HANDBUCH DER HISTOCHEMIE

HERAUSGEGEBEN VON

WALTHER GRAUMANN und KARLHEINZ NEUMANN

Giessen Köln

BAND VII

HISTOCHEMISTRY OF ENZYMES IN THE FEMALE GENITAL SYSTEM

DRITTER TEIL

Mit 200 zum Teil farbigen Abbildungen
und 22 Tabellen

GUSTAV FISCHER VERLAG · STUTTGART

BAND VII

ENZYME

DRITTER TEIL

Female Genital System

By

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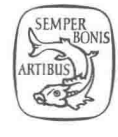

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Introduction

The histochemistry and cytochemistry of the female reproductive system comprises a subject of fascinating and enduring interest to the student who studies an area of the body which can undergo morphologic and metabolic changes of rather striking magnitude during the different phases of organismic activity. Indeed, the morphologic responses to changing hormonal environments within the body have held the attention of investigators for many decades; and perhaps, in their enthusiasm to define the complicated and relatively remarkable alterations in structure in the female endocrine targets, particular attention to specific hormonal interrelationships was delayed until relatively recently. While many of these basic endocrine reactions have been in the process of physiological definition, the new discipline of chemical morphology has come into existence and, following some initial inertia in general application, these enzymorphologic studies soon found their way into assessing such distributions; and evidently the greatest effort was directed to and expressed in the study of uterine tissues and related structures. This initial enthusiasm, manifesting itself to this day, although progressive and beneficial in its own right cannot, nevertheless, escape some censure for passively being a party to the obvious neglect of more intensive studies of other regions of the female reproductive tract. Witness, especially, the appalling state of the lack of information concerning the enzyme histochemistry of the Fallopian tube. One can only hope that the biased evolution of applied histochemical localizations within the female reproductive system soon becomes redirected into areas which have suffered considerable lack of attention and investigation. Such hopes are here expressed with the conviction that the chemical morphology of the female reproductive tract will realize its highest fruition *only* when the knowledge of any of the reproductive components is viewed and analysed in terms of the entire complex of other regions.

The reports which follow are for reasons of necessity conveniently compartmentalized under each reproductive structure. It is important to indicate, however, that this artificial listing is beset with some problems, namely those of stigmatizing the reader with a "package" of information concerning a certain region of the tract. It will be necessary to depart somewhat from this format whenever sufficient information presents itself to allow for discussion of one organ structure in relation to another. In addition, lengthy discussions of the relative adequacies or inadequacies of any given technique will not be presented in view of the fact that these technical interpretations are out of the realm of the scope of this

We acknowledge with kindest thanks the generous and indispensible help given to the authors by the many researchers who supplied data and illustrative material for inclusion in the present work. We are also most appreciative for the splendid assistance of Miss Barbara Kasprow (editorial and library research, Yale University), Messrs. Robert T. Lentz and Samuel Davis (librarians, Jefferson Medical College), Mrs. Henrietta T. Perkins (librarian, Yale University), and to Mrs. Jean Morioka (typing and library research). Messrs. Edward D'Orazio, Jerome Vernick and Marvin Hyett, medical students (Jefferson Medical College) are responsible for the localizations of SDH using TNBT (Figs. 43–45). Photostats of articles appearing in journals not in our institutional libraries were kindly supplied by the Library of Congress, USA.

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writing. Any attempt to incorporate this material into the subject matter of the text might tend to obscure the primary purpose of this literary excursion. The evaluation of methods in use both presently and in the past for the histochemical localization of enzymes is certainly a consideration in its own right and this topic enjoys a position elsewhere in the *Handbuch* series.

Nevertheless, we do not believe that the true essence of enzyme patterns of distribution both at the intracellular and histologic levels of study in these female structures can actually be attained without some inference as to the reliability of technical procedures employed in the past and also, on occasion, possible recommendations of methods presently available and their advised application to study in these areas at some future time. It must be understood, however, that any comprehensive survey of the recent literature concerning histochemical findings in the female reproductive system, or any organ system for that matter, must by necessity limit itself to studies in the past; indeed, judging from the rapid rates of evolution of many of the techniques for hydrolytic and/or oxidative enzymes (alkaline phosphatase and the succinic dehydrogenase system are classical examples, respectively) a report such as the present one will soon, and hopefully so, enter into the realm of a historical review as a result of the rapidly advancing frontiers of tissue and cellular chemical morphology.

Any consideration of an endocrine-reproductive component should for its fullest significance engage in, imply or advance those necessary endocrine postulates requisite for a comprehensive understanding of the hormonal interplay within the organism. This will be attempted, by necessity in brief form, in order to equip those readers who would extract greater significance from these data and reports if hormonal mechanisms and *interreactions* might be viewed in the light of enzyme pattern distribution and relative reactivities of areas within cellular tissues and organ structures. An effort to quantify certain of these reactions will be provided wherever possible by the inclusion of data from certain biochemical analysis. This approach is made in order to afford a more analytical correlation of the quantitative aspects with the localizations of different enzymes during the different phases of the reproductive cycle.

Finally, only very brief mention will be made where necessarily indicated, of the anatomical relationships both at the gross and microscopical levels with an occasional inclusion of special embryological considerations. When possible, however, the newer morphological treatments of electron microscopy, supported by well documented and intensive studies will be described if deemed pertinent to the topics given consideration.

I. The ovary

Introduction

The fact that new and potential life emanates from the ovary leads us to give very special consideration to the embryologic origin of the germ cells. This section will then proceed to a discussion of the development and maturation of the female germ cells, the ova. Therefore, this section will perforce be developed from a developmental viewpoint.

Considerably more is known concerning the sexual differentiation of the gonads than of the endocrine physiology of the fetal gonads (C. R. MOORE 1950, and L. J. WELLS 1950, 1959). The research reports and views by C. R. MOORE (1950) and JOST (1953, 1954) are in complete accord that there is a lack of scientific evidence concerning a satisfactory demonstration that the fetal gonads secrete native steroidal hormones. We will, however, attempt to analyze the known facts of embryonic and subsequent ovarian development, as well as its pathologic variants.

A. The enzyme histochemistry of the germ cells

Much more is known regarding the histochemical observations on the germ cells of *human* embryos than of any *invertebrate* or other *vertebrate* forms (VELARDO 1958).

While it is not the function of the present essayists to develop the story relating to the origin of the germ cells, it is nevertheless important to highlight some of the necessary embryologic background germane to a more careful appraisal of the cyto- and histochemical complexion of the germ cells. The concept that the germ cells are issued from the "germinal epithelium" has had many proponents (STIEVE 1927, NEUMANN 1929 and SIMKINS 1928). WITSCHI (1948), in contrast to the above thesis, indicated in a scholarly treatise that the germ cells of the *human* arise from the endoderm of the yolk sac or from the primitive stem cells which also are the source of the endoderm. This last report indicates that the germ cells migrate from this location to the hindgut endoderm, subsequently to the mesentery of the gut and then toward the mesonephric folds. The different theories of the origin of germ cells are reviewed by MCKAY and his collaborators (1953, 1955), F. D. ALLAN (1958) and GARCIA and ROCK (1958).

Regarding the histochemistry of the human germ cells and that of the human ovum, MCKAY (1953, 1955) and HERTIG (1958) and their co-workers deserve much credit for advancing our knowledge in this important area of human development.

1. Thirteen-day human ovum

a) Historical survey of early (very young) embryos

Thus far, six very early human embryos have been studied for their histochemical constituents. The youngest studied to date is a thirteen-day human fertilized ovum recovered at hysterectomy during its fourteenth day of development (HERTIG 1958). This specimen lies within Horizon VI of STREETER, and possesses primitive unbranched villi and a definitive yolk sac. The description by HERTIG (1958) and his colleagues state: "From its gross appearance, size, and embryologic development, it is comparable to two Carnegie specimens, No. 8672 and No. 8360. It is slightly younger than early villous ova such as Carnegie No. 7801, possessing a definitive yolk sac, and slightly

older than Carnegie No. 8330, possessing a primordial yolk sac. Hence the present specimen is the youngest thus far seen possessing a definitive yolk sac. In reference to other specimens in the literature, it is comparable to the LINZENMEIER ovum and slightly younger than the Yale (RAMSEY) ovum of 13 plus days, Carnegie No. 6734, the PETERS ovum of 14 days, and the EDWARD-JONES-BREWER ovum of 15 to 16 days' developmental age." An outline drawing showing human ova of 11 to 15 days of age or in their twelfth to sixteenth day of development may be seen on page 149 of a paper by HERTIG and ROCK (1941) which deals with two human ova of the pre-villous stage.

Since the thirteen day-old human ovum, as studied by HERTIG (1958) and his associates, is the earliest on which a whole array of histochemical tests have been applied, it seems of paramount significance to give a further description of this specimen for the purpose of orientation prior to a graphic and pictorial essay of its histochemical constituents.

Horizon VI is described more in detail as follows: "At 13 days of age the ovum is evident upon gross examination by virtue of its size and elevation. It is not completely embedded within the endometrium. Bleeding which may occur through the unhealed implantation site, arises from the increased flow of maternal blood into the lacunar spaces resulting in rupture of the thin-walled abembryonic pole of trophoblast. There is also bleeding into an occasional endometrial gland whose walls have been eroded by invading trophoblast thereby allowing the maternal blood in the lacunar spaces to flow into the gland lumen. Since this bleeding occurs at about the time of the first missed menstrual period it is an occasional source of clinical inaccuracy in foretelling the date of expected delivery.

"In the trophoblastic shell of the ovum, the cytotrophoblastic cells are rapidly proliferating so that their total mass now exceeds that of the syncytium. The latter now lines the intervillous space which contains occasional streamers of projecting syncytiotrophoblast. Some syncytium detaches as it invades the endometrial stroma and is seen as giant cells within the decidua. Primitive villi are now forming. They have a shallow core of mesoblasts and angioblasts arising from and projecting into otherwise solid cytotrophoblast which in turn is covered by syncytium. The peripheral tips of the villi are coalescing to form the cytotrophoblastic placental floor which is perforated by capillary sinusoids supplying the intervillous space with maternal blood.

"The mesoblast which lines the chorion is more abundant than in earlier stages particularly in the space between the amnion and the chorion. Angioblastic tissue, also delaminating from cytotrophoblast and lying in the mesoblast, is in various stages of early differentiation," (HERTIG et al. 1958, p. 1026).

b) Description of specimen

Age of ovum: The ovum is about 13 days of age or in its fourteenth day of development.

Size: The dimensions of this early villous ovum after paraffin embedding and serial sectioning are as follows: over-all ovum, 1.77 by 1.33 by 0.598 mm.; chorionic cavity 0.73 by 0.68 by 0.221 mm.; embryo including amnion and yolk sac, 0.196 by 0.315 by 0.076 mm.; germ disc alone, 0.196 by 0.296 by 0.044 mm. These dimensions are comparable to those previously reported lying within Horizon VI (HERTIG, ROCK and ADAMS 1956).

The endometrium: The functionalis (upper two-thirds) of the endometrium shows moderate-to-advanced progestational hyperplasia characterized by slight-to-moderate predecidua, prominent and somewhat dilated spiral arterioles, definite stromal edema, and glandular secretions. The basalis (lower one-third) of the endometrium shows compacted stroma and rather inactive glands. Directly beneath the ovum the glands contain inspissated secretory material due to blockage by the ovum whereas elsewhere the secretion appears finely granular. In one instance, the gland beneath the ovum contains recent hemorrhage due to communication of the lumen of the gland, eroded by the trophoblast, with the blood-filled intervillous space. (Hemorrhage into the

gland is normal for this stage of gestation and is comparable to the hemorrhage from the abembryonic pole of the ovum which is also normal in later stages of Horizon VI.) The surface hemorrhage plays a part in the formation of the *Schlusscoagulum*. The present specimen has a small surface coagulum without hemorrhage.

Features of the ovum: This is a normal but somewhat shallowly implanted early villous ovum possessing a bilaminar germ disc apparently without axial differentiation, but possessing a well-developed amnion and a very recently formed definitive yolk sac. Remnants of HEUSER's or the exocelomic membrane, the wall of the primordial yolk sac, are present in the chorionic cavity. The chorionic mesoblast is well formed but the chorionic villi are essentially solid epithelial structures with the earliest suggestion of mesoblastic core formation continuous with the chorionic mesoblast. Angiogenesis, in the form of solid multicellular strands, is just beginning.

The intervillous space is well formed but many lacunae of different sizes are present within the syncytiotrophoblast and are not yet incorporated into the intervillous space. The latter contains clotted and unclotted maternal blood, together with the contents of a recently eroded endometrial gland, presumably a source of nutrition for the early ovum. The syncytiotrophoblast is, in general, distally or peripherally located and is the main contact of the ovum with maternal tissue. The cytotrophoblast is more proximally or centrally situated, forming the chorionic membrane and early primordial villi whose tips are distally in contact with endometrial stroma. The trophoblast at the implantation pole is well developed but poorly so at the abembryonic pole. HERTIG et al. (1958) report that this is a function of the depth of implantation, which is subject to some variation at this time of gestation. Thus, it appears that the abembryonic pole of the ovum is covered by a thin layer of maternal epithelium of questionable viability, plus a coagulum composed of fibrin, cellular debris, and leukocytes (the *Schlusscoagulum*).

2. Histochemical features of the 1.7 mm. (13-days old) ovum

Adenosine-5-phosphatase

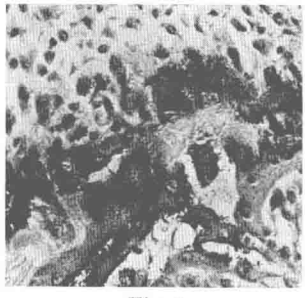


Fig. 1

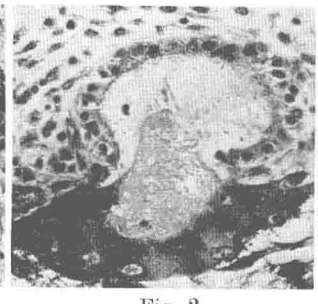


Fig. 2

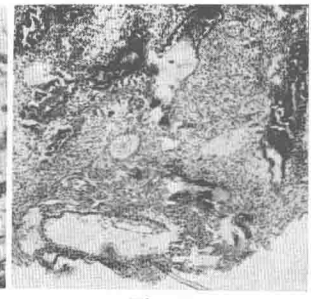


Fig. 3

Fig. 1: Adenosine-5-phosphatase (pH 7.5) showing concentration of enzyme in cytoplasm of actively invading syncytiotrophoblast. In the ovum this enzyme is confined to the cytoplasm of the distally situated syncytiotrophoblast lying in contact with endometrial stromal glands (cf. Fig. 2). × 200.

Fig. 2: Adenosine-5-phosphatase is concentrated within the cytoplasm of syncytiotrophoblast which is actively eroding, ingesting, and digesting the glandular epithelium. The remnant of the partially eroded gland is seen above; its contents lying in contact with the cytoplasm of the trophoblast. Remnants of gland cell nuclei are seen within syncytiotrophoblast at lower left. Note presence of enzyme in endometrial stromal cells immediately adjacent to trophoblast at lower left but absent elsewhere. × 200.

Fig. 3: Adenosine-5-phosphatase (pH 7.5) is confined to the actively invading portions of the syncytiotrophoblast and the endometrium at some distance from the ovum. Note especially the absence of the enzyme from the endometrium immediately around the ovum. × 40.

In their histochemical studies of the 13-days old human ovum, HERTIG et al. (1958) determined that adenosine-5-phosphatase is confined to the cytoplasm of the most active of the distally situated syncytiotrophoblast lying in contact with endometrial stroma and glands (Figs. 1 and 2). The stroma immediately surrounding the ovum is negative for this enzyme (Fig. 3), but the stroma and glands beyond this negative zone react positively for adenosine-5-phosphatase.

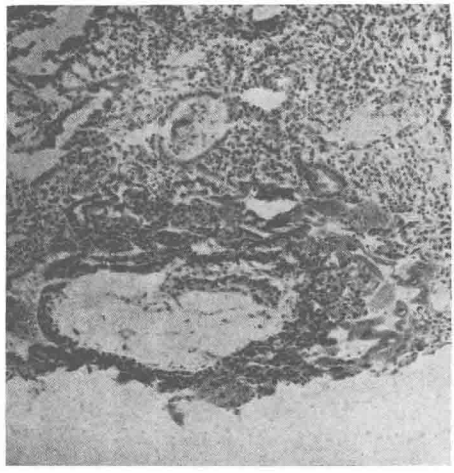


Fig. 4: Glycerophosphatase activity at this pH (7.5) is negative, thus indicating specificity seen in figure 3. Note absence of enzyme from ovum and endometrium. The dark foci in individual cells are nuclei counterstained with hematoxylin. × 45.

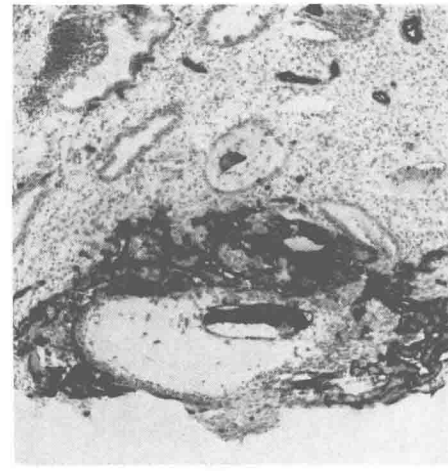
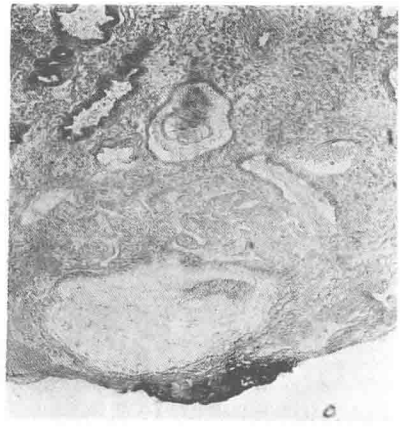


Fig. 5: Alkaline phosphatase (pH 9.4) is concentrated in the syncytiotrophoblast, more particularly in the brush border, the germ disc, and in the endothelium of the endometrial arterioles and capillaries. × 45.

Glycerophosphatase

The ovum and endometrium are negative for this enzyme (Fig. 4). Positive reactions for alkaline phosphatase were found in all of the syncytiotrophoblast, especially in the brush border, both layers of the germ disc, and in the endothelium of the spiral arterioles of the endometrium (Fig. 5).



Acid phosphatase

This non-specific phosphatase is negative in the ovum, but is present in the *Schlusscoagulum* of the healing endometrial defect at the abembryonic pole and in the endometrial glands (Fig. 6).

Fig. 6:
Acid phosphatase (pH 5.8) is absent from ovum but relatively present in *Schlusscoagulum* with lesser amounts in the endometrial glandular epithelium (Figs. 1–6, courtesy HERTIG et al. 1958).

3. The 5 mm. (28-days old) embryo

McKay, Adams, Hertig and Danziger (1955) reported that "the germ cells of this embryo are found in the connective tissue of the root of the mesentery, within and beneath the coelomic epithelium, between the epithelial cells lining the gut, within the gut lumen, and in the connective tissue and coelomic epithelium of the developing gonadal folds".

The phosphatases

The germ cells of the 5 mm. embryo are characterized by a cytoplasmic rim of alkaline phosphatase activity which sharply outlines them from the surrounding tissues. Figure 7 illustrates a transverse section through a 5 mm. human embryo showing the spinal cord, urogenital ridges and primitive gut. The primitive germ cells are outlined by the Seligman alkaline phosphatase technique and are present in the coelomic epithelium, connective tissue of the mesentery and in the developing gonadal folds. In a transverse section through the primitive gut, mesentery and coelomic epithelium covering the gonadal folds, it was determined that the alkaline phosphatase is concentrated at the periphery of the cell body and does not appear in the nucleus (Fig. 8).

Utilizing the alkaline glycerophosphatase method, McKay et al. (1953) reported that the germ cells cannot be distinguished as clearly as with the alpha naphthyl phosphatase technique. In the 5 mm. embryo, the surrounding mesenchyme has such a high activity that practically all the cells are stained and the germ cells cannot be readily outlined.

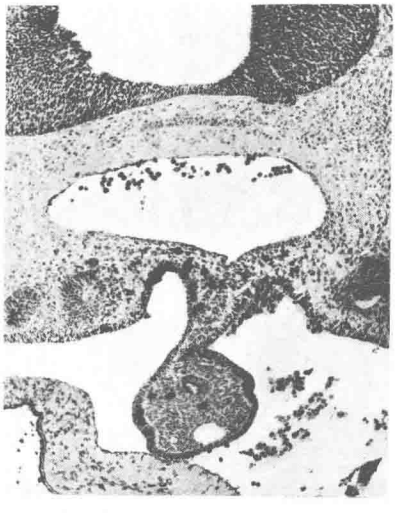


Fig. 7: Transverse section through 5 mm. human embryo to show spinal cord, aorta, urogenital ridges and primitive gut. The primitive germ cells are outlined by the Seligman alkaline phosphatase technique and are present in the coelomic epithelium, connective tissue of the mesentery and in the developing gonadal folds. × 135.

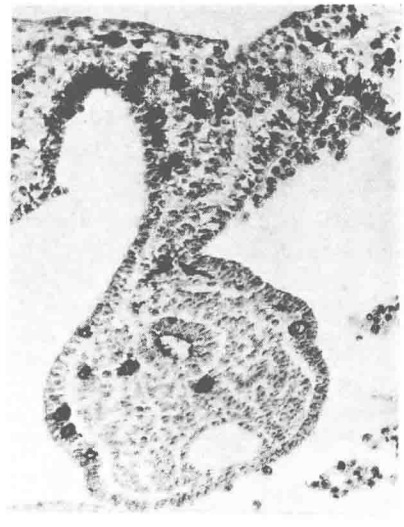


Fig. 8: Transverse section of the primitive gut, mesentery, and coelomic epithelium covering the gonadal folds of 5 mm. human embryo. Alkaline phosphatase is concentrated at the periphery of the cell body and does not appear in the nucleus (same specimen as figure 7). × 300.

Although demonstrably positive for alkaline phosphatase, the germ cells of the 5 mm. embryo do not display any activity for acid phosphatase.

Non-specific esterase and 5-nucleotidase:

The germ cells of this embryo are negative for these 2 enzymes.

Other histochemical components of the 5 mm. embryo:

In an effort to give as much detail as possible to the earliest described human embryos, it may prove of interest to include some data on parallel studies concerning the glycogen, iron and ribonucleoprotein localizations within the germ cells.

The cytoplasm of the germ cells of the 5 mm. human embryo contains visible deposits of *glycogen* which are amylase-digestable. Following treatment by amylase, there is no residual material in these cells which gives the periodic acid-SCHIFF reaction.

Minute intensities of *basophilic materials* are localized beneath the cell membrane in a few germ cells. The cytoplasmic basophilia is ribonuclease-digestable. BRACHET (1940, 1942) and LANSING and ROSENTHAL (1952) observed the localization of cytoplasmic ribonucleic acid near the cell membrane in the eggs of frogs and those of Arbacia. From their studies of ribonucleoprotein in the eggs of Arbacia, LANSING and ROSENTHAL have ascertained that the surface layer of ribonucleoprotein has a function in the transport of metabolic materials across the cell membrane, thus suggesting an active metabolic exchange between the germ cells and the surrounding tissues.

In their determinations for the presence of *iron* in this early embryo, MCKAY et al. (1953) report that there was no detectable iron present.

4. The 11 mm. (34-days old) embryo

The germ cells in this embryo are located in the connective tissue of the mesentery of the gut, in the gut endoderm, and are most numerous in the developing genital fold.

The phosphatases

The alpha naphthyl phosphatase activity is highly localized in the cytoplasmic rim and in the cytoplasm of the germ cells. The nuclei of these cells are devoid of this enzyme. Likewise, these areas and the germ cells are positive for alkaline glycerophosphatase.

The germ cells of this embryo are negative for acid phosphatase.

Non-specific esterase and 5-nucleotidase

Tests for both of these substances were negative in the germ cells of the 34-days old embryo.

5. The 13 mm. (36-days old) embryo

The germ cells in the 36-days old embryo are clearly outlined by the alkaline naphthyl *phosphatase* reaction which definitively appears concentrated in the genital ridges. A few germ cells are scattered in the connective tissue around the aorta. A larger number of germ cells are found in the connective tissue root of the mesentery around the coeliac artery near the pancreas and stomach (Figs. 9 and 10). The germ cells of this embryo are positive for *alkaline glycerophosphatase*, but are negative for *acid phosphatase*, *non-specific esterases* and iron.

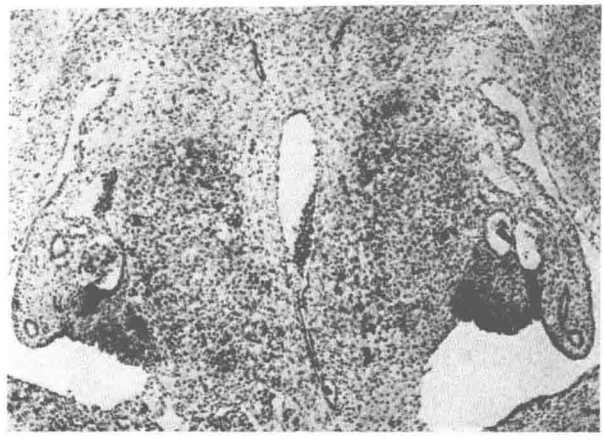


Fig. 9:
13 mm. embryo. The germ cells are most numerous in the gonadal folds but a moderate number are scattered through the connective tissue in the midline and in the developing adrenal tissues which lie medial to the mesonephric ridges. Alpha naphthyl phosphatase. × 112.5.

6. 35 mm. (52-days old) embryo

The gonad of this embryo has differentiated into an ovary which supposedly contains the great majority of germ cells at 52 days of age (Fig. 11). There are a few germ cells found in the sympathetic ganglia and nerves at the hilus of the adrenal, and in the connective tissue of the root of the mesentery (Fig. 12). Very few germ cells are found in the mesenteric connective tissue at its point of attachment to the gut.

Figure 11 shows the positive reaction for alpha naphthyl *alkaline phosphatase* in the right gonad of the 35 mm. female embryo. The positive alpha naphthyl alkaline phosphatase reaction in the few germ cells remaining in the connective tissue of the root of the mesentery is depicted in figure 12. The alkaline glycerophosphatase method reveals the same localization and intensity pattern as observed with the alpha naphthyl phosphatase reaction. As with the other embryos, the germ cells and definitive gonad of the 35 mm. embryo are devoid of *acid phosphatase*, *non-specific esterase*, and *5-nucleotidase*.

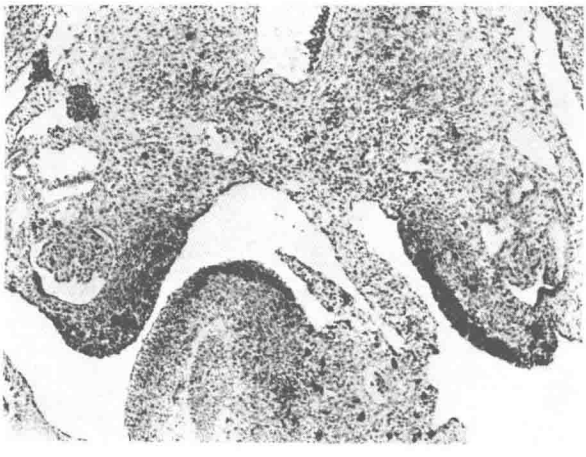


Fig. 10:
13 mm. embryo. The germ cells are concentrated in the genital ridges but are also present in the mesenteric connective tissue. Alpha naphthyl alkaline phosphatase. Same figure as figure 9. × 97.5.