ACTION OF PROSTAGLANDIN E_2 DURING EARLY PREGNANCY IN THE RAT

A Thesis

Presented to the Faculty of Graduate Studies,

University of Manitoba, in Partial Fulfillment

of the Requirements for the Degree of

Master of Sciences

by

Thomas William Suzanski

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For my mother and father

with deep appreciation and gratitude.

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ABSTRACT

Prostaglandins are a unique group of biologically active lipids that appear to be present in or can be elicited from all tissues. They have been implicated in numerous biological processes. In particular, prostaglandins involvement in female reproductive physiology has received considerable attention.

Although widely used as abortifacients the effects of prostaglandins on the developing conceptus are not known. Growth retardation, decreased litter size, increased perinatal mortality and congenital anomalies have been reported following treatment of laboratory animals with prostaglandins, whereas in other studies no such effects have been observed. The critical period at which teratogenesis is induced as well as the dose level required to produce this effect have not been determined. Furthermore, no clear relationship between animal species or routes of administration and effects observed is known.

This report deals with the influence of PGE_2 on early embryonic development in the rat.

Pregnant Sprague-Dawley rats were treated subcutaneously with PGE_2 at doses of 50, 100, and 200 µg on gestational days five through eight. Control animals received the solvent in a similar manner. The animals were divided into groups that

were killed either on day 9 or 20 of gestation. Uterine horns with intact implantation sites were examined histologically in order to assess both the embryo and the decidua.

No significant structural changes were present in the conceptuses. However, an unusually wide range of embryonic stages was detected in both the control and experimental groups of animals. The incidence of fetal resorptions was not significantly increased, and all fetuses recovered were alive and appeared normal. Visceral abnormalities and skeletal defects were not observed. Also, the mean weight of treated fetuses was not significantly different from that of the corresponding controls.

These results indicate that administration of PGE_2 during an early and critical stage of pregnancy did not prove deleterious to the offspring at term, nor did it have any significant impact on the conceptus immediately following treatment.

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1. REVIEW OF LITERATURE

1.1 Implantation and Early Embryonic Development of the Rat

Early embryonic development in the rat and mouse is similar in many respects. There have been many investigations devoted to certain aspects of both rat and mouse embryology, but few comprehensive ones. Early embryonic development to the time of mesoderm formation has been examined in the rat (Huber, 1915), mouse (Born, 1892; Duval, 1895; cited by Jenkinson, 1900). For a detailed review, reference should be made to Snell and Stevens (1966) and Rugh (1968). Subsequent studies tended to focus on specific events such as decidualization (Krehbiel, 1937; Shelesnyak, 1963; Finn, 1971, De Feo, 1967), placentation (Duval, 1891; Jenkinson, 1902; Bridgeman, 1948; Davies and Glasser, 1968; Müntener and Hsu, 1977), implantation (Mossman, 1937; Amoroso, 1952; Blandau, 1961; Enders and Schlafke, 1967), the role of the trophoblast (Billington, 1971; Alden, 1948), role of epithelium in implantation, and blastocyst endometrial relationships during implantation.

Fertilization of the ovum occurs in the ampulla of the oviduct. Blandau and Money (1944), as well as Blandau and Odor (1949), demonstrated that sperm had reached the fertilization site in all rats examined one hour after ejaculation. Similar observations have been made in the

mouse (Lewis and Wright, 1935). However, it has been noted that it may take as long as three and one-half hours before all mature ova from a single mother have been fertilized (Rugh, 1968).

In general, preimplantation development through cleavage and morula formation is similar in most mammals. The ovum develops during its passage through the uterine tubes and reaches the uterine lumen three to four days after fertilization (Psychoyos, 1969). This is subject to variation (Burckhard, 1901). Also, considerable variation with respect to development has been observed in mouse blastocysts as they reach the uterus. There may be as many as two cleavages difference between the most advanced and most retarded eggs (Rugh, 1968). These variations are thought to be due to differences in sperm penetration or cleavage time intervals.

On the late afternoon of the fifth day of pregnancy, most of the blastocysts have lost their zona pellucidae, but remain unimplanted (Dickmann and Noyes, 1961; Enders and Schlafke, 1967). It remains unknown what role the uterine environment plays, as well as the blastocyst itself, in the shedding of the zonae. Dickmann and Noyes (1960) have also found that both the blastocyst and endometrium reached a specific stage of development favorable for implantation by the fifth day of pregnancy, the uterine environment being unsuitable for implantation on the fourth and sixth days.

Implantation in the rat has been shown to depend upon the completion of a two-step hormonal sequence (Psychoyos, 1969). First, a forty-eight hour progestogenic preparation is required followed by the presence of estrogen at the end of this period. Following the completion of this sequence, in normal pregnancy, within the last six hours of the fourth day post-coitum, the uterus undergoes specific modifications leading to a receptive state. This occupies the second half of the fifth day and is characterized by a high endometrial sensitivity for decidualization. However, the receptive state lasts only a few hours, and by the beginning of the sixth day, the endometrium begins to lose its capacity for decidualization. The uterine environment becomes increasingly hostile, hence synchronization between the blastocyst and the uterus is essential.

Upon entering the uterus, the blastocysts become evenly spaced throughout its length, each finding its way into a uterine crypt located on the antimesometrial side of the uterus (Snell and Stevens, 1966). Hollander and Strong (1950) and Krehbiel (1962) have postulated that this even distribution results from a random scattering induced by the churning action of the uterus itself. This random scattering is accompanied by a non-randomized spacing

(O'Grady and Heald, 1969), and has been shown to be due to an increase in the length of the uterus at the time of implantation (Finn, 1968). This hypothesis is supported by the observation of increased mitoses occurring in uterine stromal cells located outside the developing decidual area (Finn and Martin, 1967).

The process of implantation has been divided into three stages (Enders and Schlafke, 1967). The first stage is initiated when the blastocyst becomes clasped by the endometrium, thus assuming a fixed position (evening of day five). Trophoblast cells lie in close association with uterine epithelial cells. The primitive junction formed is one of a region of close membrane apposition (Enders and Schlafke, 1969). Decidualization of stromal fibroblasts is just beginning. At this time an increased permeability occurs in the endometrial capillaries surrounding the blastocyst, and this permeability can be demonstrated by the intravenous injection of a macromolecular dye into the female (Psychoyos, 1960).

Up to this point in time there has been no increase in the total volume of the conceptus (Snell and Stevens, 1966). Cleavage has resulted in the formation of numerous smaller cells. No new protoplasm has been formed. The embryo consists of a cavity (the blastocoel) surrounded by a single layer of trophectodermal cells except at one side where cells are grouped forming a structure known as the inner cell mass (Fig. 1).

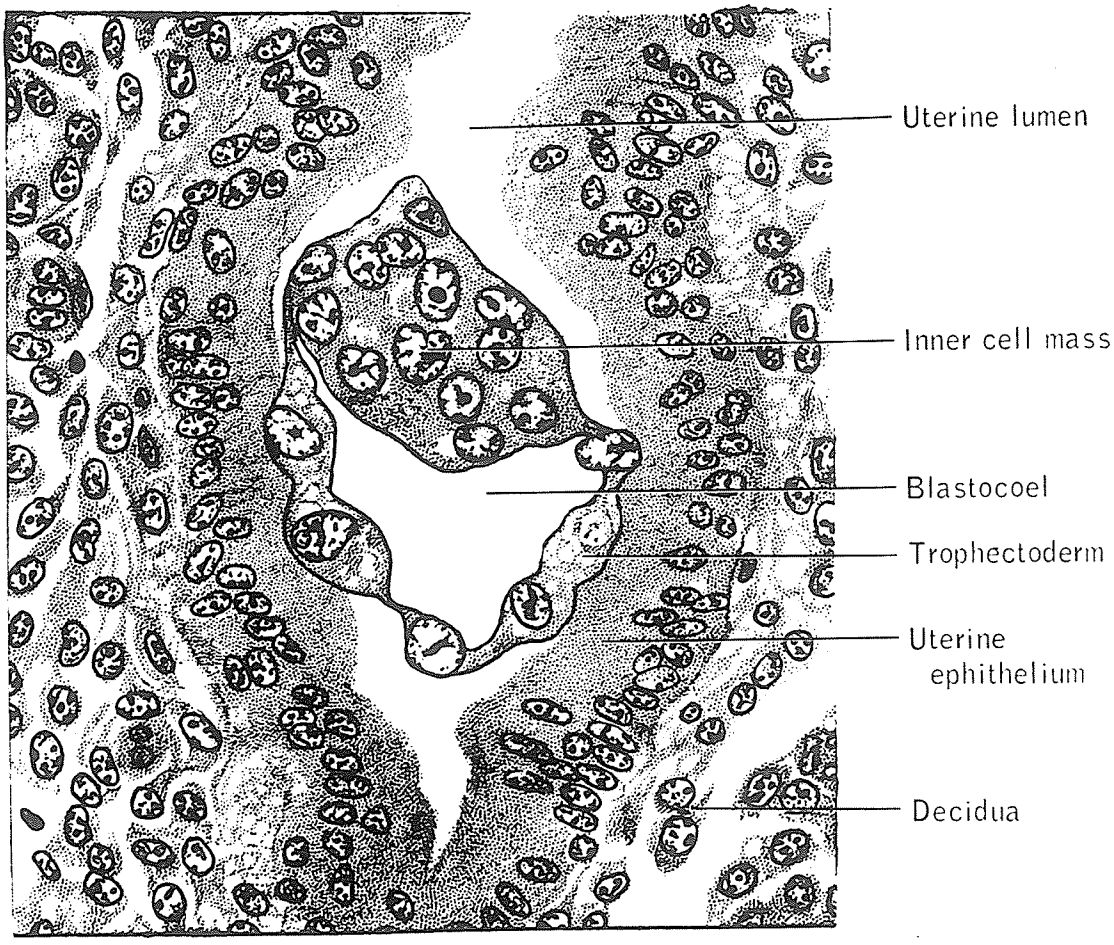


FIGURE 1.

Blastocyst lying in implantation chamber, 5 days 12 hours after copulation (From Snell and Stevens, 1966).

Immediately following this stage, mitotic activity in the embryos becomes very intense (Snow, 1976). Thus the second stage (day six) is a time of rapid change. Numerous mitotic figures may be observed in the blastocyst, stroma, and endothelial cells.

As the blastocyst begins to enlarge, the inner cell mass differentiates into two cell types. Adjacent to the blastocoel there appears a single layer of darkly staining cells, the endoderm, one of the three primary germ layers. The rest is divided into the ectoderm of the inner cell mass and the trophectoderm (Fig. 2).

Following the appearance of the endoderm, strands of cells begin to grow out from its margins along the inner surface of the trophectoderm. These cells eventually form a continuous layer around the entire inner surface of the trophectoderm. This layer is known as the parietal or distal endoderm, while the layer of endodermal cells covering the egg cylinder is known as the visceral or proximal endoderm. The egg cylinder ectoderm also differentiates into two types: a dorsal, darkly staining region with elongated nuclei and a ventral lightly staining region containing round nuclei. The dorsal region is known as extraembryonic ectoderm and gives rise to extraembryonic structures, while the ventral region is called embryonic ectoderm and gives rise to ectoderm of the embryo proper. Meanwhile growth of the inner cell mass down into the enlarged blastocoel occurs. Thereafter the blastocoel and inner cell mass are known as the yolk cavity

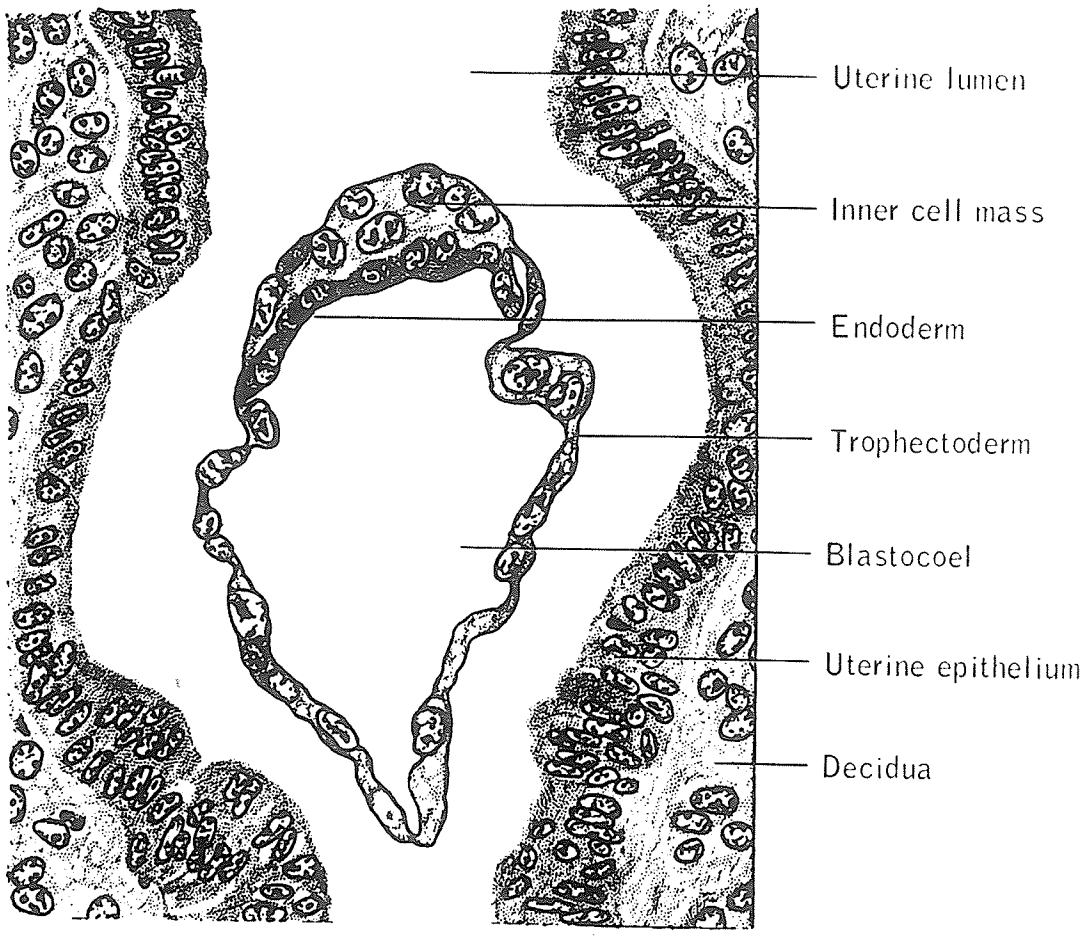


FIGURE 2.

Implanting blastocyst showing differentiation of endoderm (From Snell and Stevens, 1966).

and egg cylinder respectively (Fig. 3).

The trophoblast overlying the inner cell mass soon shows extensive changes. A burst of mitotic activity occurring in this region gives rise to a rapidly proliferating irregular mass of cells known as the ectoplacental cone (Billington, 1971). The ectoplacental cone will contribute substantially to the development of the future placenta. On the sixth day, the cells of the ectoplacental cone are full of oddly-shaped, variously-sized inclusions and vacuoles resembling those found in giant cells (Bridgeman, 1948). On the seventh day, the ectoplacental cone contains many more cells and has enlarged considerably, both in width and in length. However, there are now very few inclusions to be found in its cells. Those present are limited to the outermost cells of the cone which have now become true giant cells. Thus, Bridgeman (1948) suggests that no true cone cells were present on the preceding day, but rather undifferentiated trophoblast.

Early egg cylinders reveal small ectoplacental cones but well developed structures antimesometrially, thus indicating growth in this direction while the conceptus is tightly clasped by the uterus.

Primary trophoblastic giant cells are distinguishable for the first time on day six. They arise from trophectoderm in the antimesometrial and lateral areas of the conceptus.

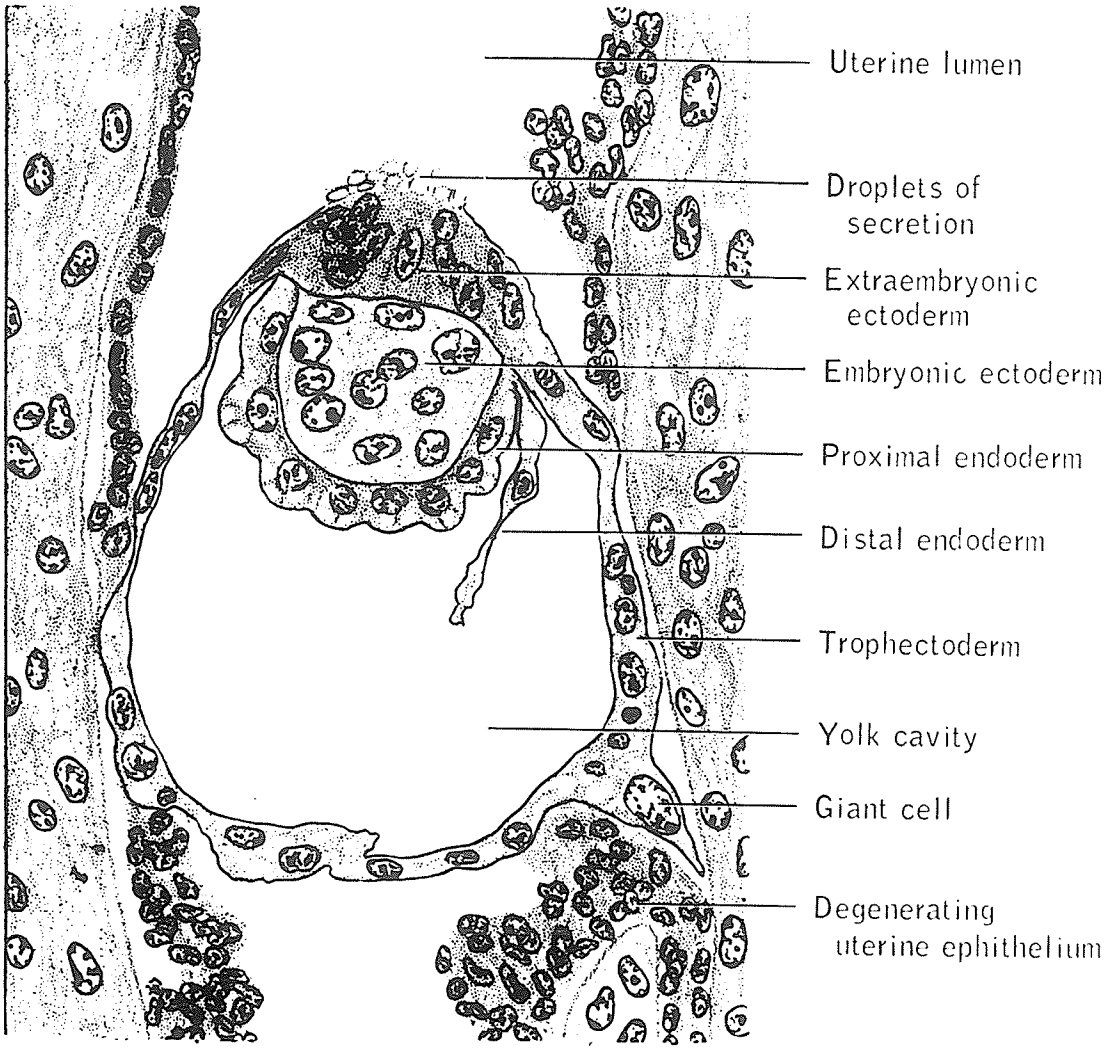


FIGURE 3.

Longitudinal section of early egg cylinder showing differentiation of distal (parietal) and proximal (visceral) endoderm, embryonic and extraembryonic ectoderm (From Snell and Stevens, 1966).

Secondary giant cells are derived from the enlargement of cells in the superficial regions of the cone. They do not make their appearance until day seven (Snell and Stevens, 1966). Orsini (1954) has described tertiary trophoblastic giant cells. These probably arise from migrating primary and secondary giant cells (Billington, 1971).

By this time decidualized fibroblasts form a cup around the luminal epithelium. There is a notable increase in the size of nuclei of these stromal cells accompanied by a substantial increase in the amount of cytoplasm present. The formation of a decidual crypt results from a rapid and eccentric growth of the decidual tissue (Blandau, 1949). The fibroblasts hypertrophy to the extent that they form a continuous layer leaving a connective tissue cleft between the basement membrane of the epithelium and the surface of the decidualizing fibroblasts. This results in the isolation of the luminal epithelium from its vascularization. This stage is well developed by the afternoon of day six (Enders and Schlafke, 1967).

In the third stage of implantation (evening of day six, morning of day seven), a subsequent extension of the cone towards the uterine lumen is associated with loosening and phagocytosis of the uterine epithelium by trophoblast of the cone, mesometrially from the egg cylinder (Finn and Lawn, 1968; Smith and Wilson, 1974). El-Shershaby and

Hinchcliffe (1975) have suggested that epithelial displacement results from an autolytic activity within the epithelium itself. Furthermore, Enders and Schlafke (1967) have found that the histolytic and invasive properties of trophoblast in the rat appear to be very feeble during implantation. Rather, it is the adherence of trophoblast cell membrane to the epithelial cells that appears to be an important process with respect to the translocation and phagocytosis of epithelial cells.

Trophoblastic cells on the lateral aspect of the egg cylinder now lie in direct contact with the residual basement membrane of the luminal epithelium and are separated by this structure and a small connective tissue cleft from the stromal cells. Subsequently, the basal lamina disappears (Tachi et al., 1970). The association between the decidua and trophoblast remains uncertain.

Stromal blood vessels re-invade and come closer to the implanting blastocyst; however, hemorrhage into subepithelial spaces at this stage is rare (Enders and Schlafke, 1967). Eventually, a periembryonic sinus will develop outside the yolk cavity. It is filled with circulating maternal blood, bathing and providing nourishment to the developing embryo (Everett, 1935).

By the morning of day seven, entypy or germinal inversion has converted the embryonic cell mass into an egg cylinder. No evidence of proamniotic cavity formation is apparent at this time (Enders and Schlafke,1967). However, it soon makes its appearance in the embryonic ectoderm (Fig. 4), followed very shortly by the appearance of a similar cleft in the extraembryonic ectoderm (Snell and Stevens, 1966).

Fusion of these two cavities converts the egg cylinder into a double walled structure enclosing a narrow lumen (Fig. 5).

Parietal endodermal cells extending along the trophoblast are flattened against it and show little evidence of the formation of Reichert's membrane until the middle of day seven (Enders and Schlafke,1967). However, there soon appears between the two cell layers a thin non-cellular, pink-staining membrane, Reichert's membrane. The thickness of trophoblast lining Reichert's membrane has been reported to vary (Reinius,1965a; Reinius,1965b). Close association of these cells to Reichert's membrane has led investigators to suggest an ectodermal origin for the membrane (Wislocki and Padykula, 1953), but the demonstration of material similar to that found in parietal endodermal cells indicates

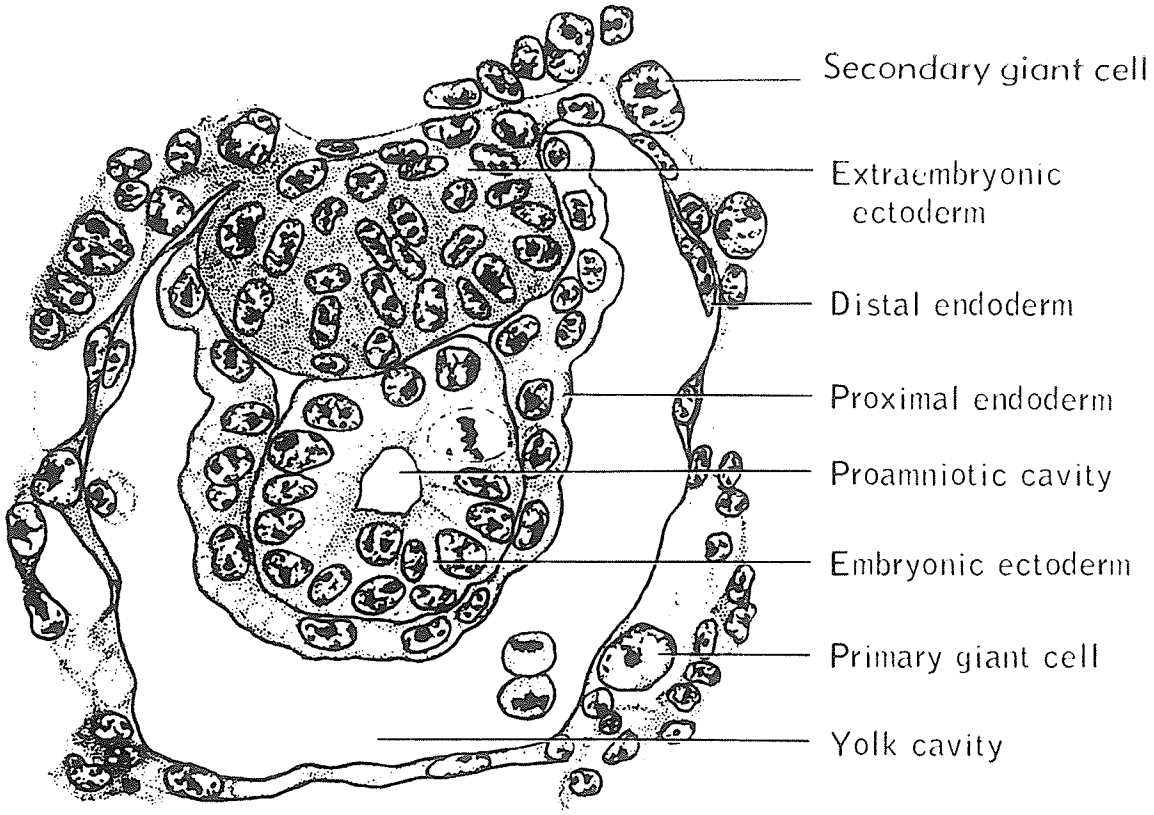


FIGURE 4.

Longitudinal section of 7-day egg cylinder showing first evidence of proamniotic cavity formation (From Snell and Stevens, 1966).

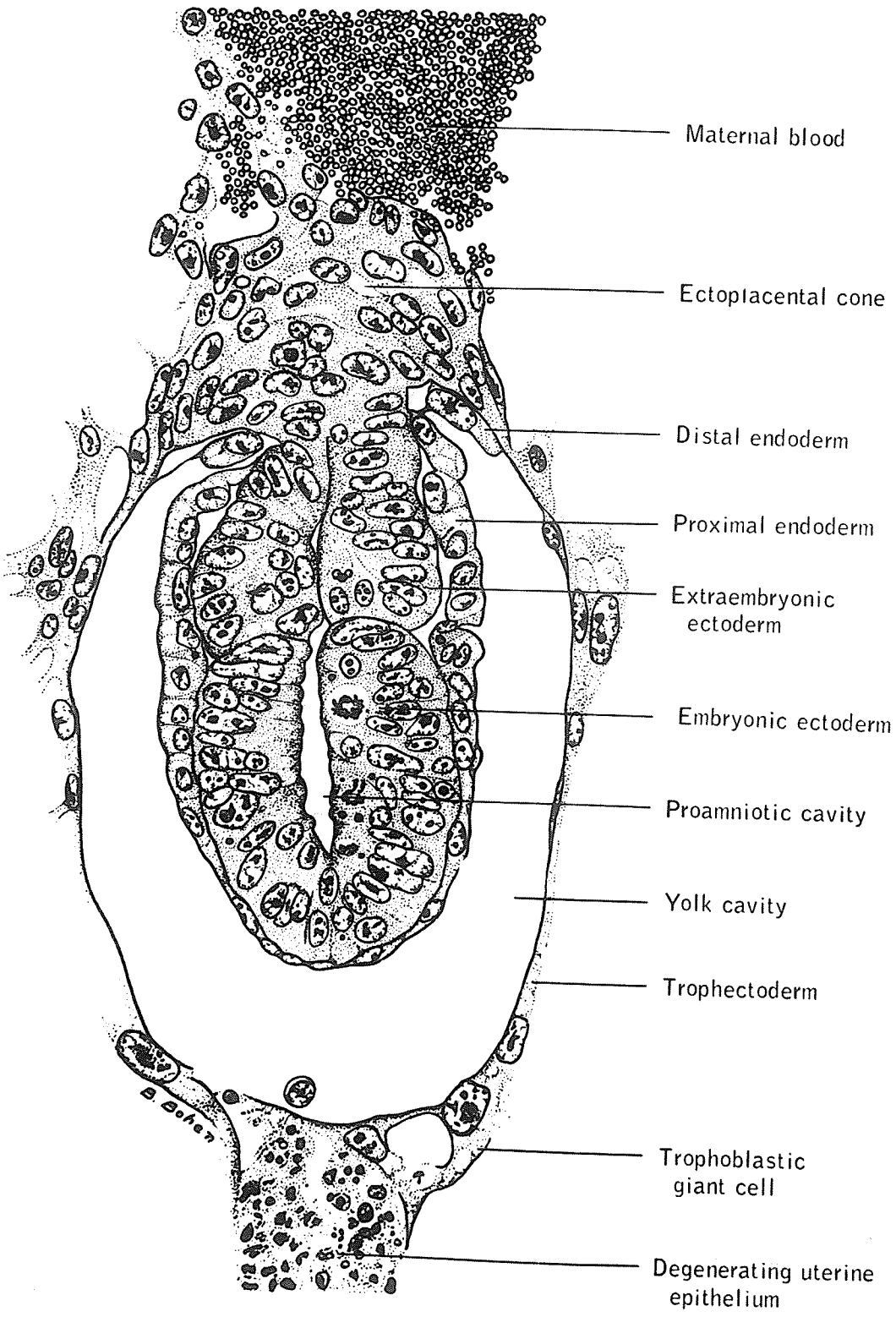


FIGURE 5.

Longitudinal section of 7-day egg cylinder. Note formation of well established proamniotic cavity (From Snell and Stevens, 1966).

an endodermal origin (Pierce, et al., 1962; Midgley and Pierce, 1963). The first signs of development of Reichert's membrane are usually at the ventral aspect of the egg cylinder. However, it soon becomes continuous over the entire surface of the trophectoderm and is reflected as a basal lamina separating the visceral embryonic and extra-embryonic endoderm from the ectoderm (Reinius, 1965b). This is supported by the observation that Reichert's membrane has the ultrastructural and immunochemical attributes of a basement membrane (Snell and Stevens, 1966).

Thus, following development of the periembryonic blood sinuses the egg cylinder is separated from the maternal circulation by a barrier composed of three layers: parietal endoderm, Reichert's membrane, and trophoblast. This barrier plays an entirely passive role in the transportation of nutrient substances, acting simply as a semipermeable membrane. Several substances have been shown to cross this barrier readily (Schultz, 1966; Seibel, 1974; Batten and Haar, 1979; Beck, 1979).

At about eight days postcoitum, the third germ layer, the mesoderm makes its appearance. It arises by delamination from a narrow strip of embryonic ectoderm extending from the line of junction of the embryonic and extraembryonic ectoderm to approximately half way to the tip of the egg cylinder (Snell and Stevens, 1966). This narrow strip of

mesoderm lying at the posterior aspect of the embryo is known as the primitive streak and once established it defines the anteroposterior axis of the embryo (Fig. 6).

The mesodermal cells wedge their way anteriorly between the ectoderm and endoderm, primarily along the line of junction between the embryonic and extraembryonic portions of the embryo. Here, the mesoderm causes the ectoderm to bulge into the proamniotic cavity giving rise to the posterior, lateral, and anterior amniotic folds. These folds create a contiguous constriction about the middle of the egg cylinder which is drawn ever tighter as the folds develop. Small cavities soon appear in the mesoderm of the posterior and lateral amniotic folds, indicative of the beginnings of the exocoelomic cavity (Jolly and Ferester-Tadie, 1936; Bonnevie, 1950) (Fig. 7).

Sometime late on the eighth day or early on the ninth fusion of the amniotic folds results in the division of the single proamniotic cavity into three cavities: the amniotic, exocoelomic, and the ectoplacental (Bridgeman, 1948). These are separated by two membranes, the chorion and the amnion, both being composed of ectoderm and mesoderm (Fig. 8).

Soon after the exocoelomic cavity becomes well established the allantois begins to grow into it from mesoderm located at the caudal end of the primitive streak (Fig. 8). This results in the flattening of the chorion against the base

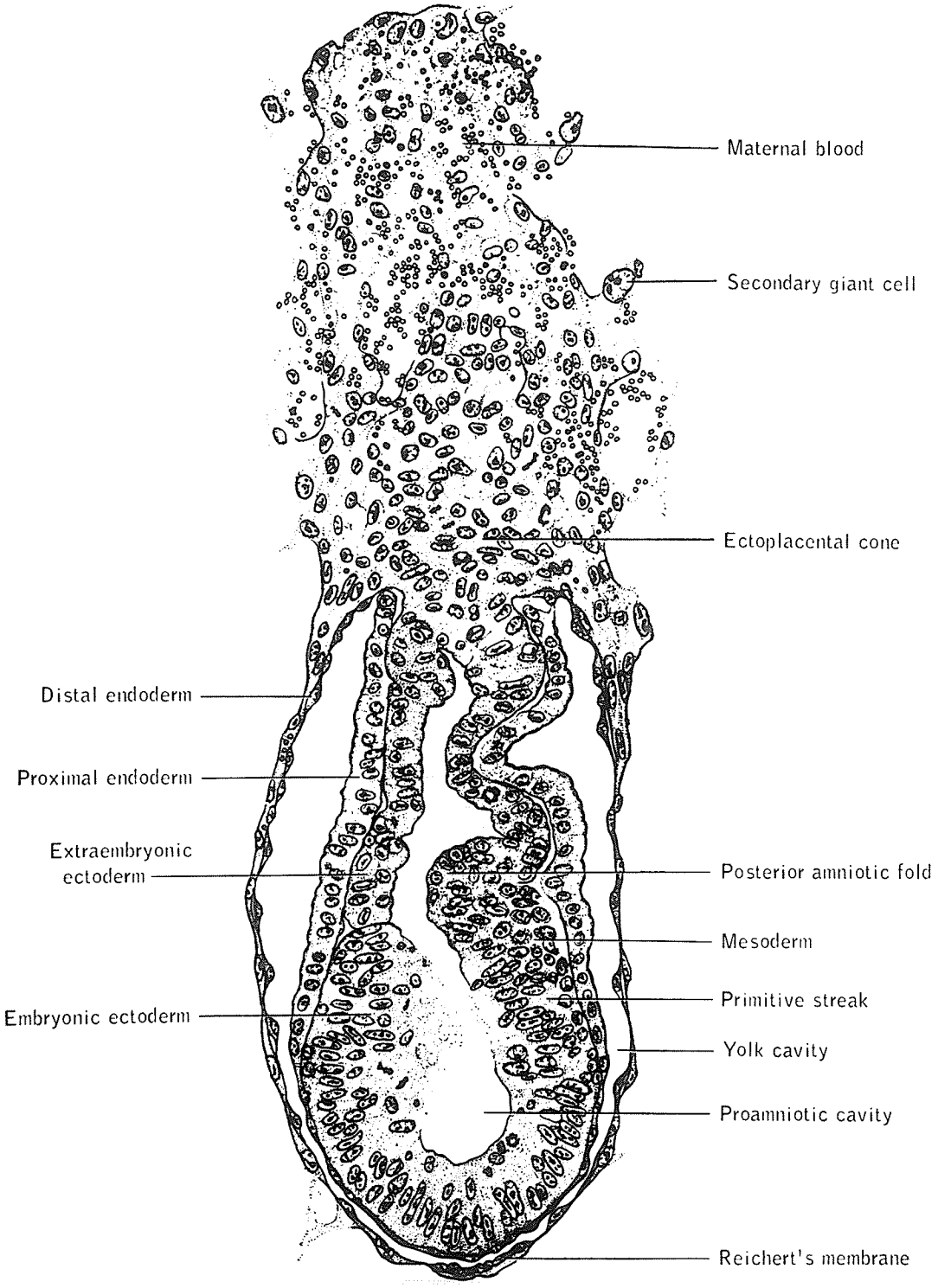


FIGURE 6.

Sagittal section of 8-day rat embryo showing early stage of mesoderm formation (From Snell and Stevens, 1966).

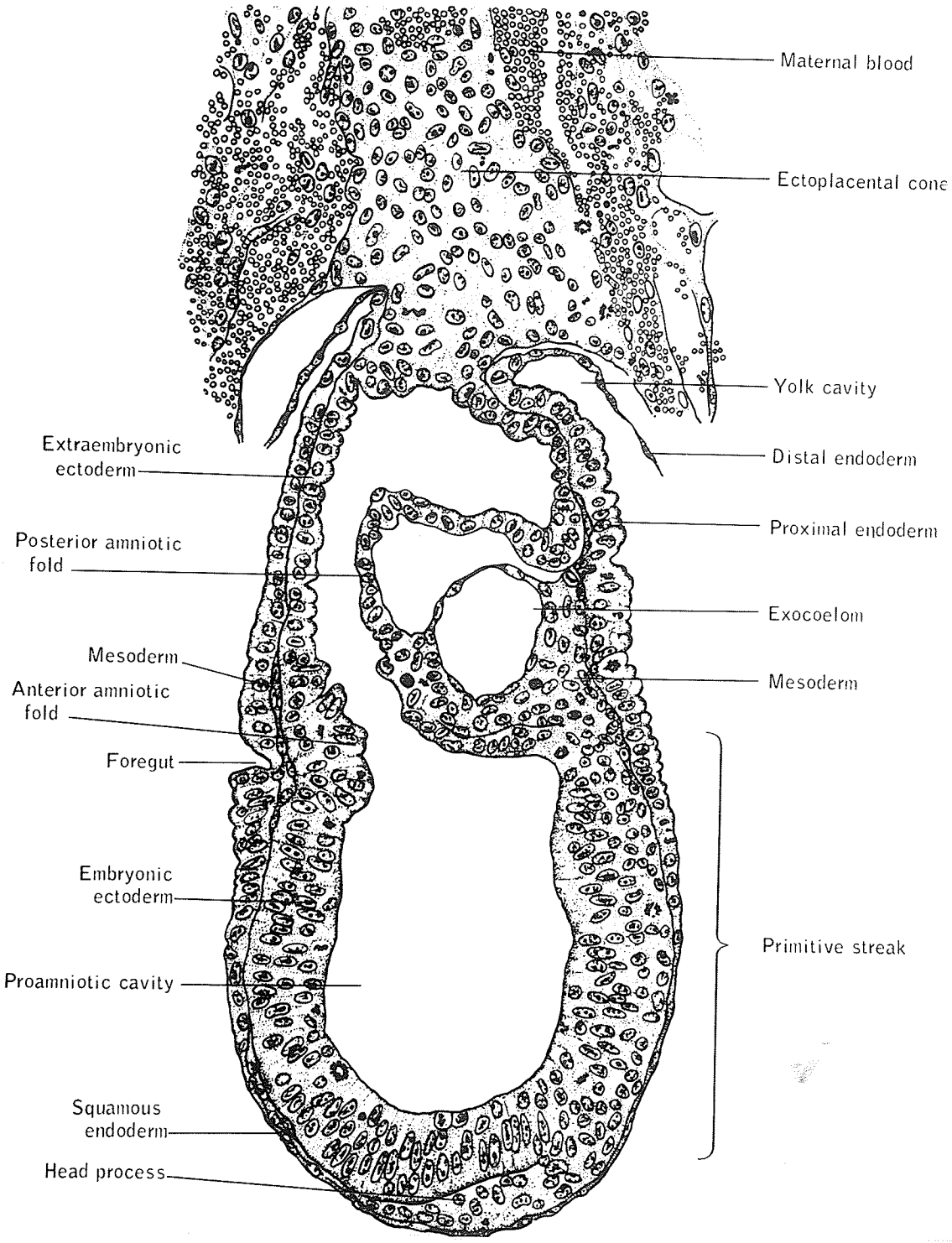


FIGURE 7.

Sagittal section of 8-day rat embryo showing development of amniotic folds and exocoelomic cavity (From Snell and Stevens, 1966).

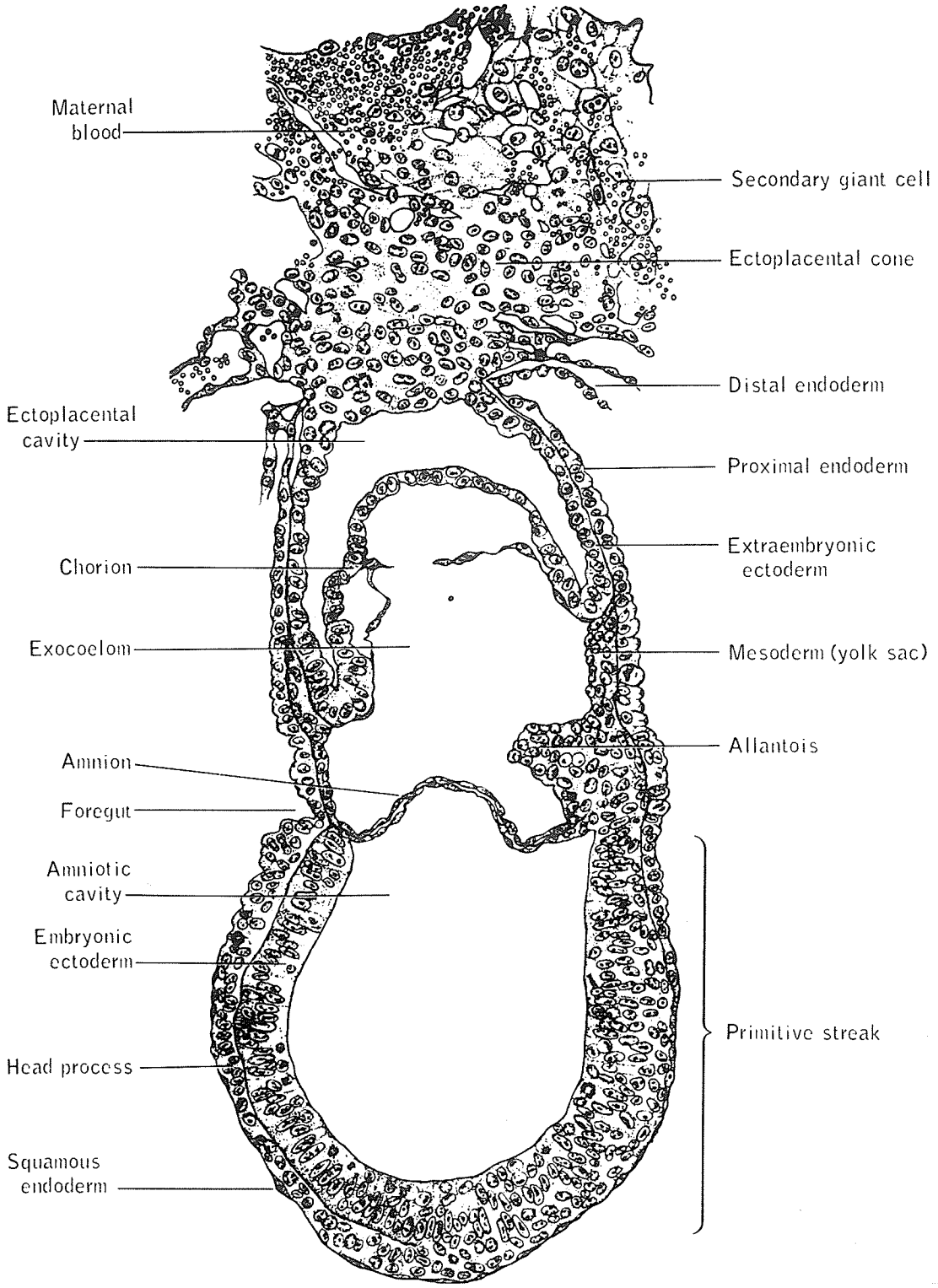


FIGURE 8.

Sagittal section of 8 to 9-day rat embryo showing completion of amnion and chorion formation (From Snell and Stevens, 1966).

of the ectoplacental cone obliterating the ectoplacental cavity. The chorio-allantoic placenta comes into existance late on the ninth day when allantoic vessels make contact with the "lame inferieur" of the ectoplacental cone thus establishing contact with the maternal circulation (Duval, 1891).

Because of the rapid changes occurring during this short period a considerable amount of variability may be seen at individual implantation sites.

1.2 Prostaglandins

1.2.1 Introduction

Smooth muscle stimulating activity of human semen was first observed in 1930 by Kurzrok and Lieb. Later, Goldblatt (1933) and Von Euler (1934) independently demonstrated that the seminal plasma was responsible for this action. Von Euler (1936) termed the active compound "Prostaglandin" in the belief that it originated from the prostate gland. However, in 1959, Eliasson showed that the active compounds, in fact, originated from the seminal vesicles. Subsequent investigations by Bergstrom and Sjovall (1960a,b), Bergstrom et al. (1962), Samuelsson (1963), and others have led to their crystalline isolation and structural identification.

1.2.2 Nomenclature

Prostaglandins are a family of naturally occurring, biologically active lipid derivatives of prostanoic acid (Fig. 9).

Structurally, the prostaglandins share certain common features, including 20 carbon atoms, a cyclopentane ring, 2 aliphatic side-chains, and a terminal carboxyl group. All possess a carbon 13, 14-trans double bond and a 15-hydroxyl function, both of which appear to be essential for biological activity. As with other organic compounds stereoisomerism is possible, but only α-isomers occur in nature.

In the past, it was customary to classify prostaglandins as being either primary or secondary, the primary prostaglandins being those derived directly from polyunsaturated fatty acids, the essential fatty acids (Fig. 10). These are PGE_1 and PGF_{1_α} derived from 8,11,14-eicosatrienoic acid (bis-dihomo-γ-linolenic acid); PGE_2 and PGF_{2_α} derived from 5,8,11,14-eicosatetraenoic acid (arachidonic acid); and PGE_3 and PGF_{3_α} from 5,8,11,14,17-eicosapentaenoic acid.

The subscripts (1,2,3) refer to the degree of unsaturation in the side chains of the prostaglandin molecule. E_1 and F_1 compounds contain only the 13,14-trans double bond. The E_2 and F_2 compounds have an additional 17,18-cis double bond, whereas the E_3 and F_3 compounds possess a further 17,18-cis double bond.

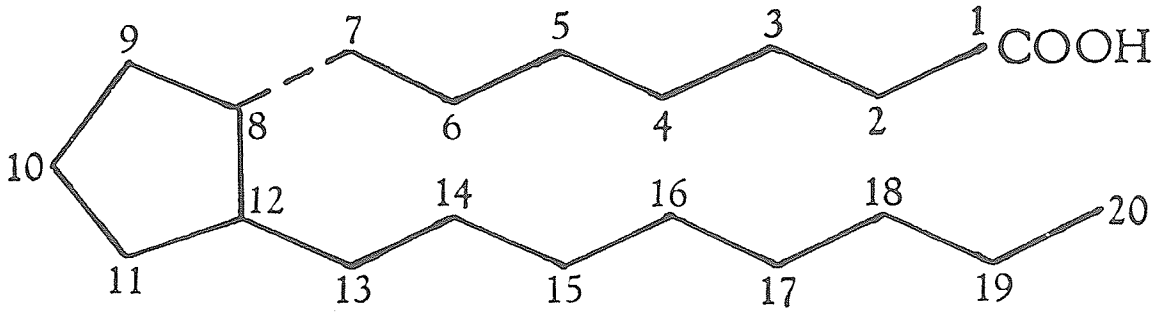


FIGURE 9.

Prostanoic acid (From Pickles, 1969).

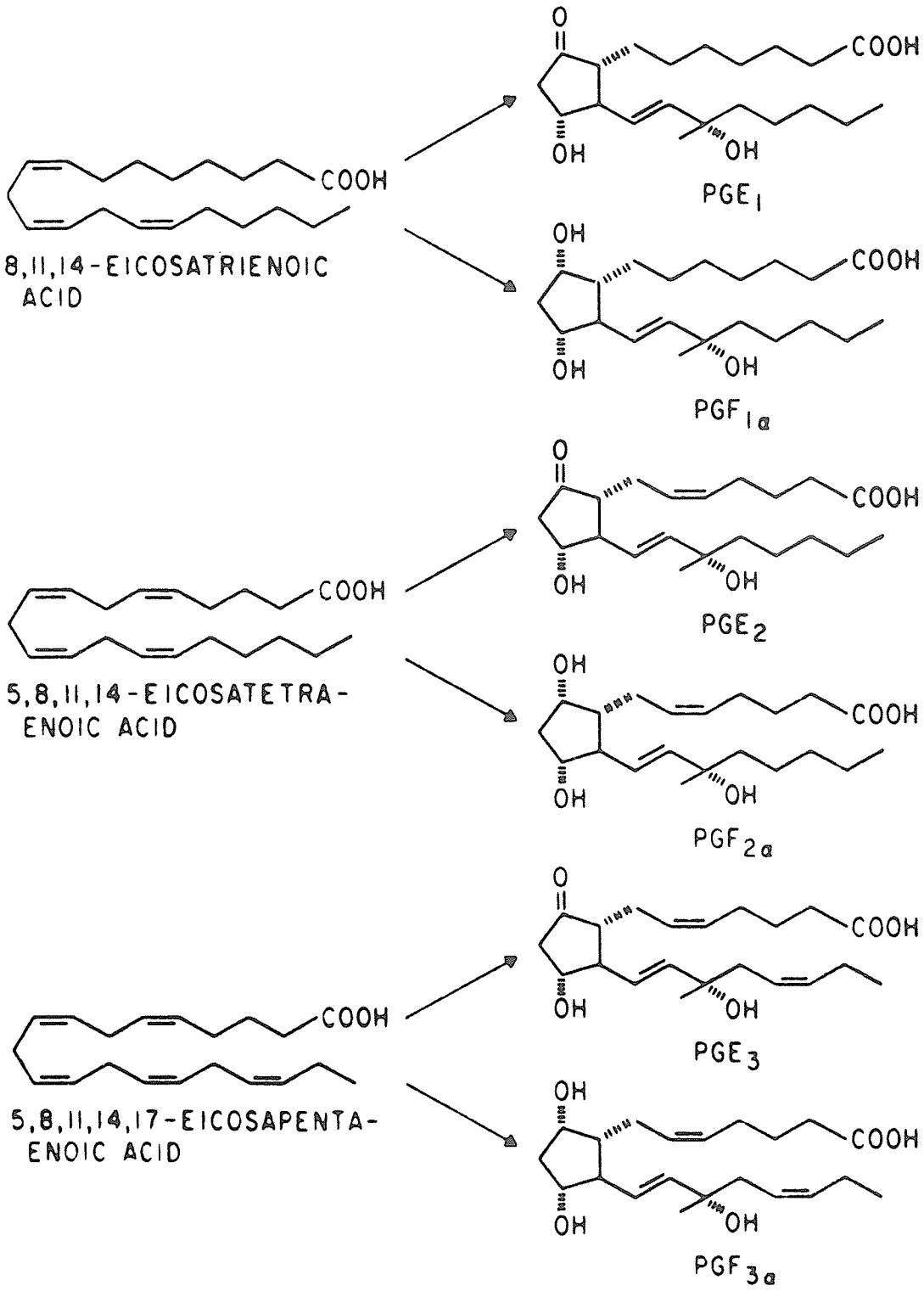


FIGURE 10.

Structural formulae of the primary prostaglandins and their precursors (From Higgins and Brunwald, 1972).

The letters E and F in prostaglandin nomenclature were derived from the early observation that prostaglandins could be partially separated by extraction of tissue homogenates with ether or phosphate buffer. E compounds were taken up into the ethereal phase, whereas F compounds remained in the aqueous phosphate (Swedish, fosfat) buffer.

Prostaglandins of the E series are further characterized by the presence of an 11-hydroxyl group and a 9-keto group; those of the F series possess 2 hydroxyl groups: one at the 9 position and the other at the 11 position.

The remaining naturally occurring prostaglandins (the secondary) are derived from the primary, A and B compounds by treatment of E compounds with either acid or base and the 19-OH series of compounds by the introduction of a hydroxyl group at position number 19 (see Curtis - Prior, 1976).

However, this classical nomenclature might become obselescent because of recent developments in the field, which include the following:

1. The discovery of some short-lived intermediates of biosynthesis (PGG's, PGH's), the endoperoxides and PGC.
2. The finding that PGE_3 and $PGF_{3\alpha}$ are of little known importance and that many of the other naturally occurring prostaglandins are only metabolites (19-OH compounds).

3. The discovery of other prostaglandins and prostaglandin-like compounds of biological and clinical importance (PGD's, PGI's and thromboxanes), all of which are derived directly from the essential fatty acids.

1.2.3 Biosynthesis

Since Van Dorp et al. (1964a,b) and Bergstrom et al. (1964a,b) demonstrated that homogenates of sheep seminal vesicle could enzymatically convert arachidonic acid to PGE_2, almost all tissues have been shown to possess the capability to synthesize prostaglandins, (Pace-Asciak and Rangaraj, 1977). The membrane bound enzyme complex responsible for their biosynthesis is termed prostaglandin synthetase.

As previously stated, prostaglandins are synthesized from three polyunsaturated fatty acids: dihomo-γ - linoleic acid, arachidonic acid, and eicosapentaenoic acid, which give rise to the mono-, bi-, and trienoic acids, respectively. These polyunsaturated fatty acids are mainly found incorporated into the cell membrane and as such cannot be utilized by the synthetase complex. The generation of free fatty acids from membrane phospholipids by the action of the enzyme phospholipase A_2 is an essential prerequisite to prostaglandin biosynthesis.

The enzymatic pathways for the diene family of prostaglandins and prostaglandin-like compounds are outlined in Figure 11. The first step involves a cyclo-oxygenase conversion (i.e., 2-fold oxygenation) to the intermediate endoperoxide PGG_2 (Hamberg and Samuelsson, 1973). A subsequent reduction of the 15-hydroperoxy group results in the formation of a second endoperoxide intermediate, PGH_2. These compounds possess a half-life of approximately 4 to 5 minutes (Svenson et al., 1975). Isomerization or reduction of PGH_2 by the appropriate enzyme results in the formation of prostaglandins D_2, E_2, or F_{2_α} (the parent prostaglandins having half-lives in vivo of approximately one minute, Hamberg and Samuelsson, 1971; Granstrom, 1972) or the prostaglandin-like compounds, the thromboxanes A_2 and B_2, and prostacyclin, PGI_2. These latter three compounds are known to play an important role in haemostasis.

1.2.4 Metabolism

A wide variety of tissues are capable of metabolizing prostaglandins; however, the major sites are in the lungs, liver, placenta, and kidney. The typical route of metabolism is as follows:

1. dehydrogenation of the carbon-15 hydroxyl group
2. reduction of the carbon-13 double bond
3. beta-oxidation of the carboxyl side chain
4. omega-hydroxylation at carbon-20
5. omega oxidation of the alkyl side-chain.

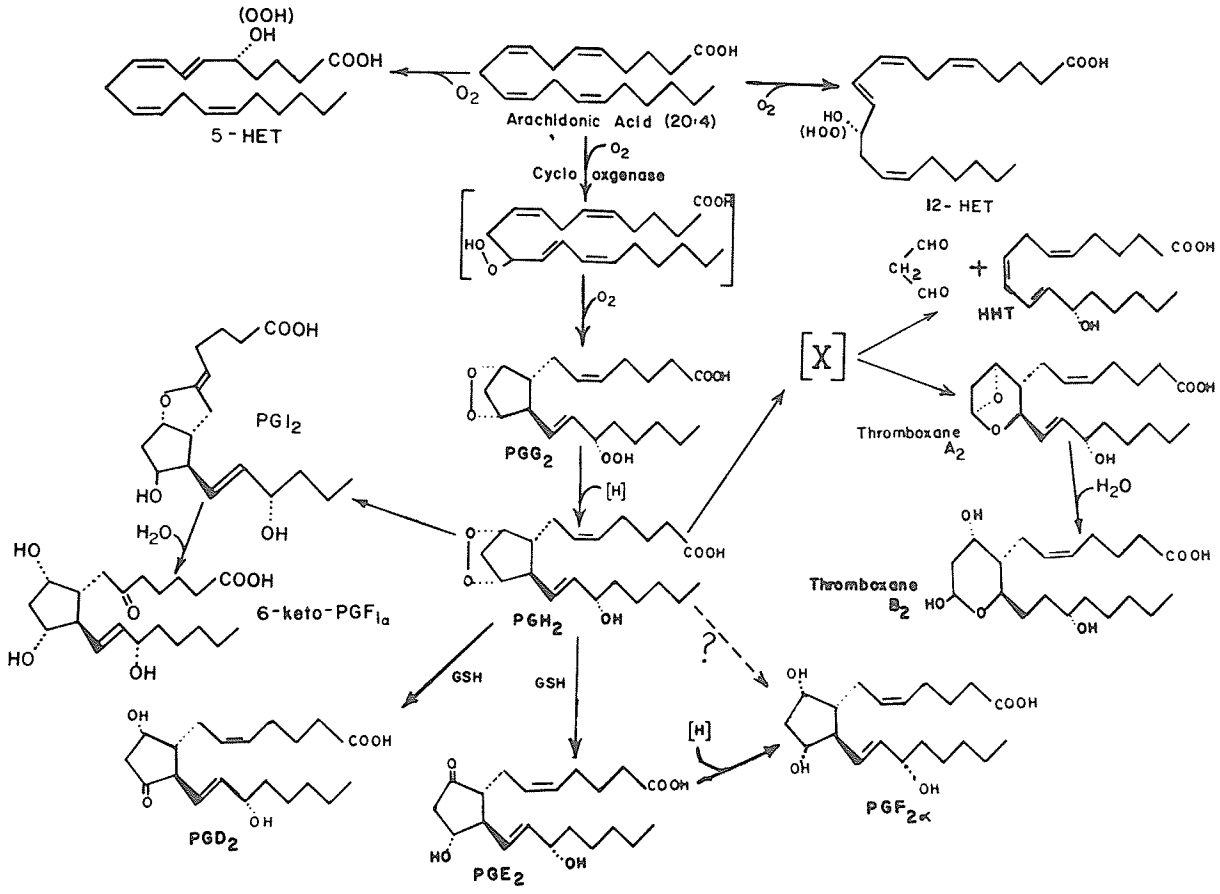


FIGURE 11.

Prostaglandin biosynthesis. Molecular oxygen is inserted into a polyunsaturated fatty acid, and the acyl chain is rearranged to form the various autacoids (From Lands, 1979).

With respect to man and PGE_2, this reaction sequence proceeds in a straightforward manner, leading to the production of a main urinary metabolite (Fig. 12) 7 $^{\alpha}$-hydroxy-5,11-diketotetranorprosta-1, 16-dioic acid (Hamberg and Samuelsson, 1971)

Dehydrogenation of the hydroxyl group at carbon-15 is accomplished by a cytoplasmic enzyme (15-hydroxy-prostaglandin dehydrogenase), first isolated from swine lung homogenate (Angaard and Samuelsson, 1966a). Furthermore, Marazzi and Anderson, (1974) have shown that it is primarily the action of this enzyme that is responsible for the inactivation of the prostaglandins. The reduction of the carbon-13 double bond is catalyzed by another cytoplasmic enzyme, prostaglandin Δ^{13}-reductase. Beta-oxidation is a non-specific reaction, typical of the metabolism of long-chain fatty acids. It consists of the cleavage of 2 carbon fragments from the terminal carboxyl group and results in the production of dinor and tetranor-prostaglandin derivatives, depending on whether it occurs once or twice. Omega-hydroxylation and oxidation involves first, the hydroxylation of the terminal end of the alkyl side chain, and a subsequent conversion into a carboxyl group. A similar reaction sequence occurs for the other prostaglandins of the E and F series.

Deviations from this sequence have, however, been described in other species. Thus, Δ^{13}-reductase activity may be absent, as in swine lung (Angaard and Samuelsson, (1966b) or, it may precede 15-hydroxy prostaglandin dehydrogenase activity (Angaard and Samuelsson, 1964) as in guinea pig lung homogenates.

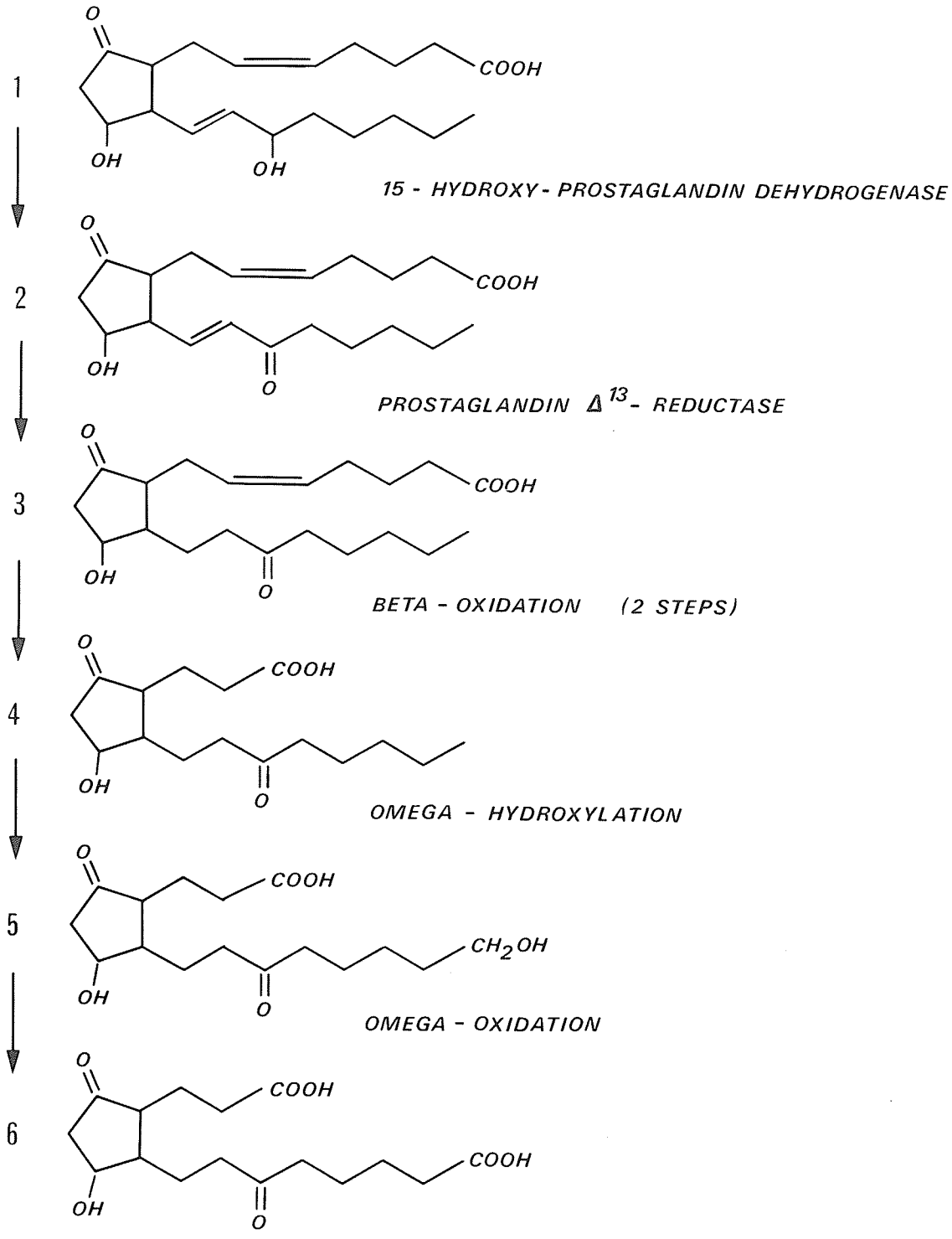


FIGURE 12.

Prostaglandin E_2 metabolism in man.

Green (1971) has shown that in the rat, PGE_2 is broken down into three groups of metabolites via three separate pathways (Fig. 13). In the first pathway, PGE_2 is broken down as in man, leading to the formation of 7 $^{\alpha}$-hydroxy-5, 11-diketotetranorprosta-1, 16-dioic acid. Secondly, there is a pathway leading to the production of a 5 β-hydroxy derivative (5β, 7 α-dihydroxy-11-ketotetranorprostanoic acid), but the reaction sequence leading to the production of this metabolite is not known. Finally, PGE_2 is oxidized forming 7α ,11-dihydroxy-5-ketotetranorprost-9-enoic acid which is then dehydrated to 11-hydroxy-5-ketotetranorprosta-4(8), 9-dienoic acid or possibly to the corresponding PGA derivative (tetranorprostaglandin A). Further oxidation to $^{\omega}$-1 and ω-2 hydroxylated compounds (11,15-dihydroxy-5-ketotetranor-prosta-4,(8),9-dienoic acid and 11,16-dihydroxy-5-ketotetranor-prosta-4,(8),9-dienoic acid) and the dicarboxylic acid, 11-hydroxy-5-ketotetranorprosta-4,(8),9-diene-1,16-dioic acid then occurs.

1.2.5 Biological Properties

Prostaglandins have been implicated in a wide range of physiological and pharmacological functions, as well as in several pathological conditions. In general, the response (physiological or pharmacological) observed depends on the type of prostaglandin administered, dose, tissue sensitivity, species of animal and whether the study has been carried out *in vivo* or *in vitro*.

Compound	
Metabolite IIa	7-Hydroxy-5,11-diketo-tetranorprosta-1,16-dioic acid
Metabolite IIb	7,16-Dihydroxy-5,11-diketotetranorpros-stanoic acid
Metabolite IIIa	11-Hydroxy-5-ketotetra-norprosta-4(8),9-diene-1,16-dioic acid
Metabolite IIIb:1	11,15-Dihydroxy-5-keto-tetranorprosta-4(8),9-dienoic acid
Metabolite IIIb:2	11,16-Dihydroxy-5-keto-tetranorprosta-4(8),9-dienoic acid
Metabolite IV	5β,7α-Dihydroxy-11-ketotetranorprosta-noic acid
Metabolite V	7α,11-Dihydroxy-5-keto-tetranorprost-9-enoic acid
Metabolite VI	7α-Hydroxy-5,11-diketo-tetranorprostanoic acid
Metabolite VII	11-Hydroxy-5-ketotetra-norprosta-4(8),9-

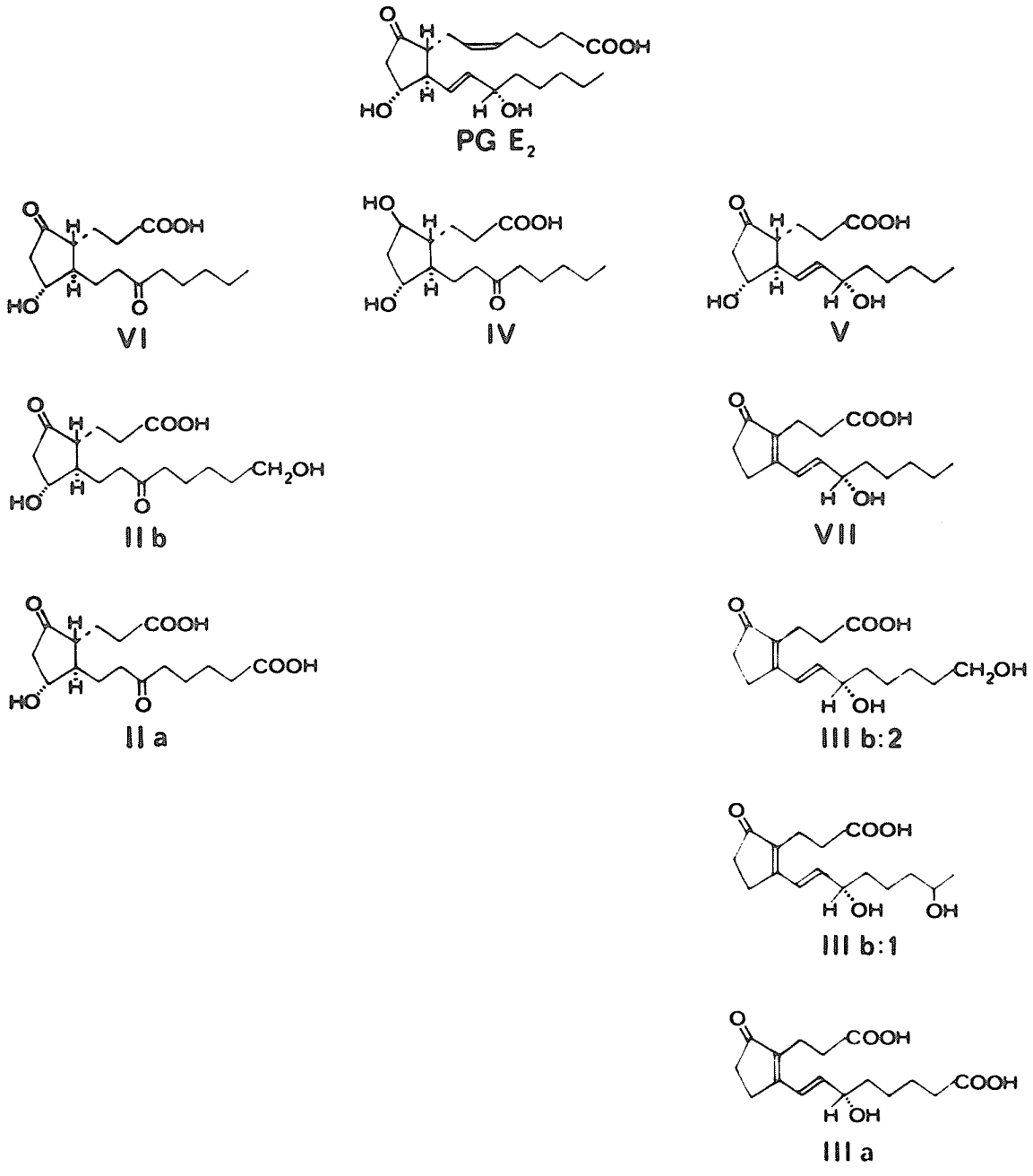


FIGURE 13.

Prostaglandin E_2 metabolism in the rat (From Green, 1971).

Most prostaglandins of a particular series seem to possess the same biological action on any one system, however, the tissue response may differ in degree depending on the type of prostaglandin used. Also, the same prostaglandins often have different biological actions on different tissues, and likewise prostaglandins of different groups may have dissimilar effects. To date, a consistent mode of action has not been elucidated.

The effects of prostaglandins are limited by their rapid inactivation (Ferreira and Vane, 1967). Over 90% of PGE and PGF compounds are metabolized during one circulation through the lungs and liver, thus they are thought to be physiologically important only as local hormones and probably act close to, or at their site of synthesis. However, it has recently been shown that some of their breakdown products which are more resistant to metabolism can be biologically active, for example, Angaard and Samuelsson,(1966b) found that dihydro-PGE_1 (a metabolite of PGE_1) had 14-35% of the activity of PGE_1 on the isolated rabbit duodenum, isolated ileum and uterus of the guinea pig. The presence of biologically active metabolites may explain why certain pharmacological effects observed after prostaglandin administration may persist for a longer period than would normally be expected.

Prostaglandins have a widespread distribution in the body; however, no tissue stores them. They are synthesized and released following physiological, pharmacological or pathological stimuli.

In addition, there seems to be distribution differences with respect to individual prostaglandins. For example, in some tissues such as: seminal plasma, vesicular glands, and thymus PGE compounds predominate (Bergstrom and Samuelsson, 1965), whereas in others like the brain and lung, the PGF compounds are present in greatest concentration. Such differences in occurrence could be due to differing rates of biosynthesis and metabolism of the individual prostaglandins in these tissues (Angaard and Samuelsson, 1967). Both prostaglandins of the E and F series possess non-vascular smooth muscle stimulating properties; however, only those of the E series show vasodepressor properties. This suggests that the potent vasodepressor activity is due to the 9-keto group. These and the other prostaglandins, and prostaglandin-like compounds exert their effects on a wide variety of systems. Many of their actions may be secondary to an effect on the adenyl cyclase-cyclic-AMP system which is the second messenger in many systems. It is the presence of prostaglandins in reproductive tissues that has raised questions concerning their physiological functions and role in reproduction and fertility.

1.2.6 Clinical Applications of Prostaglandins in Reproduction

An oxytocic activity by prostaglandins on the human pregnant myometrium was first demonstrated <u>in vitro</u> by

Bygdeman (1964) and *in vivo* by Bygdeman et al. (1968). This observation and the discovery of elevated levels of prostaglandins in amniotic fluid and peripheral plasma during spontaneous labour and abortion (Kaim, 1966, 1968; Karim and Devlin, 1967; Karim and Hillier, 1970) led to the first clinical application of a prostaglandin for the induction of labour at term (Karim et al. 1968) and for the termination of early pregnancy (Karim and Filshie, 1970a,b; Roth-Brandel et al. 1970). Since then, major advances have been made in the use of prostaglandins for the termination of pregnancy at all stages of gestation, as well as in the identification of therapeutic roles of prostaglandins, their synthetic analogues, and inhibitors of prostaglandin synthesis in several other areas of clinical importance (Table 1).

Clinical research over the past few years with respect to prostaglandin-induced abortion has aimed at:

1. defining the most effective routes of administration
2. identifying compounds with a more selective oxytocic effect, and
3. reducing gastro-intestinal and other side effects associated with prostaglandin treatment.

Five principal routes of administration have been extensively investigated: intravenous, oral, intravaginal, intra-amniotic, and extra-amniotic. Also, some analogues of the naturally-occurring prostaglandins have proven superior in terms of safety, greater uterotonic potency and duration of action, as well as

Table 1: Established and potential clinical applications of prostaglandins and prostaglandin synthesis inhibitors in relation to human reproduction (from Karim & Rao, 1975).

PROSTAGLANDINS
Induction and acceleration of labour at term.
Induction of labor in case of intrauterine fetal death.
Induction of labor in case of anencephalic pregnancy.
Cervical ripening at term.
Termination of first and second trimester pregnancy.
Termination of molar pregnancy.
Termination of pregnancy in case of missed abortion.
Menstrual induction.
Preoperative cervical dilatation in the first trimester of pregnancy.
Preoperative cervical dilatation in non-pregnant women.
Management of third stage of labor and prevention of postpartum haemorrhage.
Treatment of male infertility.
PROSTAGLANDIN SYNTHESIS INHIBITORS
Prevention of premature labor.
Prevention of spontaneous abortion.
Treatment of dysmenorrhea.
Ovulation block.
Male contraception.

having lesser side effects. (Karim and Amy, 1975; Thiery and Amy, 1975; Karim, Ng and Ratnam, 1979; Karim, 1979; Amy and Thiery, 1979, provide extensive reviews of these methods of pregnancy interruption or induction of labour and the advantages or difficulties associated with them).

In addition to being used as conventional abortifacients, prostaglandins offer a new and promising approach to fertility control. Menstrual induction refers to the termination of very early pregnancy (upper limit - 49 days amenorrhea) by the administration of drugs. Prostaglandins and prostaglandin analogues have proven to be very effective in terminating early pregnancy without producing severe complications, commonly associated with surgical approaches (reviewed by Bygdeman, 1979).

A self-administered pharmacological method of menstrual regulation is often regarded as an ideal complement to conventional contraceptive methods. Prostaglandin analogues may soon provide this potential, making this method the most attractive alternative to surgical menstrual regulation. Self-administration could, however, lead to a misuse and abuse of the drug. Furthermore, little is known of the effects of prostaglandins and their analogues on the human conceptus which could assume considerable practical importance in the event of failure of contraception.

1.2.7 PGE_2: Effects on Embryonic and Fetal Development

In view of the widespread clinical use of PGE_2, for pregnancy termination and for the induction of labour, it is suprising to find that there have been relatively few studies dealing with its effects on embryonic and fetal development. The available results indicate that PGE_2 effects on the fetus vary depending on the species of animal used, the route of administration of the drug, the dosage, and the period of gestation it is administered.

Oral administration of 6, 120 or 240 mg/kg PGE_2 to mice on the seventh through twelfth days of pregnancy has been shown to produce a dose dependent increase in fetal deaths and resorptions. This was accompanied by a significant increase in minor skeletal abnormalities. However, rats treated from the ninth through fourteenth days of gestation showed a significant increase in fetal deaths and resorptions only at the highest dose level (240 mg/kg) (Tsueneo Fujita et al. 1973). Persaud (1973a,b) demonstrated that the route of administration and day of gestation played a key role in the response. In rats, intra-peritoneal injection of PGE_2 (300 μg) on the ninth through twelfth days of gestation caused a significant increase in fetal resorptions. The same dose given on the twelfth through fifteenth days of gestation did not produce embryolethal effects, except that 18.2% of recovered fetuses showed signs of extensive edema and hemorrhagic lesions. However,

embryotoxic effects in the form of fetal death were found to occur in 100% of rat fetuses following an intraamniotic injection of 100 µg of PGE_2 on the fifteenth day of gestation.

Higher doses of PGE_2 administered intraperitoneally have been found to produce both embryolethal and teratogenic effects in the rat (L. Mercier-Parot and H. Tuchmann-Duplessis, 1977). Rats treated from days 6-10 showed a dose-dependent, progressively decreasing pregnancy rate, simultaneously accompanied by an increase in the mean resorption rate per litter from 1.2% to 4.5%. Embryonic development was impaired in 3 of the 4 treatment groups. Animals receiving 10 mg/kg showed no teratogenic effect but in those receiving 15, 20, and 30 mg/kg, 1.5%, 15% and 17% of fetuses, respectively, showed a wide variety of gross malformations. Facial anomalies were predominant.

A high embryolethality was observed in rats treated from days nine through fourteen; 5 mg/kg PGE_2 interrupted pregnancy in 93% of treated animals. The remaining 7% had only a few offspring, the mean number of fetuses per litter being 2 as compared to 8.6 in the controls. In those groups treated with 10, 15, and 20 mg/kg PGE_2 resorption occurred in all animals.

Teratogenic effects were also observed by Persaud (1975) in mice receiving 25 µg PGE_2 subcutaneously on either day 9, 12, or 15 or gestation. In addition, fetal growth was significantly

affected following treatment on either the 12th or 15th day of gestation. No significant increase in the incidence of embryonic deaths and fetal resorptions occurred. A wide variety of congenital malformations was induced including: stunting, meromelia, hydrocephalus, microophthalmia, spina bifida, polycystic kidney, absence of urinary bladder and omphalocoele. Major skeletal malformations were not present, however, a relatively high incidence of minor skeletal variations was detected in both controls and fetuses treated with PGE_2. These included incomplete sternebrae, missing phalanges, rudimentary ribs, and poor ossification of the skull, vertebrae, and ribs.

In contrast, L. Mercier-Parot and H. Tuchmann-Duplessis (1978) observed no teratogenic or lethal effects. However, they found that intraperitoneal administration of higher doses (30 and 40 mg/kg PGE_2) to mice on days 6-10 resulted in a decrease in pregnancy rate accompanied by a reduction in the average number of fetuses carried per litter. Several minor anomalies were observed, but their frequency was small and a relationship to treatment could not be established. A high embryolethality was also observed in mice treated from days 9 through 14. A dose of 5 mg/kg interrupted pregnancy in 70% of treated animals. The remaining 30% had fetuses but in much reduced numbers, 1.5 compared to 10.2 in the controls, although the mean number of resorptions was in inverse proportion, 10.3 compared to 1.1 in the controls.

Intra-amniotic administration of 25, 50, and 100 μg PGE_2 to rabbits on day 14 of gestation (Persaud, 1974) induced a significant increase in fetal resorptions. In addition, intrauterine growth was also significantly affected. An increased incidence of congenital anomalies was observed at the 25 μg dose level. Mean placental weights were significantly less than that of controls only after treatment with 50 μg, but extensive morphological changes were induced by all doses. These included marked thinning of the decidua basalis, depletion of decidual glycogen, congested and severely dilated maternal blood spaces, and extensive atrophic and degenerative changes in the labyrinthine trophoblast.

Persaud et al. (1973) have reported an increased incidence of embryonic mortality and malformations in chick embryos following treatment at 48 hours incubation with 50 and 100 μg PGE_2. Only the occurrence of abnormal embryos induced by 100 μg PGE_2 was found to be statistically significant. At 72 hours incubation, PGE_2 was not lethal to the embryos. A slight increase in abnormal embryos was noted after treatment with 100 μg but this was not statistically significant. PGE_2 had no effect on embryonic growth at either incubation period.

Subcutaneous administration of PGE_2 (1.5-15 mg) to 9 rhesus monkeys during early pregnancy for either 3 or 5 consecutive days was found to have no effect on the duration of pregnancy, nor were the offspring abnormal. Also, intrauterine growth remained unaffected (Cole and Nutting, Personal Communication, 1973).

Furthermore, it has been found that oral administration of PGE_2 at parturition produces no adverse effects on the human fetus (Barr, 1972; Craft, 1972).

The mode of action of PGE_2 in the pregnant animal is not known. The observed effects may be produced by several different mechanisms, involving the mother, the fetus, or the feto-placental unit. Persaud and Clancy (1974a) have shown that PGE_2 and/or its metabolites can readily cross the placental barrier in mice and can be detected in significant quantities in the placenta and fetus. In addition, pre-treatment of pregnant mice with PGE_2 significantly decreases the uptake of tritiated thymidine in maternal and embryonic tissues (Persaud and Clancy, 1974b). This suggests that PGE_2 is capable of interferring with DNA synthesis, either directly or indirectly.

Microscopic examination of the ovaries of rats treated with PGE_2 showed neither venous congestion nor luteolysis. Placental changes, however, were extensive and included a significant decrease in mean weight, marked inhibition of decidua formation, inhibition of trophoblastic proliferation, and focal hemorrhagic lesions. Furthermore, exogenous progesterone was unable to reduce the incidence of intrauterine deaths and fetal resorptions following intra-amniotic administration of PGE_2 (Persaud, 1973c).

These findings suggest a direct action of the prostaglandin on the developing conceptus may play an important role in accounting for the adverse effects produced by the substance during pregnancy.

2. MATERIALS AND METHODS

2.1 Animals

Albino Sprague-Dawley rats of the Holtzman strain, weighing 250-300 g, were housed in wire-mesh cages under controlled environmental conditions (temperature 72°F; 12 hour reversed day-night cycle) and maintained on standard rodent pellets and water ad libitum.

Virgin females were placed with males for a 3 hour period during the night, after which vaginal smears were taken. The presence of spermatozoa was considered to indicate day 0 of pregnancy.

2.2 Experimental Design

Experiments were designed to determine:

a) the abortifacient action of PGE_2 administered subcutaneously on days 5, 6, 7, and 8 of gestation.

b) the influence of PGE_2 on early embryonic and fetal development, and

c) the effects of PGE_2 on placental and ovarian morphology.

The animals were randomly assigned to eight groups according to the following treatment schedule:

Table 2. Treatment of female rats with PGE_2 on days 5, 6, 7, and 8 of pregnancy

Experimental group	Number of animals	Treatment	Day of gestation pregnancy was terminated
I	4	50 µg	9
II	4	100 µg	9
III	4	200 µg	9
IV	8	Saline	9
V	4	50 µg	20
VI	4	100 µg	20
VII	4	200 µg	20
VIII	6	Saline	20

The amount of PGE_2 (Upjohn Company, Kalamazoo, Michigan) to be injected was weighed out and dissolved in physiological saline. It was administered as a 0.2 ml volume solution, subcutaneously, on days 5, 6, 7, and 8 of pregnancy, to animals of groups I, II, III, V, VI, and VII. An equal volume of physiological saline was administered to the animals of groups IV and VIII. These served as controls. All animals were weighed at intervals of 2 days.

2.3 Histological Examination of Implantation Sites on Gestational Day 9

Twenty-four hours after the final treatment, the animals were anaesthetized with ether. The thorax was opened and an 18 gauge needle attached to an intravenous drip (suspended approximately 2 meters above) was inserted into the ascending aorta via the left ventricle. The right atrium was punctured and the animal was perfused with Karnovsky's fixative in Millonig's phosphate buffer. (Appendix A).

After perfusion with about 200 ml of the fixative, the uterus with the ovaries attached were removed and immersed in fresh fixative. A central slice of approximately 1 mm diameter was taken from each implantation site and placed in fresh fixative for 90 minutes. After a brief 10 min wash in Millonig's phosphate buffer, post-fixation was carried out for 2 hours in a 1% osmium tetroxide solution. The tissues were then dehydrated in an ascending ethanol-methanol series of alcohols and embedded in araldite. (Appendix B).

Sections of about 2-3 µm thickness were cut manually on an LKB Ultratome III, floated on 15% ethanol on glass slides, dried at 60°C, stained with toluidine blue for 2 minutes, and examined.

Ovaries were removed, and placed in 10% buffered formalin. These tissues were subsequently dehydrated in an ascending

series of ethanol, and embedded in paraffin. Sections of 5-6 µm thickness were cut and examined.

2.4 Fetal Studies on Gestational Day 20

Animals were anaesthetized with ether on day 20 of gestation. Fetuses were removed by hysterotomy, whereupon it was determined whether they were alive or dead. The number of resorptions was also recorded.

Two fetuses from each litter were eviscerated and placed in 95% alcohol for 2 weeks. They were then cleared in a 1% KOH solution, stained with alizarin red, and passed through an ascending series of glycerol solutions. The skeletal anatomy was then examined.

The remaining fetuses together with the ovaries, and placentas, were placed in Bouin's fixative. Fetuses and placentas were cleaned and weighed one week later. Free-hand sections with a razor blade were taken on the fetuses (Wilson, 1965) and the visceral organs were examined.

Placentas and ovaries were dehydrated in an ascending ethanol series, and embedded in paraffin. Sections of 5-6 µm in thickness were cut for routine histological studies.

2.5 Statistical Analysis

In order to determine whether the data obtained from animals treated with PGE_2 were significantly different from those animals treated with physiological saline, an analysis of

variance and Duncan's Multiple Range Test was applied to the following parameters:

1. fetal weights
2. placental weights
3. litter size - the number of live fetuses in each litter
4. prenatal loss - the number of resorptions in each litter
5. intrauterine deaths - the number of dead fetuses present at hysterotomy
6. maternal weight change - the weight change in grams over the gestational period.

The experimental data obtained from control animals were pooled.

3. RESULTS:

3.1 Long Term Assessment

3.1.1 Statistical Analysis of Fetal Data

The following parameters were studied: litter size, number of resorptions, intrauterine deaths, fetal weights, placental weights, and maternal weight gain during pregnancy. The results of the statistical analysis are presented in Tables 3-9, Graph 1.

In order to determine whether PGE_2 altered implantation rate, the total number of live fetuses, resorbed and dead fetuses (total number of implantations) present at hysterotomy were compared by Duncan's New Multiple Range Test with those of animals treated with physiological saline (Table 3).

The mean number of implantations occurring was not significantly affected in any of the groups of animals treated with PGE_2. In addition, the mean number of implantations detected on day 20 was compared with the mean number of implantations found occurring on day 9. The difference was not statistically significant.

Comparison of mean maternal weight gain over the entire gestational period using Duncan's New Multiple Range Test revealed no significant difference following treatment with PGE_2 at any of the dose levels (Table 4). All animals gained over 100 grams. However, maternal weight

gain monitored every second day indicated there was a significant decrease ($P < 0.05$) in weight gain during the treatment period following treatment with 200 µg PGE_2 (Table 5, Graph 1). Furthermore, following cessation of treatment a significant increase ($P < 0.05$) in weight gain was observed in the same group (Table 5, Graph 1).

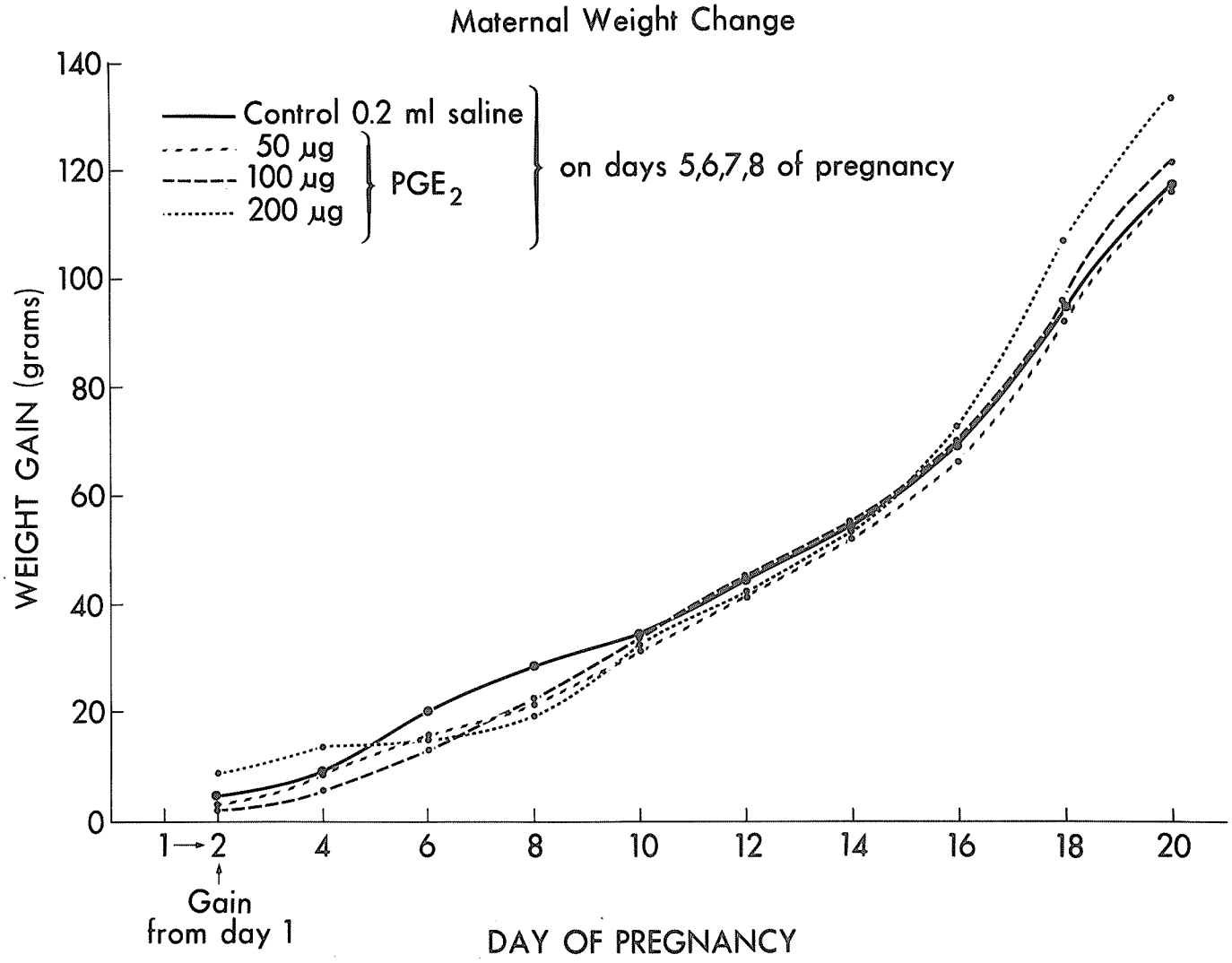
Treatment with PGE_2 at doses of 50, 100, and 200 µg on gestational days 5 through 8 did not significantly affect the mean litter size compared to the control group of animals (Table 6). All fetuses recovered at term were alive and responded promptly to mechanical stimulation. The mean number of resorptions per litter was not significantly altered by PGE_2 treatment at any of the dose levels (Table 7). There was no evidence of growth retardation.

Mean fetal weights were not altered following maternal treatment with PGE_2, compared to the controls (Table 8). In addition, the mean placental weight was not significantly altered following PGE_2 treatment (Table 9).

There were no maternal deaths as a result of the treatment. Vaginal bleeding was not observed in any of the treated animals.

GRAPH 1.

Maternal weight gain during pregnancy monitered every second day.


Maternal Weight Change
Control 0.2 ml saline
50 µg
100 µg
200 µg
PGE2
on days 5,6,7,8 of pregnancy
WEIGHT GAIN (grams)
140
120
100
80
60
40
20
0
1→2
4
6
8
10
12
14
16
18
20
Gain from day 1
DAY OF PREGNANCY

3.1.2 Morphological Evaluation

External and internal examination of the offspring recovered from both prostaglandin treated and control animals revealed no evidence of maldevelopment.

Fetuses stained with Alizarin Red S (Fig. 15) showed that PGE_2 treatment did not produce any detectable skeletal malformations or alterations in the maturity of ossification centers. All fetuses recovered from the control group demonstrated normal skeletal development.

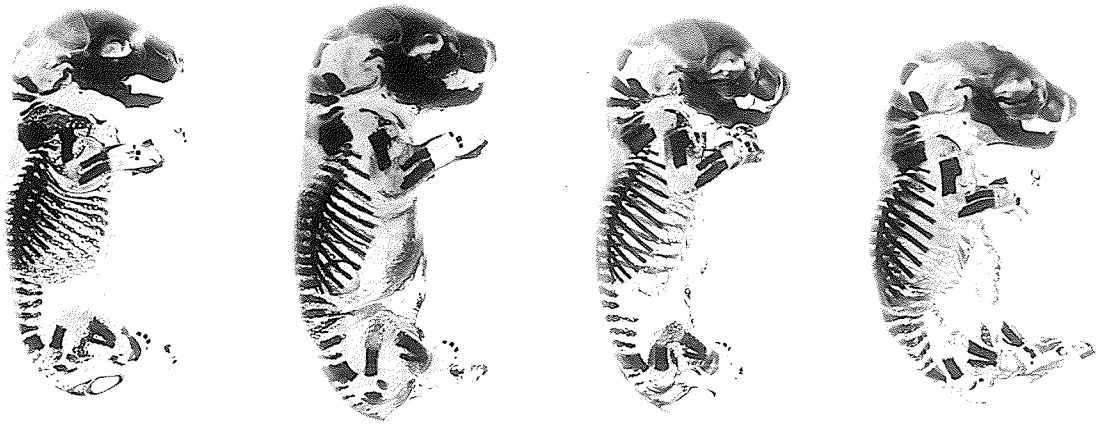
3.1.3 Influence of PGE_2 on Ovarian Morphology (old corpora lutea)

Ovaries recovered from control animals (at autopsy) near term displayed numerous corpora lutea which occupied most of any ovarian cross-section (Fig. 16). The corpora lutea were of the older type comprised of large eosinophilic granulosa lutein cells arranged in cords around branching sinusoids (Fig. 17). Blood vessels, stromal connective tissue, and interstitial cells occupy the region between corpora lutea.

The interstitial cells, morphologically similar to the mature lutein cells are found in scattered groups among the fibrous stromal cells. Developing follicles of all stages were also present in the ovaries. Graffian follicles occupied a position immediately deep to the germinal epithelium (Fig. 17).

FIGURE 15.

Alizarin-stained fetuses at day 20 of gestation. Normal skeletal development in all treatment groups was observed. A: saline-treated; B: 50 µg PGE_2-treated; C: 100 µg PGE_2-treated; D: 200 µg PGE_2-treated. X2



A B C D

FIGURE 16.

Ovary recovered from an animal treated with physiological saline. Corpora lutea (Cl) occupy a large portion of the organ. Developing follicles (F) are also evident. X70

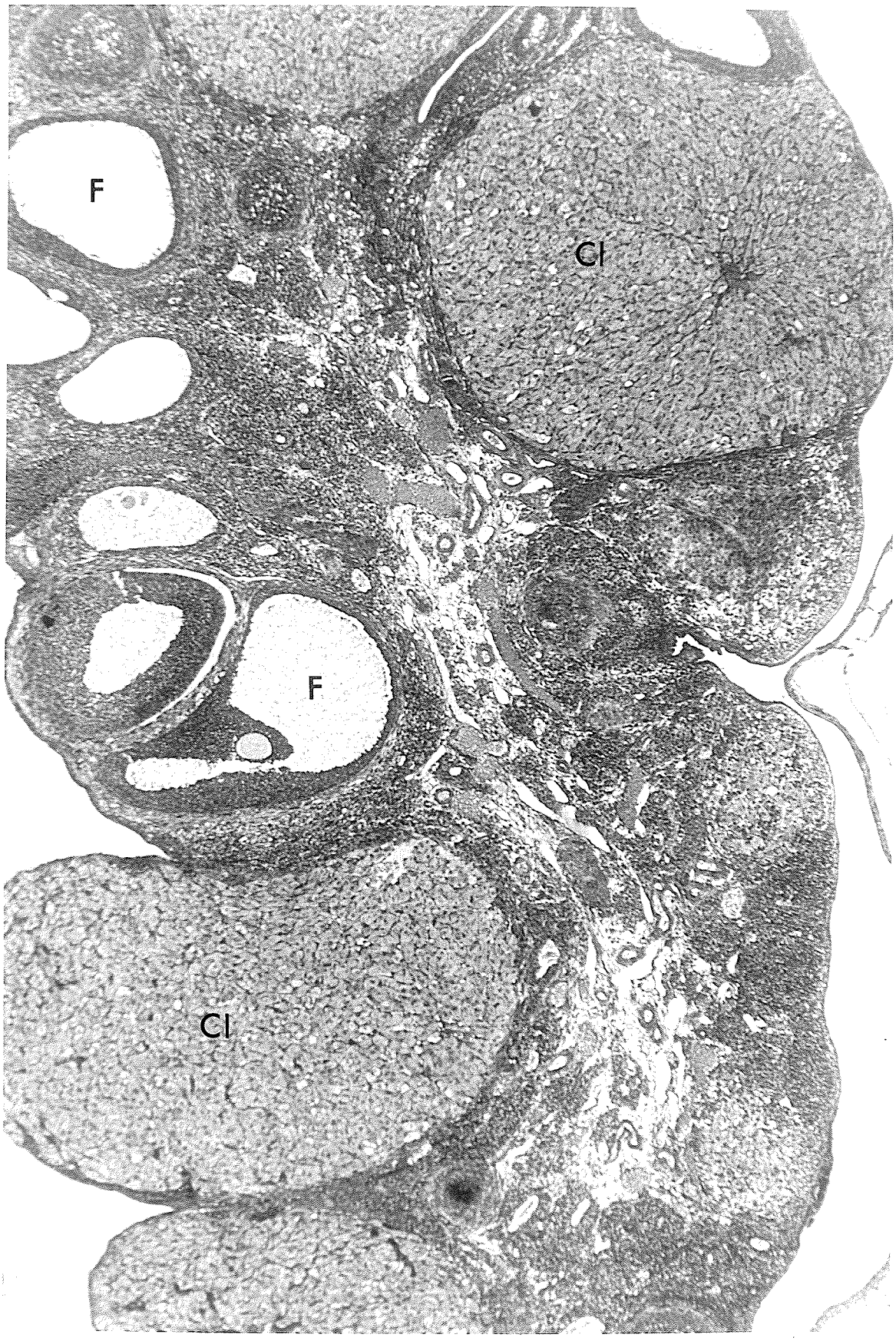
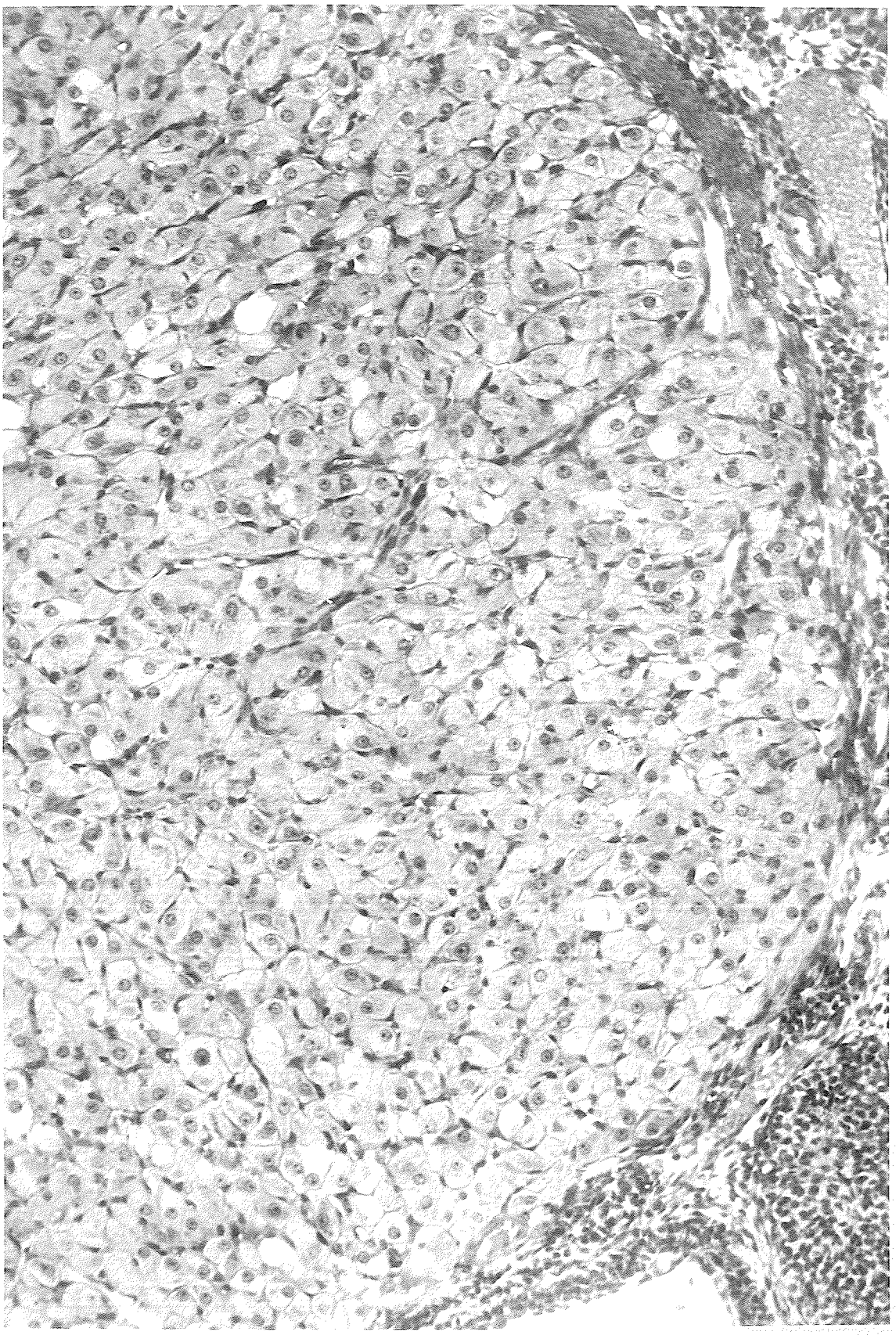

F
CI
F
CI

FIGURE 17.

High power view of day 20 corpora lutea recovered from an animal treated with physiological saline. Granulosa lutein cells are large and eosinophilic.

X280



Treatment with PGE_2 caused no apparent morphological changes indicative of degeneration or luteolysis. Recovered corpora lutea showed regular cell boundaries, eosinophilic cells with large nuclei, and very little if any fibroblastic infiltration.

3.1.4 Influence of PGE_2 on Placental Morphology

By the 20th day post-coitum, the definitive structural organization of the placenta has been achieved.

Placentas recovered from animals treated with physiological saline revealed normal histological organization, namely four zones: chorionic plate region; labyrinth; junctional zone, also called reticular zone or trophospongium; and the zone of giant cells.

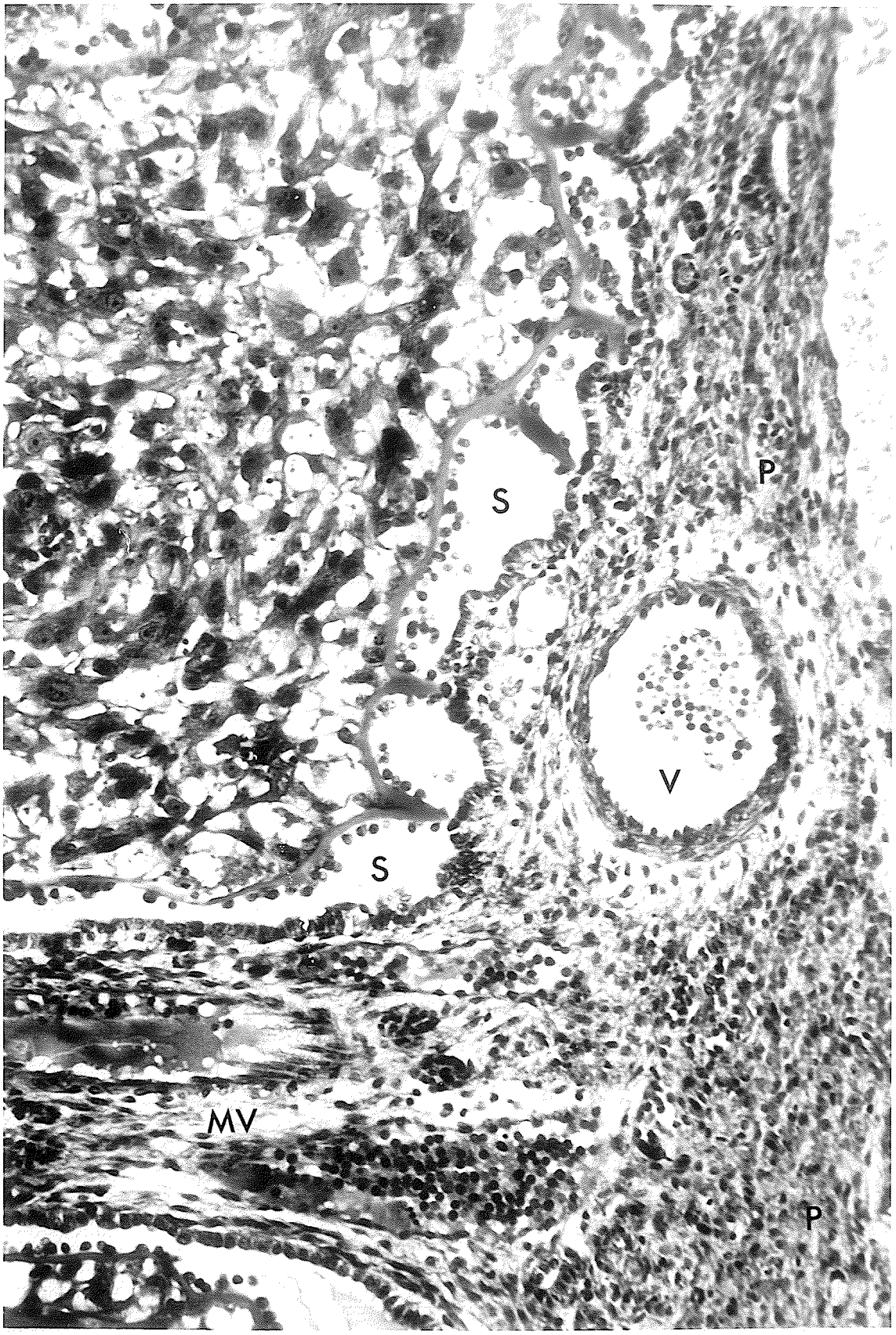
The terminology used in describing the placenta is that of Davies and Glasser (1968). The term "labyrinth" designates the zone of rat placenta in which both maternal vascular channels and fetal vessels are found. The less commital term "basal zone" designates the zone deep to the labyrinth, between it and the decidua basalis. It corresponds to the junctional zone plus the zone of giant cells. It is the zone without fetal mesenchyme and in which only maternal vascular channels are found.

The chorionic plate lying adjacent to the fetus (in utero) is largely composed of connective tissue.

Through it branches of the umbilical arteries and veins may be seen passing (Fig. 18). Adjacent to the chorionic plate a narrow labyrinthine border zone occurs which lack maternal blood spaces. It is a single narrow intraplacental space underlying the main branches of the umbilical blood vessels of the chorionic plate (Müntener and Hsu, 1977). This structure corresponds to the so-called endodermal sinuses of Duval (1892). Arising at the circular attachment of the yolk sac to the fetal placental disc, it is actually an invagination of the yolk sac into the labyrinthine region. Thus the lumen of the yolk sac extends into the placenta. The inner side of this space is lined with columnar epithelial cells of the visceral wall of the yolk sac together with fetal mesenchyme and blood vessels. The outer side is lined by flattened epithelial cells of the parietal wall of the yolk sac resting on an extension of Reichert's membrane. Extensions of the intraplacental space follow major villi into the labyrinth zone towards the maternal surface (Fig. 18). At this stage of gestation the bulk of the placenta, approximately 60%, is formed by the labyrinthine zone. This is the zone of combined fetal and maternal vascularization. Fetal capillaries branching from umbilical blood vessels within septa of connective tissue invested by a trilaminar trophoblastic epithelium form a

FIGURE 18.

The fetal surface of the placenta from an animal treated with physiological saline. Umbilical vessels (V) pass through the chorionic plate (P). The intraplacental space (S) separates the plate from the underlying labyrinthine zone. Extensions of the intraplacental space follow major villi (MV) into the labyrinthine zone. X280


S
P
V
S
MV
P

vascular network bathing in maternal blood. The details of the structural organization of this region still present unresolved problems.

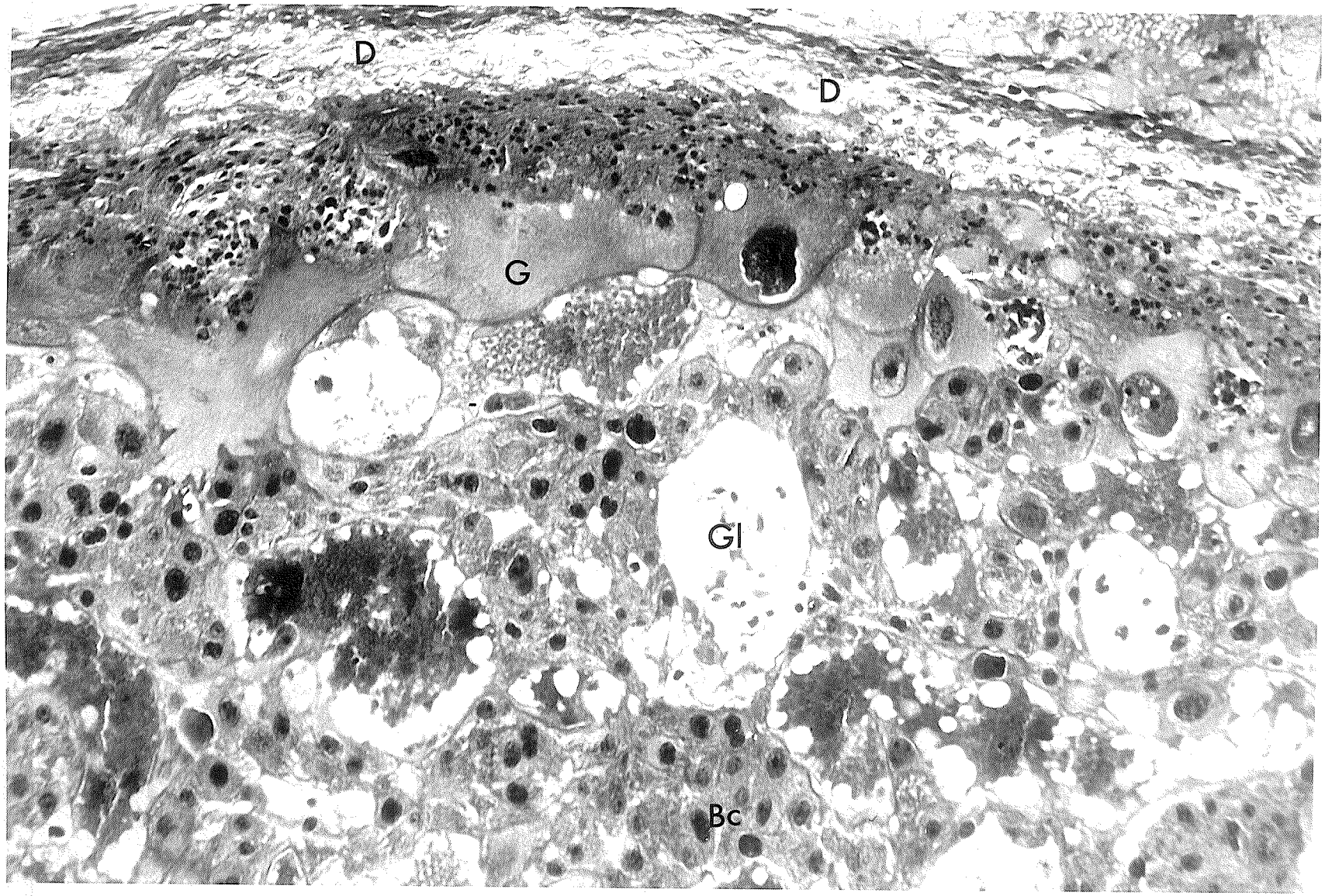
The basal zone lying between the narrow fibrotic decidua basalis and the labyrinthine zone is composed of three different cell types (Fig. 19):

1. small basophilic cells which are probably an extension of the cytotrophoblastic elements of the labyrinthine zone. These are the predominant cell type.
2. giant cells lying adjacent to the decidua basalis forming a layer several cells in thickness, especially towards the periphery of the placenta.
3. glycogen cells, so-called because of their massive accumulations of glycogen. They are characteristically arranged in groups or islands. They appear empty and display shrunken pyknotic nuclei often displaced peripherally.

The small basophilic cells and the giant cells are encapsulated by an eosinophilic type of substance termed fibrinoid. The fibrinoid is presumably a glycoprotein or mucopolysaccharide since it has been reported to be PAS positive (Davies and Glasser, 1968). Its origin has been discussed (Schiebler and Knoop, 1959).

FIGURE 19.

The maternal surface of a placenta from an animal treated with physiological saline. The basal zone lying between the narrow fibrotic decidua basalis (D) and the labyrinthine zone is composed of three different cell types; giant cells (G), glycogen cells (Gl), and small basophilic cells (Bc). X280


D
D
G
Gl
Bc

Maternal blood circulates in the basal zone in direct contact with either the giant cells or the small basophilic cells. Also, the maternal blood circulating within the labyrinth is in immediate contact with trophoblastic elements.

Placentae recovered at term from mothers treated with PGE_2 revealed the normal morphology described above. No changes were detected.

TABLE 3. Effect of PGE_2 on implantation*

Treatment	Number of implantations (Mean ± SEM)	Significance of difference
Saline	11.67 ± 0.67	
50 µg PGE_2	12.15 ± 1.38	NS
100 µg PGE_2	12.00 ± 1.47	NS
100 µg PGE_2	11.75 ± 1.11	NS

*The number of implantations is the sum of the litter size and the number of resorbed and dead fetuses.

TABLE 4: Comparison of mean maternal weight gain (grams) over the gestational period in groups of animals treated with PGE_2 or physiological saline.*

Treatment	Maternal weight gain** (grams)	Significance of difference
Saline	113.75 ± 2.49	
50 µg PGE_2	116.62 ± 8.59	NS
100 µg PGE_2	128.88 ± 5.31	NS
200 µg PGE_2	119.12 ± 14.35	NS

*Duncan's New Multiple Range Test

**Mean ± S.E.M.

TABLE 5: Comparison of mean maternal weight gain (grams) monitored every second day commencing from day two over the gestational period in groups of animals treated with PGE_2 or physiological saline.*

Treatment	Weight gain	Significance of difference
	Day 2**	
Saline	5.50 ± 2.19	
50 µg PGE_2	3.25 ± 1.70	NS
100 µg PGE_2	2.25 ± 1.75	NS
200 µg PGE_2	9.67 ± 3.48	NS
	DAY 4	
Saline	3.62 ± 1.48	
50 µg PGE_2	5.75 ± 2.74	NS
100 µg PGE_2	3.75 ± 3.75	NS
200 µg PGE_2	4.33 ± 1.96	NS
	DAY 6	
Saline	11.50 ± 1.37	
50 µg PGE_2	7.12 ± 0.85	NS
100 µg PGE_2	7.75 ± 4.25	NS
200 µg PGE_2	1.17 ± 4.78	P = 0.05

...continued

TABLE 5 Continued

Treatment	Weight gain	Significance
	DAY 8	
Saline	9.00 ± 0.54	
50 µg PGE_2	5.50 ± 1.72	NS
100 µg PGE_2	8.50 ± 2.00	NS
200 µg PGE_2	4.33 ± 2.74	NS
	DAY 10	
Saline	7.12 ± 1.20	
50 µg PGE_2	10.38 ± 1.60	NS
100 µg PGE_2	12.25 ± 1.75	NS
200 µg PGE_2	14.00 ± 1.53	P = 0.05
	DAY 12	
Saline	9.88 ± 1.20	
50 µg PGE_2	13.50 ± 1.59	NS
100 µg PGE_2	9.50 ± 0.50	NS
200 µg PGE_2	9.00 ± 0.76	NS
	DAY 14	
Saline	7.50 ± 1.57	
50 µg PGE_2	6.88 ± 1.92	NS
100 µg PGE_2	10.75 ± 0.75	NS
200 µg PGE_2	10.17 ± 0.60	NS

...continued

TABLE 5 Continued

Treatment	Weight gain	Significance
	DAY 16	
Saline	14.50 ± 2.72	
50 µg PGE_2	14.50 ± 1.50	NS
100 µg PGE_2	14.25 ± 1.75	NS
200 µg PGE_2	20.33 ± 1.34	NS
	DAY 18	
Saline	26.75 ± 0.97	
50 µg PGE_2	26.12 ± 2.49	NS
100 µg PGE_2	27.25 ± 1.75	NS
200 µg PGE_2	34.83 ± 6.60	NS
	DAY 20	
Saline	22.00 ± 2.48	
50 µg PGE_2	23.62 ± 5.08	NS
100 µg PGE_2	25.25 ± 9.75	NS
200 µg PGE_2	25.33 ± 5.02	NS

*Duncan's New Multiple Range Test

**Mean ± S.E.M.

TABLE 6: Comparison of mean litter size (number of fetuses/ litter) of groups of animals treated with PGE_2 or physiological saline.*

Treatment	Litter size (Mean ± S.E.M.)	Level of signifcance
Saline	11.50 ± 0.62	
50 µg PGE_2	12.25 ± 0.14	NS
100 µg PGE_2	12.00 ± 0.15	NS
200 µg PGE_2	11.25 ± 0.11	NS

*Duncan's New Multiple Range Test

TABLE 7: Comparison of mean numbers of resorptions per litter in groups of animals treated with PGE_2 or physiological saline.*

Treatment	Number of resorptions/litter**	Significance of difference
Saline	0.16 ± 0.16	
50 µg PGE_2	0.00 ± 0.00	NS
100 µg PGE_2	0.00 ± 0.00	NS
200 µg PGE_2	0.50 ± 0.29	NS

*Duncan's New Multiple Range Test

**Mean ± S.E.M.

TABLE 8: Comparison of mean fetal weights (grams) after maternal treatment with PGE_2 or physiological saline.*

Treatment	Fetal weights** (grams)	Significance of difference
Saline	3.12 ± 0.19	
50 µg PGE_2	3.05 ± 0.14	NS
100 µg PGE_2	3.06 ± 0.08	NS
200 µg PGE_2	3.12 ± 0.10	NS

*Duncan's New Multiple Range Test.

**Mean ± S.E.M.

TABLE 9: Comparison of mean placental weights (grams) after maternal treatment with PGE_2 or physiological saline.*

Treatment	Placental weights** (grams)	Significance of differnce
Saline	0.433 ± 0.007	
50 µg PGE_2	0.442 ± 0.025	NS
100 µg PGE_2	0.466 ± 0.020	NS
200 µg PGE_2	0.453 ± 0.024	NS

*Duncan's New Multiple Range Test

**Mean ± S.E.M.

3.2 Short Term Assessment

A statistical comparison of the total number of implantation sites recovered from saline- and PGE_2-treated rats revealed that PGE_2 had no effect on the mean number of implantations occurring (Table 10). Resorptions at this stage of development cannot be detected.

3.2.1 Histological Evaluation of Day 9 Embryos

All control embryos displayed a normal morphological appearance. However, a considerable range in the rate of embryonic development was found to exist, both between and within litters (Table 11).

Sixteen percent of embryos were found to be at the primitive egg cylinder stage of development (Table 11, Figs. 21 and 22). At this stage the embryos consisted of a double wall: ectoderm on the inside and endoderm on the outside enclosing a narrow lumen, the proamniotic cavity. The third germ layer (mesoderm) has not yet made its appearance.

Thirty-five percent of the embryos revealed the posterior, lateral, and anterior amniotic folds in a completely unfused state (Figs. 23 and 24). In 25% of embryos, the amniotic folds have begun to fuse to varying degrees, but the fusion is not yet completed (Figs. 25 and 26).

Complete fusion of the amniotic folds occurred in 24% of control embryos (Fig. 27). Three cavities are now present in the egg cylinder instead of the single proamniotic cavity. Only 11% of these embryos show a prominent allantois (Fig. 28).

Of 87 experimental embryos examined microscopically, all showed a normal morphological appearance. Subcutaneous injections of 50, 100, and 200 µg of PGE_2 on days five through eight had no adverse effects on embryonic development. However, as in the controls, a considerable range in the rates of embryonic development was seen to exist (Tables 12 through 14, Figs. 29 through 43).

In those animals treated with 50 µg of PGE_2, complete fusion of amniotic folds was observed in 37% of the embryos examined. However, a prominent allantois was present in only 20%. Incomplete fusion of amniotic folds was seen in 31% of embryos, while in 32% the folds had not started to fuse, although they were present.

Only one embryo lacked mesoderm in the group treated with 100 µg PGE_2. Twenty-two percent had amniotic folds in which fusion had not yet begun. In 33%, fusion had started. Forty-one percent of embryos displayed complete fusion of the amniotic folds, of these, 22% revealed a prominent allantois.

In all embryos recovered from mothers treated with 200 µg PGE_2, the third germ layer, mesoderm, had made its appearance. Complete fusion of amniotic folds was evident in 60% of these embryos, 36% of them showing an allantois. In 24%, the amniotic folds, although present, were incompletely fused, while in another 24% fusion had not yet started.

3.2.2 Influence of PGE_2 on Ovarian Morphology (new corpora lutea)

Examination of ovaries recovered from saline-treated controls on day nine of gestation revealed normal histological features. The cells of these newly formed corpora lutea were smaller and slightly basophilic in contrast to the larger eosinophilic cells of older corpora lutea (Fig. 44). This is because the granulosa cells have not yet fully hypertrophied. Often, a central cavity filled with fibrin (remnants of the follicular antrum) were still present. Similar morphological characteristics were observed in the ovaries of the PGE_2-treated animals. No signs of luteolysis were observed.

TABLE 10: Effect of PGE_2 on implantation*

Treatment	Number of implantations (Mean ± S.E.M.)	Significance of difference
Saline	12.75 ± 1.32	
50 µg PGE_2	14.50 ± 1.20	NS
100 µg PGE_2	12.25 ± .48	NS
200 µg PGE_2	14.00 ± .71	NS

*Duncan's New Multiple Range Test

Table 11. Range of embryonic stages in saline-treated Sprague-Dawley rats at nine days (± 3 hr.) gestation, based on 55 embryos sectioned serially.*

	Amniotic folds			
No mesoderm	Completely unfused	Fused incompletely	Completely fused	
			No allantois	Allantois present
7				
			4	1 2
1	6	1		
	2	2	1	2
	1	2		
9				
	1	5	2	
1			3	2

*Classification is based on the presence or absence of mesoderm, the state of fusion of the amniotic folds, and the presence or absence of a prominent allantois. All embryos within a box originate from the same litter.

Table 12. Range of embryonic stages at nine days (± 3 hr.) gestation, in Sprague-Dawley rats treated with 10 µg of PGE_2 based on 35 embryos sectioned serially.*

	Amniotic folds			
No mesoderm	Completely unfused	Fused incompletely	Completely fused	
			No allantois	Allantois present
	2	6		
	2	3	2	
	6	1		
	1	1	4	7

*Classification is based on the presence or absence of mesoderm, the state of fusion of the amniotic folds, and the presence or absence of a prominent allantois.
All embryos within a box originate from the same litter.

Table 13. Range of embryonic stages at nine days (± 3 hr.) gestation in Sprague-Dawley rats treated with 100 µg PGE_2 based on 27 embryos sectioned serially.*

	Amniotic folds			
No mesoderm	Completely unfused	Fused incompletely	Completely fused	
			No allantois	Allantois present
		4		4
1	3	2		1
	3	1	2	
		2	3	1

*Classification is based on the presence or absence of mesoderm, the state of fusion of the amniotic folds, and the presence or absence of a prominent allantois.
All embryos lying within a box originate from the same litter.

Table 14. Range of embryonic stages at nine days gestation (± 3 hr.) in Sprague-Dawley rats treated with 200 µg of PGE_2 based on 25 embryos sectioned serially.*

	Amniotic folds			
No mesoderm	Completely unfused	Fused incompletely	Completely fused	
			No allantois	Allantois present
	2	2		3
		4		2
			2	3
	4		2	1

*Classification is based on the presence or absence of mesoderm, the state of fusion of the amniotic folds, and the presence or absence of a prominent allantois.
All embryos within a box originate from the same litter.

FIGURE 20.

Implantation site of nine days gestation recovered from an animal treated with physiological saline. The fully implanted blastocyst (Bl) is clasped tightly by the uterus (U) and as a result of decidualization is isolated in its own implantation chamber. The ectoplacental cone (Ecp) is oriented towards the mesometrial pole of the uterus.

X70

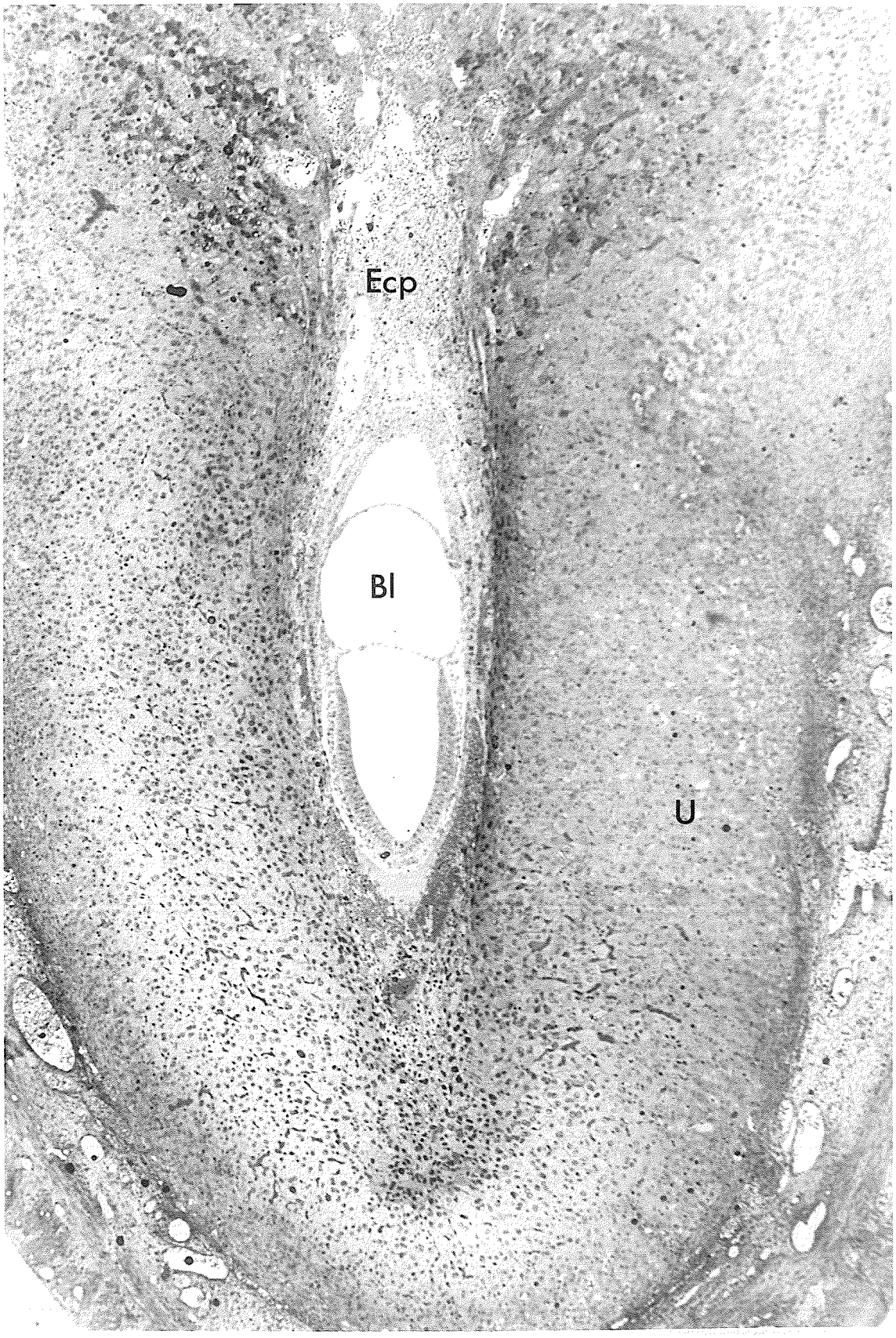

Ecp
Bl
U

FIGURE 21.

Embryo of nine days gestation recovered from an animal treated with physiological saline. At this stage it consists of a double wall composed of ectoderm and endoderm enclosing a narrow lumen, the proamniotic cavity (Pc). Embryonic ectoderm (ee), extraembryonic ectoderm (eee), visceral endoderm (Ve). X280

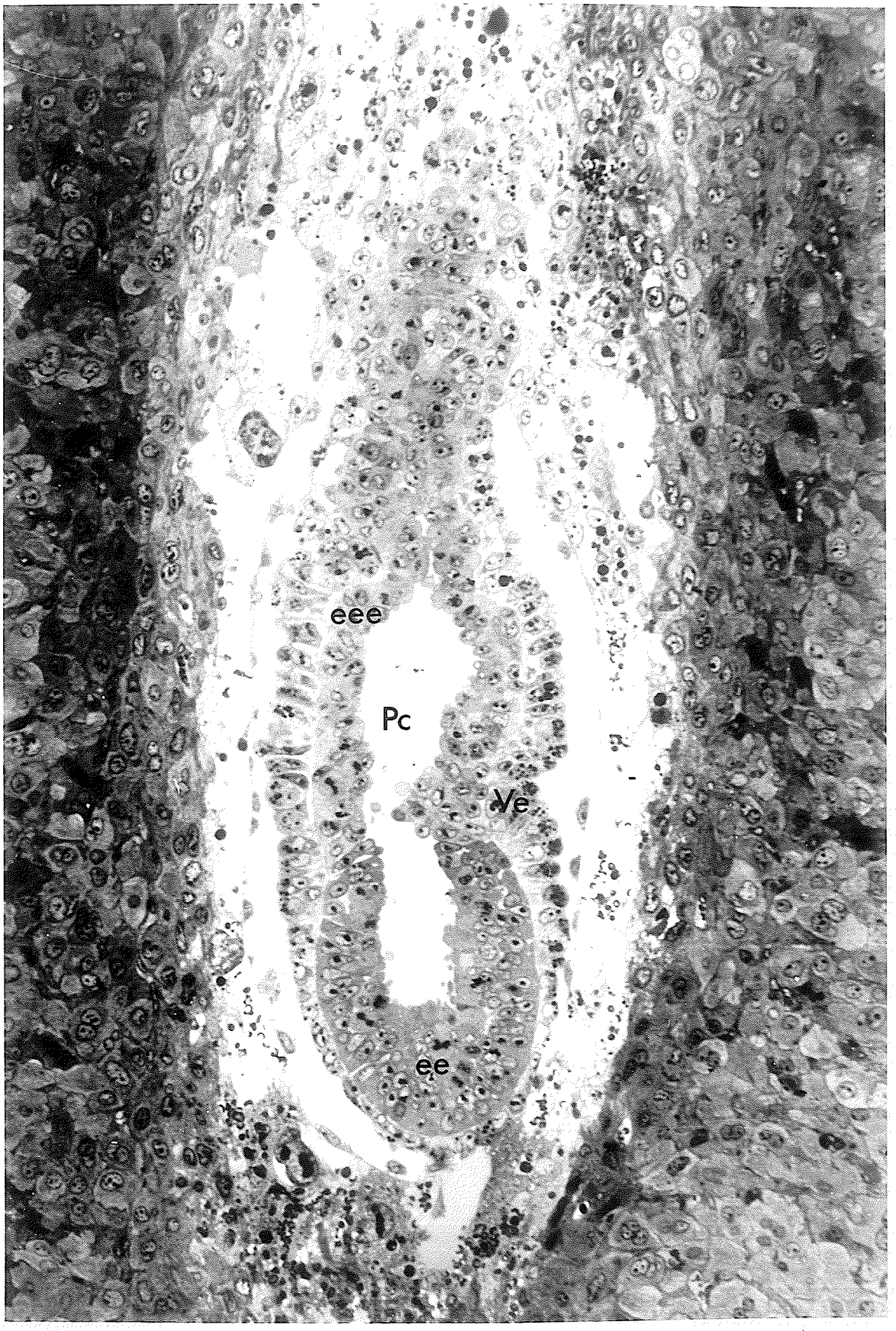

eee
Pc
Ve
ee

FIGURE 22.

Embryo of nine days gestation recovered from an animal treated with physiological saline. A slightly more advanced developmental stage is shown than in the previous figure. Embryonic ectoderm (ee), extraembryonic ectoderm (eee), proamniotic cavity (Pc). X280

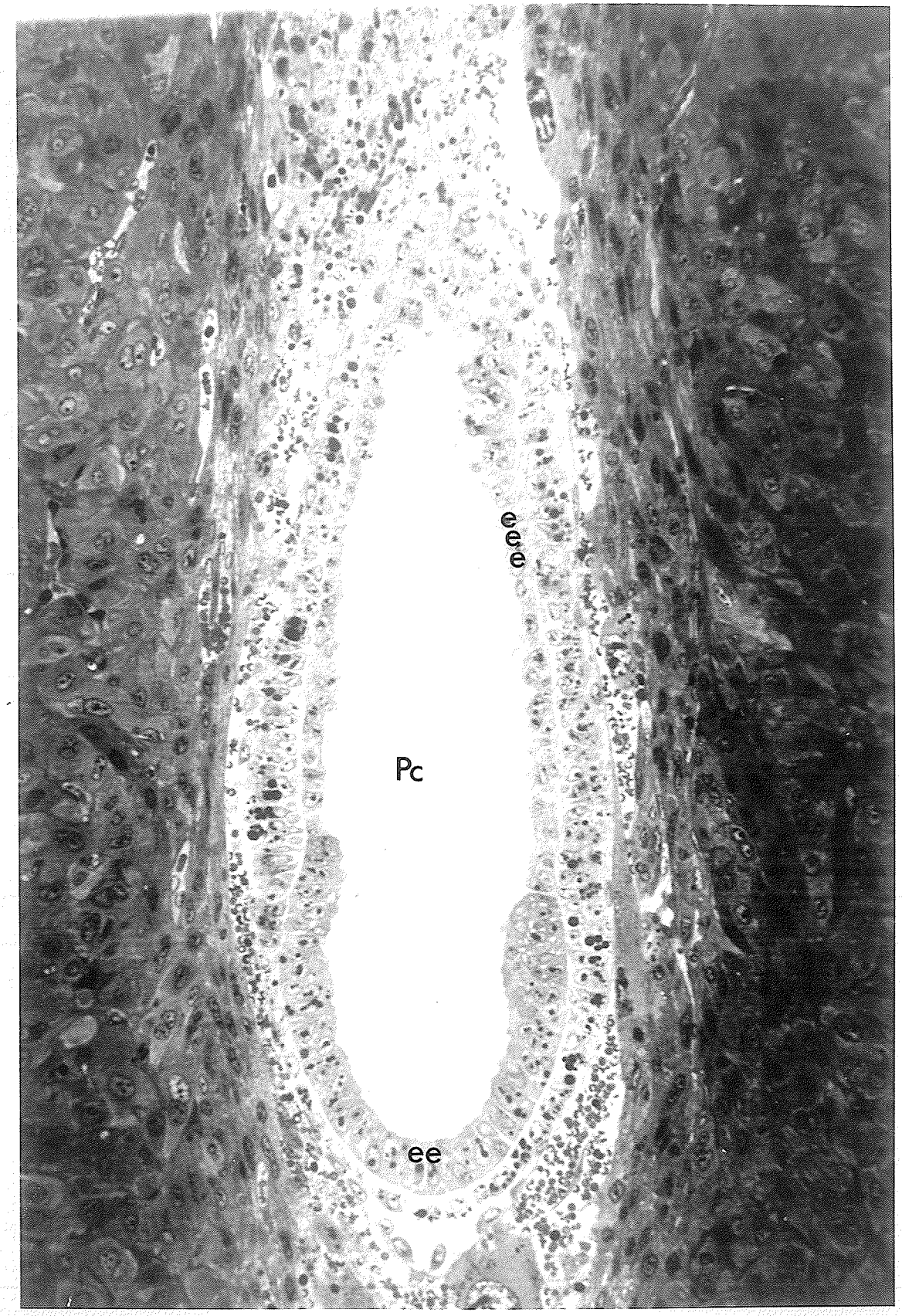

e
e
e
Pc
ee

FIGURE 23.

Embryo of nine days gestation recovered from an animal treated with physiological saline. The amniotic folds (asterisks) creating a contiguous constriction about the middle of the egg cylinder have begun to divide the proamniotic cavity (Pc). Small cavities in the folds are the first signs of the developing exocoelomic cavity; Parietal endoderm (arrowheads), Reichert's membrane (Rm), yolk sac cavity (Ys). X280

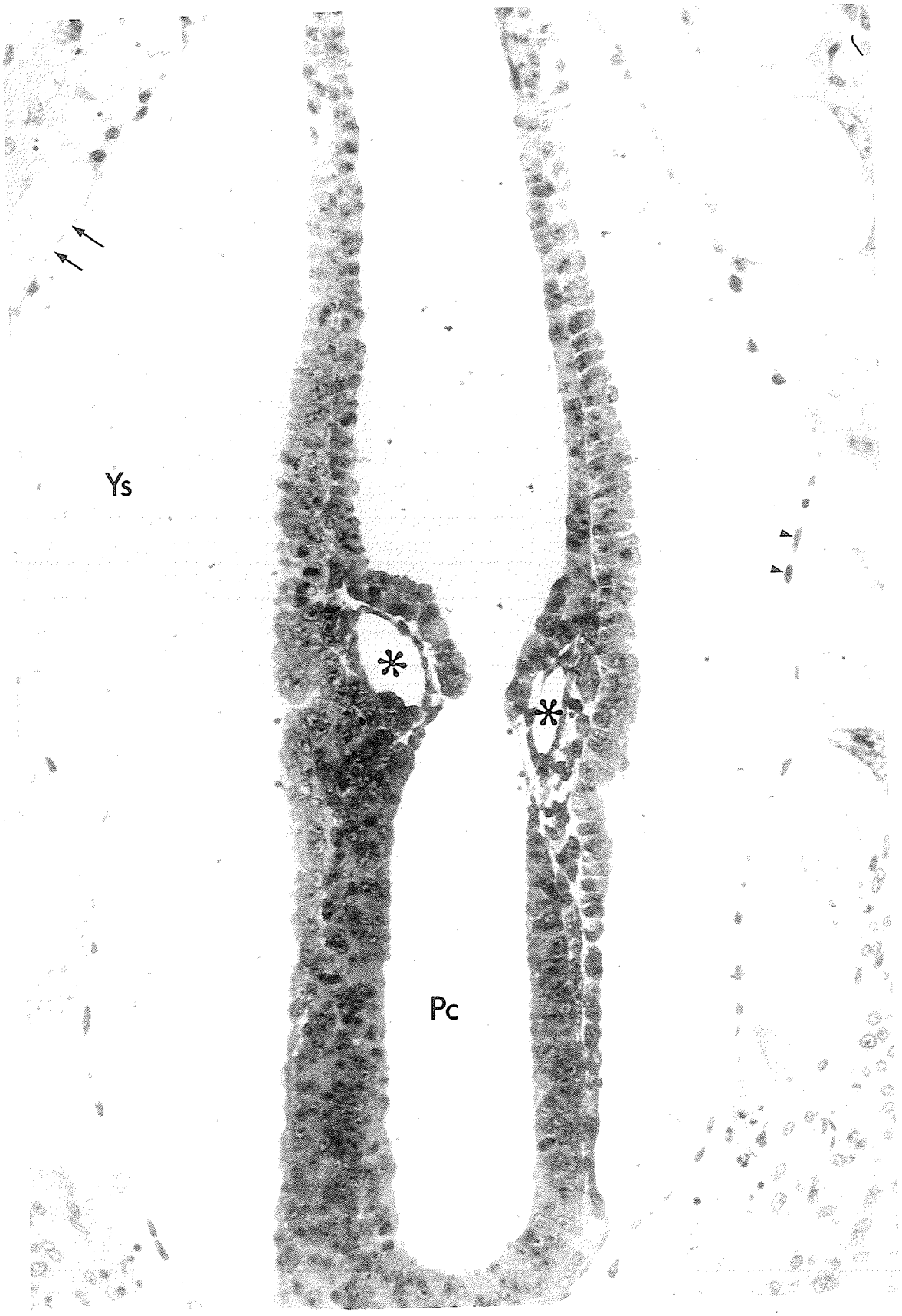

Ys
Pc

FIGURE 24.

Embryo of nine days gestation recovered from an animal treated with physiological saline. The constriction dividing the proamniotic cavity (Pc) is drawn tighter as the amniotic folds (Af) develop.

X280

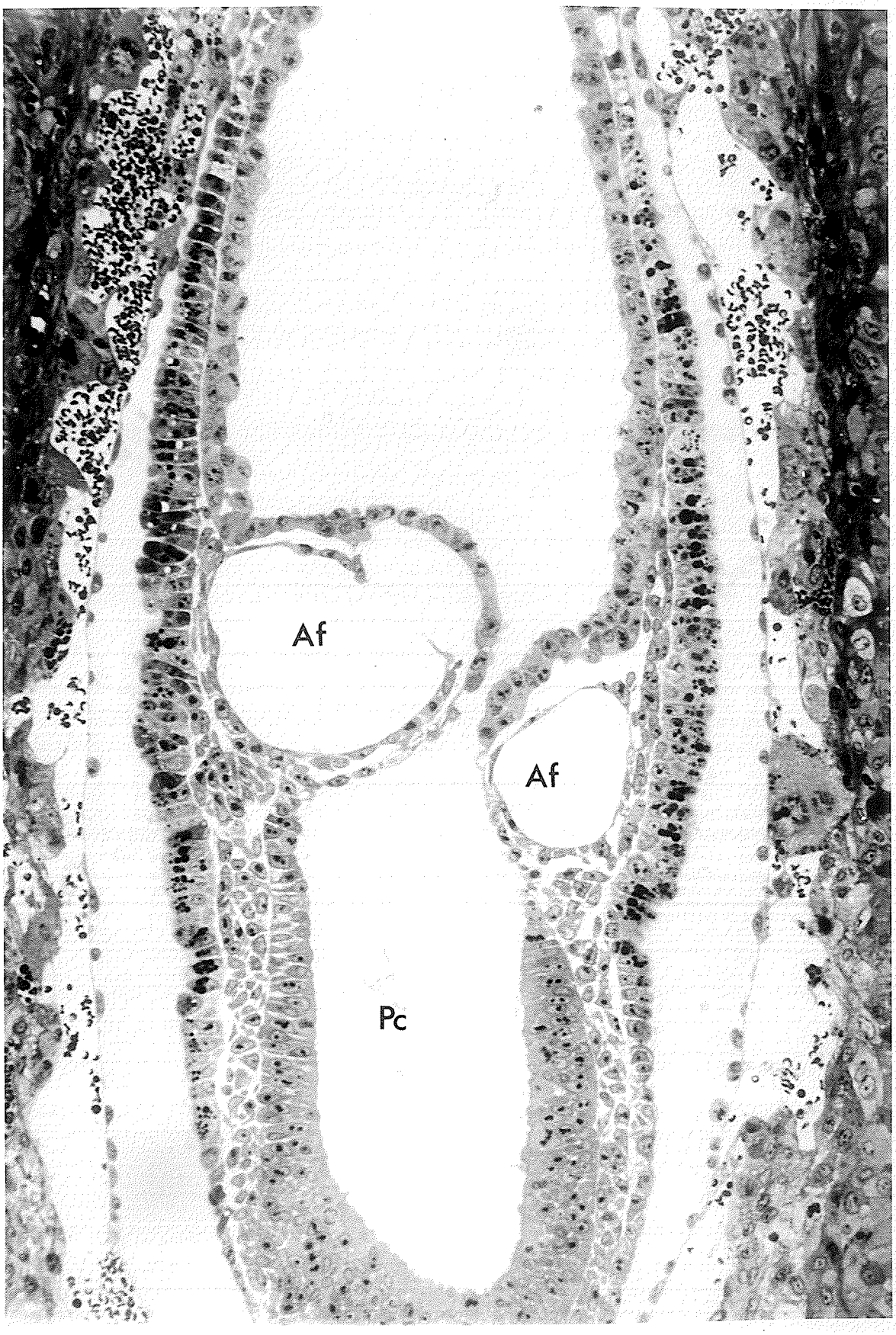

Af
Af
Pc

FIGURE 25.

Embryo of nine days gestation recovered from an animal treated with physiological saline. Fusion of amniotic folds (Af) is evident. Proamniotic cavity (Pc), yolk sac cavity (Ys). X280

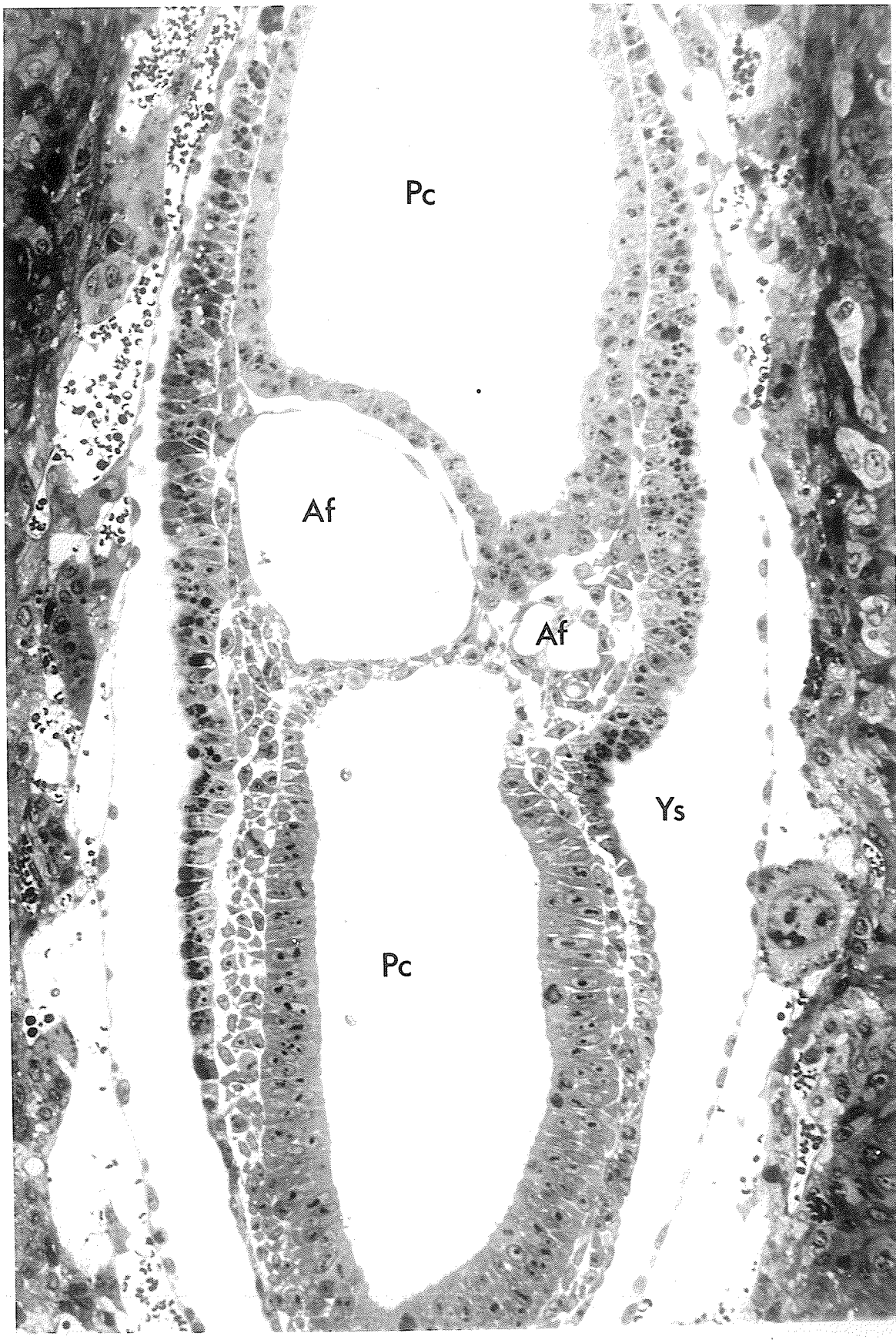

Pc
Af
Af
Ys
Pc

FIGURE 26.

Embryo of nine days gestation recovered from an animal treated with physiological saline showing almost complete fusion of amniotic folds.

X280

FIGURE 27.

Embryo of nine days gestation recovered from an animal treated with physiological saline. Complete fusion of the amniotic folds has resulted in the division of the single proamniotic cavity into three cavities separated by two membranes. Amniotic cavity (Ac), ectoplacental cavity (Ecpc), exocoelomic cavity (Exc), amnion (A), chorion (C).

X280

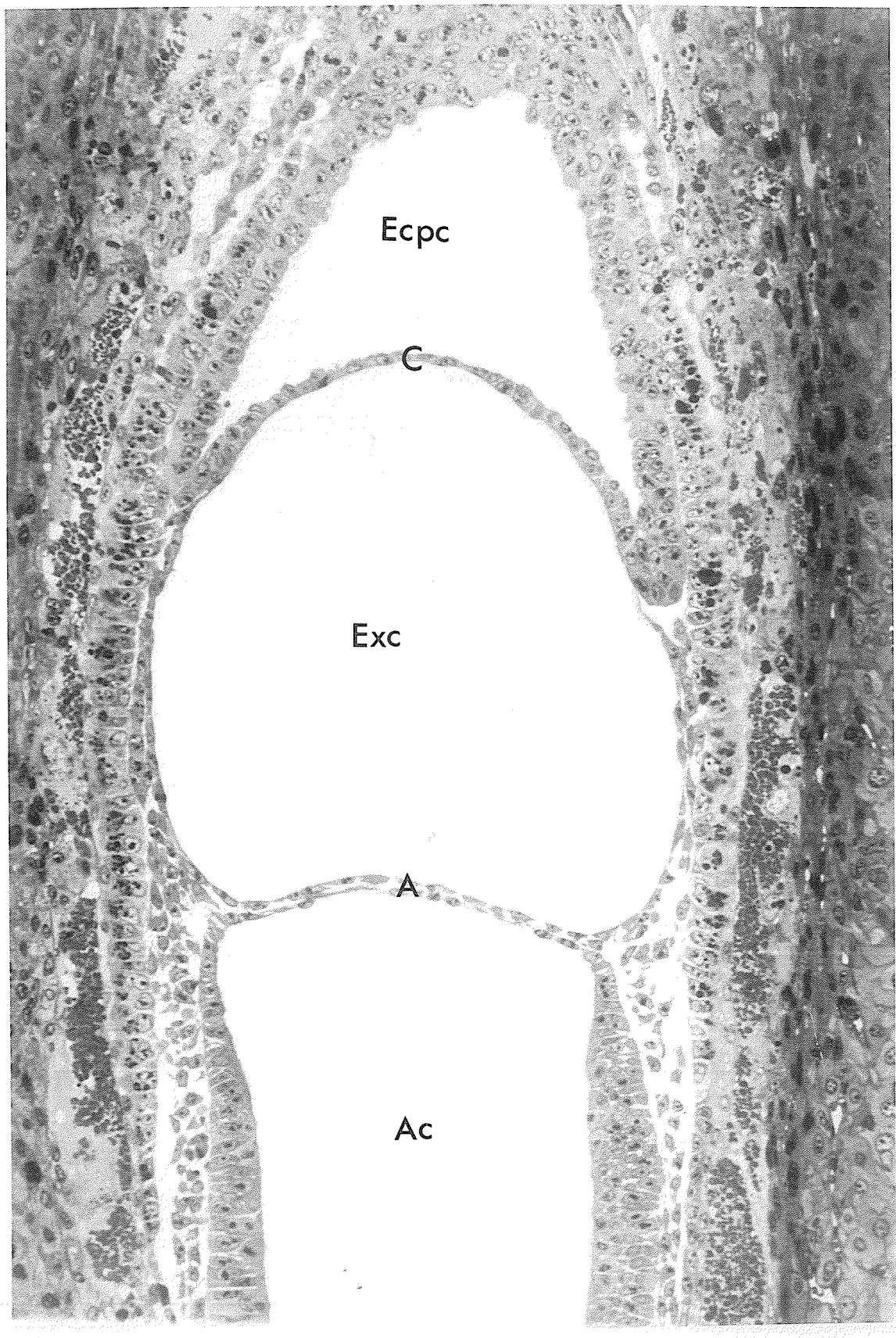

Ecpc
C
Exc
A
Ac

FIGURE 28.

Embryo of nine days gestation recovered from an animal treated with physiological saline. After the exocoelomic cavity (Exc) becomes well established an allantois (Al) begins to grow into it from the caudal end of the primitive streak. Amniotic cavity (Ac), ectoplacental cavity (Ecpc).

X280

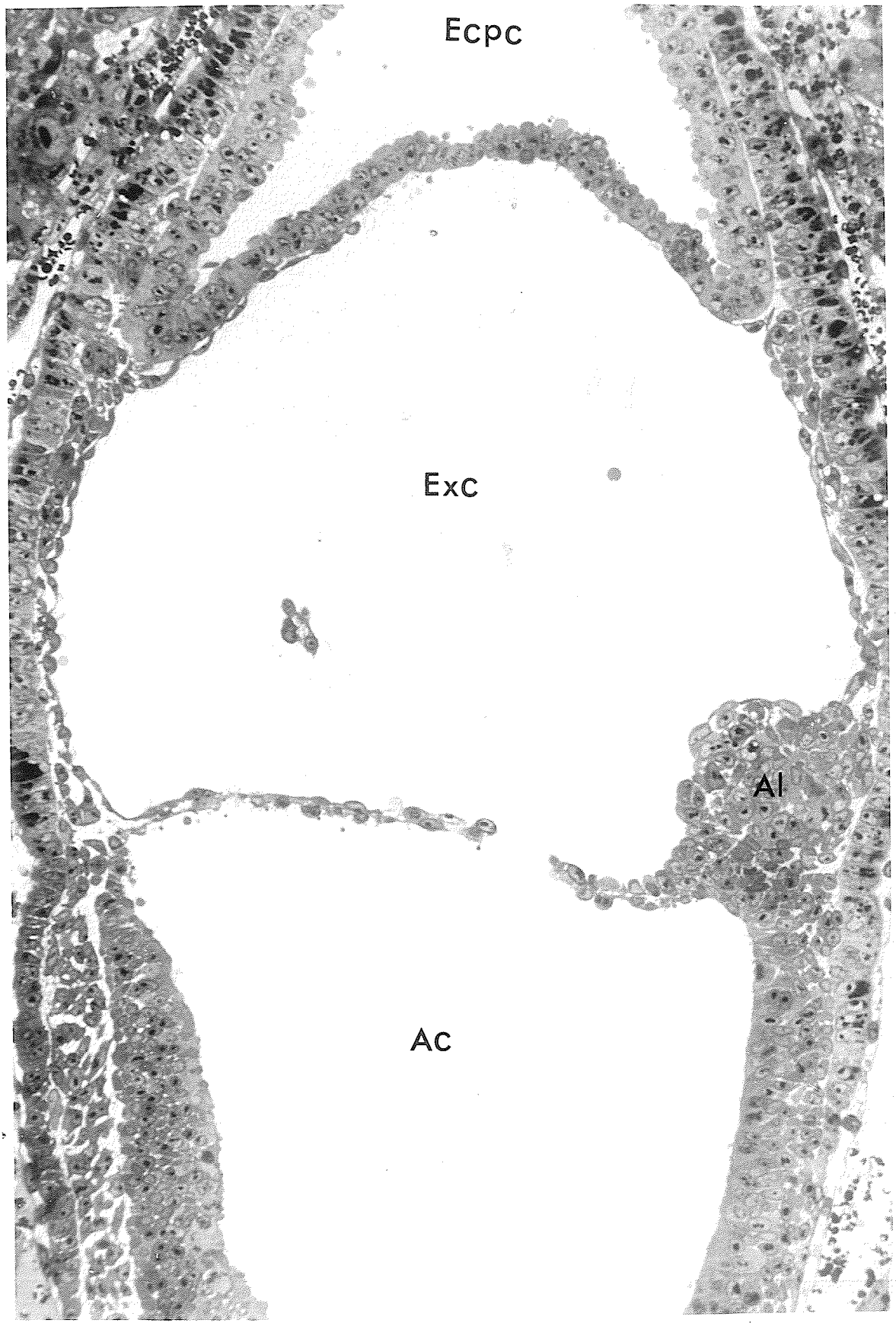

Ecpc
Exc
Al
Ac

FIGURE 29.

Embryo of nine days gestation recovered from an animal treated with 50 µg of PGE_2 on days 5, 6, 7, and 8 p.c. Amniotic folds (Af) are present but no signs of the exocoelomic cavity are evident. The flattened nuclei of the implantation zone (Iz) and the surrounding primary decidual zone (Pdz) are apparent. X280

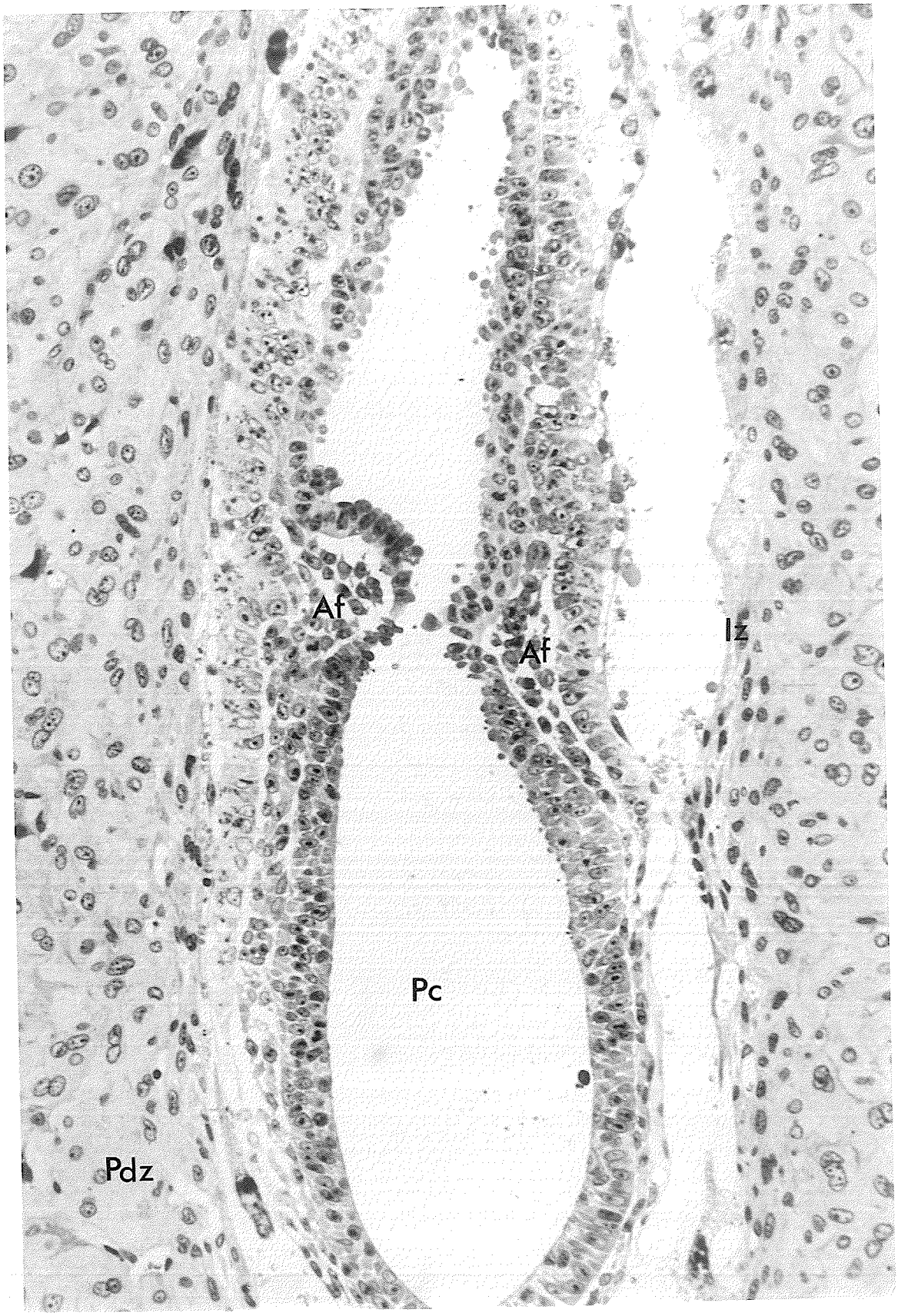

Af
Af
Iz
Pc
Pdz

FIGURE 30.

Embryo of nine days gestation recovered from an animal treated with 50 µg PGE_2. Typical morphological features of this stage of development are exhibited. Amniotic folds (Af), embryonic ectoderm (ee), extraembryonic ectoderm (eee), proamniotic cavity (Pc), Reichert's membrane (Rm), yolk sac cavity (Ys). X280

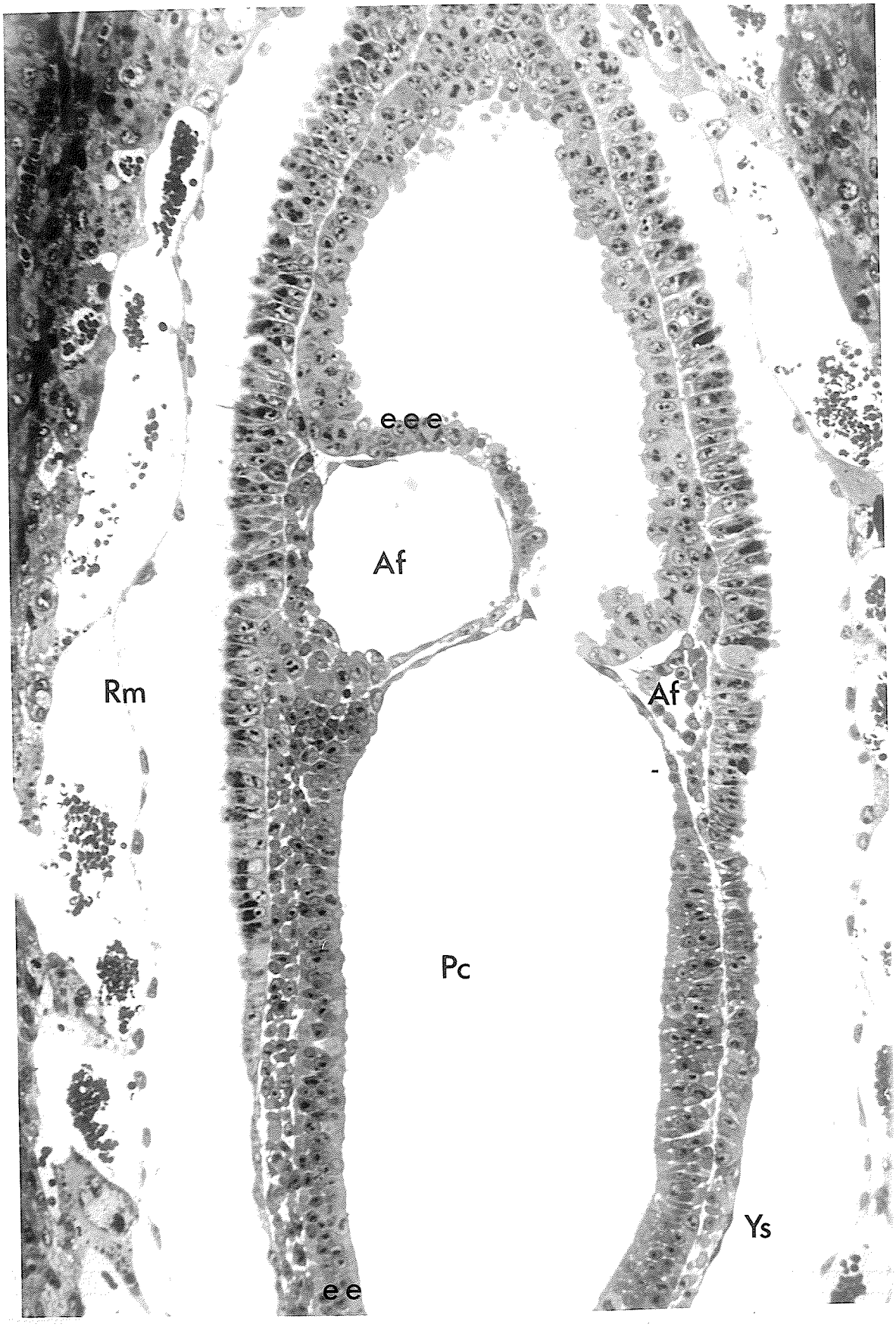

e e e
Af
Rm
Af
Pc
Ys
e e

FIGURE 31.

Embryo of nine days gestation recovered from an animal treated with 50 µg of PGE_2. Amniotic folds (Af) have almost completely fused. Growth and development appears normal. X280

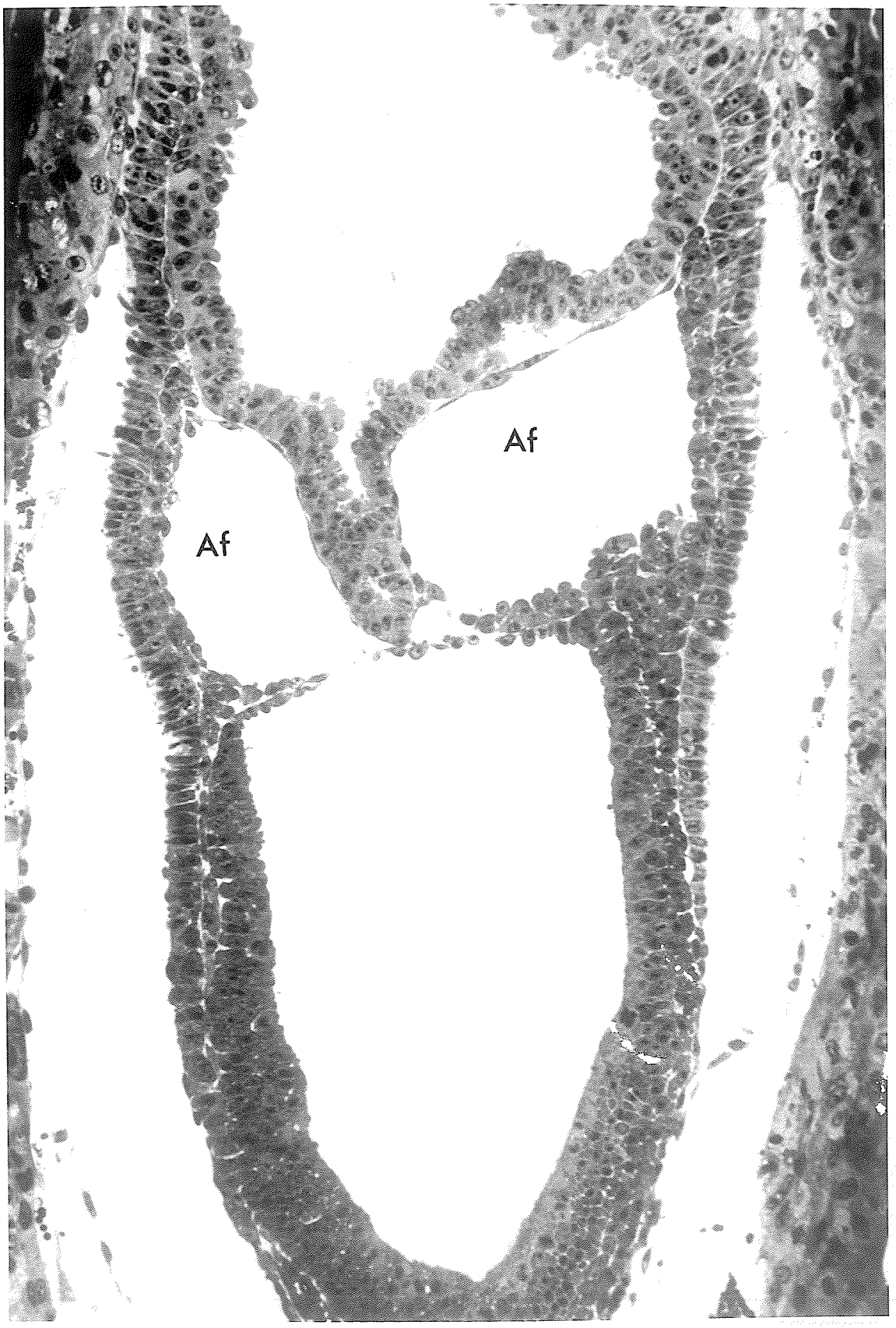

Af
Af

FIGURE 32.

Embryo of nine days gestation recovered from an animal treated with 50 µg of PGE_2. Complete fusion of amniotic folds has resulted in the division of the proamniotic cavity. Development appears normal. Amnion (A), amniotic cavity (Ac), chorion (C), ectoplacental cavity (Ecpc), exocoelomic cavity (Exc). X280

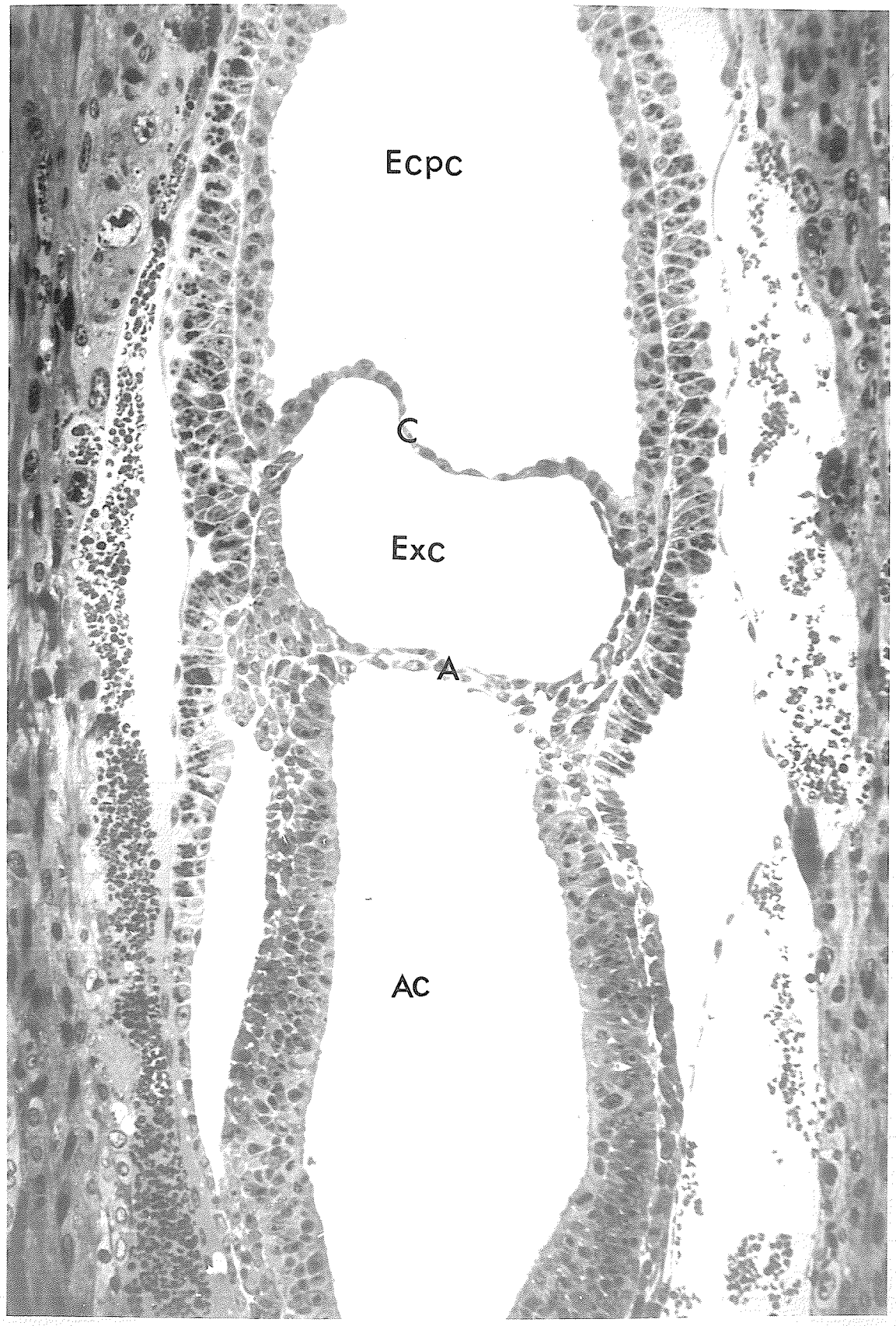

Ecpc
C
Exc
A
AC

FIGURE 33.

Embryo of nine days gestation recovered from an animal treated with 50 μg of PGE_2. The embryo shows no adverse effects in response to treatment. Typical morphology is exhibited. Note: the exocoelomic cavity (Exc) is well established and the allantois (Al) has begun to grow into it. X280

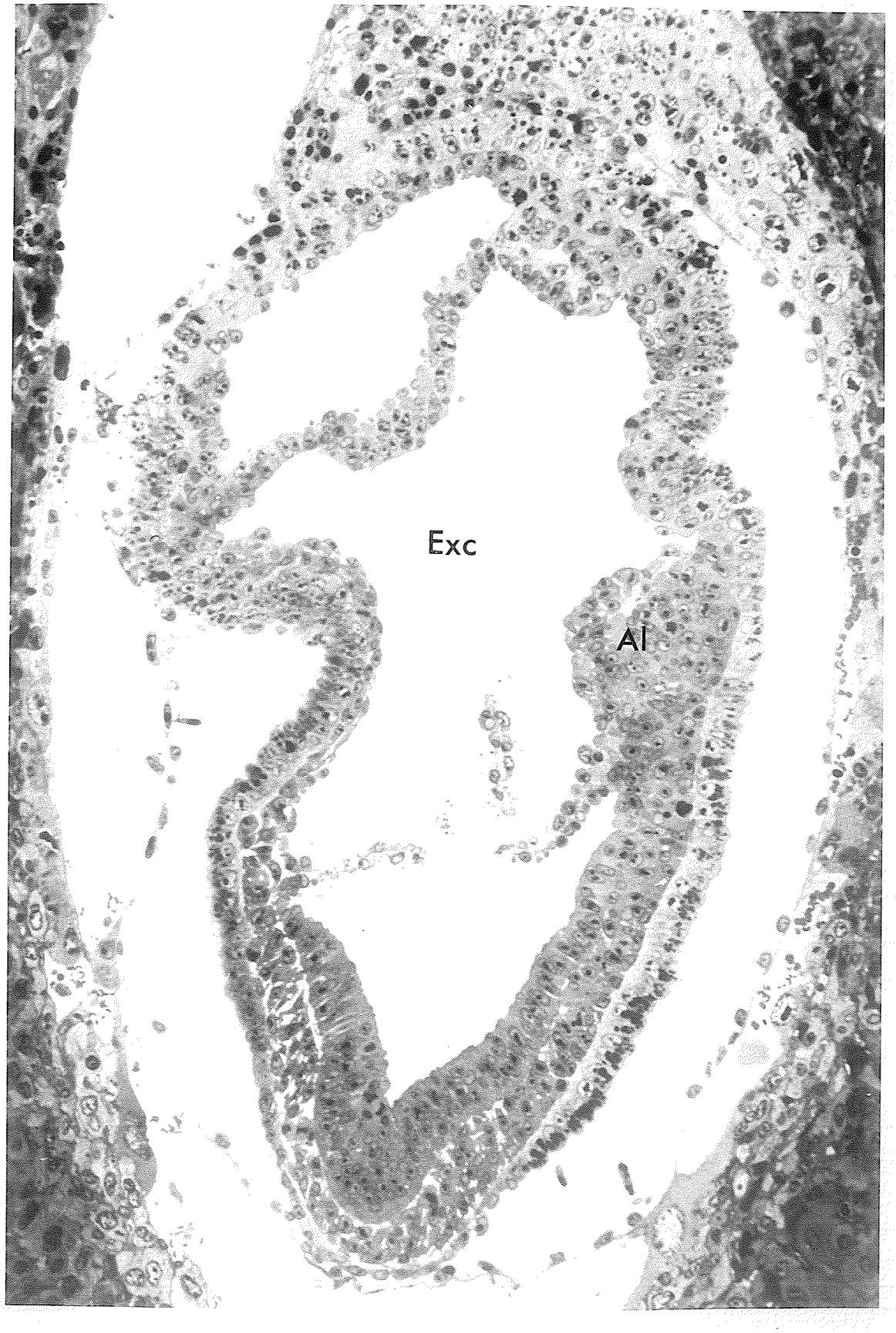

Exc
Al

FIGURE 34.

Embryo of nine days gestation recovered from an animal treated with 100 µg of PGE_2. An early stage in embryonic development is shown. The third germ layer (mesoderm) has not made its appearance. X280

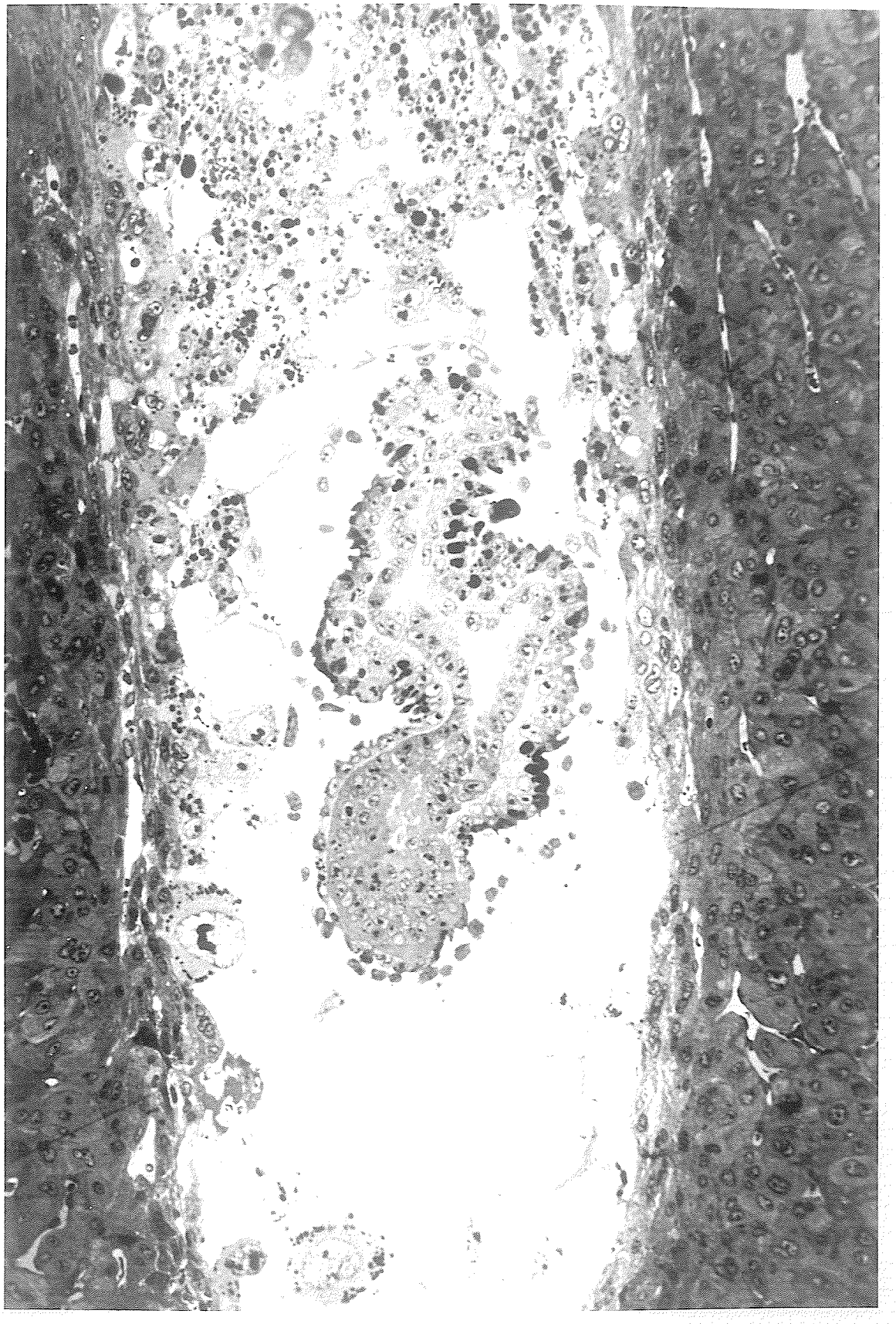


FIGURE 35.

Embryo of nine days gestation recovered from an animal treated with 100 μg of PGE_2. Amniotic folds (Af) have begun to divide the proamniotic cavity (Pc). No adverse response to treatment can be detected. X280

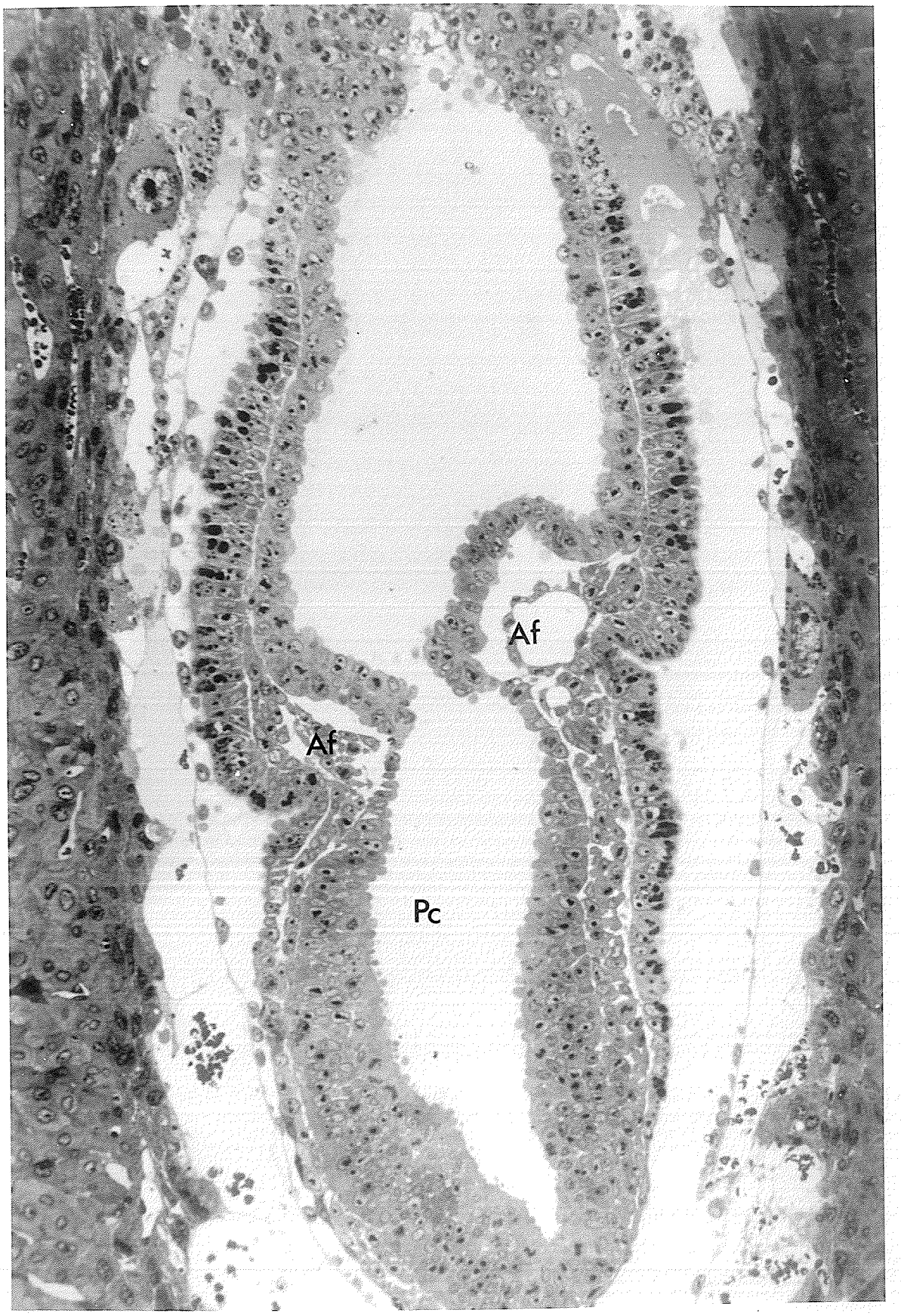

Af
Af
Pc

FIGURE 36.

Embryo of nine days gestation recovered from an animal treated with 100 μg of PGE_2. Normal morphology is displayed. Note: fusing amniotic folds (Af). X280

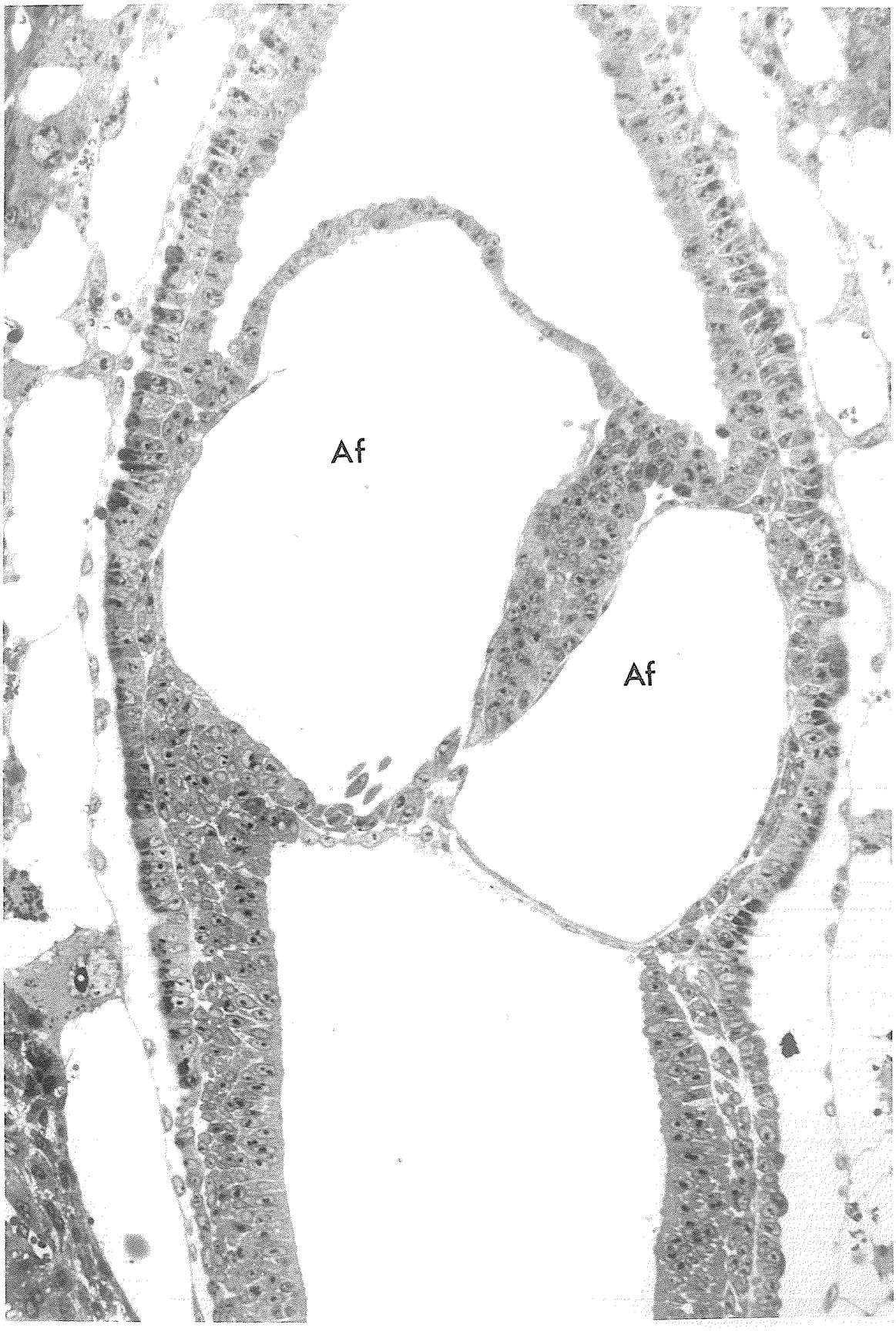

Af
Af

FIGURE 37.

A more peripheral section of the previous embryo. Note that in this plane complete fusion of amniotic folds has occurred.

X280

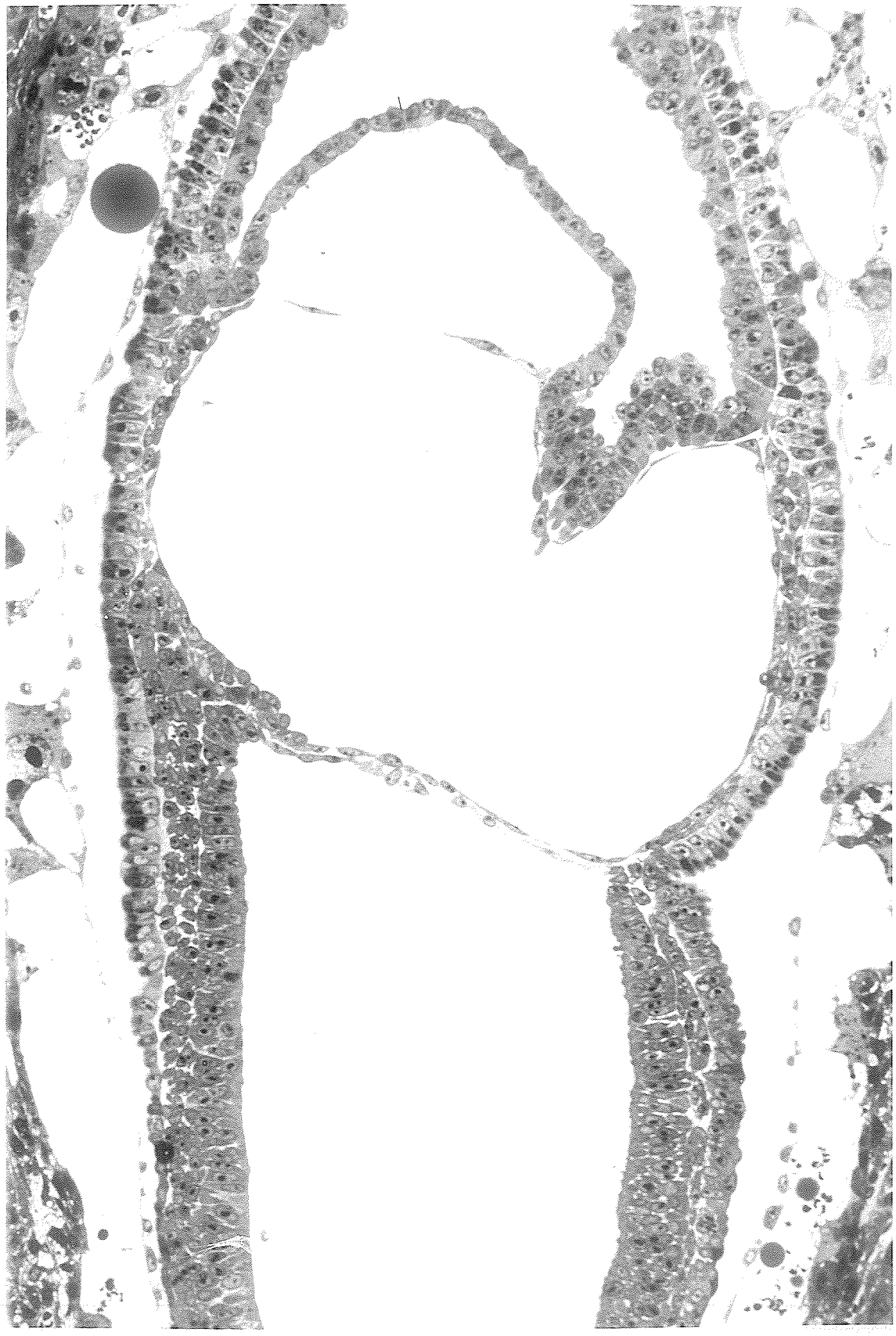


FIGURE 38.

Embryo recovered from an animal treated with 100 µg of PGE_2. Typical morphological features of this stage of development are shown. Amnion (A), amniotic cavity (Ac), chorion (C), ectoplacental cavity (Ecpc), exocoelomic cavity (Exc). X280

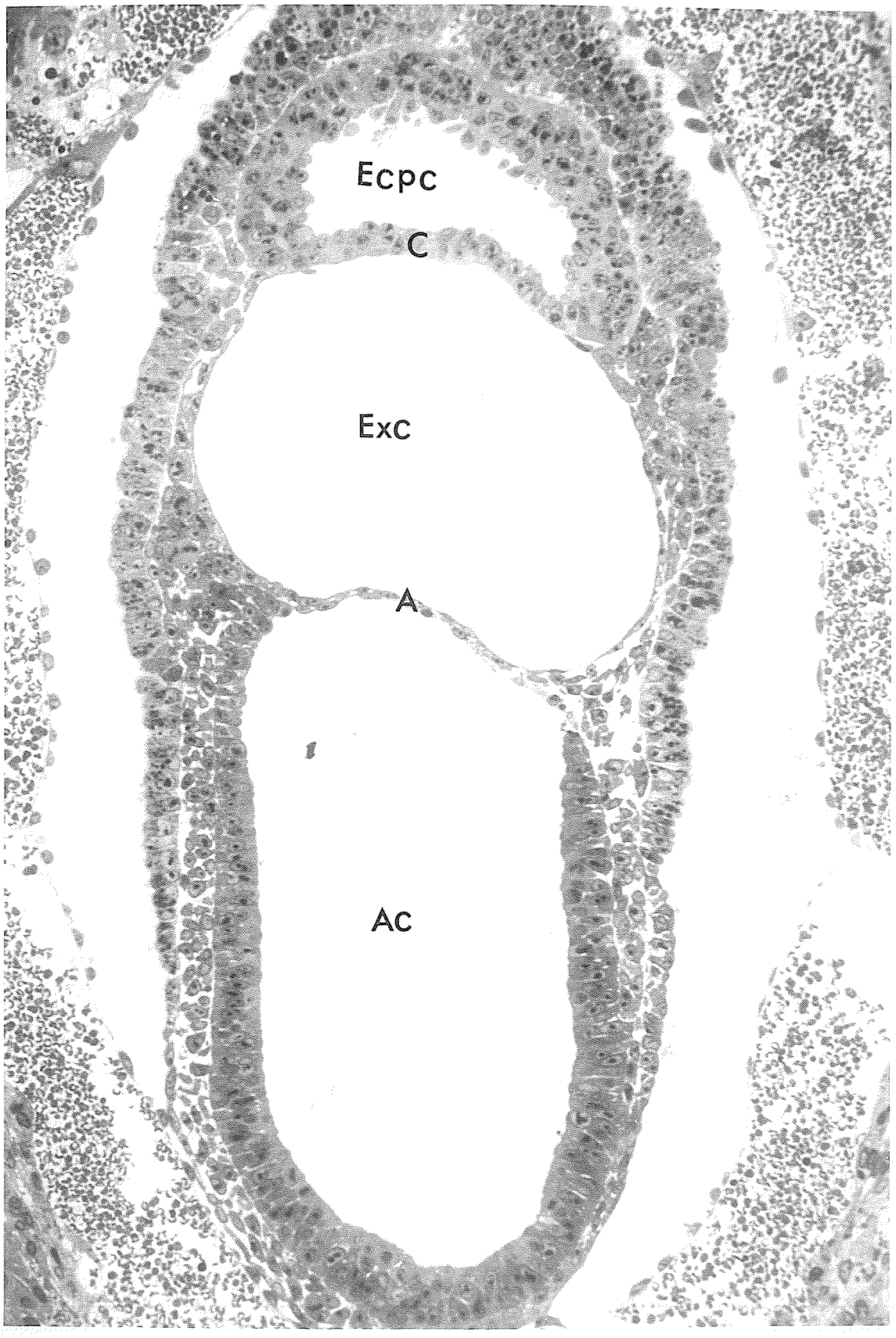

Ecpc
C
Exc
A
Ac

FIGURE 39.

Embryo of nine days gestation recovered from an animal treated with 100 µg of PGE_2. A slightly later stage than that of the previous figure. Allantois (Al), exocoelomic cavity (Exc). X280

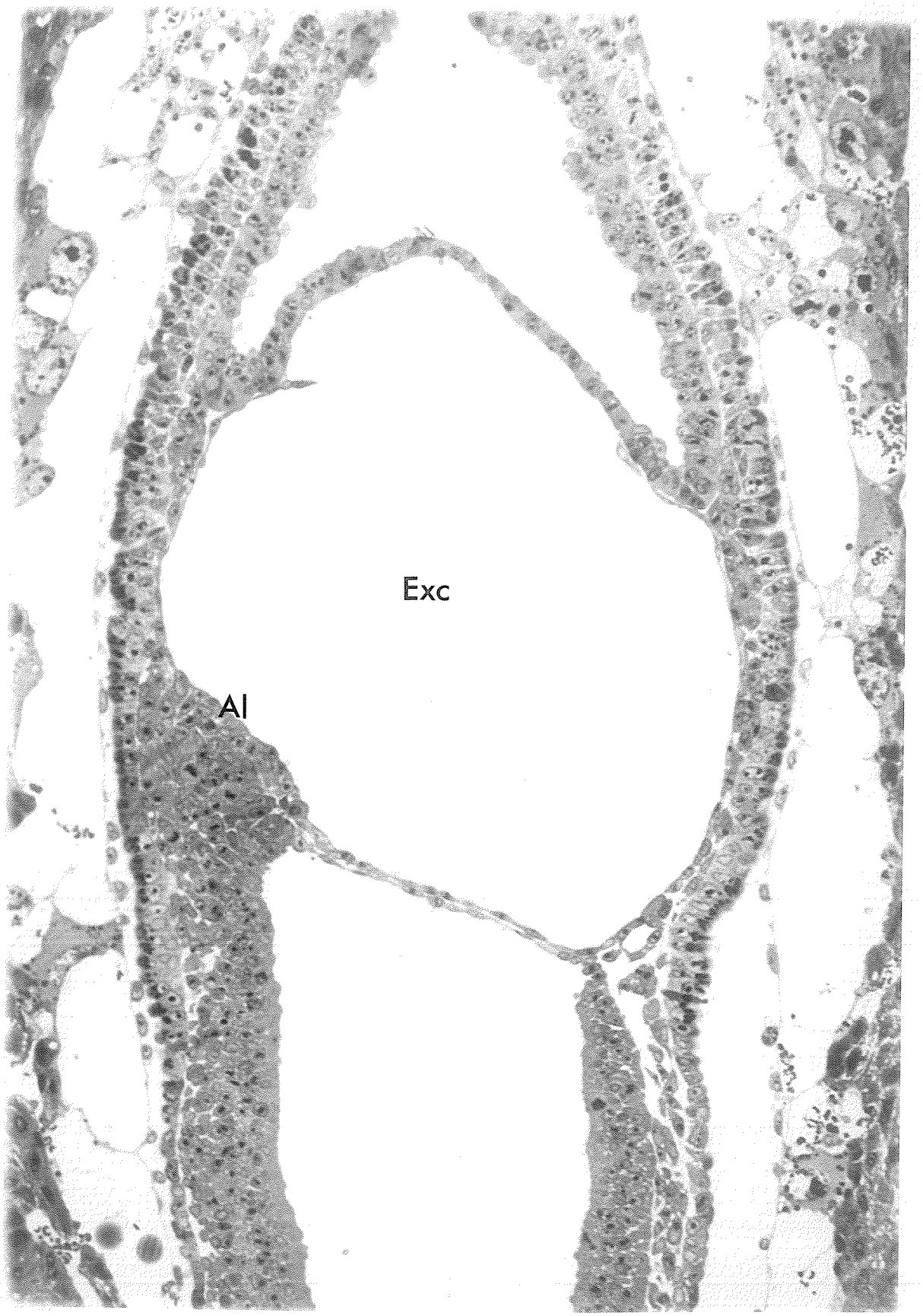

Exc
Al

FIGURE 40.

Embryo of nine days gestation recovered from an animal treated with 200 µg of PGE_2. Characteristic features at this stage of development are shown. Amniotic folds (Af), proamniotic cavity (Pc). X280

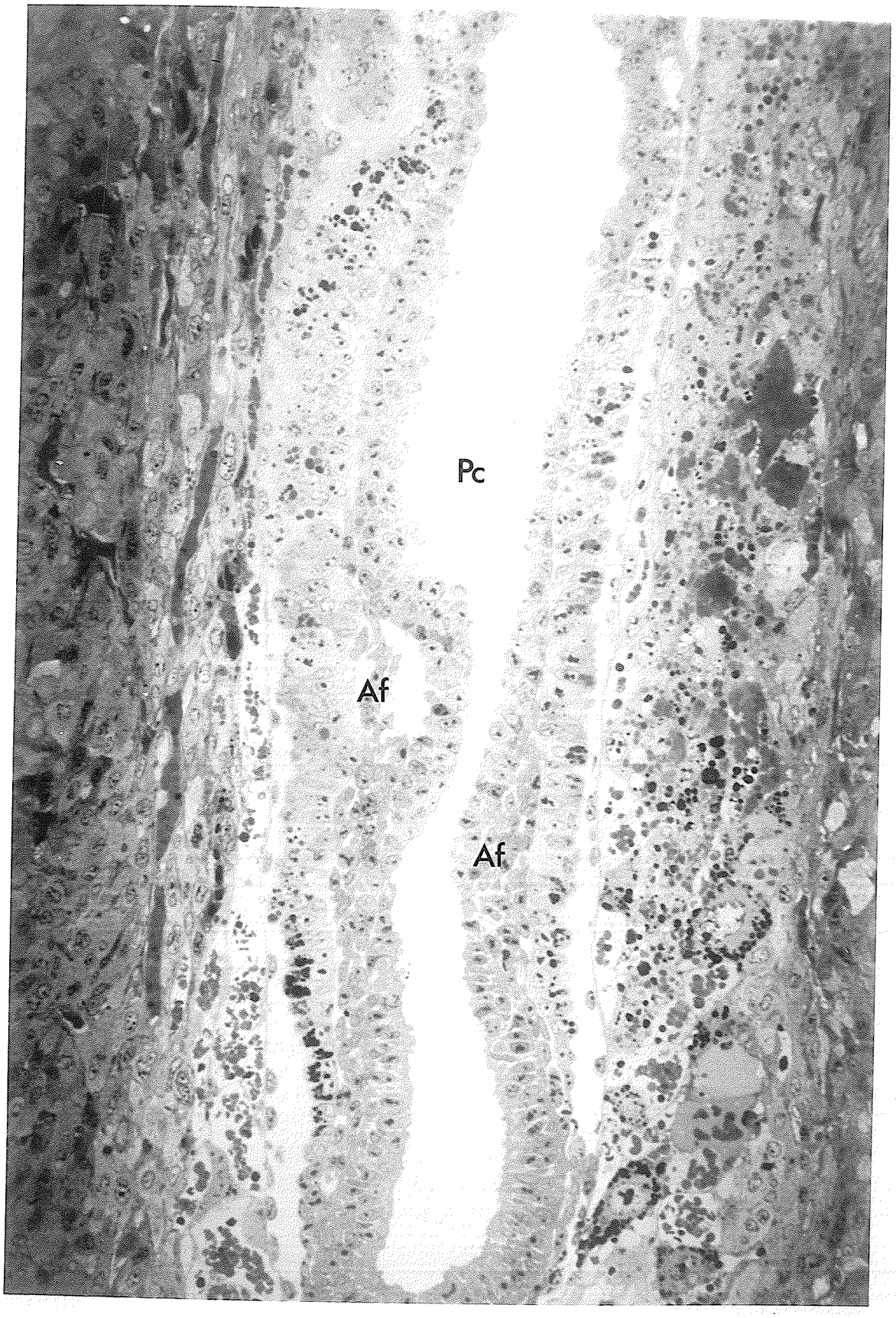

Pc
Af
Af

FIGURE 41.

Embryo of nine days gestation recovered from an animal treated with 200 µg of PGE_2. The embryo appears morphologically normal. Amniotic folds (Af), proamniotic cavity (Pc). X280

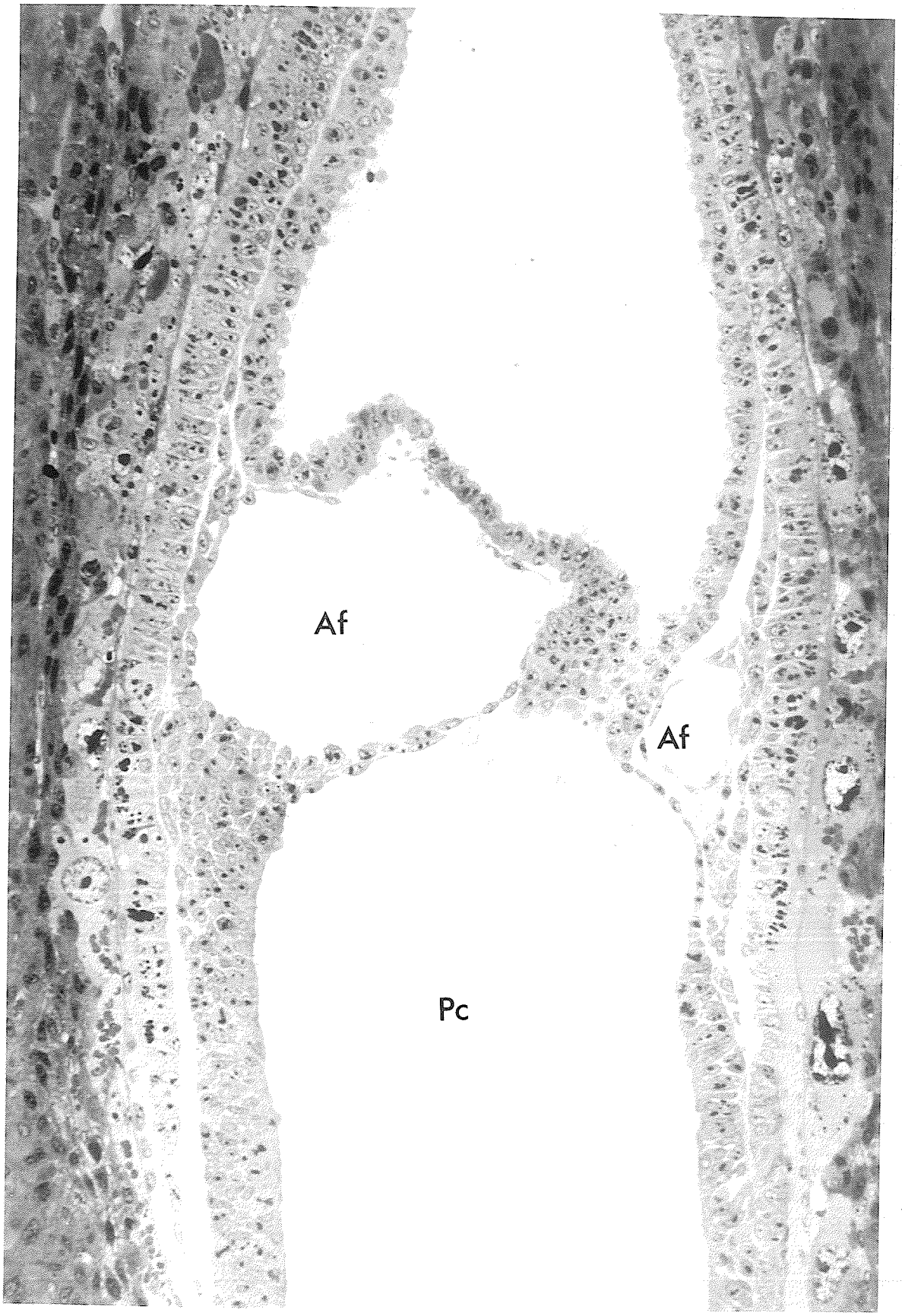

Af
Af
Pc

FIGURE 42.

More peripheral section of the previous embryo Note: It is representative of the next stage of development where complete fusion of the amniotic folds (Af) has occurred. Amnion (A), amniotic cavity (Ac), chorion (C), ecto-placental cavity (Ecpc), exocoelomic cavity (Exc). X280

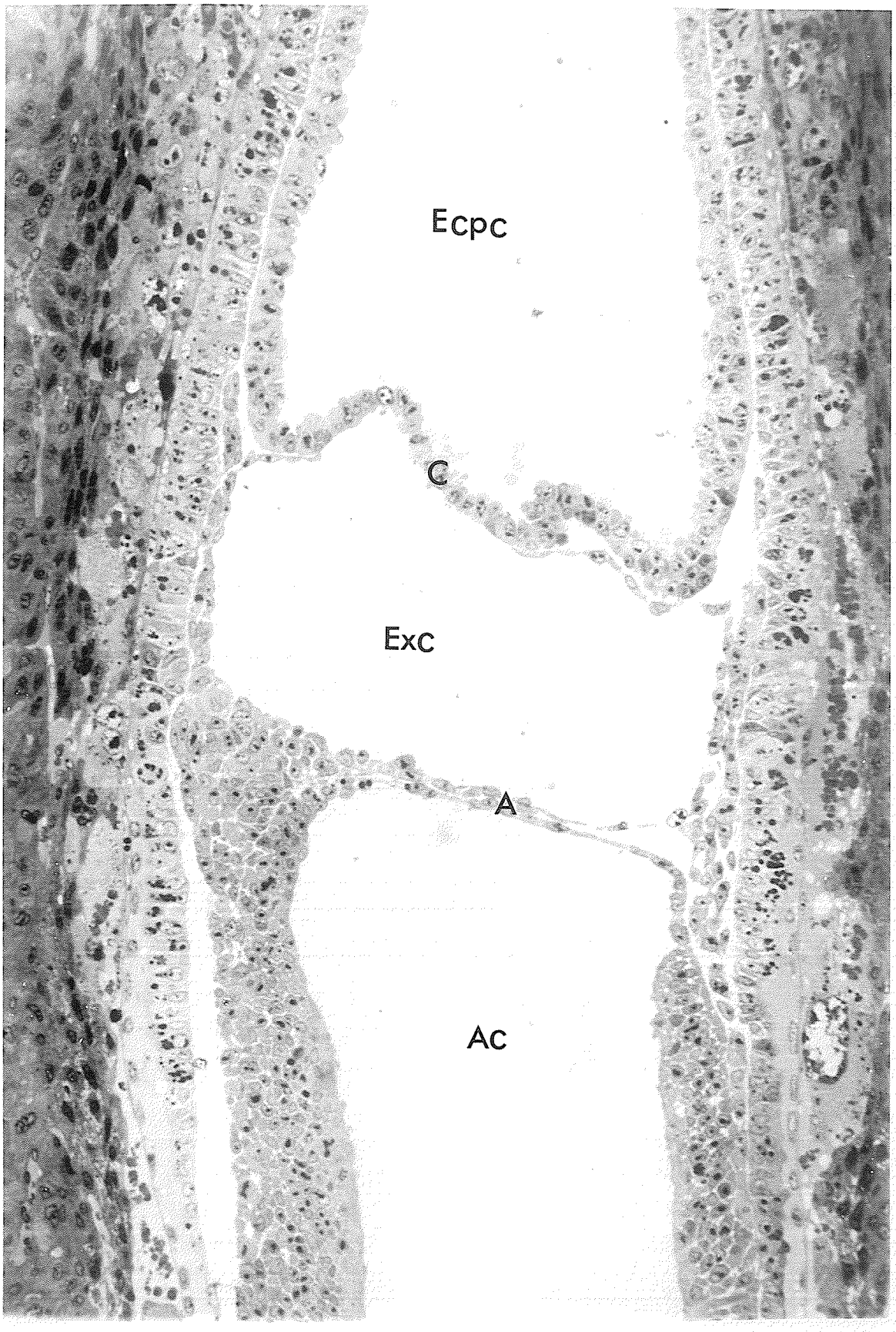

Ecpc
C
Exc
A
Ac

FIGURE 43.

Embryo of nine days gestation recovered from an animal treated with 200 µg of PGE_2. Normal morphology of a more advanced developmental stage is shown. Allantois (Al), exocoelomic cavity (Exc). X280

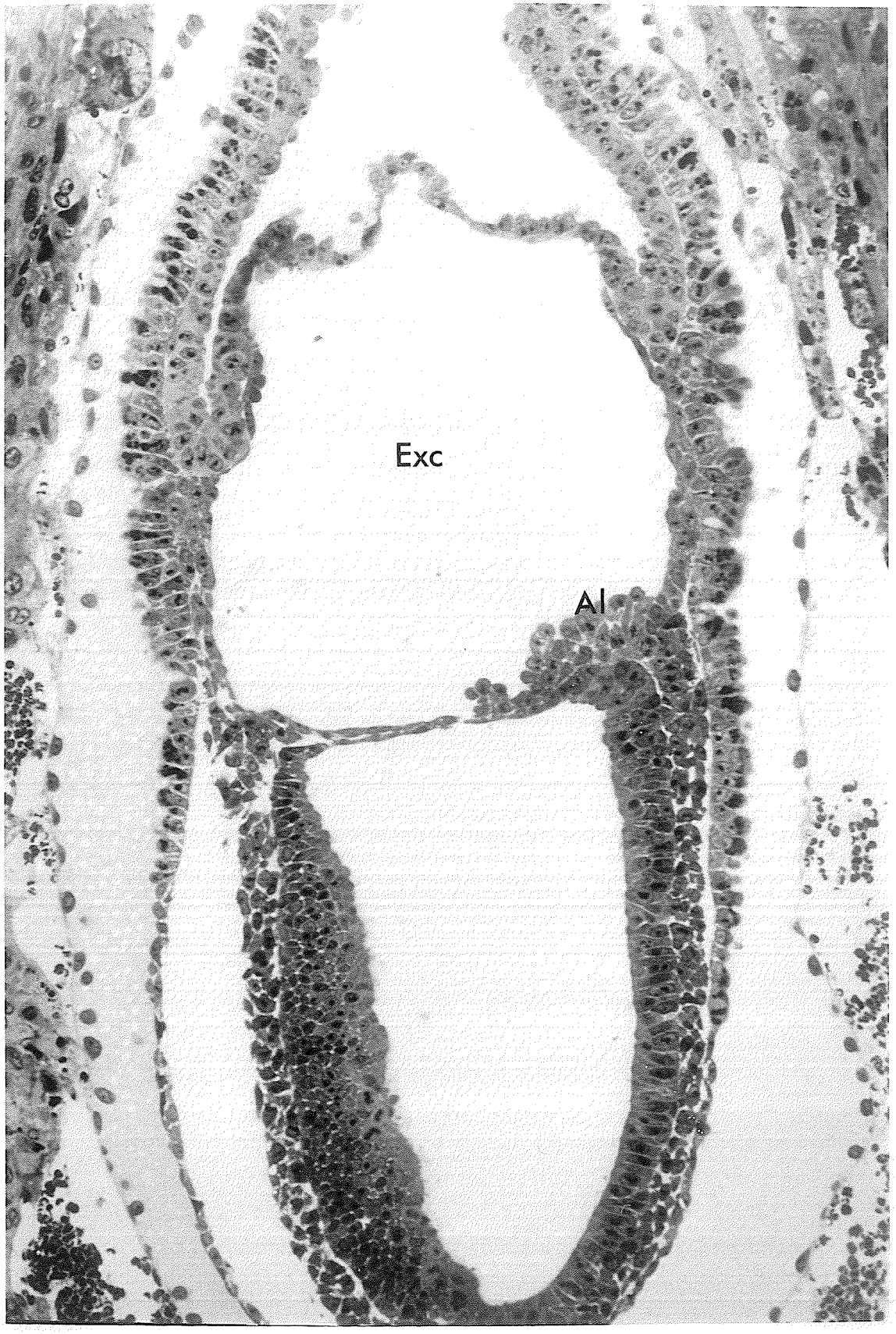
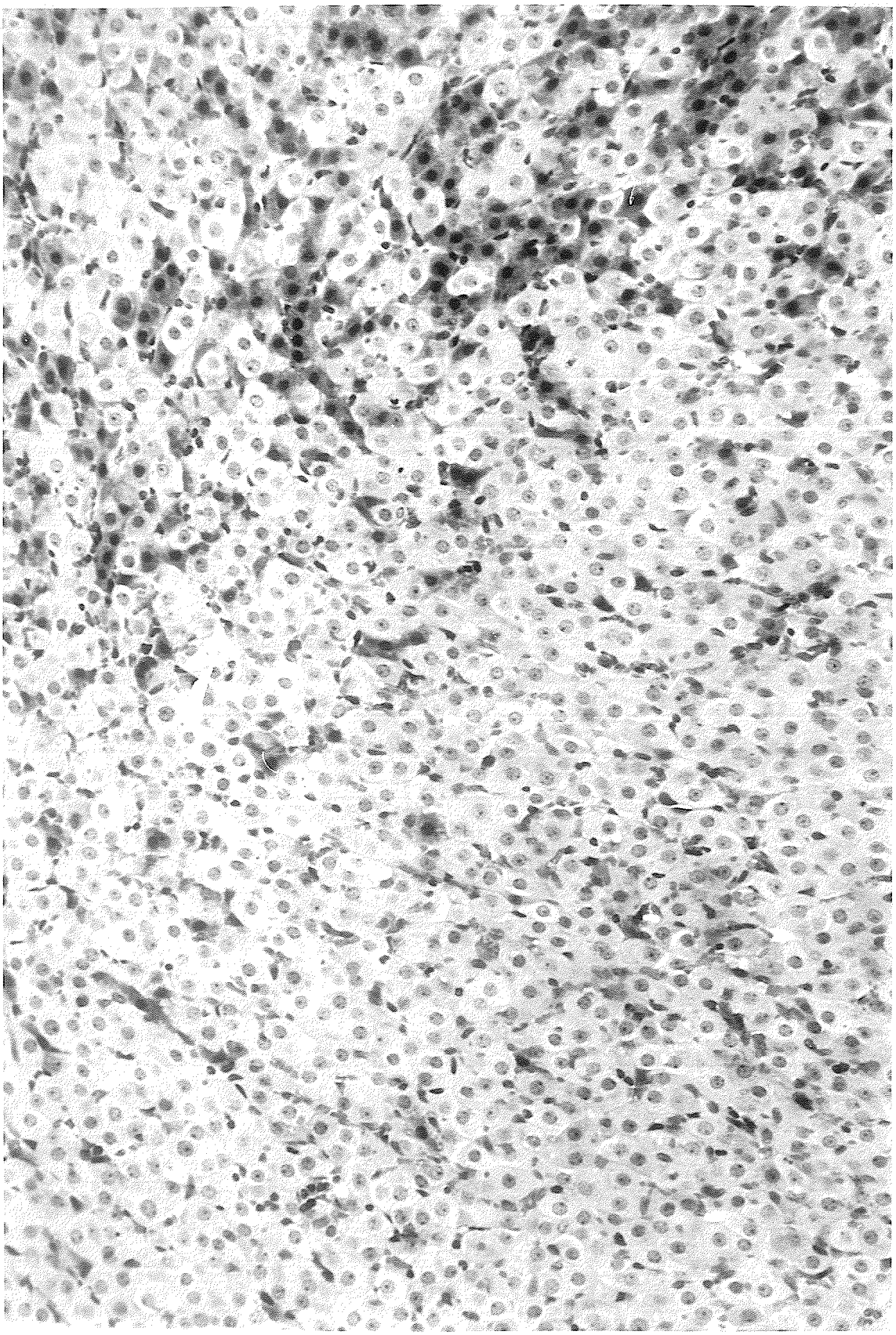

Exc
Al

FIGURE 44.

High power view of 9-day corpora lutea recovered from an animal treated with physiological saline. Note: patches of small basophilic cells indicating the nature of these newly formed corpora lutea. X280



4. DISCUSSION

It is well known that prostaglandins of both the E and F series are capable of terminating pregnancy in laboratory animals at all stages of gestation, that is, when ovarian function is necessary or not necessary to maintain pregnancy. Taking into consideration the two established physiological effects of prostaglandins, alteration of smooth muscle contractility and modulation of hormonal activity, their abortifacient action may be mediated by a number of inter-related mechanisms:

1. a direct luteolytic effect
2. an intense myometrial stimulation which results in the dislodging and expulsion of the conceptus
3. an indirect luteolytic effect (removal of luteotrophic support of the conceptus)
4. a direct action on hormone production by the placenta.

Both PGE_1 and PGE_2 are known to exert antifertility effects in laboratory animals, including the hamster (Labhsetwar, 1972a, 1973a), mouse (Marley, 1972), and rat (Nutting, 1969; Nutting and Cammarata, 1969; Labhsetwar, 1972a). In general, these compounds have been found to be much less potent than those of the F series. Moreover, marked species differences in sensitivity to the antifertility and luteolytic effects of the prostaglandins have been demonstrated,

the hamster being some 5-10 times more sensitive to PGE_2 than the rat (Labhsetwar, 1972). Similar observations have been made with respect to $PGF_{2\alpha}$ (Gutknecht et al., 1969, 1971a,b; Labhsetwar, 1972b). Furthermore, the stage of gestation has been found to play a key role in influencing the effectiveness of these abortifacients, there being periods during which sensitivity is increased.

The ability of prostaglandins to reduce progesterone secretion by the corpus luteum (luteolysis) has been reported for both the F series (Blatchley and Donovan, 1969; Pharriss and Wyngarden, 1969; Johnston and Hunter, 1970; Kirton et al., 1970; Labhsetwar, 1970; Behrman et al., 1971; Gutknecht et al., 1971a,b; Labhsetwar, 1971; Pharriss, 1971; Bartke et al., 1972; Labhsetwar, 1972b,c; Okamura et al., 1972; Shaikh, 1972; Fenner-Crisp, 1973; Labhsetwar, 1973; Fuchs et al., 1974; Keyes and Bullock, 1974; Strauss and Stambaugh, 1974) and the E series (Nutting and Cammarata, 1969; Labhsetwar, 1972a, 1973a; Chatterjee, 1974). With respect to PGE_2, these conclusions are based on the fact that treatment caused morphological degeneration of corpora lutea in hamsters; decreased peripheral progesterone levels in pregnant hamsters, pseudopregnant hamsters, and pregnant rats; inhibited decidual growth; and terminated early pregnancy which could be maintained in hamsters and to some extent in rats by the simultaneous administration of exogenous progesterone (Labhsetwar, 1972a). Fine structural

alterations indicative of luteolysis have also been observed following PGE_2 or $PGF_{2\alpha}$ treatment in gonadotrophin-treated pseudopregnant rats, including increase in lipid content, decrease in cell size, reduction in "ruffled" cell border, decrease in number and size of cellular organelles, and increase in intercellular space (Fenner-Crisp, 1973). Synthetic analogues of PGE's and PGF's with more potent luteolytic properties than the parent prostaglandins have also been reported (Labhsetwar, 1974; Karim and Amy, 1975; Kirton, 1975).

Several mechanisms have been postulated in an attempt to explain how prostaglandins exert their luteolytic effects. These include a vascular insult by these potent vasoactive compounds, a central effect, that is, an effect on gonadotrophin secretion, or a direct effect on the lutein cells themselves (see Labhsetwar, 1975 for a detailed discussion).

The corpora lutea recovered at term from animals treated with 50, 100, and 200 μg of PGE_2 showed no signs of luteolysis. The lutein cells appeared large and eosinophilic. Although morphological involution or lack of it can not be directly equated with functional luteolysis (Labhsetwar, 1975) some relationship has been found to exist (Salazar et al., 1976). Furthermore, it has been found that progesterone reduction must be greater than 80% in order to adversely

affect pregnancy (Fuchs et al., 1974). Thus, the presence of intact lutein cells at term indicates that any degree of luteolysis, while possibly occurring was not significantly extensive to be manifested at the light microscopic level, nor to cause any deleterious effects on fetal parameters.

Statistical examination of the parameters of litter size, number of resorptions, intrauterine death, total number of implantations, fetal weights, placental weights, and maternal weight gain during pregnancy indicated that subcutaneous treatment with 50, 100, and 200 µg of PGE_2 during early pregnancy (days 5-8) did not significantly alter the mean values of these measurements compared to the corresponding controls. Furthermore, no fetal anomalies (external, visceral, or skeletal) were detected indicating that the drug had no teratogenic effect at the doses employed.

Puri et al. (1976) have described the presence of vasospasm and intervillous hemorrhage in the placenta following intra-amniotic $PGF_{2\alpha}$ induced abortion, while dilated regions of fetal vessels in the labyrinthine zone, dilation of trophoblastic tissue, margination of leukocytes and occasional hemorrhage into the trophosponguim accompanied by a reduction in mean placental weight following treatment with $PGF_{2\alpha}$ has been reported to affect placental weights and cause extensive morphological changes following intra-amniotic administration in rats and rabbits (Persaud, 1973c, 1974).

No detectable morphological changes were observed in the placentae recovered at term from any of the treatment groups. These results indicate that PGE_2 administered subcutaneously from gestational days 5 through 8 at doses of 50, 100, and 200 µg had no toxic, teratogenic or antifertility action.

This system of evaluation maximizes the period during which abnormalities may develop but provides no information about the immediate impact by the drug on the embryo and any capability it may have for regulation or recovery from damage. Hence developmental disturbances could pass unnoticed. Therefore, in addition to this long-term assessment short term experiments were designed to study the effects of PGE_2 treatment on the implanting embryo, trophoblast, and decidua at the light microscopic level twenty-four hours after the final treatment.

Early teratogenic lesions could easily become obscured, not only from early resorption of affected embryos but also because of subsequent recovery from damage. Snow and Tam (1979) have shown that following treatment with mitomycin C, an antibiotic, the mouse embryo may be reduced to around 10% of its normal size at a time when it is about to begin organogenesis, but is nearly normal again before that phase of development is complete. Furthermore, most of the repair is accomplished within forty-eight hours of the damage

being inflicted. Compensatory growth in response to chemical insult following treatment of mouse embryos with $PGF_{2\alpha}$ (Scott, 1980) and 5-fluorodeoxyuridine (Langman et al., 1980) has also been reported. In addition, compensatory growth of rat blastocysts in response to ethanol treatment has been observed (Anders and Persaud, 1980). Thus it seems that during early organogenesis embryos have a remarkable capacity for recovery from severe damage and developmental retardation induced during the primitive-streak stages of development when drugs were considered more likely to be lethal than teratogenic.

Light microscopic examination of saline-treated and PGE_2-treated embryos revealed no remarkable changes. The E-prostaglandins have been demonstrated to be leukotactic (Kaley and Weiner, 1971) and other investigators have shown that leukocytes and their products are embryotoxic and arrrest development during or immediately after implantation (Parr, 1969; Smith et al., 1971; Parr and Shirly, 1976; El Sahwi and Moyer, 1977a,b). Moreover, leukocytosis has been observed to terminate early pregnancy in rats and may provide the mechanism for involution (Anderson and Alexander, 1979). There was no evidence of an inflammatory reaction in any of the treatment groups. No endometrial leukocytic infiltrate was observed and in any case, postimplantation embryos apparently are protected from the possible harmful

effects of uterine leukocytes by their fetal membranes (Anderson and Alexander, 1979). No increase in the number of dead cells and in mitotic activity was observed. An increased mitotic activity would tend to reflect accelerated compensatory growth in the embryo.

Although there was no evidence of impaired blastocyst development, embryos of the same gestational age (± 1.5 hours) recovered from both control and PGE_2-treated groups were not uniformly developed. All gestational ages were rigidly timed in an attempt to minimize variability, yet a suprising range in growth and normal morphogenesis was found to exist both between and within litters. The degree of variation appearing to be far more pronounced between litters.

It was expected that all embryos would be at the developmental stage where the allantois was evident but not yet fused with the inferior lamina of the ectoplacental cone. Instead, less advanced stages differing by as much as two days were found in the controls (compare Figs. 21 and 28) and by as much as one day in each of the experimental groups with all conceivable intervening stages noted between the two extremes. It must be stressed that this represents a normal developmental range and that sequential analysis of Figs. 21 to 28 give an overall view of early embryonic development as it normally proceeds in the rat.

That the degree of variation observed in the controls was slightly more than that of the experimental group of animals is probably not related in any way to the treatment. Furthermore it should not indicate or imply evidence of physiological importance of prostaglandins in differentiation and morphogenesis, for which there is some indirect evidence (O'Grady et al., 1972; Saksena and Harper, 1974; Persaud and Moore, 1974; Persaud, 1974).

Allan and MacDowell (1940) have reported variation ranges in mouse embryos of eight days gestation. Based on ninety-five embryos they found thirty-one had no somites (their development ranging from the primitive groove to the head fold stage) while only one embryo had eight somites. The other embryos varied between the two extremes. One litter in fact showed ranges from the primitive groove to the four somite stage. Furthermore, they found the number of embryos in the uterine horn, their position in the horn, or the size of the litter seemed to have no influence on the size and degree of development. Otis and Brent (1954) also reported variation in mouse embryos of a particular mating age; it being most apparent during the period of somite formation. At nine days gestation somite count was found to vary between nine and seventeen. In one animal, embryos of thirteen, fourteen, fifteen, sixteen, and seventeen somites were present. They also noted that variation in the presomite period is at least as great as in the somite period but it is less easily measured. One uterus was

found to have embryos ranging from the head fold stage to the early primitive streak stage. In addition, variation between surviving embryos of the same mating age decreased in the later days of gestation when there was a change in developmental rate: a very rapid differentiation of specializing tissues occurring after the fourteenth day. This may only reflect the fact that in older embryos suitably objective measurements are difficult to obtain. Considerable variations in the rate of development of mouse embryos were noted by Snell and Stevens (1966) but these investigators did not describe them, only pointing out that hybred and inbred strains may vary in developmental levels by as much as twenty-four hours.

Each developing embryo is isolated in its own implantation chamber and subject to its own microenvironment. The manner in which embryonic development is influenced by the maternal environment is not known. Likewise, neither is the extent that early differences in size and degree of development are compensated for in later development.

A statistical comparison of the total number of implantations using Duncan's New Multiple Range Test revealed that PGE_2 at any of the doses employed had no adverse effects on implantation rate. Furthermore, a comparison of the total number of implantations observed

in the long term versus the short term assessment indicated no effect by PGE_2 and that no lesions were being obscured due to early resorption of affected embryos. This is an important observation since several investigators have shown that both PGE_2 and $PGF_{2\alpha}$ are capable of interfering with implantation via various mechanisms (Labhsetwar, 1972a; Batta and Martini, 1975; Bronson and Hamada, 1978).

Normal morphological characteristics and uniform decidualization was observed in all implantation sites examined. The decidual cell reaction (DCR) consisted of a zone lying peripheral to the developing conceptus, the implantation zone in which the cells seem to lag in their development and stain more intensely. Secondly, a primary decidual zone containing large cells with large nuclei accompanied by a considerable amount of cytoplasm. Lastly, a zone lying peripheral to the primary zone characterized by many binucleated lipoid-laden cells termed the secondary decidual zone (Krehbiel, 1937). The cellular hypertrophy associated with DCR climaxes on the ninth day and clearly differentiated the antimesometrial portion of the implantation site.

Suppression of DCR to traumatization as determined by uterine weight gain in pseudopregnant rats has been reported following administration of PGE_2 and $PGF_{2\alpha}$ (Fenner-Crisp, 1973;

Castracane et al., 1974), while PGE_2 has been reported to inhibit DCR in hamsters, pseudopregnant hamsters (Labhsetwar, 1972a) and mice (Bronson and Hamada, 1978). Supression of DCR by prostaglandins has been attributed to their luteolytic effects which cause a reduction in circulating progesterone which in turn leads to inhibition of decidual growth with a resultant loss of pregnancy (Labhsetwar, 1972a,b; Chatterjee, 1972; Fenner-Crisp, 1973). The fact that PGE_2 was capable of decreasing decidual growth and circulating progesterone levels in pseudopregnant hamsters and rats and that DCR could be restored by administration of exogenous progesterone suggests that prostaglandins can exert luteolytic effects in the absence of embryos. However, there is evidence that both PGE_2 and $PGF_{2\alpha}$ may have direct effects on the uterus. Both PGE_2 and $PGF_{2\alpha}$ have been found to inhibit DCR in ovariectomized hormone-replaced mice and rats, respectively (Bronson and Hamada, 1978; Castracane et al., 1974). Thus indicating they can act independently of the ovary.

Supression of DCR has also been reported in uterine horns bearing IUD's (Margolis and Doyle, 1964; Craig, 1967; Ichikawa and Morioka, 1969; Dizzia and Bo, 1969; Bartke, 1968), and IUD's have been associated with an increased production of prostaglandins in rabbits (Saksena and Harper, 1974), rats (Saksena, et al., 1974; Chaudhuri, 1973), hamster (Saksena et al., 1974), mice (Lau et al., 1974), and sheep (Wilson et al., 1972; Spilman and Duby, 1972).

Direct embryotoxic effects leading to destruction of the blastocyst with secondary loss of decidualization seem unlikely since embryos of normal appearance have been observed within implantation sites of PGE_2-treated mice at a time when implantation weight was already significantly affected (Bronson and Hamada, 1978).

Histamine has been implicated in the process of decidualization (Shelesnyak, 1952; Ferrando and Nalbandov, 1968). PG's have been shown to modulate histamine release from dermal mast cells in the rat (Crunkhorn and Willis, 1971). In a similar manner they may also affect endometrial mast cells and in this way influence decidualization.

Conversely, Sananes et al. (1976); Tobert, 1974) demonstrated intraluminal instillation of low doses of $PGF_{2\alpha}$, PGE_2, and arachidonic acid into prepubertal rats maintained on progesterone induced deciduoma with histological and electron microscopic characteristics typical of decidualization. Tachi and Tachi (1974) have also obtained a very limited decidual response after intraperitoneal injection of $PGF_{2\alpha}$ into the pseudopregnant rat. Thus, it seems that prostaglandins at physiological concentrations are required for DCR. Further evidence comes from studies utilizing indomethacin, the prostaglandin synthesis inhibitor which has been found to inhibit DCR in progesterone pretreated

immature rats (Sananes et al., 1976), pseudopregnant rats (Tobert, 1974), and ovariectomized rats treated with estrogen and progesterone (Castracane, 1974). In fact, not only have prostaglandins been shown to play a role in decidualization, but they also have a physiologically important role in implantation. Low doses of both PGE_2 and $PGF_{2\alpha}$, in lieu of the estrogen stimulus, induce mouse blastocysts to implant in the uterus, PGE_2 being the more effective (Holmes and Gordashko, 1980). This evidence is strengthened by the finding that estrogen stimulates prostaglandin production in the uterus, particularly in decidual tissue. (Barcikowski et al., 1974; Anteby et al., 1975; Castracane and Jordan, 1975; Ham et al., 1975; Kuehl et al., 1975; Williams and Downing, 1977).

All prostaglandins are known to stimulate cAMP synthesis,E-prostaglandins being more effective than the A and F types (Kuehl et al., 1973). Also, E prostaglandins are known to stimulate cAMP formation in a dose-related manner (Wolf and Shulman, 1969; Bourne et al., 1972). Holmes and Bergstrom (1973, 1975, 1976) and Webb (1975) were able to induce implantation in delayed-implantation mice with cAMP. Thus a relationship between PGE_2 and cAMP is implied suggesting that the estrogen activating stimulus functions via a combined PGE_2-cAMP mechanism. Additional evidence for the involvement of

prostaglandins in implantation comes from observations that indomethacin is able to interfere with implantation (O'Grady et al., 1972; Lau et al., 1973; Gaven et al., 1974; Saksena and Harper, 1974; Persaud, 1974; Lau and Chang, 1975; Saksena et al., 1976; Hoffman, 1977; Evans and Kennedy, 1977, 1978; Kennedy, 1977; Holmes and Gordashko, 1980). Lau et al., (1973) also demonstrated that the anti-implantation effects of indomethacin could be reversed by administration of either PGE_2 or $PGF_{2\alpha}$.

Thus it seems that administration of low doses of PGE_2, in addition to endogenous prostaglandins normally present during early pregnancy, did not adversely affect implantation or embryonic development. The observations made when fetuses were allowed to go to term are further supported by the findings on gestational day 9. The combined results indicate thate PGE_2, injected subcutaneously at doses of 50, 100, and 200 µg, on gestational days five through eight is without detrimental effects as assessed morphologically.

5. CONCLUSIONS:

1. The results indicate that PGE_2 is not teratogenic when administered to pregnant rats at dose levels comparable to the human therapeutic range and during a relatively short gestational span. Intrauterine deaths, congenital malformations, and growth retardation, all of which are manifestations of deviant development, were not observed in any of the offspring.

2. PGE_2 has no damaging effect (direct impact) on the developing conceptus immediately following the treatment. No significant changes were present in the blastocysts or implantation sites.

3. PGE_2 did not exert an antifertility effect at the doses employed and route administered. Suppression of decidualization, luteolysis and placental damage were not evident.

4. Early blastocyst-uterine interaction showed a remarkable lack of uniformity. The wide range of developmental stages observed in the control animals has not been described before.

APPENDIX A

Millonig's Phosphate Buffer:

Solution A: 2.26% $NaH_2PO_4 \cdot H_2O$

Solution B: 2.25% NaOH

Solution C: 5.40% glucose

Solution D: Solutions A and B combined in ratio of 4.88:1

Final buffer solution: Solutions C and D combined in ratio of 1:9

Final pH: 7.3

Karnovsky's Fixative:

Combine 100 ml of 8% paraformaldehyde with 40 ml (25%) glutaraldehyde and make volume up to 200 ml with Millonig's Phosphate Buffer.

Final pH: 7.2

APPENDIX B

Dehydration and Embedding Sequence:

30% ethanol - 10 minutes - two changes

50% ethanol - 10 minutes

70% ethanol - 10 minutes

90% ethanol - 10 minutes

100% ethanol - 10 minutes - two changes

100% methanol - 20 minutes

100% methanol/propylene oxide (1:1) - 10 minutes

100% propylene oxide - 10 minutes

75% propylene oxide/25% araldite (V/V) - 2 hours

50% propylene oxide/50% araldite (V/V) - overnight

25% propylene oxide/75% araldite (V/V) - 2 hours

100% araldite - 2 hours

Embed in 100% araldite. Cure 24 hours at room temperature following by 24 hours at 60°C.

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