regards sex, and it would be impossible to say whether they are more strongly male or more strongly female. This condition usually lasts only a short time, after which one sex takes the lead over the other. When, in the course of time, an individual has become more strongly male or female, the opposite sex character gradually disappears, or at least remains undeveloped; so that if it appear at all in the adult, it is in a rudimentary or degenerate condition. This is the case, for instance, with the oocytes which are in an undeveloped condition in the adult testes. The secondary sex characters which appear in the adult are probably not hereditary characters at all, but are formed as a result of the presence of special kinds of hormones produced by the testis or ovary. These secondary characters are not present until after metamorphosis, when one or the other appears, depending upon the form of germ gland present.

As far as the primary sex characters are concerned, it appears that both male and female potencies exist in every individual from the beginning of development; that is, from the time that the egg is fertilized, and probably in both of the sex cells that are brought together in fertilization. These potencies then are transmitted from parents to offspring. It seems quite likely, however, that the two potencies are not always transferred from parent to offspring in equal strength, so that the two are not, in all cases, in a balanced condition from the very beginning. This appears from the fact that during the stage of sex differentiation all kinds of variations are found as to the quantitative appearance of the male and female characters. Since all of the larvae develop under practically identical conditions, it does not seem likely that these variations can be due to environmental factors.

Many mendelian workers have found it convenient to assume that the appearance of the two sexes in approximately equal numbers in most animals is due to the fact that one sex is heterozygous for sex, while the other is homozygous for this character. This suggestion comes from the fact that the sex ratio corresponds with the ratio obtained when a first-generation hybrid is bred to a pure recessive. In this case half of the offspring will be pure recessives or homozygotic, while the other half will be hybrid or heterozygotic with the dominant character present. From the study of the sex chromosomes it seems to have been found that one sex may produce two kinds of germ cells which are visibly different, while the other sex produces only one kind. It appears further that it is sometimes one sex and sometimes the other that is heterozygous with regard to the sex character. It has been supposed, and is still maintained by a number of investigators, that the sex chromosomes are absolute sex determiners. The idea, however, that the chromosomes act qualitatively has given way to the belief that the influence exercised by the sex chromosome is a quantitative one, and this conception has paved the way for a better understanding of the sex phenomena in forms that exhibit hermaphroditic tendencies. The conception that all individuals carry the factors of the opposite sex in a latent condition will probably prove to be correct, and it may lead to a general acceptance of the theory that sex is not unalterably fixed at the time of fertilization.

The primary difference between a male and a female in any species is not as great as one might conclude from the appearance of the adults of the two sexes. The first sexual changes usually take place in the germ gland; in the female some of the germ cells very early stop dividing and enter upon a period of growth, while in the male the germ cells continue to multiply for a long time.

Since the primary difference between the female and the male is, that in one the germ cells enter early upon a period of growth, while in the other they continue to divide, it seems probable that one or the other mode of development is the result of a difference in the body metabolism in the two kinds of individuals, and is not due to the inheritance of unalterable sex factors. If sex should prove to be the result of slight differences in metabolism, it would be easy to understand how a reversal of sex might take place under certain circumstances. It must be admitted that these metabolic differences might exist in the animals from the very beginning of development, and, in so far as they are transmitted by the parental germs which unite in fertilization, they may be said to be inherited. If the sex characters are to be compared with other so-called mendelian characters, we have to admit also the possibility of a quantitative variation in the latter, which seems to be contrary to the opinion of the majority of geneticists at the present time.

From evidence already presented we are forced to the conclusion that in many dioecious forms, at least, every individual is a potential hermaphrodite, in so far as it carries the latent qualities of the opposite sex. Whether an animal develop into one or the other sex may depend upon an inheritable quantitative relationship existing between the male and female potentialities in the fertilized egg. As has already been mentioned, there seems to be some evidence that in some forms it is one and in other forms it is the other of the two sex cells that unite in fertilization which is responsible for the quantitative difference of the sex factors. The theory advanced by Castle ('03), that both male and female cells are heterozygous with regard to sex, required the assumption that selective fertilization was necessary in order to bring about the observed results. This has been objected to on account of the lack of evidence that there is such a selection among the germ cells. Papanicolaou ('15) and Stockard and Papanicolaou ('16) have brought forward some evidence that selective fertilization might take place in the case of guinea-pigs, but the full data have not, as far as I know, been published.

If it be found necessary to consider one sex heterozygous for sex and the other homozygous, the formula that appears most applicable is that adopted by Goldschmidt, which has been accepted with some modifications by Witschi and others.

In the case of the lamprey, it does not seem necessary to tie the question of sex up with chromosomal constitution. It is easy to conceive of every fertilized egg as being practically in a balanced condition as regards sex. Some may be more strongly inclined in one direction and some in the other, and, in so far as this is so, the sex characters may be considered as inherited. But if we look upon the development of one or the other sex as a result of metabolic differences, there is no necessary reason why these differences should be referred to the chromosomal make-up of the fertilized eggs. They may equally well be the result of cytoplasmic differences in the eggs, and these may be present even before fertilization. If we consider sex from this standpoint, it is not difficult to understand how, as a matter of chance, there might be an equality of males and females when conditions of development are normal, and also to understand how, under extraordinary circumstances, sex might be altered in the developing organism. A cytoplasmic inheritance of the female characters (FF) has been suggested by Goldschmidt for the gypsy-moth. In the case of Sagitta, which is hermaphroditic, it is claimed by Elpatewsky that the development of a primordial germ cell into a male or a female cell is dependent upon the proportion of the cytoplasmic body, the so-called 'besondere Körper,' that each cell receives.

Whether we consider the chromosomes or some other part of the cell as responsible for the determination of sex, we must, in the last analysis, think of sex determination as due to the relation between two opposite potencies which are both present in the fertilized egg. In true hermaphrodites, in which male and female germ cells are matured simultaneously, the two potencies are in a state of equilibrium, so that the presence of one is not antagonistic to the other. In protandric and protogynous hermaphrodites the two sexual states seesaw back and forth so that each alternately replaces the other, while in a case like that of Crepidula, which under normal conditions is male in the young stage and female in the older stage, the male potency never reappears. In the two latter cases we may think of the antagonism between the two sexual states as the result of the action of certain hormones, secreted during the development of one or the other form of sex cell, and which inhibits the development of the other. When one set of sex cells is exhausted, the action of the hormones ceases and the other set of cells begins to develop.

In dioecious animals and plants the two forces do not exert themselves in the same individual except as the result of unusual conditions. In the free-martin, for example, the female potency is in the lead from the beginning of development; but by the action of hormones circulating through the body of the embryo, the male factor asserts itself so that the female factor is partially suppressed, even to the extent "that a gonad with a primary female determination may form a structure which is morphologically a testis" (Lillie, '17, p. 468). When the spider-crab, Inachus, is infected with the parasite Sacculina, the males, parasitically castrated, may show every degree of modification toward the female state, even to the appearance of ova in the remaining part of the testis. The females, however, are not transformed toward the male condition, and the conclusion is drawn by Geoffrey Smith that the male is heterozygous for sex and the female homozygous. Such a conclusion is hardly warranted, for the parasite does not seem to act simply by arresting the action of one sex potency, but by also elaborating certain materials which are favorable to female development. That this is so may be surmised from the fact that when immature females are infected, the effect is "to force them to assume prematurely adult female characteristics" (Smith, '10). There is no reason, therefore, why a female, when infected, should be transformed toward the male side.

In pigeons, sex seems to be a matter of metabolic difference, and a disturbance of the metabolic level may be brought about by hybridization as well as by overwork in reproduction so that a sex reversal is effected. In this case Riddle thinks he has demonstrated "that germs *normally* female-producing, have, under experiment, been made to develop males; and that germs which were prospectively male-producing have been made to form female adults" (Riddle '16, p. 410). In the case of the gypsy-moth, hybridization again seems to disturb the sex metabolism so that a prospective female develops male characters and a prospective male develops female characters. Whether or not complete reversal of sex has occurred in moths, does not appear from literature on the subject, although there are apparently cultures yielding nothing but males (Goldschmidt, '17, p. 605).

In Bonnelia the sex metabolism is disturbed by environmental conditions. This is also true in the case of Crepidula. In frogs, delayed fertilization determines the results, as possibly also does temperature to a certain extent. In rotifers the change may be the result of the amount of oxygen present in the culture (Shull and Ladoff, '16; Shull, '18). Whitney, however, has obtained different results with rotifers. From the various cases of sex reversal in plants, mentioned above, it appears that in these cases the change is effected by disturbing in one way or another the normal conditions under which the plant lives.

It seems to be amply proved that among dioecious animals and plants every individual carries the qualities of the opposite sex in a latent condition. This is a great step toward the solution of the problem of sex determination; but it remains to explain why in some cases one potency asserts itself, while in other cases the other appears. Opinions on this question converge around the conception of a variableness in cell metabolism and the action of enzymes. Riddle thinks of male- and female-producing eggs, in the case of pigeons, as different in regard to their storage capacity—a less storage capacity pertains to the male—and a high storage capacity pertains to the female-producing germ. Riddle says: "The progressive *increase* in storage capacity of the eggs during the season—under overwork—is to be interpreted as a *decrease* in the oxidizing capacity of the same eggs." This opinion is similar to that expressed by Shull in the case of rotifers.

The metabolic capacity of the germ is, of course, reflected in the adults derived from them. We can easily see how, in forms like the lamprey, the storage capacity and the oxidizing capacity may so nearly balance each other that every larva may exhibit both tendencies in different parts of the body. This, as has been suggested above, may be due to slight inequalities in the cells resulting from division or to environmental factors of some sort influencing the germ cells in different parts of the gonad. Thus we may have, in the same gonad, certain cells with high oxidizing capacity which continue to divide and form cell nests, and other cells, with a high storage capacity, which enter the growth period very early and become oocytes. After the larva has become decidedly male or female in character, as evidenced by the proportion of cell nests and oocytes, it appears that the opposite sex tendency is in decline. This can be attributed to the presence of sex-differentiating enzymes produced by the predominating sex character. This results in the arrest of the development of the opposite sex character and often in its degeneracy.

Goldschmidt has worked out a theory of enzyme action in connection with sex which seems to be in the right direction. He assumes that "in the fertilized egg the enzymes which govern the differentiation of the organism towards one of the two alternatives, maleness and femaleness, are both present." These hypothetical enzymes he speaks of as andrase and gynase. The distribution of the sex chromosome "results in the formation of two kinds of fertilized ova, differing in the relative concentration of the two enzymes." Since in "mixtures of different enzymes, every single one reacts independently, providing no interfering reaction product is formed," a decision must be reached during differentiation of the organs as to whether they shall develop along the male or the female line. "This decision must be brought about by the action of the dominating enzyme." The more nearly the two enzymes approach each other in strength, the earlier do they show their double influence on the developing organism. Such seems to be the case in the intersexual moths which show all degrees of intersexuality, from slight changes in the secondary sexual characters, which are latest to be formed, to changes in the germ gland itself, which is the first sex organ to differentiate. The same idea may be applied to the lamprey, where various grades of intersexuality are found in the germ gland, and where the sex differentiating factor seems to operate early in some of the larvae and later in others.

We are not, strictly speaking, concerned in the present work with the causes which underlie the development of the secondary sexual characters or the accessory reproductive organs, since there are none in the larval stages of the lamprey. In insects the secondary sex characters appear to be unaffected by the presence or absence of the sex glands, but in most other forms the appearance of the secondary sexual characters may be the result of certain hormones which are produced by the predominating primary sex elements. This appears to be the case in the lamprey. Sooner or later in the life of the individual the male- or the female-producing enzyme, if such it be, takes the lead so that the action of the opposite enzyme is more and more suppressed. The male or the female germ gland, which develops as a consequence of the stronger enzyme, is capable of producing certain hormones which, both at and after metamorphosis, cause the secondary characters to appear.

The above conception is not opposed to the theory that the so-called sex chromosomes are associated with the phenomena of sex in many cases. The evidence indicates, however, that they are only one link in a series of processes which result in sex determination, and that other factors may operate so as to change development, in spite of the presence of the sex chromosome. The physiological action of the sex chromosome may be fundamentally the same as that of other factors.

Finally, if we think of sex as an hereditary character, as it seems we must, then it is amply demonstrated that here we have an hereditary character that can be modified by a variety of circumstances. Unless we assign the sex character to another category than other hereditary characters, we are forced to acknowledge the possibility that other hereditary characters are modifiable also. If this should prove true, it is possible that the idea that the sex character is changeable will be accepted with less reserve than heretofore.

GENERAL SUMMARY OF OBSERVATIONS

A. Origin and early history of the germ cells

1. The germ cells are first recognizable in the American brook lamprey when the mesoderm separates from the entoderm, as large yolk-laden cells which become included in the mesoderm. Their history previous to this time could not be traced. Their large size, however, indicates that they are early segregated cells.
2. The number of germ cells that become included in the mesoderm is small. There is evidence that many of them never reach the germ-gland region. Some of these degenerate before dividing, others form cysts in other regions of the body, and the possibility is suggested that some of them may be extruded into the lumen of the intestine in early stages.
3. During the early period of their history the germ cells shift from a lateral position in the mesoderm to a median position. The change in position is accredited to a shifting of the tissues surrounding the cells and, to a lesser extent, to independent migration.
4. The germ cells begin to lose their yolk when the larva is about 5.5 mm. long, and no yolk remains in the cells when the larva is 10 mm. long. They do not begin to divide until the larva is about 20 mm. long.
5. The germ cells may be distinguished from the somatic cells by their size, structure, and location.

B. Period of secondary division

6. When the larva is about 20 mm. long the germ cells begin to divide by mitosis.
7. After each mitosis the germ cells either separate or remain together, forming cell nests. Peritoneal cells migrate in and form follicles around the individual cells and cysts.
8. An astrosphere is distinguishable in the germ cells of this stage.
9. A vitelline body is found in the cytoplasm. Its origin could not be ascertained. It becomes a very prominent structure in the growing oocyte of later stages.

10. Numerous mitochondria are present in the cytoplasm of the germ cells in most phases of their history.

11. Two plasmosomes are present in the primordial germ cells but in the growing oocyte there is only one.

12. The period of secondary division lasts until the larva is about 35 mm. long. During this period the larva appears indifferent as to sex.

C. Period of sex differentiation

13. The period of sex differentiation extends from the time the larvae are about 35 mm. in length until they are about 70 mm. long. In some larvae, however, sex differentiates much earlier than in others. During this period the sex of the larvae is indeterminate. The condition may be described as juvenile hermaphroditism.

14. A varying number of oocytes appear in practically all the glands during this period, so that a series of glands might be arranged possessing from 0 per cent to 100 per cent of oocytes.

15. The changes taking place in the oocytes during the synapsis phase are described.

16. Numerous germ cells degenerate during this period. Degeneration may take place during the synapsis phase, the growth phase, or the indifferent phase of the germ-cell history.

17. When sex is established the germ cells belonging to the opposite sex disappear or remain in the gland in a rudimentary condition.

CONCLUSIONS

The following general conclusions may be drawn from the above study of the germ cells of the American brook lamprey:

1. The germ cells are segregated very early in the life of the animal even before the germ layers are definitely established. They are first recognizable when the mesoderm separates from the entoderm.

2. The definitive germ cells take their origin from no other source than the primordial germ cells and the germ cells take no

part in the formation of somatic structures. Numerous germ cells are produced which do not become functional, and these degenerate and disappear during the process of development.

3. The germ cells of each germ gland are usually of two kinds namely, those showing a tendency toward rapid division (katabolic) and those showing a tendency for growth (anabolic). The former are regarded as having a male, the latter a female potentiality. The relative proportion of anabolic and katabolic cells determines whether the larva becomes a male or a female individual.

4. Observations seem to warrant the conclusion that each larva of this species carries the potentiality of both sexes, and that sex, therefore, is not irrevocably fixed at fertilization.

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PLATES

ABBREVIATIONS

arc., archenteron
ast., astrosphere
bv., blood-vessel
c., centrosome
ch., chromidia
co., coelom
cy., cyst
d.a., dorsal aorta
deg.cy.1., degenerating cyst, early stage
deg.cy.2., degenerating cyst, late stage
d.g., dividing germ cell
deg.g., degenerating germ cell, early stage
e.c., extruded germ cell
ex.cy., extraregionary cyst
f.c., follicle cell
g., germ cell
g.c.r., germ cell region
g.e., germinal epithelium
g. f., germ cell fragment
gon., gonad
i.e., intestinal epithelium
lu.i., lumen of intestine
m., mitochondria
m.b., midbody
m.c., mesenchyme cell
mes., mesoderm
mesn., mesonephros
m.f.c., migrating follicle cells
n., nucleus
nch., notochord
n.t., nerve tube
o., oocyte
p.c.v., postcardinal vein
pr.d., pronephric duct
st., stroma
t., tetrad
v., vitelline body
w.f., wolffian duct
y., yolk

All outlines were made with a camera lucida.

PLATE 1

EXPLANATION OF FIGURES

1 to 4 Curves showing frequency distribution of the length of larvae of Entosphenus wilderi collected during various months of the year. The ordinates represent numbers of individuals, the abscissae lengths in centimeters.

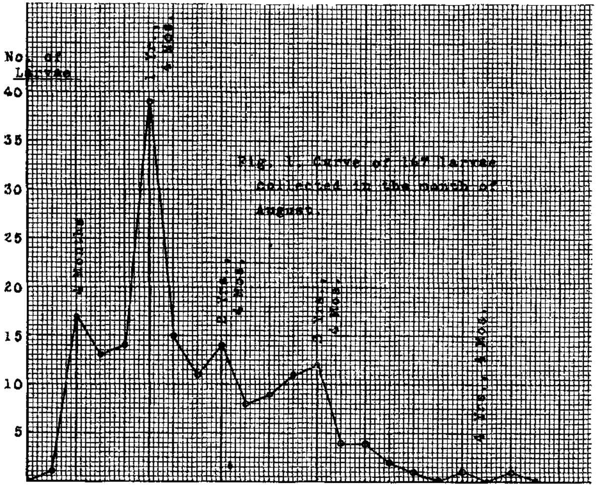


Fig. 1. Curve of 167 larvae collected in the month of August.

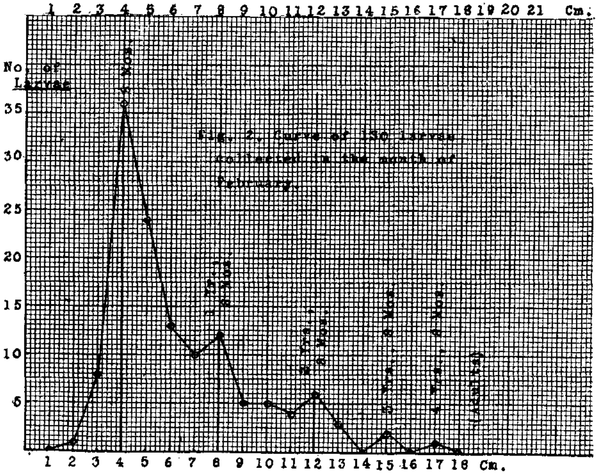


Fig. 2. Curve of 130 larvae collected in the month of February.

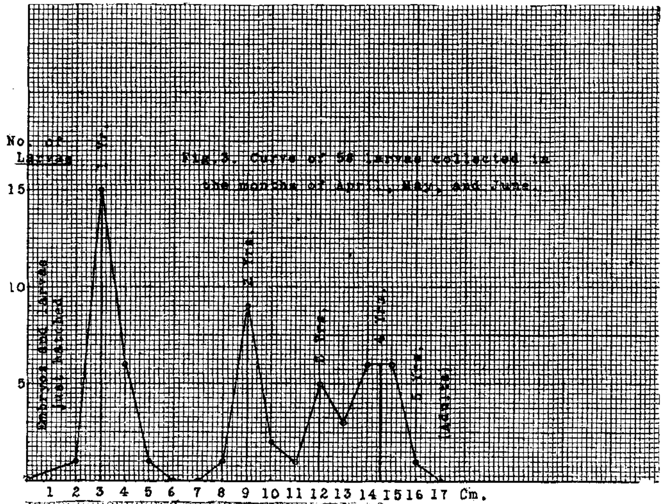


Fig. 3. Curve of 58 larvae collected in the months of April, May, and June.

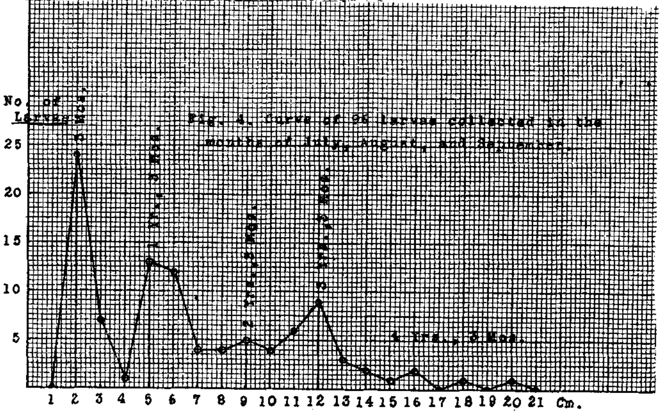


Fig. 4. Curve of 96 larvae collected in the months of July, August, and September.

PLATE 2

EXPLANATION OF FIGURES

5 Embryo of Entosphenus wilderi, 191 hours old. The black area represents the germ-cell region. The line *ab* is the plane of the section in figure 11.

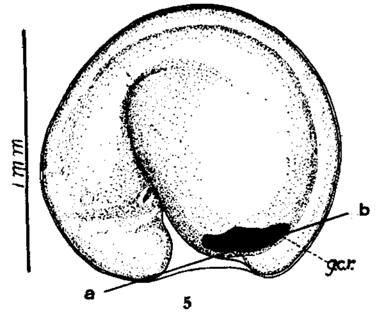
6 Embryo 238 hours old. The shaded area indicates approximately the position of the germ cells. The planes *ab* and *cd* are the planes of the sections in figures 12 and 13.

7 Larva 274 hours old. The lines *ef*, *cd*, and *ab* are the planes of the sections in figures 14, 15 and 16, respectively.

8 Larva 299.5 hours old. The line *ab* is the plane of the section in figure 17.

9 Larva 359.5 hours old.

10 Larva 8 mm. long (37 days, 14.5 hours). The distribution of the germ cells is shown by dots. Thirty-six germ cells were found in a larva of this stage. The position of the various 10μ sections craniad of the anal aperture is shown by vertical lines.



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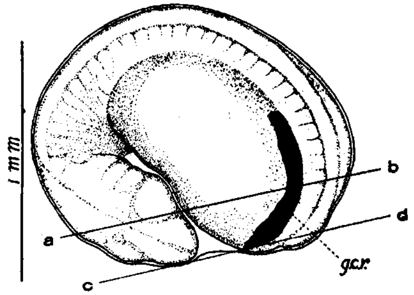

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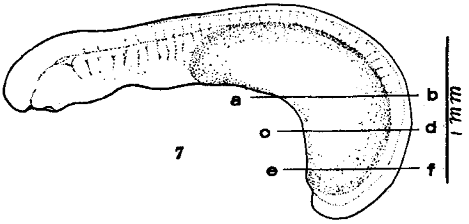

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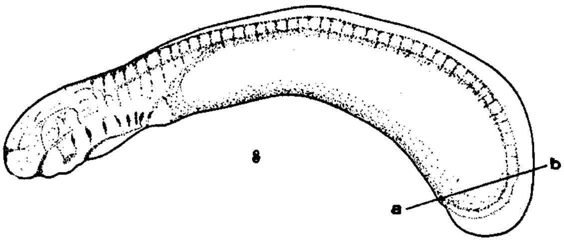

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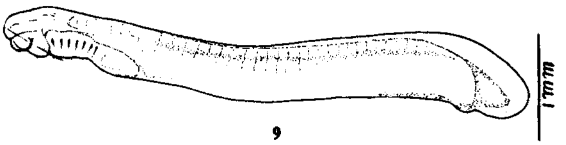

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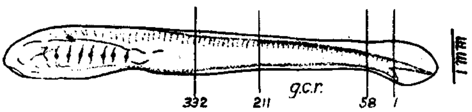

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PLATE 3

EXPLANATION OF FIGURES

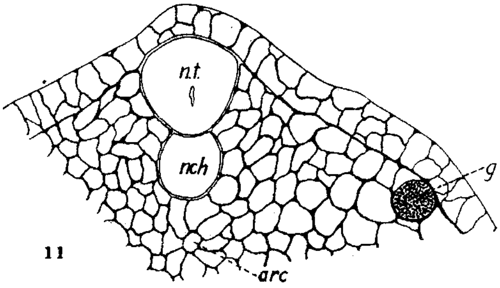
11 Section through the caudal region of the embryo shown in figure 5.

12 and 13 Sections of the embryo shown in figure 6 through the regions *ab* and *cd*, respectively.

14, 15, and 16 Sections of the larva shown in figure 7 through the regions *ef*, *cd*, and *ab*, respectively.

17 Section of the larva shown in figure 8 through the region *ab*.

18 Section of a larva 286.5 hours old.



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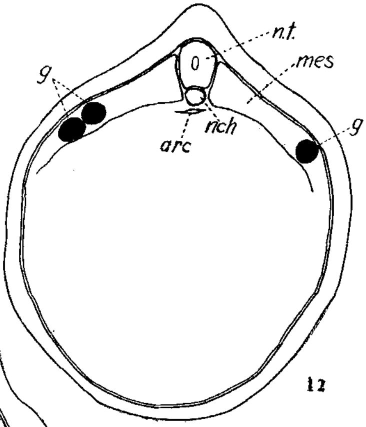

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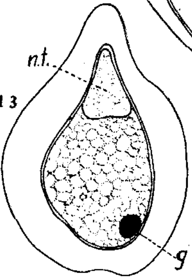

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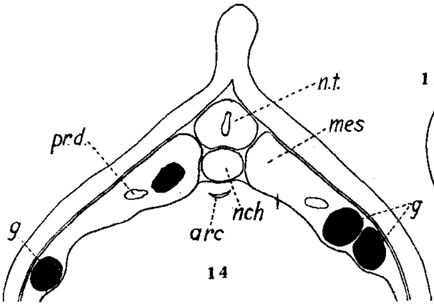

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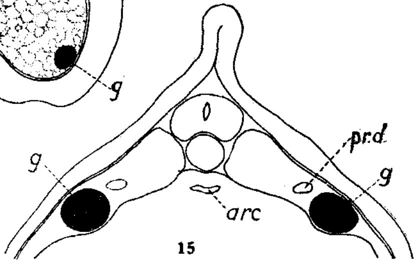

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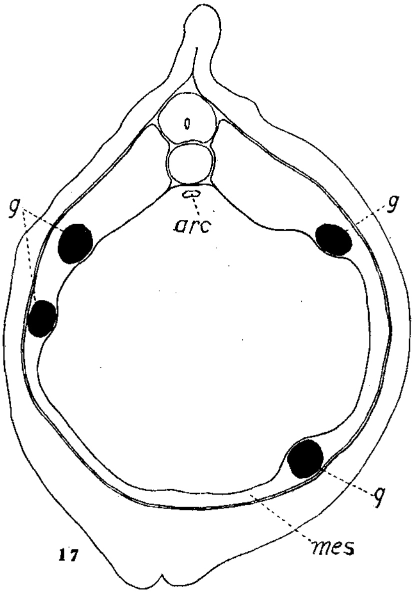

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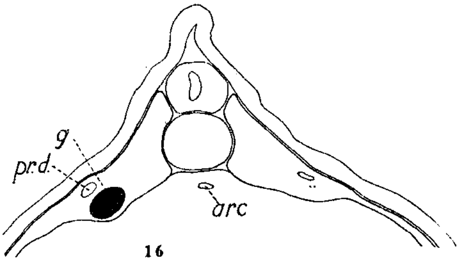

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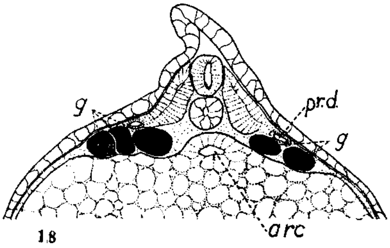

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PLATE 4

EXPLANATION OF FIGURES

19 Section through the middle of the body of a second larva 299.5 hours old (figs. 8 and 17).

20 Detail structure of germ cells from a larva 359.5 hours old. One germ cell is cut through the nucleus, the other along one side.

21 Section of a larva 373.5 hours old. The coelom is forming at this stage on each side of the intestine. One germ cell is shown near the ventral mesentery.

22 Section through the middle of the body of a larva 429.5 hours old.

23 Section through the caudal region of a larva 429.5 hours old (same larva as the preceding figure). The coëlom is forming and a germ cell (*g*) is included in the somatic layer of the mesoderm.

24 Section through the middle part of the body of a larva 478.5 hours old. The germ cell in the figure is at the cranial end of the germ-gland anlage.

25 The germ cell in figure 26 greatly enlarged. It shows disintegration of yolk globules and the elimination of chromatin-like material from the nucleus.

26 Section through the middle of the body of a larva 538.5 hours old. The yolk is beginning to disappear in the most cranial germ cells.

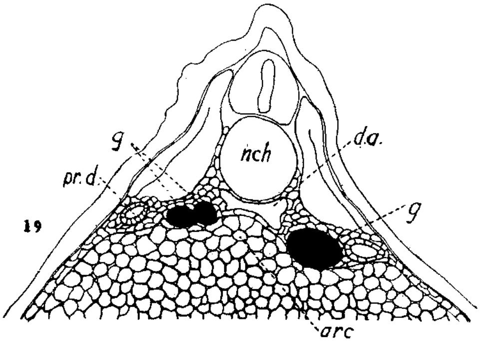
27 Section through the germ-gland region of a larva 20 mm. long. The germ ridge is forming and the germ cells are migrating into it.

28 Enlarged drawing of two germ cells from the same larva as the preceding figure, showing astrospheres and vitelline bodies.

29 Section of a larva 11 mm. long in the region of the germ gland. At this stage the germ cells have lost their yolk.

30 Enlarged drawing of the germ cells shown in figure 29. The germ cells lie above the germinal epithelium and are not a part of it. There is no germ ridge at this stage.

31 A section of the intestine near its caudal end from a 10-mm. larva. The lumen of the intestine is filled with cells that have been extruded.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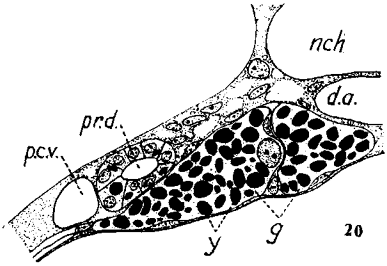
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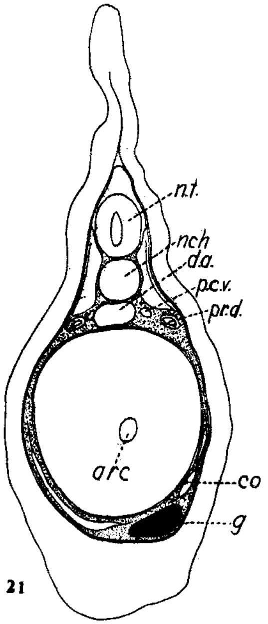
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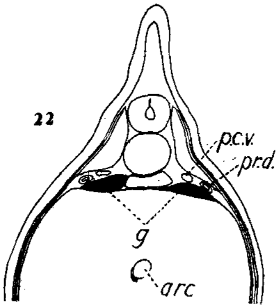
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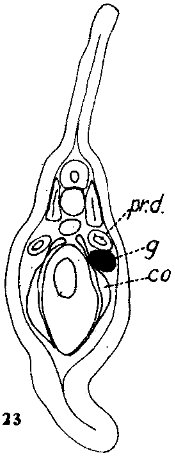
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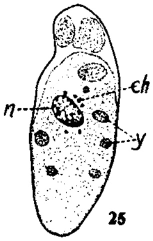
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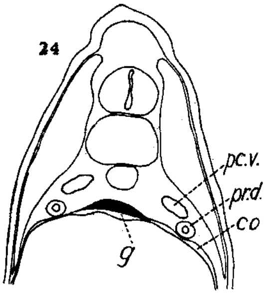
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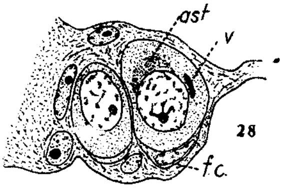
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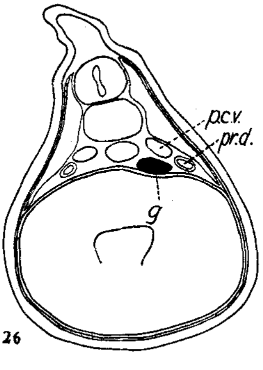
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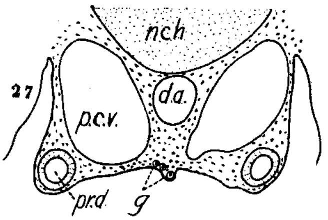
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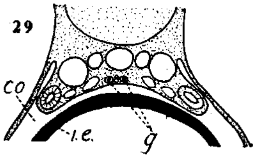
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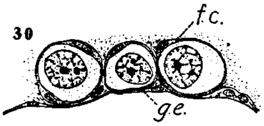
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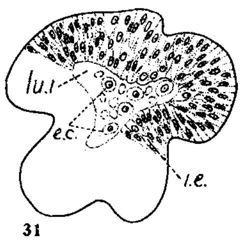
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PLATE 5

EXPLANATION OF FIGURES

32 Section through the cranial end of the coelom of a larva 35 mm. long, showing a nest of germ cells in the fat-body above the mesonephros.

33 Section through the germ-gland region of a larva 4⅜ cm. long, showing nests of germ cells in different parts of the mesonephros.

34 Section of the germ gland of a larva 21.5 mm. long.

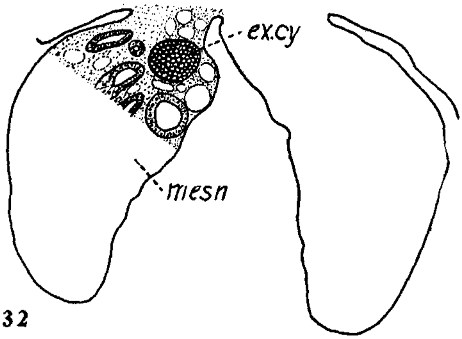
35 A magnified portion of the germ gland of a larva 25 mm. long, showing the inward migration of peritoneal cells to form follicles around the germ cells.

36 Section of the germ gland of a larva 21.5 mm. long, showing scattered germ cells and mesenchyme cells.

37 Section of the germ gland of a larva 25 mm. long.

38 Section of the germ gland of a larva 27.5 mm. long, showing a cyst of germ cells surrounded by follicle cells. The outlines of the individual germ cells are represented.

39 Section through the same gland as in figure 38 showing a cyst with dividing cells. Some of the germ cells of the cyst are in a resting stage.



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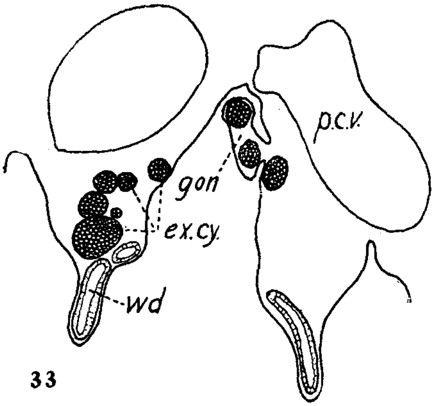

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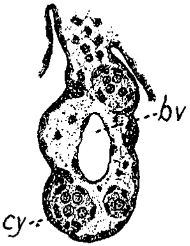

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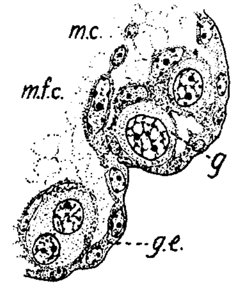

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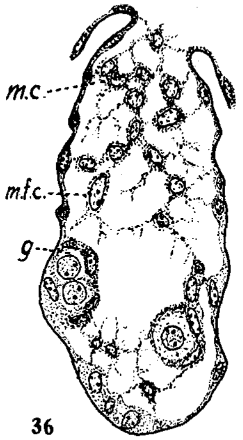

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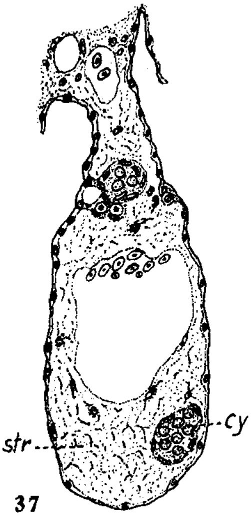

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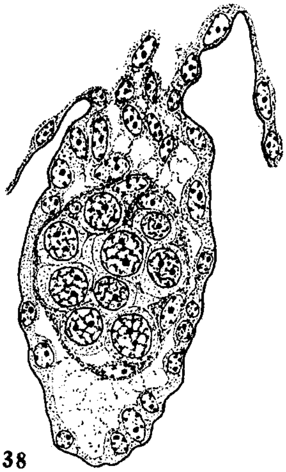

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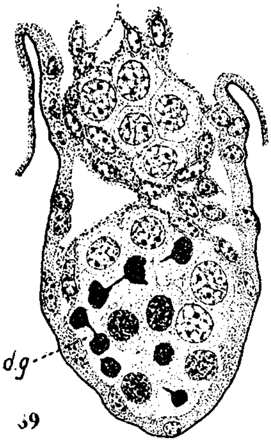

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PLATE 6

EXPLANATION OF FIGURES

40 Portion of a germ gland of a larva 30 mm. long, showing the breaking up of a cyst by the inward migration of follicle cells.

41 A resting germ cell from a larva 42.5 mm. long. The gland was fixed in Meves' solution and stained in iron hematoxylin. Mitochondria are abundant in the cytoplasm.

42 A germ cell in early prophase from a larva 30 mm. long.

43 A germ cell in prophase somewhat more advanced than that in figure 42.

44 Germ cell in middle prophase from a larva 47.5 mm. long. The chromatin network has broken up and the individual chromosomes are free. Only one nucleolus is present.

45 Side view of a cell in which the chromosomes are arranged on the equatorial plate.

46 End view of a cell in the same stage as figure 45. The individual chromosomes are still visible.

47 Early anaphase in which a chromosome-like body lies outside of the spindle.

48 Early anaphase in which the chromosomes are migrating toward the poles. Some of the chromosomes begin their migration earlier than others.

49 A germ cell in late anaphase from a larva 27.5 mm. long.

50 A germ cell in late anaphase showing a distinct midbody.

51 Telophase showing reconstruction of the nuclei. One nucleolus has made its appearance in each cell.

52 An oocyte in the early synaptic phase, from a larva 77.5 mm. long. Mitochondria are numerous at this stage.

53 Early leptotene stage from the same larva as the preceding figure. Both astrosphere and vitelline body are present.

54 Advanced leptotene stage from the same larva as the preceding figure.

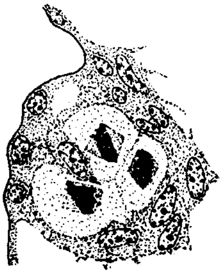
55 Synaptene stage (bouquet stage, synizesis stage), from a larva 59 mm. long.

56 Late pachytene stage from the same larva as the preceding figure.

57, 58, and 59 Various phases of the diplotene-dictyate stage. The chromosomes are paired; chromatin-like bodies (chromidia) are found in the cytoplasm; only one nucleolus is present.

60 An oocyte in the early growth phase. The vitelline body is a prominent structure at this stage.

61 A growing oocyte sectioned through the surface of the nucleus, showing the arrangement of the chromosomes in tetrads.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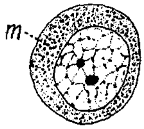

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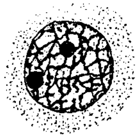
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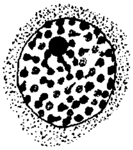
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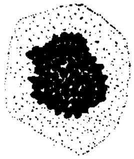
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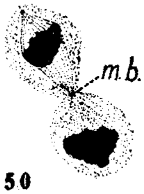

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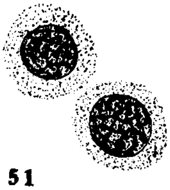
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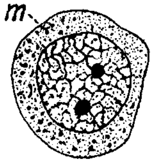

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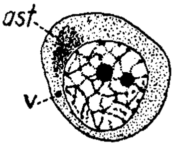

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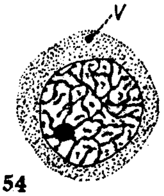

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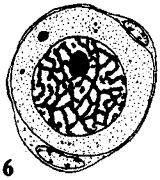
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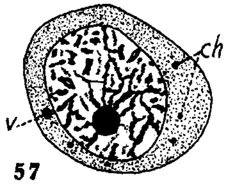

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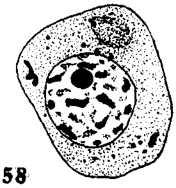
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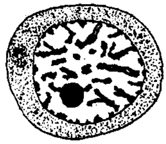
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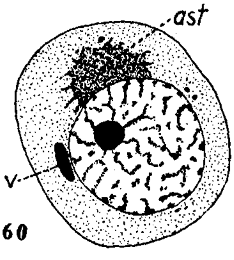

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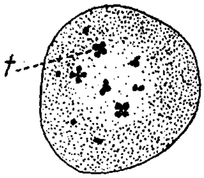

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PLATE 7

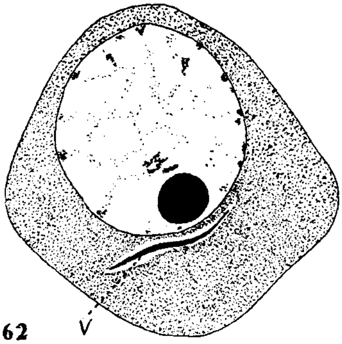
EXPLANATION OF FIGURES

62 An oocyte in the early growth phase, showing the vitelline body in the form of a spindle.

63 Section through an adult testis, showing an oocyte among the numerous cysts (not filled in).

64 Section through a cell nest containing one growing oocyte among numerous smaller germ cells.

65 Section of a degenerating cyst, with germ cells in various stages of disintegration.



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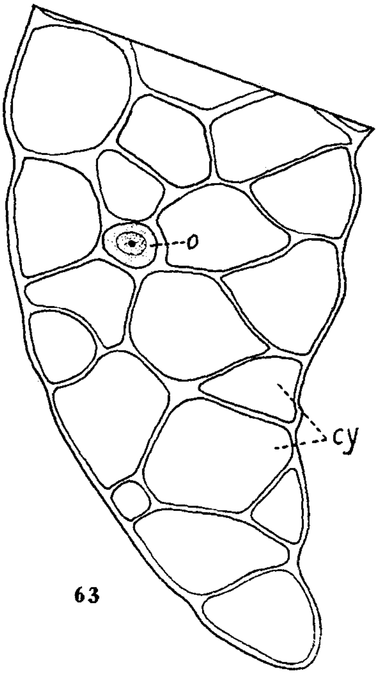

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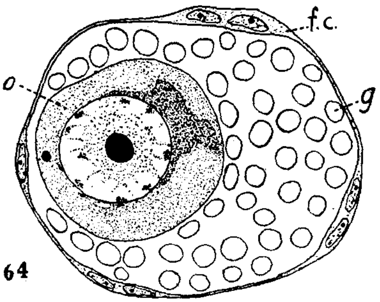

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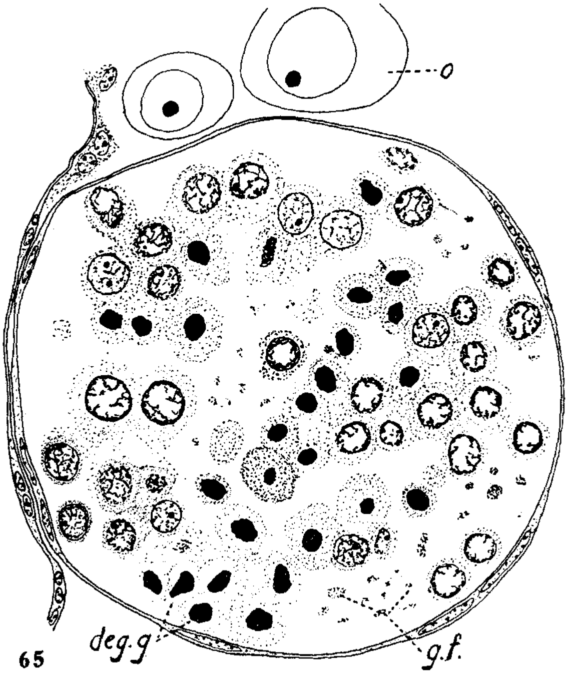

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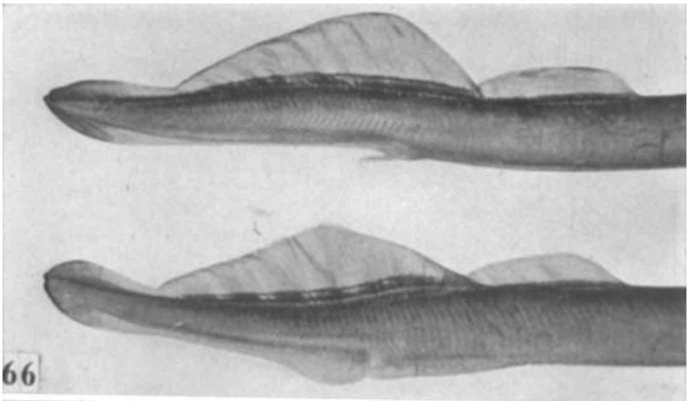
PLATE 8

EXPLANATION OF FIGURES

66 Photograph of the caudal regions of a male and a female brook lamprey, showing the external sex characters.

67 Microphotograph of a germ gland of a larva 47.5 mm. long, showing a cell nest with dividing and resting cells.

68 Section of the germ gland of a larva 54 mm. long, showing a cyst with dividing cells on the left, cysts with cells in synizesis on the right, and several growing oocytes.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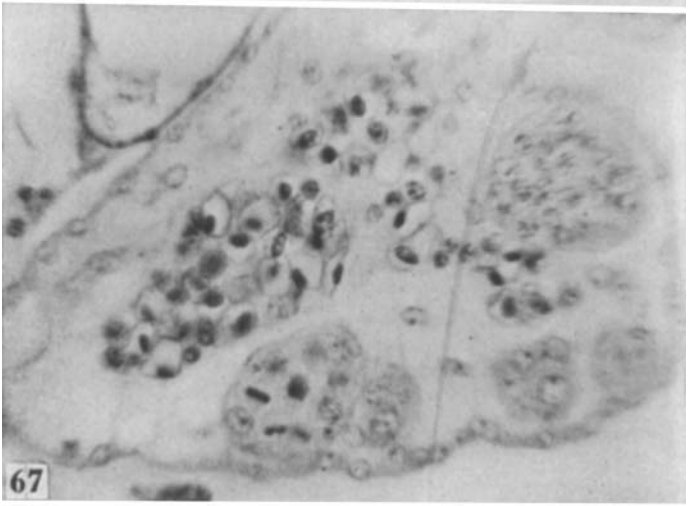
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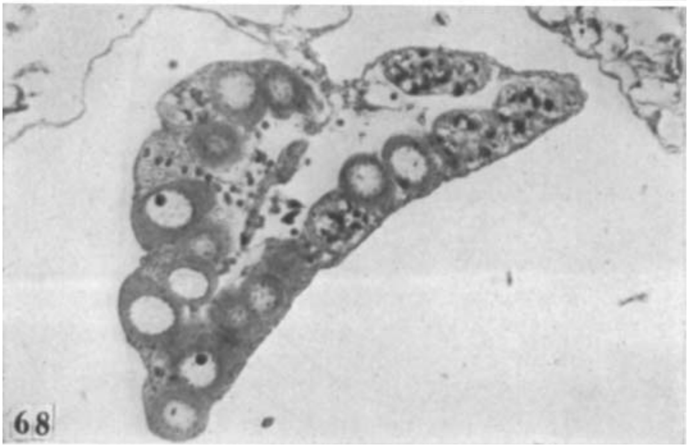
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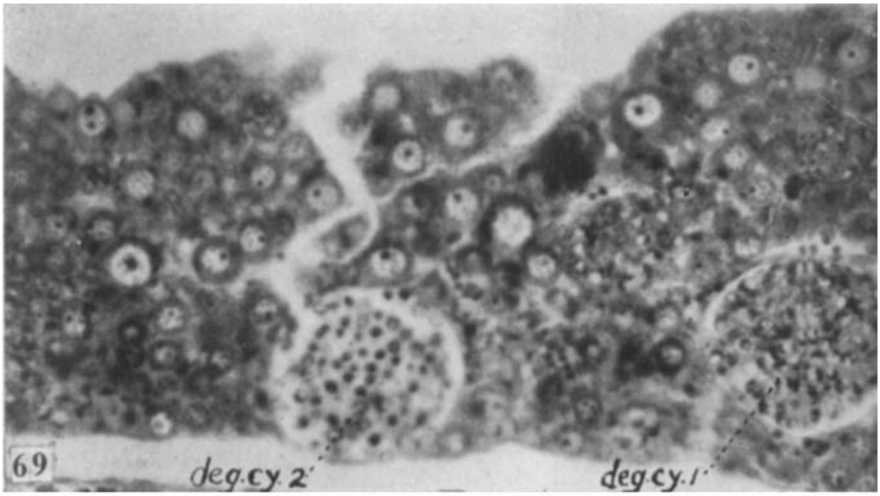
PLATE 9

EXPLANATION OF FIGURES

69 Longitudinal section of the germ gland of a larva 59 mm. long, showing two cell nests in different stages of degeneration.

70 Section of the germ gland of a larva 55 mm. long, showing cell nests and growing oocytes in about equal number.

71 Section of the germ gland of a larva 62 mm. long, containing oocytes and cell nests in about equal number.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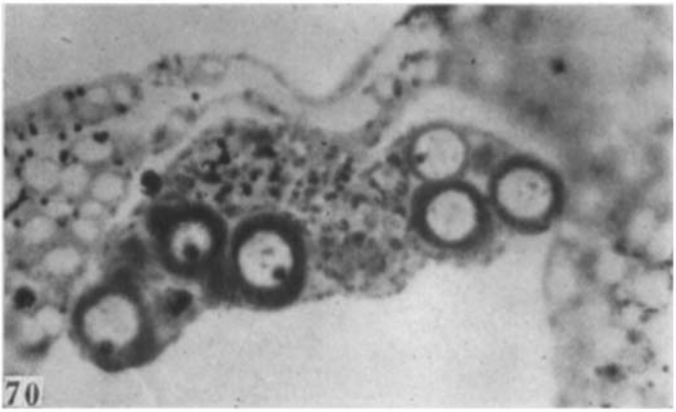
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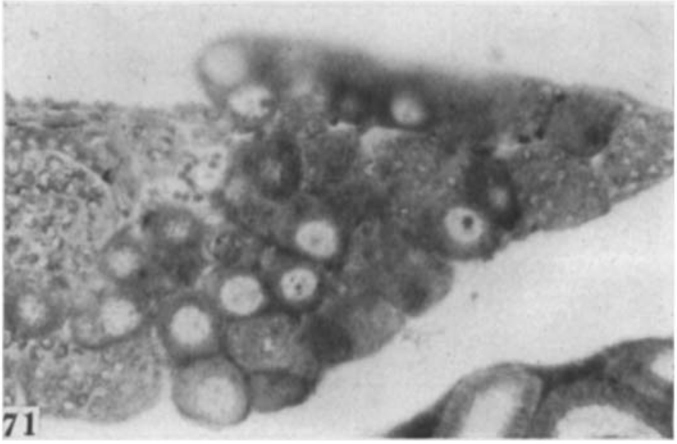
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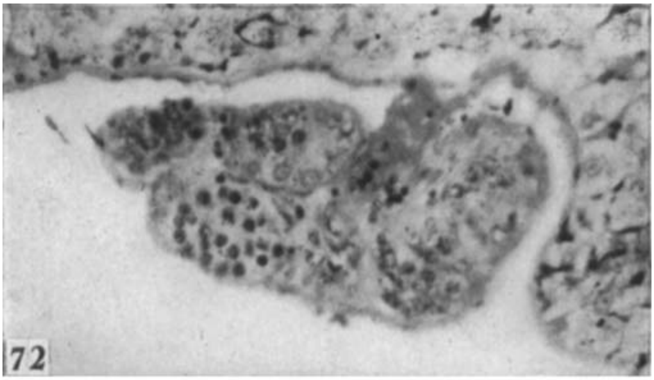
PLATE 10

EXPLANATION OF FIGURES

72 Section of the germ gland of a larva 50 mm. long, showing only cell nests.

73 Section of the germ gland of a larva 71 mm. long, showing only one growing oocyte.

74 Section of the germ gland of a larva 65 mm. long, showing very few cell nests, but numerous growing oocytes.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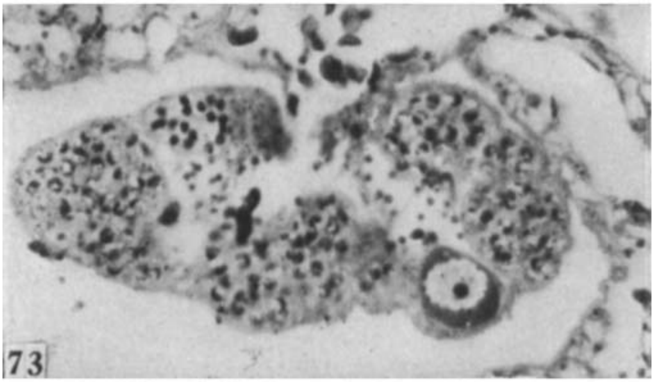
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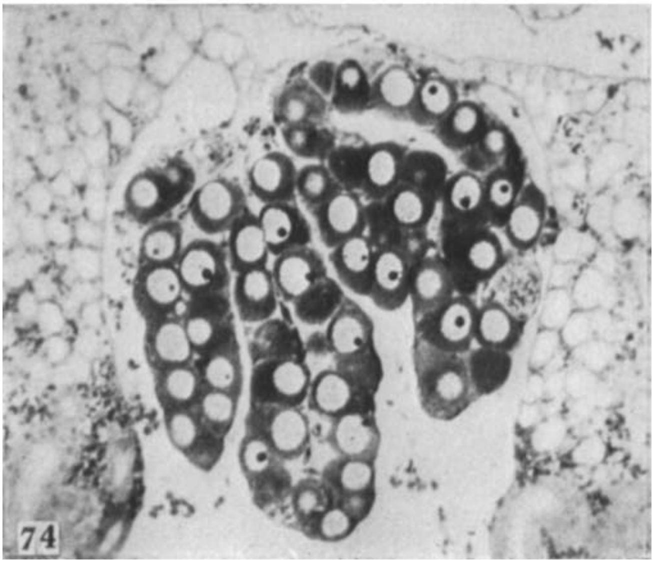
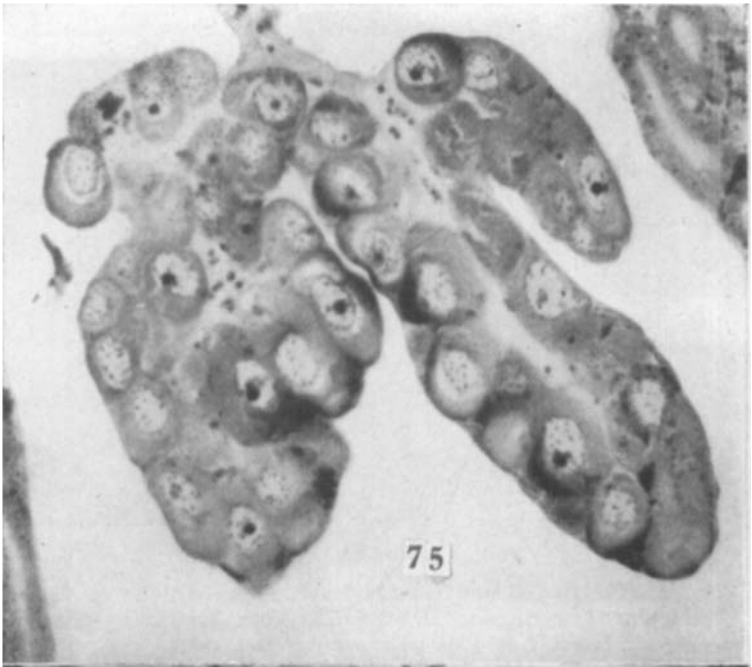

74

PLATE 11

EXPLANATION OF FIGURES

75 Section of the germ gland of a larva 63 mm. long, showing only growing oocytes.

76 Section of the germ gland of a larva 50 mm. long, showing more growing oocytes than cell nests.



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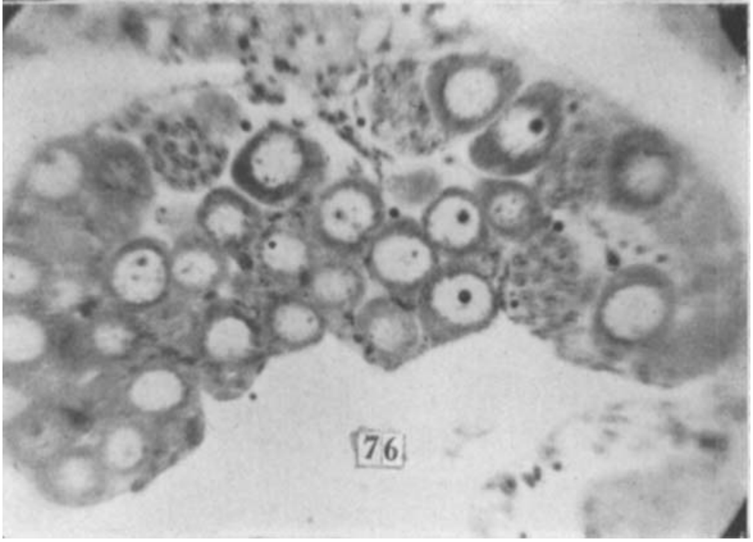


76

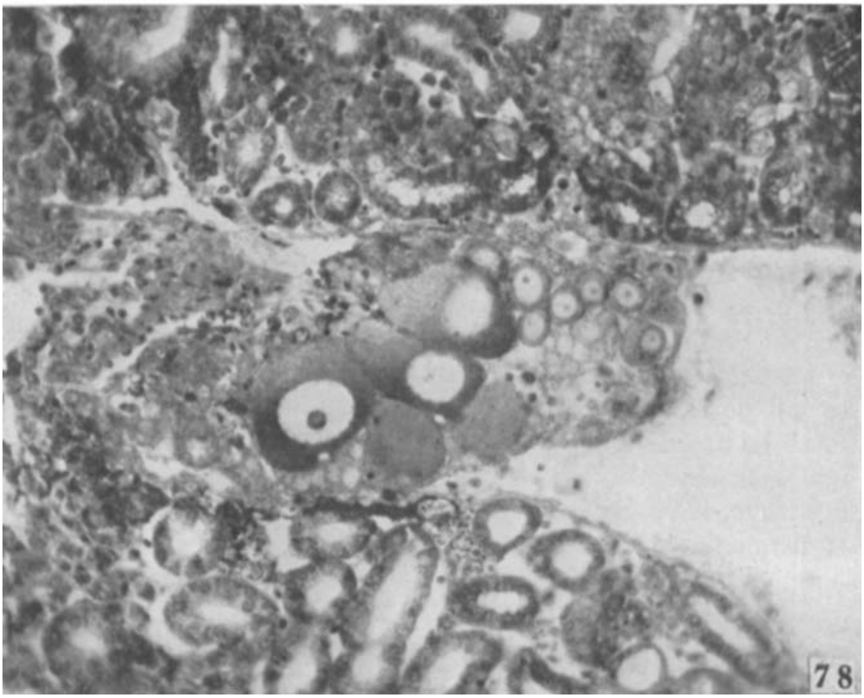
PLATE 12

EXPLANATION OF FIGURES

77 Section of a larva 60 mm. long, showing the same structure as figure 76.

78 Section of the germ gland of a larva 72.5 mm. long in which oocytes of various sizes are found.


77


78