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Acid and Alkaline Phosphatase Activity in Migrating Primordial Germ Cells of the Early Chick Embryo

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ABSTRACT Little information is available concerning enzyme activity in primordial germ cells (PGCs) of the early chick embryo. The present study is designed to examine the disposition of alkaline and acid phosphatase activity in the PGCs during their migration into the developing gonads of the early chick embryo. White Leghorn chick embryos were sacrificed at daily intervals from 1 to 6 days of incubation. Following sacrifice the embryos were fixed, dehydrated, and embedded in glycol methacrylate (GMA). Alkaline and acid phosphatases were demonstrated by the simultaneous diazo-coupling method. The embryonic tissues at the different ages were examined for PGCs and the histochemical reactions for alkaline and acid phosphatases in these cells evaluated. Acid phosphatase activity did not appear within PGCs until 3 days of incubation, and then in only a few PGCs in the active phase of their migration in the dorsal mesentery, suggesting that there is no large wave of degeneration of these cells during migration. Alkaline phosphatase activity was observed as early as 2 days of incubation in PGCs during the passive phase of their migration in extraembryonic blood vessels. Alkaline phosphatase-positive PGCs in the active phase of migration were also found in the dorsal mesentery; however, the cellular localization of this enzyme differed from that observed in the passively migrating PGCs, indicating that there are alterations in the metabolic activities of these cells during the active and passive phases of migration.

Little information is available concerning enzyme activity in primordial germ cells (PGCs) of the early chick embryo. These cells, which are formed from the hypoblast (i.e., extraembryonic endoderm) in the area of the "germinal crescent" (Swift, 1914), undergo a passive migration through the blood vessels followed by an active phase during which time they pass from the blood vessels into the embryonic mesenchyme tissue and then to the developing gonads, which is their final destination. Two enzymes which have been frequently associated with important cellular metabolic activities are acid and alkaline phosphatase. A knowledge of their presence within a specific cell can provide indications of the metabolic processes occurring therein and, in the case of the PGCs, assist in understanding what role these processes might play in the migratory activity displayed by these cells.

The histochemical localization of alkaline phosphatase has long been used as an identifying marker for PGCs in the human (McKay et al., 1953), the mouse (Chiquoine, 1954; Clark and Eddy, 1975), the rat (McAlpine, 1955), and the rabbit (Chretien, 1966). Early studies on the chick embryo using similar techniques have not been effective since with the alkaline phosphatase technique used, all cells stained for this enzyme, thus impairing its use as exclusive marker for the PGCs (Chiquoine and Rothenberg, 1957).

The classical paper by Moog (1944) on alkaline and acid phosphatase localization during the early embryonic development of the chick provides an in-depth account of the appearance and distribution of these enzymes in different organ systems of the developing chick embryo. However, no mention is made of any activity in the PGCs.

Techniques employed in these earlier studies for localizing alkaline phosphatase involved the use of paraffin-embedded tissues which

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necessitated exposing the tissue to harsh chemicals and high temperatures. Such treatment may have affected the precise appearance and distribution of these enzymes. A technique is now available for acquiring thin sections of embryonic tissue and avoiding any exposure of such tissue to harsh solvents used as clearing agents and to heat, which insures preservation of enzyme activity. This technique, which involves the use of glycol methacrylate (GMA) as an embedding medium (Nusbickel and Swartz, 1979), preserves both histological detail of the tissue and specific enzyme activity.

The purpose of this study is to examine the disposition of alkaline and acid phosphatase activity in the PGCs of the chick embryo during their migration from extraembryonic areas to the gonads in order to assess the metabolic activity of these cells during this critical period of their early development.

MATERIALS AND METHODS

Fertile white Leghorn eggs (Truslow Farms, Inc., Chestertown, Md.) were placed in an incubator at 37°C and 70% relative humidity. Eggs were opened and embryos sacrificed at daily intervals from 1 to 6 days of incubation (Stages 8–29; Hamburger and Hamilton, 1951). Following sacrifice, embryos were fixed in a solution containing 95% ETOH, 5% acetic acid, and 10% neutral-buffered formalin in a volume ratio of 85:0.4:10, respectively (Nusbickel and Swartz, 1979). After fixation for 1 hour, the embryos were dehydrated in a graded series of ethanols and embedded in glycol methacrylate (GMA). A detailed description of the embedding procedure was provided in an earlier publication (Swartz and Nusbickel, 1979).

The polymerized GMA blocks containing the embryonic tissue were trimmed and 3-μm-thick sections were cut on a Sorvall JB-4A microtome using glass knives. Each tissue section was mounted on a glass slide, air-dried, and stored at 0°C until used.

The alkaline phosphatase technique employed was the simultaneous diazo-coupling technique, using naphthol AS-MX phosphate as the substrate, at a pH of 8.4, and Fast Red TR as the coupling salt (Burstone, 1962). Acid phosphatase was demonstrated using naphthol AS-MX, but at a pH of 5.2 with Fast Violet LB as the coupling salt (Burstone, 1961). Control slides were exposed to incubation medium devoid of the substrate. Incubation of tissue sections was performed at 37°C using the drop method in a closed environment incubation chamber (Nusbickel, 1980). Times of incubation were 1 hour for alkaline phosphatase and 2 hours for acid phosphatase. Following incubation, the tissues were counterstained in 1% Fast Green and coverslipped.

The embryonic tissues at the different ages were examined for PGCs and the histochemical reactions for alkaline and acid phosphatase in these cells were evaluated.

RESULTS

Acid phosphatase

One- and 2-day embryos were devoid of any specific acid phosphatase activity. In 2-day embryos numerous PGCs were found within extraembryonic blood vessels; however, they were negative for acid phosphatase (Fig. 1). In these same embryos nonspecific acid phosphatase activity was observed in the yolk of the area opaca (Fig. 2).

By the third day of incubation PGCs were still seen within the vascular system; however, most had left the intraembryonic circulatory system and entered the dorsal mesentery. Although some blood cells in the extraembryonic circulation were positive for acid phosphatase, the PGCs still circulating were not.

Numerous PGCs were found in the dorsal mesentery at 4 and 5 days of incubation preparatory to entering the gonads. A few PGCs demonstrated a positive reaction for acid phosphatase but most of these were located some distance from the gonad (Fig. 3). Most PGCs in close approximation to the gonads were devoid of any acid phosphatase activity (Fig. 4). By the fifth day of incubation the majority of PGCs were situated within the developing gonads and there was no indication whatsoever of any acid phosphatase activity in these gonadal PGCs (Fig. 4). The results were similar in 6-day embryos.

Alkaline phosphatase

Alkaline phosphatase activity was first noted with consistency in PGCs as early as 2 days of incubation. PGCs found in the extraembryonic circulation at this stage of development exhibited a positive reaction for alkaline phosphatase activity in the form of a globular deposit(s) in the cytoplasm near the nucleus (Fig. 5). This type of reaction was still seen in circulating PGCs at 3 days of incubation.

Once the PGCs left the intraembryonic circulation and entered the mesenchyme a rearrangement in the distribution of their alkaline

phosphatase activity ocurred. Whereas its appearance in the vascular system was of a globular nature positioned near the nucleus, alkaline phosphatase activity was now found to be associated with the cell membrane found around the entire border of the cell (Figs. 6–9). This membrane-associated alkaline phosphatase activity was seen not only in those cells located in the dorsal mesentery but also in those cells already having reached their final destination in the gonads (Figs. 6, 7). This cell membrane-associated alkaline phosphatase activity was also evident in clusters of PGCs near the gonads (Fig. 8) and in PGCs undergoing mitosis (Fig. 9).

Abbreviations

BV, Blood vessel
C, Coelom
DA, Dorsal aorta
DM, Dorsal mesentery
G, Gonad
M, Mesonephros

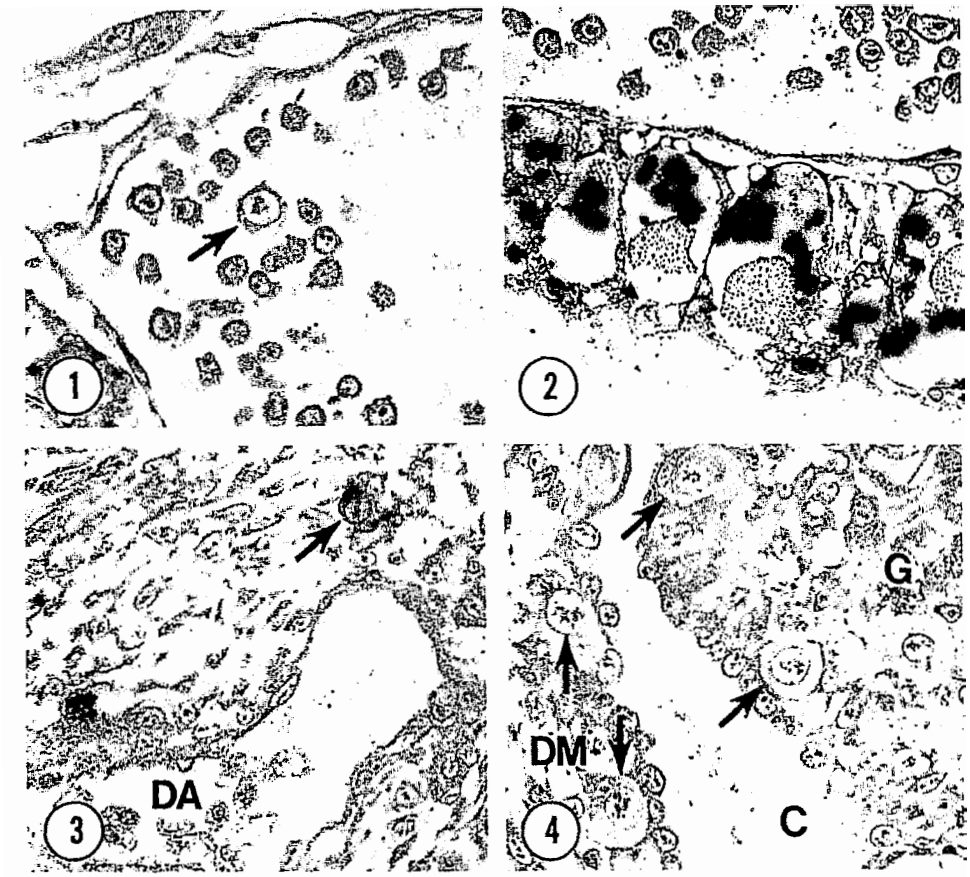

Fig. 1. Extraembryonic blood vessel of a 2-day (Stage 12) chick embryo. Note that the primordial germ cell (PGC, arrow) is devoid of acid phosphatase activity. × 500.

Fig. 2. Section through the area opaca of a 2-day (Stage 12) chick embryo. The darkened areas in the yolk below the blood vessel indicate nonspecific acid phosphatase activity. × 500.

Fig. 3. Acid phosphatase activity in a PGC (arrow) located in the mesenchyme between the notochord and the dorsal aorta of a 5-day (Stage 26) chick embryo. Positive enzyme activity and the fact that this cell is located caudal to the gonads suggests that the PGC may be undergoing degeneration. × 500.

Fig. 4. Portion of the left gonad and dorsal mesentery of a 5-day (Stage 26) chick embryo. Note the absence of acid phosphatase activity in PGCs (arrows) in both the gonads and dorsal mesentery. × 500.

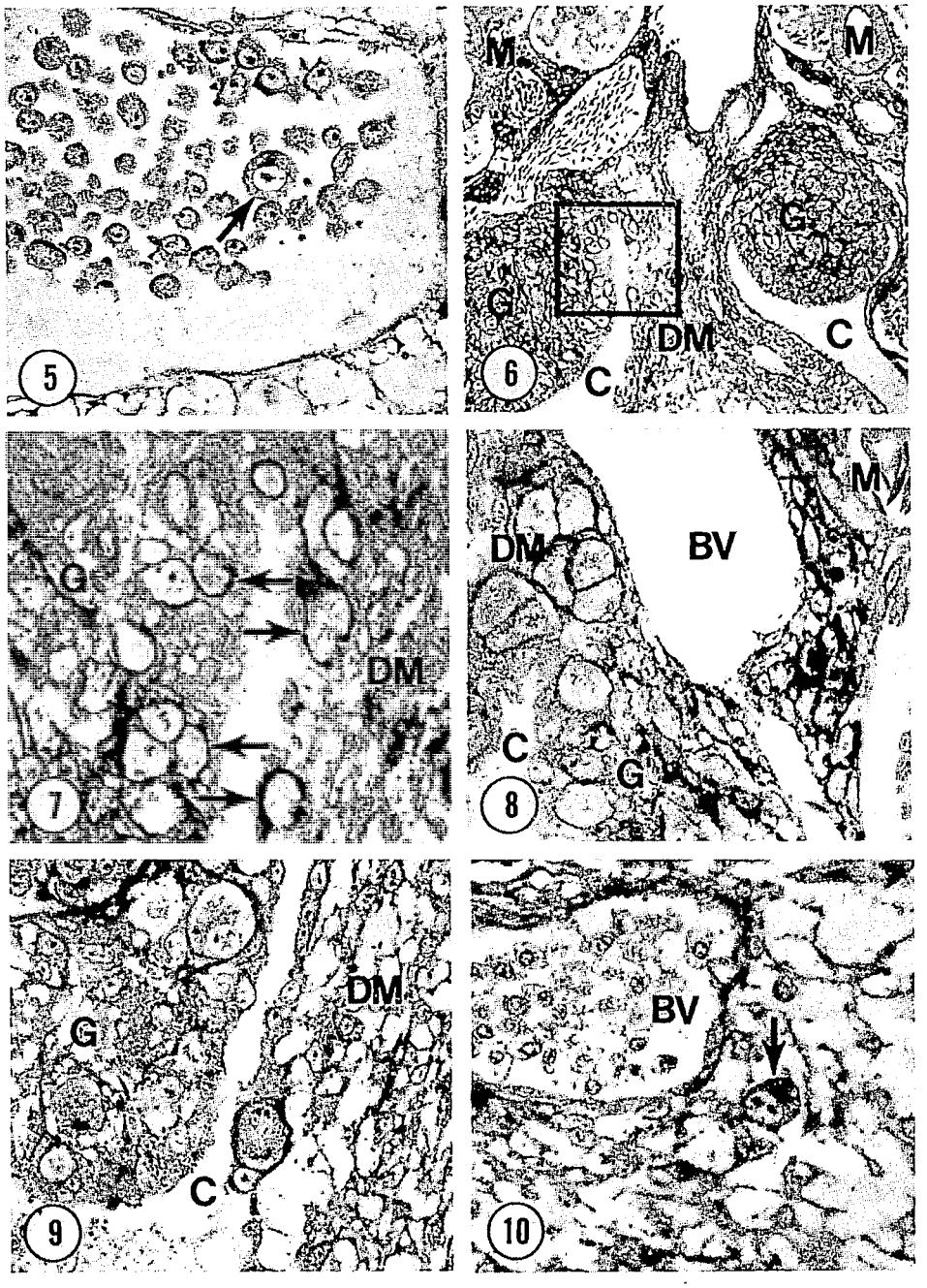

Fig. 5. Extraembryonic blood vessel of a 2-day (Stage 12) chick embryo containing a PGC (arrow). The dark area in the cytoplasm near the nucleus indicates alkaline phosphatase activity. × 500.

Fig. 6. Low-power view of the gonadal area of a 6-day (Stage 28) chick embryo. × 125.

Fig. 7. High-power view of left gonad and adjacent dorsal mesentery outlined in Figure 6. Compare the dark rim of alkaline phosphatase activity along the cell membranes of the PGCs (arrows) in both the gonad and dorsal mesentery to the globular appearance of this enzyme activity in Figures 5 and 10. × 500.

Fig. 8. Cluster of PGCs in the dorsal mesentery of a 5-day (Stage 26) chick embryo entering the gonad. Note the darkened rings along the cell membranes of the PGCs indicating alkaline phosphatase activity x 500.

Fig. 9. Alkaline phosphatase-positive PGC in mitosis in the dorsal mesentery along the coelom of a 5-day (Stage 26) chick embryo. Observe the cellular membrane-associated arrangement of the alkaline phosphatase activity. × 500.

Fig. 10. PGC (arrow) in the mesenchyme near the segmental branch of the dorsal aorta at the level of the gonads of a 5-day (Stage 26) chick embryo. Observe the globular appearance of the alkaline phosphatase activity in the cytoplasm. × 500.

Figure 10 is a photomicrograph of a PGC in the mesenchyme near a segmental branch of the dorsal aorta at the level of the gonads. Although this cell is within the dorsal mesentery, its alkaline phosphatase activity resembles that of circulatory PGCs. Because of the close approximation of this PGC to a blood vessel, it is suggested that this cell may have recently exited this vessel and may be in a transition stage with respect to its distribution of alkaline phosphatase activity.

DISCUSSION

The present report describes the localization of acid and alkaline phosphatase in migrating PGCs of the chick embryo. The PGCs of the chick are unique in that they are first observed in an area located anterior to the developing embryo called the germinal crescent, situated at the junction of the area opaca and area pellucida (Swift, 1914). These cells bud off from the endoderm and come to lie between the endoderm and ectoderm. With the development of extraembryonic blood vessels in this area the PGCs either migrate into or are trapped within these developing vessels and, thus, begin the passive phase of their migration, being propelled through the bloodstream. With the joining of the independently developing intra and extraembryonic circulatory systems at 33 hours of development (Romanoff, 1960) the circulating PGCs enter the embryo proper. The majority of PGCs then actively leave splanchonopleuric blood vessels in the area of the developing genital ridges and gain access to the mesenchyme of the dorsal mesentery from which they actively migrate to the developing gonads.

The alkaline phosphatase reaction has long been used as an identifying marker for PGCs in mammalian species (Chiquoine, 1954; Mintz, 1959; Chretien, 1966); however, because of a generalized reaction in many tissues of the avian embryo it can not be used as sole means of identification of avian PGCs (Chiquoine and Rothenberg, 1957). With the advent of the use of the periodic acid-Schiff reaction for identification of avian PGCs, resulting from their high glycogen content (Meyer, 1954), studies of alkaline phosphatase, a very important metabolic enzyme, in these avian cells ceased. With increased knowledge of the role of this enzyme in cellular activities made available, it became imperative to examine this enzyme in these unique cells.

The results presented here clearly demonstrate that the use of GMA as an embedding medium is an effective means for histochemically localizing specific enzymes in PGCs. The resulting histological sections allow for easy identification of the PGCs and precise cellular localization of acid and alkaline phosphatase activities.

Alkaline phosphatase activity is found as early as 2 days of incubation in PGCs within the extraembryonic circulation and is still present in PGCs at 6 days of incubation, the latest stage studied. However, the cellular localization of this enzyme differed significantly between those cells in the passive and those in the active phases of their migration.

Chiquoine (1954) and Mintz (1959) found alkaline phosphatase activity in the PGCs of the mouse to be evenly distributed throughout the cytoplasm. More recently, the ultrastructural investigations of Jeon and Kennedy (1973) and Clark and Eddy (1975) demonstrated this enzyme at the plasma membrane of mouse PGCs. Fujimoto et al. (1976) reported alkaline phosphatase activity in smear preparations of PGCs taken from 2-day chick embryos, but they made no mention of the cellular distribution of this enzyme activity.

In the present study alkaline phosphatase activity was also associated with the cell membrane of the avian PGCs, but not until these cells had reached the tissue or active phase of their migration. Prior to this time the PGCs are in the passive phase of their migration in which they are propelled through the extra and intraembryonic vasculature. During this vascular phase, the PGCs also show a positive reaction for alkaline phosphatase; however, it is not evenly distributed along the cellular membrane but is clumped in the cytoplasm near the nucleus. Whether this clumped reaction product originates in the Golgi apparatus and then is transported to the surface cannot be determined from this study. Examing these cells ultrastructurally would assist in elucidating this factor.

The role of alkaline phosphatase in the metabolic plan of the cell is not clear. It has been associated with the initiation of mineralization in various calcifying tissues (Boskey, 1979). This enzyme has been implicated in important cellular processes such as secretion (Bradfield, 1950), phospholipid synthesis (Malone, 1960), and carbohydrate metabolism (Moog and Wenger, 1952). It has been observed at cell membranes where active transport occurs (Kaplan, 1972) and has been considered to be involved in the transfer of metabolites across cell surfaces (Zamboni and Merchant, 1973).

The difference in distribution of alkaline phosphatase in passively and actively migrating PGCs indicates that there is a change

occurring in these cells during these two stages. Whether this change is due to an alteration of the metabolic activities of the PGCs or whether it indicates different stages in the synthesis of alkaline phosphatase cannot be determined by this light microscopical study. Certainly the metabolic needs of these cells change once they reach the tissue and begin to actively migrate. It may be that these cells are becoming more specialized as they near the gonads. This rearrangement of alkaline phosphatase localization may reflect these changing activities. There are ultrastructural differences between passively and actively migrating PGCs. Lee et al. (1978) observed chick PGCs in the blood vessels to be more spherical in shape than the tissue PGCs possessing pseudopod-like projections. The membrane-associated enzyme activity found in tissue PGCs actively migrating to the gonad might indicate an active metabolic interchange between PGCs and the surrounding tissue. This interchange may be of a secretory nature as a response to a "chemotactic factor" emanating from the developing germinal epithelium (Cuminge and Dubois, 1969).

Acid phosphatase activity in migrating PGCs was limited to a few PGCs in the dorsal mesentery. The presence of acid phosphatase has been associated with lysosomal activity (de Duve and Wattiaux, 1966) and has been used as an indicator of cell death. Karner and Leikola (1976) suggested that the presence of acid phosphatase in Henson's node of the chick embryo might facilitate cell migration through the node by breaking down epithelial-type junctions. Since acid phosphatase was present in only a few tissue PCGs in this study, it would appear that this enzyme activity is not an indicator of a migratory force, but one of possible degeneration.

This limited acid phosphatase activity in PGCs suggests that there is no wave of degeneration of these cells during their migration. Furthermore, there is no increased incidence of this enzyme activity in PGCs on the right side as opposed to the left side, which, if there were, might account for the asymmetrical distribution of PGCs in the avian species. The explanation for this asymmetry is still wanting.

Beginning with 5 days of incubation clusters of closely associated PGCs are found within the dorsal mesentery in close proximity to the gonads (Swartz, 1975). The purpose for this close association is not known. Since these cells exhibit no acid phosphatase activity but do demonstrate alkaline phosphatase activity similar to individual PGCs in both the gonads and dorsal mesentery, it appears that these cells are carrying out normal cellular activities of PGCs. Whether the PGCs comprising these cell clusters will subsequently undergo degeneration is not known. Extending the present study beyond 5 days of incubation will aid in answering these questions.

The present study has shown a limited expression of acid phosphatase activity in PGCs and a difference in the cellular localization of alkaline phosphatase activity in passively and actively migrating PGCs. Work on the status of other enzymes in these cells during this migratory period may serve to elucidate the metabolic activities of these cells and thus provide explanations for migration of these cells to the gonads and not to some other embryonic area.

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LITERATURE CITED

Boskey, A.L. (1979) Models of matrix vesicle calcification. Inorg. Perspect. Biol. Med., *2:* 51–92.

Bradfield, J.R.G. (1950) The localization of enzymes in cells. Biol. Rev., *25:* 113–157.

Burstone, M.S. (1961) Histochemical demonstration of phosphatases in frozen sections with naphthol AS-phosphates. J. Histochem. Cytochem., *9:* 146–153.

Burstone, M.S. (1962) Enzyme Histochemistry and Its Application in the Study of Neoplasms. Academic Press, New York.

Chiquoine, A.D. (1954) The identification, origin, and migration of the primordial germ cells in the mouse embryo. Anat. Rec., *118:* 135–146.

Chiquoine, A.D., and E.J. Rothenberg (1957) A note on alkaline phosphatase activity of germ cells in *Amblystoma* and chick embryos. Anat. Rec., *127:* 31–35.

Chretien, Ch. (1966) Etude de l'origine, de la migration et de la multiplication des cellules germinales chez l'embryon de lapin. J. Embryol. Exp. Morphol., *16:* 591–607.

Clark, J.M., and E.M. Eddy (1975) Fine structural observations on the origin and associations of primordial germ cells of the mouse. Dev. Biol., *47:* 136–155.

Cuminge, D., and R. Dubois (1969) Propriétes excretrices de l'épithelium germinatif de l'embryon de Poulet: Etude structurale et autoradiographique au microscope electronique. C. R. Acad. Sci. [D] (Paris), *269:* 74–77.

de Duve, C., and R. Wattiaux (1966) Functions of lysosomes. Ann. Rev. Physiol., *28:* 435–492.

Fujimoto, T., T. Ninomiya, and A. Ukeshima (1976) Observations of the primordial germ cells in blood samples from the chick embryo. Dev. Biol., *49:* 278–282.

Hamburger, V., and H.L. Hamilton (1951) A series of normal stages in the development of the chick embryo. J. Morphol., *88:* 49–92.

Jeon, K.W., and J.R. Kennedy (1973) The primordial germ cells in early mouse embryos: Light and electron microscopic studies. Dev. Biol., *31:* 275–284.

Kaplan, M.M. (1972) Alkaline phosphatase. Gastroenterology, *62:* 452–468.

Karner, J., and A. Leikola (1976) Distribution of acid phosphatase in chick Henson's node. Differentiation, *5:* 67–74.

Lee, H., R.G. Nagele, and M.M. Goldstein (1978) Scanning electron microscopy of primordial germ cells in early chick embryos. J. Exp. Zool., *206:* 457–462.

Malone, T.E. (1960) Observations on the histochemical localization of alkaline glycerophosphatase in developing corpora lutea of albino rats. Am. J. Anat., *106:* 41–53.

McAlpine, R.J. (1955) Alkaline glycerophosphatase in the developing adrenal, gonads, and reproductive tract of the white rat. Anat. Rec., *121:* 407–408.

McKay, D.G., A.T. Hertig, E.C. Adams, and S. Danziger (1953) Histochemical observations on the germ cells of human embryos. Anat. Rec., *117:* 201–219.

Meyer, D.B. (1954) The migration of primordial germ cells in the chick embryo. Dev. Biol., *10:* 154–190.

Mintz, B. (1959) Continuity of the female germ line from embryo to adult. Arch. Anat. Microsc. Morphol. Exp. Suppl., *48:* 155–172.

Moog, F. (1944) Localizations of alkaline and acid phosphatases in the early embryogenesis of the chick. Biol. Bull., *86:* 51–80.

Moog, F., and E.L. Wenger (1952) The occurrence of a neutral mucopolysaccharide at sites of high alkaline phophatase activity. Am. J. Anat., *90:* 339–377.

Nusbickel, F.R. (1980) The construction of a closed-chamber incubator for use in such techniques as enzyme and immunoperoxidase histochemistry. J. Microsc., *118:* 447–451.

Nusbickel, F.R., and W.J. Swartz (1979) Enzyme histochemical investigation of glycol methacrylate embedded chick embryonic tissue. Histochem. J., *11:* 197–203.

Romanoff, A.L. (1960) The Avian Embryo. Structure and Functional Development. The Macmillan Co., New York, Chap. 1, pp. 3–72.

Swartz, W.J. (1975) Effect of steroids on definitive localization of primordial germ cells in the chick embryo. Am. J. Anat., *142:* 499–514.

Swartz, W.J., and F.R. Nusbickel (1979) Histologic investigation of glycol methacrylate embedded chick embryonic tissue. J. Microsc., *115:* 181–185.

Swift, C.H. (1914) Origin and early history of the primordial germ-cells in the chick. Am. J. Anat., *15:* 483–516.

Zamboni, L., and H. Merchant (1973) The fine morphology of mouse primordial germ cells in extragonadal locations. Am. J. Anat., *137:* 299–366.