The Origin of Definitive Germ Cells in the Domestic Fowl

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THE ORIGIN OF DEFINITIVE GERM CELLS IN THE DOMESTIC FOWL

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INTRODUCTION

Since extensive work had been carried out in these laboratories by previous fellows, pertaining to the effects of x-ray irradiation on the gonads of the developing chick, and on the mature gonads of the adult domestic fowl, it seemed feasible that the research to be undertaken should pertain to the effects produced by the irradiation of early germ-cells in the embryonic chick. However, before active observation could be made upon the results of irradiation of these embryonic germ-cells, their definite origin had to be determined. The author has therefore, decided to investigate the origin of germ-cells. The following paper is a report of the observations made.

This work was done at the suggestion of, and in association with, Dr. J. M. Essenberg, my professor in Anatomy at Loyola University, School of Medicine, to whom I am very grateful.

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LITERATURE

With the introduction and distinction of the Weismannian concept of soma and germ plasm, the origin and history of definitive germ cells has come under active investigation.

Waldeyer (1870) was the first to investigate the history of germ cells of vertebrates. He observed definite germ cells in the germinal epithelium of chick embryos.

Eigenmann (1891) was convinced the primordial germ cells were present in late cleavage stages of the teleost, micro-metrum aggregatus. Previous to him no other investigator had been able to recognize germ cells in any vertebrate earlier than the germ layer stages.
Many contributions have appeared in more recent literature favoring continuity of germ cells from generation to generation. Other investigators find evidence in favor of the conception that in each generation, a crop of definitive germ cells arises anew from the germinal epithelium of the gonad. The investigations on germ cell origin, are grouped into four categories. Heys (1931) has classified these into the following groups:

I Those who deny early segregation of germ cells, and believe that germ cell formation results from differentiation of somatic cells.

II Those who admit early segregation of germ cells but conclude that such cells are not definitive but degenerate to be replaced by proliferations of new cells from the germinal epithelium.

III Those whose investigations have led to the conclusion that germ cells are segregated early and migrate to the site of the future gonad. These persist as definitive germ cells, but their numbers are increased periodically by a proliferation from the epithelium (germinal).

IV Those who believe that the definitive germ cells are set aside at an early stage in embryonic development not to be replaced later by transformation of differentiated peritoneal cells.

Although Heys' organization of the results of investigations of germ cell origin is for the entire animal kingdom, the same generalization can be used for a review of the literature on germ cell origin in the birds.

To Waldreyer's observation of germ cells within the germinal epithelium of the chick gonad, is added that of Semon (1881) who claimed that he saw a distinct transformation of germ cells from epithelial cells. No other investigator at that time had claimed to have seen this phenomenon.

Firket ('14, '20) advocated the early segregation of germ cells but believed that though these enter into the germinal epithelium and the cords thereof, most of the early segregated cells degenerated, and that the majority of definitive ova or spermatozoa are derived from the germinal epithelium.

The experimental demonstration of ovarian degeneration and germ cell formation from the epithelium of the ovary in studies on sex reversal, has been considered an important source of evidence in favor of the definitive germ cell origin from the germinal epithelium. Fell (1923) studied the gonads of eight intersexual birds. She found germ cells of the opposite sex
proliferating into sexual cords from the peritoneal epithelium of the degenerating ovary. Cells from the sex cords gave rise later to seminiferous tubules and finally to functional sperm.

Mihalkovics (1885) was the first to conclude that the early segregated cells of Amniotes are not definitive, but are replaced by a proliferation of new cells from the germinal epithelium in the later development of the sex gland. Minot (1894) was doubtful that these early segregated cells had any relation to the genital region, since they disappeared before definitive germ cells were differentiated.

Von Berenberg-Gossler (1912) recognized germ cells differentiating from the gonad primordium coincident with its development. He was of the opinion that any large cells present before the development of the gonads were not germ cells but retarded mesenchymal cells.

The authors who adhere to a strict independence of germ cells and somatic cells are the true upholders of the Weismannian principle, and believe that germ cells are set aside at an early stage in embryonic development. The early segregated or primordial germ cells are not replaced later by transformed peritoneal cells. Hoffman (1892), Nussbaum (1901), Rubaschkin (1907), and Tschaschin (1910) are of the same opinion.

Swift (1914, '15, '16) summarized his findings as follows: The primordial germ cells arise anterior-lateral to the embryo in a specialized region of germ-wall entoderm at the margin of the area pellucida. The area mentioned has a roughly crescentic shape and the primordial germ cells arise during the primitive streak stage and continue to do so until the embryo has attained three somites. These primordial germ cells arise before the appearance of mesoderm in this area and are seen between the ectoderm and entoderm. Later, when the mesoderm is formed and gives rise to blood vessels, the early segregated cells or primordial germ cells migrate via the bloodstream to all parts of the embryo and area vasculosa. Therefore, the distribution is general up to about 20 somites, after which stage the primordial germ cells become localized to some extent in the splanchnic mesoderm at the region of the coelomic angle. Here they remain until the formation of the gonad, into which they gradually pass. Swift (1916) in his later work pertaining to the origin of sex cords and definitive spermatogonia in the male chick, concluded that the early segregated cells of entodermal origin gave rise to definitive germ cells, while the coelomic cells of the germinal epithelium produced the supporting cells of the
Seminiferous tubules. The investigation mentioned, confirmed his work on the female chick. (1915)

"From experimental data (Willier 1931) it appears that the celomic epithelium adjacent to the developing mesonephros has the specific potencies of gonad just prior to the time that germinal epithelium is morphologically indicated. Prior to the 31 somite stage, the celomic epithelium adjacent to the mesonephros has no capacity to originate a gonad in the graft. A graft of the mesonephros during the period of morphogenesis of the germinal epithelium will develop with greater frequency (50%) than during earlier stages. At 90 hours incubation a gonad rudiment rarely fails to differentiate into a specific sexual gland."

Richards, Hulpieu, and Goldsmith (1926) restudied the germ cell history in the fowl and found the early development as described by Swift (1914, 15, 16). In the male from the eleventh day of incubation, division of sex cells (primordial germ cells) took place actively with no signs of degeneration, although their history was followed for seventy-five days. In a more complete series of the female, no transformations of peritoneal cells into germ cells were observed. These investigators are fully convinced that definitive ova are traceable to the early segregated sex cells without degeneration and without contributions from the germinal epithelium.

Goldsmith (1928) first described primordial germ cells (early segregated cells) during the primitive streak stage, anterior and antero-lateral to the head fold. Later, the primordial germ cells were incorporated into the growing mesoderm and by their amoeboid activity entered the forming bloodvessels in extra-embryonic tissues. He made known the correspondence between his observations and the findings described by Dantschakoff (1908), and Swift (1914). Wide distribution of germ cells was evident until about the forty-hour stage, when they become more numerous at the site of the gonad, which they gradually entered. Goldsmith did not observe any "wide-spread degeneration of the primordial germ cells of either sex." He believes that the primordial germ cells of the early embryonic stages form the "definitive germ plasm."

**MATERIALS AND METHODS**

The eggs of White or Brown Leghorn hens, ranging in age from 16 hours to 20 days incubation, and chicks from birth to 28 days after hatching were used in this experiment. The material comprises 85 series. Use was made also of the material prepared by Zikmund (1931-'32), a former teaching fellow in the
in the department of anatomy.

An electrically heated incubator, with a thermo-control for the maintenance of a constant temperature, was used. The temperature and humidity was kept at 101-105°F. and 65 respectively, as advised by Romanoff in his paper pertaining to effects of humidity etc., on the hatching properties of eggs. The eggs were rolled twice per day, in the morning and in the evening, so as to prevent adhesions of the embryonic membranes to the shell.

Embryos of 16 hours to 10 days incubation were sectioned whole; those of 11 days to 15 days incubation were sectioned through the gonadal region, while in embryos of later ages than 15 days, and in the hatched chicks, only the gonads were sectioned serially.

Practically all of the material was fixed in Bouin's solution, made up of 75 parts saturated aqueous solution of picric acid, 25 parts of stock formalin solution, and one part of glacial acetic acid. The routine paraffin embedding method was used in the preparation of the material for serial sectioning. The sections measured 5 and 6 μ in thickness. In instances where there was a duplication of material, the duplicate series of the later ages were fixed in aceto-osmic-bichromate solution while those of the earlier ages (17 hours incubation) were fixed in chromo-aceto-osmic solution.

With a few exceptions, the staining procedure followed was the Heidenheim's Iron Hematoxylin method. Two series, of 17 hours, and 3 days incubation, were stained by the Bensley aniline-acid fuchsin method. Two other series, each of a different age (10 and 14½ days incubation) were stained with Wright's stain.

The eggs used for incubation were obtained from a reliable source in Mulligan, Indiana, and were sent by express to our laboratory.

RESULTS

The Early Segregated Entodermal Cells

In a study of early chick embryos (16-72 hours incubation) the observations do not differ materially from those obtained by Swift. However in all of the author's material, an abundance of the primordial germ cells of entodermal origin has never been seen. The origin development and migration of early se-
aggregated cells as described by Swift is confirmed except for the specific localization of these cells within the mesenteric root. A specific localization cannot be conceded since the early segregated cells of entodermal origin are observed to be scattered throughout the mesenchyme, far removed from the area of the future gonad. Because of the increased vascularity in the region of the mesenteric root, a larger number of the early segregated or primordial germ cells are present in this locality. For a detailed description of the cytology of the primordial germ cells of entodermal origin, reference is made to Swift's article (1913).

**Destiny of the Early segregated Entodermal Cells**

In the early series, (42-72 hours incubation) the early segregated cells already show degeneration. The nucleus has not the typical appearance observed in a normal cell. The chromatic substance is clumped into an irregular mass which possesses a variable stainability. The smaller granules in the nucleus present a faded appearance whereas the nucleus as a whole is turgid and enlarged. The granular cytoplasm in many instances is withdrawn from the cell membrane, and presents liquifaction spaces along the periphery.

In later embryos (78 hours - 6 days incubation) the early segregated cells (primordial germ cells of Swift) frequently show abnormal cell division (fig. 44). The cytoplasm fails to divide during the telophase stage of mitosis, and this results in a multinucleated cell. This cell eventually degenerates and undergoes liquifaction.

Figures 11 and 26 are microphotographs illustrating the presence of multinucleated cells within the mesenteric root of a 96 hour and a 5 day chick embryo. The nuclei within the syncitial cytoplasm of the multinucleated cells are in several stages of degeneration, showing nuclear pyknosis with subsequent liquifaction.

The significance of the early segregated cells of entodermal origin is unknown. They may be of phylogenetic significance only.

**Development of the Germinal Epithelium**

An embryo of 72 hours incubation (figures 1-4) has a sufficiently thickened peritoneum in the angle of the celome to give indication of a definite germinal epithelium. This is the age at which a description of the germinal epithelium is commonly given. However, the germinal epithelium occurs at a much earlier period. In an embryo of 42 hours incubation, the lateral wings of the splanchnic mesoderm have not yet met in
the ventral midline to form the dorsal mesentery. The splanchnic mesoderm in the ceolom, caudal to the twentieth somite already shows the presence of cells actively transforming from the undifferentiated peritoneum. In an embryo of 26 somites (45-46 hours incubation) this process is further advanced. Coincident with the rapid cell proliferation of the primitive peritoneum near the angle of the ceolomes, there is a gradual accumulation of immature precocious germ cells in the splanchnic mesoderm of this area. Although other investigators have mentioned the thickening of the germinal epithelium as being caused by the activity of the primitive peritoneum, they have failed to recognize the accumulation of immature precocious sex cells of peritoneal origin as an important factor in the thickening of the simple peritoneal epithelium into a many-layered germinal epithelium.

The Origin, Development, and Passive Migration of Sex Cells

In a description of the transition stages, it is necessary to designate cell types, and hence an arbitrary letter was chosen to represent each stage. For example, the transformations follow through six stages. The germinal epithelial cell represents stage "a", and the early germ cell, stage "f", whereas the stages in between are designated by an appropriate letter (Plate X).

Stage "a" is a columnar cell, containing an elongated nucleus with a scattered chromatin content. The distribution of the chromatin is diffuse, giving the cell a darkly staining appearance. It is the darkest staining cell present in the germinal epithelium. (Plate I, figures 2, 3, 4; Plate II, figure 8; Plate IV, figures 14, 16).

Stage "b" is characterized by the more oval outline of the nucleus. The homogeneous distribution of the nuclear chromatin of stage "a" is replaced by an uneven distribution in stage "b". Two nucleoli are observed centrally, whereas at the periphery of the nucleus the chromatic substance is less dense. (Plate I, figures 2, 3, 4; Plate II, figure 8; Plate IV, figures 14, 16).

Stage "c" possesses a distinctly egg-shaped nucleus. It stains lighter because much of the chromatin material is aggregated into two distinct nucleoli. This cell is approximately twice as large as that of stage "b". (Plate I, figures 2, 3, 4; Plate II, figure 8; Plate III, figure 10; Plate IV, figures 14, 16).

Stage "d" is represented by a definitely globular or
spherical form of the nucleus. The chromatin of the nucleus is diffusely scattered in that only one nucleolus is observed, apparently from a fusion of both earlier nucleoli. There is a tendency for the chromatin particles to radiate from this single nucleolus. The size of the nucleus is greater than that of stage "c". (Plate I, figure 2, 4; Plate II, figure 8; Plate III; figure 10; Plate IV, figure 14)

In stage "e", a great change in the structure of the cell is evident. The first striking change is the delineation of a cytoplasm with a faint acidophilic affinity. The nucleus is slightly vesicular in appearance and is larger in diameter than that of stage "d". (Plate II, figure 8; Plate III, figure 10; Plate IV, figure 14)

Stage "f" is a large cell containing a very light border of cytoplasm surrounding a large vesicular nucleus. The chromatin material is being redistributed and the nucleus is assuming a vesicular character. There are usually two to three small chromatic masses from which extensions of chromatic strands radiate. The nucleus is more or less eccentrically placed. (Plate IV, figures 14, 16)

The place of origin of these sex cells is in the germinal epithelium which itself is a modification of undifferentiated peritoneal cells. Figure 5 illustrates the area occupied by the germinal epithelium. As the transforming sex cells become more mature they assume a deeper position within the germinal epithelium. It is a common finding to observe the large sex cells of peritoneal origin within the loose mesenchymal stroma of the developing gonad. (Plate III, figure 10, f; Plate IV, figures 14, 16, letters e and f) These cells are seen in the germinal epithelium, in the border of the germinal epithelium and stroma, and emerging into the stroma itself. They are left behind in the outward growth of the germinal ridge. (Plates III, IV)

Cells with large nuclei are observed scattered in the mesenchyme, in many instances, far removed from the germinal epithelium or its vicinity. The character of the nucleus varies very little from the nucleus of any celomic cell lining the peritoneal cavity, except in size. Consequently, these cells with large nuclei could be traced back to an origin from the undifferentiated peritoneum. Hargitt (1925) in his paper on the origin of germ cells in the albino rat, states that these large cells far removed from the region of the developing gonad may be new mesenchymal cells forming from the embryonic peritoneum.
The Formation of the Gonad

Indifferent Gonad

As observed by Swift, a well developed indifferent gonad is present in 4.5 and 5 day chick embryos. (figures 13, 14, 15, 16). During the 5th and 6th days of incubation the gonad differentiates either a testis or ovary.

During the indifferent stage, there is a general reduction of the mature precocious germ cells within the germinal epithelium and epithelial cords (primary sex cords). The indifferent gonad has an homogeneous appearance.

Various transition stages of the peritoneal cells into early or precocious sex cells are observed within the germinal epithelium and sexual cords. (of first proliferation) The sex cords do not consist of only two cell types as described by Swift. The cell types present are variable in size and structure and represent sex cells transforming from undifferentiated germinal epithelial cells. (figures 17, 18, 19)

Transitional cell types are also observed within the stroma. (figures 17, 18) These are transforming from the indifferent cells of the germinal epithelium which have infiltrated into the original loose mesenchyme of the indifferent gonad individually, before and during the formation of the primary sex-cords. These transitional cells located within the stroma, but outside of the sex cords, degenerate and are absorbed.

The Testis

The germinal epithelial cords of first proliferation are definitely outlined. (figures 20, 24, 45) Various cell types are observed within the primary sex cords, i.e., small germinal epithelial cells, sex cells, and the transition stages between the former and the latter. (figures 20, 21, 45) The cords appear straight at first but as the testis becomes older they become wavy and convoluted by the 11th day. The sexual cords of the testis are longer and thicker than the primary cords of the indifferent gonad. They consist mostly of undifferentiated germinal epithelial cells in the form of a syncytium. Some large germ cells (figures 24, 45, 46, 47) are present, but these occur in lesser abundance than in previous
stages. The undifferentiated cells remain close to the surrounding basement membrane of the cord, whereas the transforming cells and early sex cells when present are more centrally placed. This arrangement gives to the cords, a darkly staining periphery and a lighter staining central area. The central area is finely granular and cell outlines are not very distinct. The degeneration of the central cells in the primary sex cords adds to the process of liquifaction that takes place in later development. The large precocious sex cells are inactive, no mitoses being observed among them. The undifferentiated cells of peritoneal origin on the other hand, show active mitosis, and are responsible for the growth of the primary sex cords.

A fourteenth day chick gonad (figure 25) shows an almost complete depletion of the large sex cells within the seminiferous cords (primary sex cords), and a single layer of undifferentiated germ cells (germinal epithelial derivatives) lines the basement membrane. (figures 48, 49) There has been a degeneration of the larger transitional cell types. The center of each cord shows the large cells in the last stages of liquifaction. (figures 48, 49)

Swift and his co-workers fail to recognize the transition stages, just as they fail to observe the degeneration present. The cells present within the seminiferous cords are many and variable, but they may all be referred ultimately to the ordinary peritoneal cells for their lineage.

The Ovary

As described by Swift, Goldsmith, etc., it is customary to designate two generations of sex cords in the description of the development of the ovary. The first of these sex cords is produced by the extensive activity of the germinal epithelium about the 6-7 day of incubation. By the 8th day, there follows a disorganization of the medullary cords (cords of first proliferation in the female) so that the area originally occupied by the cords, is replaced by a homogeneous appearing portion of the medulla close to the germinal epithelium, whereas the deeper portion of the medulla acquires a tubular character.

The cords of second proliferation have not been observed in a study of the author's material. There is a gradual and uniform thickening of the germinal epithelium of the entire ovary into a definite cortex, in which the most superficial layer retains its potentiality for sex cell transformations.

In a 7 day embryo, (figures 27, 28, 29) the germinal epithelium of the ovary is composed of several cell layers. The greater number of transforming cells are located towards
the base of the germinal epithelium. Because of the infiltration of the stroma by transforming sex cells, it is common to find precocious sex cells between the medullary cords at this age. The cell components of the medullary cords are mostly of the small cell types (a, b, c). The larger sex cells (e, f), transformed from the smaller medullary cord cells have degenerated.

Ovaries of chick embryos 10-17 days old present an extensive activity of the germinal epithelium, thus elaborating a well defined cortex covered peripherally by undifferentiated cells. Situated more deeply in the cortex are the large transformation cells which give to this portion of the germinal epithelium a light staining character. As observed in this series the large cortical cells (female sex cells) show a deep red granulation of the cytoplasm, a washed out nucleus, and loss of definite cell membranes.

By the 19th day of incubation (figure 31) active transformation of germ cells from the germinal epithelium has ceased. The cortex of a 19th day chick embryo consists of medullary derivatives which surround large numbers of the transformed ova (sex cells), and isolate them into large groups. The isolated cortical areas consist primarily of large ova in later stages of transformation and mitosis. However, degeneration of the large ova is very evident; many of them present a pyknotic nucleus and variable degree of cytoplasmolysis.

In later stages, (chicks 1, 2, 3 days after hatching, figures 32, 33) there is a continuance of cortical degeneration and infiltration by medullary derivatives to such an extent that the cortex is now represented by masses of degenerating ova surrounded by smaller undifferentiated cells. In a chick of 7 days only a few of the early transformed ova remain. These few precocious ova acquire a peripheral layer of indifferent cells of medullary origin. This is the initiation of a primitive ovarian follicle. (figure 34)

In chicks 18-20 days of age, the scattered cortical remnants contain ova in early stages of follicle formation, whereas the rest of the cortex is replaced by undifferentiated medullary and germinal epithelial derivatives.

The number of precocious follicles is smaller in the 20 day chick ovary than in younger ovaries (18 days, etc). The older specimen also shows a more extensive degeneration of the follicles. (Plate XIV) Although the cytoplasm of the ova has accumulated yolk, the nuclei show karyolysis (figures 54, 55).
Some follicles contain only follicle cells and a few cytoplasmic remnants; a cytolysis of the ovum has occurred. At birth or shortly after, all the precocious ova and follicles degenerate completely.

Degeneration of Sex Cells of Peritoneal Origin

The precocious sex cells are transformed from the germinal epithelium of the indifferent gonad, and later from the seminiferous cords (primary sex cords) of the testis, medullary cords (primary sex cords of the ovary) and cortex of the ovary. Degeneration may occur (1) when the germinal epithelial cell has reached the last stage of transformation (stage f) or (2) after the transformed sex cell has undergone mitosis into two daughter cells. The daughter cells degenerate by liquifaction and absorption. (figure 16) However, the activity of the germinal layer is so pronounced that at no time is the germinal epithelium devoid of large genital cells.

Following the formation of the sexual cords in the indifferent gonad, the site of degeneration is transferred to the epithelial cords. The degeneration is especially extensive in the germinal epithelium of the 5½ day chick embryo. (figure 19)

Within the immature seminiferous cords of the embryonic testis, the large transition stages do not stain distinctly. They are located centrally within the sex cord. In the following liquifaction of the cord, the large sex cells that have not already been absorbed are now dissolved (figures 48, 49). By the 7th-12th day after hatching the indifferent cells lining the basement membrane of the primary sex cord again undergo an extreme transformation into germ cells (figures 45, 46). Following this increase of germ cells within the seminiferous cord, there is a drastic degeneration of the sex cells centrally placed, so much so, that the testis cords of a 14 day chick gonad (figures 25, 48) are again reduced to a single layer of dark staining undifferentiated cells surrounding the pale and vacuolated center of the sex cord. Thus the development of the seminiferous tubule is marked by intervals of transformation and degeneration up to 20 days after hatching. (figures 26, 49)

In the ovary, although degeneration is observed within the medullary cords, the site of greatest degeneration is in the cortex of later ages. (Plate XIII, figures 50, 51, 52; Plate XIV)
Evidence that Early Segregated Cells of Entodermal Origin do not give Rise to Definitive Germ Cells

The early segregated cells described by Swift as primordial germ cells, were first described by Dantschakoff in 1908. The entodermal origin of these cells is conceded by both Dantschakoff and Swift, but these authors vary in their belief as to the ultimate fate of the large entodermal cells. Dantschakoff called them "entodermal wandering cells." The wandering cells enter the mesoderm in embryos older than the primitive streak stage, and are distributed by means of blood vessels to tissues where they degenerate. That the large entodermal cells take no part in tissue formation was believed by Dantschakoff. Swift (1914) ignores these earlier findings of degeneration completely. Likewise he does not observe degeneration previous to the ninth day among the early segregated cells which have failed to reach the "gonad rudiment" (germinal epithelium).

The author agrees with the entodermal origin of the early segregated cells but cannot conceive of them as giving rise to definitive germ cells in the light of his own investigations wherein he substantiates the finding of Dantschakoff, namely, that there is an early degeneration of these cells derived from the area pellucida.

Swift's conception involves an entodermal origin of germ cells; in practically all other amniota there is a mesodermal origin. Why should the chick vary from the other amniotes in the origin of definitive germ cells?

The migration of the early segregated cells of entodermal origin to their final location in the mesenteric root has been observed, but the entrance into the gonad has not been observed by any investigator. The migration into the gonad is inferred by Swift and those who agree with him from the presence of amoeboid processes on the cell and from the change in position of the early segregated cells (primordial germ cells). If Swift's conclusions are correct there should be observed a definite streaming of the early segregated cells into the gonad at some definite age of development. Also it stands to reason that there would be a gradual depletion of these cells within the mesenteric root. The author has not observed a migration of the early segregated cells into the developing gonad. A study of table I will show that there is
a constant increase of genitaloid cells within the mesentery coincident with the formation of the germinal epithelium. The increase cannot be attributed to mitosis since the cells of entodermal origin in these early stages are more or less quiescent; the few instances of mitoses observed cannot account for the large increase of genitaloid cells. There is no doubt that most of the cells outside the gonad region are the result of a passive migration from the "gonad rudiment" (germinal epithelium). Many of these extra-gonadal cells are in various transition stages, the immature cells being in closer proximity to and within the germinal epithelium, whereas the mature types are located deeper in the extra-germinal mesenchyme.

The accumulation of large genitaloid cells in the base of the mesentery is not proof that migratory cells enter the gonad. It is, however, a proof of cell formation within the gonad. Table I shows that there is a constant increase of germ cells within the mesenteric root, as the development of the gonad progresses.

The disappearance of the early segregated cells from the root of the mesentery is not the result of migration into the gonad, but the result of degeneration. The early segregated cells of entodermal origin when observed in the mesentery, do not appear normal. This is substantiated by observations of degeneration and abnormal cell division in older embryos. Figures 11 and 29 are microphotographs of multi-nucleated cells undergoing degeneration. The multi-nucleated cells are the results of abnormal mitotic divisions of the early segregated cells (primordial germ cells of Swift).

The presence of early transformation cells of peritoneal origin before the occurrence of early segregated cells within the vicinity of the splanchnic plate, lateral to the celomic angle and caudal to the heart, gives to the early segregated cells of entodermal origin an insignificant meaning. Embryos of 36 hours incubation show the transition of peritoneal cells into early sex cells, which is significant since the segregated cells have as yet been unobserved within the mesenteric root. Caudal to the fusion of the omphalomesenteric veins and the heart, the splanchnic mesoderm in the angle of the celome shows evidence of cell transformation. Inter-spersed among the ordinary mesodermal cells of the splanchnic plate are observed slightly larger cells, ovoid in appearance. The nuclei of mesodermal cells contain definitely outlined, dark compact, spherical or ovoid nucleoli, whereas in contrast the larger ovoid cells possess nucleoli which are assuming a more irregular outline, in some instances indicating a lobulation. In these cases, the chromatin of the nucleolus is
more diffuse. The nucleus, as a whole, is slightly larger and lighter than that of an ordinary splanchnic mesodermal cell.

Goldsmith, in a restudy of the germ cell history in the fowl, figured early segregated cells in the bloodvessels of the splanchnopleure of a 24 hour embryo, and at various locations in later stages. In this connection he points out the correspondence between his observations and the findings described by both Dantschakoff (1908) and Swift (1914). Thus a wide distribution was evident until about the 40 hour stage when the early segregated cells became more numerous at the site of the gonad. The author has observed early segregated cells of entodermal origin within the mesenteric root of a 42 hour embryo, but the number observed was very small. The comparison in number between early segregated cells in the mesenteric root and the transforming cells within the splanchnic mesoderm (early germinal epithelium) shows the transition stages from the very first exceed the early segregated cells. Another important observation is that of cell transformation at 36 hours incubation, before the occurrence of early segregated cells at the site of the developing germinal epithelium.

The author makes note of the lack of correspondence between the occurrence of transition stages and the time of origin of the specific potencies of the gonad. Just prior to the time that the germinal epithelium is structurally indicated, the celomic epithelium, adjacent to the developing mesonephros has the "specific potencies of the gonad (Willier 1934). Prior to the 31 somite stage (52 hours) the celomic epithelium adjacent to the mesonephros has no capacity to originate a gonad in a graft." This problem has not been completely investigated since the cases observed are too few to be certain of interpretation.

The idea of dedifferentiation of early segregated cells of entoderm origin into small cells indistinguishable from peritoneal cells cannot be entertained. If this phenomenon was to occur, it would necessitate a localization of the early segregated cells prior to the transformation of the celomic cells. The earliest localization of early segregated cells in the splanchnic mesoderm of the gonad region occurs in a 40 hour embryo (Goldsmith 1928). Transformation stages are observed within the embryo as early as 36 hours of incubation, earlier than the localization of early segregated cells. Hence the earliest indication of germ cells is in the intra-embryonic splanchnic plate of mesoderm, just lateral to the nephrotome.
Transitional Cell Types in the Germinal Epithelium

The cells described as transition stages represent true modifications of the germinal epithelial cells. The gradual increase in size and conformity of the nucleus is characteristic of cell transformation. The gradual and uniform redistribution of the nucleolar particles and the chromatic granules embedded within the nucleoplasm of the transforming cell can be identified in specific transitional cell types. There is likewise a gradual increase in the size of the cell and a definite transition of the cell as a whole from a small elongated columnar form into a large spherical cell characterized by a vesicular appearance. Six stages can be definitely identified, the earliest being the germinal epithelial cell and the latest being a fully formed germ cell. The cytology for each stage has been described in the results; the cells representing these stages, have a definite morphology, depending upon the degree of transformation. The time and place of origin is proof that the sex cells are cells transformed from mesodermal derivatives.

Evidence in Favor of Cell Transformation

That the definitive germ cells arise from the undifferentiated peritoneal epithelium is brought out by the following:

The occurrence of transforming sex cells in the superficial layer of the splanchnic mesoderm, just lateral to the celomic angle, earlier than the localization of early segregated cells in the region of the gonad.

The early occurrence of transformation stages within and only within the splanchnic mesoderm of young embryos (36, 38, 40 hours incubation). There are no transition cells and no early segregated cells outside of the splanchnic mesoderm in these early embryos.

There is an ever-increasing number of genital cells within the peritoneum and immediate vicinity of older embryos. If there had been a migration of early segregated cells into the gonad, there should have been a depletion within the mesenteric root. The opposite condition was encountered (Table I).

Degeneration of the early segregated cells occur rather early, which is contrary to Swift's observation of primordial germ cells persisting as late as nine days of incubation without any degeneration occurring. The interesting observation
of multinucleated cells has already been mentioned. These attest to the abnormal character of the early segregated cells; likewise they illustrate various grades of degeneration within one syncytial mass (multinucleated cell). The activity of the early segregated cells is very much reduced; only in a few instances were mitoses observed.

Gatenby (1924) observed the transition of peritoneal epithelial cells into germ cells in Gallus bankiva. In a fowl that possessed some male characteristics, he observed an adenomatous and atrophic ovary. Within this "effete ovary" the peritoneal epithelium had begun to elaborate new testicular tissue. He noticed the transition of avian peritoneal cells into germ cells similar to that described in his work on amphibia. He also calls attention to the work of Fell, (1923) who has described the metamorphosis of peritoneal cells into germ cells.

Greenwood (1925) studied gonad grafts in the fowl. He observed the structure of activated right gonads in ovarioto-mized hens, and saw a secondary invasion of sex cords. Testicular tubules were derived from a further differentiation of the invading sex cords. The presence of one or two small invaginations from the germinal epithelium indicated a germinal epithelium origin. In a distal portion of the medulla, aggregations of small cellular cords suggested a possible origin from medullary cords, which in themselves were products of the germinal epithelium.

Fell (1923), in her work on intersexual birds was able to show definitely that sex cords sometimes proliferate from the peritoneal covering of the gonad. She also admitted the possibility of medullary cords contributing to these, since any influence on the germinal epithelium might be assumed to effect also its intra-ovarian derivatives. Some of the germinal cords in the intersexual birds had developed into differentiated tubules showing active spermatogenesis.

Finlay (1925) observed that in an ovarioto-mized fowl, after the influence of the female hormone had disappeared the new tissue growth in the right gonad represented a new development of testicular tissue.

Degeneration of Germ Cells

The so-called, fully formed germ cells of the immature testis or ovary are probably degeneration stages. That the early formed sex cells (precocious germ cells) never reach maturity has already been mentioned, consequently the functional germ cells are derived at a later period of development.
A dormancy of germ cells does not occur, as is indicated by the development of undifferentiated cells of peritoneal origin into germ cells which degenerate. The above finding is to be regarded as an indication of the potentiality of the coelomic epithelium to form germ cells. The author's material does not permit him to accept the idea that germ cells are able to stop at some stage of development and then later continue development into functional germ cells. Gatenby has contrasted the number of oocytes within the amphibian ovary, with the number of eggs laid, and has definitely shown that the number of eggs laid exceeds the number of oocytes found within the ovary.

If there is a degeneration of germ cells in the developing gonad, then where do the definitive or functional germ cells arise?

The definitive germ cells of the testis are transformed from the undifferentiated germinal epithelial cells situated in the testis cords of a chick, after the 20 day of hatching. Previous to this age (20 days) the germ cells produced are precocious sex cells; and degenerate.

In the development of the ovary the author describes an infiltration of the stroma by germinal epithelial cords, which then disorganize. In this way the stroma of the medulla becomes infiltrated with germinal epithelial derivatives. The large early sex-cells degenerate and contribute nothing to the formation of the medulla. With the development of the cortex from the germinal epithelium and a subsequent degeneration of the early sex cells, there occurs an infiltration of the area originally occupied by the cortex, with medullary and germinal epithelial derivatives. Since both are derivatives originally from the germinal epithelium they are potential germ cells and can by a process of transformation give rise to the definitive ova of the mature ovary, or there may be a transformation from the mesoblastic lining of the ovary itself.

CONCLUSIONS

1. The early segregated entodermal cells of Swift are probably expressions of recapitulation in the ancestral history of the gonad.

2. The early germ cells of entodermal origin do not add to the structure of the germinal epithelium and never reach expression as definitive germ cells.
The transformation of ordinary peritoneal cells within the splanchnic mesoderm just lateral to the celomic angle occurs very early in the development of the chick, before there is any distribution of the "Swift cells" by way of the bloodstream. (36-38 hours)

There is no dedifferentiation of the segregated entodermal cells into small peritoneal components, but an early degeneration outside the germinal epithelium occurs.

There is a gradual transformation and degeneration of the early sex cells within the germinal epithelium.

The development of the male gonad or testis takes place as observed by Swift, with the exception that none of the early segregated cells of entodermal origin ever enter into the development. The testicular epithelium is purely of peritoneal origin.

In the development of the chick ovary, the medulla is produced by the cords of first proliferation from the germinal epithelium and contains no early segregated entodermal cells.

The cortex of the early ovary is produced during a second period of extensive transformation of the germinal epithelium, resulting in a displacement and infiltration at the periphery of the medulla. There follows a severe degeneration of all the early ova, and an infiltration of the area originally occupied by the cortex with undifferentiated medullary components.

The functional ova of the ovary are purely of peritoneal origin, and are the result of transformation of peritoneal derivatives just before the ovary reaches maturity.

Only a few of the early ova reach the stage of early follicle formation. Ultimately, these also degenerate, without ever ovulating.

The ova which finally reach the peak of normal development and ovulate are produced by a transformation of the undifferentiated medullary derivatives of peritoneal origin or of the mesoblastic layer covering the ovary, or from both of these.

The definitive germ cells are not early segregated cells but are the products of differentiation from the germinal epithelium.
### TABLE I

Distribution of Germ Cells of Peritoneal Origin in 4 Chick Embryos

<table>
<thead>
<tr>
<th>Stage</th>
<th>Mesenteric root region</th>
<th>Somatopleure</th>
<th>Superficial Splanchnopleuric layer (future germinal epithelium of both sides included)</th>
<th>Subgermial region</th>
<th>Multinucleated cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>46 hours</td>
<td>2</td>
<td>2</td>
<td>37</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>72 hours</td>
<td>93</td>
<td>--</td>
<td>195*</td>
<td>39</td>
<td>3</td>
</tr>
<tr>
<td>77 1/2 hours</td>
<td>121</td>
<td>--</td>
<td>257*</td>
<td>33</td>
<td>5</td>
</tr>
<tr>
<td>83 hours</td>
<td>316</td>
<td>--</td>
<td>617*</td>
<td>256</td>
<td>4</td>
</tr>
</tbody>
</table>

*Only large typical cells were counted; the smaller transition stages were not included.*
ABBREVIATIONS

a., peritoneal cell
b,c,d,e,f, transforming sex-cells
b.m., basement membrane
ao. d., dorsal aorta
ang. c., angle of the celome
ect., ectoderm
ent., entoderm
g. e., germinal epithelium
g. r., genital ridge
l. lumen
l. s., liquifaction spaces
m. medulla
m. d., medullary cells of peritoneal origin
mes. r., mesenteric root
mult. c., multinucleated cell
o. v., ovarian cortex
p.g.c., primordial germ cell of Swift
pr. f., precocious follicle
s. c., sex-cord
st., stroma
deg. c., degenerating cell
x, degenerating ovum
y, degenerating follicle

All figures are mounted dorsal side up.

All figure represent sections of the left gonad testis, or ovary.

Limits of germinal epithelium indicated by red line.
PLATEX

Fig. 35  a
Fig. 36  b
Fig. 37  c

Fig. 38  d
Fig. 39  e
Fig. 40  f

Fig. 41  f_inmitosis
Fig. 42
Fig. 43  d
Fig. 44

**All figures magnified 970 x**
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