(Zoology Department, University of Michigan.)

CONTRIBUTIONS ON THE DEVELOPMENT OF THE REPRODUCTIVE SYSTEM IN *STERNOTHERUS ODORATUS* (LATREILLE).

I. THE EMBRYONIC ORIGIN AND MIGRATION OF THE PRIMORDIAL GERM CELLS. [1]

By

Paul L. Risley.

With 19 figures in the text.

General introduction and statement of problem.

Among the many controversial problems which have aroused bio-
logists to intensive investigation during the past half century, none
perhaps is more prominent than that of the origin of the germ cells.
Years of research on the development of many organisms have resulted
in the accumulation and presentation of much information and numerous
conflicting conceptions. Certainly the majority of investigators who
have studied the problem in invertebrate animals are agreed that the
germ cells arise from early blastomeres of the embryo (See Hegner,
1914). Among the workers on vertebrates, however, there remains an
active conflict of opinion (See Heys, 1931), and the exact status of the
question has remained more or less doubtful since its origin. The pro-
blem is concerned principally with the question of a somatic or an in-
depedendent origin of the reproductive cells. Those who claim an in-
dependent origin, or origin from the primary blastomeres of the embryo,
have difficulty in conceiving of an origin of reproductive elements from
differentiated somatic elements. This obstacle is not apparent to those
who, with Child (1906) and others, can even picture a dedifferentiation
of somatic cells. It is readily believable that the early tissues may be
made up in part of undifferentiated or embryonic cells from which the
germ cells take their origin. Furthermore, the hereditary elements may
remain unaltered in somatic cells, and thus the apparent difficulty of
a conception of a transformation of somatic cells to cells of reproductive

---

[1] Contribution from the Zoological Laboratory, University of Michigan.

function is overcome. If we accept the chromosome theory of heredity, the Weismannian conception of a "Keimbahn", or continuity of a germ cell lineage from one generation to the next, seems to lose much of its significance. Nevertheless, the idea of germ cell integrity is, contrary to the statement of HARGITT (1926) that biological sciences would profit by discarding it, of importance in many philosophical and unifying concepts of life and life processes. The problem will always retain a significant and fundamental place in embryological investigations, as well as those related to reproduction and sex.

Numerous other embryological problems are so interrelated with studies of germ cells that it is advisable to consider them all at the same time, insofar as it is possible. Among these are the problems concerned with gonadogenesis, which involves the histological changes occurring during the development of the genital ridges and the formation of the testis or ovary. Controversial questions still exist in regard to the origin of the rete-cords, sex-cords, and other components of the sex-glands.

Sex differentiation presents still another problem of interest and importance. In a great many animals, the sex-chromosomes are, partially at least, responsible for the determination of the sex of the individuals. In some forms, these elements do not seem to play an important part in sex differentiation. In either case, the early developmental stages are similar in the two sexes, but sooner or later the development of the reproductive organs proceeds along one of two alternative directions. In some forms (Cyclostomata, Pisces, Amphibia), this period is marked by the appearance of certain hermaphroditic tendencies, characterized by the simultaneous presence and growth of structures representing both sexes. In other forms, the change of the sexually indifferent embryo to a definitive male or female occurs rather abruptly.

Of all vertebrate classes, the Reptilia alone have not contributed their proper share to our knowledge of the germ cells or of other morphological problems. PAINTER (1921) has already pointed out that they offer a virgin field to the cytological investigator. Very little is known about the cytoplasmic constituents or the chromosome complex (See MATTHEY, 1931) of these forms, and there are but few accounts dealing with the interesting morphology of the reptilian group. Also, in view of the fact that the literature reveals very little about sex determination and sex differentiation in Reptilia, it seemed probable that some interesting conditions in regard to the prevailing problems of sex might be uncovered in such an investigation.

The primary purpose of this work lies in an attempt to place our knowledge of the development of the reproductive system of a chelonian upon a basis which will allow satisfactory comparisons with the facts already known concerning related questions in other vertebrate forms. Because of the phylogenetically intermediate position of the reptiles

in relation to the lower and higher vertebrates, one might reasonably expect to find significant correlations between the distinct types of reproductive systems. The following discussion constitutes the first of a series of papers which consider several phases of the development of the reproductive system as determined from a study of the common Musk Turtle, sometimes known as the "Stink-pot Terrapin", Sternotherus odoratus (LATREILLE).

My sincere appreciation and thanks are extended to Professor PETER O. OKKELBERG of the Department of Zoology of the University of Michigan, under whose direction this investigation was begun and carried out. I wish also to thank Professor EMIL WITSCHI of the Department of Zoology of the State University of Iowa, who has kindly read the manuscript.

I. The embryonic origin and migration of the primordial germ cells.

A. Introductory statement.

Particular emphasis in the following observations and discussion is directed toward the following problems: the primordial germ cells and their extraregional embryonic origin, their migration to the gonad region, and their behavior in the undifferentiated germ glands. The question of germ cell origin in reptiles remains unsettled, especially since the investigations of SIMKINS (1925) and SIMKINS and ASANA (1930). The same argument exists among workers on reptiles as among workers on other vertebrates. The majority of investigators are agreed that the germ cells are segregated early in the life of the embryo, and that they persist to give rise to the definitive germ cells of sexually mature animals. SIMKINS and ASANA are apparently the only investigators in this field at the present time. They are emphatic in their position that there never are any such cells as primordial germ cells, and that germ cells are not present in the reptilian embryo until the formation of the genital folds when they arise from the cells of the germinal epithelium. The literature will be considered more fully in the discussion of our results.

B. Material and Methods.

The material used in this study consists of a complete series of embryos collected by the writer during several summers of observation upon the habits and embryology of the Musk Turtle, Sternotherus odoratus (LATREILLE). For further information regarding the natural history of this turtle, the reader is referred to another publication by the writer (RISLEY, 1933). Due to various difficulties encountered in obtaining the eggs from a normal environment at the times when embryos were desired, the eggs were collected during the egg-laying season and brought to the laboratory, where development proceeded very satisfactorily.

30*

The embryos were removed from the eggs at the desired intervals and fixed in various solutions. BOUIN's fluid gave the most consistent and best results. ZENKER's, CHAMPY's and cold FLEMMING were also used occasionally. For all general purposes, BOUIN's fluid gives very satisfactory preparations of reptilian material. HEIDENHAIN's Iron Haematoxylin was used almost exclusively in staining, and only occasionally was it followed with a counterstain of Eosin or Licht Grün. Other stains used were EHRLICH's Haematoxylin, and MAYER's Phosphomolybdic Acid Haematoxylin, especially applicable with the younger specimens and for the demonstration of cell membranes.

It is well known that, in the developmental stages of reptilian embryos, there is a remarkable degree of variation. Collections of Sternotherus embryos during three summers have disclosed no entirely satisfactory way of designating the exact stage of any given embryo. It has been found best to group the embryos according to size and approximate age. The stages to be described are given in terms of the antero-posterior length in millimeters, as measured after fixation. The cervical and caudal flexures appear early, and measurements are made in terms of the distance between the points of flexure. Only representative stages are considered in the observations recorded.

C. Observations.

1. Origin of the primordial germ cells.

Embryos of approximately 72 hours after laying (1.6 to 1.75 mm. in length). These embryos are characterized by the presence of the open neural groove, and the first to the third somites. The head-fold is well-defined, and the amnion extends well over the embryo although its degree of development is somewhat variable (fig. 1).

The entodermal cells underlying the embryo proper anterior to the neurenteric canal have lost most of their yolk granules and cellular differentiation is progressing rapidly. In a typical transverse section just anterior to the neurenteric canal of an embryo of this stage (fig. 2), the entoderm may be divided into three regions: 1. That which underlies the embryo proper and extends laterally to the edge of the area pellucida — the definitive entoderm. It consists of a layer of one or occasionally two cells in thickness, and the cells are characterized by a noticeable absence of yolk granules. 2. Towards the margin of the area pellucida, the entoderm gradually increases in thickness, becoming from three to six cells deep. The cells in this intermediate zone contain numerous small yolk granules, which vary from 3 to 9 micra in diameter. 3. In the area opaca, the entodermal (vitellin entoderm) cells are much larger, with vacuolated cytoplasm and many large yolk granules. The yolk granules of the yolk-sac entoderm may sometimes even reach a diameter of 27.5 micra.

The intermediate region of the entoderm will be known as the *germ-cell zone*, for it is here that the germ cells are first recognized. An examination of the serial sections reveals the position of the germ-cell zone in relation to the blastoderm (fig. 1). From the neurenteric canal, the germ-cell zone extends cephalad on each side of the area pellucida to a point about one-third (500 micra) of the distance between the neurenteric canal and the cephalic end of the embryo. As the arms of the zone are followed anteriorly, they become smaller, taper off and disappear. In the region of the neurenteric canal, the lateral arms gradually converge posteriorly towards the median line, where they meet and become continuous for several sections at the ventral surface of the primitive plate. ALLEN (1906) already has described this region as horse-shoe shaped.

Fig. 1. Photomicrograph of a three somite embryo. The germ cell zone is drawn in outline in its approximate position with respect to the remainder of the embryo. (× 9.)

The individual cells of the germ cell zone are characterized by the presence of numerous yolk granules in the cytoplasm (fig. 9). This is their most pronounced character at this time, primarily because of the absence of the yolk granules in most of the other cells of the embryo
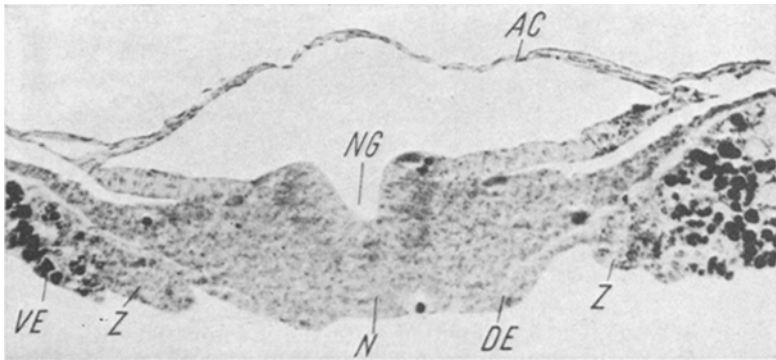

Fig. 2. Photomicrograph of a transverse section through the germ cell zone of a 1.75 mm. embryo (3 somites), showing the relations of the entodermal parts. (× 52.)

proper. Because of the crowded arrangement of the cells the cell outlines are irregular in many instances; the cell membranes are also more distinct than those of adjacent entodermal elements. The germ cells appear in compact groups, and entodermal cells, which are usually somewhat flattened so as to remind one of follicle cells (fig. 6), surround them. In cases where the cytoplasm may be seen, it is of a clear homogeneous nature, distinctly different from that of the smaller cells of various

other regions of the embryo in which the cytoplasm is more granular and more deeply stained. The nuclei of the primordial germ cells differ from those of the entodermal cells in the immediate vicinity of the germ cell zone mainly in their general spherical shape, in the presence of one or two large nucleoli, and in the more indistinct chromatin. The
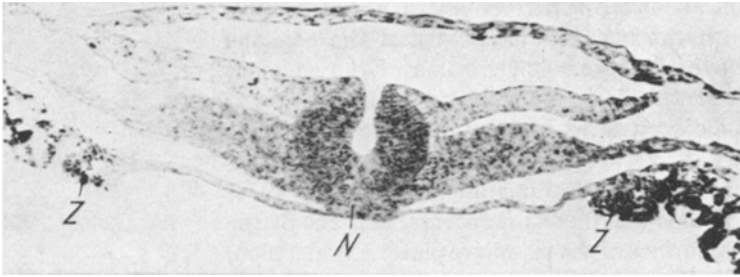

Fig. 3. Photomicrograph of a transverse section of a 5 somite embryo showing the position of the germ cell zones. They are now separated from the vitellin entoderm and lie in the definitive entoderm. (× 52.)

nuclei may be central in position but they are often crowded to one side of the cells by yolk granules. They are approximately 10 micra in diameter, while the cells themselves vary some in size but average about

Fig. 4. Photomicrograph as in Fig. 2, showing the presence of the germ cell zone on one side only. Note the haemoblasts in the entoderm of the same region. In adjacent sections, the isolated blood cells are connected with the entoderm. (× 52.)

17 micra in diameter. A primordial germ cell is compared with an entodermal cell of this region in fig. 7. Fig. 8 shows a cell from the ventral region of the primitive plate of an earlier stage, in which the cells are undifferentiated. Its character is very similar to that of the primordial germ cell.

Occasionally, the primordial germ cells are found in mitosis during this stage (fig. 6). During the earlier periods when embryonic cells are morphologically similar, cell division apparently occurs more or less uniformly in all regions of the embryo, except in certain growth centers where it is more rapid than in others, such as in the walls of the blastoporic canal, the dorsal lip of the blastopore, and the primitive plate.

Since numerous primordial germ cells are present, mitoses must have occurred among them. Their divisions in the early developmental stages are looked upon as constituting a primary period of multiplication.
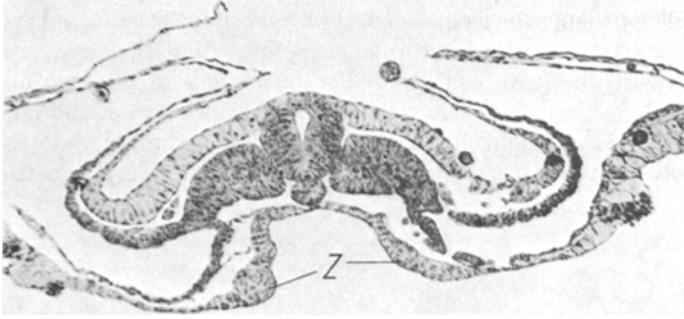

Fig. 5. Photomicrograph of a transverse section of an embryo of ten somites. The germ cells are located in two regions of the entoderm on either side of the median line. (×52).

Nearby, but lateral to the germ cell zone, certain cells, which are taken to be angioblasts, appear among the cells of the vitellin entoderm (fig. 12 and 13). The cells are small and their staining capacity is
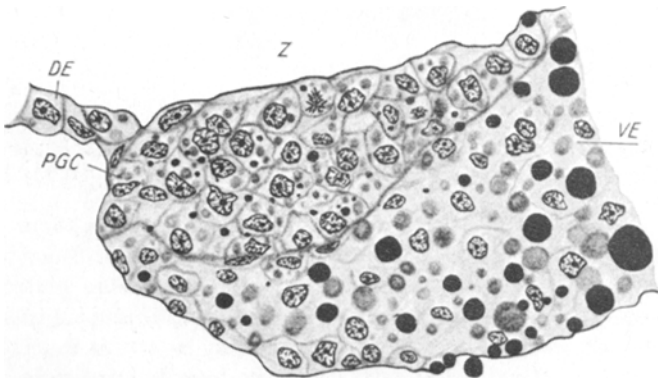

Fig. 6. Drawing of the germ cell zone of the right side of an embryo of 1.75 mm. *PGC* Primordial germ cell. *DE* Definitive entoderm. *VE* Vitellin entoderm. *Z* Germ cell zone. *A* Angioblasts. *AC* Amnion and Chorion. *N* Notochord. *NG* Neural groove.

decidedly more basophilic than that of the surrounding cells. They are grouped together exactly as the cells of the blood islands which are just appearing between the splanchnic mesoderm and the vitellin entoderm of the area opaca. Transitional forms between the cells of this region and primitive blood cells are also demonstrable. The cells at the edge of the pellucida undergo a decrease in size, become more basophilic in staining reaction, and increase in number by rapid cell divisions.

ALLEN (1906) states that, "The cord-like anlage of the red-blood corpuscles appears immediately above the sex-cell zone." He was undecided, however, as to the question of the origin of the angioblasts, although he was inclined to consider them as derivatives of the mesodermal layer. There is no evidence of the presence of blood cells within the area pellucida at this stage, except for the ones encountered in the margin in close relation with the germ cell zone. The blood cells of the area opaca are apparently derived from the mesoderm. The presence of the blood cells in the entoderm might be accounted for by assuming that they had migrated from the mesoderm into the entoderm. They find their way
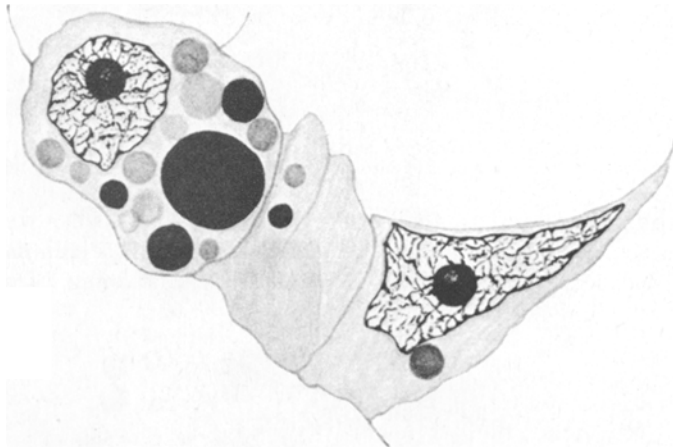

Fig. 7. A drawing of a primordial germ cell and a nearby entodermal cell of the same embryo as Fig. 6.

into the blood stream later, but at this particular time, there is little or no activity on the part of the angioblasts themselves. Since the cells in question are found among the deeper entodermal cells, ventro-lateral to the germ cell zone, and since transitional cells are present in this region, it appears that, in this form, the entoderm may be a contributing factor in blood cell formation. RÜCKERT (1906) has described an origin of blood cells from the entoderm in embryos of the Selachia and the Reptilia. JORDAN and FLIPPIN (1913), however, did not find vascular elements among the yolk-laden cells of the vitellin entoderm in turtle embryos.

2. *Migration of the primordial germ cells.*

(1) Embryos of 2.0 mm. total length (approximately 4 days following laying). In embryos of this stage, the neural groove is partially closed. The notochord is completely set off from the entoderm, and five somites are visible.

The germ cell zone is no longer closely attached to the large cells of the yolk-sac entoderm, but is separated from them by a few entodermal

cells which resemble the cells of the definitive entoderm between the germ cell zone and the median axis of the embryo (fig. 3). Little change occurs in the position of the germ cell zone in relation to the remainder of the embryo. The germ cells are still dividing although probably very slowly, judging from the number of mitotic figures present; it is, however, very difficult to distinguish a dividing primordial germ cell from an entodermal cell in mitosis. The separation of the germ cell zone from the vitellin entoderm in this stage is more likely due to the widening of the area pellucida rather than to an actual shifting of the germ cell zone as a mass toward the median line.

A variation which seems especially worthy of notice is the absence of the germ cell zone on the right side of an embryo of four somites. The germ cell zone of the left side is distinctly hypertrophied, probably compensating for its
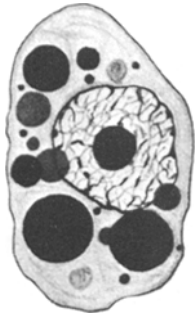
Fig. 8. Drawing of an early embryonic cell from the floor of the primitive plate of an embryo at gastrulation.
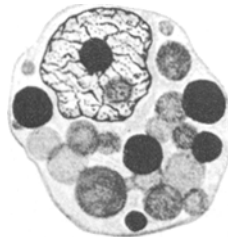
Fig. 9. Drawing of a primordial germ cell.

Fig. 10. Drawing of a primordial germ cell from the mesentery of a 25 somite embryo, showing amoeboid character.

absence on the opposite side (fig. 4). It is a common occurrence to find the germ cell zone of the left side of the embryo somewhat larger than the right one; the opposite condition has never been encountered.

The resting primordial germ cells are now very easily distinguished from the surrounding entodermal cells (fig. 11). They may be identified by their spherical, oval, or sometimes irregular contours, which make them stand out sharply in contrast to the elongated columnar elements of the entoderm proper. Definite cell membranes form cytoplasmic boundaries which allow no doubt concerning their identification. Irregularities in the cell outlines at this time are due to the compact arrangement of the groups of cells. The nucleus is almost spherical in shape, and is enclosed by a deep-staining nuclear membrane, showing a concentration of chromatin around the periphery. A fine granular network of chromatin extends irregularly throughout the nucleus and surrounds a slightly excentric nucleolus. Yolk granules are typical structures of the cytoplasm, but, in some of the cells they have already disappeared, and in many of them, they are now decreasing in number and size. A new structure appears in the cytoplasm as a crescent of deeply stained granules

in close juxtaposition to the nucleus, forming a nuclear cap at one pole of the cell. It represents the centrosphere, together with mitochondria and minute yolk granules, and will be designated as the attraction sphere. The attraction sphere can only be observed in those cells which have lost or are losing their yolk granules, and is correlated in some way with the disappearance of the yolk. It persists and forms a most valuable criterion for the identification of the primordial germ cells in the later stages.

(2) Embryos of approximately 2.5 mm. total length (about 6 days after laying). In embryos of about ten somites, the primordial germ cells have become somewhat separated in the anterior portions of the germ cell zone, although they retain a cord-like arrangement in the greater part
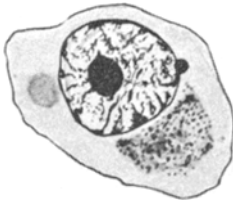
Fig. 11. Drawing of a primordial germ cell from the germ cell zone of a 2.0 mm. embryo. Note the large attraction sphere and the presence of small yolk granules.

Fig. 12. Drawing of an angioblast from an early embryo.

of their antero-posterior distribution. The germ cell zone appears as two cords, one on each side of the embryo. The cords are not continuous in every section, but the apparent segmentation does not in any way correspond to the segments of the embryo, and only calls to mind the "Gonotome Theory" of RÜCKERT (1888). The germ cells lie either singly or in groups in the entoderm about midway between the median axis and the margin of the area pellucida. They are found as far forward as the sixth somite and may be observed in almost every section as far back as the neurenteric canal where they become more scattered. There is still some indication of the posterior connection of the two lateral arms of the germ cell zone. Coincident with the elongation of the embryo, there has been a slight increase in the craniocaudal extent of the germ cell zone, which is now about 710 micra in length.

A comparison of the embryos at this stage of development shows that variation is quite extensive. Consequently, an exact position and distribution of the germ cells can not be accurately given. These differences are probably due to variations in the rates of growth and differentiation of the individual embryos. The folding of the splanchnopleure and the widening of the area pellucida results in a shifting of the germ cell zone towards the mid-line of the embryo (fig. 5). Generally speaking, the primordial germ cells lie in the entoderm on each side of the embryonic

axis between the median line and the outer edge of the area pellucida, and in the posterior third of the blastoderm.

(3) Embryos of 3.25 mm. total length and approximately 8 days after laying (14 somites). In embryos of fourteen somites, the most cranial germ cells are in the region of the tenth somite. The cord-like germ cell zones have become broken up and are less continuous than in early stages. The primordial germ cells are now often found as single cells in the entoderm; this presages the beginning of an independent migration of each individual cell. The breaking up of the groups of primordial germ cells is more pronounced at the anterior end of the germ cell zone than at the posterior, where the cords of cells still remain intact. The zones now extend over a distance of about 800 micra. Only occasional germ cells
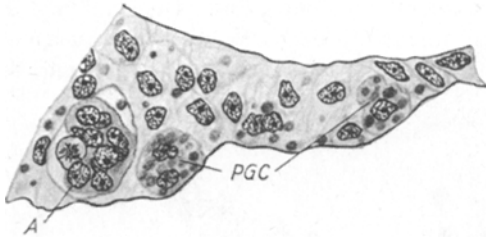

Fig. 13. Drawing of a portion of the entoderm of a 3-somite embryo, showing the primordial germ cells and the angioblasts in the germ cell region.
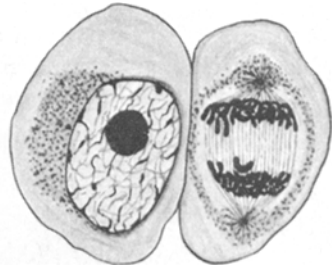
Fig. 14. Drawing of a resting and a dividing primordial germ cell from the genital ridge of a 5.0 mm. embryo.

appear in the vicinity of the neurenteric canal, and one has been found in the splanchnic mesoderm. DUSTIN (1910) and JORDAN (1917) have both seen primordial germ cells in the splanchnic mesoderm during the early migration stages, but this is apparently the result of a deviation from the normal migration path. There has been a progressive decrease in the yolk content of the germ cells, and in many of them there is no longer any trace of the yolk granules. The cells often have irregular outlines, indicative of an amoeboid character.

With the increase in the length of the embryonic axis, the region of the germ cell zone which extended across the embryo, connecting the two antero-lateral arms just posterior to the neurenteric canal on the ventral surface of the primitive plate, disappears. It has been stated by ALLEN (1906) that, in embryos of about this stage, there are no germ cells to be found in the yolk-sac entoderm of the blastoderm posterior to the embryo proper. He suggests several reasons for their absence: "This may be due to a folding forward of the entoderm of that region to assist in the closure of the alimentary tract, coupled no doubt with independent migration of the sex cells. — It may be possible that at least some of the sex cells may degenerate in situ or be transformed into somatic cells, although I think it unlikely that such is the case."

In order to answer this question if possible, I have examined these stages very carefully. It seems evident that the disappearance of the yolk-laden cells of this region is correlated with the elongation of the embryo posteriorly, and that it occurs before the formation of the hind-gut. Furthermore, the cells in the posterior region of the germ cell zone are still arranged in compact groups and have not reached a stage of independent migration. None of these cells appear to be degenerating. It is possible that the primordial germ cells may become entodermal cells of the hind-gut, but there is no evidence for such a view. It seems, rather, that the primordial germ cells of the posterior curve of the germ cell zone are forced to a lateral position as the embryo elongates. One embryo, in which the hind-gut has formed unusually early, shows the presence of a few of the primordial germ cells in the entoderm of the hind-gut, as

Fig. 15. Drawing of a degenerating primordial germ cell from the indifferent gonad of an 8.0 mm. embryo.

Fig. 16. Photomicrograph of a transverse section through the mesentery of a 4.9 mm. embryo, showing the passage of the primordial germ cells from the entoderm to positions in the mesentery and the peritoneal epithelium. (× 293.)

well as some in the yolk-sac entoderm posterior to the embryo. It seems unquestionable that growth factors involved in the elongation of the embryo are responsible for the lateral position of the primordial germ cells in the posterior part of the embryo.

(4) Embryos of 3.8 mm. total length and about 9 days after laying (18 somites). In eighteen somite embryos, the neurenteric canal is still open and the hind-gut may or may not have begun its development. The germ cells lie in the entoderm on both sides of the open gut below

the lateral walls of the aorta. They appear as two more or less closely packed groups of cells in the fold of the entoderm, which is being carried ventrally by the folding of the splanchnopleure. Some of the cells may lie in the entoderm as isolated units. The germ cell zones extend anteriorly from the neurenteric canal for a distance of about 1000 micra. No change is noted in the structure of the primordial germ cells.

(5) Embryos of 4.9 mm. total length (11 days incubation, 28 somites). In the 28 somite stage, the primordial germ cells have reached the median axis of the embryo, where they are scattered among the entodermal cells and in the dorsal mesentery. The mesentery is short and thick and is in the process of formation. Fig. 16 illustrates the migration of the primordial germ cells from the entoderm into the splanchnic mesoderm of the mesentery. A few of the germ cells are encountered as far forward as the tenth somite. Just posterior to the omphalomesenteric arteries they become very numerous. None of them are in mitosis, and practically no structural
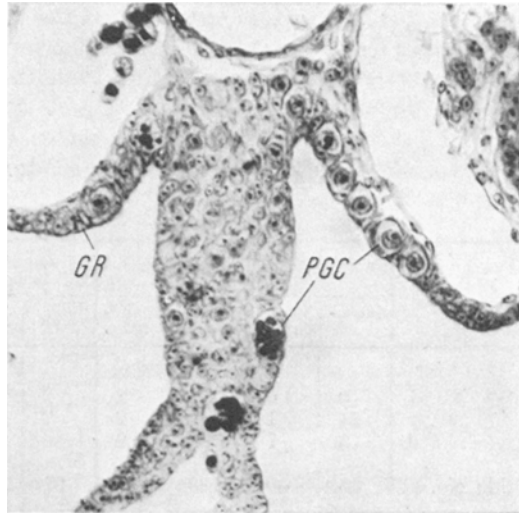

Fig. 17. Photomicrograph showing the primordial germ cells in the mesentery and in the genital ridges. Yolk granules may be either present or absent in the primordial germ cells of these latter stages. (× 293.)

changes appear in this stage. In embryos fixed in ZENKER's fluid and stained in MAYER's Phosphomolybdic Acid Haematoxylin, the cells stand out very clearly from the mesodermal elements of the mesentery. The cell outlines are distinct and definite; often irregular contours demonstrate amoeboid characters (fig. 10).

(6) Embryos 5.0 mm. in length from the cervical to the caudal flexure (about 12 days incubation). Important changes in the form of the embryos have occurred in the eleventh and twelfth days of the developmental period. The cervical flexure becomes accentuated and the caudal flexure appears. A single section through the mid-gut region shows the presence of primordial germ cells in the entoderm, in the elongating dorsal mesentery, and in the genital ridges which are just beginning to appear. The genital ridges are made up of one or two layers of epithelial cells among and beneath which the primordial germ cells are

located (fig. 17). Mitotic figures in the peritoneum and in the underlying mesenchyme indicate the beginning of the formation of the indifferent gonads. The mesenchymal cells are few in number, apparently due to the presence of the large subcardinal veins immediately dorsal to the genital ridges.

Statistical studies of the number of primordial germ cells have heretofore proved relatively unprofitable, but they have shown that there is an extreme range of variation. ALLEN (1907) found the number to vary from 302 to 1744, averaging about 700 for each embryo. DUSTIN (1910) and JORDAN (1917) have found the number to be much smaller, the average being about 300. In the first embryo counted by the writer, approximately 984 were found, while in the second, there were 1532. Table I illustrates the distribution and approximate number of primordial germ cells in the embryo under discussion. Because of the large variation, only two counts were made.

Table 1.

Embryo No. 109	Gut		Mesentery		Genital Ridges		Abnormal in Position		Total
	Ento-derm	Mesen-chyma	Meso-thelium	Mesen-chyma	Left	Right	Position	No.	
Anterior									
Slide No. 8	0	0	2	2	2	1		0	7
Slide No. 9	11	1	7	2	7	9		0	37
Slide No. 10	5	1	28	29	46	41	Mesenchyme of Body	3	153
Slide No. 11	45	40	130	264	91	78	Near Aorta	1	648
							Sympathetic Ganglion	1	
							In Aorta	1	
							mesenchyme of Body	2	
Slide No. 12	193	153	33	210	45	44	Coelom	2	680
Slide No. 13	2	2	0	1	1	1		0	7
Posterior									
Total	256	207	200	508	192	172		10	1532

Table illustrating the number and distribution of the primordial germ cells in an embryo of 5.0 mm. in length from the cervical to the caudal flexure. Approximately 42 sections at 10 micra per slide.

Primordial germ cells are found in mitosis as soon as they reach the vicinity of the genital ridge (fig. 14). Some of them are in the early prophases of divisions while still in the dorsal part of the mesentery. The small number of mitotic figures encountered in the mesenteric region renders it probable that divisions of the primordial germ cells are relatively infrequent during the migration period. The resumption of cell division, although slow at first marks the beginning of a so-called secondary period of multiplication.

Certain of the primordial germ cells are found in distinctly abnormal positions (Table 1). These will be considered more fully later, since they are more commonly observed after the majority of the germ cells have reached the developing sex-glands.

3. *The germ cells of the indifferent gonads.*

During the early formative period of gonadogenesis, the morphological pictures are complicated to a marked degree by the development of the numerous blood vessels of the mesonephric region, the mesonephroi and their glomeruli and capsules, the appearance of the urogenital connections, and the adrenal tissues. All of these, together with the growing genital ridges and the mesenchymal elements, contribute to the confusion which exists concerning the problem of early gonado-genesis and the origin of the various elements of the germ-glands. This has been pointed out by FIRKET (1914), who says: «La principale cause de l'obscurité qui règne encore sur cette question réside dans le peu de netteté des images que l'ébauche présente.» The indistinct appearance of the numerous cord-like lumenless structures of these early stages has resulted in as many conflicting views on the origin and growth of the urogenital connections and the sex-gland components as there are possibilities. The observations recorded here are concerned primarily with the primordial germ cells and their subsequent history during the indifferent period of gonadogenesis, which signifies that period of development during which the genital ridges proliferate and enlarge before the appearance of definite sex characters, such as sex-cords, spermatogonia or oogonia, etc. Briefly stated, the period constitutes one in which the genital ridges undergo an organization into definite medullary and cortical primordia in preparation for sex-differentiation.
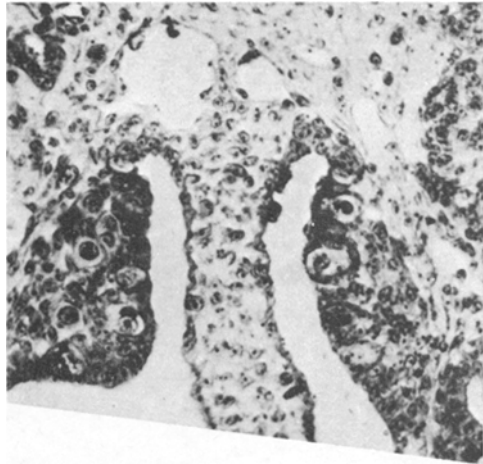

Fig. 18. Photomicrograph of a transverse section through the indifferent gonads of an embryo of 6.0 mm. length (cervical to caudal flexure). Note the definite cell outlines of the primordial germ cells and the presence or absence of yolk granules, also the absence of degenerating germ cells or the transformation of epithelial elements into germinal cells. (× 208.)

(1) Embryos of 5.0 to 7.5 mm. in length (cervical to caudal flexure; 12 to 18 days after laying). Except for occasional mitoses, the primordial germ cells are relatively inactive during this period of germ-gland

organization; at least they show no outstanding morphological modifications. They retain the same clear cytoplasm with definite cell boundaries, the characteristic nuclear shape and number of nucleoli, the attraction sphere, and other characters which have designated them as cells of a special and distinct type from those of the mesonephric region and the coelomic epithelium. Some yolk granules are retained by some of the germ cells throughout the entire period, and some have been found with yolk granules in much later stages (embryos in sex differentiation — 11.0 mm. carapace length). A few cells are apparently unable to
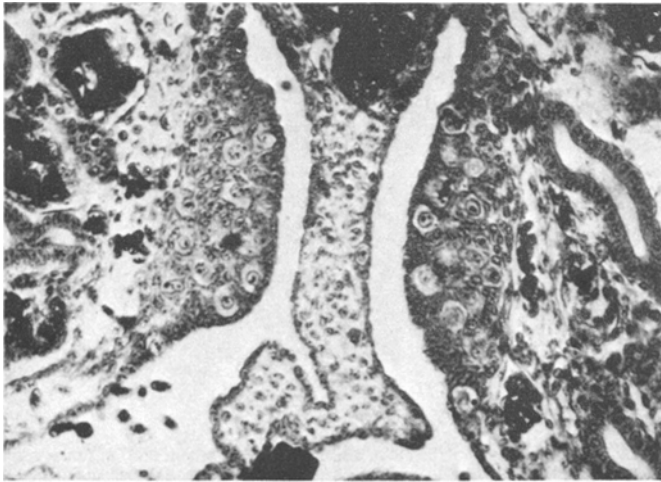

Fig. 19. Same as 18, but of an embryo of 7.5 mm. length. The primordial germ cells are about equally distributed in the epithelial layer and in the inner portion of the gonad. (× 208.)

assimilate the yolk and carry on; these undergo degenerative changes (fig. 15). The degenerating cells are never very numerous, however, and degeneration of the primordial germ cells is not a general feature of this period of development, as postulated by some authors [DUSTIN (1910) and SIMKINS and ASANA (1930)].

The most prominent change during this period is the increase in the size of the genital ridges (figs. 18 and 19). Cell divisions occur in large numbers in the germinal epithelium and in the underlying mesenchyme, producing a thickening of the germinal epithelium, which becomes two or three cells deep. It appears as if cells from the epithelial layer break away and enter the inner portion of the dense mass of cells and there is no very definite line of demarcation between the epithelial and inner portions. Whether additional cellular elements are added to the complex from the mesonephric tissue is extremely difficult to determine, because of the great similarity of all of the elements

including the mesenchymal cells of the region. It seems likely that both sources contribute to a certain extent, but the problem will be dealt with in more detail in a later paper of this series. At the beginning of the indifferent period, the germinal ridge extends posteriorly over ten or eleven somites, beginning cranially in the region of the eleventh primary Malpighian corpuscle of the mesonephros. Toward the end of the period, they are slightly longer, and can be measured upon dissection under the binoculars; they are then about 1.1 mm. in length. There is practically no appreciable difference in the width of the ridges until somewhat later, and no differences in the general size and appearance of the left and right gonad anlage have been observed.

Among the increased number of cells of the indifferent gonads are numerous primordial germ cells. The additional ones are the results of divisions of early arrivals in the gonadal region, and many of them are newcomers from the entoderm by way of the dorsal mesentery. The migration is entirely completed by the end of this period, at which time the primordial germ cells are about equally distributed among the cells of the thickened germinal epithelium and the cells of the inner medullary portion of the gonad (fig. 19). There is no evidence that indicates a transformation of cells of the germinal epithelium into "primary germ cells", as SIMKINS and ASANA (1930) and SIMKINS (1925) have attempted to demonstrate.

D. Literature and Discussion.

1. Historical résumé.

In 1870, WALDEYER was the first to advance a theory of the embryonic origin of vertebrate germ cells. He observed the presence of large spherical cells in the coelomic epithelium of the genital ridge of a chick embryo, and believed them to be early developmental stages of ova ("Ureier"). The peritoneal epithelium of this region of the coelom was designated by him as the "Keimepithel", or germinal epithelium, from which the so-called germinal epithelium theory of germ cell origin has taken its name and significance. Numerous investigators have ardently given their support to the idea that the germ cells are derived from the mesodermal elements of the peritoneal or germinal epithelium of the growing gonads. Most of these have made their investigations upon mammalian forms (HARGITT, SIMKINS, STIEVE and others), although not exclusively so. In fact, some of the authors [WALDEYER himself (1903), FELIX and BÜHLER (1906), and FELIX (1912)] suggest that the germ cells may arise earlier in vertebrates below the mammals phylogenetically, but insist that, in mammals, the germ cells arise only from the epithelium of the germ-gland.

Evidence favoring an early segregation of germ cells has slowly, been accumulating. In certain invertebrates, Sagitta [O. HERTWIG (1880)]

Ascaris [BOVERI (1887)], certain Diptera, Coleoptera, and Hemiptera [HEGNER (1914), HASPER (1911)], Drosophila [HUETTNER (1923)], and many others, it has been possible to show that the germ cells are derived from cells of early cleavages. In the vertebrates, studies in cell lineage are much more difficult, and the material does not lend itself to such accurate demonstration of the history of single cells in early cleavage stages. EIGENMANN (1896) believed that in Cymatogaster the close similarity in size and structure of the germ cells to the blastomeres of the 32-cell stage indicated a segregation at that time. It has been adequately demonstrated that the germ cells of vertebrates, at least those below the mammals phylogenetically, originate extraregionally and migrate to the sex-gland anlage. The, are usually located in some special zone of entoderm or mesoderm, and are distinguished in early stages principally by their location and the presence of large numbers of yolk granules. It is impossible to mention here all of the investigators who have favored the theory of early segregation. NUSSBAUM (1880), WEISMANN (1885), BEARD (1904), WOODS (1902), ALLEN (1906, etc.), KING (1908), WITSCHI (1914), SWIFT (1914), OKKELBERG (1921), SWINGLE (1925), HANN (1927), GOLDSMITH (1928), and CHENG (1932), as well as many others have given accounts which are interpreted as favoring early segregation.

Many modifications of the above theories have been presented. While some workers (RUBASCHKIN, ALLEN, WITSCHI, OKKELBERG, HANN, SWINGLE and others) have been able to show that the primordial germ cells actually give rise to the definitive germ cells of the adult, others have assumed that such is the case without examination of later developmental stages. Or some [FIRKET (1920), McCOSH (1931)] believe that a few of the primordial germ cells may possibly become definitive gonia, but that the majority of the definitive germ cells are derivatives of mesodermal elements of the germinal epithelium. VON WINIWARTER et SAIMONT (1909), KINGERY (1917) and HARGITT (1926) believe that the so-called primordial germ cells are only transient structures that later degenerate; the definitive germ cells are then derived from the germinal epithelium. KUSCHAKEWITSCH (1910) thinks that, in Rana esculenta, the oogonia are derived from the primordial germ cells, but that the spermatogonia are transformed mesodermal cells from the axial mesenchyma. VON BERENBERG-GOSSLER (1914) questions the identity of the primordial germ cells as germinal elements, and concludes that they are merely entodermal wandering cells which participate in a late period of mesoderm formation. According to GATENBY (1916), there is a seasonal transformation of peritoneal cells into definitive germ cells in Rana, although later (1924) he admits that the primordial germ cells may form some of the definitive reproductive elements. BUTCHER (1928) found that both primordial germ cells and

cells from the germinal epithelium form the definitive germ cells of the lake lamprey.

Many excellent reviews of the literature have been made during the past ten years. The reader is referred to OKKELBERG (1921) and HEYS (1931) for more detailed references and literature lists concerning the general problem.

The very early investigators of reptilian material were primarily interested in the general developmental history of the reproductive organs rather than in the germ cell history, and only cursory attention was given to the problem of germ cell origin. BRAUN (1877), working on embryos of Lacerta agilis and Anguis fragilis, observed large cells, "Primordialeier", in the germinal epithelium, from which he believed they were derived. VON MIHALKOVICS (1885) also observed the same cell types in Lacerta agilis, but he called the large cells "große Geschlechtszellen", pointing out that it was impossible to distinguish whether or not they were primitive ova. HOFFMANN (1889), also working on Lacerta, observed large cells in the peritoneal epithelium of the splanchnopleure in very young stages, but was unable at that time to trace them to the sex-gland anlagen. This is apparently the first intimation that, in the Reptilia, the large cells of the germinal epithelium might have an extraregional origin.

In 1906, B. M. ALLEN published the results of an investigation on the origin of germ cells in the painted turtle, Chrysemys marginata. In a 1.7 mm. embryo, he was able to locate the primordial germ cells in the entoderm between the area pellucida and the area opaca, entirely outside of the embryonic area and opposite the posterior third of the embryo. The primordial germ cells are readily distinguished from the surrounding entodermal units by their large nuclei with a single nucleolus, their clear cytoplasm and well-defined cell boundaries. Another important characteristic for their identification is the presence of yolk granules in the cytoplasm. In later stages, the cells migrate toward the median line of the embryo, where they enter the mesentery. The migration of the primordial germ cells continues dorsally through the mesentery to the genital ridge. He was able to show that these migrating cells persist through the later stages to give rise to the definitive germ cells of the adult. In a later paper (1907), ALLEN describes a large variation in the number of germ cells present in each embryo. It was possible to determine that a more restricted range of variation occurred in embryos of the same parentage than in embryos from different parents, thus suggesting that variation in germ cell number is possibly hereditary in nature.

JARVIS (1908) described an early segregation of germ cells in Phrynosoma cornutum. However, only two stages of development were considered, and they show no fundamental differences from the condition

in Chrysemys. No transitional cells between purely entodermal cells and primordial germ cells were observed. The migration path agrees with that described by ALLEN. GASPARRO (1908) demonstrated the presence of the large migrating primordial germ cells in embryos of *Gongylus ocellatus,* and traced them from a position in the entoderm to their final location in the indifferent gonads, thereby confirming the results of ALLEN.

Although DUSTIN (1910) confirmed ALLEN's observations on Chrysems, his conclusions differed from those of ALLEN. After studying the embryonic stages only up to the embryo of 8.5 mm. in length, he concluded that the primordial germ cells degenerate and are replaced by peritoneal cells which become the definitive germ cells of the adult, making two generations of germ cells. Controversial papers were published by DUSTIN (1911) and ALLEN (1911). Because of the completeness of ALLEN's investigation, it must be given the greater attention.

VON BERENBERG-GOSSLER (1914) has drawn some unique conclusions after a study of the migrating primordial germ cells of *Lacerta agilis.* According to him, the cells in question are not to be considered as germ cells at all, but as mesoderm-forming cells. He interprets the migration of the so-called primordial germ cells from the entoderm as a method of late mesoderm formation, and presents evidence for such a view. Instead of finding all of the "entodermal wandering cells" entering the sex-gland anlage as germ cells, he has observed many of them contributing to the formation of the WOLFFIAN duct, which is in itself a unique condition. This conception must be discarded much as the "Gonotome Theory" of germ cell origin [RÜCKERT (1888)] has been.

According to H. E. JORDAN (1917), the origin and migration of the primordial germ cells in *Caretta caretta* follows the same general plan as described by ALLEN, DUSTIN, JARVIS and GASPARRO. The primordial germ cells migrate from the yolk-sac entoderm in to the lateral border of the area pellucida on each side of the embryonic disc during the second day. By the beginning of the third day, they become segregated into two bilateral cords situated in the entoderm of the area pellucida. With further development, these cords migrate toward the median line of the embryo where they enter the mesoderm of the mesentery or the splanchnopleure. The migration continues to the end of the sixteenth day, when the majority of the sex cells are found in the undifferentiated germ-glands.

In a more recent paper, SIMKINS (1925), working in Trionyx (species not mentioned), has reopened the question. He states that there is no evidence to indicate an extraregional origin of primordial germ cells in this species. After a survey of embryonic stages up to hatching, he concludes that, "The gonocytes arise from the fundaments of the sex-glands during the development of the mesonephros, either from the

stroma or from the germinal epithelium." This is the only case in which the large yolk-laden and migrating cells have not been found in reptilian embryos, except for the early reports of BRAUN and MIHALKOWICZ, who were unaware that such cells existed. VON BERENBERG-GOSSLER was later successful in finding them in *Lacerta agilis*. SIMKINS' conclusions were arrived at apparently without reference to an earlier paper by ALLEN (1904), in which ALLEN states that he found the large primordial germ cells seemingly migrating through the splanchnopleuric mesoderm to the point where the germ-glands are about to develop. These cells were observed by ALLEN in a 3 mm. embryo of Trionyx, the same stage of the same form in which SIMKINS was unable to find them.

Later, on the germ cells of Calotes, SIMKINS and ASANA (1930) describe the occurrence of certain large yolk-laden cells in the mesentery and in the genital ridge, which they call "Nurse-cells". The origin of the "Nurse-cells", which are probably none other than the primordial germ cells of other authors, has not been traced in stages previous to the appearance of the genital ridge. According to these authors, the "Nurse-cells" degenerate in the third week of incubation without undergoing mitosis or without giving rise to any germinal elements. The primary germ cells of the embryonic gonads are described as differentiating from the cells of the germinal epithelium. They say: "The preponderance of opinion now supports the contention of WALDEYER that the reproductive elements arise from the germinal epithelium, by a process of differentiation of somatic cells into germ-cells." From the above review of the literature regarding the germ cells of Reptiles, it is quite obvious that such a broad statement cannot be applied to the status of the problem in reptilian species, since it is not in agreement with any of the recorded evidence.

2. The origin of the primordial germ cells.

The time, place, and manner of origin of the primordial germ cells in *S. odoratus* corresponds closely to that described by ALLEN, DUSTIN, JARVIS and JORDAN. Before the primordial germ cells can be distinguished, a localized region of relatively undifferentiated embryonic cells, which resemble in all characters the cells of the primitive knot, is located in the entoderm. This region has been designated as the germ cell zone; it surrounds the posterior part of the embryo and continues anteriorly on both sides along the zone of junction of the area pellucida and the area opaca. JORDAN (1917) has observed that little difference exists between these elements in the early stages. He states that "the resemblance signifies, most probably, a similar low grade of differentiation from the original blastomeres. — These two types of cells are similar because they are practically undifferentiated". OKKELBERG (1921), working on the lamprey Entosphenus, has said that, "In one sense,

all the yolk-bearing cells of the entoderm may be considered as undif-
ferentiated cells." The loss of yolk from the entodermal cells between
the bilateral germ cell zones produces changes which distinguish them
from and demonstrate the presence of the still undifferentiated pri-
mordial germ cells. Likewise, certain changes in the cells of the yolk-sac
entoderm lateral to the germ cell zones at about the same time makes
it possible to recognize the small group of primordial germ cells when
seen in cross-section. It seems that the time when the primordial germ
cells first appear is closely associated with the assumption of a nutritive
function upon the part of the cells of the yolk-sac entoderm, and the
loss of nutriment from the cells of the embryo proper. Practically all
authors have regarded the presence of yolk granules in the cytoplasm
of the primordial germ cells for relatively long periods as a manifestation
of their early origin and primitive condition. It is most certainly true
that a description of the primordial germ cells at the earliest stage at
which it is possible to recognize them, would also apply to many cells
of the still earlier stages, particularly the cells of the primitive plate
and the floor of the posterior part of the blastoporic canal. It is quite
apparent that certain cells remain localized and relatively inactive
during early differentiation. When these cells are ·followed in their
later history, they may be traced to the sex-gland anlage, where they
form the reproductive elements of the early gonads. The evidence derived
from this study must be interpreted as favoring the idea of an early
segregation and extraregional origin of the primordial germ cells.

The work of VON BERENBERG-GOSSLER (1914) on the lizard has
been criticized by JORDAN (1917), who states that VON BERENBERG-
GOSSLER's observations and conclusions are unique in the literature
on germ cell origin. According to VON BERENBERG-GOSSLER, the major-
ity of the migrating entodermal elements become transformed into
mesodermal cells and thus disappear. However, he also was of the
opinion that some of the wandering cells found their way into the peri-
toneal epithelium of the genital ridges, and there gave rise to definitive
germ cells. The inclusion of the migrating cells in the WOLFFIAN duct,
as described by him, was probably due to an individual abnormal distri-
bution, since it does not conform to any accepted idea of the formation
of the duct. In Sternotherus, there is certainly no great tendency for
the primordial germ cells to be found in places very remotely removed
from the genital ridges, except previous to and during the very early
migration stages, when they are still in the entoderm. No evidence that
the primordial germ cells transform into somatic cells has appeared in
our preparations.

While VON BERENBERG-GOSSLER and DUSTIN (1920) were both able
to identify the primordial germ cells in their migration, and even though
their interpretations of the significance of the migration and the origin

of the definitive germ cells were at variance with other authors, SIMKINS' opinion that the migrating cells are not primordial germ cells and never become reproductive elements seems to be unsupported in the literature. The evidence that somatic cells of the germinal epithelium transform and differentiate into cells of reproductive function seems to be inconclusive and quite untenable in view of the imposing array of data in favor of the opposing theory.

3. Criteria for the identification of the primordial germ cells.

The appearance of the so-called primordial germ cells in an extra-regional position outside of the embryo and far removed from the genital ridge has led some investigators to conclude that they are not germ cells. VON BERENBERG-GOSSLER has spoken of them as entodermal wandering cells which move to various locations and eventually form mesodermal elements. SIMKINS believes that they have been confused with early blood cells which, according to him, agree with the usual descriptions of the primordial germ cells. He states, however, that the nuclei of the blood cells are smaller than the nuclei of cells of other tissues, an entirely correct observation. The small nucleus of the hemo-blast is one of the best possible characters on which to base the distinction between blood cells and the primordial germ cells, as well as other cells. The statement of JORDAN, that "...no confusion between blood-cells (hemoblasts) and germ cells seems possible", is likewise applicable to my material.

SIMKINS has made statistical studies of the diameters of nuclei of cells in various parts of the embryo, especially in the mesentery, and has based his measurements upon diameters only. His observations that "At no time is there a variation of more than 1 micron — a figure well within the limit of error. There is no evidence from the relative size of the nuclei that germ cells ever pass through them." are probably correct insofar as the measurements are concerned. However, the data as presented are too incomplete to permit the conclusions which have been drawn. It is difficult to understand how SIMKINS' measurements of the diameters of nuclei of entodermal or mesodermal cells were made, for there is a pronounced tendency of the nuclei of these two types of cells to be elongated or oval in shape. Whether the long or the short diameters were measured is not stated. Attempts have been made in this study to verify the data obtained by SIMKINS by the use of his method, but too many uncertainties are involved to render it of value. If the long diameters of the mesodermal elements were used, there would probably be no differences between them and the diameters of the nuclei of the primordial germ cells. The only accurate statistical method of determining differences in the sizes of the nuclei of mesenteric and primordial germ cells would rest in the computation of the nuclear volumes. In embryos of 5.0 mm. in length, the nuclei of the primordial

germ cells compare favorably in size with those of the entodermal cells, and both are decidedly larger than the nuclei of the mesodermal cells of other embryonic regions. It is probable that the failure of SIMKINS (1925) to recognize the primordial germ cells during the migration stages was largely due to the method of identification used. He apparently neglected the cytoplasmic characters, which are decidedly more valuable in distinguishing the germ cells from other cellular units of the embryo than are the nuclei, just as they are more valuable in identifying any other type of cell. Differences in nuclear size in the germ cells and in other cells of the embryo do not form good criteria for their recognition as distinct units.

The criteria which have been most widely used in the identification of the primordial germ cells are mainly cytoplasmic in nature. The presence of yolk granules in the cytoplasm in early stages and the retention of the yolk material for a long period has been cited as evidence that the cells are relatively undifferentiated. The clear cytoplasm and definite cell membranes are both definitive characters. The criticism of JORDAN's figures by SIMKINS, who says that the cell membranes are indistinct, does not seem justifiable. SIMKINS' (1925) own figures (see his fig. 14), illustrating the indefiniteness of the cell membranes of the germ cells found in the developing gonad, seem to demonstrate a clear demarcation of the lighter staining cytoplasm of the germ cells from the more deeply staining cytoplasm of the somatic cells. If such definite differences in the cytoplasmic portions of the two types of cells exist as figured, limiting boundaries appear to be present and must be interpreted as such.

The primordial germ cells of a 2.0 mm. embryo show the appearance of an additional cytoplasmic structure, the exact nature of which is uncertain. It has been called the attraction sphere. SWIFT (1914) used the attraction sphere as one of the useful characters in the identification of the primordial germ cells of chick embryos. JORDAN (1917) also demonstrated the presence of an attraction sphere in the primordial germ cells of the Loggerhead Turtle after FLEMMING's fixation. He does not, however, show the attraction sphere in his figures of the germ cells of young embryos, nor does he mention its occurrence. His attraction sphere apparently refers to the central bodies and centrosphere only, and not to a densely granular portion of the cytoplasm which lies in a close juxtanuclear position, as I have used the term. It has been impossible for me to identify the attraction sphere in stages earlier than the 5-somite embryo. Whether this is due to the methods used, or to the presence of the numerous yolk granules in the earlier stages has not been determined. It seems probable that the formation of the attraction sphere is correlated with the disappearance of the yolk, since the last traces of the yolk granules are always within the granular crescent of

the attraction sphere. The structure is of great importance in distinguishing primordial germ cells from other embryonic cells, because of its large size and its prominent appearance.

BOUNOURE (1927, 1929) has described a similar structure in the primordial germ cells of Rana temporaria and calls it the "chondriome". By using it as a criterion for identifying the primordial germ cells, he claims to have traced the primordial germ cells to earlier stages than have previous investigators who have used more ordinary methods. In regard to the nature of the juxtanuclear crescent, he states that, «Pour le moment, on peut supposer qu'il s'agit d'un cytoplasme spécial, riche en substance lipoidiques, structuré primitive homogène et dans lequel s'organiseraient peu à peu des mitochondries», SWIFT (1914) has noted that a certain relationship seems to exist between the attraction sphere and the distribution of the yolk granules, which arrange themselves about the attraction sphere as they decrease in size. The diplosome forms a part of the attraction sphere, and appears to vary in its relation to the granular portion and to the nucleus. With the decrease in the amount of yolk, the granular crescent becomes more prominent and distinct than in somatic cells. BOUNOURE's description of the "chondriome" is probably applicable to the attraction sphere present in the primordial germ cells of the birds and reptiles.

Numerous investigators have compared the mitochondria of the germ cells with those of the somatic cells in an attempt to show that the mitochondria of the germ cells are of a specific form and arrangement. RUBASCHKIN (1910, 1912) was the first to state that the mitochondria of the germ cells were in the form of small granules, while those of the somatic cells were thread-like in character. TSCHASCHIN (1910) came to the same conclusions with regard to the germ cells and somatic cells of the chick. VON BERENBERG-GOSSLER (1912), SWIFT (1914) and FIRKET (1914, 1920) were unable to confirm these observations. KINGERY (1917) was also unable to find definite mitochondrial characteristics which separated the primordial germ cells from cells of non-reproductive function. RAUH (1928), who worked on the rat, believes that the mitochondria and the chondriome are both good criteria for the identification of germ cells. He states that, „Sie haben ein charakteristisches Chondriom, welches nur körnige Bestandteile (Plastochondrien) aufweist. Das Chondriom ist durch seinen Formenzyklus ein relatives Merkmal der primordialen Genitalzellen und ihrer Abkömmlinge." His conclusions are in direct opposition to those of HARGITT (1925, 1926), who claims that no primordial germ cells appear, and that the definitive germ cells are produced from the germinal epithelium after the degeneration of several previous generations of germ cells.

It is possible to identify the primordial germ cells only by the use of the sum total of all the characters of the cell, namely: its large size

and definite cell boundaries; its prominent nucleus, the size of which is accentuated by the clear lightly staining cytoplasm; the attraction sphere and chondriome; the yolk granules; and the position of the cell in the embryo.

4. The migration of the primordial germ cells.

The fact that, during progressive embryonic stages, the primordial germ cells appear first in the extra-embryonic entoderm, in the entoderm of the median line of the embryo proper, then in the splanchnopleure as well as in the mesenteric mesoderm, and finally in all of the above positions and the genital ridges, indicates that a migration and shifting of position does actually occur. The above statement describes the actual route of migration, which agrees closely with that described by previous authors for other turtles.

Although the actual manner of migration has never been observed, there are certain indications that at least two factors are involved in the action. VON BERENBERG-GOSSLER emphasizes the fact that migration is passive, resulting from a mechanical process of unequal growth. HUM-PHREY (1925), on the basis of his conclusions that the primordial germ cells of all vertebrates are derived from the germ ring in the Amphibia, or the equivalent blastodermic margin in the Reptilia and Aves, believes that the mechanical growth factors of various forms are responsible for the diverse situations of the primordial germ cells in the vertebrates at the time when the cells are first recognizable as such. To be more specific, the mechanism of gastrulation and germ-layer formation in different vertebrates determines the primary position of the primordial germ cells at the time of first discovery. He believes that migration of germ cells is due to "growth shiftings of related parts". Undoubtedly growth is an important factor in the change in position of the primordial germ cells, particularly during the early stages. It has been shown that the primordial germ cells of reptiles are located in the entoderm as two bilateral cords. It seems impossible that these cords would be able to move as a whole through the entoderm toward the mid-line. Certain growth processes can account for the apparent change in position of the cords during the early migration. The widening of the area pellucida and the recession of the area opaca together with the lateral growth of the embryo proper produces an apparently closer location of the germ cell cords to the median axis. In reality when the distance between the two germ cell cords is measured, little difference exists. The bending inward and downward of the splanchnopleure to form the embryonic gut may also assist in accounting for a closer position of the cords. How-ever, these processes do not seem to account for the breaking up of the cords, and for the transposition of the germ cells from the entodermal layer to the mesodermal cells of the elongating mesentery. A somewhat new conception of the origin of the gonad primordia is presented by

DANTSCHAKOFF (1931), who speaks of „die Verschiebung der Gonaden-anlage als Ganzes". She states that, in the chick, „Die Gonadenanlage bildet sich im Mesoderm der Splanchnopleura, also im ventralen Blatt des Mesoderms aus. In ihrer endgültigen Lage nimmt sie den medialen Teil der Somatopleura ein, somit liegt sie im dorsalen Blatt des Meso-derms". In explaining the changing position, which assists in the trans-portation of the primordial germ cells along the borders of the mesentery, she says, „Dagegen wird die endgültige Lage der Gonadenanlage durch Wachstumsvorgänge der Mesonephroi und des Darms bedingt". Al-though this introduces the idea that the gonad primordia or genital ridges are present in earlier stages than heretofore accepted, it may be a more true representation of the actual state of affairs than the older belief that the genital ridge first appears on the ventro-medial wall of the mesonephros. If such is the case and it is applicable to the rep-tilian embryo as well as the chick as would be expected, the migration of the gonad primordia from a position in the splanchnopleure along the mesentery dorsally to the mesonephric wall adequately explains the process of migration of the primordial germ cells through the mesen-tery.

As indicated above, it seems necessary to look for some other factor to account for certain stages in the migration. ALLEN, JORDAN, SWIFT, and others have suggested that an inherent factor of the primordial germ cells themselves, amoeboid movement, accounts for the migratory nature of the cells. There is considerable evidence that an amoeboid movement actually occurs, especially in the chick embryo, where the migration of the primordial germ cells via the blood vessels allows the best observational possibilities. ALLEN (1906) and JORDAN (1917) both believe that the irregular shape of the primordial germ cells and the compressed nature of the cells surrounding them indicates their amoeboid nature. JORDAN figures two such cells (see his fig. 3, aa and ba). WOOD-GER (1925) has demonstrated pseudopodia of the primordial germ cells of the chick, and so has DANTSCHAKOFF (1931). SWIFT (1915) believes that different numbers of primordial germ cells in the assymetrical ovaries of the chick give positive proof that the cells migrate to the left side in preference to the right. It is in reality most difficult to prove that an amoeboid movement does occur, since the usual fixing fluids do not, as a rule, preserve pseudopodial processes. It seems probable, and there is some evidence in the irregular character of some cells, that an amoeboid factor plays an important part in the migration. On the evidence cited above, it seems that both embryonic growth changes and inherent capacities of the primordial germ cells for migration are the factors which produce the translocation of the primordial germ cells from their extraregional positions to their place of residence in the germ-gland anlage.

5. Multiplication and degeneration of primordial germ cells.

It has been observed by SWIFT (1914) that the primordial germ cells divide before as well as during the migration stages. BECCARI (1921), in anurans, has also observed them in division before the migration begins. In *S. odoratus*, the primordial germ cells divide before the migration begins. However, it is very difficult to distinguish a dividing primordial germ cell from entodermal cells in mitosis. Although JORDAN (1917) has apparently observed divisions during the migration through the mesentery, I have been unable to determine that divisions do occur at that time. Towards the end of migration, divisions are relatively frequent in the dorsal part of the mesentery and the genital ridge. Appa-' rently there is a slowing up of divisions during the migration period, which might be explained by assuming that the cells are utilizing their available energy for the movements required in moving through the tissues.

The primordial germ cells of the indifferent gonads have been observed to undergo no changes other than that of normal mitotic division and an occasional degeneration. At the end of the indifferent period the yolk granules have practically disappeared, but there are a few cells which retain the yolk until later stages. Up to this time there is no difficulty whatsoever in distinguishing the reproductive elements of the germ-gland from the somatic or epithelial elements. No intermediate forms appear. Even SIMKINS (1925) has been unable to demonstrate transformation stages between the cells of the germinal epithelium and the germ cells in Trionyx. He admits that "These large cells do not betray their derivation from the germinative epithelium directly". In an attempt to explain the absence of such transitional forms, he says, "It is quite possible to assume that they were derived from that layer by a process of downgrowth in the form of epithelial cords, which subsequently enlarged in place, but no such clear-cut indications of such a process of epithelial proliferations are discernible,......". It is extremely difficult to understand how such a proliferation of cords of cells from the germinal epithelium could result from or have anything to do with the enlargement of one cell of a cord to form a single cell, germinal or otherwise. In their investigation of the germ cells of Calotes, SIMKINS and ASANA (1930) have described the presence of large yolk-laden cells in the indifferent genital ridge, but they have not traced the origin of these so-called "Nurse-cells". They also have never observed any division figures in the "Nurse-cells". It is singularly odd that the disappearance of the "Nurse-cells" coincides with the appearance of new and smaller elements, „primary germ cells" according to their nomenclature, and that their entire discussion makes no mention of the possibility of cell divisions. The writer finds it difficult to understand the absence of divisions in their material. for they are decidedly plentiful

in the indifferent gonads of *Sternotherus*. A comparison of the cells in their figure 5, plate 4 (Compare with fig. 13, pl. 2 of this paper) shows that the two smaller cells are obviously the result of a division of one of the larger cells, that should be considered as one of the large "Nurse-cells" which has assimilated the yolk content of earlier stages. Their denial of the migratory nature of the "Nurse-cells" is not supported by evidence, since the earlier stages which should show the migration and origin of these elements, if they are the primordial germ cells of other writers, were not examined by them. The arrangement of the germ cells of the indifferent gonads in groups of twos and fours can only be explained by the division of larger pre-existing elements, the only ones present being the primordial germ cells. This interpretation is supported by the almost identical size of the adjacent germ cells. Isolated germ cells are, on the other hand, somewhat larger than those arranged in groups; they are approximately the same size as the primordial germ cells at the time they enter the genital ridge. During this period, the number of primordial germ cells in the gonads increases not only by cell division, but also by the late entrance of additional cells from the mesentery. There is no evidence that a differentiation of epithelial or somatic cells into germ cells at this period adds to the number of germ cells present in the indifferent sex-glands.

6. Experimental results on the origin and fate of the primordial germ cells.

The use of experimental methods in attempts to confirm the results of morphological investigations on the early embryonic origin of the primordial germ cells is only beginning to show results. In only four instances have such attempts been made on vertebrates. REAGAN (1916) operated on chick embryos which were still in the pre-somite stages. He succeeded in removing the crescent-shaped area of the proamnion, in the entoderm of which, according to SWIFT and others, the primordial germ cells first make their appearance. None of the embryos upon which the operation was performed survived longer than five days, and examination of the gonads at that time showed only the presence of stromal tissue with its peritoneal covering. The gonads were completely sterile. The possibility remains, however, that if the embryos had been kept alive somewhat longer, the gonads might have shown the origin of secondary germ cells as described by FIRKET (1914, 1920).

In the Amphibia, KUSCHAKEWITSCH (1910) and WITSCHI (1914, 1924) were able to prevent the germ cells from entering the germinal ridges by modifying the developmental processes through delayed fertilization of over-ripe eggs. Sterile genital ridges were produced which, somewhat later in development, showed the presence of germ cells, which KUSCHAKEWITSCH believed were derived from the indifferent cells of the testicular ampullae by transformation, and which WITSCHI believes are late arrivals of the primordial germ cells which were slowed

up in their migration by the experimental procedure. HUMPHREY (1927 a) attempted to duplicate the results of KUSCHAKEWITSCH and WITSCHI by using operative and chemical methods to suppress the migration of the primordial germ cells from the entoderm of anuran embryos. Unsatisfactory results were obtained, for apparently the elimination of the primordial germ cells was incomplete in many instances. At a later time, using Amblyostoma embryos (1927 b), he was able to remove the intermediate mesoderm, in which the primordial germ cells are found before the migration to the germinal ridges, from the seventh to the sixteenth somites on one side of the embryo. In cases of complete unilateral extirpation of the primordial germ cells, no gonad developed on the operated side in 34 animals killed at intervals of from 33 to 290 days following operation. This result is the most satisfactory of the experimental data on Amphibia.

The recent results of DOMM (1929), who obtained spermatogenesis in the right ovary of the chick after removal of the left ovary before the primordial germ cells completely disappeared from the right, would seem to offer evidence to show that the primordial germ cells were necessary for the formation of the definitive germ cells. DANTSCHAKOFF (1931), in an extensive series of experiments using operative techniques, was successful in demonstrating that the primordial germ cells of the entoderm of the proamnion were the same cells which are to be seen in the genital ridge of later stages. She also demonstrated that the blood vessels are followed in the migration from the proamnion to the region of the genital ridge. The evidence that a "Keimbahn" is present in the chick seems to be founded on adequate morphological and experimental evidence. Further attempts along experimental lines are desirable upon suitable material, but until more successful methods of experimental approach are devised, the principal evidence on the origin of the germ cells must come from morphological observations, upon which the truth of the "Keimbahn" theory is largely based at the present time.

A recent investigation of the origin of the germ cells of the chick embryo by MATSUMOTO (1932) has been brought to my attention since the completion of my manuscript. This interesting paper describes a different place of origin and a different mode of migration than those described by previous investigators on the chick (SWIFT, GOLDSMITH, DANTSCHAKOFF). This author finds the primordial germ cells in the developing primitive streak of the blastoderm of 10—14 hours incubation. They remain in the primitive streak until the 26-hour stage, when they are given off into the medial portion of the proliferating lateral mesoderm. With the formation of the coelom, the majority of the germ cells are carried into the splanchnic layer, but some also enter the somatic layer of mesoderm. Those of the splanchnic layer are displaced dorsally during the elongation of the mesentery, and are eventually included

with the others of the somatic mesoderm in the developing genital ridge. Certainly these observations are more closely related with observations made on other forms than those of an aberrant extraregional origin of the primordial germ cells in the proamnion area, and the migration through the blood vessels to the genital ridge of other authors. The observations coincide with those on reptiles and teleosts, and permit much greater generalization than has heretofore been possible. It is obvious that the entire problem needs further consideration, especially in relation to the earlier stages of primitive streak formation and germ layer differentiation. This I am attempting to do, and a future paper will consider the relation of the primordial germ cells to the primitive plate and the differentiation of the germ layers.

E. General Conclusions and Summary.

(1) In Stenotherus odoratus (LATREILLE), the primordial germ cells are recognized first, in embryos of 1.6—1.75 mm. total length (1—3 somites), as large undifferentiated elements which are similar in character to the cells of the floor of the primitive plate. They are located in a definite horseshoe-shaped germ cell zone. The bend of the horseshoe is in contact with the ventro-median part of the primitive plate, and the two lateral arms of the zone lie in the entoderm at the junction of the definitive entoderm and the vitellin entoderm, opposite the posterior third of the embryo. An anomaly appears in certain embryos in which the germ cell zone lies on only one side, the left.

(2) The primordial germ cells may be distinguished from other cells of the embryo by their large size, clear cytoplasm, definite cell membrane, the presence of yolk granules, in later stages by the presence of the large attraction sphere, and their embryonic position.

(3) Succeeding developmental stages show that the primordial germ cells move from their lateral extraregional positions to a median one in the entoderm. They then pass into the mesoderm of the mesentery and splanchnopleure, from where they migrate dorsally and laterally through the mesentery into the genital ridge. The transposition of the primordial germ cells is primarily the result of certain mechanical alterations in the embryonic tissues during growth and differentiation, but is also probably due to amoeboid migratory movements on the part of the individual germ cells.

(4) The early divisions of the primordial germ cells during the period prior to their recognition and their migration out of the entoderm constitute a primary period of multiplication. Divisions during the migration stages are rare. Towards the end of migration, mitoses in the primordial germ cells begin to reappear; this marks the beginning of a secondary period of multiplication. Throughout the indifferent period

of gonadogenesis, the primordial germ cells divide at a slow rate. The amount of degeneration is negligible.

(5) The time of arrival of the primordial germ cells at the genital ridges coincides with the beginning of germ-gland formation.

(6) In many of the primordial germ cells, the yolk granules disappear early, but they may still be found in the germ cells of embryos of 11.0 mm. carapace length. With the disappearance of the yolk granules, a large attraction sphere appears in the cytoplasm (as early as embryos of 5 somites).

(7) No uniformity in the number of primordial germ cells appears in different individuals.

(8) The germ cells of the indifferent gonads are derivatives of primordial germ cells which arise in early stages in an extragonadal position.

Literature cited.

Allen, B. M.: The embryonic development of the ovary and testis of the mammalia. Amer. J. Anat. 3 (1904). — The origin of sex-cells of Chrysemys. Anat. Anz. **29**. (1906) — A statistical study of the sex-cells of Chrysemys marginata. Anat. Rec. 1 (1907 a); Anat. Anz. 30 (1907 a). — An important period in the history of the sex-cells of Rana pipiens. Anat. Anz. 31 (1907 b). — The origin of the sex-cells of Amia and Lepidosteus. J. Morph. a. Physiol. 22 (1911). — The origin of the sex-cells in Chrysemys (A reply to Dustin). Anat. Anz. 39 (1911). — The origin of the sex-cells in Necturus. Science, N. S., **33** (1911). — **Berenberg-Gossler, H. von:** Die Urgeschlechtszellen des Hühnerembryos am 3. und 4. Bebrütungstage mit besonderer Berücksichtigung der Kern- und Plasmastrukturen. Arch. mikrosk. Anat. 81 (1912). — Über gitterkapselartige Bildungen in den Urgeschlechtszellen von Vogelembryonen. Anat. Anz. 40 (1912). — Bemerkung zu einem Referat von W. Felix über meine Arbeit: „Die Urgeschlechtszellen des Hühnerembryos am 3. und 4. Bebrütungstage" usw. Anat. Anz. 45 (1914). — Über Herkunft und Wesen der sog. primären Urgeschlechtszellen der Amnioten (vorläufige Mitteilung). Anat. Anz. 47 (1914). — **Bounoure, L.:** Le chondriome des gonocytes primaires chez Rana temporaria et la recherche des éléments génitaux aux jeunes stades du developpement. C. r. Acad. Sci. Paris 185 (1927). — Sur un caractère cytologique essential des gonocytes primaires chez la Grenouille rousse. C. r. Soc. Biol. Strasbourg 101 (1929). — **Boveri, T.:** Über Differenzierung der Zellkerne während der Furchung des Eies von Ascaris megalocephala. Anat. Anz. 2 (1887). — **Braun, M.:** Das Urogenitalsystem der einheimischen Reptilien. Arb. zool.-zoot. Inst. Würzburg 4 (1877). — **Butcher, E. O.:** The origin of the germ cells in the Lake Lamprey (Petromyzon marinus unicolor). Biol. Bull. 56 (1929). — **Child, C. M.:** The development of germ cells from differentiated somatic cells in Moniezia. Anat. Anz. **29** (1906). — **Dantschakoff, Wera:** Keimzelle und Gonade. Die entodermale Wanderzelle als Stammzelle in der Keimbahn. Experimentelle Beweise. Vorläufige Mitteilung. Z. Zellforsch. 14 (1931). — **Dantschakoff, Wera, Dantschakoff, Wera Jr. u. Bereskena, Lydia:** Keimzelle und Gonade. IA'. Identität der Urkeimzellen und der endodermalen Wanderzellen. Experimentelle Beweise. Z. Zellforsch. 14 (1931). — **Dodds, G. S.:** Segregation of the germ-cells of the teleost, Lophius. J. Morph. a. Physiol. 21 (1910). — **Domm, L. V.:** New experiments on ovariotomy and the problem of sex inversion in the fowl. J. exper. Zool. 48 (1927). —

Spermatogenesis following early ovariotomy in the brown leghorn fowl. Proc. Soc. exper. Biol. a. Med. 26 (1929). — Dustin, A. P.: Recherches sur l'origine des gonocytes chez les Amphibiens. Archives de Biol. 23 (1907). — L'origin et l'evolution des gonocytes chez les Reptiles (Chrysemys marginata). Archives de Biol. 25 (1910). — A propos de l'origine des Sex-cells. (Response to B. M. ALLEN.) Anat. Anz. 40 (1911). — Eigenmann, C. H.: On the precocious segregation of the sex-cells in Micrometrus aggregatus Gibbons. J. Morph. a. Physiol. 5 (1892). — Felix, W. u. Bühler, A.: Die Entwicklung der Keimdrüsen und ihrer Ausführungsgänge. O. HERTWIGS Handbuch der vergleichenden und experimentellen Entwicklungslehre der Wirbeltiere, Bd. 3. 1906. — Felix, W.: Development of the urogenital organs. In KEIBEL and MALL's Manual of Human Embryology, Vol 2. 1912. — Firket, Jean: Recherches sur l'organogénèse des glandes sexuelles des Oiseaux (Note préliminaire). Anat. Anz. 46 (1913). — Recherches sur les gonocytes primaires (Urgeschlechtszellen) pendant la période d'indifférence sexuelle et le développement de l'ovaire chez le poulet. Anat. Anz. 44 (1913). — Recherches sur l'organogénèse des glandes sexuelles chez les oiseaux. Archives de Biol. 29 (1914). — Recherches sur l'organogénèse des glandes sexuelles chez les oiseaux. Archives de Biol. 30 (1919). — On the origin of the germ-cells in higher vertebrates. Anat. Rec. 18 (1920). — Gasparro, E.: Osservazioni sull'origine delle cellule sessuali nell Gongylus ocellatus. Monit. zool. ital. 19 (1908). — Gatenby, J. B.: The transition of peritoneal cells into germ cells in some Amphibian Anura, especially in Rana temporaria. Quart. J. microsc. Sci. 61 (1916). — The transition of peritoneal epithelial cells into germ-cells in Gallus bankiva. Quart. J. microsc. Sci. 68 (1924). — Goldsmith, J. B.: History of the germ cells in the domestic fowl. J. Morph. a. Physiol. 46 (1928). — Hann, H. W.: The history of the germ cells of Cottus bairdii Girard. J. Morph. a. Physiol. 43 (1927). — Hargitt, G. T.: Germ cells of Coelenterates. VI. General considerations, discussion, conclusions. J. Morph. a. Physiol. 33 (1919). — Germ-cell origin in the adult salamander, Diemyctylus viridescens. J. Morph. a. Physiol. 39 (1924). — The formation of the sex-glands and germ cells of mammals. I. The origin of the germ cells in the albino rat. J. Morph. a. Physiol. 40 (1924). — The formation of the sex-glands and germ cells of mammals. II. The history of the male germ cells in the albino rat. J. Morph. a. Physiol. 42 (1926). — The formation of the sex-glands and germ cells of mammals. III. The history of the female germ-cells in the albino rat to the time of sexual maturity. J. Morph. a. Physiol. 49 (1930). Hasper, M.: Zur Entwicklung der Geschlechtsorgane von Chironomus. Zool. Jb. 15 (1911). — Hegner, R. W.: Studies on germ cells. I. The history of the germ cells in insects with special reference to the Keimbahn-Determinants. II. The origin and significance of the Keimbahn-Determinants in animals. J. Morph. a. Physiol. 25 (1914). — Hertwig, O.: Die Chäetognathen. Eine Monographie. Jena. Z. Naturwiss. 14 (1880). — Heys, Florence M.: The problem of the origin of the germ cells. Quart. Rev. Biol. 6 (1931). — Hoffmann, C. K.: Zur Entwicklungsgeschichte der Urogenitalorgane bei den Reptilien. Z. Zool. 48 (1889). — Étude sur le développement de l'appareil urogénital des oiseaux. Verh. koninkl. Akad. Wetensch. Amsterdam, Tweede Sectie, 1 (1893). — Huettner, A. J.: The origin of the germ cells in Drosophila melanogaster. J. Morph. a. Physiol. 37 (1923). — Humphrey, R. R.: The primordial germ cells of Hemidactylium and other Amphibia. J. Morph. a. Physiol. 41 (1925). — Modification or suppression of the so-called migration of primordial germ cells in anuran embryos. Anat. Rec. (abstract) 35 (1927 a). — Extirpation of the primordial germ cells of Amblystoma; its effect upon the development of the gonad. J. of exper. Zool. 49 (1927 b). — The early position of the primordial germ cells in Urodeles: Evidence from experimental studies. Anat. Rec. 42 (1929 c). Jarvis, Mary H. The segregation of the germ cells of Phrynosoma cornutum. Biol. Bull. 15 (1908). — Jordan, H. E. and Flippin, J.: Haematopoiesis in Chelonia. Folia haemat. (Lpz.) 15 (1913). — Embryonic history of the germ cells of the

Loggerhead Turtle (Caretta caretta). Publ. Carn.-Inst. Wash. No. 251, 1917. — **Kingery, H. M.:** Oogenesis of the white mouse. J. Morph. a. Physiol. 30 (1917). — **Kuschakewitsch, S.:** Die Entwicklungsgeschichte der Keimdrüsen von Rana esculenta. Festschrift für (60.Geburtstag) R.Hertwig, Bd. 2. (1910). — **McCosh, Gladys M.:** The origin of the germ cells in Amblystoma maculatum. J. Morph. a. Physiol. 50 (1930). — **Matsumoto, Tohru:** On the early localization and history of the so-called primordial germ cells in the chick embryo (Preliminary Report). Sci. Rep. Tôhoku Imperial Univ., 4. S., Biology, 7 (1932). — **Matthey, R.:** Chromosomes de reptiles Sauriens, Ophidiens, Cheloniens. L'évolution de la chromosomiale formule chez les Sauriens. Rev. Suisse Zool. 38 (1931). — **Mihalkovicz, G. von:** Untersuchungen über die Entwicklung des Harn- und Geschlechtsapparates der Amniotes. Internat. Mschr. Anat. 2 (1885). — **Okkelberg, P.:** The early history of the germ cells of the brook lamprey, Entosphenus wilderi Gage, up to and including the period of sex differentiation. J. Morph. a. Physiol. 35 (1921). — **Rauh, W.:** Das Chondriom in den ersten Keimzellen der Ratte. Eine Keimbahnuntersuchung. Z. Anat. I 89 (1929). — **Reagan, F. P.:** Some results and possibilities of early embryonic castration. Anat. Rec. 11 (1916). — **Richards, A. and Thomas, J. T.:** The migration of the primary sex cells of Fundulus heteroclitus. Biol. Bull. 40 (1921). — **Risley, P. L.:** Observations on the natural history of the Common Musk Turtle, Sternotherus odoratus (LATREILLE). Papers Mich. Acad. Sci. 17 (1933). — **Rubaschkin, W.:** Über das erste Auftreten und Migration der Keimzellen bei Vögelembryonen. Anat. H. 35 (1907). — Über die Urgeschlechtszellen bei Säugetieren. Anat. H. 39 (1909). — Chondriosomen und Differenzierungsprozesse bei Säugetierembryonen. Anat. H. 41 (1910). — Zur Lehre von der Keimbahn bei Säugetieren. Anat. H. 46 (1912). — **Rückert, J.:** Die Entstehung des Blutes und der außerembryonalen Gefäße in den meroblastischen Eiern. I. Selachien. O. HERTWIGs Handbuch der Entwicklungslehre der Wirbeltiere, Bd. 1 (1906). — III. Entwicklung der extraembryonalen Gefäße der Reptilien. O. HERTWIGs Handbuch der Entwicklungslehre der Wirbeltiere, Bd. 1. — **Simkins, C. S.:** On the origin and migration of the so-called primordial germ cells in the mouse and the rat. Acta Zool. (Stockh.) 4 (1923). — The origin of the germ cells in Trionyx. Amer. J. Anat. 36 (1925). — Origin of germ cells in man. Amer. J. Anat. 41 (1928). — **Simkins, C. S. and Asana, J.:** Development of the sex-glands of Calotes. I. Cytology and Growth of the Gonads prior to Hatching. Quart. J. microsc. Sci. 74 (1930). — **Swift, C. H.:** Origin and early history of the primordial germ cells of the chick. Amer. J. Anat. 15 (1914). — Origin of the definitive sex cells in the female chick and their relationship to the primordial germ cells. Amer. J. Anat. 18 (1915). — Origin of the sex cords and the definitive spermatogonia in the male chick. Amer. J. Anat. 20 (1916). — **Swingle, W. W.:** The germ cells of anurans. I. The male sexual cycle of Rana catesbiana larvae. J. of exper. Zool. 32 (1921). — Germ cells of anurans. II. An embryological study of sex-differentiation in Rana catesbiana. J. Morph. a. Physiol. 41 (1926). — **Weismann, A.:** Die Kontinuität des Keimplasmas als Grundlage einer Theorie der Vererbung. Jena: Gustav Fischer 1885. — The Germ plasm: A theory of heredity. New York: Charles Scribner's Sons. 1893. **Witschi, E.:** Experimentelle Untersuchungen über die Entwicklungsgeschichte der Keimdrüsen von Rana temporaria. Arch. mikrosk. Anat. 85 (1914 a). — Die Entwicklung der Keimzellen der Rana temporaria. L. Erster Teil: Urkeimzellen und Spermatogenese. Z. Zellenlehre 1 (1924). — **Wolf, L. E.:** The history of the germ cells in the viviparous teleost Platypoecilus maculatus. J. Morph. a. Physiol. 52 (1931). — **Woodger, J. H.:** Observations on the origin of the germ cells of the fowl (Gallus domesticus) studied by means of their Golgi bodies. Quart. J. microsc. Sci. 69 (1925). — **Woods, F. A.:** Origin and migration of the germ cells in Acanthias. Amer. J. Anat. 1 (1902).