Resumen por el autor, W. W. Swingle,
Princeton University.

Las células germinales de los Anuros.

I. El ciclo sexual del macho de Rana catesbiana.

Las células germinales aparecen primeramente bajo la forma de una cresta mediana de células semejantes a las del endodermo, situado encima del techo del arquenterio, en los embriones de 7 mm. La cresta está separada del endodermo subyacente por: (1) La oclusión de las placas laterales y la formación del mesenterio, y (2) Por la emigración activa de las células germinales. La cresta germinal se divide longitudinalmente, y las mitades se separan para formar las crestas gonadiales pares de la larva. En la larva de la primera estación (de cuatro a seis meses de edad) las gonadas son simplemente sacos huecos cuyas paredes están formadas de una capa sencilla o doble de células sexuales. A pesar del carácter no diferenciado de las gonadas y de la falta de madurez de las larvas, las células sexuales pasan por un ciclo sexual muy precoz y abortivo, el cual termina con la degeneración y reabsorción de las células.

El fenómeno de la maduración es normal hasta la primera división de maduración, cuando la fragmentación del centrosoma, con formación consiguiente de poliasters, tiene lugar acompañada de la destrucción de los cromosomas. Se forman unas cuántas espermátidas gigantes mediante crecimiento de una fibra axial que crece del centrosoma de los espermatocitos primarios no divididos. Las células y los cromosomas se parecen mucho más a las de los Urodelos que a las células y cromosomas de los anuros adultos. Unas cuántas espermatogonias, descendientes lineares de las células derminales primordiales, persisten sin cambiar durante el ciclo sexual abortivo y producen una segunda generación de células germinales en las larvas de dos años de edad. Muchas de estas células pasan por un segundo ciclo de desarrollo y dan lugar a espermatozoides en el renacuajo. Por consiguiente, en la larva de la rana toro existen dos ciclos sexuales: El primero es muy precoz y abortivo, el segundo es normal. El autor interpreta este fenómeno como una recapitulación en el ciclo de las células germinales de condiciones filogéneticas que han pasado en los anuros.

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THE GERM CELLS OF ANURANS

I. THE MALE SEXUAL CYCLE OF RANA CATESBEIANA LARVAE

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TWO TEXT FIGURES AND FIFTEEN PLATES (ONE HUNDRED AND THIRTY-ONE FIGURES)

CONTENTS

INTRODUCTION

Several years ago, while engaged in experimental work involving the germ glands and germ cells of anurans (Swingle, '17, also '17–'18), the writer was somewhat hampered by lack of definite criteria for differentiating the sexes in young larvae. In so far as the cytological conditions presented by the germ cells were concerned, it was impossible at that time, to distinguish clearly male from female tadpoles. The literature concerning sex in larval Anura was found to be voluminous and contained a great variety of opinions, many of which were mutually exclusive, others evidently based upon scanty evidence of somewhat dubious value, and none in any sense adequate to account for the conditions presented by my material. In the summer of 1917, therefore, an attempt was made to clear up the puzzling question of sex differentiation, but the effort proved abortive owing to lack of sufficient material. Certain cell stages occurred in my larval material which had been a source of mystification to the writer and to many others as well who had examined the material; these stages had apparently never been observed or at any rate reported by previous workers on anurans. Fortunately, an opportunity soon presented itself of working with Prof. E. G. Conklin, of Princeton University, who made a suggestion that further investigation has since shown to be correct, i.e., that I was dealing with a precocious maturation cycle in anuran larvae. Professor Conklin's suggestion throws an entirely new light upon the question of sex differentiation and development in the Anura, and brings the sexual conditions of these forms more nearly into line with those described for other vertebrates.

It is a pleasure to acknowledge my indebtedness to Professor Conklin for this illuminating suggestion, and for many others as well, which have made this work possible, for the time he has spent looking over material, and for the keen interest displayed in the progress of the work.

To Prof. N. P. Sherwood and Dr. Cora Downs, of the Department of Bacteriology of the University of Kansas, I am greatly indebted for aid in collecting 2000 tadpole specimens from the outlying districts of Douglas County, Kansas, during the summer

of 1918. To Professors Allen and W. R. B. Robertson, of the Department of Zoology, the University of Kansas, I am also indebted for aid in collecting tadpoles and newly metamorphosed bullfrogs at various times.

DIVISION OF THE PROBLEM

The subject of sex in larval anurans is such a complex one and the literature on the question so vast, that no attempt will be made to deal with all the aspects of the problem in this paper. Instead, the material has been so arranged that different phases will be taken up and discussed separately in a series of papers. This paper is concerned chiefly with the more usual phases of the sexual cycle of the male Rana catesbeiana, both in the larvae and newly metamophosed animals, with especial reference to chromosomal conditions. The broader questions of hermaphroditism, alleged to exist normally as a developmental phase of anurans, reversal of sexuality, anomalous sex ratios and their experimental modification, Bidder's organ, and other interesting problems will not be touched upon here, save perhaps incidentally, and then only in the briefest fashion. It will be recalled that Pflüger reported years ago, that there occur normally in newly metamorphosed frogs three kinds of individuals, males, females, and hermaphrodites, the two latter forms much more numerous in early stages than the males. In the course of further development the hermaphrodites become either definitely male or female, as the sex ratio for adult frogs is approximately 50–50. The investigations of R. Hertwig, Kuschakewitsch, and Witschi not only confirmed Pflüger's work, but extended it by showing that anurans apparently first develop solely as females and sexual intermediates, the males only later differentiating from the females and hermaphroditic forms. Moreover, these investigators described in great detail modification of the sex ratios by environmental changes, such as extremes of temperature and late fertilization. All of these alleged facts have given rise to the belief that anurans in their sexual development differ greatly from other vertebrates. These questions are reserved

for a later paper, which will be a consideration of the developmental history of the male and female sex glands, neoteny, Bidder's organ, and an attempt at a reinterpretation of the problems stated in the light of certain phenomena described below. The writer regards the second part of this work as perhaps the most interesting from a theoretical standpoint and as comprising the main portion; however, for sake of clarity in presentation, division of the subject has been found essential. It is necessary to give in detail the normal germ-cell cycle before discussing its aberrations or more unusual modifications.

MATERIAL AND METHODS

During the course of the work only one species of anuran has been employed to any extent, i.e., Rana catesbeiana. Other forms have been examined for comparison with the bullfrog, but not for the phase of the problem treated in this paper, so they need not concern us here. Rana catesbeiana in its larval stage has no equal among other frogs in respect to the peculiar fitness of its germ cells for this sort of study. The sex cells of the Urodela have long been noted for their size and fitness for cytological study, whereas the cells of adult frogs and toads have received scant attention. Yet it is a fact that the germ cells of larval bullfrogs, in regard to the size of cells and chromosomes, are little surpassed by even the best urodele material, and in this respect they more nearly resemble the caudate forms than the conditions presented by adults of their own species. In the adult frog or in newly metamorphosed animals the size of cells, nuclei, and chromosomes is distinctly less than in the larvae. The germ cells of sexually mature bullfrogs are in this respect like those of a different animal group when compared with larval stages. The explanation of this peculiarity will be discussed in its proper place.

Another interesting feature about the bullfrog that makes it an especially favorable object for study is its remarkable long larval life. This species usually spends several seasons as a larva, and is a tadpole for approximately two years. Sometimes these animals pass through almost three years as tadpoles, though this is

a rare condition and probably a result of defective thyroid development. The animals are abundant, are easily caught, and readily adapt themselves to laboratory conditions. Tadpoles caught in the autumn need not be fed more than once a month throughout the winter to keep them in good condition. First-season tadpoles rarely attain a greater length than 35 to 40 mm.; second-season specimens average 65 to 85 mm.; mature tadpoles, 100 to 154 mm. It is rare to find larvae with a greater length than 145 mm., though the writer recently caught two male specimens measuring 159 and 165 mm., respectively, from snout to tip of the tail; both had ripe spermatozoa in the gonads.

It will be shown later in this paper that the long larval life of Rana catesbeiana is correlated with a very interesting and suggestive phase of the germ-cell cycle—a phase which, while normally occurring in other anurans and probably in many other vertebrate forms, is brief, and apparently obscured by other developmental phenomena, hence not so easy of interpretation as the same condition in the bullfrog larva.

It should be stated here that there is apparently no seriation of germ-cell stages anteroposteriorly in the testis of larval or newly metamorphosed Rana catesbeiana such as has been described for various urodeles. The testis of a 40 to 50-mm. larvae is a narrow, flat, ribbon-like structure, gray-white in color, somewhat convoluted, attached by a mesentery to the inner edge of the ventral surface of the mesonephros. It bears little resemblance to the testis of the adult and is longer than the gonads of newly metamorphosed frogs. The relation of the glands of first-year animals to those of second-year larvae and newly metamorphosed frogs is indicated in text figure 1. The internal structure of these gonads is indicated in photographs (33 to 35, explanation of figures), where it will be readily seen that the center of the gonad consists of a large hollow (secondary genital cavity) surrounded by a germinal epithelium consisting of a single or double layer of germ cells in 40 to 50 mm. tadpoles and of many layers of cells in 80 to 90 mm. animals. In mature larvae and newly metamorphosed frogs the central cavity of the testis is obliterated at definite intervals by migration of

mesodermal cells from the mesentery and mesonephros (the so-called sex cords, a misnomer for they are in reality the anlagen of the rete or efferent apparatus).

Various fixatives have been employed, such as Flemming, Bouin's, Ezra Allen's ('16) modification of Bouin's fluid, and others. The best results were obtained with the last two fluids. The mesonephros was usually left attached to the testis. Sections were cut at a thickness of 8 to 10 μ. Even at this thickness it

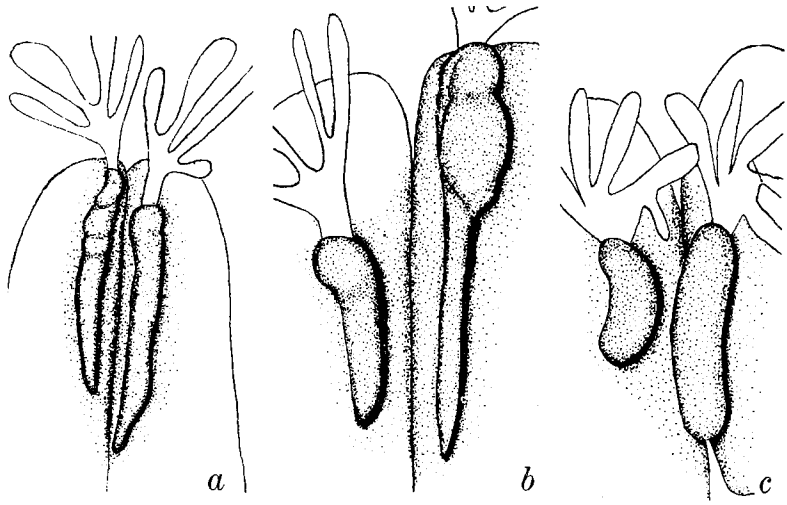

Fig. 1 *a*) Gonads of animal of first year. Average total length of larvae, 40 to 50 mm.; *b*) gonads of tadpoles 70 to 95 mm. total length; *c*) gonads of tadpoles nearing metamorphosis; total length, 120 to 150 mm.

is necessary to reconstruct the nucleus in most cases because of the large size of the cells. All spermatogonial counts, however, were made from complete cells.

The larvae used for the present work were taken from various localities and during different seasons of the year. Four hundred larvae measuring from 70 to 110 mm. were taken from the ponds of the State Fish Hatchery, Pratt, Kansas, during the month of September, 1917; 300 larvae averaging 100 mm. were taken from pools in the vicinity of Lawrence, Kansas, in the fall of 1916; a group of 1500 larvae averaging 70 mm. was caught in August,

1918, from a pool in Douglas County, Kansas; 1700 larvae measuring from 60 to 165 mm. total length were taken from a pond on the University Campus at Princeton during the months of July, August, and October, 1919. Only a comparatively small number of animals from these various groups were examined microscopically, the remainder were preserved for a study of the sex ratios and so-called hermaphroditism at various developmental stages—phases of the subject not dealt with here, but which make up the subject-matter of a later communication.

The size or length of tadpoles is not a good criterion of their age because of the size variability shown by anuran larvae of similar age, reared under identical environmental conditions. The writer was, until last year (1919), unable to get the eggs of the bullfrog in sufficient quantity to rear the tadpoles artificially. Hence the age of the older larvae given in this account is only approximate, for they are classified according to size and stage of development, as first- and second-year tadpoles.

RÉSUMÉ OF A FEW OF THE MORE IMPORTANT POINTS IN THE DEVELOPMENTAL HISTORY OF THE GERM GLANDS AND GERM CELLS OF RANA CATESBEIANA LARVAE

A brief summary of the developmental history of the gonads and sex cells may prove useful in elucidating some of the peculiarities of the sexual cycle described later in the paper. Only a few of the more important stages will be considered here, and then only in a very brief and sketchy way.

1. The primordial germ cells of the embryo are first distinguishable from other yolk-laden entoderm cells as a ridge just dorsal to the cavity of the archenteron and ventral to the aorta, separating the two lateral plates of mesoderm (text fig. 2, A). The medial growth of the two lateral plates and formation of the mesentery together with probably an active migration dorsally of the germ cells themselves, cuts off this germ-cell ridge from the underlying entoderm (text fig. 2, B and C). As development proceeds this median ridge of germ cells splits longitudinally and the cells of the two halves then migrate laterally on either side to form two independent ridges invested with a cov-

ering of peritoneum. In cross-section each ridge is seen to be made up of several large yolk-laden germ cells and a few small deeply staining peritoneal cells.

2. The two germ ridges enlarge considerably by proliferation of the cells and also by migration of mesoderm cells into the

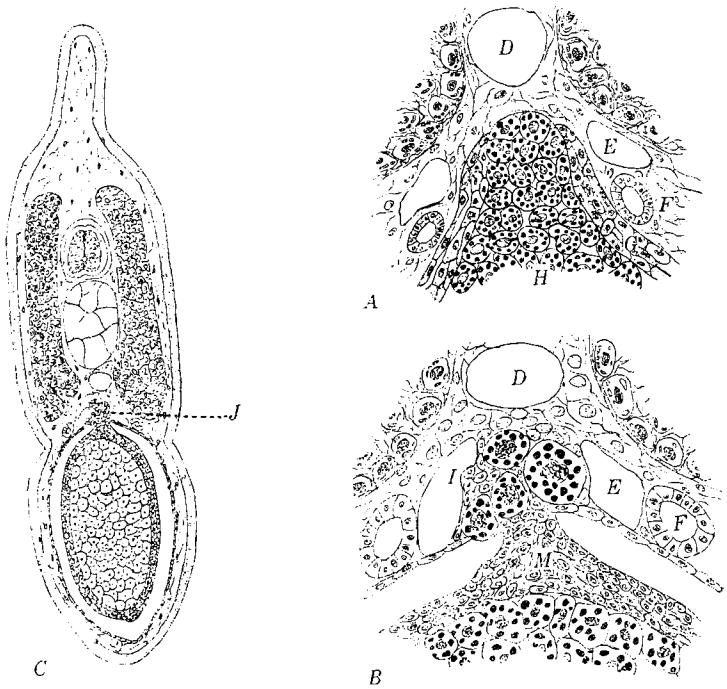

Fig. 2 Origin of the germ-cells. A. Cross section through germ-cell region of 7-mm. larvae. *D*, the aorta; *E*, cardinal veins; *F*, wolffian duct; *G*, lateral plate; *H*, entoderm cells. B. Transverse section through germ-cell region of 8-mm. larvae. *I*, germ-cells containing yolk; *M*, mesentery. C. Low magnification of stage shown in B. *J*, germ cells.

ridges from the mesonephros and peritoneum. The ridges project into the body cavity and take on the character of germ glands. The germ cells lose their yolk at about this time and divide actively.

3. As development progresses, the glands grow rapidly, the number of germ cells greatly increasing. Large cavities are

formed in the gonads, the so-called secondary genital spaces, lined by small non-sexual cells which have migrated into the gland from the mesonephros by way of the mesentery. At this stage the gonads of both sexes are hollow sacs (surrounded by peritoneum, the so-called germinal epithelium), the walls of which consist of one, two, or three layers of sex cells, depending upon the stage studied (figs. 33 and 34).

In female larvae the cavity is later obliterated by growth of the oocytes. In male animals, these secondary genital spaces persist until shortly before metamorphosis, when they also are obliterated, chiefly by increased division of the germ cells, and the ingrowth of cells from the mesonephros, which form anastomosing cords throughout the testis, the future rete or efferent ducts (fig. 35 shows the obliteration of the testis cavity).

4. In young first-season tadpoles, the sexes are indistinguishable, though later males and females are easily separated by microscopic examination. The female glands grow very fast and greatly enlarge, owing to oocyte formation, becoming irregular in outline. On the other hand, the male gonads remain small, are fairly regular in outline, but do not generally assume the shape characteristic of the adult testis until some months previous to metamorphosis, i.e., until the larvae are about two years of age (fig. 35). Also text figure 1, C.

5. The germ cells of larvae, taken in summer of the second season, both male and female are found to be undergoing simultaneous maturation changes. This is a most unusual phenomenon, and so far as the writer is aware, unique among the vertebrates, though common enough perhaps among the invertebrates. In no other group of the Chordata has anything analogous to the simultaneous maturation changes of male and female germ cells of larval anurans, such as here described, been reported, although on certain theoretical grounds based on a study of the sexual cycle of the larval bullfrog, the writer ventures to suggest that analogous phenomena are likely to be found in the myxinoids, larval petromyzonts, and eels.

The early maturation stages preceding the growth period of the oocyte in female animals, such as leptotene, amphitene, pachy-

tene, and diplotene, are said to occur normally extremely early in most vertebrates, in some mammals before birth. In male individuals, according to the usual accounts in the literature, the same stages of the maturation cycle do not usually take place until shortly before the attainment of sexual maturity.

It has long been known that the germ cells of female frogs undergo the earlier maturation changes while the animal is still a tadpole. The growth period of the oocyte in this group of amphibia is assumed to last a very long time, though further work may show this not to be altogether true. According to several investigators of European frogs, the eggs are not ready for fertilization until the fourth or fifth season after metamorphosis, when the first polar body is extruded shortly before fertilization. In this connection Gatenby ('14) says of Rana temporaria:

> Though one cannot be certain, I believe that an oöcyte takes two years at least, and more probably three to become mature. It is evident, therefore, that the young oöcytes formed in April or May in the adult will not be used for spawning next March, but certainly for a spawning several years ahead. The first ova derived from primordial germ cells would not be spawned till three years after the hatching of the tadpole, since the frogs around Oxford seem to become mature in three years.

However this may be, the germ cells of male Rana catesbeiana larvae enter maturation simultaneously with those of female tadpoles—long before metamorphosis, or before the gonads have even differentiated sufficiently to resemble a testis. Until the onset of maturation the gonads of the two sexes are morphologically identical. Following the precocious maturation cycle, it becomes easy to differentiate the sexes, as the female germ cells soon enter the growth period and become oocytes. The gross appearance of the glands of the two sexes changes at this time. The male germ cells pass through all stages of maturation, leptotene, amphitene (synapsis), pachytene, diplotene, tetrad formation, up to the first maturation division in a perfectly normal manner. During the anaphase of the heterotypic mitosis, or at the earlier period of spindle formation, the spermatocytes

undergo degeneration owing to fragmentation of the centrosome and consequent formation of polyasters which lead to aberrant divisions. Practically all of the first generation of male germ cells, i.e., those derived from the primordial germ cells of the entoderm ridge, pass through this abortive larval sexual cycle and degenerate in the act of division. A few of these cells give rise by direct transformation, without the intercalation of the first or second maturation division, to gigantic spermatids with axial filaments. Such spermatids possess fourteen tetrads. A very few of the primordial germ cells fail to pass through the precocious maturation cycle, and probably persist unchanged, apparently giving rise later by repeated division to a second generation of germ cells in the male. It may be remarked here that many cells of this second generation look as if they take origin from germinal epithelium elements, i.e., appear to be transformed mesothelial cells. This point, however, is still under investigation as morphological methods are not sufficient to determine whether or not such transformations actually occur. The mode of origin of the definitive germ cells of the adult is not strictly germane to this particular paper, and the question must be left undecided, pending results of experimental investigation.

This second generation of male sex cells, and this is the important point here, no matter whether they be lineal descendants of the primordial germ cells or transformed mesothelial elements, undergo a second maturation (sexual) cycle in larvae just ready for metamorphosis, i.e., in second-year tadpoles, and this generation of cells, oddly enough, gives rise to normal sex products, spermatids, and spermatozoa. The maturation cycle is normal in every respect. From the time of metamorphosis on to sexual maturity the young male frog apparently ripens his sex products continuously—this despite the fact that for a year or so, owing to his small size contrasted with that of mature females, he is probably unable to copulate.

It will be recalled that in the female sexual cycle the stage corresponding to the first spermatocyte division of the male is the stage of polar body formation which occurs normally at the time of copulation, presumably several years after metamorphosis.

Just why there should be this difference in time of maturation between the male and female sexual cycles of the tadpoles the writer is unable to say, though from certain data to be considered hereafter, obtained from studies on birds and mammals, it would not be surprising if the young female frog some months after metamorphosis likewise showed an abortive maturation cycle culminating in degeneration of the oocytes.[1] This point is now under investigation. Bearing in mind, then, this outline sketch of the developmental history of the gonads and germ cells of both sexes in the bullfrog tadpole, the following detailed account of the cellular changes involved in the larval maturation cycle of the male becomes more intelligible.[1]

OBSERVATIONS. SEXUAL CYCLE FIRST-YEAR LARVAE. PRIMARY AND SECONDARY SPERMATOGONIA

The primary spermatogonia found in such gonads as shown in figures 33 and 34 and text figures 1 A and B are much larger than the later generation of cells to which they give rise. In general these primary cells are more lightly staining than other elements of the gonad, and are peculiar, moreover, in that they are usually surrounded by a follicle made up of small, flattened, deeply staining stroma or peritoneal elements separating them one from another. This is true of this generation of cells in both larval and adult frogs.

The primary spermatogonial nuclei are large and very irregular in outline, presenting marked lobulations and indentations—the so-called polymorphism of the nucleus. Study of these polymorphic nuclei during early prophase stages of division has led to the conclusion that the lobulations and consequent polymorphism are due merely to large chromosomal vesicles or to the partial fusion of such vesicles, for from each of these lobulations a chromosome or pair of chromosomes appear in division prophases. The resting nucleus contains considerable karyolymph,

[1] Recently the writer has observed typical tetrad formation and a few first-maturation spindles and chromosomes in oocytes of female larvae. Such cells degenerate in the act of division just as do the laval spermatocytes of the male tadpole of the first year.

and an irregular linin network upon which is scattered chromatin granules of various size and shape, together with one or more nucleoli. The nuclear size is in many instances enormous, completely filling the cytoplasm except for a narrow peripheral border (figs. 1, 3, 115 and 117).

The character of the attraction sphere and centrosome presents nothing unusual and conforms to the type described for amphibians by earlier workers, hence it need not detain us here.

Division of the primary spermatogonia is always mitotic, and amitosis, though described for this type of cell in amphibians by La Valette St. George ('85), Meves ('91), Benda ('93), and McGregor ('99), has not been observed in Rana catesbeiana.

The somatic or diploid number of chromosomes in the male bullfrog larva is twenty-eight, and presumably this number is characteristic of the adult also, though no counts have been made on metamorphosed animals. A few years ago the writer found that twenty-six is the male diploid number for Rana pipiens, the leopard frog (Swingle, '17). Parmenter ('20) has recently confirmed this count for parthenogenetic frogs of the same species. According to King ('07), the somatic number in Bufo is twenty-four. This last number has also generally been regarded as characteristic for urodeles, such as Triton and Salamandra. Recently Snook and Long ('14) described twenty-eight chromosomes in the urodele Aneides lugubris, and Parmenter ('20) finds the same number in the larva of Ambystoma tigrinum. Levy ('14–'15) states that the diploid number in male Rana temporaria is twenty-five. The writer does not regard Levy's evidence as above criticism, and is much inclined to consider this statement as possibly a mistake. It would be odd if the males of all other amphibians, both urodeles and anurans so far studied, possessed an even number of chromosomes, and one species, Rana temporaria, possessed an odd number. Levy regards this species as having an accessory chromosome.

The writer described an odd chromosomal body in the germ cells of Rana pipiens as an accessory chromosome (Swingle, '17), but has since been in doubt in regard to this matter. The body described by me is probably a precociously dividing chromosome,

one-half of which sometimes migrates toward the pole of the spindle more quickly than does the other half to the opposite pole. The figures of Levy indicate that the body described by him as the sex chromosome is in all probability of the same nature as the precociously dividing chromosome described by myself. Further work on Rana temporaria will in all likelihood bring it into line with other species in regard to chromosomal constitution.

The twenty-eight somatic chromosomes of Rana catesbeiana may be divided into four groups: 1) Large V- or J-shaped elements; 2) intermediate sized J's; 3) small J's and, 4) slightly curved rods. These chromosomes appear to be definitely paired according to size and shape, and in this respect resemble those of other amphibians. It should be stated, however, that the chromosomes, though occurring in pairs in regard to size and shape relations, are not always found side by side within the nucleus. Many times the members of a pair are widely separated and may be on opposite sides of the nucleus. In general, though, the two homologues are usually near one another. Certainly, the intimate pairing of somatic chromosomes, such as described by Metz ('14) for Drosophilia and by Whiting ('17) for the mosquito, does not occur in the Anura (figs. 4 to 6).

The size and shape of the spermatogonial chromosomes vary somewhat with the fixative used, particularly if the fixation is not of the very best. The size variation is due to the preserving fluid and not to any real variation of chromosomal size or shape in the living tissue. In extreme cases the chromosomes may appear as short blocks, and their characteristic shape is entirely lacking (fig. 5). It is interesting in this connection to compare King's ('07) figure 10, plate 1, with my figure 5. King regards the chromosomes figured by her as those by young spermatocytes before the stage of reduction (p. 368). They look very much like the short dumpy chromatin blocks of my figure 5. This cell is an ordinary spermatogonium in prophase, in which the spireme segments have either been greatly condensed by imperfect fixation or else the cell was abnormal, probably the latter is the case, as such cells appear in otherwise excellently

fixed material. The chromatin masses are readily counted and are of the diploid number. This type of cell is unusual in the larvae, and has never been observed in metamorphosed frogs.

In metaphase the apices of the J-chromosomes are oriented toward the center of the spindle, and spindle fiber attachment is non-terminal.

The spermatogonial chromosomes are occasionally split into two elements twisted about each other as apparently is the usual condition in Ambystoma (Parmenter, '20).

Variations in the chromosome number have been observed in but two cases: once in a spermatocyte which contained eighteen tetrads, possibly the result of fusion of two adjacent cells, and once in a spermatogonium containing thirty-six or more chromosomes (fig. 19).

It is doubtful if variation of chromosomal number occurs in normal cells within one and the same individual, save perhaps in those cases where a single chromosome may occasionally undergo fragmentation. Even in such cases there is apparently no real variation in quantity of chromatin mass. Such chromosomal fragmentation as is described by recent writers, notably Hance ('18), has not been observed in the bullfrog except in degenerating first spermatocytes where the multipolar spindles literally tear the chromosomes to pieces (fig. 115).

The multiplication of secondary spermatogonia in the larval gonad, and this is especially true of first-year tadpoles, does not continue long enough to obliterate the lumen of the gland or to crowd the cells together owing to greatly increased numbers. During the second season the spermatogonial divisions come to a close and maturation begins in the type of gonad shown in figures 33 and 34, also text figure 1, A.

Last spermatogonial division. First-year tadpoles

The telophases of the last larval spermatogonial divisions differ in no respect from other similar stages in the mitosis of the primary and secondary spermatogonia. The period of nuclear reconstruction, however, presents marked structural changes differentiating it from all previous stages, in that the nucleus enters

the so-called 'resting' period, preparatory to undergoing the complex phenomena of maturation. As stated before, the nuclei of the primary and secondary spermatogonia soon become polymorphic in character, following division, and the chromatin material is found scattered throughout the nucleus in bead-like masses or granules, attached to an irregular linin network. In sharp contrast to this type of nuclear reconstruction, nuclei of the last spermatogonial telophase are round or oval in shape and of small size. The chromatin is in the form of small lumps or blocks (Janssens, '03) somewhat irregular in outline. In especially favorable cells the number of these blocks can be made out with a fair degree of accuracy. Their number is certainly diploid. The writer is inclined to regard these chromatin blocks as representing individual chromosomes at this stage. In early stages they are independent of one another, but very soon anastomosing linin fibrils appear between them.

The preleptotene period (Grégoire, '07) marks the first indications of resolution of the blocks. They become woolly or mossy in appearance, delicate, much coiled, and tangled thread-like processes appear, as if spinning out from the chromatin material in the mass. During the course of these changes the nucleus increases in size (figs. 8 and 47). These tangled threads so characteristic of the preleptotene elongate, lose their spiral-like character, and extend across the nucleus in loops. At this period there is no definite orientation of the leptotene filaments. Apparently, for the writer cannot speak with certainty on this point, each of the chromatin blocks of the telophase nuclei gives origin to a single thread. It is difficult, if not impossible, to unravel the snarl of elongating threads crowding the nucleus at the time of their first appearance. Wenrich ('16) has been able to trace the origin of the leptotene threads with considerable clearness in Phrynotettix magnus, and he is of the opinion that a single chromatin block gives rise to a single filament. This view seems very probable, when consideration is taken of the fact that the number of leptotene filaments is diploid and corresponds closely, if not exactly, to the number of blocks. Judging from Wenrich's figures, conditions in Phrynotettix at this period are much more favorable for study than in Rana catesbeiana tadpoles.

Shortly after their formation the leptotene threads tend to show a definite orientation of their free ends toward the centrosome and sphere in many cells, giving the appearance of a series of delicate loops. Janssens ('05) has characterized this orientation as the bouquet grêle or leptotene bouquet. The chromatin portion of the looped threads is in the form of very minute particles distributed at more or less regular intervals along a central linin core or fibril. Usually at this stage one or more nucleoli are present, though they differ from ordinary nucleoli in being in intimate connection with the chromosomes. These bodies have been termed chromoplasts by Eisen ('00), who first studied them in Batracoseps. We shall have more to say about these bodies later (figs. 10 and 55).

Following the period of the leptotene bouquet, there occurs in amphibians an extremely important and interesting stage, first observed by Janssens ('05) and named by him amphitene. This stage marks the first formation of the thickened pachytene thread and corresponds to the zygotene of Grégoire's ('07) terminology. In Rana catesbeiana larvae the amphitene constitutes a very definite and well-marked period in the maturation process—one that is easily differentiated from the leptotene preceding or the pachytene following. Judging by descriptions of various investigators of the zygotene in different animal groups, the amphitene of the amphibian germ-cell cycle is a prolonged transition stage between leptotene and pachytene. In typical amphitene nuclei one finds the nucleus marked off into two more or less distinct portions by the type of chromatin thread present. At the proximal pole of the nucleus, i.e., that side nearest the centrosome and sphere, the delicate leptotene filaments have disappeared, and one finds only the thickened pachytene threads; conversely, at the distal pole of the nucleus, i.e., the pole opposite the sphere, the leptotene condition persists. By focusing through a single cell, it is possible to bring into view now a leptotene, now a pachytene condition (figs. 11 to 13, 36 to 38). The explanation of this apparently anomalous condition is simply that the thick pachytene threads of the proximal pole of the amphitene nucleus represent the longitudinal fusion (parasynapsis) of two originally distinct leptotene

filaments. The side-by-side fusion or synapsis begins at the ends of the threads nearest the centrosome, and extends distally until fusion is complete throughout the length of the conjugants. Thus the amphitene is essentially a transition period in which the pairing of chromosomes in the stage of leptotene filaments is progressing. In the distal portion of the nucleus, where typical leptotene conditions persist, parasynapsis has not yet occurred.

The evidence for this point of view is quite conclusive in Rana catesbeiana larvae: *a*) The leptotene threads are certainly nearer the somatic number than the haploid number; *b*) the thickened pachytene loops represent the haploid or reduced number; *c*) the thickness of the pachytene elements is just twice that of a single leptotene filament, *d*) and, perhaps most conclusive, it is not difficult in studying amphitene nuclei, to trace the two unpaired ends of the leptotene threads from the distal pole into a single thickened pachytene thread at the proximal pole (figs. 11 and 12). Janssens ('05) has figured this stage clearly in his figures 20, 21, 22, and 23, plate IV. Wilson ('12) observed the same thing in Batracoseps material obtained from Janssens, and states that the conditions described are even clearer than Janssens figured them. Apparently analogous conditions are figured by Wenrich ('16) (fig. 77) and designated by him as zygotene stages showing incomplete conjugation of chromosomes.

The leptotene threads appear to coil or twist about each other corkscrew fashion so tightly that all trace of their double nature is lost and the resulting thickened thread appears single. (Fig. 12.)

Many investigators of amphibian spermatogenesis have described other methods of synapsis for this group of vertebrates. However, the period assigned, which has usually been considered as identical with synapsis in urodeles and anurans, is in all probability an artifact due to imperfect fixation of material, poor staining, or both. This so-called synaptic period corresponds to what McClung has termed synizesis or the "unilateral or central contraction of the chromatin in the nucleus during the prophase of the first spermatocyte." Nuclear conditions are at this time extremely difficult to make out, to say nothing of interpreting correctly. The pachytene spireme is considered as evolving out of this contracted nuclear condition (King, '07, figs. 24 and 25).

Janssens ('01) first called attention to this condition in urodeles and considered it a definite stage of the germ-cell cycle. Later ('05) he reversed his earlier opinion and stated the condition described earlier was due to poor fixation.

In the bullfrog larva there can be no question that synizesis is an artifact due to poor penetration of fixatives. For instance, in well-preserved material it is impossible to find contraction stages; where large pieces of the gland are used, generally the peripheral portion of the tissue will show no synizesis, whereas the central portion will show numerous contraction figures. A comparative study of reagents, such as Bouin's or, better, Ezra Allen's modification of Bouin's fluid without urea which is a good fixative for frog material, with Flemming's osmic fixative, a rather poor penetrant, on similar sized pieces of gonad, gives illuminating results in regard to contraction stages. In Rana catesbeiana and Rana pipiens slow penetration of fixatives clumps the delicate loops of the leptotene bouquet into a typical synizesis figure.

The condition described here for anurans possibly is not comparable to a somewhat similar clumping of nuclear contents in other forms described by various investigators. The writer has had the opportunity, through the courtesy of Dr. E. L. Shaffer, to examine synizesis stages in Cicada material. The conditions presented by this form are hardly comparable to those described here for anurans, and it may well be that in certain groups synizesis is a definite stage in the maturation cycle.

The partial synapsis of leptotene threads in the amphitene is completed in the pachytene stage which immediately follows. The threads of this period are thickened throughout uniformly and usually show no trace of their dual nature, save perhaps in respect to size. It is odd that in a fully formed pachytene spireme there is usually no indication of the leptotene threads which entered into its formation (fig. 39, also 11). Most animals show distinct traces of a primary longitudinal split or line of fusion between the conjugants. For example, Wenrich ('16), describing the pachytene stage in Phrynotettix magnus, states: "The line of separation between the threads which have conjugated (i.e the primary longitudinal split) remains visible throughout the pacytene stage."

It is generally only in the amphitene and diplotene that the line of fusion of the conjugants is visible in the bullfrog larvae. In this connection it is interesting to note that Janssens ('05), in his study of Batracoseps, was unable to detect any indication of a paired condition. Wilson ('12), in his examination of the same animal, agrees with Janssens that the pachytene threads appear as if single.

The pachytene period in anurans larvae is in all respects like that described for urodeles. In many cases the free ends of the thickened threads are applied close to the nuclear membrane at the proximal pole, the broad loops extending distally, thus giving rise to the pachytene bouquet. Janssens ('05) divided the pachytene in Batracoseps into two distinct periods: the 'bouquet orienté,' corresponding to the condition just described, and the 'bouquet transverse,' in which the nuclear contents have apparently rotated in relation to the sphere, so that the bouquet instead of being oriented toward the centrosome and sphere is turned at right angles to it. The writer is unable to say definitely whether the period of the transverse bouquet does or does not represent a well-marked stage in the maturation cycle of Rana catesbeiana. Very probably this stage is more marked in urodeles than in anura.

In Bufo, King ('07) derives the pachytene spireme from the irregular, deeply staining, confused chromatin mass of the synizesis period. Her figure 25, plate 1, is a clear expression of her idea regarding the derivation of the pachytene threads. According to her account, it is a continuous spireme, does not show any evidence of longitudinal splitting, and later in the course of development segments transversely into the reduced (haploid) number of chromosomes. If this account of conditions in Bufo is correct, then this anuran differs from other amphibians, both caudate and tailess, in respect to formation of the pachytene spireme and the tetrads. The writer is under the impression that the difference between Bufo and other forms rests upon a misinterpretation of synizesis and synapsis, and if reexamined Bufo will very likely be found to conform to the amphibian type of maturation cycle.

Following the pachytene is the period of exconjugation or disjunction of the homologous chromosomes, i.e., the longitudinal splitting of the thick double threads into two thin threads which diverge in the center, but remain united at both ends (figs. 14, 15, and 40 to 51). This stage corresponds to the diplotene of Winiwarter ('00) or the prostrepsinema of Janssen ('05). The pachytene threads split longitudinally, the split first appearing apparently at the distal pole of the nucleus and extending proximally. The line of cleavage might possibly be looked upon as marking the earlier line of fusion of the two originally unpaired leptotene threads, and hence be regarded as the line of disjunction (figs. 14 and 15, also 40 to 51). This is only guesswork, however, because in general the fused leptotene threads show no sign of separation in the pachytene as they do in other forms; i.e., the primary longitudinal split is usually invisible at this stage.

The diplotene stage in Rana catesbeiana larvae is marked by extreme growth of the cell, especially the nucleus which reaches gigantic proportions in many instances. In general the cells of the pachytene stage, though larger than those of the leptotene, do not present such marked size differences over leptotene stages as do the diplotene nuclei over both pachytene and leptotene.

In early diplotene, when the primary longitudinal split is just making its appearance, there is somewhat superficial resemblance to the amphitene stage. The similarity is, however, slight, and one could hardly confuse the two periods. The longitudinal split of the diplotene appears first at what corresponds to the distal pole of the double thread. In the amphitene just the reverse condition is presented, the initial pairing of the leptotene filaments begins first at the proximal pole. The very obvious difference in the size of the nuclei of the two periods is an excellent criterion for distinguishing the two stages. Also separation is never complete in the diplotene, as the homologues remain united at their ends; conversely, in the amphitene the unpaired leptotene threads at the distal pole of the nucleus diverge widely from one another.

Shortly after the process of disjunction, a secondary longitudinal splitting of each member of the pair appears, forming the

tetrad-complex, made up of four chromatids (McClung, '00) united at the ends. This secondary split marks the line of separation of the chromatids in the subsequent equational or homotypic division, i.e., the second maturation division. In each chromosome this second split is apparently at right angles to the primary split. Coincident with the appearance of the secondary split is a process of shortening and thickening of the diffuse, thread-like tetrads. This shortening and condensation of the chromosomes marks the end of the diplotene (figs. 16 and 17).

Following the stage just described, there occurs a series of transition stages leading up to the complete formation of the heterotypic tetrads on the mitotic figure. These stages are known by various names, but for present purposes Häcker's ('95) term 'diakinesis' will be employed as including that period in the germ-cell cycle, from the first formation of the tetrads to their definitive arrangement upon the first maturation spindle.

Diakinesis—formation of the tetrads

The ring tetrads, so characteristic of the Amphibia, are formed by the disjunction of the homologous chromosomes that paired during the amphitene and pachytene and separated during the diplotene, except at their ends which remained in contact (figs. 14, 16, 41 to 51). Thus, in the writer's opinion, the annular space represents, in Rana catesbeiana, the space between homologous chromosomes. In other words the space between the rings represents the 'primary longitudinal split' and probably the original line of fusion in parasynapsis of the autosome pairs. The first maturation division in the bullfrog larva is heterotypic or reductional for most of the tetrads in the sense that entire chromosomes are separated. This conception has been held by various workers on urodele spermatogenesis. Thus Janssens holds this view for the urodele Batracoseps and the anuran Alytes and Montgomery for Plethodon cinereus and Desmognathus fuscus, though the latter writer arrived at this conclusion by assuming telosynapsis occurs first. He interpreted the pachytene loops correctly as bivalent chromosomes, but he misinterpreted the nature of the double spireme, in considering each loop as two

univalent chromosomes united telosynaptically at the angle of the loop. According to this point of view, the space between the two arms of the loop is the space between two univalent chromosomes, but does not represent the line of fusion of originally separated leptotene threads. This view, while erroneous (admitted to be so by Montgomery himself, '12), leads to essentially the same end results as those stated by the writer.

There are fourteen typical rings and crosses plainly of tetrad nature in the spermatocytes of the bullfrog larvae and in some cells a large rod-shaped body may appear (fig. 26 to 28). The rings are of large size as compared with similar chromosome stages of adult frog material and are practically identical with those of urodeles in regard to size and shape. In the larvae these rings can be grouped according to their size relations—one very large ring (fig. 18), five intermediate in size (fig. 20), and eight smaller ones (fig. 21). The size relations of these rings in various cells is apparently constant for the species in cells of the same size, and this is an important point, for there is a variation of chromosomal size in cells of different size. The amount of volume of cytoplasm has much to do with the size of the chromosomes. Figures 29, 30, 31, and 32 bring out clearly this difference in chromosome size when two cells in identical stages but of different size are compared. The thinness of the chromosome group in figure 78 is not entirely due to stretching on the spindle. The chromatin mass varies in proportion to cellular size, i.e., the larger the cell the larger the chromosomes, and vice versa. Conklin ('12) has clearly shown and discussed this point also in numerous other papers. The extremely large cells shown in plates 7 to 14 have relatively large tetrads, conversely the smaller cells figured in plate 15 have much smaller tetrads. The photographs of plates 7 to 14 are of larval cells, those of plate 15 of the germ cells of animals at the time of metamorphosis.

Ring tetrads in amphibia have been described by several previous writers, so only a brief discussion is needed here. Following the separation save at the synaptic ends in the diplotene of the paired elements, and the appearance of the secondary split, the rings open in two planes at right angles to each other: 1) In the

center probably along the original line of fusion of the homologues; 2) along the line of the secondary split after the fashion described by Robertson ('14) and Wenrich ('16) for the Orthoptera. Condensation of the chromatin begins at this stage. In early stages of ring formation the tetrads stain rather lightly and are somewhat (fig. 17) diffuse, but as condensation proceeds they readily take up the basic dyes. The tetrad character is obvious from a study of the synaptic ends of the homologues. The larger rings in middle prophase stages are generally in the form of figure 8's, and this character may be maintained up to the metaphase. The smaller tetrads early assume the character of the rings; other shapes, such as crosses and y's, and in some cells a rod, appear. There is usually a single Y and a single cross-shaped tetrad in every spermatocyte, though these may appear much like small rings. Other shapes that appear are transitory stages in ring formation, or else portions of rings viewed from various angles. Large crosses, for instance, sometimes appear in early prophase, and are generally true rings viewed 'en face,' the arms of the cross being the long synaptic ends of the paired chromosomes. Such crosses are not comparable to or to be confused with true cross-shaped tetrads (figs. 19).

In sections slightly overstained, the smaller rings appear solid, the synaptic ends being represented merely by rounded knobs. At times such rings may even appear like dumbbells, and this is notably true of the second-year spermatocytes, i.e., those that give rise to true spermatozoa at the second ripening of the germ cells of the larvae (figs. 120 to 128). Indeed, it is not improbable that those investigators of anuran spermatogenesis who have described prophase tetrads as solid and of dumbbell shape were perhaps dealing with either overstained or imperfectly fixed material.

Spindle-fiber attachment is non-terminal usually, but may occur anywhere in spermatocytes of the first-year larvae showing centrosomal fragmentation. There are no normal spermatocytes in the first maturation cycle, so any discussion of spindle-fiber attachment is useless (figs. 29, 31, and 32).

During late stages of diakinesis the cells in many cases become greatly enlarged and in many instances are of giant proportions (figs. 64, 67, 112). The increase in volume may affect either nucleus or cytoplasm or both. It is rather common in my material to find over one-half or two-thirds of a nucleus in a single section because of the size, and it may be added that my material was sectioned at a thickness of 8 to 10 μ. These large spermatocytes of the larvae resemble those of urodeles more than adult anurans. It is an interesting and suggestive fact that near the period of metamorphosis the elongated, more or less ribbon-like testis becomes transformed into a very small typically shaped frog testis. The shortening process may require a considerable time, though the writer is inclined to doubt this on account of the absence of transition stages. The shortening progresses from posterior to anterior and may amount to as much as 1 mm. Figure 35 is a section through gland from a newly metamorphosed animal; figure 34 a gland of the second season (before metamorphosis); figure 33 a section of a gland of a first season larva.

Examination of the small, fully formed testes (full formed except for the efferent or rete apparatus) of recently metamorphosed animals reveals some interesting size differences of the cells compared with those of the gonads of young larvae of the first season. The cellular elements of the small gonads are more nearly like those of the adult, and it is rare to find the giant spermatocytes of the type figured in plates 7 to 13. The primary spermatogonia are of about equal size with those of the younger tadpoles, the chief differences are in the spermatocytes and diplotene and pachytene nuclei. These small testes first appear in tadpoles measuring 120 mm. or more from snout to tip of tail. Such animals are about a year and a half old or perhaps somewhat younger and are due to metamorphose the following summer. The hind legs are on the average about 25 to 30 mm.; the fore limbs are not visible. Now, oddly enough, some of the male animals are mature, in so far as the possession of ripe spermatozoa is concerned. And, as we shall shortly see, this character marks off this type of gland from those of the first-season larvae. As was stated before, the bullfrog tadpole passes approximately two

years as a larva, and each year is marked by a seasonal ripening of sexual products. Attempts of first-year tadpoles to ripen their sex products is abortive; the second year's attempt is successful (plate 15) at the time of metamorphosis.

The heterotypic mitosis of first-year larval spermatocytes

First maturation division metaphases are very abundant in first-year larvae, especially in young larvae 45 to 60 mm. total length which have passed through one winter as larvae. In such animals entire cysts are found with completely formed tetrads and spindles all ready for division, and many in the act of dividing; yet, oddly enough, careful examination of many hundreds of sections of such larval gonads fails to show stages of the first maturation mitosis beyond very early anaphases (plates 9, 10, 11). This is an interesting fact, and it is strange to see entire cysts of apparently normal spermatocytes in metaphase or early anaphase, and yet never find telophases of such divisions, interkinesis stages, or any indications of secondary spermatocytes. Cells which have developed thus far in a perfectly normal manner, save for precocity of the maturation cycle and are apparently in possession of the requisite mechanism for cell division, are unable to complete the process. During late prophase and metaphase the achromatic elements, that is, the machinery of cell division and chromosomal separation, break down and the tetrads go to pieces before the telophase. It is rare to find complete separation of the homologous components of the tetrads. Sometimes the smaller ring elements do separate (figs. 29 and 30) and in rare cases an early anaphase is reached. In general the degenerative processes set in shortly after the time of spindle formation when the chromosomes are arranged in a typical metaphase plate, their long axis parallel to the long axis of the spindle, or else when they are scattered irregularly through the cytoplasm, following the disappearance of the nuclear wall (figs. 100 to 110). The spermatocytes may even go to pieces in late diakinesis, though such cases are not common.

The obvious cause of degeneration of the spermatocytes of first-season larvae is to be found in the abnormal behavior of the centrosomes. Very early in the work it was observed that multipolar mitotic figures were exceedingly frequent, and, indeed, these came to be regarded as the rule rather than the exception in the maturation cycle of first-year tadpoles. Triasters and tetrasters with striking and bizarre chromosomal arrangement proved so common that attention was focused upon the centrosome as the primary seat of degenerative processes. It may be added here that these aberrant polyasters are very favorable objects of study in Rana catesbeiana larvae because of their size and number and should prove of interest to anyone concerned with cellular mechanics.

Fragmentation of the centrosome of first-year larval spermatocytes

A study of the centrosome of the first-season spermatocytes proved very fruitful in several respects: 1) it gave the clue to correct interpretation of the anomalous behavior, i.e., the failure to divide of the spermatocytes; 2) the results of such study explained on sound mechanical grounds the presence of the polyasters; 3) it led to the discovery of certain giant spermatid-like structures.

In most, if not all, of the first-season larval spermatocytes, the centrosome behaves abnormally, rarely does it pass through the normal cycle and give rise to a typical bipolar spindle. The usual thing is fragmentation of one or both halves of the divided centrosome; figures 18, 21, 79 show such fragmentation. There may be a central granule, surrounded by four or five others, all connected to one another by very delicate filaments. Each of these granules may or may not form a tiny aster in the cytoplasm. In other cells a typical spindle may be formed at one pole with numerous smaller spindles at the other pole. There are several variations of this type (figs. 18, 21, 79).

Perhaps the most peculiar condition noted in the centrosomal behavior of the spermatocytes was the tendency to form axial filaments or tails. It required much searching to find anything

like 'tailed cells,' and in the writer's experience they occur rather infrequently, although on theoretical grounds one would expect to find them numerous. In 'tailed cells' the centrosome, instead of fragmenting or forming a multipolar spindle, sends out a long, somewhat spiral filament that grows outside the cell like the axial fiber of a spermatid. These filaments are extremely delicate structures and difficult to make out. The stage at which these axial fibers grow out from the centrosome may vary somewhat. In figure 23 the filament had evidently formed during late diakinesis, as there is a nuclear wall present. Figure 22 shows the fiber extending out from the periphery of the nucleus—an unusual condition. Some of these figures correspond to, in fact are practically identical with, Broman's ('00) drawings of giant spermatids in adult Bombinator ingenus material. Compare my figures 22 and 23 with his figures.

The type of cell represented in these figures is very abundant in larval material, especially following the period of greatest abundance of aberrant spermatocyte divisions. Not all such cells show axial filaments, indeed, they are rare. Such cells with filaments growing from the centrosome may be regarded as giant spermatids resulting directly from transformed first spermatocytes which have not undergone either first or second maturation division. Comparison of the stages figured in plate 13 brings out this point clearly. The same type of cell but without axial filaments is quite abundant; these originate in the same manner as described above, but cannot be spoken of as spermatids in the absence of the axial fibers. Broman has observed several filaments growing out from a single cell in his adult toad material; so far such cases have not appeared in my material, and it has been a source of some wonder on my part why such cells are not of greater frequency. Cellular conditions in the first-season larvae are ideal for the development of such structures in abundance. The relative infrequency of the tailed cell may perhaps be correlated with the fragmentation of the centrosome.

The giant spermatids are non-functional and usually undergo no further metamorphosis, but degenerate and are resorbed. Stages in the process are shown in figures 103 to 110. In very

rare cases these abnormal spermatid-like bodies apparently give rise by condensation of the nuclear material and elongation of the cytoplasm to structures bearing a faint resemblance to the apyrene spermatozoa of certain prosobranchs.

The degeneration of the first-season spermatocytes at the metaphase is somewhat analogous to the degeneration of that type of ova that requires the stimulation of a spermatozoon to enable it to complete the developmental cycle. Mead ('98) and Conklin ('05) both observed ova with perfectly formed spindle and chromosomes go to pieces at this stage without further development unless fertilized. In the case of the larval bullfrog spermatocytes, fragmentation of the centrosome is the immediate cause of the failure of the cells to divide and of the resulting degeneration. Professor Conklin has suggested that perhaps the non-fertilized ova observed by him also go to pieces because of centrosomal fragmentation. It is known that the entering spermatozoan brings in a centrosome which takes part in the cleavage process. In regard to the abortive maturation cycle of the first-year bullfrog larvae it is possible that it is a vestige of an early reproductive cycle inherited from remote ancestors. Centrosomal fragmentation is merely the more obvious morphological cause of the degeneration of the spermatocytes and itself a symptom of a deeper-seated derangement of cellular life.

Cytoplasmic and nuclear changes in the degenerating spermatocytes of first-year larvae

The nuclear changes are more or les characteristic of degenerating cells in general, including those just described as ultimately destined to form the spermatid-like bodies. The initial stage in degeneration apparently first affects the centrosome, the chromosomes and cytoplasm are later attacked. To take a typical example of degeneration in a spermatocyte (omitting those polyasters where the chromosomes are pulled to pieces), there is first fragmentation of the centrosome and formation of polyasters, followed by shortening and thickening of the tetrads accompanied by increased staining capacity. The chromosomes soon lose the ring-tetrad structure, and the annular space entirely dis-

appears as though there had occurred a running together of the chromosomes. The lugs or knobs marking the synaptic ends of the chromosomes round off and disappear, leaving an oval-shaped shiny mass, resembling a heavily stained oil drop. Such masses tend to run together, forming larger units, until in final stages of the process the original fourteen tetrads are represented by three or four large deeply staining spherical masses (fig. 22 and 23). During this time the nuclear wall may or may not have disappeared, depending upon the age of the spermatocyte when degeneration began. Where this process sets in after spindle formation there is no nuclear wall. In cases where the nuclear membrane is present, the entire nucleus is excentric in position (fig. 106). In those cases where degeneration begins after the complete formation of the mitotic figures, and after the tetrads are arranged on it, the history of the degeneration is slightly different from that just described. The essential difference is that there is no nuclear wall present, the spindle apparatus is resorbed into the surrounding cytoplasm, and the tetrads go to pieces in situ. The latter take on the appearance of oily masses which may or may not fuse together. In final stages, all that remains of the fourteen tetrads and mitotic figure is a group of oily vacuoles grouped together much in the same fashion as the chromosomes were grouped on the spindle. In many instances the number, and even the size relations of the vacuoles corresponds to the first-maturation chromosomes (figs. 109 and 110).

The cytoplasmic changes accompanying these regressive nuclear pnenomena are interesting. The normal, clear, lightly staining protoplasm becomes yellow in color, much vacuolated, and numerous spherical droplets of yolk-like substance appear. In this connection it is interesting to note that an essentially similar yolk-like material has been described and figured by practically all workers on apyrene spermatozoa. For instance, Gould's ('18) description and figures for Crepidula plana and Reinke's ('12) figures of Strombus. However, as the substance in question occurs in degenerate and functionless cells in all cases, the presence of similar degeneration by-products is to be expected and has no special significance. The writer doubts if this substance is yolk, though it does resemble it.

Maturation cycle of second-year larvae and formation of functional spermatozoa

Much that should more properly have been discussed in this section has been referred to here and there earlier in this paper in order to clear up certain sources of confusion which might arise.

The second-year larval sexual cycle differs from that of the first year in two ways: 1) The germ cells of the second maturation cycle are considerably smaller, the tetrads are consequently much smaller than those of the first maturation cycle, and have less the appearance of rings than of dumbbell-shaped bodies when attached to the first maturation spindle; 2) mature spermatozoa are produced, there is little cell degeneration, but few polyasters occur, and hence few cases of fragmentation of the centrosome. All maturation divisions are normal. It is obvious that there is a vast difference between the first and second maturation cycles of the male larvae; the first is aberrant, the second normal; one culminates in degeneration, the other in the production of functional male sex cells.

The smaller size of the germ cells of second-year larvae is not difficult to explain. During the period of the first sexual ripening practically all of the germ cells in the gonads are affected, and consequently destined to degenerate and disappear. There are, however, a few primary spermatogonia with polymorphic nuclei, lineal descendants of the primordial germ cells, scattered here and there through the gonad, which fail to undergo the precocious maturation cycle. These cells are generally, though not always, found near the sex cord region. In the interval between the first and second larval sexual cycles these cells apparently divide rapidly and spread through the gonads. It is probably the repeated division of these cells, and their progeny that brings about the marked reduction in cell size, so noticeable at the second maturation cycle. The proliferation of germ cells is so extraordinarily rapid in the gonads of tadpoles just about to metamorphose that the cellular size becomes reduced to a size scarcely larger than that characteristic of the larger stroma or peritoneal cells. Indeed, conditions are such in

the gonads at this time, and especially in certain individuals, that a great many of the definitive sex cells appear to arise by an actual transformation of mesothelial elements into germ cells. This question is still under investigation, for it is exceedingly difficult to determine definitely whether this is or is not the case from morphological data alone. Certainly in my material there is very suggestive morphological evidence that such transformations may possibly occur, but whether such transformations actually do occur is an entirely different thing.

Another factor to be considered in regard to the reduction in cell size of the first-year germ cells is the fact that the entire gonad undergoes a striking diminution in size during the second year of growth, taking on the character of the adult testis. There is a great loss of water from the tissues at the time of metamorphosis and consequent shrinkage of the cells of the animal in volume.

The size of the chromosomes depends upon the volume of the surrounding cytoplasm and of the nucleus, hence the smaller size of the second maturation cycle tetrads. These tetrads are of the short dumpy type found normally in adult frogs and toads (figs. 120 to 128).

The interesting fact that the sex products of the first larval sexual ripening are all abortive, while those of the second larval cycle are normal is something of a puzzle, and the only explanation occurring to the writer is based upon the phylogenetic history of Anura and will be discussed later in this paper along with some data of a somewhat similar nature regarding mammals and birds.

There is one type of cell in the gonads of second-year larvae and metamorphosed frogs that may remain about equal in size to the germ cells of the younger larvae—the primary spermatogonia. This type of cell is large in frogs and larvae of any age.

During the month of August, 1919, several very large tadpoles were captured with a total length of 140 to 160 mm. Examination of the testes of male individuals showed many normal spermatocyte divisions, spermatids in all stages of development, and a few mature spermatozoa. At this time the efferent ducts of the testis were not yet fully developed. The gonads were extremely small and immature looking. Female gonads of larvae of similar size showed only oöcytes undergoing growth.

The age of these animals could only be estimated by their size and developmental stage, and were probably about two years old. Some of the tadpoles would have undergone metamorphosis within a short time but for the lateness of the season. The writer has known for several years that young male bullfrogs shortly after metamorphosis are sexually mature, though in regard to size they are pygmies compared to the adults and it is difficult to see how they could possibly copulate with mature females. The female Rana catesbeiana apparently does not become sexually mature and ready for copulation until several years after metamorphosis, and in this respect resembles the European frogs, such as Rana esculenta. According to the observations of R. Hertwig, Witschi, and others, the female of Rana temporaria and Rana esculenta does not become fully mature and ready for copulation until the fifth season after metamorphosis. Despite the fact that the females are sexually immature, the young male bullfrog apparently ripens his sexual products continuously, beginning with the first year of larval life, though the first-year sexual cycle is abortive.

The writer has observed somewhat similar phenomena in the leopard frog, Rana pipiens. It is not uncommon to find very small immature looking individuals of this species with sperm in their testes. It has been known for several years that prolongation of the larval life of tadpoles of this species by thyroid extirpation does not prevent the normal seasonal ripening of their sex products. Such ripening corresponds to the second larval sexual cycle in Rana catesbeiana, for it is probable that the tadpoles of this species (i.e., Rana pipiens) undergo a very precocious and abbreviated maturation cycle very early in larval life. This early cycle in Rana pipiens would correspond to the first sexual cycle of Rana catesbeiana; the prolonging of the larval life of the leopard-frog tadpoles leads to the second seasonal ripening and production of normal spermatozoa just as in the bullfrog, though in the latter species the period of larval existence covers the second seasonal ripening of the germ cells.

The germ-cell cycle of the second year in Rana catesbeiana larvae, as was previously stated, is normal in every way, hence no description will be given of the process here. The secondary

spermatocytes give rise to normal spermatids and some spermatozoa, and the writer has nothing to add to this phase of the subject that has not been described many times before in papers concerned with the spermatogenesis of other amphibians. Unquestionaly, these larval sperm cells are functional, because morphologically they are indistinguishable from spermatozoa of adult frogs (figs. 118 to 131).

DISCUSSION OF OBSERVATIONS

1. Amitosis in anurans

It has frequently been stated by earlier investigators working with amphibian material that amitosis occurs quite commonly in the testis cells of urodeles and anurans. Several writers have even asserted that at certain seasons of the year amitosis is the sole method of division (La Valete St. George, Meves, Benda and McGregor). It has even been seriously stated that the primary spermatogonia not only divide amitotically, but the results of such direct division become functional spermatozoa. Meves and Benda state that amitosis occurs by means of the constrictive force of a ring-shaped centrosome in Salamandra. McGregor states that the nucleus is divided by a cleft into two approximately equal parts. Oddly enough, in view of these positive statements, the writer has never observed anything in primary or secondary spermatogonia or in follicle or stroma cells that is comparable in any way to amitosis. The polymorphic nuclei of the spermatogonia do somewhat superficially seem to be constructed into two halves at times, and the constriction may be deep enough to give (fig. 1) the appearance of separate nuclei in the same cell. Careful study reveals connecting portions lying at deeper levels. It is evident from descriptions of amitosis in amphibians that polymorphism of the nucleus has been mistaken for direct division. The writer takes the position, perhaps extreme, that in the spermatogenesis of anura, amitosis does not normally occur, and if it ever occurs in these forms, it is an extremely rare and aberrant condition, save in senescent cells such as those of Bidder's organ, and even in this degenerate structure direct division is uncommon.

2. The polymorphic nuclei of amphibians

Practically all investigators of the germ cells of urodeles and anurans have described and figured the bizarre and striking lobulation of the spermatogonial nuclei of these forms, but few have attempted any explanation of the peculiarity. Most of the earlier investigators regarded the nuclear polymorphism as stages in amitotic division. The writer has observed, however, that the striking nuclear lobulations of the spermatogonial nuclei in Rana catesbeiana larvae, are nothing more or less than chromosomal vesicles and fusions of such vesicles. In reality, the polymorphism of nuclear structure is due to these vesicles, remaining more or less distinct and independent from one another. That this view is essentially correct is readily seen by examination of early prophase and late telophase spermatogonial divisions. In early prophases the individual chromosomes arise by condensation of the chromatin material of the vesicles. Later telophases clearly show the formation of the vesicles which increase greatly in size as the cell grows.

Conklin ('02) has called attention to the occurrence of such chromosomal vesicles in Crepidula. He states in this regard:

> 1. The chromosomes, consisting of chromatin enclosed in a linin sheath, divide and move to the poles of the spindle, where they partially surround the spheres. 2. Here they become vesicular, the interior of the vesicle becoming achromatic, though frequently containing a nucleolus-like body, while the wall remains chromatic. 3. These vesicles continue to enlarge and then unite into the 'resting' nucleus. The nuclear membrane is composed of the outermost walls of the vesicles, while the inner walls stretch through the nucleus as achromatic partitions.

Again on page 47 of his ('02) communication, Conklin writes:

> It sometimes happens, especially in eggs in which more than the normal number of centrosomes and asters are present, that some or all of the chromosomal vesicles do not fuse, but remain distinct through the whole of the resting period. In such cases each of the vesicles behaves like a miniature nucleus, absorbing achromatic material and forming a net-work of chromatin either within the vesicle or on its walls. In this growth and differentiation the vesicles keep pace, step by step with the normal nucleus, so that one must regard the resting nucleus as virtually composed of vesicles, though their union may be so intimate as to hide this structure.

The resting nucleus is not, therefore, a single structure any more than is the equatorial plate. It is composed of units, each of which, so far as known, has the properties of the entire nucleus, and the union of these vesicles into a single one may be considered as a secondary character. It is altogether probable that the chromosomes, and hence the chromosomal vesicles preserve their identity throughout the resting period, and I venture the suggestion that the daughter chromosomes will be found to arise within the chromosomal vesicles.

The description just quoted, of the formation and behavior of chromosomal vesicles in gasteropod molluscs, applies equally as well to the conditions in the bullfrog larva, and certainly cannot be better stated than Professor Conklin's description (figs. 1, 2, 3).

Häcker ('95) reported that the chromosomes of the early cleavages of Cyclops brevicornis formed two groups of vesicles, one group from the paternal, the other from the maternal pronuclei.

More recently, Wenrich ('16) has reported that each chromasome in Phrynotettix becomes surrounded, as early as the anaphase, by a hyaline region; that this region expands in the telophase; that the chromatin of each chromosome becomes diffused within its own region; that a membrane becomes formed at the boundary between the hyaline region and the cytoplasm, producing the chromosomal vesicle. The nuclear membrane consists of the outer walls of the vesicles at the periphery of the nuclear group. Wenrich concludes that the hyaline region is formed at the expense of the cytoplasm and that the material of each chromosome tends to remain within the space of its own vesicle, a core of chromatin being particularly noticeable in the center of this region, and that the prophase chromosome subsequently formed, is developed out of the substance of one, and only one, of the previously existing telophase chromosomes.

Conditions in Rana catesbeiana larvae, while not so clearly marked, in regard to individual chromosome vesicles, as those described for Orthopteran material, nevertheless strongly indicate that the 'polymorphic' nucleus of amphibians is nothing other than a group of large chromosomal vesicles, more or less independent, the outer walls of the outermost vesicles forming the nuclear membrane.

Some of these vesicles are of exceedingly large size in the primary spermatogonia, and represent possibly, two or more individual vesicles pressed so tightly together as to appear as a single vesicle. At times the individuality of the larger number of vesicles is obscured or may disappear altogether, not to appear again until the early prophase of the succeeding division when the chromosomes reform within the vesicles. Figures 1 and 2 give a good idea of the enormous size attained by these vesicles in certain cells. The combined size of the vesicles is so great that the nucleus completely fills the cytoplasm except for a narrow peripheral border.

These chromosomal vesicles are the means by which the individuality of the chromosomes is maintained from cell generation to cell generation. During the so-called 'resting' stages of the cell, when the chromosomes appear to have lost their identity, and merged with the other elements of the nucleoplasm, they are in reality diffused within little sacs or vesicles, and probably thus remain entirely separated from one another throughout this diffuse period. No one nowadays seriously maintains that chromosomes maintain a strict morphological identity, i.e., appearance, throughout all stages of cell life, but that they do maintain a genetic continuity or individuality throughout 'resting' stages of cellular life by means of chromosomal vesicles cannot be seriously questioned.

The chromosomes that arise from chromosomal vesicles during prophase stages of mitosis are the same chromosomes that went into them during the telophase of the preceding division. The writer can speak only for the conditions presented by the frog, but the accounts in the literature indicate that this statement probably holds true for a great many forms, possibly all. The fact that such chromosomal vesicles have not been found in certain groups, as for instance, coelenterates, according to G. T. Hargitt ('20), is no sure indication they do not exist in that group.

3. Synapsis

This question needs little discussion here, considering the beautiful work of Janssens ('05), Janssens and Willems ('08), the Schreiners ('06), and Snook and Long ('14) on various amphibian types. The conditions in Rana catesbeiana larvae are essentially similar to those described by these works for the urodeles.

Some of the earliest observations on the problems of synapsis were made on amphibian material, chiefly urodeles, and an end to end conjugation of chromosomes or telosnapsis was generally conceded to occur. Janssens ('05), working with Batracoseps, demonstrated parasynapsis, and later, working in collaboration with Willems, showed this to be true of the anuran Alytes. Wilson ('12) after a study of Janssen's material, agrees with this author regarding parasynapsis in Batracoseps. Montgomery ('11) who previously ('03) had described telosynapsis in urodeles, reversed his earlier opinion and states (p. 753): "During the past year I have also convinced myself of the occurrence of parasyndesis in Plethodon, such as Janssens had described for this object and the Schreiners for Salamandra." Snook and Long ('14) describe the same kind of evidence for parasynapsis in Aneides lugubris as that presented by Janssens and the Schreiners.

It is interesting to note that King ('07) denies the existence of parasynapsis for Bufo lentiginosus, and states that telosynapsis is the method of chromosome pairing in this form. King regards the period of synapsis in Bufo as occurring coincidently with synizesis—a condition now generally regarded as an artifact, at any rate in Amphibia. The tetrad chromosomes of the first maturation mitosis, King thinks, arise by transverse segmentation of the thick spireme. If this description of conditions in the toad is correct, then this form differs markedly from other amphibians. Recently, however, the writer has had an opportunity of examining preparations of Bufo, and is convinced that parasynapsis occurs in this animal as the normal method of chromosome conjugation. Amphitene stages are abundant in the material examined by me, and this is the true period of synapsis in anurans (figs. 11, 12, 13, 36 to 38).

The twisting together of the leptotene threads to form the double pachytene spireme which occurs during the amphitene in anura (fig. 12) seems to the writer to be the period when the mechanical conditions for the 'chiasma-type' theory of Janssens are present, and not during the later stage figured by this writer. This theory of 'Chiasma-type' has been extensively employed by Professor Morgan and his co-workers to explain 'crossing-over' in Drosophila. It has repeatedly been observed that genetic factors belonging to a certain group, and presumably carried by a single chromosome, go into a mating together, but do not always reappear together, as they should, if carried by a single chromosome that has maintained its individuality throughout. Janssens endeavored to explain the anomalous genetic behavior of such factors on mechanical grounds, i.e., by showing that in the behavior of the chromosomes, at certain stages in the maturation cycle, it is possible for actual 'crossing-over' of parts of homologous chromosomes to occur, and this exchange of parts of chromosomes he termed 'Chiasmatype.' This theory is based upon a study of certain postspireme (strepsinema) stages in the spermatogenesis of the urodele Batracoseps. In this form, after the secondary longitudinal split (equational split) has taken place, the tetrads are composed of four separate strands or chromatids. These strands may cross each other at certain places, and, owing to strains or weakness at the point of contact, break, subsequently recombining in such a manner as to form threads composed of parts of both original strands. That is to say, parts of the two strands 'crossed over' and became incorporated as a portion of the opposite chromatid. Janssens has carefully figured many such apparent 'cross-overs' in the postspireme stages of Batracoseps.

There can be no reasonable doubt of the accuracy of the genetic evidence for 'crossing-over,' nor of the general truth involved in the chiasma-type theory. The point to be considered here is whether or not the cytological evidence for this view is not more convincing if a stage in the maturation cycle of the chromosomes is used as the basis of cross-over, earlier than the early tetrad state employed by Janssens. The mechanical conditions furnished by the amphitene period in the bullfrog, for crossing over of

parts of homologous chromosomes is well-nigh perfect, certainly more so than the later stages. In the first place, the side-by-side pairing of the two leptotene threads is accomplished by a process of twisting together, and not merely by a side-by-side union; secondly, the twisting of the homologous threads is so tight that all trace of their double nature is lost and the two elements appear as one. It is only later at the time of separation during the diplotene that the dual character of the pachytene thread again becomes apparent. It would be odd if during the process of separation of the tightly twisted threads 'crossing-over' did not sometimes occur (figs. 11, 12, 13, 36 to 38).

Aside, however, from the ideal conditions presented by the amphitene stage for exchange of parts of chromosomes, the period figured by Janssens as furnishing actual cytological evidence of such exchange is not entirely satisfactory. The chief objection here is that study of the postspireme chromosomes of the maturation cycle in the larval bullfrog fails to support the contention that breaking and recombination of the chromatids occur at this particular period. The strepsinema stages of the bullfrog tadpole are very similar to those figured by Janssens, but the writer is of the opinion that crossing-over does not occur here; at any rate, no good evidence for it has been observed. The tetrads are of the non-cross-over type like those figured by Wenrich ('16) and Robertson ('14) for grasshoppers.

Strepsinema stages of tetrad formation are perfectly definite and characteristic periods in the maturation cycle, and so far as my$_7$material is concerned, the chromatids appear to preserve their identity through this period (figs. 16, 40 to 63).

There is certain genetical evidence indicative of chromosomal 'crossing-over' during the early synaptic stages of the oöcytes of Drosophila, such, for instance, as Plough's ('17) experiments. He found that environmental changes such as low or high temperature markedly increased the percentage of 'cross-overs' in the second chromosome of Drosophila melanogaster (ampelophila). The temperature apparently increased the amount of 'crossing-over' at a definite stage of oogenesis, and Plough's evidence suggested strongly that the chromosomal exchange takes

place at the stage when the chromosomes of Drosophila are known to be finely drawn-out threads. In other words, he localizes the period of 'crossing-over' in the stage of oogenesis when twisting together of the homologous threads is possible.

It matters little, in so far as the validity of the genetical evidence is concerned, at exactly what stage in the germ-cell cycle 'crossing-over' may take place, for that such a process does occur can scarcely be denied in view of the mass of positive evidence. It is not impossible that the phenomenon may take place at several different stages.

It is an odd fact that 'crossing-over' of genetic factors apparently does not occur in the male Drosophila, but is confined solely to the female. Oddly enough, the chiasmatype theory invoked to explain it is based upon conditions observed in male Amphibia. So far no one has advanced a satisfactory explanation to account for the apparent absence of this phenomenon in the male Drosophila. Nabours has reported evidence for 'crossing-over' in the males of grouse locusts, Castle for the male rat, hence it is evident that it is not confined solely to females.

4. The chromoplasts: (?) karyosomes

Regarding the true nature of these bodies and their relation to the chromosomes, the writer is in doubt, Janssens ('05) who has made a careful and detailed study of the origin and fate of these structures in Batracoseps states:

> Que le chromoplaste prend naissance aux derniers télophases spermatogoniales et qu'il résulte d'un empâtement dû an dépôt d'une substance sidérophile entre les pointes des V chromosomes aux pôles de la figure.
>
> Qu' à mesure que le chromosome se remplit de substance sidérophile le chromoplaste diminue de volume. Il est donc naturel de la considérer comme une substance destinée à être absorbée par les chromosomes à la fin du stade auxocytaire comme il semble qu'elle à être excrétée par eux au commencement de ce stade.

This view is an interesting one; however, the writer has not paid sufficient attention to the chromoplasts and nucleoli in Rana catesbeiana to make any statement regarding the origin and fate of these structures.

The view of Eisen ('00) that the definitive chromosomes of the spermatocyte are derived from the chromoplasts and that "chromoplasts guide the formation of the chromosome just as the archosomes guide the formation of the spindles," does not seem to be entirely substantiated by conditions in the bullfrog tadpoles. In this form the chromoplasts appear to have little to do with the origin of the definite chromosomes in so far as the chromatin material is concerned, for this originates from the preexisting chromatin blocks of the last spermatogonial telophases by a spinning-out process of the leptotene threads. But it is very likely, and my own observations bear this out, that the chromoplasts do give up substance to the chromosomes, though just what the nature of this substance is the writer is unable to say. It is doubtful if the chromoplasts are composed of true basi-chromatin.

In early stages of chromosome formation, such as the preleptotene and leptotene, the chromoplasts are usually large heavily staining bodies, to which are attached several chromatin threads. As development of the threads proceed, the chromoplasts become smaller and take the stain with less avidity. In still later stages they become vacuolated as if being drained of their contents by the growing threads. In final stages these bodies disappear.

5. Significance of the maturation cycle in the larvae

It is possible that the precocious, seasonal ripening of the male germ cells of larval bullfrog represents a recapitulation in ontogeny of a primitive, phylogenetic sexual cycle of ancestral forms, when the Anura were sexually mature and reproduced as larvae, much in the same fashion as does the axolotl to-day. Few biologists would hesitate nowadays to deny that the latter is not merely a neotenous, gigantic, sexually mature larva of the urodele Amblystoma tigrinum, in view of the work of Chauvin ('75) and Duméril ('65). The question why this animal sometimes fails to undergo metamorphosis in certain districts does not concern us here (Swingle, '19). Besides the axolotl there are numerous other instances on record of neotenous, sexually mature amphibians that have failed to metamorphose at the proper time. So far as the writer is aware, such individuals are confined

to the urodeles, with the exception of the bullfrog larvae described here. In other anurans permanent retention of larval characters may be experimentally produced by prolonging the larval life by thyroid extirpation; the retention of the larval somatic characters has no effect upon the germ cells. Similar results were obtained by the writer (Swingle, '17–'18), where it was shown that acceleration of metamorphosis by thyroid feeding does not accelerate the normal course of events in the germ-cell cycle.

It appears possible that the precocious seasonal ripening of the male tadpoles germ cells is a recapitulation, just as the tadpole soma is possibly a recapitulation of an earlier phylogenetic stage when the present-day Anura were more like the Urodela than they are at present, both in regard to body form and sexual conditions. It would be interesting to know whether or not larval urodeles show any such precocious sexuality as described here for anurans. It is not improbable that other vertebrates with larval periods of development, such as some of the eels and petromyzonts, will be found to present analogous conditions to those described for the bullfrog larva. In fact, judging by certain facts to be presented hereafter, it seems likely that all the vertebrates present some such precocity of the germ-cell cycle as described here. If the phenomena described here are in any way rooted in past phylogenetic conditions, it is a much more remote past than anything represented by any living Urodele type.

6. Is there a precocious sexual cycle in other anurans?

This question must be answered at once in the affirmative.

In Rana catesbeiana the larval period is the longest of any other anuran known, and, as a consequence, the precocious sexual cycle of the tadpole is carried farther than in other frogs. There is no question but that if other anurans presented conditions in their germ-cell cycle as marked and unmistakable as those in the bullfrog, such conditions would have been reported years ago. One could not easily overlook cysts of spermatocytes in which the tetrads are of sufficient size to permit counting with a one-sixth Leitz objective and a no. 5 ocular. Although conditions in other frog species are not so plain and easy of interpretation as those presented by the bullfrog larvae, yet nevertheless such species

apparently show essentially identical phenomena as described by myself. The trouble heretofore has been one of interpretation. In Rana temporaria and Rana esculenta tadpoles the same precocity of the sexual cycle, as is presented by the bullfrog has been described many times by various investigators of these European frogs, but has been interpreted in a manner entirely different from the explanation here given. The figures and descriptions of Bouin, Kuschakewitsch, Witschi, Schmidt-Marcel, and others on the history and development of the germ cells of these frogs plainly indicate the precocious maturation process in the larvae. However, the ripening of the tadpole germ cells of the species studied goes only up to and including the pachytene, according to their figures. These writers probably misinterpreted the sexual conditions, and this has led to some bizarre theories of sex differentiation in frog larvae. According to the interpretation of this school, all larvae whose germ cells presented auxospireme, i.e., leptotene and pachytene maturation stages, are to be regarded as females, because it is only female animals that show such maturation changes in larval or embryonic life. Similar maturation stages of male sex cells do not occur until near the period of sexual maturity, according to them. In the anurans studied by these writers the precocity of the sexual cycle is very marked, and the germ cells do not go beyond the pachytene and form tetrads and first-maturation spindles as normally occurs in Rana catesbeiana. Consequently they were not aware that the male larvae exhibits a precocious maturation cycle coincident with that of the females, when the cells of the latter go through the early stages of oöcyte formation. As a consequence of this developmental peculiarity, i.e., curtailment of the maturation cycle to the early stages of the process, without exception these writers, being unable to differentiate male from female, concluded that all frog tadpoles first develop as females, then later half of the female tadpoles must transform into males, because the sex ratio of adult frogs is approximately 50–50.

Their conclusions were logical enough, even though probably erroneous, considering their premise that early maturation stages, leptotene and pachytene, are solely characteristic of female animals, when found in immature or embryonic forms. From a

study of the serm cells of Rana pipiens the writer arrived at essentially the same conclusions, and only after a study of the germ-cell cycle of the bullfrog was it possible to unravel the puzzle. The writer does not believe that females transform into males or vice versa, nor that tadpoles develop solely as females during early stages. A correct interpretation is possible only by comparing the bullfrog tadpole with other forms. In the germ-cell cycle of the larvae of most species of anurans, for example, forms like Rana pipiens with short periods of larval life, the precocity of the maturation cycle is apparently very marked and evanescent, hence it is more obscure and difficult to interpret than that of the bullfrog. But perhaps the most remarkable example of precocity of the larval germ-cell cycle is presented by Bufo. In the toad the early maturation phenomena of the germ cells, i.e., leptotene, amphitene, and pachytene stages, appear in extremely young larvae, about two weeks after hatching and are confined chiefly to the anterior end of the male and female gonads, i.e., that portion which develops into the organ of Bidder. The ripening process does not go beyond the pachytene stage apparently before the cells become senescent. The question whether or not Bidder's organ is or is not a rudimentary hermaphrodite gland is reserved for discussion in another paper. A very brief and curtailed sketch of the writer's view of this structure in the toad and the so-called hermaphroditism of the Anura will be found in the American Naturalist for July-August, 1920.

There appears to be some sort of correlation in anurans between length of larval life and stage in development to which the ripening of the germ cells is carried before degeneration. The whole problem of sex development and sex differentiation of anuran larvae needs reinvestigation in the light of these observations on the bullfrog tadpole.

7. Significance of degeneration of sexual elements derived from primordial germ cells

It is with considerable reluctance that the writer touches upon this particular phase of the problem in this paper, which is to be regarded as a cytological introduction to a more extensive report

later upon the entire developmental history of the male and female sex glands and cells in anurans. The problem stated in the heading of this section is of such importance as to deserve a much more detailed discussion than is possible here. However, a brief statement of the more important theoretical considerations suggested by the results obtained in the study of the larval sexual cycle of the bullfrog cannot well be avoided. There are certain obscure and little-known phenomena occurring in several other classes of vertebrates of a similar, if not identical nature with those reported here for the Anura. When the germ-cell cycle of some of the higher vertebrates is correlated with the maturation cycle of the larval frog, there is much that suggests to the writer that possibly we are here dealing with a fundamental principle in germ-cell development, of widespread, perhaps of universal occurrence among the vertebrates. Let us examine some of this evidence.

There are two important theories concerning the origin of the germ cells of vertebrates, each backed by considerable amounts of evidence not lightly to be disregarded: 1) The first view is that the definitive sex cells of the gonads are derived from primordial germ cells which have originated elsewhere in the organism, probably from entoderm, and have migrated into the genital ridges and there differentiated into oöcytes or spermatocytes as the case may be. These primordial germ cells of the embryo are distinct from other surrounding mesothelial cells and have a separate origin from cells of this type. The advocates of this view are many, and a great deal of valuable data in support of this theory has been collected of late years. 2) The second view regards the germ cells as differentiated products of the germinal epithelium, i.e., that they arise by direct transformation of sexually indifferent cells of mesodermal origin.

The advocates of the first point of view, we may, for the sake of convenience, term the 'entodermists,' though not all believers in the Keimbahn theory are agreed that the germ cells take origin from entoderm; the adherents of the second theory we shall call 'mesodermists.' Between these two groups there is little or no common ground, but instead a great deal of controversy.

The first view can be traced back to Nussbaum ('80) who claimed that germ cells are not derived from the soma, but are early differentiated segmentation products which take no part in body formation and retain their primitive embryonic character. Many facts supporting this view have come to light through study of the origin of the germ cells in almost all classes of vertebrates. There can be no question that in most vertebrates the sex cells are early set aside during development, and later migrate into the germ ridges. In the embryo frog this process is readily traced through every stage; the same is true of other forms, also, as, for instance, certain ganoids, reptiles, and birds.

The second or mesodermal view, viz., that the germ cells arise from an original sexually indifferent germinal epithelium originated with Waldeyer ('70) and has been held by a great many observers to be the true method of germ-cell origin. Oddly enough, practically every animal claimed by the 'entodermists' as illustrating their view has also been claimed by the 'mesodermists' as illustrating their theory. For instance, take the Amphibia: Allen, King, and Witschi hold the view that the primordial germ cells arise from the entoderm and give rise to the definitive sex cells; on the other hand, Semon, Bouin, Dustin, Kuschakewitsch, Champy, and Gatenby are all equally certain that the definitive sex cells of the amphibia arise from the germinal epithelium. These views are diametrically opposed, consequently both cannot be either entirely true or entirely false, and the problem is to point out if possible the source of confusion. To take the case of the frog again, in this form the primordial sex cells unquestionably arise from the entoderm. There is absolutely no indications of germ cells arising from the germinal epithelium in early larval stages. The two types of cell in the gonad, mesothelial and sexual, are entirely distinct and it would be difficult to confuse them. All increase in germ cell number is by mitotic division of preëxisting, differentiated primordial germ cells. This state of affairs persists until the tadpole is about 45 to 60 mm. total length. Thus far it is obvious that the evidence derived from the frog is decidedly in favor of the 'entodermists.' However, practically all of the germ cells derived from the ento-

derm in the male bullfrog larva undergo a precocious and abortive maturation cycle ending in degeneration and absorption. A very few descendants of the primordial line of cells fail to maturate or degenerate, and apparently give rise to a new generation of sex cells in the tadpole. Whether or not this second generation of germ cells is derived entirely from the few left-over cell descendants of the primordial line is difficult to say, for in my material at this time there is a marked increase of germ cells which looks suspiciously like an active transformation of epithelial elements into sex cells. The new germ-cell generation undergoes another maturation cycle at the time of the metamorphosis of the tadpole, or very shortly afterward, and gives rise to normal sex products. Thus it is to be seen that the advocates of the germinal epithelium theory may not be entirely mistaken, for in the light of events in the germ cycle of the Anura, it is not sufficient to trace the primordial sex cells into the genital ridges and there leave them. Without following their later history, it is unjustifiable to assume that they do give rise to the definitive sex products. This is what most workers on the origin of germ cells in vertebrates have done. On theoretical grounds the writer doubts if mesothelial cells transform into sex cells but the morphological evidence from my material does not rule out the possibility of such transformations.

The evidence presented by the precocious sexual cycle of the bullfrog leads the writer to believe that further investigation may perhaps show the existence of analogous phenomena throughout the various groups of Chordata. It may not be too much to state as a sort of working hypothesis for further investigation of germ-cell development in the vertebrates, that most of the primordial germ cells, i.e., those arising in early stages of embryonic development, undergo a precocious and abortive developmental cycle, culminating in degeneration, or else degenerate before the precocious maturation cycle has had sufficient time to manifest itself. Furthermore, that the abortive developmental phenomena are perhaps evidences of a phylogenetic regression in the germ-cell cycle to remote ancestral conditions.

The question immediately arises if such an hypothesis, no matter how tentatively stated, is in any sense justified by evidence

presented by merely a single group of vertebrates. The answer is, that scattered here and there through the literature is considerable evidence of an extremely suggestive nature, derived from study of the germ cells in widely separated groups of chordates, which, when taken in conjunction with conditions existing in anurans, suggest at once the working hypothesis stated above. Some of this evidence will be briefly reviewed here.

In 1908 Von Winiwarter and Sainmont described two proliferations of cells from the germinal epithelium of the cat ovary; the first formed the medullary, the second the cortical cords. According to their account, the germ cells and follicle cells of the first proliferation and the sex cells of the second all degenerate and disappear when the young kitten is only a few months of age. About three and a half to four months after birth, the germinal epithelium of the ovary shows marked activity, and proliferates a third generation of germ cells, from which the definitive sex cells of the adult are derived. This third generation forms the cortex of the ovary in grown animals. In a short paper published at nearly the same time as their monograph they state that the definitive ova of the sexually mature cat are derived either entirely from this third proliferation of the germinal epithelium, or else partly from it and from undifferentiated cells left over from the second proliferation, i.e., those that failed to degenerate. For example, these authors state (p. 616):

> Es tauchen nun jetzt in den Epithelhaufen und Strängen der Corticalis kleine Gruppen von Zellen auf, deren Kerne im staubförmigen oder deutobrochen Stadium sind. Diese Formen waren schon seit langer Zeit nicht mehr vorhanden, und da sie den ersten Stufen des Wachstums des Oocyten entsprechen, ist es augenscheinlich, dass sie mit einer Neubildung von Eiern zusammenhängen . . . . Wir glauben bewiesen zu haben, dass in Säugetierovarium nicht nur sämtliche Markstränge, sondern auch alle Eier und Follikel der primitiven Corticalis dem Untergang anheimfallen. Die definitiven Eier entstammen entweder von undifferenzierten Zellen der zweiten Proliferation (Pflügersche Schläuche) oder von Zellen der dritten Wucherung oder Invaginationsepitheles. Es ist uns nicht möglich, wenigstens morphologisch, die Elemente der einen und anderen zu unterscheiden.

Now, oddly enough, all of the embryonic (primordial) germ cells described by Winiwarter undergo the characteristic nuclear

changes of oöcytes, such as leptotene, pachytene, and diplotene, before undergoing degeneration.

Rubaschkin ('12) confirmed the conclusions of Von Winiwarter and Sainmont that the cells of the first and second proliferations in the cat degenerate. In the ovary of the guinea-pig this same investigator observed a third proliferation of germinal cells from the germinal epithelium which occurs before birth and which he considers the source of the definitive sex cells.

Firket ('14) using female chick material, showed that the primordial germ cells pass through the first stages of maturation previous to oöcyte formation, leptotene, pachytene, etc., enter the growth period and then degenerate. They all disappear in the chick fourteen days after hatching. The oöcytes of the cortical zone (second embryonic proliferation) practically all degenerate, although he states that he cannot be sure that they all do. There is a new formation of germ cells in the cortical region, from cells derived from the germinal epithelium, and from these the definitive oöcytes develop; but it is not improbable, at least, that a small number of the primordial germ cells are differentiated into definitive ova. One of the conclusions Firket ('14) draws from his work is of considerable interest from the standpoint of the results on the frog recorded here:

> Il faut, donc, morphologiquement parlant, considérer les gonocytes primaires des Vertébrés comme étant un rappel phyolgénique des gonocytes définitifs des classes inférieurs, notament des Cyclostomes et des Acraniens. L'epuisement gradual, dans la série phylogénique des éléments de cette lignée a nécessité l'apparition, au cours de l'ontogénèse, d'une seconde lignée de gonocytes, moins précoces (pp. 330, 331).

Recently there came to my attention an abstract of a paper as yet unpublished by this same author. I shall quote the abstract entire because of the striking similarity of the conclusions of this author, to some recorded by myself in this paper, both independently conceived:

> In the testis and the ovary of the chick there are two generations of germ cells: primary germ cells, which appear in very early stages, before the genital ridge is formed, and secondary germ cells, which are derived from the so-called 'germinal epithelium.' The former are able to become oöcytes, or spermatocytes, but while most of them

degenerate, it is not possible to determine if any of them give rise to definitive germ cells, because at a certain stage it is impossible to distinguish the former and the latter from each other. In the white rat (male) the same two generations occur, but primary germ cells degenerate before they reach the period of growth and only secondary cells become the definitive germ cells. That primary germ cells disappear, in the ontogenesis, earlier in mammals than in birds, seems to show that they must be considered as being cells in 'phylogenetic regression.'

Two interesting papers by Kingery on the female white mouse show that the phenomenon of primordial germ-cell degeneration is found in the mouse, and perhaps even more important in this connection is the fact that certain degenerating cells of this primitive germinal line may undergo abortive maturation stages even to the formation of first polar bodies.

This author ('14) in a study of the so-called parthenogenesis in the mouse found that the degenerating primordial germ cells, i.e., those of embryonic origin, undergo a degenerative fragmentation and may even form a first polar body and second polar spindle, and may even break up into fragments with or without nuclei in much the same fashion as described by me for the larval spermatocytes of the frog. It is interesting to compare the figures in Kingery's paper with those of my own in degenerating spermatocytes.

In a later paper by this same author ('17–'18), evidence of the kind described for the cat by Winiwarter and Sainmont ('08) and Rubaschkin ('12), for birds and the white rat by Firket ('14, also '20), and the male mouse by Kirkham ('16) is presented. The first or embryonic set of germ cells in the female mouse pass through early maturation stages, leptotene, pachytene, and diplotene, enter the growth period of the oöcyte, then degenerate. The second generation of germ cells arise from the germinal epithelium after birth and give rise to the definitive sex cells of the adult female. He says:

> The evidence shows that all these germ cells formed before birth degenerate and are resorbed, none of them developing into definitive ova. This degeneration takes the form of atrophy and resorption in some cases, but in others there may occur atresia folliculi; accompanied by the formation of a first polar body, and a degenerative fragmentation of the egg-cells, simulating more or less closely a parthenogenetic cleavage.

Kirkham ('16) observed that the primordial germ cells of the mouse first appear on the eleventh day after fertilization. In male embryos these primitive germ cells all degenerate, and none persist by the eighth day after birth. The definitive sex cells of the male arise from undifferentiated epithelial elements according to this account, whereas the definitive oögonia are direct descendants of the primordial germ cells. It will be seen that Kingery's account of the definitive germ cells of the female mouse agrees with Kirkham's account for the corresponding conditions in the male.

Felix states that in the human embryo the primordial germ cells degenerate (no details given) and a new generation of sex cells arises from the germinal epithelium which give origin to the definitive sex cells (Keibel and Mall, Embryology).

Now it is obvious that evidence of this sort obtained by different investigators, working on vertebrate forms as widely separated as amphibia and mammals, must be of some significance. In all vertebrates a definite Keimbahn probably exists; this is certainly true of the frog, but the important question is, do the primitive products of the keimbahn and their lineal descendants in these vertebrate forms early undergo an abortive maturation or developmental cycle which ends in degeneration such as occurs in the bullfrog tadpole? Certainly, the evidence looks suggestive. Apparently in the male bullfrog larvae this precocious sexual cycle is carried further than in any other form so far reported. The figures of Kingery for the female mouse and of Winiwarter and Sainmont for the female cat indicate plainly that the primordial germ cells are undergoing a precocious maturation cycle. These figures show every phase in the maturation cycle of normal eggs, such as leptotene, pachytene, diplotene, and growth of the oöcyte, yet, just as happens in the male tadpole, these early maturating cells degenerate. The same condition is reported in birds. It is difficult to avoid the suspicion that we are here concerned with a fundamental principle of germ-cell development. The question arises, why should practically all of the primordial germ cells of vertebrate undergo an abortive sexual cycle long before the animal is mature and ready for

reproduction, and then degenerate? The evidence from the maturation cycle of the tadpole is again suggestive on this point.

In the tadpole we may assume, in so far as it is safe to assume anything in biology, that the abortive and precocious sexual cycle is possibly a case of phylogenetic regression to ancestral conditions when the Anura were permanently of the caudate type and lived and reproduced normally as Urodele-like creatures. The carrying over into the ontogeny of the anuran larva's sexual cycle of this phylogenetic vestige is not surprising, considering the heavy impress of phylogeny upon the tadpole soma. Though this explanation may be involved with much plausibility to explain the larval sexual conditions of the bullfrog, is it in any sense adequate to account for the apparently analogous germ cycle of the Sauropsida and mammals, forms which do not have a larval period? I believe the same explanation applies to these forms also, and that the precocious developmental cycle and degeneration of the primordial germ cells described in the Amniota differ not in kind, but merely in the degree to which the maturation cycle is carried from the larval sexual cycle of the Anura.

In the Amniota we cannot speak of a precocious and abortive larval germ-cell cycle, but we can speak of an abortive embryonic sexual cycle, which, like that of the tadpole, possibly bears the impress of past phylogenetic conditions. And why not? If the embryo of the higher vertebrates can develop gill clefts and a thousand and one other evanescent phylogenetic vestiges in the course of somatic development, it should not be regarded as extraordinary if the germ-cell cycle likewise presented similar 'ancestral reminiscences,' and did a little recapitulating on its own account.

However, it must be confessed that 'phylogeny,' 'recapitulation,' 'ancestral reminiscences,' and other vague and more or less mystical terms of a kindred nature are after all merely convenient pegs upon which to hang our ignorance. There are immediate physicochemical reasons for the degeneration of the primordial germ cells or their abortive sexual cycle, but what these reasons are is unknown, and in view of a better or more plausible, hypothesis to account for this phenomenon, the one presented above is advanced tentatively.

Another point is worthy of consideration here, and that is the possibility of bringing about some measure of reconciliation between the 'entodermists,' or advocates of the Keimbahn, and the 'mesodermists.' In view of the evidence presented by study of germ-cell origin in all classes of vertebrates, there can be no reasonable doubt that the primordial sex cells are products of the entoderm, and probably migrate into the germ ridges at an early period of development. However, according to the hypothesis advanced here these primordial cells, after a period of multiplication, undergo an abortive developmental cycle and for the greater part degenerate—perhaps, in mammals, entirely degenerate. The new cell generation destined to give origin to the definitive sex cells may possibly arise in part from the germinal epithelium by direct transformation of mesothelial elements. The evidence for this point of view is suggestive, at any rate, judging by reports on conditions in the birds and mammals, and there is little evidence to the contrary, but many pure assumptions.

In the bullfrog the writer prefers to believe that some cells of the primordial germ-cell line persist unchanged through the phase of maturation and degeneration, and ultimately, by repeated mitosis are the chief, and probably only contributors to the cells of the definitive sexual line. There is considerable evidence for this view, because a few primordial spermatogonia or at any rate lineal descendants of these cells can be traced through the sexual cycle easily enough, but it is by no means certain that they are the sole contributors to the definitive line of germ cells.

Thus it appears possible that there is some basis here for reconciliation between the entodermists and mesodermists regarding germ-cell origin and development. The former have been at fault by contenting themselves with tracing the primordial sex cells into the genital glands and there leaving them, with the assumption that they persist and form the sexual elements of the adult organism. The mesodermists, working chiefly on mammals, have for the most part ignored the contributions of the 'entodermists' because they have been unable to trace the germ cells back to the very earliest stages such as described for the lower vertebrates.

No investigation of the germ-cell cycle in the Chordata should be regarded as complete or as being more than a half-truth which does not take into consideration the entire history of the germ-cell cycle, from the origin of the primordial germ cells to the formation of the definitive sexual elements of the adult. The investigators of the keimbahn have not gone far enough, for between the origin of the primordial germ cells and the formation of the ripe sexual products there is a critical stage in the germ-cell cycle, characterized by a precocious and abortive maturation, degeneration, and reformation of a new line of germ cells, perhaps by transformation of mesothelial elements, but more probably by active mitosis of a few left-over cells of the primordial line.

According to Hegner (Germ-cell cycle of animals, p. 99), germinal epithelium theories of germ-cell origin have little if any evidence in their favor, since no one has actually observed a transformation of peritoneal or mesoblast cells into germ cells. "On the other hand there is an abundance of proof that these cells (germ cells) migrate from some distance into the position of the sex glands."

The writer is quite in agreement with Hegner regarding the existence of a keimbahn in vertebrates, but is not so sure that no one has actually observed a transformation of mesoblast cells into germ cells in late stages of development or that germinal-epithelium theories have little if any evidence in their favor. My observations on the larval bullfrog have taught me caution in regard to dogmatizing on this problem. Odd as it may seem, it is not impossible, in the light of conditions described above for the bullfrog larvae, that the primordial germ cells of vertebrates, i.e., the keimbahn elements discussed by Hegner, may possibly be found upon further investigation to contribute little if any to the definitive sex products of the adult organism. Further investigation of the germ-cell cycle of the chordates may possibly enable the 'mesodermists' to turn the tables on the 'entodermists' with a vengeance by showing that no one has actually observed a transformation of keimbahn cells into definitive sex products. Though regarding himself as an entodermist, and taking the point of view that the keimbahn is probably continuous in

vertebrates, and that there is no actual transformation of mesothelial elements into sex cells, the writer admits that conditions are such in the bullfrog that it is impossible to state positively that the primordial germ cells of the bullfrog tadpole do give rise to the definitive sex cells of the adult frog. Certainly, this is the more probable view, though the burden of proof rests with those of us who hold that the keimbahn is continuous.

It would seem from this that the crux of the whole problem is to determine whether or not germ cells can develop in an organism after the primordial germ cells have been destroyed. If they do develop, then the doubtful question of transformation of mesothelial cells into germ cells is settled in favor of the mesodermists, but if they do not develop, and the gonad is sterile and remains so up to the period of sexual maturity, then the decision is in favor of the entodermists. It is not sufficient to extirpate the primordial germ cells or otherwise destroy them, as was done by Reagan in the embryo chick, and then report the resulting sterile gonad as conclusive evidence against the idea of a transformation of epithelial elements, because proof positive can only be had by rearing the animals to sexual maturity.

The only adequate method of attack upon this problem is by experimental methods. Morphological methods are not sufficient to determine whether or not a germ cell in the germinal epithelium or sex cord tissue is a transformed epithelial element or a small germ-cell descendant of the primordial line. Transition stages, nuclear configuration of the cell, size, position, and such like may be illusory. In my material there is apparently every transition stage between peritoneal and true germ cells, use whatever morphological criterion you please, at certain developmental stages of the tadpoles, and such transition stages almost fill the gonads, but always the question arises upon examining these apparent transition stages—they look exactly like mesothelial cells transforming into germ cells, but are they? If one must judge from morphological data alone, the answer is that they could very readily be taken for mesothelial elements transforming into germ cells, but, as stated before, the morphological criterion alone does not furnish sufficient evidence to permit one to make a definite answer.

SUMMARY OF CONCLUSIONS

A. The origin and fate of the primordial germ cells

1. The primordial germ cells of the embryo bullfrog are first distinguishable from other entodermal elements in embryos of 7 mm. total length. They arise from the entoderm as a median ridge of yolk-laden cells just dorsal to the roof of the archenteron, ventral to the aorta, and separating the two lateral mesodermal plates from each other.

2. In embryos of 8 mm. total length, the germ-cell ridge becomes separated from the underlying entoderm forming the roof of the archenteron, partly by the median growth of the two lateral plates which pinch off the ridge and also by active migration of the germinal elements themselves. In cross-section at this stage, the germ cells are found at the root of the forming mesentery as an unpaired ridge, consisting of two or three large yolk-laden cells.

3. As development progresses, this median ridge of germ cells splits longitudinally and the cells of the two halves migrate laterally on either side to form two independent ridges, invested with peritoneum. This stage is represented in embryos of 9.5 mm. total length.

4. The two germinal ridges project into the coelomic cavity and enlarge considerably by increase in number of their cellular elements. The primitive sex cells actively divide and there is also a migration of mesenchymal cells into the ridges from the mesonephros and peritoneum. These conditions are found in 14 to 15 mm. tadpoles. The germ cells have lost their yolk in the meantime.

5. The gonads greatly increase in size. Large cavities are formed, the secondary genital spaces, lined by small non-sexual cells which have migrated into the gonads from the mesonephros by way of the mesentery suspending the gland. When the tadpole has attained a length of 30 mm., the gonads are hollow sacs surrounded by a single layer of peritoneum and one or two layers of germ cells.

6. All increase in the number of germ cells in male larvae up to the 40-mm. stage is beyond question, by mitotic division of the preexisting sexual elements derived from the primordial germ cells of the entoderm ridge. A 40-mm. larva is about one year of age.

7. At the 40-mm. stage, despite the fact the tadpole is an immature larva and the gonads mere hollow sacs and in no way resemble testes, the germ cells enter maturation and pass through every stage of the maturation cycle in a normal manner, up to the first maturation division. In the act of division, the spermatocytes go to pieces and are resorbed.

8. Practically all of the germ cells derived from the primordial sex cells pass through this abortive maturation cycle and degenerate. A very few germ cells lineal descendants of the primordial embryonic sex-cell line persist unchanged, i.e., remain as spermatogonia through the maturation cycle and do not degenerate. Later these few cells give rise to a second generation of smaller germ cells.

9. This second generation of germ cells shortly before metamorphosis of the larvae undergoes a second sexual cycle, characterized by the production of normal spermatozoa. Thus there are two larval sexual cycles: one occurring in immature larva of 40 to 60 mm. and ending in degeneration, the other appearing shortly before metamorphosis, i.e., in larvae 140 mm. total length and ending in the production of normal sex products.

10. In the interval between the first and second larval sexual cycles following the degeneration of large numbers of maturation cells the gonads become filled with small cells which, because of their size, nuclear structure, and staining capacity, appear as transition stages between mesothelial cells (germinal epithelium and sex cord elements) and true germ cells. The later history of these cells shows them to be germ cells, but their origin is open to two interpretations and is not as clear as could be desired. The writer considers these cells as small germ-cell descendants of the primordial sexual elements, and not as transformed germinal epithelium elements, but admits that the evidence from his material is equally strong in support of the germinal epithelium view-point.

11. The sexual cycles of the larval bullfrog are tentatively interpreted, in lieu of a more satisfactory hypothesis, as recapitulations of the germ-cell cycle to past phylogenetic sexual conditions when the vertebrates ripened their sex products at an earlier developmental stage than at present.

12. An analogous precocity of the maturation cycle probably exists in all of the vertebrates, Amniota as well as Anamnia. Evidence for this hypothesis is presented in detail.

B. The chromosomes and larval sexual cycles

1. The diploid number of chromosomes in the male larva is twenty-eight. The elements are J- and V-shaped and curved rods. Portions of certain chromosomes do not take the stain under any circumstances and may give the appearance of fragmenting into two or more parts. Such appearances of fragmentation are illusory.

2. Spindle-fiber attachment is non-terminal.

3. The chromosomes exist in definite pairs according to size and shape; i.e., there are fourteen pairs of homologues.

4. The homologues of any pair are not invariably found side by side within the nucleus, though in general they are near together.

5. The size and shape relations of the chromosomes are perfectly definite throughout all cell generations, and this is probably true not only for the individual, but for the larvae of the species as a whole.

6. As an illustration of the statement just made (number 5), see chromosome pair marked A in figure 6. These chromosomes are peculiar in that the knob-like end-piece is separated from the main body of the chromosome by a clear, non-stainable area. This peculiarity is probably constant in the cells of the larvae, and has been observed in the spermatogonia of twenty-nine individuals of various ages and stages of development.

7. The resting nucleus of the Anuran germ cell is a polymorphic, much-lobulated structure, made up entirely of chromosome vesicles which are incompletely fused, and in many cases the vesicles are

entirely independent. By means of these vesicles the chromosomes preserve their identity through the so-called resting stages.

8. The cells and chromosomes of the larvae are considerably larger than those of the adult frog, and more nearly resemble the cells and chromosomes of urodeles than those of the adult of their own species.

9. This size difference between the larval and adult cells and chromosomes is explained in detail in the text and is considered to be due in part to the number of intervening cell divisions, with reduction of cell and chromosome size the greater the number of divisions, and to reduction in cell size at the time of metamorphosis due to loss of water from the tissues.

10. The haploid number of chromosomes in the larvae is fourteen. The tetrads of the first larval sexual cycle are extremely large and of the open-ring type characteristic of urodeles. They differ markedly from the type of tetrad appearing in the second larval sexual cycle.

11. Conjugation of the chromosomes is by parasynapsis, and occurs in the amphitene stage, when the leptotene threads twist together to form the pachytene.

12. Evidence of 'crossing-over' during diakinesis, such as figured by Janssens for Batracoseps, has not been observed, or rather, has been observed but not interpreted as such. The chiasma-type which appears during diakinesis stages of the bullfrog larvae has been interpreted by the writer as tetrads opening out in two planes at right angles to one another thus giving the *appearance* of 'crossing-over' of the chromatids.

13. It is suggested that 'crossing-over' occurs during the amphitene stage, when the conjugating leptotene threads coil tightly about each other corkscrew fashion.

14. The first larval maturation cycle is normal in every respect save for the size of the cells and chromosomes, up to the formation of the first maturation spindle. The spermatocytes degenerate in the act of division.

15. The cause of the degeneration of the larval spermatocytes is the abnormal behavior of the centrosome which fragments, forming accessory asters and spindles. It is recognized that the

centrosomal behavior is but a symptom of a deep-seated protoplasmic disorganization of the larval sex cells.

16. Giant spermatid-like structures are formed by the suppression of the first and second maturation divisions and the growth of an axial fiber from the centrosome. These bizarre structures degenerate.

17. The cells of the first larval sexual cycle degenerate and disappear gradually. A few cells, lineal descendants of the primordial germ cells, persist unchanged through the cycle of maturation and degeneration, and give rise by repeated mitosis to a second germ-cell generation in larvae just about ready for metamorphosis. This second cell generation is small in size.

18. Shortly before metamorphosis, this second generation of germ cells undergoes a second sexual cycle, characterized by the formation of normal spermatozoa. The cells and chromosomes are comparable in every way with those of the adult frog and are smaller than the larval cells and chromosomes.

19. The second larval sexual cycle is normal in every respect. There is no degeneration of the sexual elements. The maturation cycle is normal, as are also the spermatozoa, despite the fact the animal is a larva with the efferent ducts of the testis incompletely formed.

20. In so far as the possession of ripe spermatozoa is concerned, the larval bullfrog at metamorphosis may be said to be mature, and in this respect resembles the axolotl.

21. The germ cells of female larvae at the time of metamorphosis are not mature, but are young oöcytes undergoing growth. The writer has some evidence that, like the male, the female larvae may also show a precocious and abortive maturation cycle. This point is now under investigation.

22. The question of hermaphroditism and the sex ratios of the Anura is not dealt with in this paper, but forms the subject-matter of a later communication.

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EXPLANATION OF PLATES

All drawings were outlined with camera lucida; 2-mm. oil-immersion objective used, ocular 12; hence are of the same magnification. Plates 1, 2, and 3 have been reduced one-third.

PLATE 1

EXPLANATION OF FIGURES

1 Primary spermatogonium surrounded by follicle. Note polymorphic nucleus, i.e., chromosomal vesicles. From first-year larvae.

2 Diagram of early division prophase of primary spermatogonium. The chromosomes appear in vesicles which make up the entire nucleus and give it the lobulated appearance.

3 Diagram of later prophase. Chromosome vesicles have disappeared.

4 and 6 Equatorial plates showing twenty-eight chromosomes. Note Chromosome pair marked A. The peculiarity of an end-piece attached by a non-staining area is constant.

5 Odd type of spermatogonial prophase chromosomes may appear in best fixed material. Chromosomes appear as solid balls. Abnormal cell evidently degenerating.

7 Odd cell division. Spindle oriented in short axis of cell.

8 Resting nucleus after last spermatogonial telephase. Note the chromatin blocks and linin fibrils. Larvae 45 mm. total length.

9 Preleptotene stage showing resolution of the chromatin blocks into fine threads. Larvae 40 mm. total length.

10 Large cell with leptotene threads. Note the chromoplasts with attached fibrils.

11 Amphitene nucleus. Pachytene loops at proximal pole, unpaired leptotene filaments at distal pole. This cell illustrates the formation of the pachytene bouquet. See plate 5, fig. 39. Larva 45 mm. total length.

12 Isolated pachytene threads showing unpaired leptotene filaments at distal ends. Compare with figures 36 to 39, plate 5.

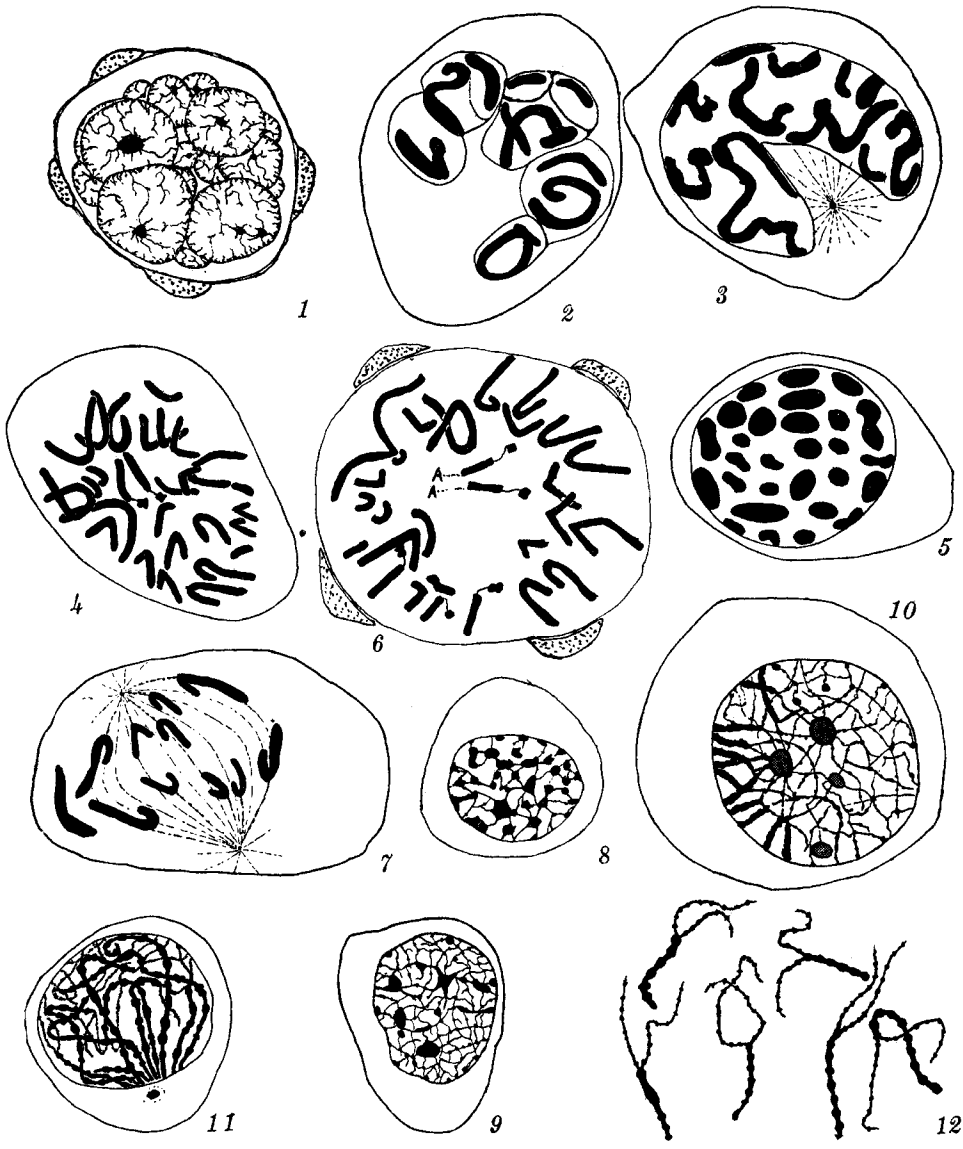

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PLATE 2

EXPLANATION OF FIGURES

13 Amphitene nucleus. Larva 45 mm.

14 Diplotene stage showing splitting of the thick pachytene threads and their separation in the middle. Note attachment at synaptic ends.

15 Diplotene nucleus. Compare with figures in plates 5 and 6. Larvae 45 to 60 mm.

16 Diplotene nucleus showing early formation of tetrads. Larvae 45 to 60 mm. No evidence of crossing-over.

17 Condensation of the type of tetrad shown in figure 16. Note the woolly appearance. Larva 74 mm.

18 First spermatocyte tetrads and tripolar spindle. Fourteen tetrads present. First-year tadpole. See figure 79.

19 Spermatocyte with eighteen tetrads. Very unusual condition. Larvae 80 mm. total length.

20 Tetrads of larvae of first year. See plates 9 to 11. *X* in figure 20 indicates persisting karysomal structure of unknown origin and fate.

21 Spermatocyte with multiple asters. First-year larvae 40 to 50 mm. total length.

22 Giant spermatid-like body resulting from degeneration of larval spermatocytes like those shown in figures 106 and 108. See also plate 13.

23 Different type of spermatid-like body. The black masses represent the ring tetrads which have run together. See plate 13 also. Larva 80 mm.

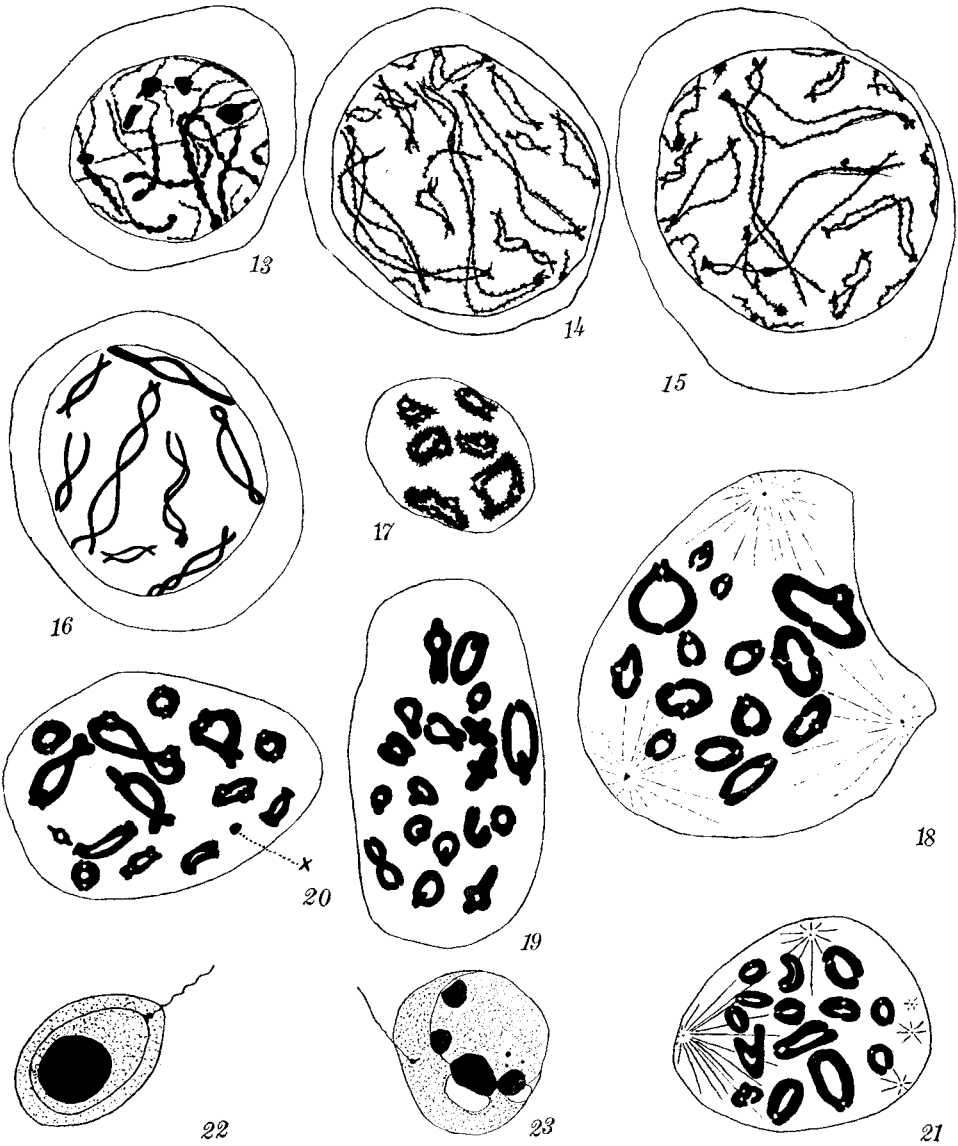
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PLATE 3

EXPLANATION OF FIGURES

Fig. 24 and 25 Large larval spermatocyte. Cell cut. Both figures are of one cell. Note the body resembling a yolk nucleus in figure 25. Larva 100 mm.

26 and 27 Both figures of same cell. Note rod-shaped tetrad in figure 26. Unusual condition. Larva 80 mm.

28 Giant spermatocyte of first-year larva. Note rod tetrad and extreme nuclear size. This cell shown in photographs 64 and 67. Larva 80 mm.

29 and 30 Sections of the same cell. Note the Y-shaped and cross-shaped tetrads. Cell of unusual size. Note similarity of tetrads to those of urodeles. Same cell shown in photographs 87 and 88. Larva 100 mm.

31 and 32 Sections of same cell. Note the rod-shaped and cross tetrads. Cell of unusual size. Figure 114 is a photograph of a portion of this cell. Larva 95 mm.

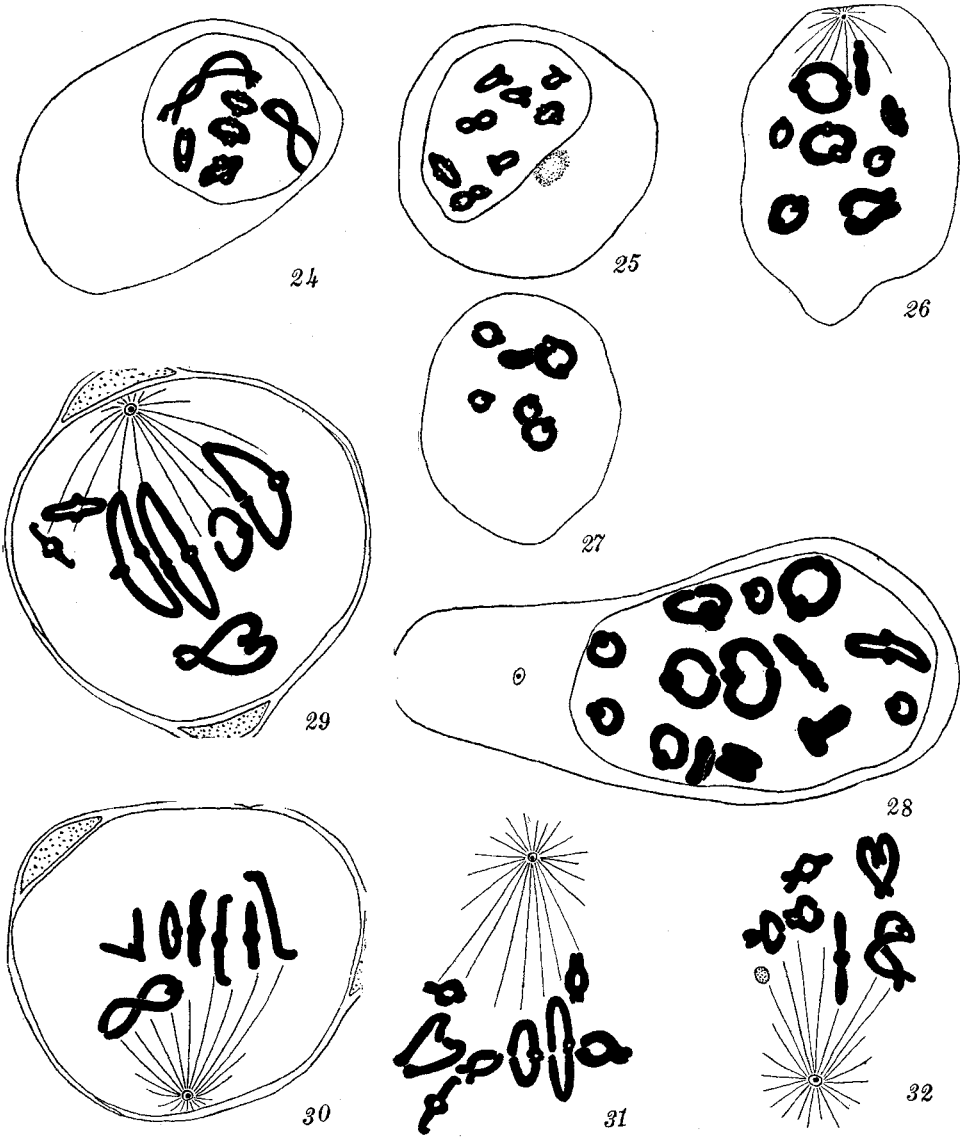
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PLATE 4

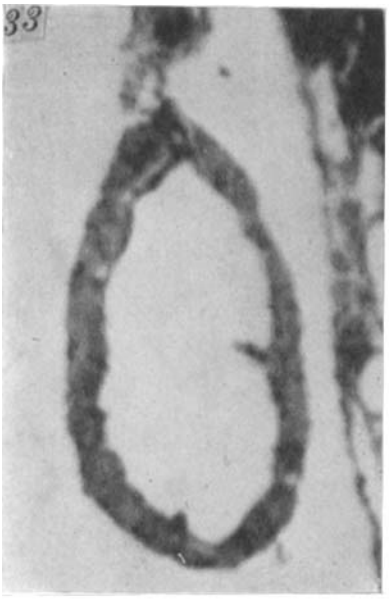
EXPLANATION OF FIGURES

Microphotographs on this plate made at magnification of 50 diameters. No reduction.

33 Transverse section through male gonad shown in text figure 1, a. Note the very large secondary genital cavity surrounded by double layer of germ cells. Animal 40 to 50 mm. total length. Maturation of the germ cells begins in many instances in undifferentiated glands of this type.

34 Cross-section through male gonad of first-year tadpole (text fig. 1, b). The large cavity is disappearing, owing to rapid proliferation of germ cells. The cavity is lined by non-sexual mesodermal cells which have migrated in from the mesonephros. Practically all the germ cells in this type of larvae gonad are maturating. Animal 70 to 95 mm. total length.

35 Section through male gonad of second-year tadpole approaching metamorphosis (text fig. 1, C). The cavity has disappeared. Note the testicular ampullae. The second sexual cycle occurs in this type of testis. Animals 120 to 150 mm. total length.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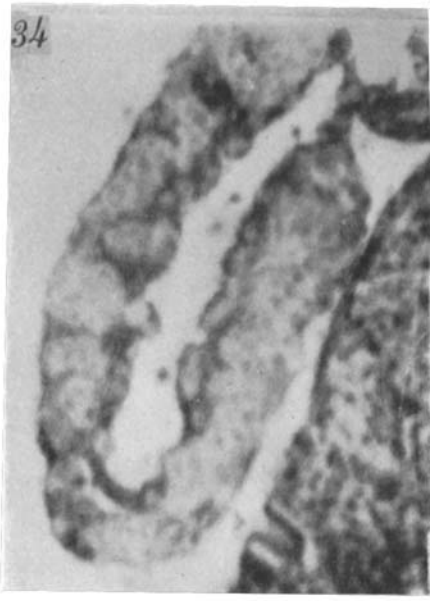
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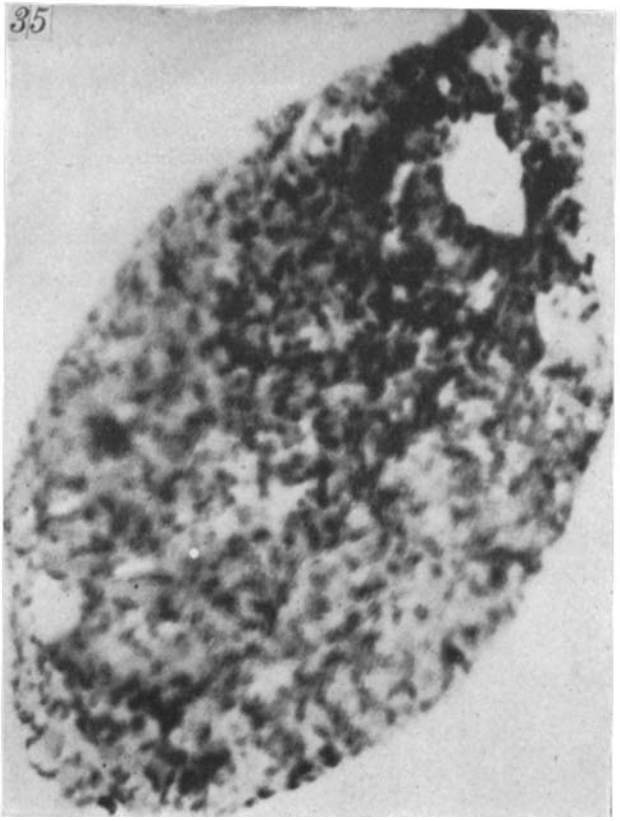
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PLATE 5

EXPLANATION OF FIGURES

Microphotographs made at a magnification of 1500 diameters. No reduction.

36 Amphitene nuclei. Note the thick pachytene thread at one pole and the fine leptotene filaments at the opposite pole. Synapsis occurs at this stage.

37 and 38 Amphitene nuclei from first-year larvae. No effort has been made to show the cytoplasm.

39 Pachytene nuclei. Synapsis complete. The threads appear single.

40 Transition stage from pachytene to diplotene nuclei. The thick pachytene threads of figure 39 are in figure 40 splittion into two thin threads.

41 Diplotene stages showing the disjunction of the leptotene threads paired in figure 39. Note at *X* the split in the thick thread. This is the primary longitudinal split.

42 Diplotene stage showing splitting of the thick pachytene threads.

43 and 44 Diplotene stages showing the figure-8 configuration of the splitting threads.

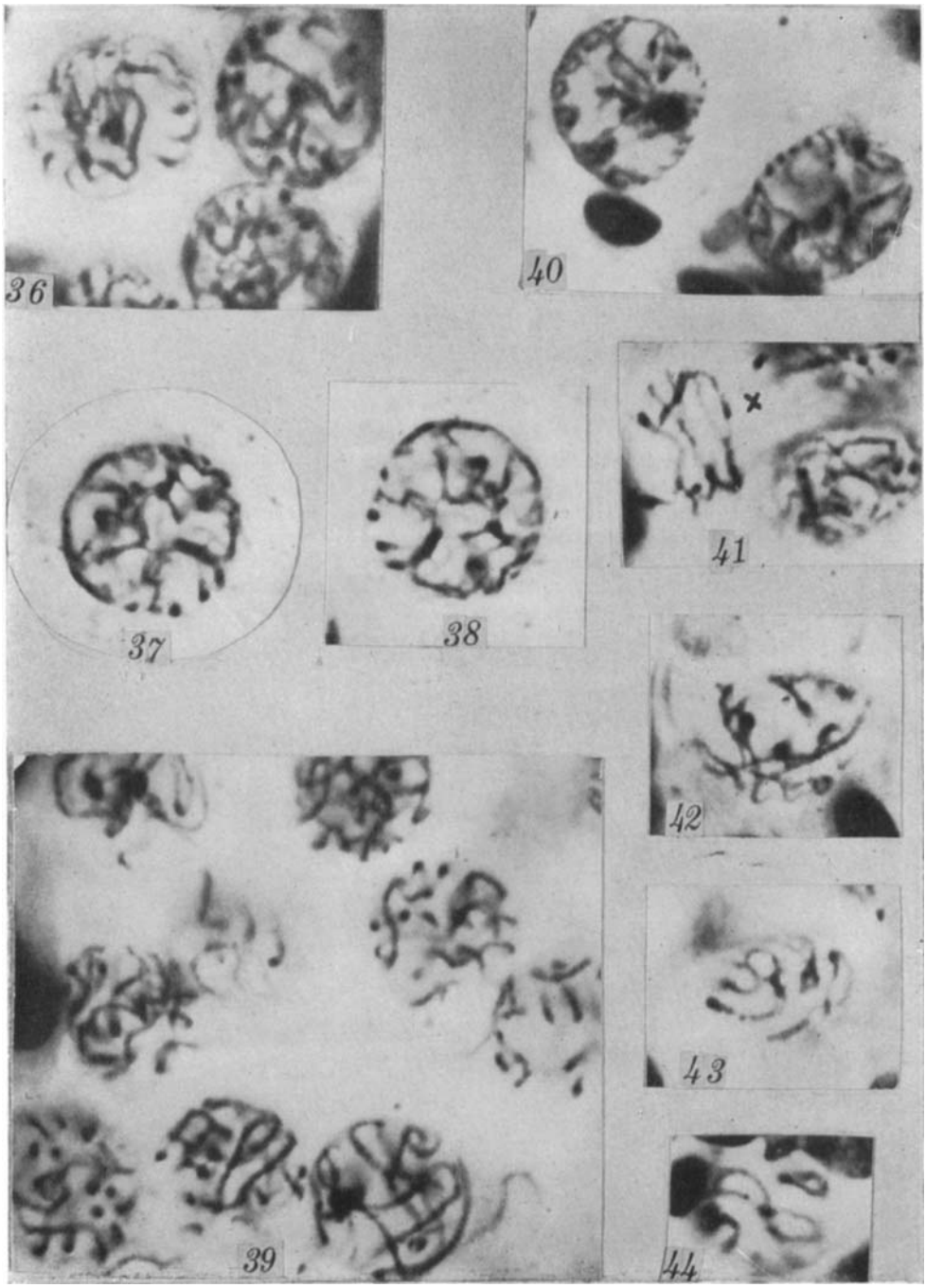
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PLATE 6

EXPLANATION OF FIGURES

45 The large cell near the upper central edge of the photograph is a stage in middle diplotene, showing the disjunction of the homologous chromosomes in the shape of fine threads. The threads, it will be observed, are united at their ends. Several pachytene nuclei are visible at the lower left-hand corner. In the lower right-hand corner are shown portions of three ring tetrads.

46 Gigantic diplotene cell. This type of cell is very common; the size of the nuclei is remarkable in some instances. Note the split threads extending across the nucleus, but united at the ends by two dark staining knobs.

47 Portion of a diplotene nucleus together with two early preleptotene nuclei. Note the size difference.

48 Diplotene nucleus.

49 Part of a diplotene nucleus, showing splitting of the threads.

50 and 51 Large diplotene nuclei. Larva of 80 mm.

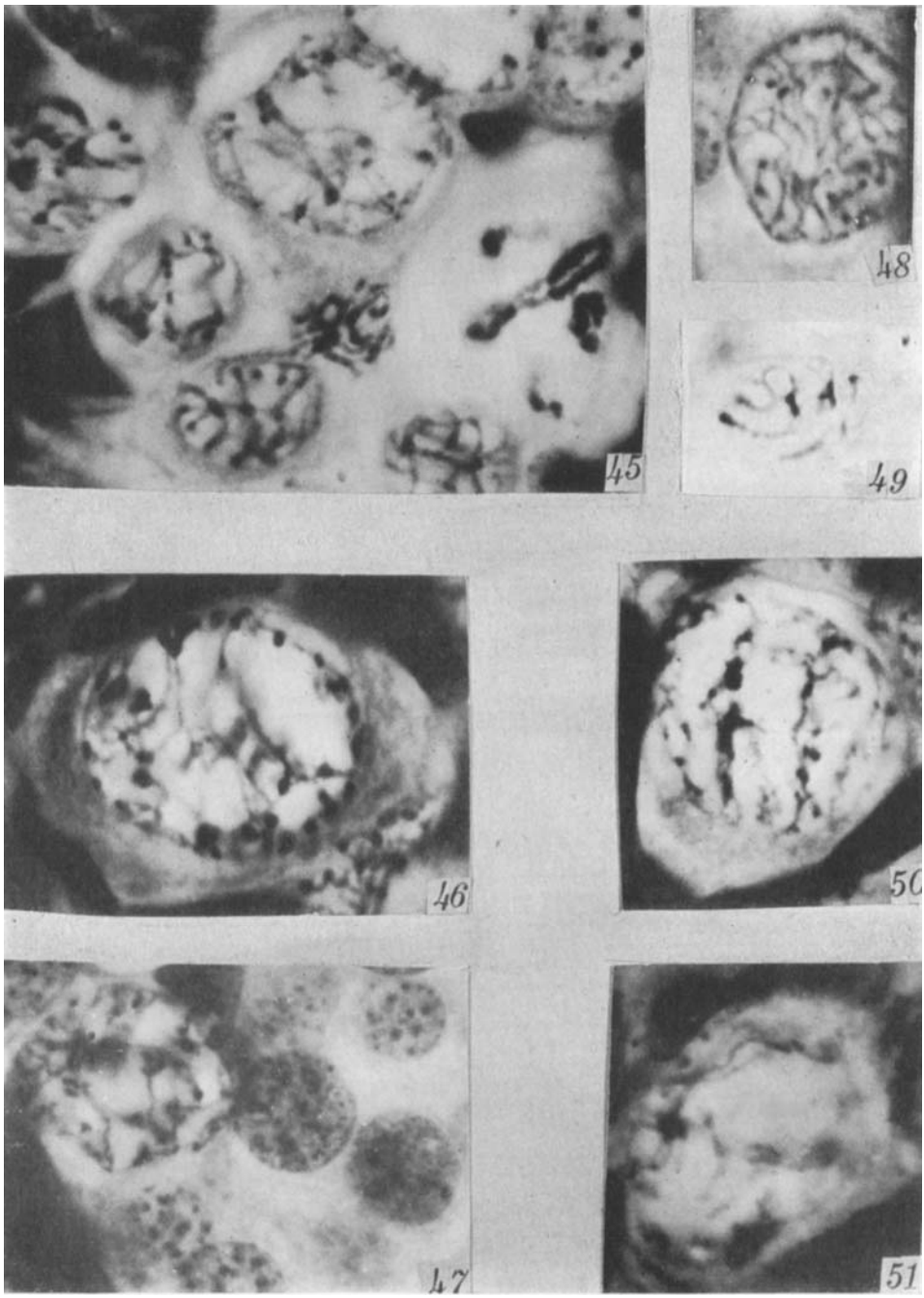
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PLATE 7

EXPLANATION OF FIGURES

52 to 54 Portions of large diplotene nuclei, showing condensation and great thickening of the threads to form the tetrads. Note the size of the chromosomes. All are cut in these photographs; the size of the nuclei is so great that each one of these cells is cut into three parts when sectioned at a thickness of 8 to 10μ. In figure 54 note that the condensing chromosomes are split again—equational split.

55 and 56 Portions of large cells in diakinesis. The ring tetrads are shown.

57 Large diplotene nuclei. Nucleus almost completely fills cytoplasm.

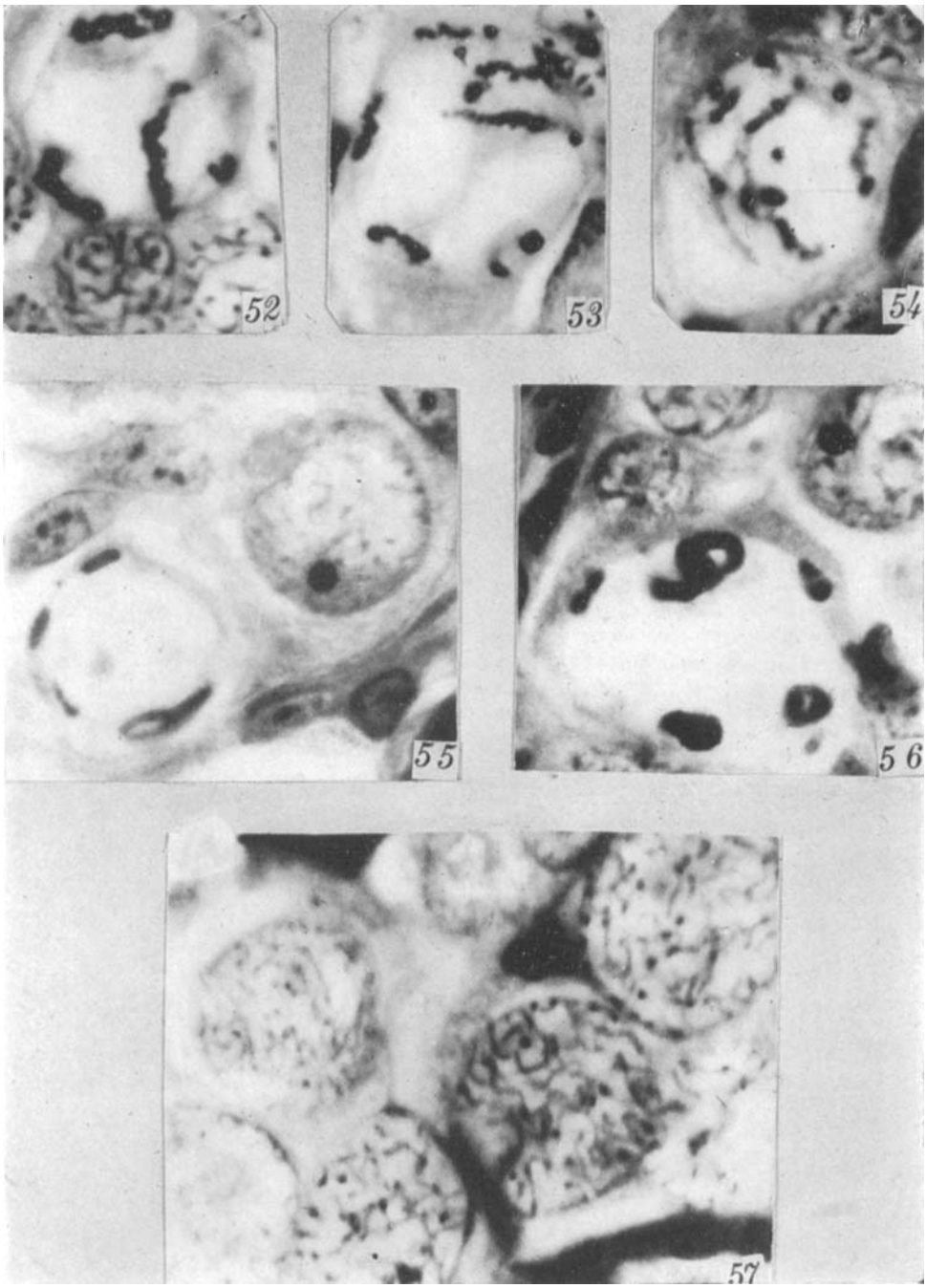
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PLATE 8

EXPLANATION OF FIGURES

58 Portion of large nucleus in diakinesis stage. Practically no cytoplasm surrounding nucleus.

59 to 63 Smaller cells in diakinesis. Note figure-8 chromosomes.

62 Portion of large nucleus in diakinesis. Note the extraordinary large figure 8 in this cell.

64 Portion of large spermatocyte, showing ring tetrads.

65 and 66 Portions of spermatocytes showing chromosomes.

67 Same cell as figure 64 photographed at a different focus. Note follicle cells and nuclear size. Compare these cells with those of plate 15.

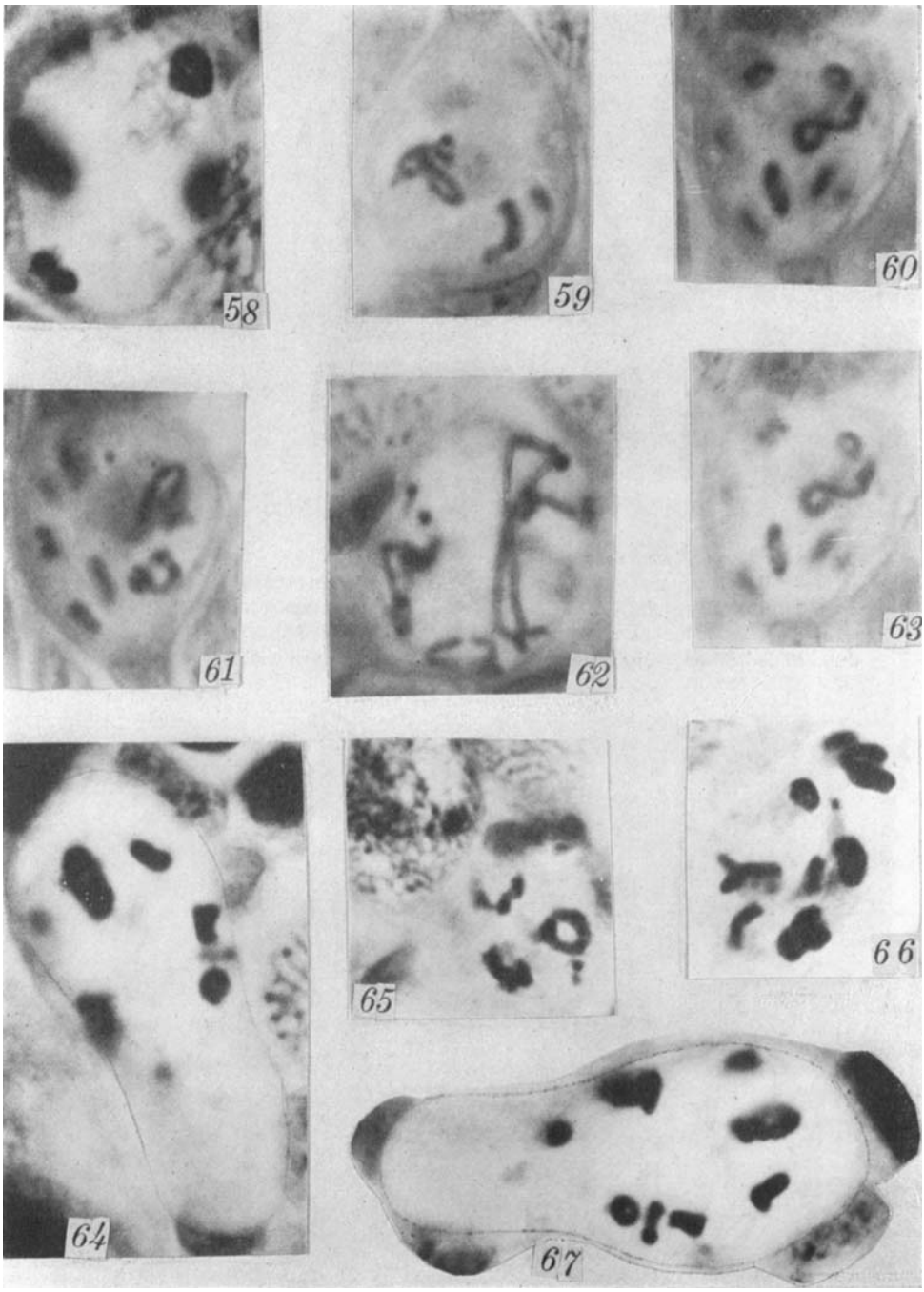
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PLATE 9

EXPLANATION OF FIGURES

68 Portion of large spermatocyte nucleus.

67 to 76 Ring tetrads of first-year larval spermatocytes. Note the large open rings and especially the size of certain of the tratrads. In adult frogs the tetrads on the spindle are not in the form of open rings of this type, but resemble dumbbells and are very much smaller in size. Compare with plate 15.

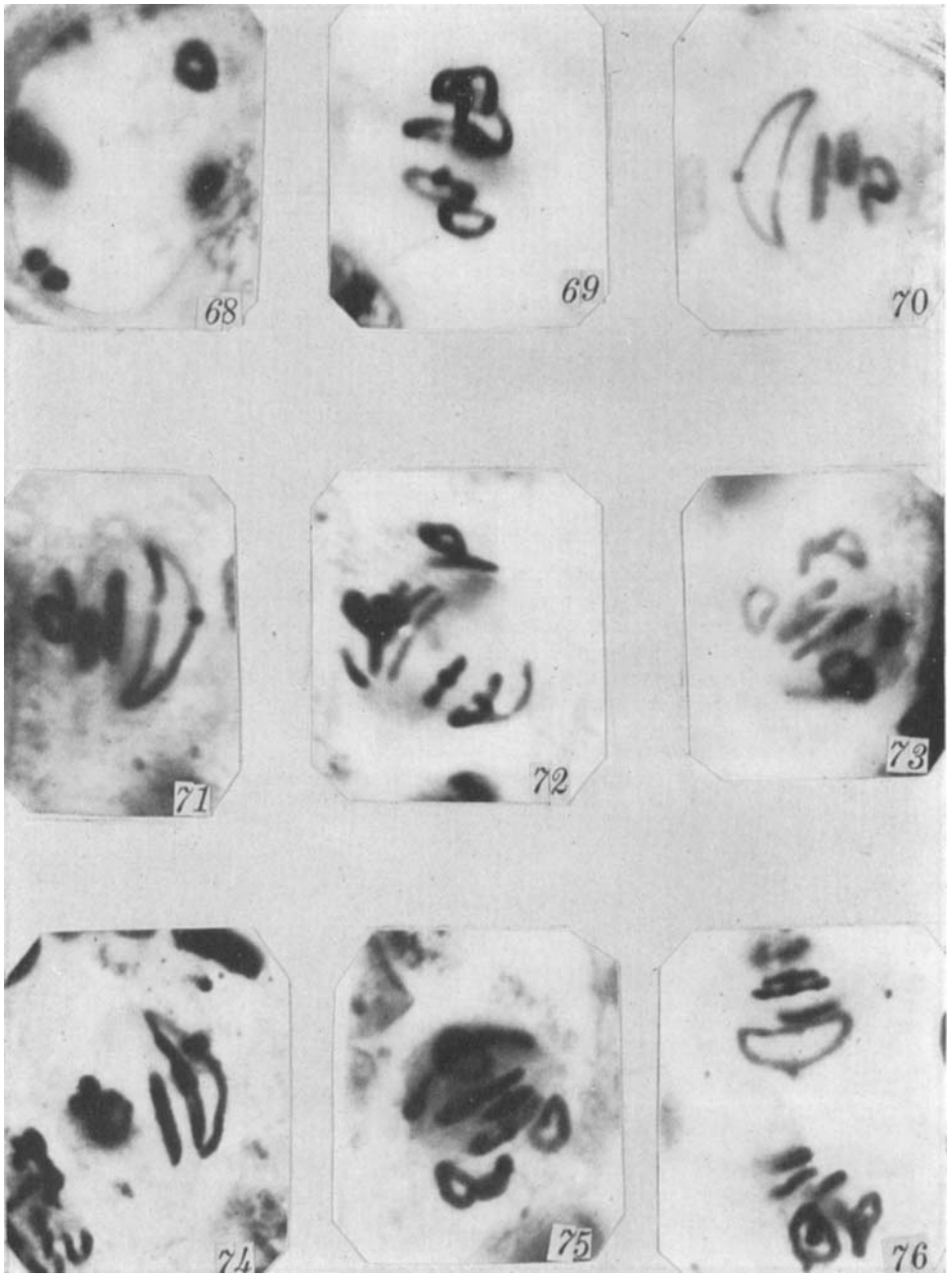
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PLATE 10

EXPLANATION OF FIGURES

77 Large first spermatocyte in act of division. Note the size of the tetrads and spindle. These cells degenerate in the act of dividing at this stage.

78 to 80 Large spermatocytes of first-year larvae in act of division. Figure 79 shows a tripolar spindle and irregular arrangement of the tetrads.

81 and 82 Larval spermatocytes. Figure 82 is of a cell in process of degeneration. The tetrads have lost their annular appearance.

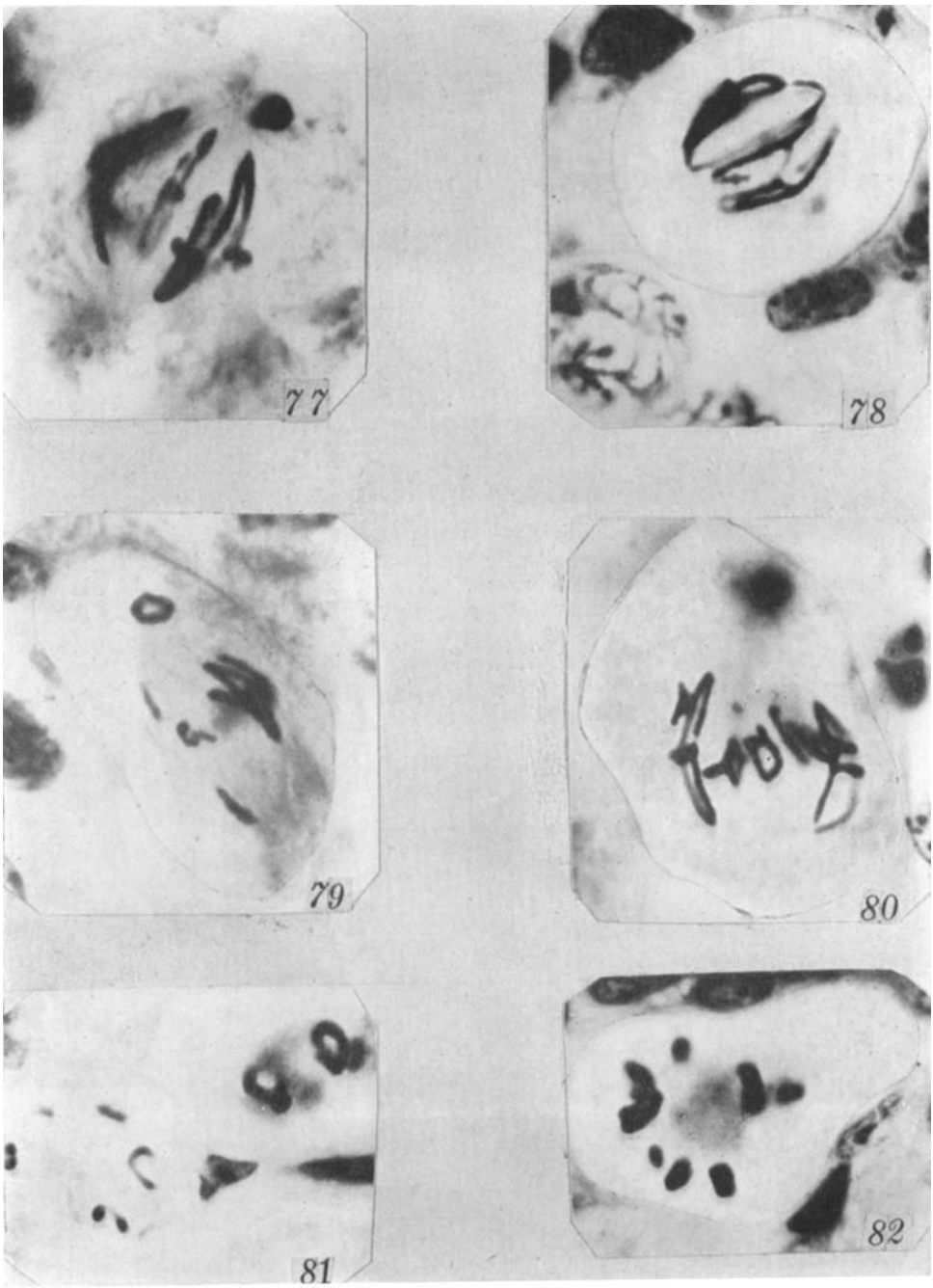
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PLATE 11

EXPLANATION OF FIGURES

83 Giant spermatocyte in division. Note size of the cell and its follicle.

84 A portion of large spermatocyte preparing for division. All such cells degenerate before completing the process.

85 to 88 Large larval spermatocytes preparing for division. All degenerate before completing the process.

89 and 90 Are isolated tetrads of larval germ cells in similar stages of development. Note variation in size of the tetrads in these cells. Figure 90 is from animal in second year. It is rare to find this type of tetrad in second-year larvae. Compare with plate 15.

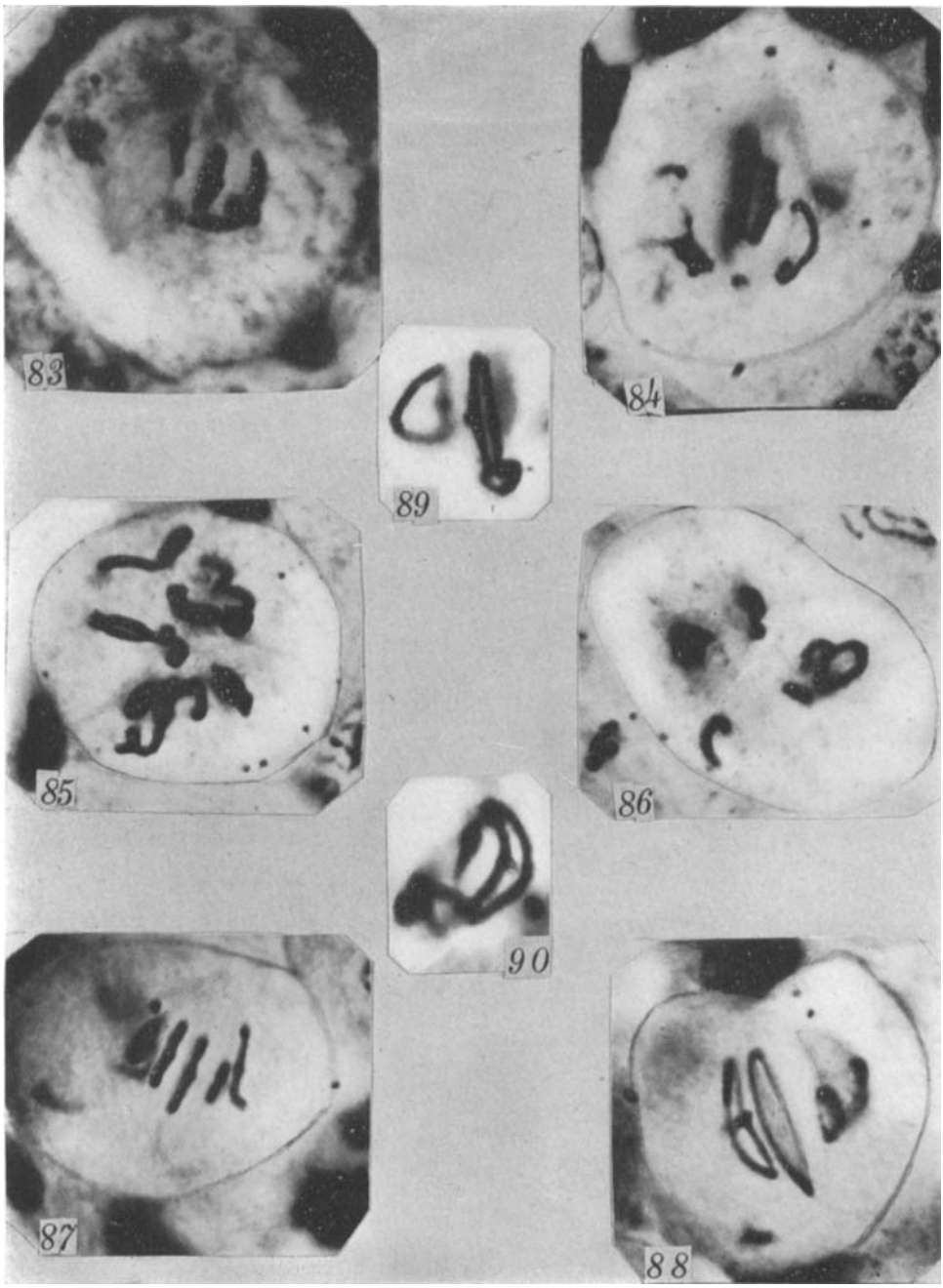
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PLATE 12

EXPLANATION OF FIGURES

91 to 94 Larval spermatocytes dividing.

95 Single large ring tetrad. Note the size and the lugs marking the synaptic ends of the homologous chromosomes.

96 to 99 Larval spermatocytes dividing abnormally. Note in figure 97 the great size and thickness of the central chromosome. This tetrad is cut in half longitudinally.

100 and 101 Abnormal spermatocyte. Many tiny asters scattered through the cytoplasm.

102 Large ring tetrad from second-year larvae. Just beneath is a small germ cell of the second year; note the size difference. The spermatocyte in which this tetrad was photographed is not shown here and was in early stages of degeneration. This type of tetrad rare in second-year tadpoles and found only in degenerating cells, which persist from first sexual cycle.

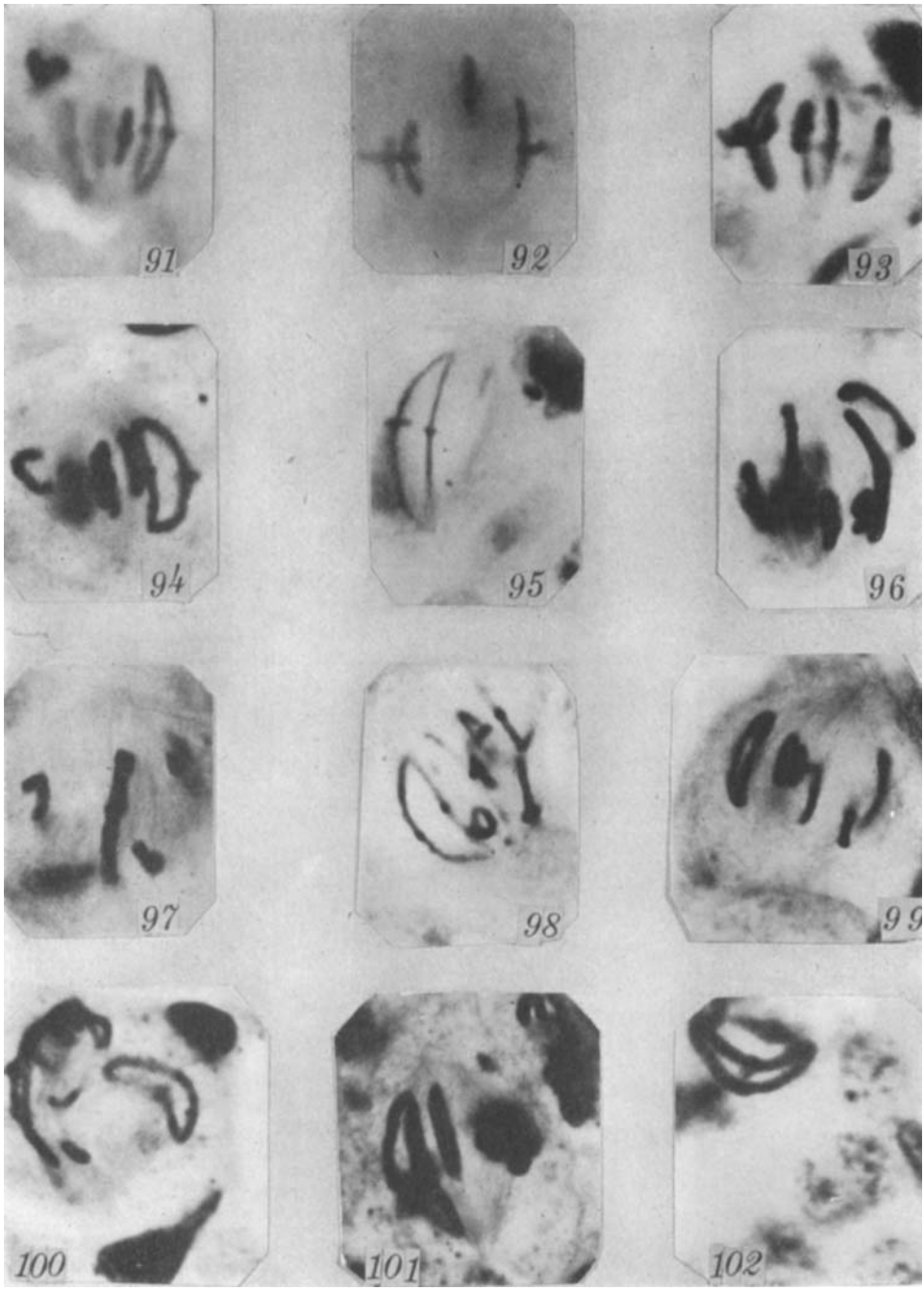
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PLATE 13

EXPLANATION OF FIGURES

103 Larval spermatocyte in early stages of degeneration. Tetrads condensing or running together.

104 and 105 Further stages in spermatocyte degeneration.

106 to 108 End stages of degeneration of larval spermatocytes. The type of cell depicted in figures 106 and 108 sometimes show long axial filaments as outgrowths of the centrosome. The black balls are the remains of the tetrads.

109 and 110 Stages in the degeneration of the larval spermatocytes of the first sexual cycle. Note in figure 109 the condensed group of vacuoles in the large clear area. The clear area represents the original size of the cell; the vacuoles are the remains of the tetrads that went to pieces in situ on the first maturation spindle.

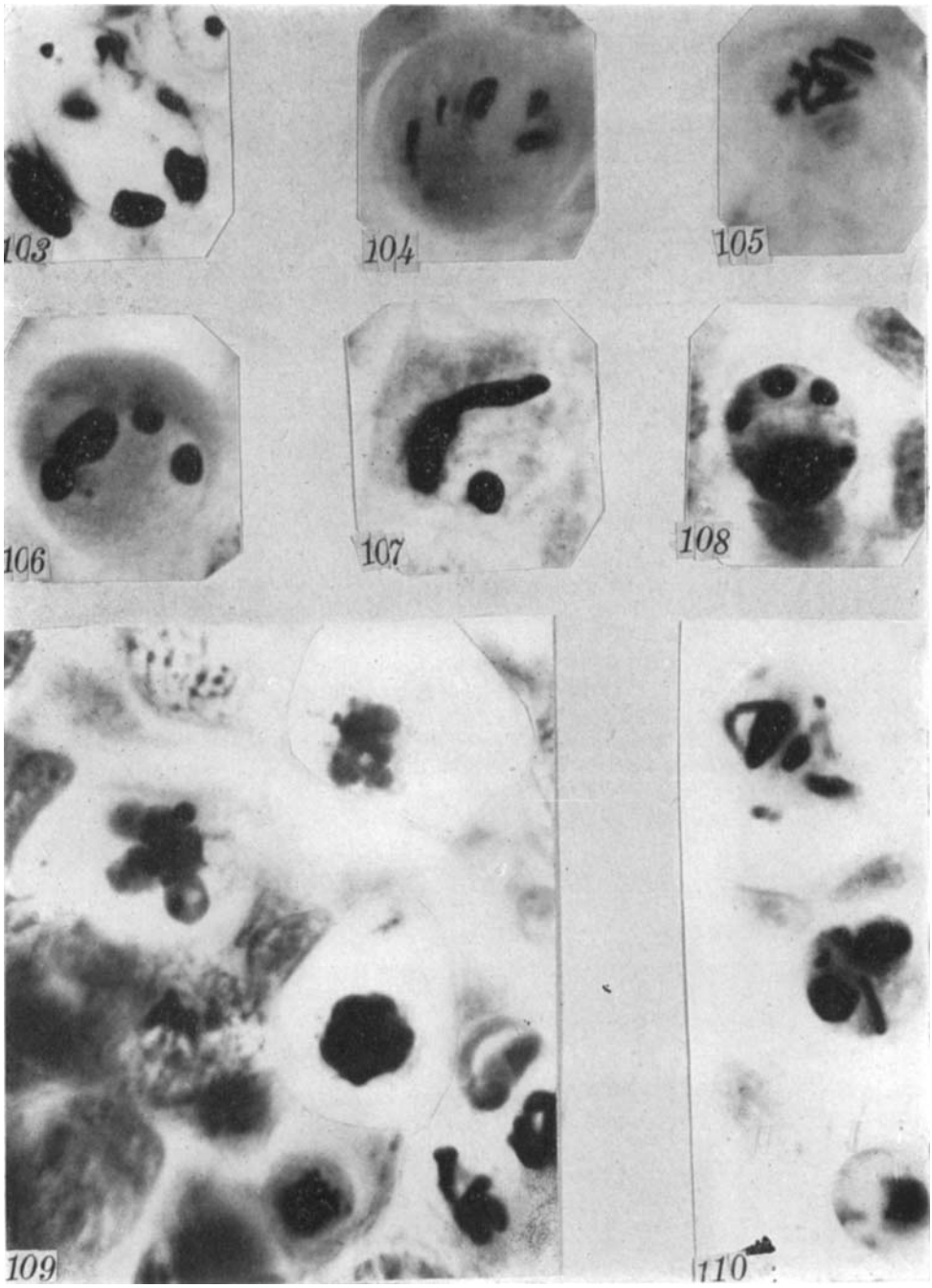
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PLATE 14

EXPLANATION OF FIGURES

111 So-called 'oocyte' in larval testis. The character and history of these cells will be discussed in another communication.

112 Giant larval spermatocyte, showing size of the spindle.

113 Giant spermatogonium from testis of first-year larvae.

114 Large spermatocyte dividing. Only half of the cell is shown. Note the extreme length of spindle.

115 Spermatogonium showing polymorphic nucleus. At left of picture is an abnormal spermatocyte division. The chromosomes are torn to pieces by polyasters in the cytoplasm.

116 Gigantic spermatocyte dividing, showing tripolar spindle. Note distribution of chromosomes on the two spindles.

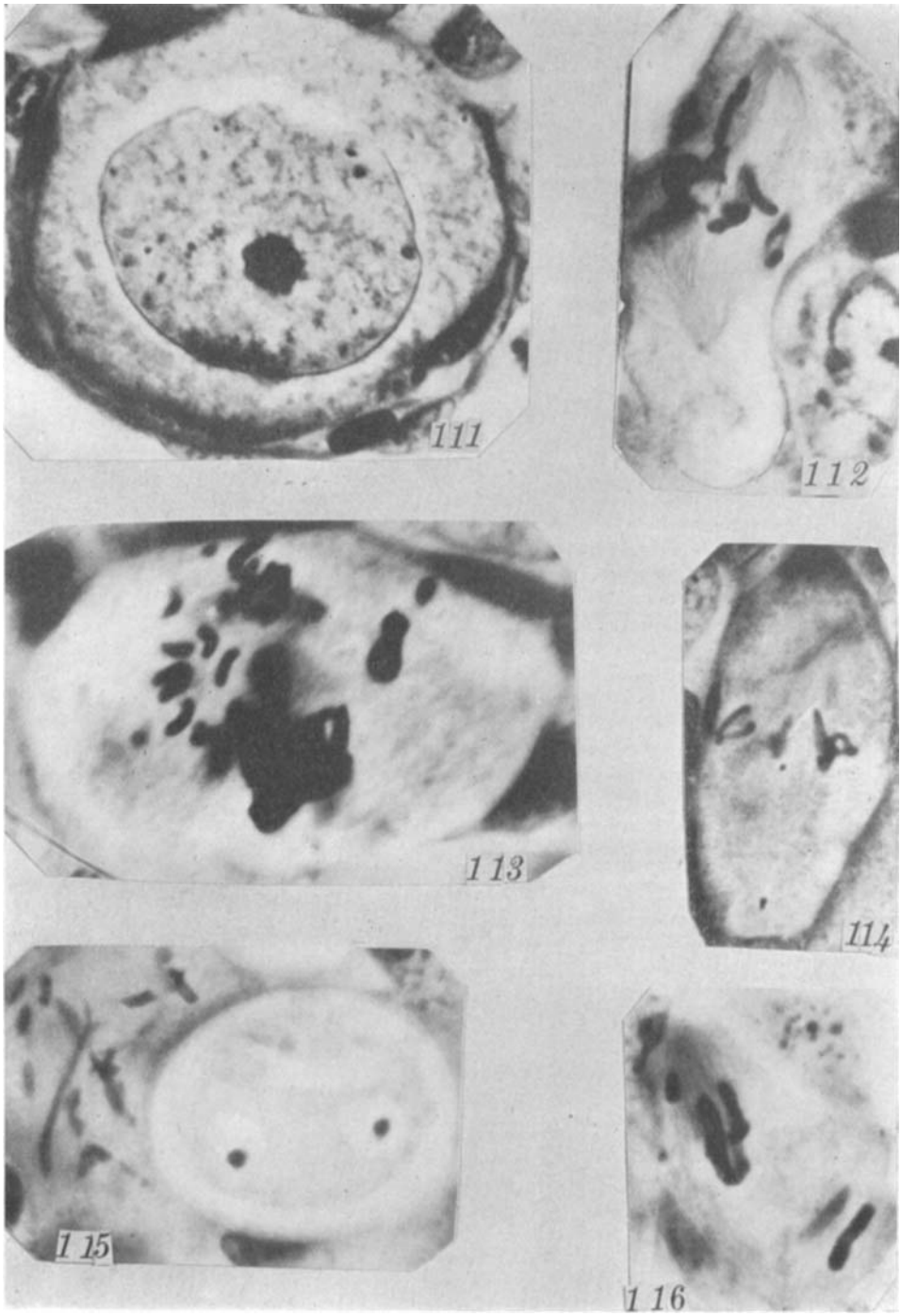
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PLATE 15

EXPLANATION OF FIGURES

117 Primordial spermatogonium with large vesicular nucleus. This type of cell, in very small numbers, persists unchanged through the first-year maturation cycle of the larvae, and by repeated divisions probably contributes the cells of the second sexual cycle of the larvae.

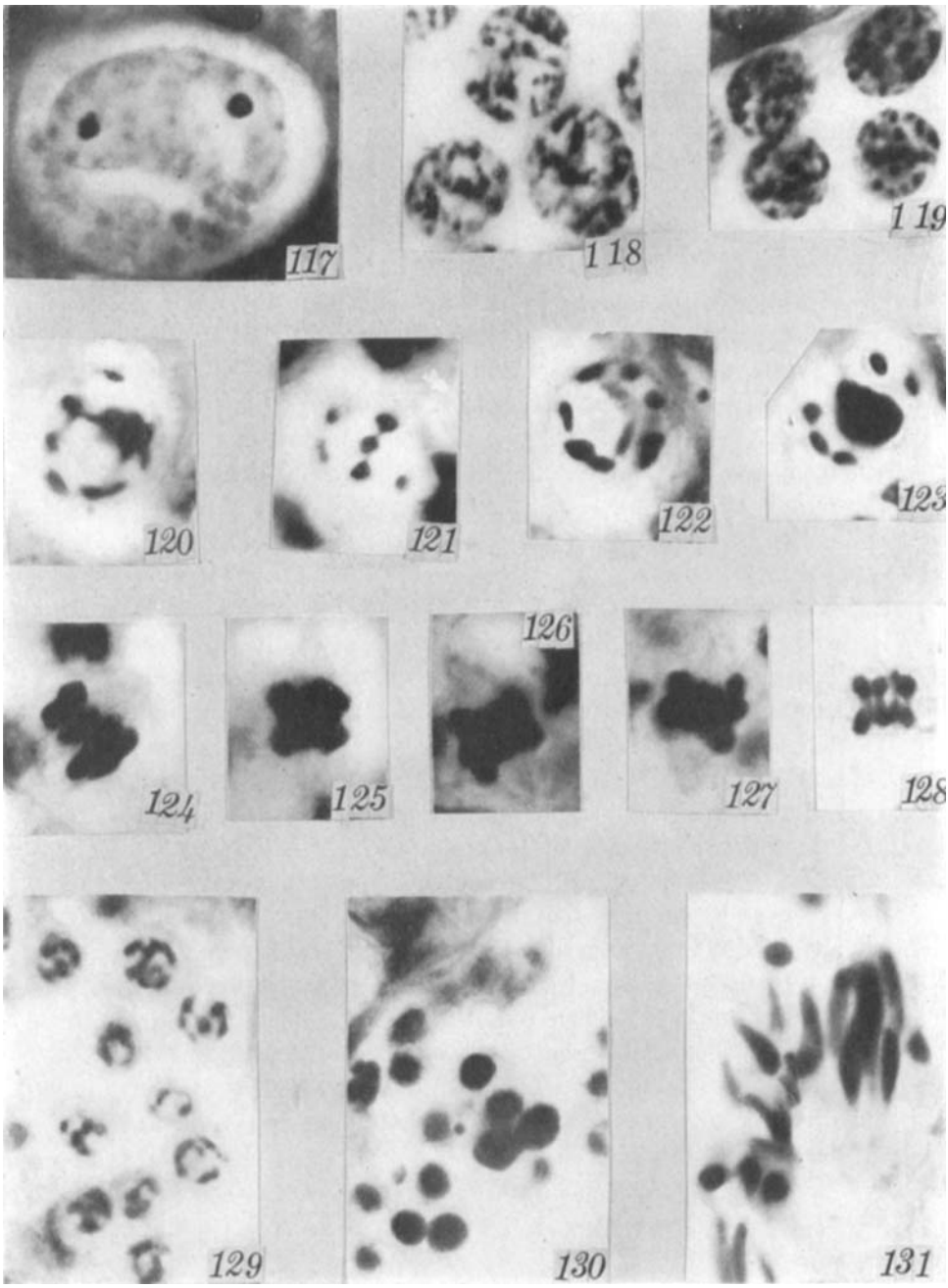
118 and 119 Small germ cells of second-year tadpoles preparing for the second sexual cycle. Compare these cells with figure 117. Both types of cell are found in the same gonad. The smaller elements appear to originate in part from transformed mesothelial cells (?).

120 to 123 First spermatocyte prophases. Note the small size of the cells and tetrads. Compare with figures 112 or 114 or any cell on plates 7 to 13.

124 to 128 First spermatocyte divisions of second larval sexual cycle, or newly metamorphosed frogs. Note extremely small size of cells and tetrads. Compare with plates 7 to 13. Clearness of detail of the chromosomes has been sacrificed by overdevelopment to show the spindles and cell outlines.

129 and 130 Spermatids of second sexual cycle.

131 Spermatozoa of second larval sexual cycle. These are sometimes formed in large numbers in the larvae, but are more numerous shortly after metamorphosis.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