THE HISTORY OF THE PRIMORDIAL GERM CELLS IN THE LOGGERHEAD TURTLE EMBRYO

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In view of the wide discrepancies in the published accounts of the germ-cell history in various vertebrates, it seems very desirable that the problem should be reinvestigated among a greater range of forms, and by additional workers.

The present study represents an attempt to trace the complete early history of the genital cells in a peculiarly favorable form, and, in the light of this data, to correlate the mass of apparently discordant observations recorded for other vertebrates.

The material employed consists of embryos of the loggerhead turtle, *Caretta (Thallassochelys) caretta*, from the two- to the thirty-two day stage of incubation. Parallel series were prepared by the Flemming-iron-hematoxylin and the Helly-Giemsa technics.

This material possesses the following combination of unusual advantages for such study: (1) it can be procured in great abundance; (2) the developmental process is relatively slow (the incubation period extends through fifty-six days), yielding thus a closely graded series of stages; and (3) the germ cells are abundant and at all stages sharply demarked, by size, shape, and nuclear and cytoplasmic characteristics, from the cells with which they seemed possibly to have been confused in some forms, especially the less differentiated yolk-laden entoderm cells, and certain blood granulocytes (eosinophiles).

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The fundamental controversy concerning the history of the germ cells in vertebrates centers about the question of their relationship to the so-called 'germinal epithelium' of the genital ridge. Waldeyer[1] maintains that in the chick certain cells of the portion of the peritoneal epithelium covering the primitive gonad differentiate into germ cells. Nussbaum[2] was the first to controvert this theory, claiming that the germ cells are early segregated (in trout, frog and chick embryos) from the soma cells and that they subsequently wander from originally widely scattered regions into the differentiating sexual glands.

These two rival theories have had their adherents to the present day. Gatenby,[3] for example, claims that in certain amphibia (*Rana temporaria*, *Salamandra*) the definitive germ cells arise by process of transformation of peritoneal cells, as first described by Waldeyer; in similar forms (*Rana pipeins; Bufo lentiginosus*) Allen[4] and King[5] claim that the definitive germ cells (gonia) and the primordial germ cells are the same, the former being genetic derivatives of the latter, and that neither are genetically related to the peritoneal epithelium.

A number of investigators, e.g., Felix,[6] Firket[7] and Dustin[8] claim the existence of two generations of germ cells, the primary and secondary genital (sex) cells or gonocytes. The earlier generation is supposed to have only a phylogenetic significance and to early disappear after a more or less nomadic career; while the later generation is claimed to have its origin from the cells of the peritoneum covering the gonads.

As regards the chick, Nussbaum's original claim is now fully sustained both on the basis of morphologic and of experimental data. Swift[9] has discovered the original entodermal locus of the primordial germ cells in this form in a cephalic crescentic area, in the early primitive-streak stage. As the spreading mesoderm invades this region the germ cells become largely involved in the blood vessels, by which they are carried to the splanchnic mesoderm of the hind-gut region; from whence, by ameboid activity, they wander up the forming mesentery and across the celomic angle into the developing gonads. An unbroken lineage has now been traced by Swift[10, 11] between these primordial germ cells of the gonads and the oögonia and spermogonia of the ovary and testis respectively, without any contribution from the peritoneal epithelium. Reagan[12] has put Swift's observations to a crucial experimental test by removing the crescentic germ-cell area of the primitive-streak stage, and reincubating such operated specimens. Sections of such chick embryos revealed a complete lack of germ cells.

Subsidiary more important matters concern the migration route of the primordial germ cells, and the possibility of a persistence of latent 'stray' germ cells to serve as possible future foci for neoplasms. In the chick (Swift) and in the duck (von Berenberg-Gossler[13]) the germ cells are said to migrate largely via the blood vessels. These observations involve the possibility of a confusion with hypertrophied blood granulocytes. Moreover, von Berenberg-Gossler[13] records the anomolous observation that in the lizard, *Lacerta agilis*, 'primordial germ cells' ('entodermal wandering cells') contribute to the formation of the caudal extremity of the Wolffian duct. He regards these cells as representatives of a dilatory process of mesoderm derivation from entoderm.

As regards amphibia, the later observations incline Allen[14] to conclude that in anurans the germ cells have an entodermal origin (i.e., original segregation), in urodeles a mesodermal origin.

The germ-cell history of *Caretta* is very similar to that first described by Allen[15] for *Chrysemys marginata*—and more recently confirmed by Dustin[16] —and to that described by Woods[17] for the dogfish. This may be summarized as follows:

1. The primordial germ cells in *Caretta* migrate during the second day (5 somites; 2 mm. length) from the yolk-sac entoderm, where they were more or less widely scattered caudally, into the lateral border of the area pellucida on each side of the embryonic disc. Here they become sharply segregated by the beginning of the third day (10 somites; 3 mm. length) into bilateral cords situated in the entoderm of the area pellucida laterally, in the caudal half of the disc. In the two-day embryo they extend from the neurenteric canal to the end of the primitive streak; in the three-day embryo from the sixth somite to the caudal extremity of the streak. The cords become more medially placed, make a linear connection with the overlying visceral mesoderm, and their cells migrate during the fifth day into this mesoderm, and thence medially (during the sixth and seventh days) towards the root of the forming mesentery of the closing hind-gut. Individual cells migrate medially also within, or back into, the entoderm of the gut. The germ cells in the medial entoderm become included in the tunica mucosa of the closed hind-gut, those in the mesoderm in the enveloping mesenchyma and the gut end of the mesentery. From these locations the majority of the germ cells subsequently (seventh to twelfth day) migrate up the mesentery and across the celomic angle into the future sexual gland. They become incorporated among the mesenchymal cells of the gland and the covering peritoneal epithelium, where they suffer no striking change in form, size or content at least as late as the thirty-second day of incubation.

2. The germ cells migrate by ameboid activity, assisted in small part probably by the factor of unequal growth, involving the shifting of the medial portion of the splanchnopleure to the mesentery, and the dorsal portion of the mesentery to the gonads.

3. The migration period is not sharply limited. It is at its height from the seventh to the twelfth day, and practically ceases about the sixteenth day. But occasional extra-regional cells may still be found in the gut and mesentery at the thirty-second day stage, usually, however, showing signs of degeneration.

4. A certain number of germ cells migrate out of the regular germ-cell route and go astray. Such 'strays' are especially numerous in the periaortic mesenchyma where they may become incorporated among the neuroblasts of the developing peripheral sympathetic ganglia. The majority of these strays probably degenerate *in situ*, but some may possibly persist to form, under the proper pathologic stimulus, a focus of neoplastic growth. An occasional cell is found also in the blood vessels of this region. Such may be carried by the blood stream to distant regions and perhaps again enter the mesenchyma or degenerate within the vessels.

5. The total number of primordial germ cells counted in a twelve-day embryo is 352, the number within the gonads being about equally divided between the two (118, left to 127 right).

6. Occasional cells may divide by mitosis or undergo degeneration at any stage of their history or at any point of the route. Mitoses are relatively more numerous during earlier stages and among the entodermal cells; degeneration is more general during the later stages and in the mesenchyma of the closed hind-gut.

7. No germ cells were found contributing to the formation of the Wolffian duct. There is no evidence in this form in support of von Berenberg-Gossler's claim, on the basis of his observations on the lizard embryo, that the so-called primordial germ cells represent simply a belated stage of mesoderm formation from entoderm.

8. The germ cells do not differ from young somatic cells in the character of their mitochondrial content. The mitochondria include granular as well as beaded rod and filamentous forms.

9. No transition stages between celomic epithelial cells and germ cells appear up to the thirty-second-day stage. From the sixteen-day stage on, when the nuclei of some of the germ cells within the gonads became coarsely granular and the reticulum stains more deeply, apparent transition stages occur between the larger of the mesenchymal cells and the smaller included subepithelial germ cells. But no secure histologic basis can here be found for separating the germ cells of the gonads into large "primary genital cells" and smaller "secondary genital cells" (Felix) or gonocytes (Dustin), derived by process of differentiation from the cells of the germinal (peritoneal) epithelium or the subjacent mesenchyma. The size variations among the germ cells of the gonads of the older stages are no greater than in the original cords of the area pellucida or in the subsequent early stages; and the cytologic similarity between the two dimensional grades of cells is much closer than between the larger mesenchymal cells and the smaller germ cells.

10. The evidence derived from a study of the *Caretta* embryos is

in complete harmony with the idea of a single uninterrupted line of sex cells from primordial germ cells to oögonia and spermagonia, and with the hypothesis of a vertebrate 'Keimbahn' or continuous germinal path.

11. The variations in the distribution of the primordial germ cells during earlier embryonic stages described by various investigators for a number of vertebrate forms—as pertains both to their presence in blood vessels (chick, Swift; duck, von Berenberg-Gossler) and in various regions and tissues remote from the more direct and more usual germinal route (Wolffian duct and somatopleure in the lizard, von Berenberg-Gossler; and sympathetic ganglia in the loggerhead turtle, Jordan); and to their apparent primary (urodeles) or secondary (anurans and other vertebrates) derivation from the splanchnic layer of the lateral mesoderm—are incidental to their original location with respect to the embryonic area and the vascularizing mesoblast of the blastoderm, and to their ameboid capacity. Since the primordial germ cells are genetically directly related to neither of the secondary germ layers their origin in either (entoderm or mesoderm) has no fundamental significance. Since they are capable of ameboid activity, and may become included in blood vessels, they may migrate anywhere, and so occur in any location, from where they may subsequently migrate again to the more direct germinal path, or perhaps disintegrate. The fact of fundamental significance with respect to the primordial germ cells is their original extra-regional distribution and their direct genetic independence of the soma cells.

A more complete paper, with illustrations, and a fuller review of the literature, will appear in a forthcoming volume from the Tortugas Laboratory of the Carnegie Institution of Washington.

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