A STUDY OF PRIMORDIAL GERM CELL MIGRATION AND GONADOGENESIS IN NORMAL AND STERILE *STEEL* MUTANT MICE

A STUDY OF PRIMORDIAL GERM CELL MIGRATION AND GONADOGENESIS IN NORMAL AND STERILE STEEL MUTANT MICE

By

JOHN ADRIAN McCOSHEN, B.Sc., M.S.

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Author: John Adrian McCoshen, B.Sc. (Saint Francis Xavier University)

M.S. (State University of New York)

Supervisor: D. J. McCallion, Professor of Anatomy

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Abstract

The development of murine gonads commences in both males and females as indifferent appearing blastemata around the 10th day of embryogenesis. Within the following two days, the gonadal blastemata became heavily populated with the primordial germ cells which migrate in from extra-gonadal sites.

The process of gonadal sex differentiation is first evident in males where the appearance of sex cords marks testicular histogenesis. Ovarian development is largely marked by the fact that female gonads do not resemble testes and also by the early transformation of oogonia into oocytes.

It has been proposed by a number of investigators that the absence of the primordial germ cells in the gonadal blastemata during the indifferent stage of gonadogenesis would result in the failure of sex cord differentiation to take place within the gonadal soma. Other investigators have proposed that gonadal sex differentiation is not dependent upon the presence of germ cells within the gonadal soma.

To resolve this controversy mice carrying the alleles Steel/Steel Dickie ($\underline{Sl/Sl^d}$) were selected since the Steel mutation is known to severely affect the primordial germ cell line prior to its populating the gonadal ridges before sex cord differentiation. The experiment therefore is a natural one not requiring chemical or physical ablation of

the germ cell line at the time it is first histochemically identifiable in sites far removed from the gonadal anlages.

Primordial germ cells were identified histochemically by an azo dye technique in 27 day 9, 33 day 10, 33 day 11 and 21 day 12 embryos which were obtained from matings between mature WC/ReJ-Sl/+ females and C57Bl/6J-Sl^d/+ males. One fourth of the embryos were expected to carry the homozygous Sl/Sl^d mutation. Failure of the germ cell population to increase after 9 days gestation occurred in 26% of the embryos. These embryos were classified as the mutant Sl/Sl^d group. The primordial germ cells of the mutants, though few in number, were found to follow a normal migratory pattern to the gonadal ridges. Gonadogenesis was studied in 227 fetuses of 12-18 days gestation and in animals from birth to maturity. The genetic sex of fetuses was determined by the presence or absence of sex chromatin in amnion cells. Prior to day 14, genotypes were established as normal or mutant according to the germ cell population present in one gonad from each fetus. After day 14 the genotypes were determined from red blood cell samples, the Sl/Sl^d fetuses displaying the macrocytic anemia characteristic of this mutation.

The ratio of mutant males to mutant females was the normal 1:1, and the number of mutants identified was within the expected 25% frequency. Despite a paucity of germ cells, mutant gonads differentiated according to their genetic sex. Although mutant gonads are composed almost entirely of somatic tissue they grow at the same rate as the somatic component of

normal gonads up to day 16 post coitus in males, and to the day of birth in females. The overall pattern of growth was similar to that seen in normals. The Mullerian and Wolffian ducts as well as the external genitalia also developed according to gonadal sex in the mutants.

In those mutant gonads which contained few germ cells, they grew and differentiated in the same manner as did those of the normals up to birth. By maturity, no mutant germ cells were found to have differentiated beyond prophase of meiosis I.

It is concluded that sexual differentiation and gonadogenesis can take place in the absence or at least in the near absence of germ cells. The Steel mutation appears to act in preventing the proliferation of the primordial germ cells and their capacity to complete meiosis I of gametogenesis. The mutation, however, does not affect the evolution of the germ cells from their source nor the capacity of the germ cells to migrate to the gonadal blastemata.

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INTRODUCTION

Sexual differentiation of mammalian gonads is characterized by the development of somatic sex cords within the gonadal blastema. For most mammalian species this event occurs during early organogenesis in the embryonic phase of development. In male embryos, primary sex cords extend from the coelomic (germinal) epithelium to just below the mesonephros where they anastomose to form the rete testis. The primary sex cords then become separated from the coelomic epithelium by the tunica albuginea and are called seminiferous cords, In females, the primary sex cords are not prominent as in the males and they develop into the rete ovarii which is separated from the cortex by the tunica albuginea. In the female gonad, the cortex develops secondary sex cords which contain most of the germ cells. In ovaries, the secondary (cortical) sex cords appear to fragment into isolated units whereas the primary sex cords of testes will eventually form continuous tubules. Prior to the establishment of the gonadal primordia the primordial germ cells first become identifiable in regions far removed from the site where gonadogenesis will take place. Subsequently, the primordial germ cells migrate toward the gonadal blastemata and infiltrate them by the time they are recognizable as gonadal ridges. Immediately

following this event, the process of sexual differentiation begins, first in male gonads and later in female gonads. (Brambell, 1927; Torrey, 1944; Ohno, 1967; Peters, 1976). Whether or not the germ cells are a principal factor in the sexual differentiation of mammalian gonads and in the subsequent development of the gonadal soma is the subject of this thesis.

Based on histological observations of human embryos, Witschi (1948) concluded that the primordial germ cells (PGCs) first arise in the yolk sac endoderm even before the appearance of the gonadal anlage. This report marked the end of a long debate as to whether the PGCs arose extragonadally or intrinsically within the coelomic epithelium covering the gonadal anlage (see Nieuwkoop, 1949; and Witschi, 1948; 1956 for reviews). The concept that the PGCs originate in the covering coelomic epithelium of the gonads resulted in the misnomer "germinal epithelium" (Falin, 1969).

Everett (1943) and Wolff and Haffen, (1952) were first to experimentally demonstrate the extragonadal origin of the primordial germ cells. Both investigators found that in the mouse, removal of the gonadal ridges prior to the time of PGC infiltration resulted in "more or less sterile gonads" when cultured either <u>in vivo</u> or <u>in vitro</u>.

The primordial germ cells are readily identifiable by selective histochemical staining for the enzyme alkaline phosphatase in such mammalian species as mouse (Chiquoine,

1954), rat (McAlpine, 1955), calf (Ohno and Gropp, 1965) and man (Baxter, 1950). The primordial germ cells have been reported to be first identifiable in the mouse in the caudal end of the primitive streak, allantoic bud and yolk sac splanchnopleure (Chiquoine, 1954). Mintz and Russell (1957) also reported the primordial germ cells to be first seen in those regions. Perhaps because of Witschi's (1948) earlier work on human embryos, these authors concluded that the PGCs originate in the yolk sac endoderm. Ozdzenski (1967) re-examined this question in the mouse using the azo-dye method for alkaline phosphatase to identify PGCs. By examining embryos at earlier stages than previous authors, Ozdzenski (1967) concluded that the PGCs of mice are first identifiable in the allantoic mesoderm. PGCs were next identified in the posterior end of the primitive streak and only later were they seen in the yolk sac endoderm. Since the allantois is partly a derivative of the primitive streak, he concluded that the PGCs originate in the posterior end of this region.

From a study on the ultrastructure of the primordial germ cells in mouse, Spiegelman and Bennett (1973) have reported that some PGCs in the hindgut endoderm had the appearance of splanchnic mesodermal cells. Recently Clark and Eddy (1975) have concluded from their ultrastructural studies of early mouse PGCs, that the germ cells appear not to be endodermal in origin. Also, the fact that the PGCs of mice are rich in alkaline phosphatase when first identifiable may

in itself indicate a differential response to a new tissue environment (Kaplan, 1972; Clark and Eddy, 1975). This might then imply that the PGCs do not arise in situ in the yolk sac endoderm, but rather the endoderm would represent a secondary site in which PGCs are found.

The migration of the germ cells to the gonadal ridges has been well documented for the mouse, (Chiquoine, 1954; Mintz and Russell 1957; Zamboni & Merchant, 1973;) rat, (McAlpine, 1955; Eddy, 1974; Merchant, 1975) rabbit, (Chretien, 1966) calf, (Jost and Prepin, 1966) man, (Witschi, 1948; McKay et al, 1953; Falin, 1969). The path of migration of PGCs appears to be common to all these species. From their site of origin in the posterior end of early somite embryos, the germ cells migrate anteriorly through the wall of the hind gut, then into the dorsal mesentery and laterally around the coelomic angles into the gonadal ridges.

Witschi (1948) proposed that the PGCs reach the gonadal primordia by ameboid activity since they possessed processes reminiscent of pseudopodia. Subsequently, Blandau et al (1963) reported that mouse PGCs in vitro were in fact capable of ameboid activity. At the ultrastructural level, both Spiegelman and Bennett (1973) and Zamboni and Merchant (1973) have reported that the PGCs of the mouse embryo possess pseudopods while in the gut region of the migration path. Fukudu (1976) has also found that in man, pre-gonadal PGCs possess pseudopods.

It is a characteristic of PGCs that during migration many of these cells wander into sites considered to be ectopic to the normal migratory pathway (Mintz and Russell, 1957). Such sites include the placenta, umbilicus, tail bud, posterior limb bud and base of the dorsal aorta (Chiquoine, 1954; Mintz and Russell, 1957; Mintz, 1959; 1960; Falin, 1969; Ozdzenski, 1969). In transplants of mouse hind gut containing PGCs into the anterior chamber of the eye Ozdzenski (1969) observed that the primordial germ cells failed to differentiate and soon lost their alkaline phosphatase activity. Although he concluded that the primordial germ cells cannot survive in ectopic sites, it is well known that germ cells normally lose their alkaline phosphatase even within the gonads (Mintz, 1960). Thus it would seem that the fate of ectopic germ cells is not yet known.

Witschi (1951) argued that the gonadal blastemata actively attract migrating germ cells by producing a substance he termed "telepheron". Similarily, Simon (1960) and Dubois (1968) thought that a chemotactic attraction exists between the extragonadal PGCs and the gonadal primordia in the chick embryo. Germ cells from the germinal crescent have experimentally been shown to pass directly into genital ridge tissue in the chick (Dubois, 1968; Rogulska, 1969). By transplanting mouse hind gut containing PGCs intracoelomically into early chick embryos, Rogulska et al (1971) found the murine PGCs to have entered the chick tissue in the region

of the genital ridge. Blandau et al (1966; and see Baker 1971) demonstrated, using cinematography, the ameboid activity of early mouse PGCs in vitro. When gonadal ridge tissue was placed in the culture dishes, the PGCs migrated towards it. It is generally agreed that PGCs are actively attracted by the gonadal ridges but the nature of the attractant (telepheron) is not known.

Germ cell degeneration is a common feature of both immature and mature sex cells (Baker 1972; Roosen-Runge, 1973). Among the mammals studied, massive germinal cell loss has been reported during fetal life (Beaumont and Mandl 1962, 1963; Chretien 1966; Baker 1963; Huckins and Clermont 1968). However, germ cell degeneration does not appear to any significant degree until the time of cessation of mitoses in the germ cell line. This event takes place after sexual differentiation of the gonads has occurred. There are no reports of excessive germ cell degeneration during the migration phase among the mammalian species studied, however, some does occur (Mintz and Russell, 1957).

In mice of eleven days gestation bilateral elongate coelomic swellings appear in the dorsal wall of the coelomic cavity just lateral to the dorsal mesentery of the gut and in close proximity, buf ventro-medial to the mesonephros. These swellings are recognized as the early formation of the genital ridges (Rugh, 1968). At this time some PGCs are found in the newly forming gonadal ridge and in adjacent

areas (Mintz & Russell, 1957). It is generally agreed that these events mark the onset of gonadogenesis. One day later, on day 12, the first signs of sexual differentiation are apparent. The arrival of the germ cells and the appearance of the genital ridge occur simultaneously. Almost immediately after these events, the process of sexual differentiation of the gonads takes place. The question therefore arises as to whether or not the germ cells are an intrinsic requirement for the differentiation of the somatic cells of the gonads towards sexual differentiation and growth.

It is known that in the amphibians (Bounoure et al., 1954) and in the chick (Burns, 1971), gonadogenesis will commence after selectively eliminating the germ cells by either U.V. or X-irradiation prior to their populating the gonadal blastema. Furthermore, sexual differentiation has been found to take place in the experimentally produced sterile gonads. In mammals, similar results have been obtained when gonadal ridges were excised and cultured in vitro prior to the PGCs populating the ridges and prior to the onset of sexual differentiation (Wolff and Haffen, 1952; Asayama & Furusawa, 1961). These latter investigators explanted mouse gonadal ridges from as early as 10 days gestation and found that sexual differentiation took place. Earlier, Everett, (1943) who was first to experimentally demonstrate the extra-gonadal origin of mouse PGCs, was unable to demonstrate sexual differentiation to occur in pre-germinal gonadal

ridges cultured *in vivo*. The cultures of gonadal ridge and adjacent mesonephros were allowed to grow for at least 14 days under adult kidney capsule. During this time, the adjacent sex ducts differentiated but the gonadal tissue failed to do so. Everett concluded that since sex cords did not appear, the differentiation and growth of the gonads was dependent upon the presence of the primordial germ cells.

Strains of mice carrying mutant genes which result in sterility have been investigated during the germ cell migration phase to late gonadogenesis. In the case of the *W/W* locus, a defect in the ability of the germ cells to proliferate and migrate has been reported in embryos homozygous for the mutation. (Coulombre & Russell, 1954; Mintz and Russell, 1957; Mintz, 1959) Similarily, Bennett (1956) has reported that mice homozygous for the gene, Steel (Sl) are also sterile due to a failure in the germ cells to proliferate and migrate to the gonadal ridges. Sexually differentiated gonads have been reported to be present in these mutant mice (Coulombre and Russell, 1954; Bennett, 1956). In the case of the *Sl* mutants the gonads were found to be completely agametic at 15 days gestation and at birth (Bennett, 1956). Coulombre and Russell (1954) have reported some germ cells to be present in the gonads of post partum mice although few in numbers. Some gonads were observed to be entirely agametic.

Merchant (1975) has experimentally tested the problem

of gonadal sex differentiation in the agametic state in the rat. He treated rat embryos while in utero by maternal injections of busulphan (1,4- butanedial dimethanosulphanate), which is known to selectively destroy PGCs, Hemsworth & Jackson, 1963), at gestational stages prior to their populating the gonadal ridges. By this method nearly agametic and some entirely agametic gonads were produced. Busulphan at dosages adequate to eliminate the germ line appears not to cause any obvious fetal malformations (Hemsworth & Jackson, 1963).

After examining treated gonads from 11 days to birth, by light and electron microscopy, Merchant (1975) concluded that the presence of germ cells are not a prerequisite for the differentiation of sex cords. Both testes and ovaries which lacked germ cells were found. The precocious appearance of sex cords in treated embryos was indicative of testicular differentiation which is found in the normal state. Qualitative data on the genetic sex of the fetuses, germ cell numbers and gonadal sizes were not reported by Merchant, but his evidence does indicate that mammalian gonadogenesis is not germ cell dependent during the period of sexual differentiation.

In contrast to the concept of somatic tissue independence in gonadogenesis and sexual differentiation, Goodfellow et al (1965), Steward (1965) and Fechheimer et al (1963) have proposed that not only is the presence of PGCs a determ-

inant in sexual differentiation but also the genetic sex of these cells will direct the course of differentiation to either the male or female line. In their studies on freemartinism these authors concluded that the virilization of freemartin ovaries is a result of male germ cells migrating into these glands from the male co-twin via the placental anastomosis. Although the circulatory migration of calf PGCs between freemartins has not yet been adequately proven, Ohno (1969) has histochemically demonstrated the presence of a few cells identified as PGCs in the dorsal mesenteric blood vessel of a calf embryo. However, Jost (1970) studied the gonads of 35 freemartins from the time of sexual differentiation of the gonads to the establishment of the genital tract (days 44-110) and found that during this time none of the ovaries had formed ovotestes. Ovotestes apparently arise only later on in development. This evidence does not favour a germ cell influence on the early development of the gonads (Jost et al 1973). Although XX germ cells have been identified in the testes of neonatal bulls twinned to a freemartin (Ohno et al 1962; Teplitz et al, 1967), the converse of male germ cells in freemartin gonads has not been demonstrated. From observations in mouse (Tarkowski, 1970; McLaren, 1972; 1975) and mormoset (Hampton, 1973), meiotic germ cells resembling the female line have been found in chimaeric male testes. However the functional gamete of XX/XY chimaeras have been found to be of one class

only, that of the phenotypic sex of the chimaera (McLaren, 1972). Only one exception has thus far been reported. Ford et al., (1975) have recently revealed that an XX/XY female mouse chimaera formed by fusing AKR/J (albino) to CBA/H-T6 (agouti) gave birth to an XXY albino after mating with a BALB/c (albino) male. The Y chromosome was identified as that of the AKR type. Even should Ford's (1975) report prove to be true and repeatable, it would not favour the concept of germ cell influence in the fetal gonad but rather it would imply an overbearing influence of the gonad on the germ cells.

Earlier work on chimaeric mice (Tarkowski, 1970; McLaren, 1972) had indicated that there exists a certain degree of germ cell independence during fetal development. These reports indicate that female line germ cells in testicular tissue are able to enter meiosis prenatally as in the normal state, but around the time of birth or shortly after, the presence of these germ cells is not apparent. It must, however, be kept in mind that some of the somatic tissue of the testes is also XX and this in itself may be demonstrative of a somatic-germinal interdependence rather than an independence (McLaren, 1972).

Changes in gonadal volume have been measured in chick (Mittwoch et al, 1971), mouse (Jean, 1971; Jean and Berger, 1972; Mittwoch 1974), rat (Beaumont and Mandl, 1962; 1963), calf (Jost et al, 1973) and human (Mittwoch and Kirk.,1975). In all

cases studied, it is apparent that the gonads of the heterogametic sex grow at a rate greater than that of the homogametic sex. In mammals, it has been found that testicular growth greatly exceeds ovarian growth from very early on in gonadal development. Mittwoch et al (1969) and Mittwoch (1971; 1973) explain the increased growth differential of mammalian testes and avian ovaries to be due to a higher mitotic rate of the heterogametic gonads which carry about 2% less chromosomal material than their counterparts. (Mittwoch, 1970).

In two studies on the rat, the volume of germinal tissue occupying the gonads has been calculated (Beaumont and Mandl, 1962; 1963). These authors have shown that the amount of somatic tissue of testes far exceeds that of ovaries as development proceeds in the fetal rat. The difference was obvious even from 14.5 days post coitus which is equivalent to a 13 day mouse embryo in development. Ovarian volume was found to increase more rapidly just prior to birth whereas the testes followed a near logarithmic growth rate throughout fetal and neonatal life.

A substantial volume of fetal testes (rat, Beaumont and Mandl, 1963) and ovaries (calf, Erickson, 1966; rat, Beaumont & Mandl, 1963; human, Baker, 1963) is occupied by germ cells. As the fetal gonads grow, the mean volume of the germ cells increases. At the same time, the population of germ cells also increases up to late stages of fetal development (Beaumont and Mandl, 1962; 1963; Chretien, 1966;

Baker, 1963) after which a steady decline in the germ cell population takes place due to atresia (see Baker, 1972 for review). Beaumont and Mandl (1963) found that the germ cell population of fetal male rats was always greater than that found in their female siblings. One possible explanation may be that the female germ line enters prophase of meiosis I fairly early in fetal life. The stages of prophase I of meiosis are fairly well synchronized in both rats (Beaumont & Mandl, 1962) and mice (Peters, 1970).

Recently Byskov (1974, 1975) has implied that the rete ovarii plays a significant role in controlling meiosis and follicular cell formation in mice, cats and minks. In male rats and mice, meiosis does not commence until after the first week past partum (Nebel et al 1961). In the mouse, Jean (1971) reported that the fetal ovary showed very little growth during development and in fact, his measurements showed a decline in volume at the time of birth. Testicular growth greatly exceeded ovarian growth. The volume of germinal tissue and germ cell numbers were not calculated in this study. In a later study, Jean and Berger (1972) found that testicular growth continued up to 90 days post partum after which their size remained constant until the end of the first year. After this time the testes appeared to atrophy.

A significant asymmetry in gonadal size is reported for the chick (Mittwoch, et al 1971) with the left side the

dominant one, but this was not the case in fetal rats (Beaumont and Mandl,1962, 1963). The number of germ cells in chick gonads has also been found to be asymmetrical as would be expected with a large size discrepency (Satoh,1974). Size asymmetry has also been reported in human ovaries by Mittwoch et al (1975). In the chick in which the female is the heterogametic sex, the ovary exceeds testicular size from as early as 8 days of incubation (Mittwoch et al 1971). McLaren (1963) noticed a pattern in mature female mice whereby the right ovary produced more ovulations than the left. Much variation in this bilateral asymmetry was evident but when tested statistically the difference was not significant, thus suggestive that each ovary contains an equivalent number of germ cells.

During gonadogenesis the secondary sexual characteristics develop in close proximity to the gonads. In mammals the mesonephric duct (Wolffian duct) and a paramesonephric duct (Mullerian duct) give rise to the male and female extragonadal sex organs respectively. The rudiments of the two organ systems sppear in both male and female embryos. The mesonephric duct appears about the time of sexual differentiation followed shortly after by the rudiment of the paramesonephric system. In a sense the early fetus is bipotential as to sex (see Fort, 1970).

In a review of his own early work on sex duct differentiation in rabbit, Jost (1953) clearly elucidated the fact

that fetal testes support mesonephric ducts and inhibit the paramesonephric ducts. In the female, there is a failure of support for the mesonephric ducts to differentiate and a differentiation of the paramesonephric system which is independent of ovarian influences. It is now generally accepted that in the male, it is testosterone which supports the differentiation of the mesonephric duct into the male duct system (Neumann et al, 1970; Jost,1972a,1972b), and that the paramesonephric system is inhibited also by a testicular factor, the mullerian inhibiting factor (MIF). Although MIF has yet to be identified, recent work by Blanchard and Josso (1974) and Josso (1972a, 1972b,1974); and Picon (1970, 1971) has shown that MIF is not species specific and that it is probably a product of the Sertoli cells.

After rendering fetal calf testes nearly agametic by X-irradiation Josso (1974) determined that these testes were still capable of antimullerian activity which indicated that the germ cells are not the source of MIF. The nature of MIF seems to be that of a proteinaceous macromolecule (Josso, 1971,1972a,1972b, 1974).

The continuity of the germ cell line from the migrating germ cells of the early embryo to the mature sex cells of the adult has been the subject of numerous observations and experimentations (see Mintz 1960 for review). There no longer seems to be a controversy concerning this matter although an occasional opinion favouring a "germinal" epithelial

origin is reported (Schaepman-van Geuns, 1972). Through the use of X-irradiation (Mintz, 1960; Beaumont 1962), autoradiography (Borum, 1961, 1966; Kennelly and Foote, 1966; Peters and Crone 1967; Huckins and Clermont, 1968) and mutations in mice which result in sterility due to a PGC defect (Mintz, 1957, 1960; Bennett 1956), the evidence that the early migrating cells (PGCs) first seen extragonadally are the progenitors of the adult sex cells is overwhelming.

If the early germ cell line is considered to be a tissue, then it is one of only a few types of tissue which functions during its early life history as independent cellular units. Only well after these cells populate the gonads are they found intimately connected in nests by intercellular bridges (Gondos, 1973; Fawcett, 1975). During migration close associations between germinal and somatic cells have been described by Zamboni and Merchant (1973), but it is not known whether one cell type plays a dominant role over the other type or if there is a mutual influence between the two cell lines. The process of induction by one class of tissue to direct a further differention of another tissue is well known (Davidson, 1969; Hamburgh, 1971). Since the early work of Spemann & Mangold (1924) the role of inductors in development has been accepted as a normal and necessary part of organogenesis. Concerning the process of gonadogenesis, one might question whether the germ cells are a factor in the process of sexual differentiation and gonadal

growth. On the other hand, the converse could be the question: what is the role of the somatic elements of the gonads on subsequent germ cell differentiation? Undoubtedly, the germinal and somatic cell lines influence each other since ectopic germ cells do not induce the formation of a gonad in the alien tissue they come to lie in. Nor do these ectopic germ cells exhibit independent differentiation towards becoming mature germ cells (Falin, 1969).

One method of testing for the presence of tissue specific interactions is to remove one of the suspect cell lines and then observe the subsequent developmental events of the other tissue. In mammalian systems, it is difficult to intervene in the process of embryogenesis during the time when the germ cells are populating the gonads and still expect normal events to occur since the culture of mammalian embryos at this time is technically difficult (Hsu, 1973). X-irradiation (Mintz, 1960) and specific chemical inhibition (Hemsworth & Jackson,1963; Merchant 1975) have been used in mammals to ablate the early germ cell line. These methods of course expose themselves to the somatic tissue as well and therefore they may be said to be non-selective in that they may well be influencing the very tissue to be studied.

The present study was initiated to investigate the development of murine gonads in the absence of germ cells. A genetic mutation carried by mice which is known to affect early germ cells was chosen to produce a mammalian model

having agametic or nearly agametic gonads. The gene Steel (Sl), first described by Sarvella and Russell (1956), arose spontaneously in an inbred strain of C3H mice at the Oak Ridge National Laboratory, Oak Ridge, Tennessee. Steel has also been reported to have arisen spontaneously in a colony of wild mice from Peru (Wallace, 1971). The effects of Steel in the homozygous state are pleiotropic in that complete sterility, macrocytic anaemia and lack of hair pigmentation results (Sarvella & Russell, 1956; Bennett, 1956; Mayer & Green, 1968, Russell & Bernstein, 1966). To date, little attention has been directed at the problem of the sterility resulting from this mutation. Bennett (1956) studied the germ cells in 8 to 9-1/2 day embryos and concluded that the germ cells of mutants arise in numbers equal to that of the normals but they failed to proliferate and migrate which resulted in agametic gonads. This conclusion was based only on the examination of some 14 and 15 day gonads which were found not to contain germ cells. Similarly, male and female gonads of newborn Sl/Sl pups were found to be agametic. Later, Younglai and Chui (1973) incidentally reported in a study on the hormone levels in mature Steel males that the mature testes from Steel homozygotes were entirely lacking germ cells. These reports served as the basis for choosing the mutation Steel as a model for this study.

Since Sl/Sl has been found to drastically affect viability from 16 days post coitus (Bennett, 1956), the less

severe Steel/Steel dickie (Sl/Sl^d) combination was developed (Russell & Bernstein, 1966). Each of these genes has been transferred to non-isogenic backgrounds to produce hybrid vigor and to reduce non-viability in offspring from heterozygate matings (Russell and Bernstein, 1966).

Since there were no previous reports prior to the present study concerning germ cells and gonadogenesis in mice bearing the $\underline{Sl/Sl^d}$ mutations, the first part of this work was directed at the question of when and how many germ cells were present when they first arose. Once this was determined the next step was to determine whether the mutant germ cells proliferated and/or migrated towards the gonadal anlage. This latter point of course was dependent upon devising a means of identifying mutant embryos. If $\underline{Sl/Sl^d}$ gonads proved to be agametic, then the status of sexual differentiation of the gonads could be examined next. Once this question was determined, the growth of $\underline{Sl/Sl^d}$ gonads was compared to that of the normals. These questions form the basis of this study. Essentially it is a description of the morphological events resulting from a mutation which is known to produce sterility. Because the genetic effects of $\underline{Sl/Sl^d}$ act on the early germ cell population, then mice carrying this mutation serve as a tool to further understand the processes involved in sexual differentiation and gonadogenesis.

MATERIALS & METHODS

Mature virgin female mice of the strain WC/ReJ-Sl/+ were caged overnight with C57Bl/6J-Sl^{d}/+ males. Both strains were obtained from The Jackson Laboratories in Bar Harbor, Maine. The presence of a vaginal plug at 0800 hours was considered to be day zero of gestation. Pregnant females were sacrificed at exactly 9, 10, 11 and 12 days after finding a vaginal plug. The pregnant females were sacrificed by cervical dislocation and their uteri were exposed, the location of the conceptuses and resorbing moles in the uteri was recorded and the uteri were then removed and placed in cold Hank's Balanced Salt solution (GIBCO).

Deciduomas (conceptuses) of nine days gestation were carefully dissected free from the uterus and immediately fixed whole in cold 95% EtOH. Deciduomas of ten and eleven days gestation were similarly dissected free from the uterus. Each embryo was then exposed by cutting a slit in the yolk sac and staged by counting the somite pairs. The placenta, yolk sac and embryo were kept intact and fixed in cold 95% EtOH. Embryos of twelve days gestation were dissected free from placenta and yolk sac. Somite pairs were counted on the 95% EtOH fixed embryos which were then decapitated to reduce the amount of material to be sectioned. All tissue was kept in fixative at 10° C for 24 hours with one change

of fresh 95% EtOH. The tissue was next dehydrated in two one hour changes of cold 100% EtOH. Following this, tissue was cleared in two - one hour changes of benzene.

A low melting point (50-52°C) was used for infiltration. Tissue was transferred to a mixture of one part 45°C M.P. paraffin and one part 56° M.P. paraplast (Sherwood) and kept under high vaccuum for 20 minutes at 52°C. The tissue was next embedded in 56° paraplast and immediately solidified on a cold plate.

Blocks were sectioned at 10μ, mounted on albuminized slides and allowed to dry overnight. Slides were deparaffinized in three 60 second changes of xylene and hydrated in distilled water via one minute changes in 100%, 80%, 50% and 40% acetone solutions.

Sections were stained for alkaline phosphatase based on the azo-dye technique of Gomori (1951). Fast Red TR Salt (Sigma) (250mg), and α-Napthyl phosphate sodium salt (Sigma) (400mg), were added to 500ml fresh Tris buffer at pH 9.4 just prior to staining. The Fast Red TR salt and α-Napthyl phosphate sodium salt were kept stored in a dessicator at -20°C when not being used. After 5 minutes mixing the solution was fast filtered into staining jars. Sections were allowed to stain for 15 minutes and then returned to distilled water. All slides were cover-slipped with glycerine jelly (1 part gelatin, 6 parts H_2O, 7 parts glycerine, 1 phenol cystal) as a mounting medium (Mintz, 1957).

After staining, 9 day embryos were staged by counting somite pairs from the serial sections. The location and number of primordial germ cells (PGCs) were determined by examining every second section. The number of PGCs for each of the 9, 10 and 11 day embryos was also determined by a second independent observer without having prior knowledge of the first counts. Counting by both observers commenced from the first section showing germ cells and then every second section after that. In this way a standard starting point was used by both observers. Any counts differing by more than 5% were redone by both observers. When counts were within this limit the first count was used in the analysis.

Sexing

The genetic sex of all fetuses of 12 days and older was determined according to the method of Vickers (1967). The amnion was removed and spread flat on a clean glass slide and stained with a few drops of 2% aceto-arcein (GIBCO). After staining for 15 minutes, the slides were cover-slipped and blotted to remove excess stain. The presence or absence of a sex chromatin body was scored for fifty randomly selected cells in each amnion at a magnification of 1000 x. The histological sex of 13 day fetuses and older were checked against the chromatin sex scores. From birth on, the animals were sexed by gross inspection of the distance between the anus and urinary papilla and the size and position of the gonads.

Fetal Size

Crown rump lengths of all 13 day fetuses and older were measured under a Wild stereoscope fitted with a calibrated occular micrometer. The fetuses were entirely covered with Hank's Balanced Salt solution when measured. Crown rump lengths and body weights were also measured for all animals from birth onwards.

Volumetry

Gonadal volumes were estimated according to the methods of Beaumont and Mandl (1962,1963) Weibel et al. (1966) and Mittwoch (1969). Depending upon the size of the gonad to be measured, every 5th, 10th or 20th section was examined under a binocular compound microscope (Wild) fitted with a calibrated ocular disc with a grid etched onto it. The grid consists of 11 x 11 equally spaced lines intersecting at right angles to each other giving a total of 121 equally spaced points of intersection. At a magnification of 100 x the points are spaced from each other at a distance of 70μ and therefore each point represents an area of $(70\mu)^2$ in the field. Since the number of points superimposed on a section is proportional to the area of that section (Weibel et al, 1966), then the volume of a sectioned organ can be estimated (Mittwoch, 1969). For example, given a section thickness of 8μ and by counting every 10th section, then the distance between sections equals 80μ. Therefore, by counting the number

of points which are superimposed on every 10th section, a volume can be determined by the formula $V(\mu^3) = (70\mu)^2 \times 80\mu \times n$ where n is the total number of points scored for a gonad. The volumes of gonads of $WCB6F_1$ - +/+, (Sl/+ or $Sl^d/+$) and Sl/Sl^d offspring were calculated for gestational days 12 to birth.

The percentage of gonadal tissue occupied by germ cells was estimated by the method of Chalkley (1943). In this method sections of gonadal tissue were brought into focus at a magnification of 600x or 1000x. The same grid having 121 intersecting points was superimposed within the perimeter of a gonadal section. According to Chalkley (1943) the number of points or "hits" which lie on a particular cell type will represent the proportion of that cell type in the tissue being examined. In this study "hits" were scored as either germinal or somatic cells. Sections from the beginning to the middle of each gonad inspected were scored. The grid was superimposed on sections so as to cover an area from the perimeter to the center of the section. At least 500 hits were scored for each gonad measured and the number of germinal hits was expressed as a percentage of the total number of hits.

By knowing the gonadal volume and the percentage of somatic tissue, an estimate of the total volume occupied by somatic tissue can be calculated (Beaumont and Mandl 1962,1963) by simply multiplying the total gonadal volume x the percentage of somatic tissue. In addition, the calculated volume of

germinal tissue has been used in estimating the number of germ cells in developing testes and ovaries.

In dividing the gonadal volume occupied by germinal tissue by the mean germ cell volume of that gonad the total number of germ cells can be derived. Sections from fetuses of 13 days to birth were examined at a magnification of 1500x and the maximum diameter of germ cells was measured by means of a calibrated ocular micrometer having 100 divisions of 1.0μ each. Germ cells were selected on an imaginary line from the periphery of the section to the center. Only cells showing a distinct nucleolus, or in the case of some stages of prophase I of meiotic oocytes, a nucleus which identified the stage of meiosis, were measured. Twenty germ cells were measured in each of those gonads examined.

Gonadogenesis

Gonadogenesis was studied in 227 fetuses of 12-18 days gestation. The genotype of day 12 and 13 fetuses was determined by removing one gonadal ridge or early gonad from each fetus and processing the tissue for alkaline phosphatase staining by the Gomori Azo-dye technique as described earlier. Germ cells were counted on every second section and recorded. The contralateral gonad was removed and fixed in aqueous Bouin's fixative for 24 hours. After bleaching for three hours in three changes of 70% EtOH, the tissue was dehydrated in three one hour changes of 1, 4-Dioxane (Fisher) and para-

plast infiltrated (M.P. 56°C) in a vacuum oven for 40 minutes. Serial sections were cut at 8μ (AO Spencer 820 microtome) and stained with either hematoxylin (Harris) and eosin or Mallory's Azan stain according to Pearse (1968).

Fetuses of 14 days and older can be genotyped according to the method described by Chui & Loyer (1975_a). After the amnion was removed for sexing and crown-rump measures taken, each fetal-placental unit was rinsed in Isoton red blood cell diluent (Coulter Electronics) to remove any maternal blood cells which may have adhered to the fetus. Each fetus was placed in a clean Falcon dish (35 x 10 mm), and decapitated with iridectomy scissors. The head of each fetus was then dropped into a vial containing Isoton and allowed to bleed for a few minutes. The head and any small pieces of tissue were removed from the vial which was then shaken gently to suspend the red blood cells in the Isoton.

Within 30 minutes of collection, the mean cell volume of the red blood cells was analysed on a Coulter counter Model B (Coulter Electronics). The results of each analysis were plotted on a Coulter channel analyser Model P64 and the results for each embryo within a litter were compared against each other.

After genotypes were determined, the gonads from each fetus were removed. One gonad was fixed for alkaline phosphatase staining while the other was processed for H & E staining. Some gonads were selected for staining with Mallory's Azan stain.

Neonates were identified as Mutant (Sl/Sl^d) or normal (+/+, Sl/+ or Sl^d/+) according to body and liver pallor. By one week after birth the genotype of each pup could be determined according to coat color.

Mature WCB6F$_1$ - +/+ and Sl/Sl^d males and females were obtained from the Jackson Laboratories. These animals were sacrificed between 8 - 12 weeks of age. The gonads from these animals, and in some cases the entire reproductive tract of one side, were processed for histological examination. Body weight, crown-rump length and gonadal measures were recorded. Genotypes were determined according to coat color.

Photography

All photomicrographs were taken on a Wild M2-104 microscope equipped for photomicrography. Kodak Panatomic X film (ASA 32) was used and prints were made on Kodabromide F-3 paper.

RESULTS

Mice carrying the genes $\underline{Sl/Sl^d}$ are sterile, therefore matings were carried out between the fertile heterozygotes, WC/ReJ - $\underline{Sl/+}$ x C57BL/6J - $\underline{Sl^d/+}$. The resulting offspring from such crosses could be any one of $WCB6F_1$ - $\underline{+/+}$, $\underline{Sl^d/+}$, $\underline{Sl/+}$ and $\underline{Sl/Sl^d}$. The combination of Sl/Sl^d is expected to occur at a frequency of 25 percent.

In order to test the hypothesis that the Steel mutation in the homozygous condition affects either germ cell proliferation or a reduction in the germ cell population and an impaired ability of these cells to migrate, three litters of day 9 post coitus (p.c.) four day 10, four day 11 and three day 12 litters were obtained for histology. The number of implants and resorbing moles was recorded for each litter (Table 1). The mean number of implanted embryos/litter was 9.50 while the mean number of viable embryos/litter was 8.14.

Although embryos were collected at one day intervals, they were classified according to the number of somite pairs in order to prevent the germ cell counts of developmentally retarded embryos from misleading the interpretation of the results.

Table 1

The number of implants, resorbing moles and viable embryos from litters of 9, 10, 11 and 12 days gestation

Day	Litter No.	No. Implants		No. Resorbs		No. Embryos Viable
		L	R	L	R	
9	1	3	7	0	1	9
9	2	4	5	0	2	7
9	3	5	4	0	0	9
		12	16	0	3	25
10	1	3	8	0	0	11
10	2	6	5	3	1	7
10	3	3	8	0	0	11
10	4	3	5	1	1	6
		15	26	4	2	35
11	1	3	8	1	1	9
11	2	4	7	0	0	11
11	3	4	3	2	1	4
11	4	6	4	1	0	9
		17	22	4	2	33
12	1	3	6	2	1	6
12	2	7	1	0	0	8
12	3	4	4	1	0	7
		14	11	3	1	21
TOTALS	14	58	75	11	8	114

Only two of the 114 embryos of days 9 - 12 p.c. had to be reassigned to another day. They occurred on day 10 and had somite counts falling in the day 9 range (Table 2). The final numbers of embryos assigned to each of the four days examined were, 27 day 9, 33 day 10, 33 day 11 and 21 day 12.

The total number of PGCs counted for each of the embryos of days 9 - 12 are presented in Tables 3, 4, 5 and 6. If indeed the PGCs in mutant embryos were unable to proliferate after their appearance around day 9 p.c., then it was expected that 25% of the embryos of days 10, 11 and 12 would show a PGC population which was no greater than the highest number of germ cells found in any day 9 embryo.

After all the PGC counts were completed, it was found that 26.4% of all the 10, 11 and 12 day embryos contained less than 126 PGCs, the number 126 being the highest PGC count found at 9 days. The 26.4% level was found to be well within the expected 25% frequency (X^2 = 8.925, $0.96 > P > 0.98$).

A chi-square goodness of fit test showed that at 10 days, the number of embryos having 126 or less PGCs (11/33= 33.3%) does not differ significantly from the 25% expected frequency (X^2 = 2.86, $0.5 > P > 0.1$, 3 d.f.). Similarly at 11 days, 9/33 or 27.3% of the embryos have less than 126 PGCs and this number does not differ significantly from the expected frequency of 25% (X^2 + 5.077, $0.5 > P > 0.1$, 3 d.f.). At 12 days 3/21 or 14.28% is also within an expected 25% frequency (X^2+0.988, $0.9>P>0.5$, 2 d.f.).

Table 2

Mean number of somite pairs and somite pair ranges from embryos of 9, 10, 11 and 12 days gestation arranged according to germ cell counts from 0-126 (presumed mutants) and 127 and greater (presumed normals). PGCs = primordial germ cells.

	0 - 126 PGCs			127 PGCs +		
Day	No. Embryos	Mean	Range	No. Embryos	Mean	Range
9	25	12.8	9-16	0	0	0
10	12	25.5	8-32	23	28.6	24-33
11	9	40.8	36-43	24	39.3	35-42
12	3	52.7	46-53	18	50.8	39-57

Table 3

The number of germ cells scored from embryos of each of three litters of 9 days gestation and arranged in ascending order.

Litter No.	No. Embryos	Germ Cells Nos.
1	9	31,38.45,48,49, 69,79,96,126
2	9	23,52,78,79,79 80,85,94, 115
3	7	39,47,52,65,74, 93,106

Table 4

The number of germ cells scored from embryos of each of four litters of 10 days gestation and arranged in ascending order. Asterisk (*) denotes 2 embryos with somite counts equivalent to day 9.

Litter No.	No. Embryos	Germ Cell Nos.
1	11	16,44,49,69,133,137,142 169,173,231,266
2	7	90,97,104,189,260,275,371
3	11	53*,82,92,96,127,178, 196,218,219,239,272
4	6	84*,118,138,164,208,249

Table 5

The number of germ cells scored from embryos of each of four litters of 11 days gestation and arranged in ascending order.

Litter No.	No. Embryos	Germ Cell Nos.
1	9	52,162,305,521,601,618,690 923,1230
2	9	10,20,70,78,99,309,681,698 829
3	4	362,683,759,941
4	11	16,26,31,141,238,300,311, 323,419,578,1215

Table 6

The number of germ cells scored from embryos of each of three litters of 12 days gestation and arranged in ascending order.

Litter No.	No. Embryos	Germ Cell Nos.
1	6	103,144,871,1077,1444,1552
2	8	0,815,962,1034,1154,1369, 1417,1500
3	7	50,191,1188,1661,1927,2213, 3417

Based on these data, embryos having a PGC count of 126 or less were considered to be the $\underline{Sl/Sl^d}$ mutants. The litter mates would then be $\underline{+/+}$, $\underline{Sl^d/+}$, or $\underline{Sl/+}$ and will be referred to as normals as a distinction between these genotypes could not be determined. The mean number of PGCs for both normal and mutant embryos of days 9 - 12 are presented in Table 7.

Table 7

Mean number of PGCs for day 9 embryos and day 10, 11 and 12 presumed normal and mutant embryos.

		Genotype		
Day	Total	Undetermined (No.)	Normal (No.)	Mutant (No.)
9	27	69.6 (27)	-- -- --	-- -- --
10	33	-- -- --	206.9 (22)	77.9 (11)
11	33	-- -- --	576.5 (24)	44.7 (9)
12	21	-- -- --	1329.8 (18)	51.0 (3)

Using the criterion that embryos having 126 or less germ cells at 10 days gestation and older are mutant, the mean number of somite pairs counted for the presumed mutants and normals was compared. Referring back to Table 2, it can be seen that mutant embryos were developmentally equivalent to the normals when judged by somite aging. As mentioned previously, two embryos of 10 days gestation were analysed with the day 9 group in order not to bias the results pertaining to germ cell migration. Those two embryos also presented as day 9 embryos with respect to their overall state of organogenesis.

Germ Cell Migration

The first appearance of the primordial germ cells and their subsequent migration has been well documented for the mouse (Chiquoine, 1954; Mintz and Russell, 1957; Mintz, 1957; Jeon & Kennedy, 1973; Spiegelman and Bennett, 1973; Zamboni and Merchant, 1973). The primordial germ cells (PGCs) are first obvious between 7.5 to 8.5 days of gestation (Chiquoine, 1954; Ozdzenski, 1967). At this time they are found exclusively in the caudal end of the primitive streak, allantoic mesoderm and yolk sac splanchnopleuce. On day 9 the PGCs are found primarily in the hind gut splanchnopleure although many occupy sites ectopic to this region such as the placenta, allantoic bud and the yolk sac and its forming stalk. During this time, those germ cells in the gut are almost exclusively located laterally and ventrally.

During the tenth day the PGCs are found ventrally, laterally and in the dorsal aspects of both gut endoderm and mesoderm. (Figure 1) They also occupy the developing dorsal mesentery, the coelomic angles and the newly forming gonadal ridges. Most germ cells along the path of migration are found in the dorsal mesentery, its root and the coelomic angles. Fewer germ cells are located in the gut at 10 days than at 9 days. Some PGCs are found in ectopic sites such as the skin ectoderm (caudal) and posterior limb buds.

On day 11 most germ cells are in the gonadal ridges and the adjacent coelomic angles, mesenteric root and dorsal mesentery. Some germ cells are still located in the gut and ectopic sites.

By day 12 almost all the germ cells are in the gonadal blastema (Figure 2). It is at this time that the migration of the germ cells is said to be completed. Few germ cells are reported to be outside of the gonadal blastema by day 12 (Chiquoine, 1954; Mintz, 1957).

The number of PGCs reported by Chiquoine (1954) range from 100 - 150 at 8 days, 400 - 480 at 9 days, 900 - 1000 at 10 days, 1575 - 1625 at 11 days, and an estimated 2000 - 2500 at 12 days. Mintz has reported the following ranges: Day 8, 15 - 76; day 9, 149 - 379; day 10, 339 - 677; day 11, 863 - 4022 and day 12, 2582 - 5711. From preliminary work for this study in which normal Swiss Albino mouse embryos were used, the ranges of germ cells fit a pattern similar to those of Chiquoine and Mintz (Table 8).

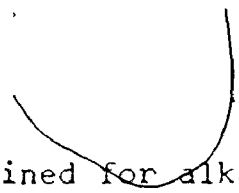


Figure 1. Primordial germ cells (arrow) stained for alkaline phosphatase in the gut endoderm of a 10 day embryo. x 100.

Figure 2. Gonadal ridge of a 12 day fetus containing primordial germ cells. x 100.

1

2

Table 8

The range of numbers of PGCs and the mean number in normal Swiss Albino mouse embryos of gestational days 8-10.

Age	No. Embryos	PGC range	Mean no. PGCs
8	3	5 - 25	13
8.5	3	62 - 96	74
9	22	178 - 404	287
10	11	451 -1138	827

These data demonstrated the outstanding increase in the germ cell population from one day to the next. More importantly, there is no overlap in the ranges of germ cell numbers. The preliminary data also showed that 73% of the germ cells at day 9 are located in the gut whereas less than 3% are located there by day 10. By day 10 almost 95% of the germ cells were found in the dorsal mesentery, coelomic angles and gonadal ridges.

If the germ cell defect in $\underline{Sl/Sl^d}$ mutant embryos was one of proliferation and/or retarded migration then it was postulated that it would reveal itself in the form of low germ cell numbers and a non-progressive effort to reach the gonadal ridges. If the $\underline{Sl/Sl^d}$ germ cells were incapable of proliferating, then throughout gestation, no mutant embryo was expected to have a germ cell population in excess of the highest number observed at 9 days gestation.

Once a criterion had been established for the identif--

ication of the Sl/Sl^{d} embryos, the location of their germ cells was compared to that of the normals. At day 9 the total number of germ cells counted for all 27 embryos examined ranged from 23 - 126 with a mean of 69.6. The percentage of PGCs in the gut ranged from 15 - 90% with a mean of 56%. In all cases germ cells were found resident in the gut region.

From day 10 to 12 all embryos having a germ cell count of less than 126 were presumed to be the Sl/Sl^{d} mutants and those with counts above 126 were considered to be the normals. This latter group had from 10 - 138 PGCs located in the gut with a mean of 45 or 22%. A mean of 108 (range 31 - 222) or 53% were found in the dorsal mesentery, the mesenteric root, coelomic angles and gonadal ridges. In the mutants, from 1 - 44 were situated in the gut with a mean of 29 or 39%. An average of 22 or 30% (range 7 - 48) were in the dorsal mesentery, root, angles and ridges at this time.

On day 11, a mean of 16 (range 3 - 33) or 3% of the PGCs in normals are located in the gut while 83% or a mean of 480 (range 43 - 1121) are in the mesentery, root, angles and ridges. In the mutants a mean of 9 (range 0 - 22) or 20% were found in the gut and a mean of 16 (range 1 - 55) or 20% were in the mesentery, root, angles and ridges.

On the 12th day less than 1% of the germ cells in normals were found in the gut and only 4% in the mutants.

Germ cells were found residing in the yolk sac, yolk sac stalk, allantois and placenta in both normals and mutants from day 9 through 11. Germ cells were also found within the embryos of days 9 - 12 in other sites ectopic to the migration path. The mean numbers and their percentage of the total PGCs are presented in Table 9.

Occassionally germ cells were found tightly clustered together in both normals and mutants (figure 3). These tight clusters, or clumps, were almost always situated in the allantoic mesoderm and placenta or in the umbilicus of older embryos.

Table 9

The mean number of PGCs and their percentage of the total number of PGCs scored in the yolk sac and its stalk plus allantois - placenta (extraembryonic) and ectopic sites within the embryos of days 9 - 12 p.c.

Day	Extraembryonic		Ectopic	
	Mean	%Total	Mean	%Total
9	12	17	15	21
10 Normal	36	17	28	13
Mutant	29	37	6	8
11 Normal	58	10	60	>1
Mutant	19	42	<1	<2
12 Normal	--	--	46	<4
Mutant	--	--	<1	<1

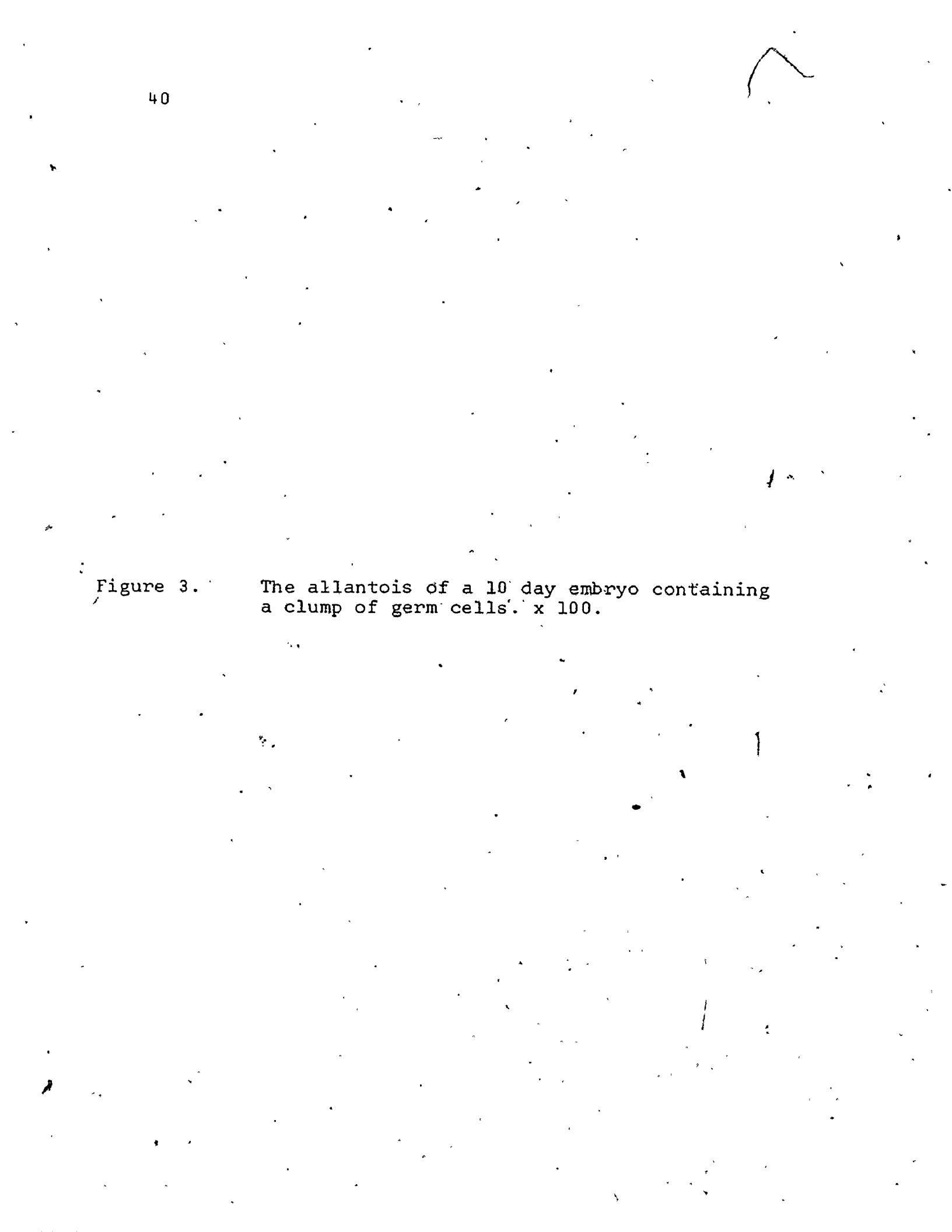


Figure 3. The allantois of a 10 day embryo containing a clump of germ cells. x 100.

Half of the mutant embryos and one quarter of the normals had some clumped germ cells, however these clumps were of no value in identifying mutants. In only one instance were germ cells found clumped in a site considered to be the path of migration. One 10 day embryo (mutant) contained a clump in the hind gut region. Ectopic germ cells within the embryo were found in bizzare sites such as the metanephros, adjacent to the dorsal aorta and somitic region. Of those found in the posterior limb buds, most were in the ectoderm.

The mean number of total PGCs of the day 9 counts (Figures 4 and 5) and the counts of the presumed mutants of days 10, 11 and 12 do not differ significantly according to a one-way analysis of variance ($F=2.09$, $P>0.25$). However, a dramatic difference exists between the day 9 mean and the means of the day 10, 11 and 12 normal embryos, the three latter representing a three-, eight-, and nineteen- fold increase respectively. There was no significant reduction in the total number of PGCs of mutant embryos during the migratory period between days 9 - 11 of gestation. However, during this time, a reduction in the PGC population in the region of the gut (Figure 6) is seen in both normal and mutant embryos. At the same time, a great increase is seen in the region of the mesentery, mesenteric root, coelomic angles and gonadal ridges in normal embryos (Figure 7). When compared to the normal embryos, these same areas in mutant

Figure 4. The percent frequency of total primordial germ cell (PGC) counts as arranged on a logarithmic scale from embryos of 9, 10 and 11 days gestation. Using the number 126 as a cut off point, it is seen that two populations are evident on days 10 and 11.

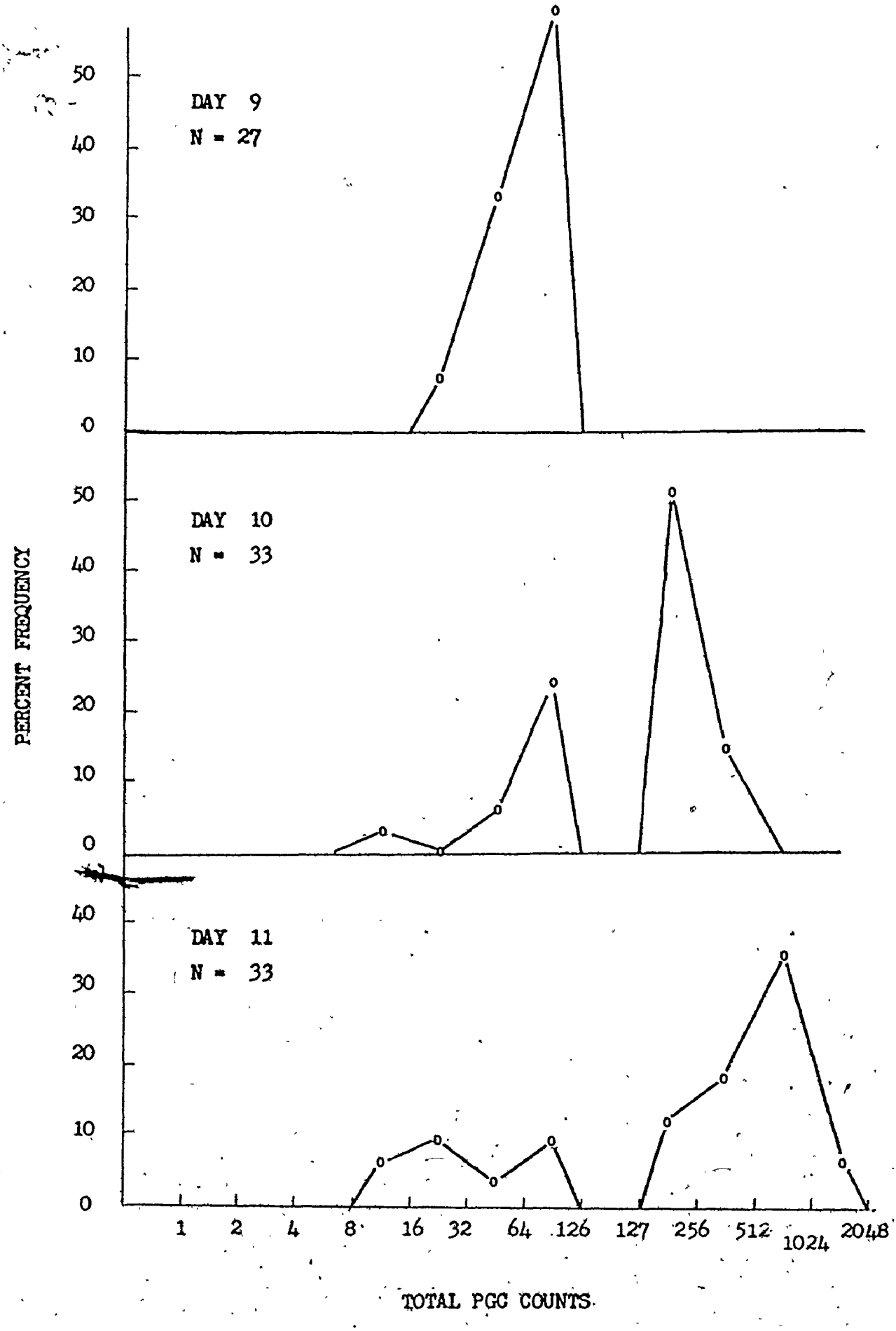

DAY 9
N = 27
DAY 10
N = 33
DAY 11
N = 33
PERCENT FREQUENCY
0
10
20
30
40
50
1
2
4
8
16
32
64
126
127
256
512
1024
2048
TOTAL PGC COUNTS

Figure 5. An increase in the mean number of primordial germ cells (PGCs) is seen for days 9 through 12 for normal embryos whereas the mutant PGC population remains the same. Error bars= standard deviations.

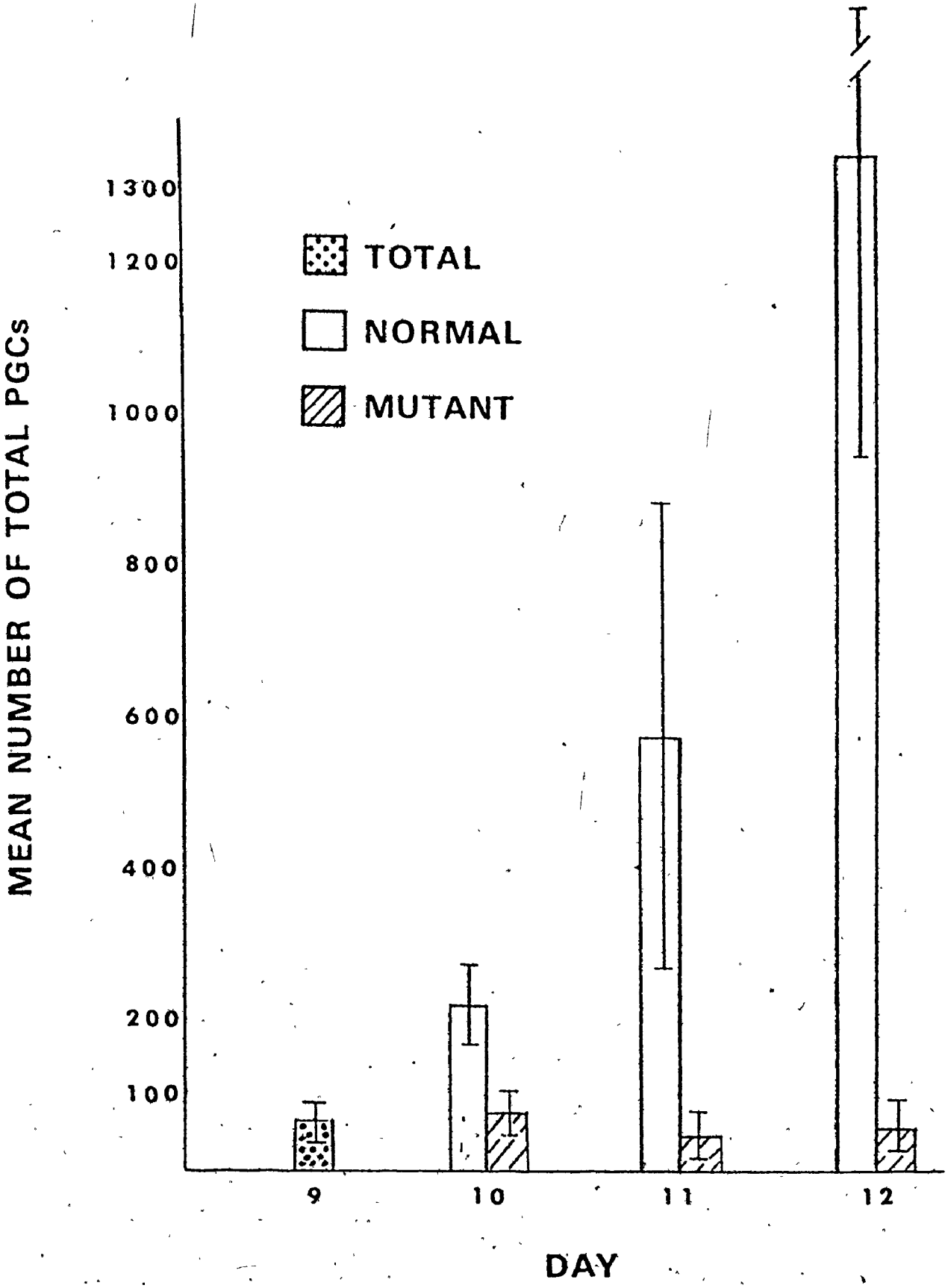

MEAN NUMBER OF TOTAL PGCs
1300
1200
1000
800
600
400
200
100
TOTAL
NORMAL
MUTANT
9
10
11
12
DAY

Figure 6. A decrease in the mean number of primordial germ cells (PGCs) from the gut endoderm in both mutant and normal embryos indicates an exodus of germ cells from this region after day 9 of gestation. Error bars=standard deviations.

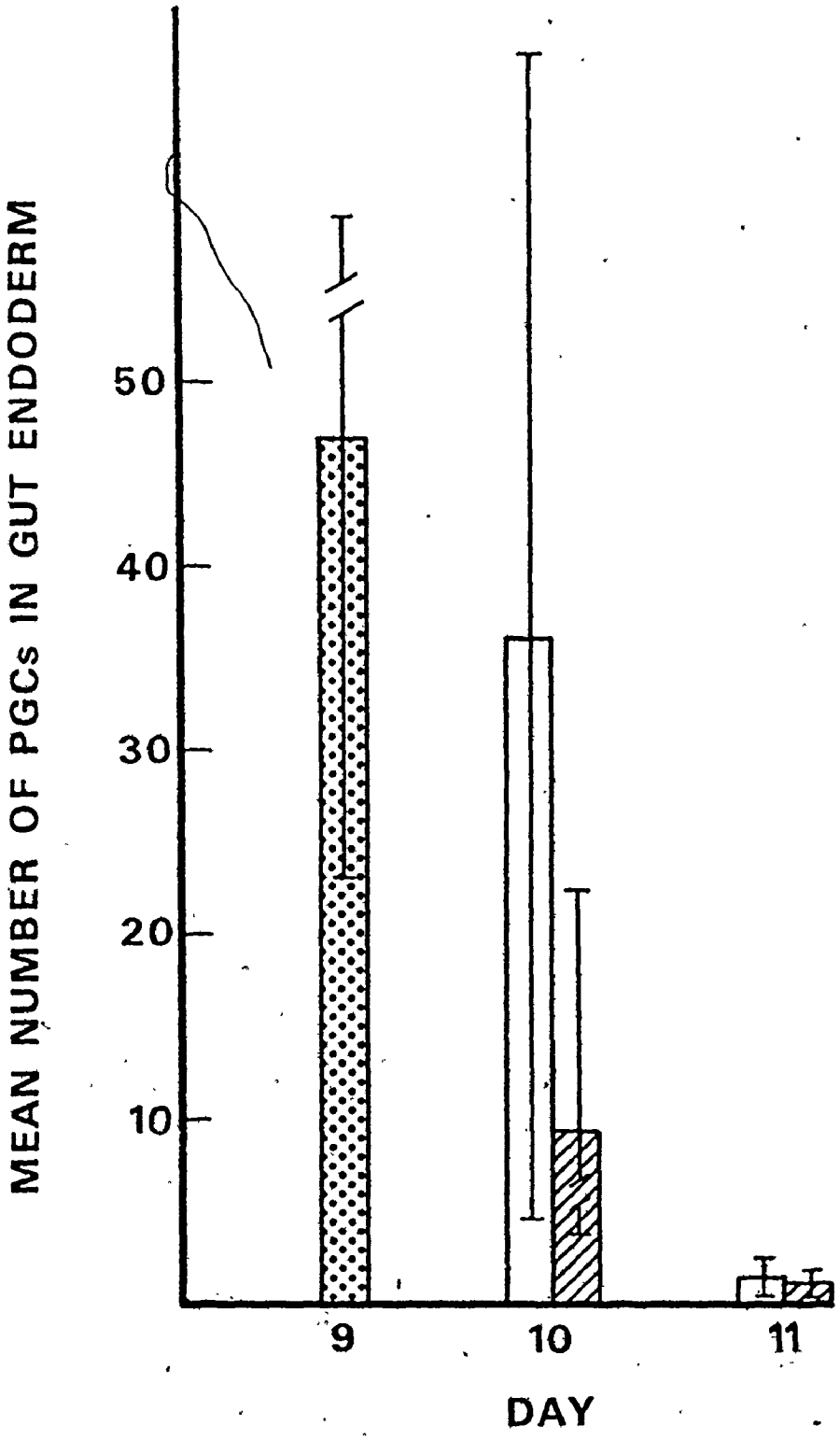
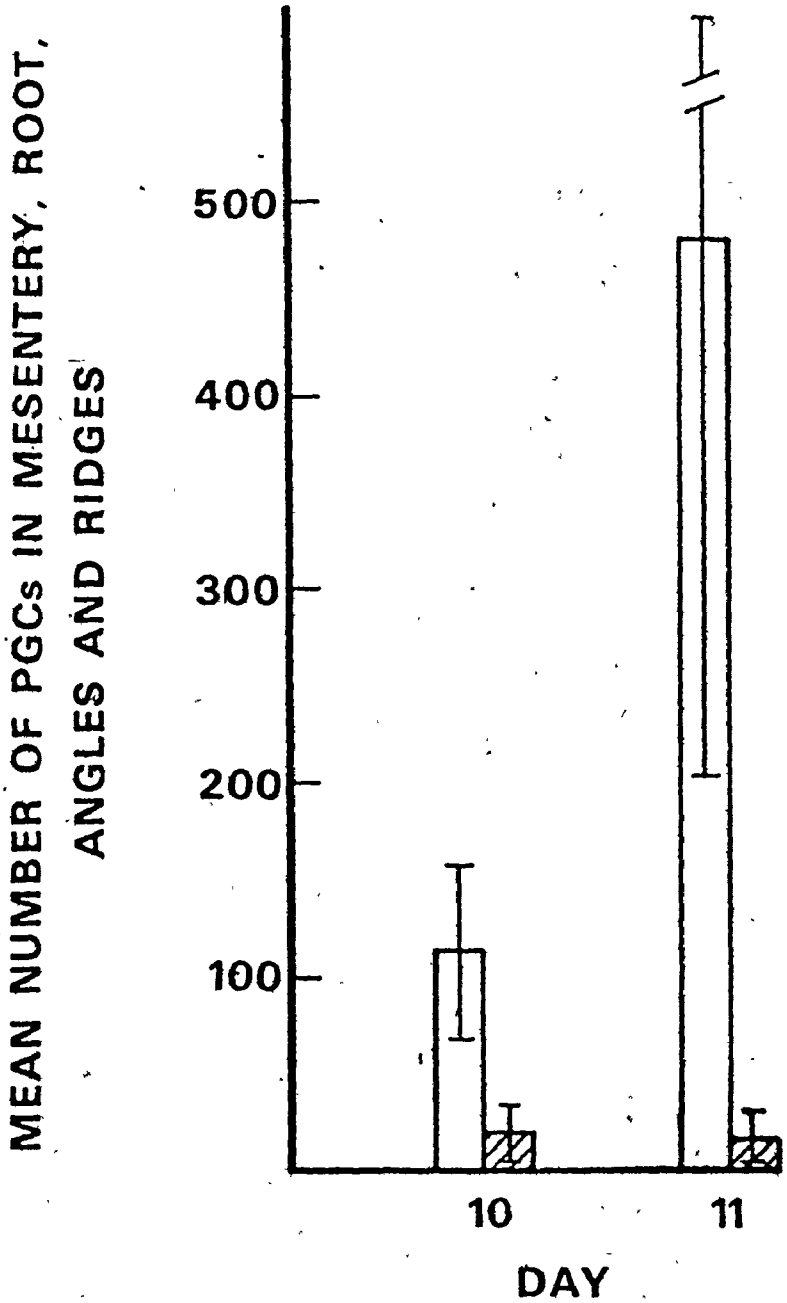

MEAN NUMBER OF PGCs IN GUT ENDODERM
50
40
30
20
10
9
10
11
DAY

Figure 7. An infiltration and proliferation of the primordial germ cells (PGCs) into the mesentery, mesenteric root, coelomic angles and gonadal ridges of normal day 10 and 11 embryos. Mutant PGCs are in these same locations on the same days. Error bars = standard deviations.


MEAN NUMBER OF PGCs IN MESENTERY, ROOT, ANGLES AND RIDGES
500
400
300
200
100
10
11
DAY

embryos are also populated with PGCs (Figure 7).

The mean number of PGCs scored in the gonads of day 12 embryos was 1138 for the normals and 51 for the mutants, one of which contained no PGCs throughout the entire embryo. The PGCs of mutants, although few in number, appear in the same locations on the same days as do normal PGCs. Less than 2% of the normal germ cells reached the gonadal ridges by day 10, whereas over 70% populate the ridges after 11 days. In mutant embryos, 23% of the germ cells reached the ridges by day 11 and an additional 12% were found in the adjacent mesenteric root and coelomic angles.

Also, 44% of the mutant germ cells were found in sites ectopic to the normal migration path. The remaining 21% were found in the mesoderm surrounding the gut. Considering that there is no apparent proliferation of mutant PGCs, it would appear that their rate of migration up to 11 days is equivalent to the normals. In other words, at least 63% of the PGCs in the normal migration path will reach the gonadal ridges.

Two of the three mutant embryos of 12 days gestation also support this view. In one case 89.3% of the germ cells were in the gonadal primordium or immediately adjacent to it. In the second case 100% of the germ cells were either in the gonads or immediately adjacent to them. One of the mutants had no germ cells whatsoever (Figure 8). In the normals, anywhere from 87.1% to 99.4% are found in the same regions.

Figure 8. A 12 day gonadal ridge (arrow) from an embryo identified as a mutant because of a lack of primordial germ cells. Compare to figure 2. x 100.

8

Ovarian Gonadogenesis (Day 12 - Birth)

The growth of normal and mutant ovaries was studied in terms of gonadal volume, percent germinal tissue, germ cell volume and numbers of germ cells. The sex of all fetuses studied was determined by the sex chromatin test of Vickers (1967).

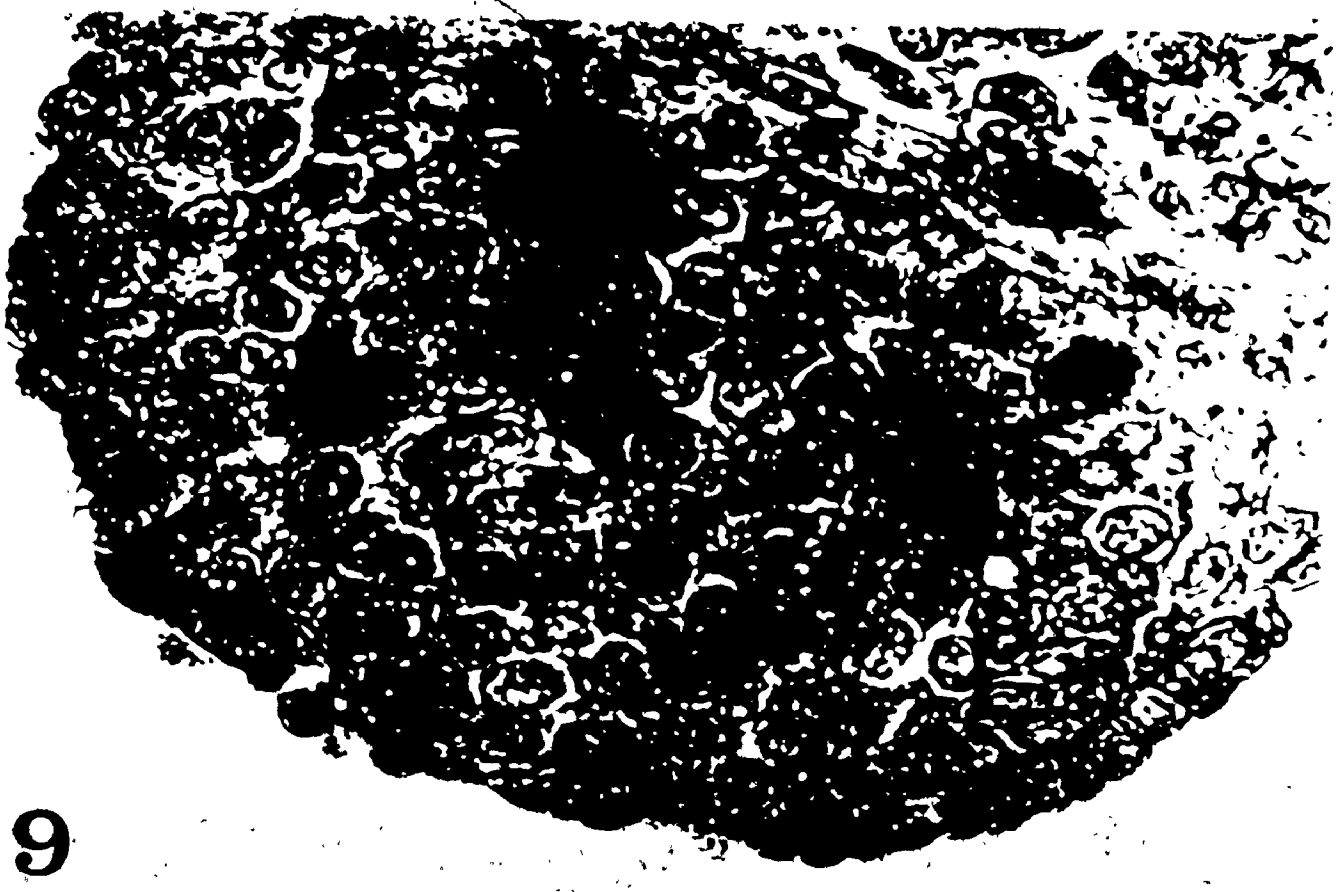
On day 12 p.c., gonads are indistinguishable as to sex by histological criteria (Figures 9 and 10), whereas by day 13 the development of sex cords in male gonads is apparent in both normals and mutants (Figures 11 and 12). From day 12 to 13 there is a two-fold increase in ovarian volume in the normals after which there is only a slight increase in the total volume up to day 15. From day 15 - 18 the ovarian volume remains quite constant (Table 10). By birth the ovarian volume again shows a dramatic increase (Table 10 and Figure 13).

In Sl/Sl^{d} ovaries, an increase of approximately 1.5 fold is seen from day 12 to 13 p.c. After this time the ovarian volumes remain constant up to birth (Table 10). Only by day 14 is there a significant difference ($t=3.1130$, $P<0.01$) between normal and mutant ovaries with respect to their volumes. This significant difference continues up to birth.

The volume of the somatic elements occupying normal ovaries was determined by deducting the calculated volume of germinal tissue occupying these organs from day 13 to birth. The resulting values were tested against the ovarian volumes

Figure 9. An indifferent gonad from a normal 12 day fetus identified as a male by the sex chromatin test. x 400.

Figure 10. An indifferent gonad from a normal 12 day fetus identified as a female by the sex chromatin test. x 400.



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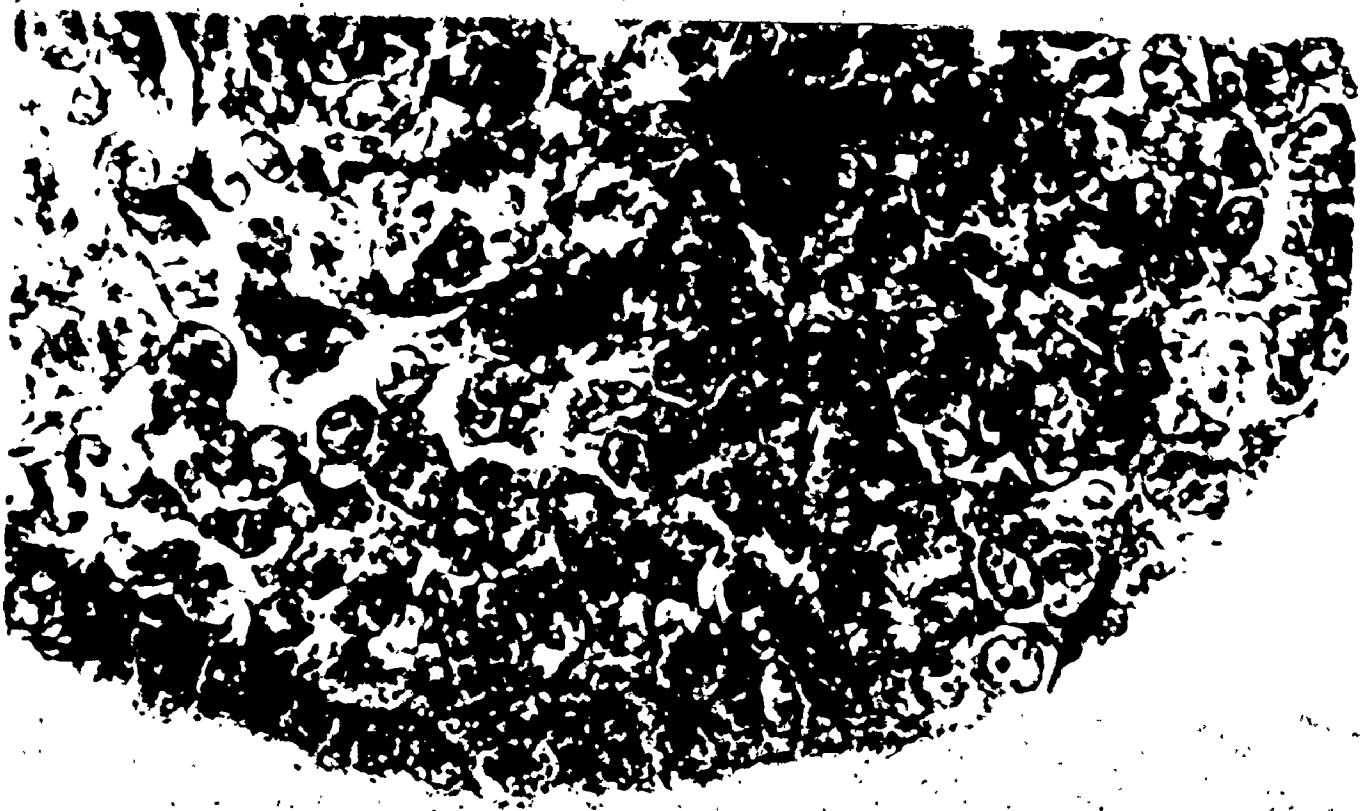
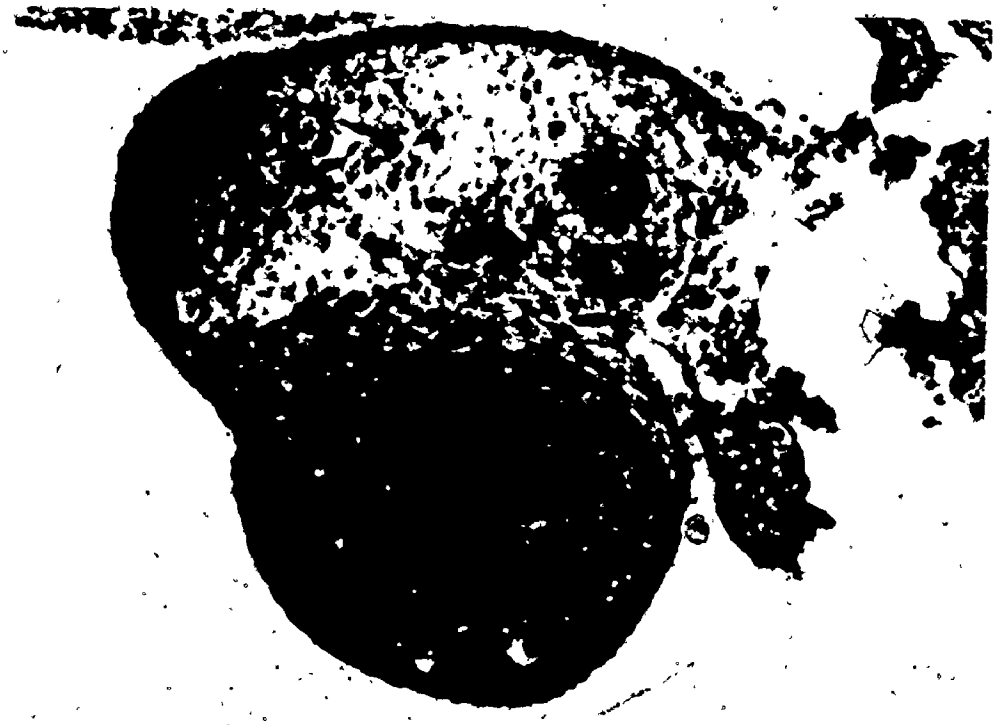


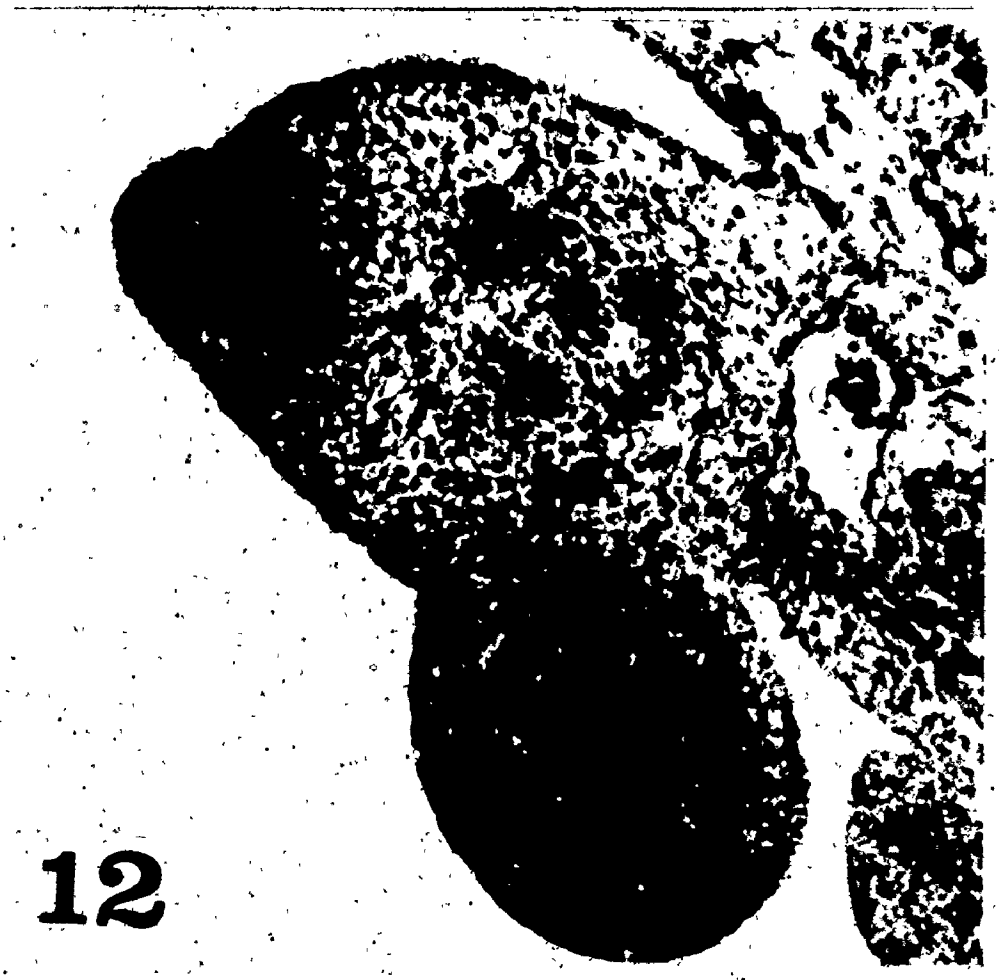
10

Figure 11. Sex cord differentiation (arrow) seen in a 13 day genetic male of normal genotype. Mallory Azan, x 100.

Figure 12. Sex cord differentiation (arrow) seen in a 13 day genetic male of $\underline{Sl/Sl^{d}}$ gentotype. Mallory Azan, x 100.



11



12

obtained for mutant gonads.

There was no significant difference between the volume of somatic tissue in normal ovaries from days 13 - 18 versus the mutant ovaries which are greater than 99% somatic tissue only. Only on the day of birth was the volume of somatic tissue in normal ovaries significantly greater than that of the mutants ($t=5.2917$, $P<0.001$; Table 11 and Figure 13). It is at this time that follicular development becomes very apparent in normal ovaries.

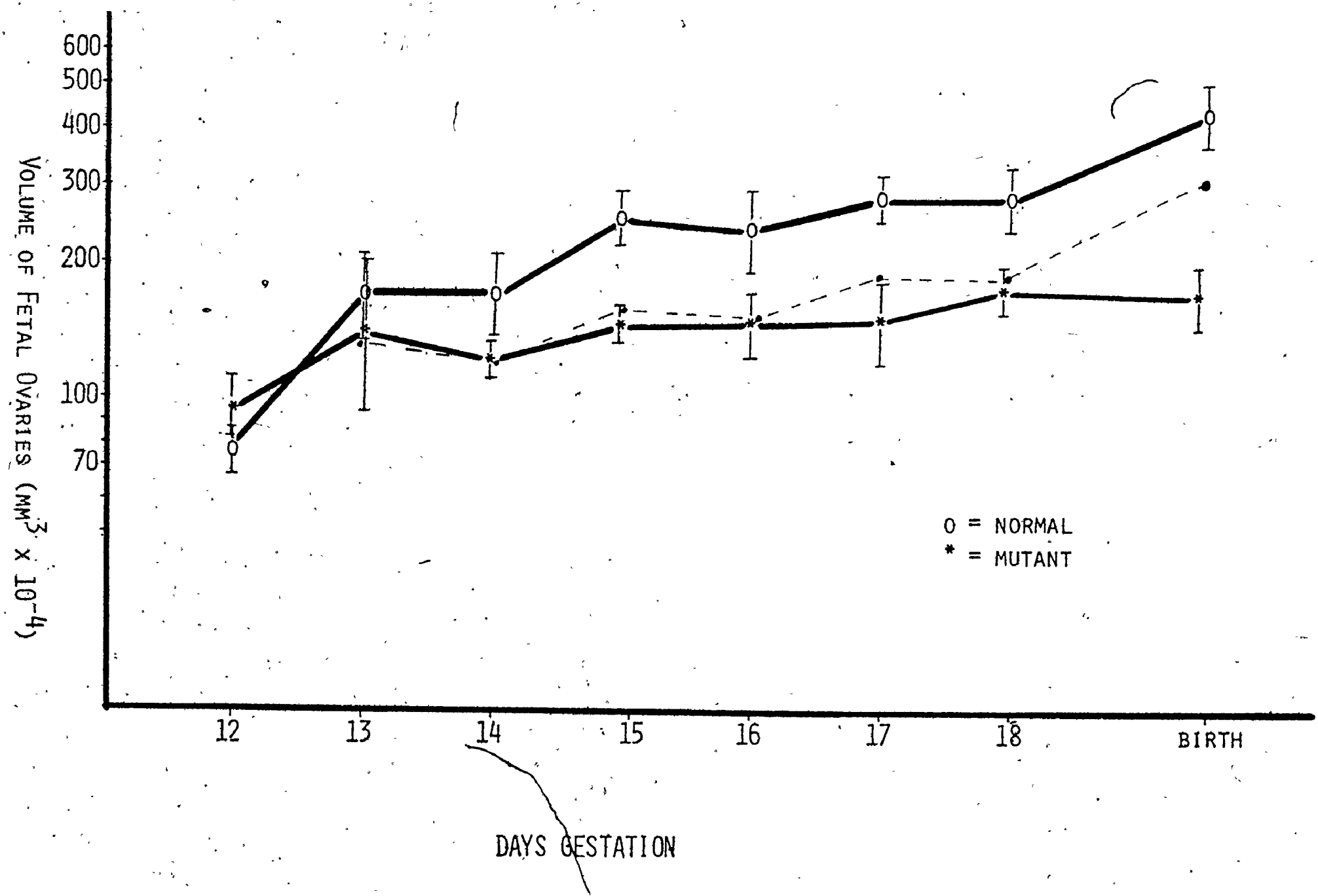
Table 10

Gonadal volumes from normal and mutant female fetuses from day 12 of gestation till birth.

	Mean Volume ($mm^3 \times 10^{-4}$)						
Day	Normal	SD	Mutant	SD	d.f.	t	P
12	79	± 6.00	98	±16.97	4	2.20	>.05
13	180	±36.09	151	±57.98	5	0.8255	>0.5
14	178	±38.12	128	± 9.91	12	3.1130	<.01*
15	264	±41.78	157	± 3.54	6	3.4711	<.02*
16	255	±57.59	152	±28.63	11	4.3233	<.01*
17	299	±33.45	158	±34.70	4	5.0671	<.01*
18	298	±52.63	186	±16.82	8	4.5086	<.01*
Birth	466	±78.07	176	±30.60	7	7.6821	<.001*

* significant difference

Figure 13. A graphic illustration of the growth of normal ovaries (open circles), normal ovaries minus the volume occupied by germinal tissue (closed circles with dashed lines) and mutant ovaries (asterisks) from days 12 to birth as plotted on a log scale. Error bars=standard deviations.


600
500
400
300
200
100
70
VOLUME OF FETAL OVARIES (MM³ X 10⁻⁴)
12
13
14
15
16
17
18
BIRTH
DAYS GESTATION
O = NORMAL
* = MUTANT

During days 13 - birth the percentage of germinal tissue occupying normal ovaries increases steadily from 19.50% at day 13 to 38.13% by day 16. After this time the percent germinal tissue decreases to 28.10% by birth (Table 12). The mean volume of the germ cells increases steadily from day 13 - birth. The calculated mean number of germ cells increases from 2626 ± 726 on day 13 to a peak of 7861 ± 1244 on day 15. After this time the population of germ cells steadily decreases to 3625 ± 687 by birth (Table 13).

The number of germ cells encountered in mutant ovaries were few. Germ cells were counted directly from histosections of mutant ovaries and a summary of these counts is presented in Table 14.

Table 11

A statistical comparison between the mean volume of somatic tissue in normal and mutant ovaries from 13 days gestation to birth. (SD = standard deviation, d.f. = degrees freedom, t = Student's t values, P = probability).

	Mean						
Day	Normal	S.D.	Mutant	S.D.	d.f.	t	P
13	141	± 28.45	151	± 57.98	5	0.3224	>0.5
14	130	± 24.71	127	± 9.91	12	0.1858	>0.5
15	168	± 21.06	156	± 3.54	6	0.7305	>0.4
16	151	± 39.23	153	± 28.63	11	0.1040	>0.9
17	199	± 29.57	158	± 34.70	4	1.5450	>0.1
18	193	± 31.14	186	± 16.82	8	0.4170	>0.5
Birth	334	± 57.76	176	± 30.60	7	5.2717	<0.001*

Table 12

Percent gonadal tissue occupied by germ cells in normal ovaries from dav 13 of gestation to birth.

Day	%Germinal Tissue
13	19.50
14	28.09
15	36.17
16	38.13
17	33.60
18	32.39
Birth	28.05

Table 13

The mean germ cell diameters, volumes and calculated number of germ cells in normal ovaries from day 13 of gestation to birth. (S.D. = Standard Deviation, GC = germ cell).

Day	Mean GC diam. (μ)	SD	Calculated Mean Vol (μ^3)	SD	Calculated Mean #GCs	SD
13	12.28	± 1.52	969	± 4	3,626	± 726
14	12.55	± 1.39	1035	± 51	4,830	± 1034
15	13.24	± 1.54	1215	± 59	7,861	± 1244
16	13.68	± 0.98	1339	± 52	7,261	± 1640
17	14.16	± 0.97	1488	± 50	6,753	± 756
18	15.24	± 1.95	1852	± 173	5,213	± 920
Birth	19.02	± 3.20	3606	± 624	3,625	± 607

Table 14

A summary of the number of germ cell counts in mutant ovaries for days 14, 16, 18 and birth:

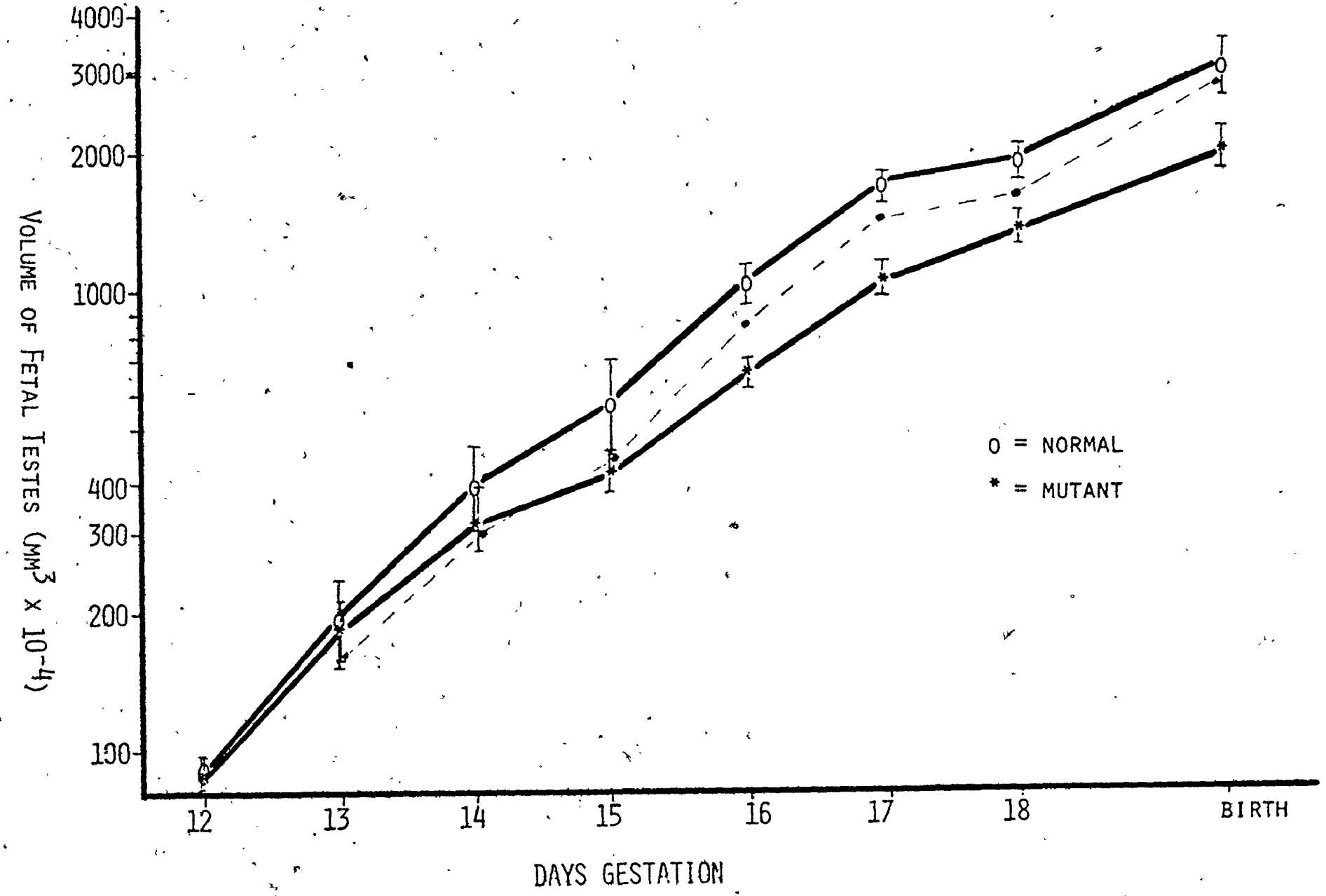
Day	No.	Range	Mean
14	6	22 - 116	60
16	8	47 - 260	127
18	5	17 - 140	89
Birth	3	0 - 54	18

A comparison between the mean number of female gonadal germ cells of day 12 (41) and the mean number of germ cells of day 14 and day 16 was made. An unpaired t-test revealed that between day 12 to day 16, the number of germ cells in the gonads increased significantly ($P < 0.05$).

Testicular Gonadogenesis (Day 12 - Birth)

On day 12 p.c. there is no significant difference between the normal indifferent gonads and mutant gonads (Table 15). Not until day 16 is the difference between normal testes and those of the mutants significant ($t = 6.6935$, $P < 0.01$). This difference continues to birth. In both normal and mutant gonads the increase in volume is approximately two-fold from day 12 to 13, after this time, normal testes increase in size at a greater rate than the mutant testes (Figure 14).

Figure 14. A graphic illustration of the gnowth of normal testes (open circles), normal testes minus the volume occupied by germinal tissue (closed circles with dashed lines) and mutant testes (asterisks) from days 12 to birth as plotted on a log scale. Error bars = standard deviations.


4000
3000
2000
1000
400
300
200
100
VOLUME OF FETAL TESTES (MM^3 x 10^{-4})
12
13
14
15
16
17
18
BIRTH
DAYS GESTATION
O = NORMAL
* = MUTANT

The volume occupied by germinal tissue in normal testes was determined (Table 16). The percentage of germinal tissue was found to increase from 10.30% on day 13 to a maximum of 20.83% on day 15. After this time the percent germinal tissue steadily decreased to 5.67% by birth.

The volume of somatic tissue occupying the total testicular volume was estimated in the same manner as for ovaries (Table 17). Somatic volumes did not differ significantly between normals and Sl/Sl^{d} testes until day 16. This is the same day on which the total volume of normals becomes significantly different from that of mutants. On day 18 no significant difference was found although the P value was found to be just above the 0.05 level.

Germ cell volumes were found to increase steadily from day 13 (633 μ^3 $\pm$ 88) to birth (1941 μ^3 $\pm$ 239). The calculated mean number of germ cells was also found to increase from day 13 (3142 μ^3 $\pm$ 707) to a peak of 16,562 μ^3 $\pm$ 1307 on day 16. The number of germ cells decreased thereafter to 9196 μ^3 $\pm$ 1658 by birth (Table 18).

Unlike the ovaries, the testes were found to continually increase in size from day 12 onwards. This was characteristic of both normals and mutants. The number of germ cells counted directly from testicular histosections from mutants is presented in Table 19.

A comparison between the mean number of male gonadal germ cells of day 12 (53) and the mean number of germ cells

Table 15

A comparison between the gonadal volumes from normal and mutant males aged 12 days gestation to birth. (d.f. = degrees freedom, t= Student's t value, P = probability).

	Mean Volume ($mm^3 \times 10^{-4}$)					
Day	Normal	SD	Mutant	SD	d.f.	t	P
12	90	± 8.00	91	± 2.12	3	0.2472	>0.5
13	193	± 43.44	183	± 27.84	12	0.3857	>0.5
14	371	± 82.06	314	± 54.96	10	1.4625	>0.1
15	567	± 125.34	406	± 45.25	5	1.6934	>0.1
16	1058	± 83.49	634	± 26.87	5	6.6935	<0.01*
17	1699	± 120.40	1129	± 155.68	6	5.7873	<0.01*
18	1869	± 174.77	1417	± 90.47	4	3.9692	< .02*
Birth	3148	± 567.69	2072	± 265.03	6	3.4357	< .02*

*Significant difference.

Table 16

Percent germinal tissue occupying normal testes from 13 days gestation to birth.

Day	% Germinal Tissue
13	10.30
14	13.99
15	20.83
16	17.11
17	12.08
18	10.94
Birth	5.67

Table 17

A statistical comparison between the mean volume of somatic tissue in normal and mutant testes from 13 days to birth (SD = standard deviation, d.f. degrees freedom, t= Student's t values, P= probability)

	Mean volume ($mm^3 \times 10^{-4}$)				
Day	Normal SD	Mutant SD	d.f.	t	P
13	159 ± 34.87	183 ± 27.84	14	1.0857	> 0.2
14	311 ± 60.14	313 ± 54.96	10	0.0692	> 0.5
15	448 ± 91.05	406 ± 45.25	5	0.5982	> 0.5
16	872 ± 54.12	634 ± 26.87	5	5.7176	< 0.01*
17	1492 ±122.46	1129 ±155.68	6	3.6879	< 0.02*
18	1662 ±190.94	1417 ± 90.47	4	2.0057	> 0.05
Birth	3013 ±456.77	2071 ±265.03	6	3.5657	< 0.02*

Table 18

Mean germ cell diameters, volumes and number of of germ cells in normal testes from 13 days gestation to birth (SD = standard deviation, GC= germ cell).

Day	Mean GC diam (μ)	SD	Calculated Mean Vol (μ^3)	SD	Calculated Mean #GCs	SD
13	10.65 ±	1.47	633 ±	88	3,142 ±	707
14	11.76 ±	1.22	852 ±	80	6,090 ±	1348
15	12.37 ±	1.35	991 ±	72	11,915 ±	2634
16	12.78 ±	1.41	1093 ±	91	16,562 ±	1307
17	13.47 ±	1.55	1279 ±	265	16,047 ±	1137
18	14.52 ±	1.60	1603 ±	60	12,752 ±	1193
Birth	15.48 ±	1.68	1941 ±	239	9,196 ±	1658

Table 19

The mean number of germ cells and their range as counted in mutant testes of days 14, 16, 18 and birth.

Day	No.	Range	Mean
14	7	14 - 67	42
16	2	59 , 137	98
18	3	0 -198	85
Birth	2	23 , 32	28

of day 14 and day 16 was made. An unpaired t-test showed that between day 12 to day 16, the difference in the number of mutant germ cells increased significantly ($P < 0,02$). A germ cell in mitosis in a mutant 16 day testes can be seen in Figure 23.

Segregation data

The homozygous wild type (+/+), heterozygotes (Sl/+ or Sl^d/+) and homozygous mutants (Sl/Sl^d) could be distinguished from days 14 to 18 post coitus. The segregation data of 21 litters representing 134 fetuses is presented in Table 20. For technical reasons, 49 fetuses from 5 litters were classified as normal (wild type or heterozygous) or mutant according to the germ cell population as observed in histosections of the gonads (Table 21).

A total of 183 fetuses ranging in age from 14 to 18 days post coitus were genotyped according to the RBC method

Table 20

Segregation data representing the number of 14 to 18 day old fetuses classified as +/+, Sl/+ or Sl^d/+, and Sl/Sl^d genotypes which were determined according to the mean size of the cells within the RBC population.

Day	No. Litters	+/+		Sl/+ or Sl^d/+		Sl/Sl^d		Total
		♂	♀	♂	♀	♂	♀	
14	4	2	3	4	4	3	6	22
15	3	5	4	7	4	0	1	21
16	5	3	3	12	12	2	7	39
17	4	3	4	8	5	3	3	26
18	4	3	7	3	3	5	5	26
TOTAL	20	16	21	34	28	13	22	134

Table 21

Segregation data of five litters of 15, 16 and 17 day old fetuses genotyped as normal (+/+, Sl/+ or Sl^d/+) and mutant (Sl/Sl^d) according to gonadal histology.

Day	No. Litters	Normal		Mutant		Total
		♂	♀	♂	♀	
14	1	2	3	4	1	10
15	1	4	2	2	1	9
16	1	5	3	0	1	9
17	3	12	8	1	0	21
Total	6	23	16	7	3	49

(134) or from gonadal histology (49). In the former method, 25% or 34 fetuses were expected to be $\underline{Sl/Sl^d}$. The number of fetuses identified as $\underline{Sl/Sl^d}$ was found to be 35 (Table 20). Of the 49 fetuses examined for mutants from the gonadal histology, 12 were expected to have a germ cell defect. Ten out of the 49 fetuses were classified as mutants (Table 21). Of the total of 183 fetuses examined 138 were classified as normal ($\underline{+/+}$, $\underline{Sl/+}$ or $\underline{Sl^d/+}$) and 45 were classified as mutants ($\underline{Sl/Sl^d}$). In both methods employed, the ratio of normals: mutants was well within the expected 3:1 ratio ($X^2 = 0.016$, $P > 0.5$). In the RBC method, the ratio of the (+/+): (Sl/+ and $Sl^d/+$): Sl/Sl^d was also found to be well within the expected 1:2:1 ratio ($X^2 = 0.806$, $P > 0.5$).

Fetuses of 12 and 13 days were genotyped as normal or mutant according to the number of germ cells present in one gonad which was stained for alkaline phosphatase. Twenty-seven fetuses were obtained from 3 litters of 13 days of age. Six of these fetuses had germ cell counts ranging from 0 - 64 (as counted from every 2nd section.) The range of the number of germ cells from one gonad of each of the remaining 20 fetuses was 522 to 1312, also counted from every 2nd section. The ratio of 20 normals to 6 mutants falls well within the expected 3:1 ratio ($X^2 = 0.051$, $P > 0.5$). Seventeen fetuses of 12 days of age were obtained from 4 litters. Five of these fetuses were found to have germ cell numbers ranging from 0 - 38 in one gonadal ridge. The range of germ cells from one

ridge of each of the remaining 13 fetuses was 684 - 782. The ratio of 13 normals: 5 mutants also falls well within an expected 3:1 ratio (X^2 = 0.074, P > 0.5). The total number of fetuses genotyped from litters ranging from 12 days to 18 days of age was 227. Of these, 171 were classed as normal (+/+, and Sl/+ or Sl^d/+) and 56 were classed as mutant (Sl/Sl^d). These numbers fit an expected 3:1 ratio (X^2 = 0.004, P > 0.9).

Sex ratio data

The genetic sex of each fetus was determined according to the presence or absence of sex chromatin bodies in the cells of the amnion. Fetuses of 14 days of age and older were also sexed according to gonadal morphology and histology. Of the 227 amnions recovered, 2 were accidentally lost prior to staining, one from a 14 day +/+ male and one from a 16 day Sl/+ or Sl^d/+ male. These 2 fetuses were sexed according to gonadal histology. A summary of the results of sexing is presented in Table 22. The total number of males and females was well within the expected 1:1 ratio (X^2 = 0.071, P > 0.5). Of the 56 mutants, 27 males and 29 females were found. These numbers fall well within the expected 1:1 ratio (X^2 = 0.991, P > 0.1)

The number of germ cells counted in the left and right gonads of the day 12 fetuses were compared to determine whether or not an asymmetry of the germ cell population existed. Each fetus had been previously sexed according to

Table 22

The numbers of male and female fetuses in litters from day 12 - 18 and the ratio of mutant males to females.

Day	Total	Males	Females	Mutants		
				Number	Male	Female
12	17	9	8	5	2	3
13	27	20	7	6	5	1
14	32	15	17	14	7	7
15	30	18	12	4	2	2
16	48	22	26	10	2	8
17	47	26	21	7	4	3
18	26	11	15	10	5	5
Total	227	121	106	56	27	29

the method of Vickers (1967). From Table 23 it is seen that the mean number of germ cells in the left and right gonads of both females and males does not differ significantly, nor is there a significant difference between the mean number of germ cells/gonad of females versus males.

Testicular Histogenesis

Testes from normal mice of 14 days gestation, when stained for alkaline phosphatase (AP), show a positive reaction for this enzyme in the surrounding coelomic (germinal) epithelium. The underlying tunica albuginea appears to be AP negative. Some interstitial cells were found to be slightly AP positive. The basement membrane surrounding the seminif-

Table 23

A statistical comparison between the germ cell population in left and right gonads from 12 day female and male fetuses and the total number of germ cells/gonad of females versus males. (P = probability)

No. Gonads	Mean No. Germ Cells	t value	P
	Female		
	L R		
12	670 ± 370 775 ± 437	0.449	> 0.5
	Male		
	L R		
20	538 ± 175 627 ± 217	1.017	> 0.2
	Total / Gonad		
	Male Female		
32	722 ± 390 582 ± 197	1.349	> 0.2

erous cords were also found to be slightly AP positive, while within the seminiferous cords the sustentacular cells failed to stain for alkaline phosphatase.

The germ cells could still be identified by the alkaline phosphatase reaction with Fast Red stain, but they appeared to stain less strongly than in earlier stages. The golgi body of germ cells still showed a strongly positive AP reaction in most germ cells despite an overall weaker staining within these cells. Some AP positive cells (germ cells) were occasionally seen extragonadally in the forming

mesorchium and mesonephic regions. These cells appeared clumped and much smaller than gonadal germ cells.

Testes from the mutant Sl/Sl^d fetuses also show an AP positive coelomic epithelium. The underlying tunica albuginea contains some slightly AP positive cells but not to the same extent as seen in the coelomic epithelium. A weak AP positive reaction is seen in some of the interstitial cells. The basement membrane of the seminiferous cords are alkaline phosphatase positive but within the cords there is an almost complete lack of an alkaline phosphatase reaction. Seldom are AP positive cells (germ cells) seen within the cords. Those few germ cells encountered show a weak AP reaction as is the case in the normal testes. At this stage mutant testes appear the same as do normal testes except for the lack of germ cells (Figures 15 and 16 and compare to figure 2).

Normal 14 day testes stained with H & E present with a well defined coelomic epithelium and tunica albuginea. The seminiferous cords are very apparent as is a surrounding basement membrane. Germ cells are well defined by a coarsely granular chromatin and a granular cytoplasm. The sustentacular cells have a finely granular nucleoplasm and fairly clear cytoplasm. The germ cells contain from three to five prominent nucleoli. Both germ cells and sustentacular cells are found intermingled within the cords but the majority of the germ cells are within the center of the cords. The basement membrane is lined principally with sustentacular cells although

Figure 15. Testis from a normal 14 day male fetus stained for alkaline phosphatase. Arrow indicates germ cells within a sex cord. The germ cells at this age stain more lightly than at 12 days. Compare to figure 2.

Figure 16. A testis from a 14 day mutant fetus stained for alkaline phosphatase. Germ cells are absent from the sex cords. The tunica albuginea is stain positive lying just beneath the coelomic epithelium. x 100.

15

16

some germ cells are also found adjacent to it. Mitotic figures within the cords are common. A dense interstitial tissue is present and an extensive vascularization is apparent at this time.

The testes of 14 day mutants when stained with H & E also show a distinct coelomic epithelium and underlying tunica albuginea. Seminiferous cords are no less apparent than that seen in normal testes. Mitotic figures are also common within the cords which are occupied by sustentacular cells having a normal appearance. A distinct basement membrane is also present around these agametic cords. Apart from the absence of germ cells, these testes appear histologically the same as those of the normals (Figures 17 and 18).

Testes from normal fetuses of 15 days gestation showed an AP reaction similar to normal 14 day testes except that the golgi body of germ cells is seldom seen now. Almost all of the germ cells were seen to be centrally located within the seminiferous cords. The overall staining of the germ cells for AP appears to be lighter than at 14 days. The coelomic epithelium is still AP positive and the underlying tunica albuginea still contains some AP positive cells. The interstitial tissue is now AP negative. Mutant testes of 15 days show an AP reaction similar to the normals except for the absence of germ cells. The seminiferous cords are as well defined in mutants as in normals with a surrounding AP positive basement membrane.

In H & E stained sections the germ cell population

Figure 17. A section of the seminiferous cords of a 14 day normal testis. Arrows indicate the germ cells within the cords. H and E, x 400.

Figure 18. A section of the seminiferous cords of a 14 day $\underline{Sl/Sl^d}$ testis. Note that the cords contain sustentacular cells only. H and E, x 400.

1

18

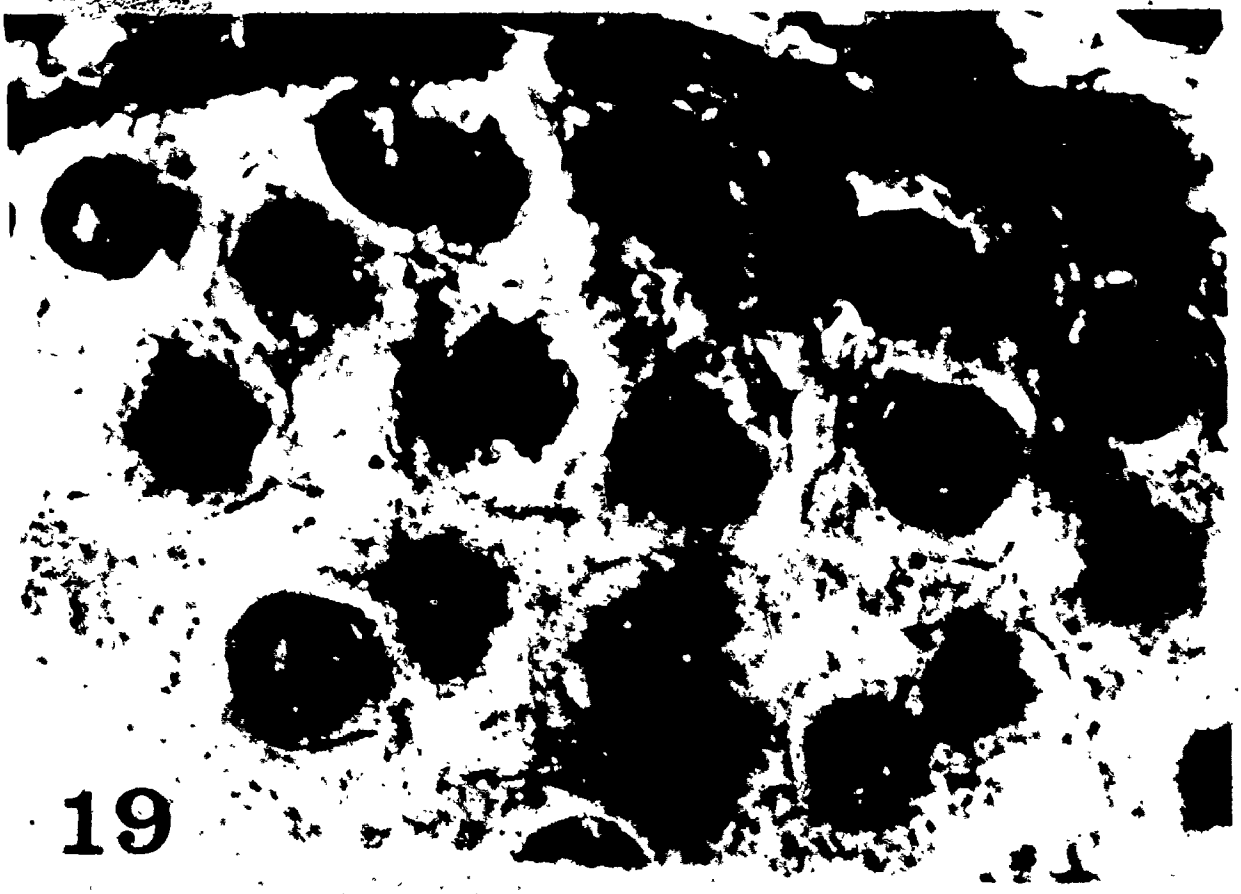
in 15 day normals is more abundant within the seminiferous cords. The cords themselves appear to have a larger diameter than one day earlier. The germ cells almost exclusively occupy the center of the cords. The coelomic epithelium is very well defined and the tunica albuginea appears to be thicker. Some interstitial cells now show a granular eosinophilic cytoplasm. The stroma within the center of the testes is now less dense and mesenchymous in appearance.

The H & E stained testes of mutants have a similar appearance to that of the normals except for an absence of germ cells and smaller diameters of the seminiferous cords. Where the few germ cells present are seen they also occupy the center of the cords. Both mutant and normal germ cells show up as having a less coarsely granular nucleoplasm than seen at 14 days, and 2 - 3 prominent nucleoli are present (Figures 19 and 20).

Normal 16 day testes stained with Fast Red maintain an AP positive tunica propria about the cords. The coelomic epithelium is now only lightly stained while the tunica albuginea has maintained its AP content. The germ cells are even less AP positive than one day earlier, they are still centrally located and the golgi body is no longer seen according to the AP reaction. Mutant testes appear the same as the normals except for the lack of germ cells. In both mutants and normals, the sustentacular cells have taken on a light Fast Red stain.

Figure 19. High power view of a section of seminiferous cord from a 15 day normal testis. Note the abundance of germ cells. H and E, x 1000.

Figure 20. High power view of a seminiferous cord from a 15 day mutant testes. Note the smaller size of the cord compared to that of the normal testes. In this view only 2 germ cells are present within the seminiferous cord (arrows). H and E, x 1000.



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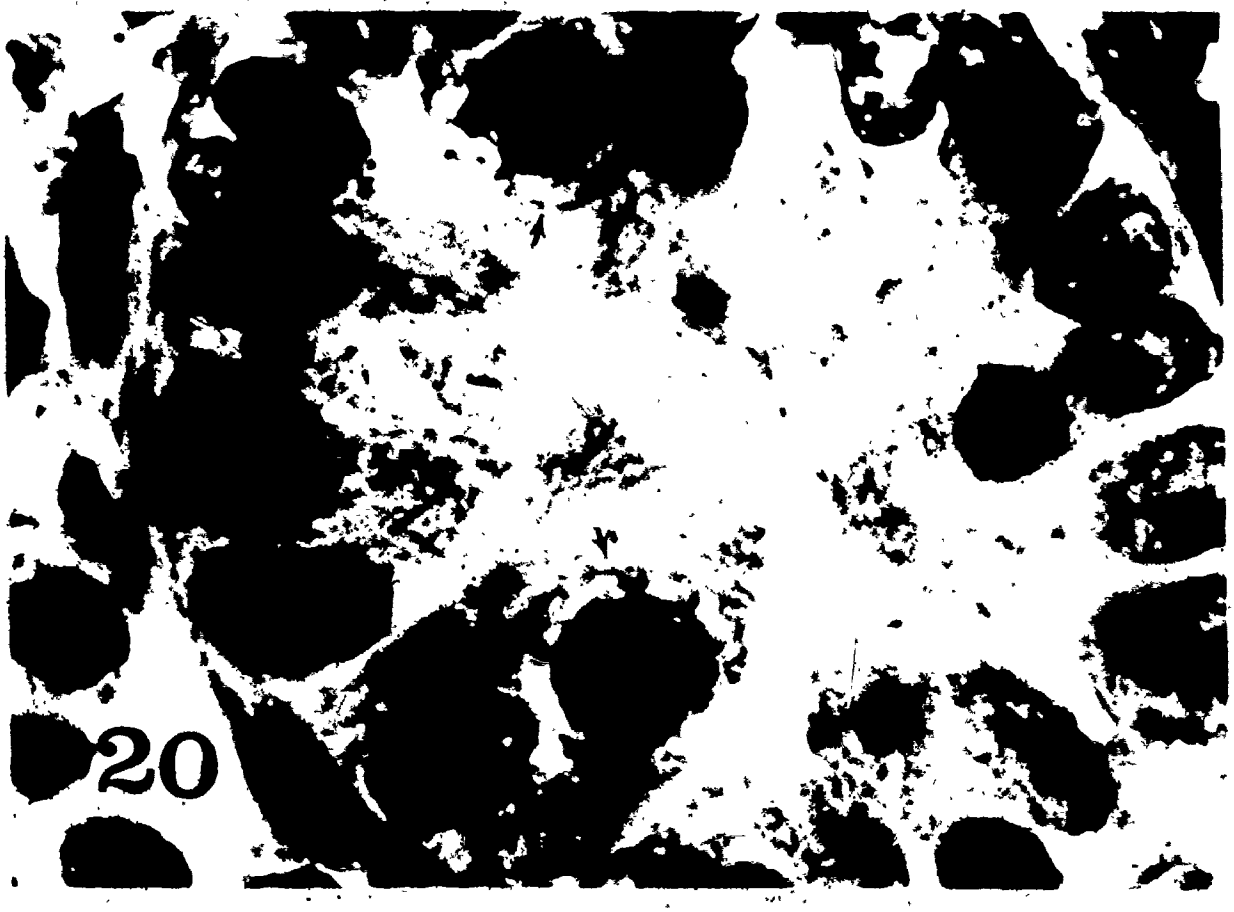

20

The normal testes of 16 days gestation when stained with H & E shows up as having larger cords with a single layered supporting epithelium. Germ cells are abundant within the centre of the cords.

The germ cell nuclei appear even more finely granular than earlier and contain 2 - 3 prominent nucleoli. Only rarely are germ cells seen adjacent to the basement membrane. Mitoses are still seen in some cells of the supporting epithelium but rarely are they seen among the centrally located germ cells. The differentiating Leydig cells are more numerous than at 15 days and there also appears to be more mesenchymous stromal cells.

In mutant testes of this age, the seminiferous cords are smaller than in normals. The supporting cells, are aligned along the basement membrane forming a distinct epithelium as in the normals. Counts of the number of supporting cells within circular sections of the cords showed that both normal and mutant cords contained equivalent numbers of these cells (24 ± 3.6 and 24.7 ± 2.8 respectively) and the difference was insignificant ($t=0.507$, $P > 0.5$). Early stages of Leydig cell differentiation is also apparent in mutants. (Figures 21 and 22). In one mutant testis, a cell resembling a germ cell in mitosis was encountered (Figure 23).

At 17 days, the normal germ cells are almost entirely devoid of AP. Only the tunica albuginea and basement membrane

of the seminiferous cords still show an AP positive reaction. In the mutant, the supporting cells are still lightly stained as they were one day earlier.

The coelomic epithelium of 17 day normal testes now consists of elongated spindle shaped cells and the cells of the tunica albuginea appear loosely arranged and fibroblastic in appearance. The Leydig cells appear to be more numerous than at 16 days. The germ cells are still not in contact with the basement membrane.

The histology of the mutant testes of 17 days resembles that of the normals except for the paucity of germ cells. Where germ cells are seen there are both dark and lightly stained nucleated ones present as is also the case in normals. Leydig cells are also numerous. Mitotic figures among the supporting cells are seen in both normals and mutants.

The AP reaction in the 18 day normal and mutant testes is confined to the tunica albuginea, the coelomic epithelium now entirely lacking a reaction. The basement membrane of the cords is still AP positive in both types of testes.

Apart from the lack of germ cells, both mutant and normal 18 day testes appear much the same when stained with H & E. The Leydig cells now appear larger. Mitotic figures in the supporting epithelium are still present and the germ cells are not as yet seen to be aligned along the basement membrane.

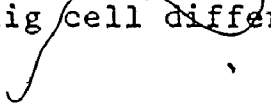

Figure 21. High power view of the interstitial tissue of a normal 16 day testis. Arrows indicate early Leydig cell differentiation. H and E, x 1000.

Figure 22. High power view of the interstitial tissue of a mutant 16 day testis. Early Leydig cell differentiation (arrows) is also apparent in mutant testes. H and E, x 1000.

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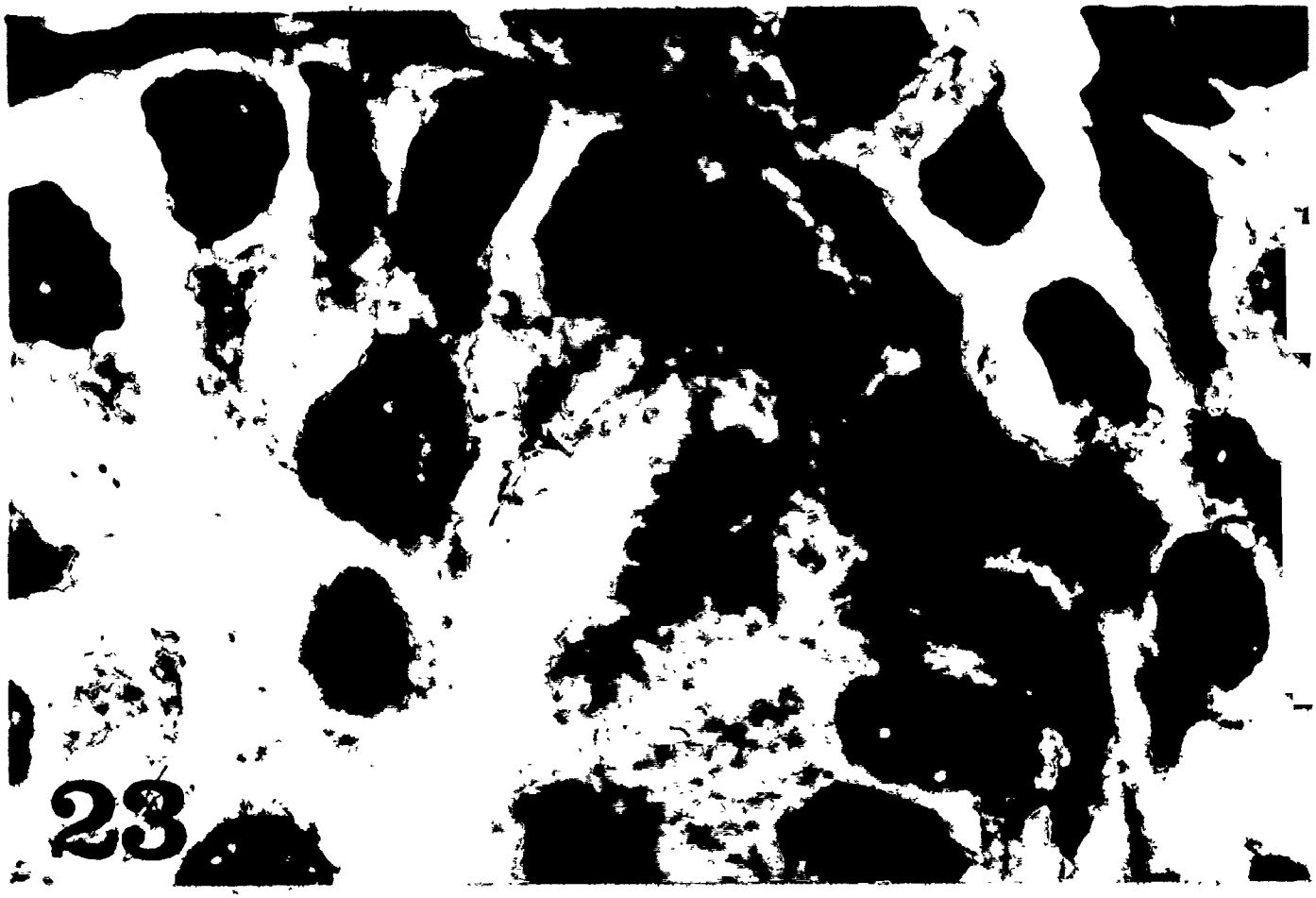
Figure 23. A single germ cell in mitosis (arrow) taken from a mutant testis at day 16 of gestation. H and E, x 1000.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By birth the testes of normals and mutants appear the same as at 18 days with regard to Fast Red staining for alkaline phosphatase. H & E sections of normals show a dramatic decrease in the germ cell population and an increase in seminiferous cord size. Many germ cells in normals are now seen in contact with the basement membrane. (Figure 24) Although degenerate germ cells were encountered at earlier stages they now appear more numerous. The mutants appear to differ only in their lack of germ cells. Where germ cells were encountered in mutant testes, they also were found in contact with the basement membrane of the seminiferous cord (Figure 25).

Ovarian Histogenesis

Normal ovaries of 14 days gestation when stained for alkaline phosphatase show that this enzyme is present primarily in the germ cells. The germ cells stain strongly for AP and the golgi body is still very prominent in some of them. Germ cells are very numerous and often found located in the coelomic epithelium. Most extend throughout the whole gland and are found in clusters or nests. In both mutants and normals the coelomic epithelium is only very slightly AP positive. Where germ cells are present in mutant ovaries they are usually found as small clusters (Figures 26 and 27).

In normal H & E stained ovaries of 14 days, a distinct coelomic epithelium is present. The development of

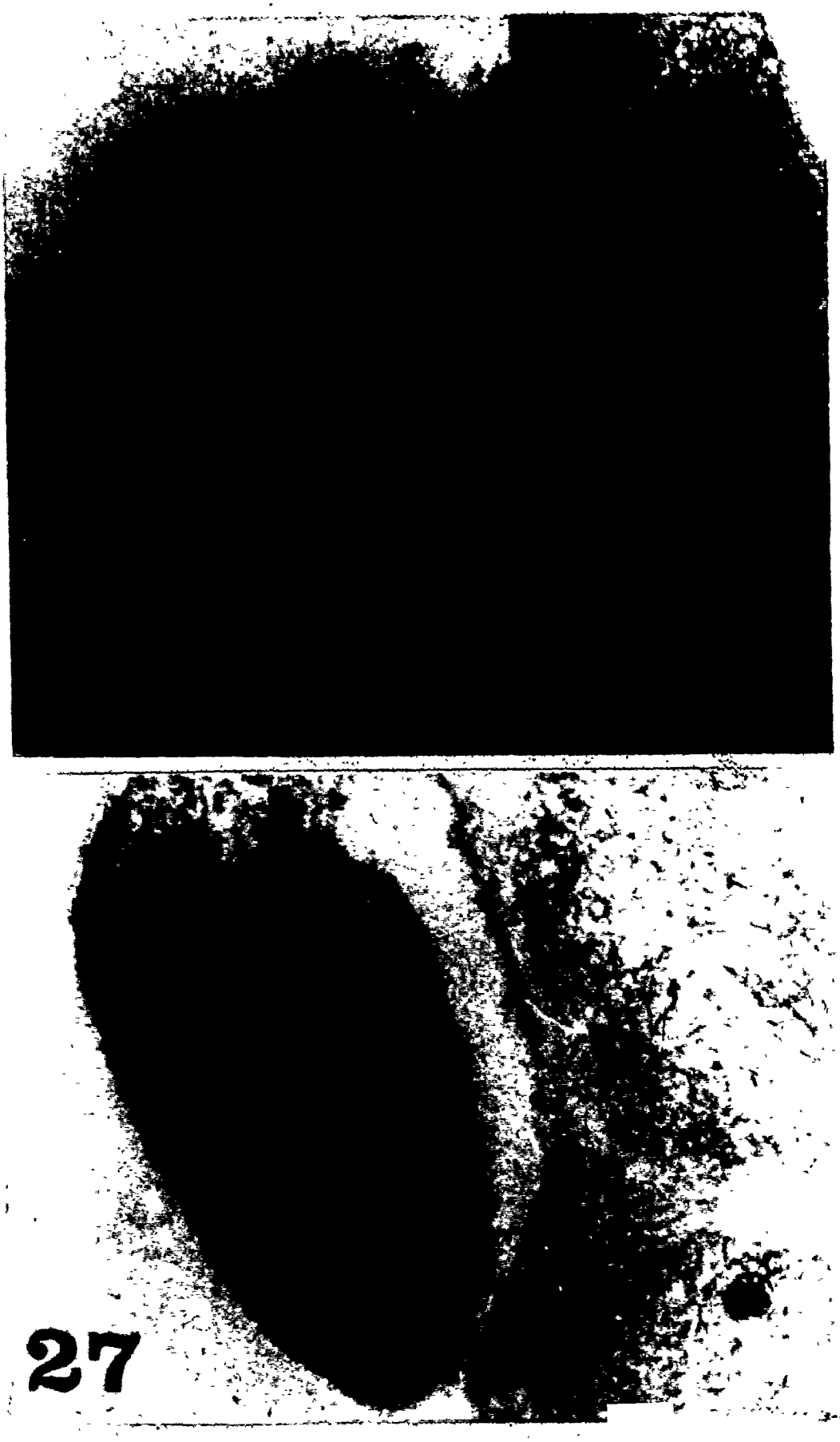
Figure 24. Section of seminiferous cord from a normal testis at birth. Note the germ cells to be located on the basement membrane (arrow). H and E, x 1000.

Figure 25. Section of seminiferous cord from a mutant testis at birth. A single germ cell in the cord (arrow) is seen to lie on the basement membrane. H and E, x 1000.

25

Figure 26. A mutant ovary of 14 days stained for alkaline phosphatase. Two dark staining germ cells are seen within the ovary. x 100.

Figure 27. A normal ovary of 14 days stained for alkaline phosphatase. Note the large population of dark staining germ cells. x 100.



27

sex cords is apparent at this time within which nests of germ cells are present. The germ cells have a coarse chromatin with 3 - 5 nucleoli in the nucleus. The cytoplasm appears finely granular and distinct. Many germ cells are in meiosis I prophase at the leptotene stage. Oogonia are still seen and there are also some signs of cell degeneration within the cords.

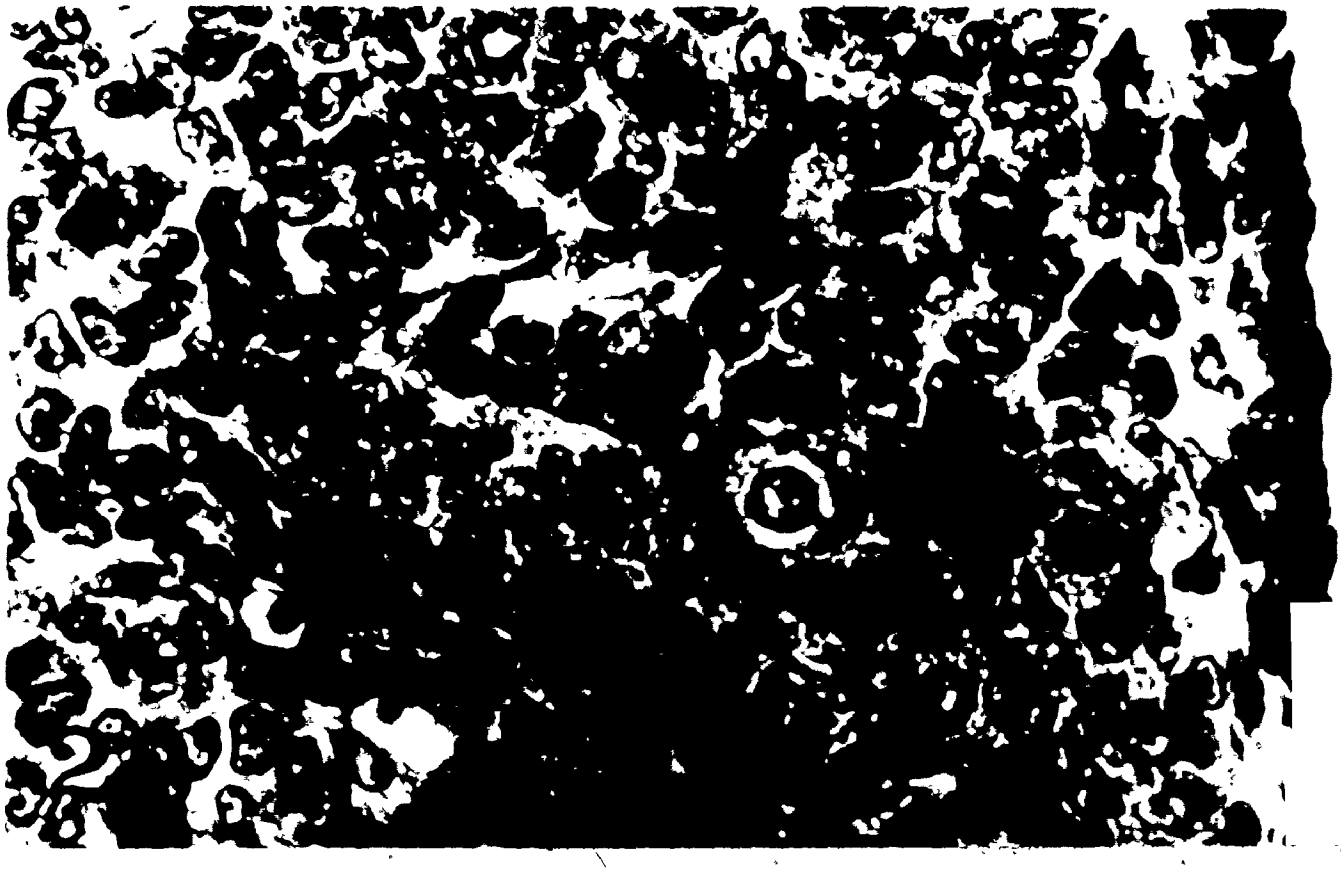
A distinct coelomic epithelium is also present in the mutant ovaries. Where germ cells are encountered they appear normal and within a sex cord. There are no signs of massive germ cell degeneration (Figures 28 and 29).

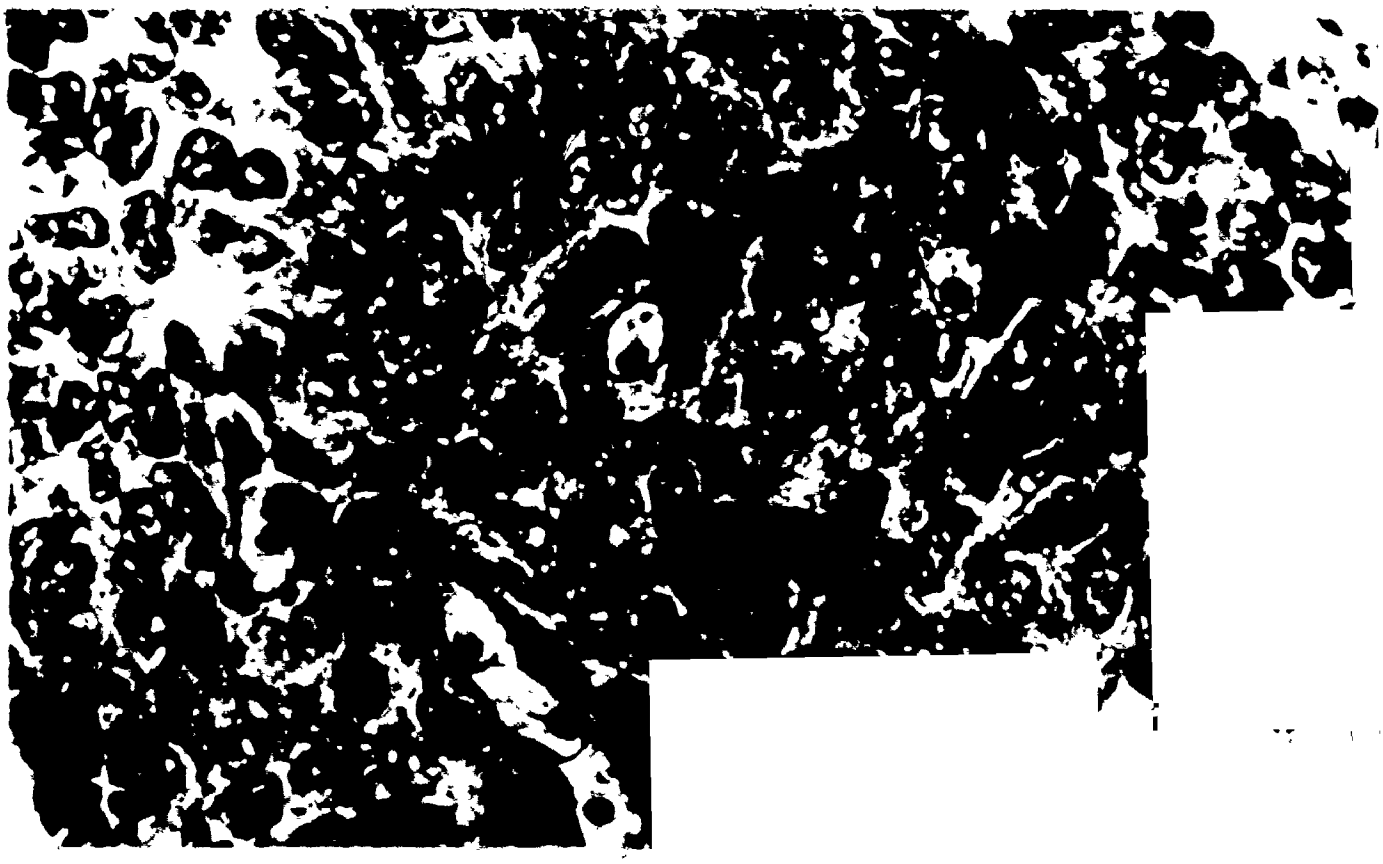
The 15 day normal ovaries do not differ markedly from those of 14 days except that most germ cells are now in leptotene and zygotene of meiosis. Few oogonia are now present and they are located just under the coelomic epithelium. Most germ cells appear to be at the same stage of meiosis within a particular nest. Extensive germ cell degeneration is not apparent at this time. Where germ cells are seen in mutant gonads, they are also in either leptotene or zygotene of meiosis.

By day 16, the AP reaction appears to be less strong in many germ cells of normal ovaries as compared to earlier days. Cross sections show that most of the germ cells are cortically located. In H & E sections it is seen that most germ cells are now in zygotene of meiosis. Degenerate cells, although present, are not abundant. Mutant ovaries are not

Figure 28. A section of normal ovary of 14 days showing the presence of germ cells within the somatic stroma. H and E, x 400.

Figure 29. The histological appearance of an agametic ovary of 14 days. Note the complete absence of germ cells in the stroma. H and E, x 400.





markedly different except for lack of germ cells. Those few seen are also in zygotene.

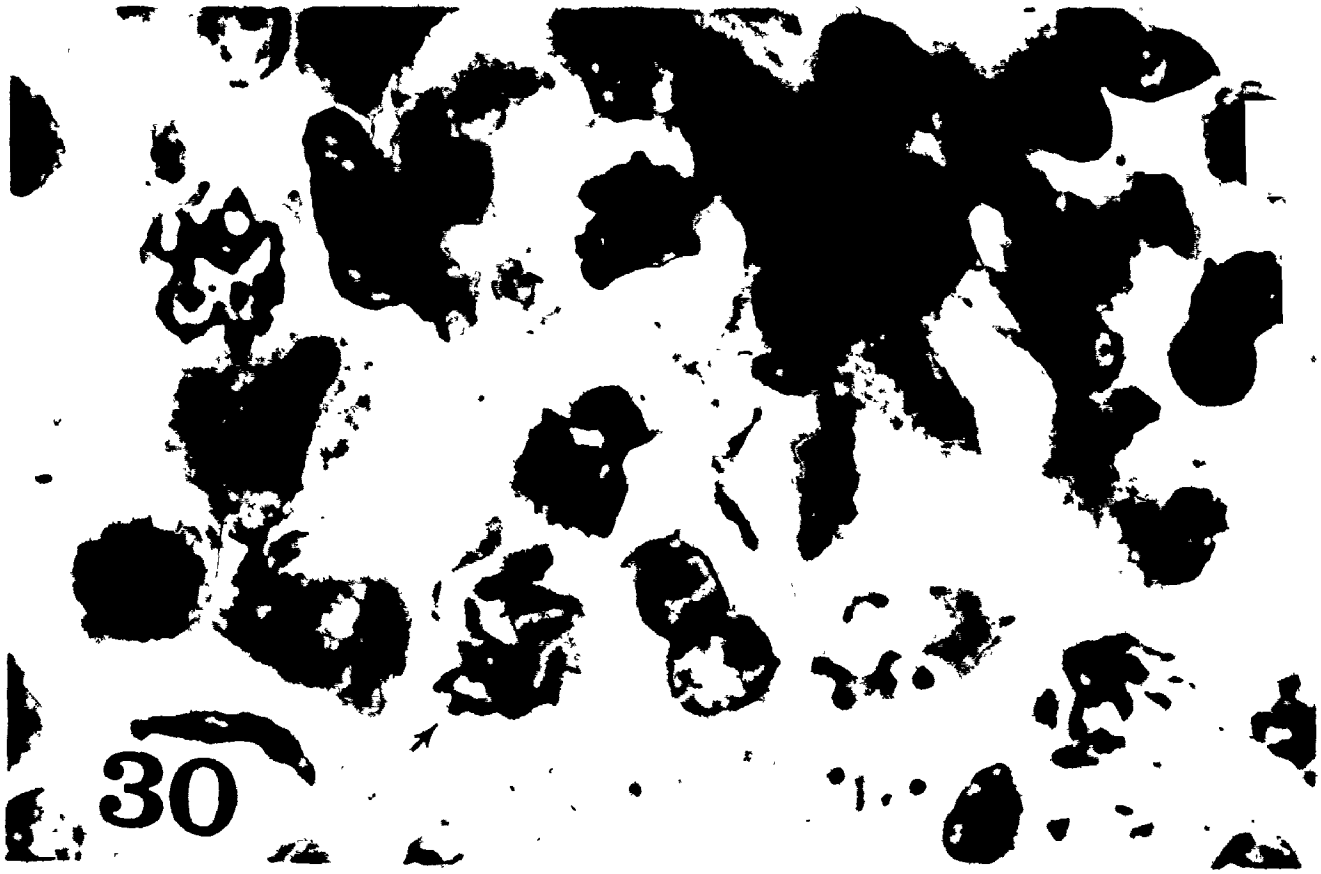
In normal ovaries of 17 days, numerous germ cells are seen in a state of degeneration. The majority of normal germ cells are in early pachytene although some are still seen to be in zygotene. No oogonia were observed to be present at this stage. Of the few germ cells seen in mutant ovaries, they were in either zygotene or pachytene. (Figures 30 and 31).

Oocytes from normal 18 day ovaries were less AP positive than earlier, particularly those in the medullary region of the gonad. Most oocytes are located cortically. The majority of oocytes were in pachytene while a few were identified as being in diplotene. Degenerating oocytes were quite numerous at this stage also. Where germ cells were seen in mutant ovaries they too had stained lightly for alkaline phosphatase, and were located in the cortex just beneath the coelomic epithelium.

At birth, the most significant change in normal ovaries is seen in the growth of follicular cells. The oocytes which were for the most part in nests prior to this stage are seen to be separated from each other by somatic cells. The majority of germ cells are in diplotene and numerous degenerate oocytes are present. In mutant ovaries little change is seen except for the presence of a few germ cells which are also in diplotene. These cells are also surrounded by early

Figure 30. Pachytene oocyte (arrow) in a normal ovary of 17 days. H and E, x 1000.

Figure 31. A single oocyte in a mutant ovary of 17 days (arrow). Note the oocyte to also be in pachytene. H and E, x 1000.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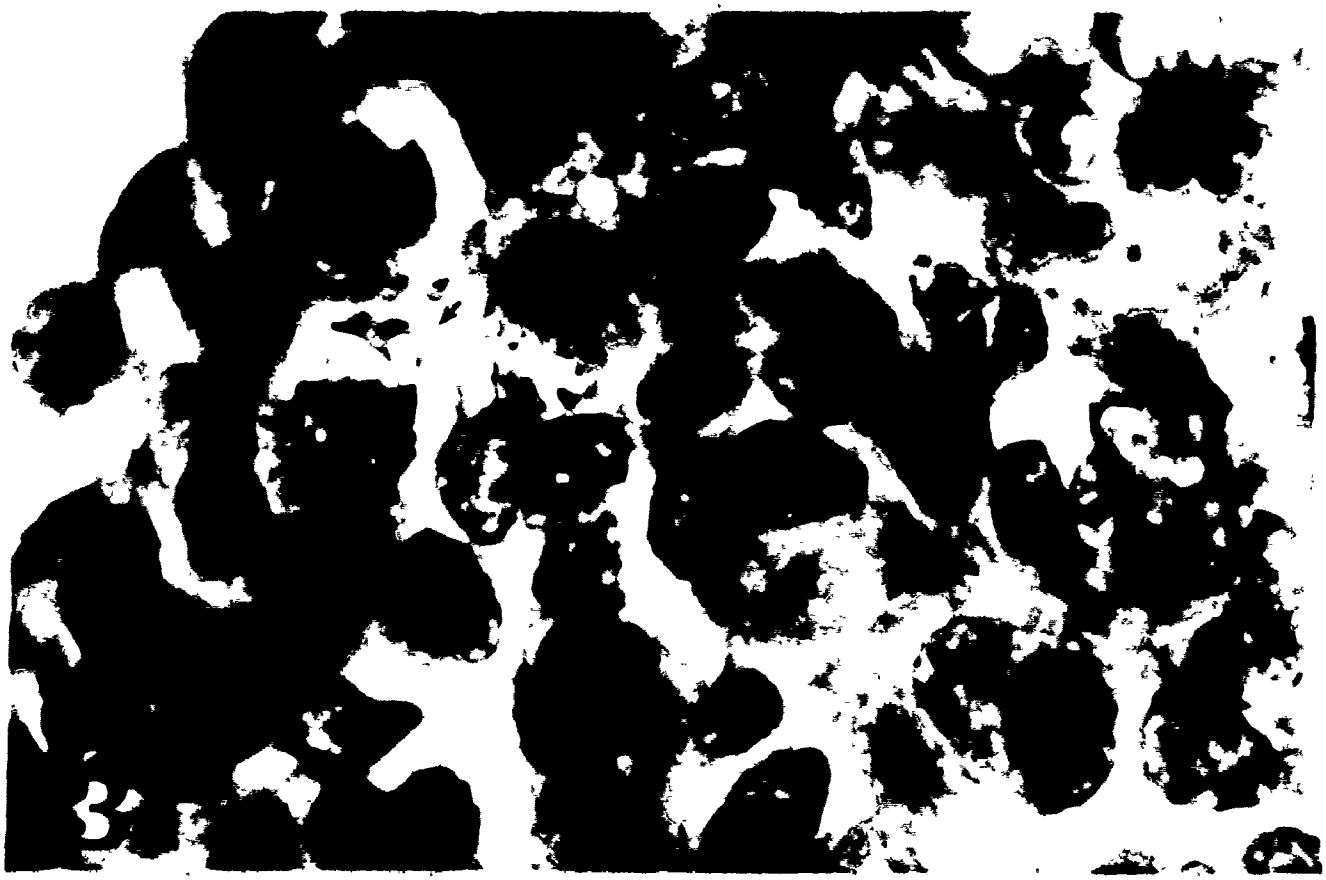

Figure 32. The first stages of follicle formation around oocytes in normal ovaries at birth. H and E, x 1000.

Figure 33. An oocyte in a mutant ovary also showing early follicle formation. H and E, x 1000. Oocytes are rare in mutant ovaries of this age but where they are found, follicle formation is apparent.

2

stage follicular cells (Figures 32 and 33).

Crown Rump Lengths and Body Weights

The crown rump lengths were measured in fetuses aged 13 days to birth and are presented in Table 24. The mean length of the mutants was consistently less than that of normals on each day, but an unpaired t-test revealed a significant difference only on day 17 and the day of birth. The P value of day 18 although not significant is less than that of day 13 to 16. The body weights of normals and mutants at birth were also found to be significantly different ($t=4.88$, $P < 0.001$).

The mean body weights of normal males and females were found to differ significantly only in the mature animals (Table 25). In the case of mutant males and females no significant difference in mean body weight was detected even at maturity (Table 26).

It was also found that by the time of maturity, although the normals still outweigh the mutants, the difference is not a significant one ($t=1.88$, $P < 0.05$) although it is close to the 0.05 level. Mutant females were also found to differ less, regarding body weight, from normal counterparts at maturity although the difference was still greater than the $P=0.05$ level ($t=3.56$, $P > 0.05$; also compare Tables 25 and 26).

Table 24

The mean crown-rump lengths (mm) ± the standard deviations (S.D.) of normal and mutant fetuses of 13 days old to birth. t=t value, P= probability, d.f.=degrees freedom.

Day	Mean length (mm) Normal ± S.D.	Mutant ± S.D.	d.f.	t	P
13	9.71 ± 0.66	9.48 ± 0.33	14	0.90	> 0.2
14	11.22 ± 0.62	10.91 ± 0.64	30	1.29	> 0.2
15	13.44 ± 0.97	13.03 ± 1.20	19	0.83	> 0.4
16	15.30 ± 0.80	15.06 ± 0.77	36	0.72	> 0.4
17	18.13 ± 0.93	16.85 ± 1.33	31	2.94	< 0.01*
18	20.47 ± 1.84	19.44 ± 1.29	22	1.46	> 0.1
Birth	28.86 ± 0.85	26.40 ± 0.96	10	4.68	< 0.01*

*Significant difference

Table 25

The mean body weights (gms) of post natal normal males and females at 1 week, 3 weeks and maturity (8-12 weeks). S.D.= standard deviation; t=t value; P=probability.

Age	Mean Weight (gms) Male ± S.D.	Female ± S.D.	d.f.	t	P
1 week	4.00 0.27	3.72 0.26	11	1.86	> 0.2
3 weeks	12.01 3.81	9.25 2.37	8	1.43	> 0.1
Mature (8-12 weeks)	24.03 1.99	19.29 3.40	15	3.56	< 0.01*

*Significant difference

Table 26

The mean body weights (gms) of post natal mutant males and females at 1 week, 3 weeks and maturity (8 - 12 weeks), S.D.= standard deviation; t=t value; P=probability.

Age	Mean Weight (gms) Male ± S.D.		Female ± S.D.		d.f.	t	P
1 week	2.13	0.66	2.11	0.43	5	0.03	0.9
3 weeks	5.00	0.91	5.10*	----	-	----	---
Maturity (8-12 wks)	19.52	6.91	14.73	2.94	8	1.54	0.1

* One female only

Post natal Gonadal Volumes and Weights

The mean gonadal volume of one side only from 1 week, 3 week and mature (8 - 12 weeks) normal and mutant mice is presented in Table 27. In both the normal and mutant males and females, a trend of continual growth of both testes and ovaries is apparent. The degree of increase of normal testes (from 1 week to maturity) is over 6 times greater than that of the mutants whereas the normal ovaries increase only 1.3 times more than mutant ovaries.

At maturity, the volume of mutant testes is only 7.8% that of the normals and mutant testes weigh only 9.3% that of normals. The volume of mutant ovaries is only 10.5% that of normals and they weigh only 12% as much as the normal ovaries at maturity (Table 28).

Table 27

The mean gonadal ($mm^3 \times 10^{-4}$) volume (one side only) from 1 week, 3 week and mature (8 - 12 weeks) post natal mice. SD=standard deviation.

	Volume ($mm \times 10^{-4}$)			
	Testes		Ovaries	
Age	Normal ± SD	Mutant ± SD	Normal ± SD	Mutant ± SD
1 week	16242 ± 1486	7509 ± 2527	1491 ± 741	203 ± 53
3 weeks	191460 ± 12796	14455 ± 4920	7448 ± 256	306 *
Mature	423286 ± 53330	32886 ± 19700	11451 ±2643	1201 ±408

*one value only

Table 28

Mean gonadal weight (mg) ± standard deviation (S.D.) of normal and mutant testes and ovaries (one side only) from mature (8-12 week) mice.

	Gonadal weight (mg)			
	No.	Normal ± SD	No.	Mutant ± SD
Testes	13	94.6 ± 12.7	7	8.8 ± 4.1
Ovary	6	2.5 ± 0.5	4	0.3 ± 0.07

The Mature Gonads

The histological appearance of mutant testes from mature animals (8 - 12 weeks) showed the seminiferous tubules to consist of Sertoli cells only. Occasionally spermatogonia were encountered as well as a few primary

spermatocytes (Figures 34, 35 and 36). Mutant testes are reminiscent of the Sertoli-cell only syndrome of humans (Figure 37). Secondary spermatocytes were never encountered in any mutant testes. The seminiferous tubules were smaller in mutants than in normals. Where germ cells were seen they occurred in small clusters, seldom were germ cells seen isolated singly. In one prepuberal/8 day specimen, a single cell resembling a mitotic germ cell was observed (Figure 38). The interstitial tissue of mutant testes contained abundant Leydig cells as do normal testes. Secondary spermatocytes were never seen in mutant testes. Where germ cells were seen they invariably rested upon the basement membrane of the seminiferous tubules. The epididymis and vas deferens of mutants appeared histologically normal but smaller than that seen in normal males.

Ovaries from mature mutant females were also smaller than normal. Although in most cases a few primary oocytes were present, follicular development was never seen to have reached the Graffian follicle stage, nor were corpora lutea ever encountered in mutant ovaries (Figures 39, 40 and 41). As in the males, the sex ducts appeared histologically normal but smaller than the fallopian tubes and uteri from normals. Also, mutant ovaries were entirely encapsulated by an ovarian bursa as are ovaries from normal mice.

Figure 34. Appearance of a section of normal fertile seminiferous tubule from a mature male. H and E, x 1000.

Figure 35. A section of sterile seminiferous tubule from a mature mutant testis. A germinal epithelium is absent and the tubules contain only Sertoli cells (arrow), H and E, x 1000.

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35

Figure 36. A section of mature mutant testis containing a few germ cells. Upper arrow indicates a primary spermatocyte, lower arrow shows a spermatogonium. H and E, x 1000.

Figure 37. A section of human seminiferous cord taken from a sterile patient having the Sertoli cell only syndrome. Note the absence of a germinal epithelium. H and E, x 400.

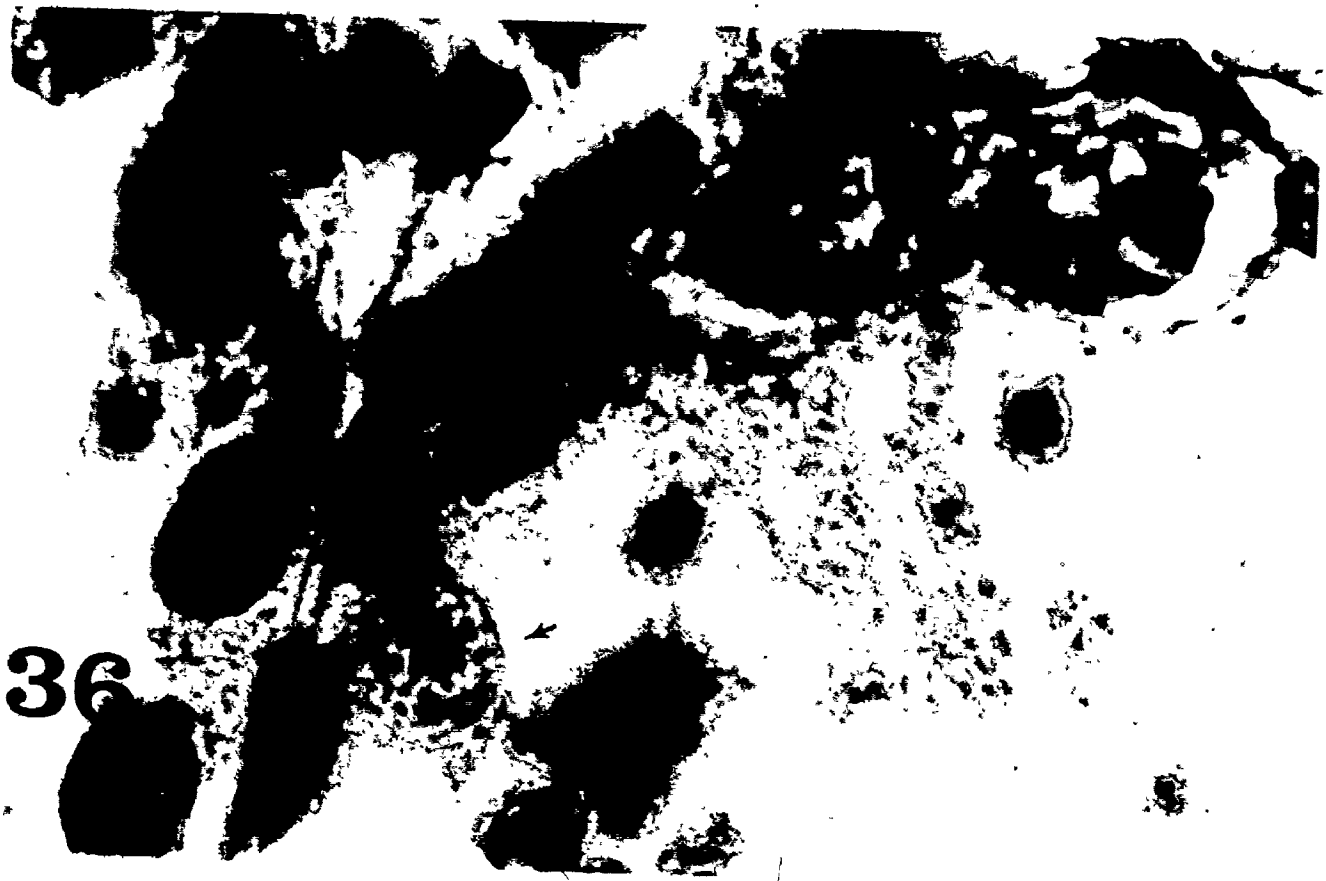

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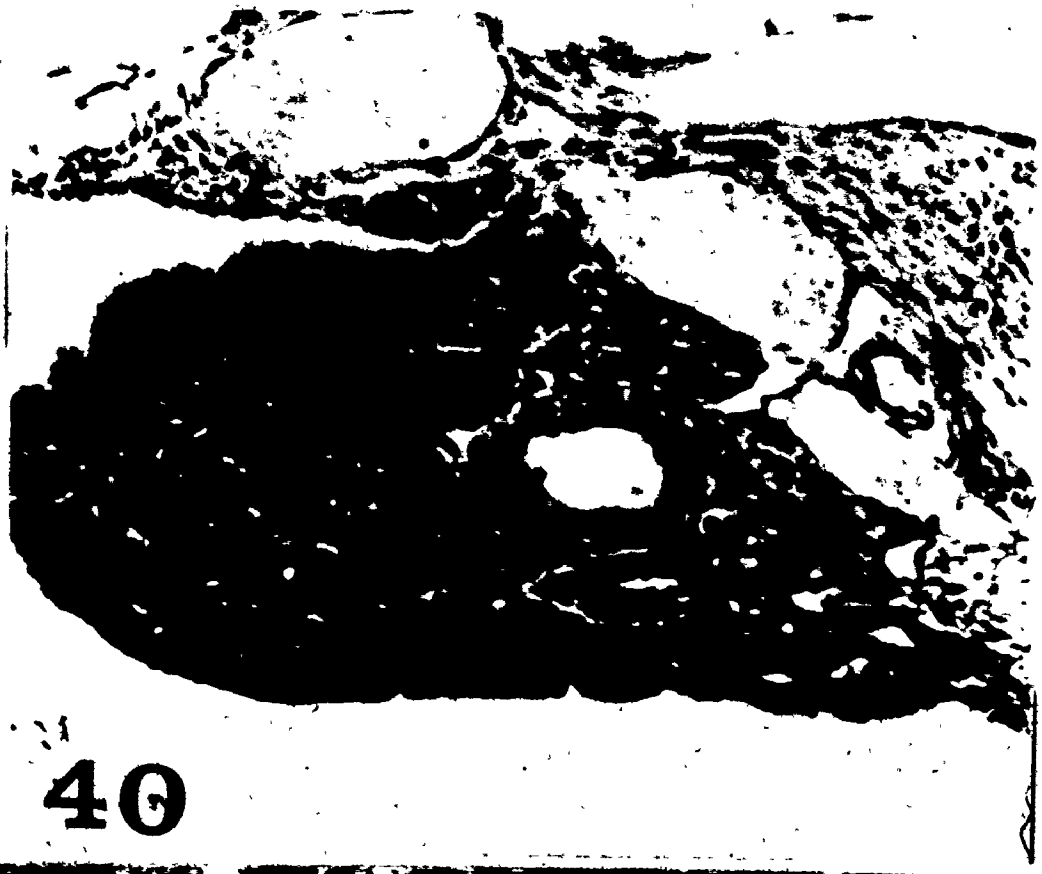
Figure 38. A single germ cell in mitosis in an 8 day post partum testis from a mutant male. H and E, x 1000.

Figure 39. The appearance of a normal ovary from a mature female. Note the various stages of follicle development. H and E, x 30.

39

Figure 40. A mutant ovary from a mature female. Note the absence of germinal tissue. The structure near the center had a cystic appearance and did not contain an oocyte, H and E, x 100.

Figure 41. An oocyte found in a primitive follicle in a mature mutant ovary. Mature follicles were never encountered in these ovaries. H and E, x 1000.



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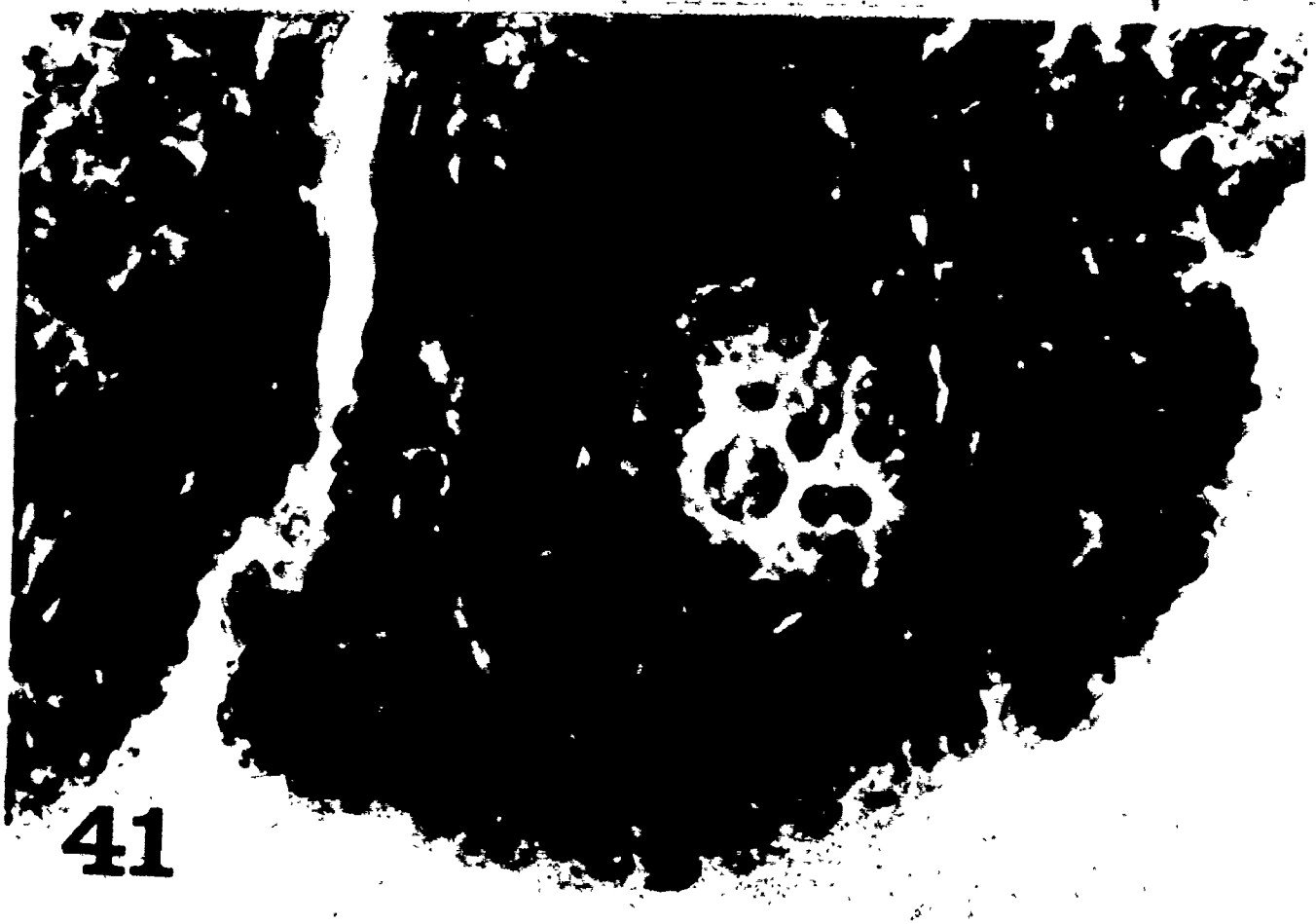

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DISCUSSION

The role of the germ cells in sexual differentiation during gonadogenesis is one of major importance in developmental biology. In most vertebrates (Blackler, 1970; Burns, 1971; Baker, 1972; Hardisty, 1967; Swift, 1914) the primordial germ cells can first be identified in yolk sac by the early somite stage. These cells then proliferate and migrate to the gonadal anlage. There has been considerable interest as well as controversy regarding the question as to whether the germ cells exert a prerequisite influence on the gonadal blastemata in the differentiation of the sex cords and the growth of the gonadal soma. Similarly, the possible influence of the gonadal soma on the maturation of the germ cells is also important and presently unresolved.

Adult mice carrying two mutant genes at the Steel (Sl) locus on linkage group IV are sterile due to the absence of germ cells. In fetuses of these mutant animals, the differentiation and migration of the germ cell line is defective prior to gonadogenesis (Bennett, 1956), and therefore this single locus mutation in mouse provides a useful experimental model to study and investigate the interaction between germ cells and gonadal blastemata during embryogenesis.

Since the $\underline{Sl/Sl^d}$ alleles cause sterility in mice, matings were carried out between the fertile WC/ReJ - $\underline{Sl/+}$ females and C57Bl/6J-$\underline{Sl^d/+}$ males. The offspring from these matings could be any one of $WCB6F_1$ - $\underline{+/+}$, $\underline{Sl^d/+}$, $\underline{Sl/+}$, or $\underline{Sl/Sl^d}$, each one of which is expected to occur at a frequency of 25%. Identification of $\underline{Sl/Sl^d}$ embryos is possible as early as day 10 of gestation by the small number of germ cells present (McCoshen and McCallion, 1975). However, a distinction between the $\underline{+/+}$, and heterozygous combinations is not possible until day 13 of gestation at which time some degree of the macrocytic anemia characteristic of this mutation is expressed in the heterozygotes (Chui and Russell, 1974; Chui and Loyer, 1975a). The mature animals are easily identified as $\underline{Sl/Sl^d}$ mutants by their white coat color whereas the $\underline{+/+}$ wild type are black. Heterozygotes also possess a black coat with a white spot either on the head or abdomen. Their tail and foot pads are lighter than those of the wild type.

Germ cell counts done on Swiss albino mouse embryos of days 8 - 10 of gestation demonstrated that the germ cell population increases steadily from one day to the next even before the infiltration of the gonads by these cells (Table 8). Since Bennett (1956) reported that the $\underline{Sl/Sl}$ mutation prevents the germ cells from proliferating and also retards their ability to migrate, it was expected that the $\underline{Sl/Sl^d}$ combination would also express itself in the same manner. Thus, the mutants would be identifiable on the basis of low

germ cell counts. Somite counts were done on all embryos in order to avoid developmentally retarded embryos from biasing the results. There was no indication that any group of embryos was developmentally retarded (Table 2). Only 2 of 114 embryos examined were reclassified to an earlier day due to slow development. This indicated that during early development, mutant embryos keep pace with the normals.

The germ cell counts from the day 9 - 12 embryos showed that at 9 days gestation, mutant and normal embryos cannot be distinguished by this criterion. Although the mutants cannot be identified at this early stage, the germ cell counts do show that the $\underline{Sl/Sl^d}$ mutation does not effect the evolution of the germ cells from their site of origin. By day 9, the germ cells are already found in locations which include the path of migration; i.e. the ventral aspect of the hind gut. By day 10, among normal embryos, one would expect to find that the mean number of germ cells had increased significantly over that of one day earlier. The same would be expected for days 11 and 12. From matings between the $\underline{Sl/+}$ x $\underline{Sl^d/+}$ animals, 25% were expected to be Sl/Sl^d and thus express the mutation by having low germ cell counts. In fact, an increase over the mean number of germ cells from day 9 embryos was not expected since Bennett (1956) reported mutant germ cells to be non-proliferative. Therefore, the number 126, the highest number of germ cells found at 9 days gestation, was chosen as a standard cut-off point.

Embryos having germ cell counts equal to or less than 126 from day 10 and after were considered to be the mutants. In testing this assumption, it was indeed found that from days 10 - 12, 26.4% of all the embryos did have germ cell counts of 126 or less. Two embryos of 12 days gestation had counts of 144 and 191 respectively, the next lowest count was 815. These 2 embryos were probably mutants, but they were not treated as such in terms of the statistics in order to test the hypothesis that mutant germ cells do not proliferate. However, they were included in a separate analysis of variance to test the mean number of germ cells from the total day 9 group and the presumed mutants of days 10, 11 and 12 which included the two embryos having germ cell counts of 144 and 191. The ANOVA test showed that there is no significant difference among the means due to a "day effect" ($0.10 > P > 0.05$). These data demonstrate that there is no recognizable increase in the number of mutant germ cells from one day to the next during the migratory phase of their development.

Between days 9 - 12 of gestation embryos were genotyped as normal or mutant according to the number of germ cells which were identified cytologically by their ability to stain for alkaline phosphatase. Based on the absolute numbers of germ cells it appears that the $\underline{Sl/Sl^d}$ mutation severely effects the capacity of mutant germ cells to proliferate. However, the total number of germ cells depends upon proliferation and cell loss. The possibility that mutant germ cells

disappear at a rate comparable to proliferation is recognized, but it is equally possible that those cells in mutants fail to divide yet retain a viable integrity. Mintz and Russell (1957) reported the presence of positively stained fragments in the W/W embryos which they interpreted to be dead germ cells. In this study, few stain-positive fragments were encountered in the mutants. Also, evidence from germ cell counts from later stages of mutant gonads stained with H and E failed to reveal any signs of excessive germ cell death (Tables 14 and 19). These data are interpreted as evidence that the mutant germ cells are severely inhibited from dividing, yet germ cells were found along the path of migration. McCoshen and McCallion (1975) reported earlier that the germ cells of mutants between 9 - 11 days gestation, were indeed capable of migration. In fact the evidence indicated that there is no obvious retardation of migration in mutant germ cells. By extending this study to include day 12, it was found that among the mutants, well over half of the germ cells reach the gonadal primordium. Considering that the mutant germ cells do not increase in number as do the normals, then a lower number of germ cells along the migration path is not indicative of retarded migration.. Mutant germ cells reach the gonadal ridges at the same time as do those of the normals. Furthermore, the percentage of ectopic germ cells among the mutants is not very different from that of the normals (Table 9). Figures 2 and 3 also indicate that the mutant germ cells

not only migrate, but they apparently keep pace with the migration process as seen in the normals. From these data it is concluded that the $\underline{Sl/Sl^{d}}$ mutation does not inhibit the migratory capacity of the primordial germ cells.

The initial localization of the germ cells in extragonadal sites supports the work of other investigators in that the germ cells do not arise from the gonadal epithelium (Chiquoine, 1954; Mintz and Russell, 1957; Ozdenzski, 1967; Spiegelman and Bennett, 1973; Jeon and Kennedy, 1973; Zamboni and Merchant, 1973; Clark and Eddy, 1975). During the subsequent migration of the primordial germ cells, they are invariably found in the gut endoderm and the surrounding gut mesoderm (Zamboni and Merchant, 1973).

From Figure 6 it is apparent that on day 9 the majority of the germ cells are located in the gut endoderm. Whether the germ cells randomly migrate into the gut tissue or are attracted to it is not known, but the possibility exists that these cells must pass through the gut endoderm in order to continue differentiation and to successfully reach the gonads. The fate of ectopic germ cells is not known, but there is no evidence that these cells ever differentiate beyond the primitive state (Mintz and Russell, 1957; Mintz, 1960; Falin, 1969). In addition, it appears that from the bizarre sites where ectopic germ cells are found, such as the allantois, placenta, limb bud ectoderm and tail bud, it is improbable that they are ever in contact with the gut endoderm

during their life history.

If the germ cells are actively attracted to the gonadal ridges as suggested by Witschi (1948, 1951) and Blandau et al (1963), then one may question why ectopically situated germ cells are not also attracted to the ridges. From Figures 4 and 5 it can be seen that in the mutants, the mean number (9) of germ cells found in the gut endoderm at day 10 corresponds closely to the number (10.3) found in the gonadal ridges by day 11. From Table 9 it is also apparent that the number of ectopic germ cells in the normal embryos increases only 4 times between day 9 to day 11 whereas the total number of germ cells shows an increase of over 8 times during the same period (Table 7). Since the majority of germ cells on day 9 are found in the gut endoderm (56%) then it is quite possible that the gut endoderm serves as an inducing agent for a germ cell response to an attracting agent from the gonadal ridges. The gut endoderm may also act in increasing the capacity for germ cells to divide at a higher rate once having contacted it. However, experimental evidence in support of endodermal induction of the germ cells is still lacking.

The presence of germ cells found existing in tight clumps (figure 3) was found to be of no value in identifying mutants. In the W mutation which shows the same pleiotropic effects as Steel, Mintz and Russell (1957) also reported clumped germ cells. The signficance of these cells is not known but they may represent aberrent or incomplete divisions

of some germ cells. Clumps were found in half the mutants and one quarter of the normals and the degree of clumping was the same in both. The clumped cells were almost invariably found in the umbilical mesoderm or in the region of the allantois Some authors have speculated that the ectopic cells retain their multipotentiality and are responsible in producing spontaneous teratomas (Stevens, 1967; Falin, 1969; Damjanov and Solter, 1974).

By day 12, the mean number of germ cells in the gonadal ridges of mutant embryos was approximately 45. In one case no germ cells were encountered. Despite the paucity of germ cells, histological examination of day 13 normal and mutant embryos which were genetically sexed by the sex chromatin test of Vickers (1967), showed that among the males, sex cord differentiation was apparent in both cases (Figures 11 and 12). Both normal and mutant female gonads retained an indifferent appearance at this time. The histological appearance of the mutant gonads corresponded to the genetic sex in all cases from day 12 and thereafter (Table 22, Figures 11, 12, 28 - 33). On no occasion was an ovotestes or sex reversed gonadal differentiation encountered. These observations support the view that the sexual differentiation of the somatic elements of the gonads is not germ cell dependent. These results also support the same view held by other authors for the amphibia (Bounoure, 1950), chick (Willier, 1939; Simon, 1960; and see Burns, 1971) and mammals (Wolff and Haffen, 1952; Merchant, 1975). After

reviewing the literature on the experimental results of germ cell ablation in amphibians, Burns (1971) concluded that the germ cells are not even required in the formation of the genital ridge, the appearance of the ridge being conditioned by regional influences.

In Merchant's (1975) recent report on rat gonadogenesis, busulphan, which is known to specifically destroy primordial germ cells (Hemsworth and Jackson, 1963) was used to ablate these cells prior to gonadal ridge formation and germ cell infiltration. Merchant found that sexual differentiation occurred among either the male or female line in gonads having few germ cells or no germ cells at all. In his study, Merchant (1975) did not sex the fetuses nor was a quantitative study of the gonads performed. However, his conclusion was that the sexual differentiation of the gonads is not germ cell dependent which further supports the findings in this investigation.

The model used in this study did not require chemical ablation of the primordial germ cells, but rather they were naturally prevented from proliferating after their initial appearance due to the genetic affect of the Steel mutation. All fetuses were genetically sexed from the time of sex cord differentiation onwards thus providing the opportunity to compare the histological appearance of the gonads to the sex of each fetus. In addition to the gonadal histology of mutants, the results presented here are even more supportive

of gonadal differentiation in the agametic state since in all cases the gonads corresponded to their genetic sex. One argument which may be raised against these conclusions is that mutant gonads did contain a few germ cells in most cases. Thus it may be that only a few germ cells are required for sex cord differentiation. This argument cannot be accepted because if the germ cells were capable of such a strong inducing influence one would expect that in totally agametic male gonads, they would have the appearance of indifferent gonads and this was never encountered. Also, one would not expect a few germ cells to have an effect on the whole gland. If the germ cells exerted even a regional effect, then a number of ovotestes would be expected to develop and this was not the case. The previously mentioned evidence from other species does not support the view that the germ cells induce gonadal differentiation, nor do ectopically situated germ cells ever induce gonadal tissue to develop even when in close proximity to the gonadal anlage. It is still reported in fairly modern literature that the germ cells induce gonadal ridge formation and sexual differentiation (see Gier and Marion, 1970; Hamilton, Boyd and Mossman, 1972). These views appear to be based on the older literature and probably are representative of the small amount of information which has been generated from work on mammalian species over the past many years (see Willis, 1962).

The volumetric changes of the normal and mutant

gonads were measured from day 12 of gestation to birth. The total ovarian volumes from normal fetuses increased to day 15 after which it remained constant until day 18. By birth the volume once again showed an increase. (Table 10 and Figure 13). This pattern of increase in ovarian volume is similar to that reported by Jean (1971) for Swiss fetal mouse ovaries up to day 18. Jean (1971) further reports that ovaries from neonates show a tendency to decrease in volume. His findings are inconsistent with the volumes of ovaries reported in this study for mice of 1 week, 3 weeks and maturity (Table 27) as well as with the findings of Beaumont and Mandl (1962) for the rat, where a continual increase is evident. Jean (1971) was unable to explain the decrease in the volume of neonatal ovaries in his study. The volume of mutants ovaries, which are between 98 - 100% germ cell free, were found to significantly differ in size from that of normal from day 14 and thereafter (Table 10 and Figure 13). When the calculated percentage of germinal tissue (Table 12) was deducted from normal ovaries, the volume of somatic tissue of both normal and mutant ovaries was found to be equivalent up to day 18. The difference only became significant ($P < 0.001$) among the neonates (Table 11). The increase in the amount of somatic gonadal tissue of the normals is attributed to the neoformation of follicular cells which are apparent at birth (Figures 32 and 33). The formation of primitive follicles is also reported to occur at birth in the mouse by Peters (1969) and Pederson

(1969).

From Table 12, it can be seen that the percent germinal tissue increases steadily to day 16 in ovaries after which it declines. The calculated mean number of germ cells (Table 13) was found to reach a maximum on day 15 after which the number of germ cells declined until birth. From birth to old age Jones and Krohn (1960) have shown that the number of germ cells in mouse ovaries follows the pattern of continual decline characteristic of females. The mean germ cell volume (Table 13) was found to steadily increase up to birth. Although the number of germ cells declines after day 15 post coitus, the relative volume of germinal tissue up to birth does not. In rat ovaries Beaumont and Mandl (1962) also found that even after the germ cell numbers began to decline, the mean oocyte volume continued to increase. However, by full term, the relative volume of germinal tissue did show a decline in the rat.

The growth of normal and mutant testes also paralleled each other from day 12 and after (Table 15 and figure 14). The volumetric difference was found to be significant at the $P < 0.01$ level by day 16. When the volume of germinal tissue (Table 16) was deducted from the normal testes, the volume of somatic tissue in normals also differed significantly from that of the mutants, but the growth of mutant testes continued even up to maturity as did the normals. Fetal testicular growth was equivalent to that reported by Jean (1971),

and in both mutants and normals the testes grew at a dramatically greater rate than ovaries of the same ages. These observations are in accord with the growth of testes in the rat as reported by Beaumont and Mandl (1963) and Mittwoch (1969, 1970) and Jean (1971) for the mouse. Part of the difference in the volume of normal and mutant somatic tissue may be explained by the difference in the crown rump size of mutants and normals. This point will be treated in more detail later.

The number of germ cells in normal males increased to a peak by day 16 after which a decline is also consistent with observations reported in the rat (Beaumont and Mandl, 1963; Huckins and Clermont, 1968;) and rabbit, (Chretien, 1967) in which quantitative measurements were done. The number of germ cells in mutant testes up to birth ranged from 0 - 198. The mean number of germ cells in normal males was approximately the same as that found in females up to day 13 (3626 and 3142 germ cells respectively) after which the number of germ cells found in testes exceeded the female line by almost 2 - 3 times (Tables 13 and 18). However, the female germ cells begin to enter prophase of meiosis I on day 13 and thus the number of oogonia available for further division becomes less. The mean size of spermatogonia also increases in size at least up to birth as was the case for oogonia and oocytes during the same period. Both oogonia and oocytes are larger than spermatoginia of the same age. In the rat, Beaumont and Mandl (1962, 1963) observed similar

size changes and discrepencies between males and females during fetal development. The size of the germ cells of mutant males and females also showed an increase during development and appeared to keep pace with the normals.

From Tables 14 and 19 which lists the numbers of germ cells counted from every second section in mutant ovaries and testes it is obvious that the number of gonadal germ cells has increased over the mean numbers found at day 12 (females = 53, and males = 41). The mean numbers of gonadal germ cells in normal females and males at day 12 are 1186 and 1023 respectively. In both mutants and normals the numbers represent the mean number of germ cells in both gonads, whereas the numbers in Tables 14 and 19 are representative of one gonad only, the other side having been processed for alkaline phosphatase staining. Therefore, the means in tables 14 and 19 may be doubled for comparison with the day 12 numbers. The same holds true for the numbers of normal germ cells displayed in Tables 13 and 18.

When the number of mutant germ cells counted in the day 12 and day 14 gonads was subjected to an unpaired t-test, the difference was not significant, but when compared to the day 16 counts it was found that the difference is significant at the $P < 0.05$ level for ovaries and $P < 0.02$ level for testes. These tests indicate that between day 12 and 16 there is some increase in the number of gonadal germ cells in mutants after which they apparently decline as do the normals. The degree

of increase in the number of germ cells in mutants though is 4.8 times for both sexes compared to 16.2 and 6.12 times for normal males and females during the same period of time. These data imply that in the mutants, once the germ cells become resident in the gonadal anlage they are capable of some degree of mitotic activity. In Figure 23, taken from a mutant testis of day 16 of gestation, a large cell have the appearance of a spermatogonium is seen in mitosis.

The gonads chosen for H & E staining and analysis were from either the left or right side. From the day 12 data of germ cell counts it was found that the number of germ cells in the left gonad did not differ statistically from that of the right (Table 23). A similar finding was reported by Beaumont and Mandl (1962, 1963) and McLaren (1963). Also, the number of germ cells in female gonads was not statistically different from that of males at day 12 (Table 23).

The histological changes of the normal testes from day 14 to birth coincided well with the report of Sapsford (1962) for the mouse and rat. The differentiation of the somatic elements of mutant testes corresponded in time and appearance with the normals. Even the mean number of Sertoli cells in a circular cross section of seminiferous cord was equivalent in mutants and normals. Leydig cells were also present in the mutant testes (Figures 21 and 22). Testes stained for alkaline phosphatase were also equivalent with respect to the

location of the enzyme. Except for the lack of germ cells, mutant gonads appeared histologically the same as the normals. Normal germ cells lose their alkaline phosphatase by day 17 which corresponds to the time when the germ cell population shows a decline. Those germ cells present in mutant testes also show a loss of alkaline phosphatase. Also, the changes in the appearance of mutant germ cells corresponds to that seen in the normals (see Figures 19 and 20). By birth many germ cells in normals are seen to lie on the basement membrane of the seminiferous tubules. A few germ cells in mutant testes were seen in this location also (Figures 24 and 25). Nebel et al (1961) suggest that this is a prerequisite for further differentiation of the spermatogonia. The alignment of the spermatogonia on the basement membrane takes place only after the temporary cessation in the mitotic activity of the gonial cells in the fetal period (Peters, 1970). In this study, germ cell proliferation was estimated to cease between the mid-fifteenth to sixteenth days. This would place the estimate at least one day later than the time reported by Peters (1970). This discrepancy of one day may be due to a strain difference and the techniques employed. However, the two estimates are very close to each other.

The histogenesis of the ovaries also followed a similar pattern of differentiation between mutants and normals except for a lack of germ cells in mutant ovaries. In the female germ line, the oocytes retain their alkaline phosphatase

for a longer time than do the spermatogonia. This observation was also noted by Mintz (1960) for fetal mouse gonads.

The most interesting aspect of ovarian gonadogenesis is the early transition of the oogonia into oocytes. By day 13, early prophase of meiosis I is seen in some germ cells. Having entered meiosis I, female germ cells are called primary oocytes by birth (Brambell, 1927; Borum, 1961; Peters, 1970). In this study, the germ cells of normal ovaries followed the schedule of meiosis I prophase as expected. From day 13 to birth the stages of leptotene, zygotene, pachytene and diplotene were each seen to predominate from one day to the next according to the order reported by Borum (1961; 1966), Peters et al (1962) and Peters (1970). Where germ cells were seen in mutants, they too were seen to have entered meiosis in synchrony with the normals (see Figures 30 and 31).

The germ cell population of both normal females and males was found to decline after day 15 and 16 respectively (Tables 13 and 18). This reduction in the germ cell population is in accord with descriptions reported for other species (rat; Huckins and Clermont, 1968; Beaumont and Mandl, 1962, 1963; rabbit, Chretien, 1967; human, Baker, 1963; cow, Erickson, 1966; monkey, Baker, 1966; and guinea pig, Iannou, 1964).

There is some evidence from the decrease in the mean number of germ cells found in mutant gonads, that the process of germ cell atresia corresponds in time of onset to that

seen in the normals (Tables 14 and 19). In the ovary germ cell atresia continues with advancing age (Jones and Krohn, 1959; Baker, 1963) whereas in the testes, the spermatogonia resume mitotic activity prepubertally and a massive repopulation of the germ line occurs (Oakberg, 1956; Nebel et al., 1961; Dym and Clermont, 1970). It is not known what causes germ cell atresia but there is evidence that it is under the control of the pituitary gland (Baker, 1972). Removal of the pituitary in the mouse results in a decrease in the rate of atresia of follicular oocytes (Jones and Krohn, 1961). Atresia may also be related to an elimination of genetically abnormal germ cells (Hendersen and Edwards, 1968). Whatever the role of germ cell atresia, it is a normal part of gametogenesis among the vertebrates (Roosen-Runge, 1973).

It appears then that those factors which control germ cell atresia, are operative in mutant gonads as well as the normals. From these histological observations, it is apparent that the differentiation of the somatic elements of mutant gonads takes place despite a severe germ cell depletion. The differentiation of mutant germ cells, seen to occur in synchrony with the normals, is taken as meaning that the Steel defect affects the ability of germ cells to divide, but does not affect their capacity to differentiate to meiotic cells according to a fixed temporal schedule. The differentiation and growth of the somatic elements of the gonads appear to be independent of a germ cell influence at least up to near

birth. At this time, the growth of follicular cells requires the presence of germ cells (Peters, 1969; Pedersen, 1969; Short, 1972).

One function of fetal mutant gonads can be expressed in terms of sex duct differentiation. This criterion is really one of testicular function since it is well known that Wolffian duct differentiation and Mullerian duct inhibition is dependent on testicular hormones. In the agonadal state, Mullerian duct differentiation and Wolffian duct regression is the norm (Jost, 1953; 1970; Jost et al, 1973). The differentiation of the Wolffian ducts is testosterone dependent (Jost, 1955; 1970; Jost et al., 1973; Neumann et al., 1970). The inhibition of the Mullerian ducts results from an as yet unidentified substance, the Mullerian inhibiting factor (MIF) (Josso, 1971; 1972a;1972b; Picon, 1971). In both mutant males and females, the sex ducts differentiated according to the gonadal sex. Gonadal sex also corresponded to genetic sex in the mutant fetuses (Table 22). In no instance was the state of sex duct differentiation seen to be bisexual. These results indicate that mutant testes produce testosterone and MIF as do the normals. The timing of sex duct differentiation in the mutants also corresponded to that seen in normals. The external genitalia differentiated according to gonadal sex as well and in the mutant males the testes descended normally.

The differentiation of the Wolffian ducts and cor-

responding inhibition of the Mullerian ducts in the male mutants supports the findings of Josso (1974) in that the germ cells are not involved in the secretion of the Mullerian inhibiting hormone. Whereas Josso (1974) reduced the germ cell population of human testicular fragments to as low as 3% of controls by X-irradiation, the testes of mutants in this study contained only from 0 - 2% the normal germ cell numbers. The fact that Mullerian duct inhibition took place in the mutant males strongly supports Josso's (1974) hypothesis that the germ cells do not influence the development of the male reproductive tract.

The growth of mutant gonads continued up to maturity for both males and females (Tables 27 and 28) as was also evident in the normals. The volume of mutant gonads however, was only about one tenth that of the normals. The weight of the mature mutant ovaries was 12% of the normals and the mutant testes weighed only 7.8% of the normals (Table 28). From the histological appearance of the mutant testes it is obvious that these organs are aspermatogenic (compare Figures 34 and 35). Examination of complete serial sections failed to reveal any mature germ cells whatsoever. But, some sections of seminiferous tubules did contain large cells characteristic of spermatogonial stem cells and primary spermatocytes. In all cases, the germ cells were found lying on the basement membrane where stem cells and early spermatocytes are normally found (Figures 34 and 36). No secondary spermatocytes were

ever encountered. This finding indicates that the Steel mutation also prevents the completion of meiosis I. Whether this effect is a primary or secondary one is not known. The appearance of the mature mutant testes is also reminiscent of the appearance of the Sertoli-cell-only syndrome of the human (Figure 37). Leydig cells were also seen in mature mutant testes. Recently, YoungLai and Chui (1973) have reported that Steel testes produce significantly higher levels of testosterone than their normal siblings.

YoungLai and Chui (1973) also reported that the adult Steel testes were completely agametic. In this study, spermatogonia and some immature spermatocytes were evident. There is also some evidence that some of the spermatogonia are capable of mitosis during embryogenesis and early neonatal periods (Figures 23 and 38). This finding is consistent with the evidence from fetal stages that some mutant germ cells are able to divide in the gonads. There is no evidence that the few spermatogonia are capable of adequately populating the mature gonad. It is known that after X-irradiation the germinal epithelium loses most of the germ cell population save for the stem cells. The stem cells of the irradiated testes are then capable of restoring the germinal epithelium to the level of spermiogenesis. (Dym and Clermont, 1970). For some reason in the Steel mutant, the few germ cells present are incapable of generating a normal germinal epithelium.

In the females, mature mutant ovaries were almost all entirely agametic (Figures 39 and 40). In some cases, germ cells were seen in primitive follicles (Figure 41). Never were mature Graffian follicles seen. In fact, the oocytes encountered appeared as diplotene cells. Again this would seem to imply that the Steel mutation acts also on the meiotic apparatus of the germ cells. Unlike the Steel testes, reports on the endocrine function of Steel mutant ovaries has not appeared in the literature.

The crown rump lengths of mutant fetuses from day 13 to birth were consistently less than that of normals (Table 24). The difference however was significant only on day 17 and birth. The difference in body weight of normal and mutant neonates was also found to differ significantly ($P < 0.001$). Chui and Loyer (1975a) found that the mean body weight of mutant fetuses between day 13 to 17 was only 10% less than that of their normal littermates. They also noted that the fetal brain weights did not differ between mutants and normals of 17 days gestation, whereas the liver weights from fetuses of 13 to 17 days gestation were considerably less. Probably a goodly portion of the difference in body weight and crown rump lengths can be accounted for by the smaller livers and gonads of mutants and their reduced number of circulating erythrocytes. The overall rate and degree of growth is however similar to the normals (Chui and Loyer, 1975). It seems that the smaller size of the mutants can be

mostly accounted for by the defective organs rather than a slower growth rate affecting the developmental rate of the gonads and liver. The fact that the sex cords differentiate and germ cells of mutants differentiate on schedule also supports this view.

The body weights of the post-natal normals fitted the growth curve for mice as reported by Theiler (1972). The mutants weighed less than normals through to maturity although the difference was not as great by maturity. Thus there seems to be a tendency for the mutants to catch up to their normal counterparts with time (Tables 25 and 26) at least with respect to body weight.

It is not known whether the sterility of Steel mutant mice results from an expression of the mutation within the germ cells, the tissues through which the germ cells migrate and reside, or other factors. Evidence from studies on hematopoiesis and melanoblast development in Steel mutants would seem to favour a defective microenvironment. The mutation Steel in the homozygous state is pleiotropic in that sterility, macrocytic anemia and lack of hair pigmentation results (Sarvella and Russell, 1956; Bennett, 1956).

Chui and Russell (1974) have demonstrated that the fetal erythropoiesis in mutants is defective at least as early as day 13 of gestation as evidenced by significantly fewer circulating erythrocytes than normal littermates. Hepatic erythropoiesis at this time is also abnormal in that

the number of mature erythroblasts containing hemoglobin is much less in mutants than in normals. The yolk sac derived erythrocytes appear to be affected little or not at all by the mutation (Chui and Loyer, 1975a). These cells proliferate and mature while within the vasculature thus implying that the Sl/Sl^d defect is not expressed within the circulation (Chui and Russell, 1974).

It has been demonstrated that irradiated normal spleens are able to support the proliferation of Sl/Sl^d hemopoietic stem cells but the proliferation of normal stem cells in irradiated Sl/Sl^d spleens is inhibited (McCulloch et al 1965). Alleviation of the anemia in Sl/Sl^d animals is possible by the transplantation of normal spleens from isogenic mice (Bernstein, 1970), whereas the transplantation of Sl/Sl^d spleens to normal mice does not result in the growth of spleen colonies (Altus et al, 1971).

The structure of erythroid precursor cells from fetal mutant livers has been found to be the same at the ultrastructural level as that of cells from normal littermates (Chui and Russell, 1974). Also, the number of circulating red blood cells in mutant fetuses is much less than in normals during prenatal development (Chui and Russell, 1974; Chui and Loyer, 1975a; Cole et al, 1974). However, the relative number of immature hemopoietic precursor cells in mutant fetal livers is higher than in normals (Chui and Russell, 1974). In the mature adult mutants, Chui and Loyer (1975b) have found

some cells from mutant bone marrow which normally do not respond to erythropoietin *in vivo*, do show a response *in vitro*. Wolf (1974) has reported that the spleen stroma, which has an erythrocytic proliferative function, is either lacking or very weak in Sl/Sl^d mutants. The Sl/Sl^d stem cells, though, are capable of colonizing normal irradiated spleens (McCulloch et al, 1965). These data are strongly suggestive of a microenvironmental defect in the mutant stromal tissue associated with erythropoiesis.

Results from studies on the lack of hair pigmentation in Steel mutants support the hypothesis that the Steel defect is a microenvironmental affect on the melanoblast population during development. By grafting normal and mutant embryonic skin ectoderm with neural crest from which the melanoblasts are derived, it has been found that Sl/Sl^d melanoblasts migrate into normal skin ectoderm and form pigment, but reciprocal grafting fails to form pigment (Mayer and Green, 1968; Mayer, 1970, 1973, 1975). The Steel mutation was found to block pigment production by melanoblasts in both the dermal mesoderm and epidermal ectoderm (Mayer, 1975).

It has been proposed by Mayer (1975) that the mechanism of the Steel mutation is to prevent the migration of melanoblasts into the mutant tissue environment. The melanoblasts themselves are capable of melanin synthesis in tissue from normal embryos (Mayer and Green, 1968; Mayer, 1970; 1973).

Although the nature of the germ cell defect is not

known, it is possible that it is of the same basic nature as that of the erythropoietic and melanoblast systems. It is known from this study that Sl/Sl^d germ cells are capable of migration and differentiation up to the primary oocyte and spermatocyte level. There is also some evidence that once the germ cells become resident in the gonads, they are capable of some proliferation. However, save for deletions, no gene mutation effects are absolute and this may explain why there is some apparent proliferation of mutant germ cells.

The model used in this study has been valuable in demonstrating that sexual differentiation of embryonic gonads is possible in the agametic state. Thus the gonadal soma is not dependent upon the presence of germ cells for sex cord differentiation to follow according to the genetic sex of the embryos. Also, the growth of the gonadal soma is not dependent upon the presence of germ cells except in females from birth onwards when follicle formation only takes place about the oocytes.

Sex duct differentiation also follows a normal course of development in agametic fetuses. The inhibition of the Mullerian duct system and the differentiation of the Wolffian ducts is not influenced by the presence of germ cells.

The growth and differentiation of the germ cells however, is dependent upon their residing in the gonadal soma. Extragonadal germ cells were not seen to have differentiated beyond the primordial germ cell level. The more striking

influence in the process of gonadogenesis appears to be inherent in the somatic tissue of the gonads except for the genesis of follicle formation in the female line.

BIBLIOGRAPHY

Altus, M.S., S.E. Bernstein, E.S. Russell, A.L.Carsten and A.C. Upton. 1971. Defect extrinsic to stem cells in spleens of steel anemic mice. Proc. Soc. Exp. Biol. Med. 138: 985-988.

Asayama, S. and M. Furusawa. 1961. Sex differentiation of primordial gonads of the mouse embryo in vitro. Jap. J. exp. Morph. 15: 34-40.

Baker, T.G. 1963. A quantitative and cytological study of germ cells in human ovaries. Proc. Roy. Soc. 158(B): 417-433.

Baker, T.G. 1966. A quantitative and cytological study of oogenesis in the rhesus monkey. J. Anat. (Lond.) 100 (4): 761-776.

Baker, T.G. 1971. "Primordial germ cells", in Germ Cells and Fertilization, ed. Austin, C.R., Cambridge Univ. Press. pp. 1-12.

Baker, T.G. 1972. Gametogenesis
Acta Endocrinol (suppl) (Kbh) 166:18-41.

Baxter, J.S. 1950. Alkaline phosphatase in primordial germ cells in a 10 mm. human embryo (Abst.) Int. Anat. Cong., Oxford p. 17-18.

Beaumont, H.M. and A.M. Mandl , 1962. A quantitative and cytological study of oogonia and oocytes in the foetal and neonatal rat. Proc. Roy. Soc. B, 155, 557-579.

Beaumont, Heather and Anita M. Mandl. 1963. A quantitative study of primordial germ cells in the male rat. J. Embryol. exp. Morph. 11: 715-740.

Bennett, Dorothea, 1956. Developmental analysis of a mutation with pleiotropic effects in the mouse. J. Morphology 98 (2): 199-233.

Bernstein, S.E. 1970. Tissue transplantation as an analytic and therapeutic tool inhheriditary aneamias. Am. J. Surg. 119:448-451.

Blackler, A.W. 1970. The integrity of the reproductive cell line in the amphibia. Curr. Top. Dev. Biol. 5:71-87.

Blanchard, M.G. and N. Josso. 1974. Source of the anti-mullerian hormone synthetized by fetal testis. II. Mullerian-inhibiting activity of fetal bouine sertoli cells in tissue culture. Pediat. Res. 8:968-972.

Blandau, R.J., B.J. White and R.E. Rumery. 1963. Observations on the movements of the living primordial germ in the mouse. Fertil. Steril 14:482-489.

Borum, Kirstine. 1961. Oogenesis in the mouse, a study of the meiotic prophase. Exptl. Cell Res. 24:495-507.

Borum, Kirstine. 1966. Oogenesis in the mouse (A study of the origin of the mature ova) Expt. Cell. Res. 45: 39-47.

Bounoure, L. 1950. Sur le developpment sexuel des glands genitale de la grenouille en l'absence des gonocytes. Arch. Anat. Microsc. Morphol. Exp. 39:247-256.

Bounoure, L., R. Aubry and M. Huck. 1944. Nouvelles recherches experimentales sur les origines de la ligner reproductive chez la genouille rousse. J. Embryol. exp. Morph. 2:245-263.

Brambell, F.W.R. 1927. The development and morphology of the gonads of the mouse. Part I. The morphogenesis of the indifferent gonad and of the ovary. Proc. Roy. Soc. B. 101:391-409.

Burns, R.K. 1971. Urinogenital system, in Analysis of Development pp 462-491.

Byskov, Anne Grete S. 1974. Does the rete ovarii act as a trigger for the onset of meiosis? Nature 252:396-397.

Byskov, A. 1975. The role of the rete ovary in meiosis and follicle formation in cat, mink and ferret. J. Reprod. Fert. 45(2):201-214.

Chalkley, H.W. 1943. Method for the quantitative morphologic analysis of tissues. J. Nat. Cancer Inst. 4:47-53.

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

Chretien, F.C. 1967. Etude de l'arigine, de la migration et de la multiplication des cellules germinales chez l'embryon de lapin. J. Embryol. exp. Morph. 16:591-607.

Chui, David H.K. and Elizabeth S. Russell. 1974. Fetal erythropoiesis in Steel mutant mice. I. A morphological study of erythroid cell development in fetal liver. Dev. Biol. 40:256-269.

Chui, David H.K. and Brian V. Loyer. 1975a. Foetal erythropoiesis in Steel mutant mice. II. Haemopoietic stem cells in foetal livers during development. Br. J. Haematol. 29:553-565.

Chui, D.H.K. and B.V. Loyer. 1975b. Erythropoiesis in Steel mutant (Sl/Sl^d) mice. Effects of erythropoietin in vitro. Blood. 45: 427-433

Clark, J.M. and E.M. Eddy. 1975. Fine structural observations on the origin and associations of primordial germ cells of the mouse. Dev. Biol. 47(1):136-155.

Cole, R.J., R.E. Tarbutt, E.M. Cheek and S.L. White. 1974. Expression of congenital defects in the hemapoietie micro-environment: Prenatal erythropoiesis in anemic steel (Sl^J/Sl^J) mice. Cell Tissue Kinet. 7(5):463-477.

Cole, R.T., T. Regan, S.L. White and E.M. Cheek. 1975. Expression of congenital defects in the haemapoietic micro-environment. Cell Tissue Kinet. 8(5):479-489.

Coulombre, J.L. and E.S. Russell. 1954. Analysis of the pleiotropism at the W-locus in the mouse. The effects of W and W^v substitution upon post-natal development of germ cells. J. Exp. Zool. 126:277-296.

Crone, M. and Levy,E. and Peters, H. 1965. The duration of the premeiotic DNA synthesis in mouse oocytes. Expt. Cell Res. 39:678:688.

Damjanov, I. and D. Solter. 1974. Experimental teratoma. Curr. Top. Path. 59:1-52.

Davidson, Eric H. 1969. Gene Activity in Early Development. Academic Press, N.Y. pp 165-246.

Dubois, R. 1968. La colonisation des ebauches gonadiques par les cellules germinales de l'embryon de poulet, en culture in vitro. J. E. Exp. M. 20:189-213.

Dym. M. and Y. Clermont. 1970. Role of spermatogonia in the repair of the seminiferous epithelium following X-irradiation of the rat testis. Am. J. Anat. 128:265-282.

Eddy, E.M. 1974. Fine structural observations on the form and distribution of nuage in germ cells of the rat. Anat. Rec. 178(4): 731-758.

Erickson, B.H. 1966. Development and radioresponse of the prenatal bovine ovary. J. Reprod. Fert. 11:97-105.

Everett, H.B. 1943. Observational and experimental evidences relating to the origin and differentiation of the definitive germ cells in mice. J. Exp. Zool. 92:49-91

Falin, L.I. 1969. The development of genital glands and the origin of germ cells in human embryogenesis. Acta. Anat. 72:195-232.

Fawcett, Don W. 1975. The mammalian spermatozoon. Dev. Biol. 44:394-436.

Fecheimer, N.S., M.S. Herschler and L.O. Gilmore. 1963. Sex chromosome mosaicism in unlike sexed cattle twins. Proc. 11th Intl. Congr. Genets. Hague. Lond. p. 265.

Ford, C.E., E.P. Evans, M.D. Burtenshaw, H.M. Clegg, M. Tuffrey and R.D. Barnes. 1975. A functional 'sex reversed' oocyte in the mouse. Proc. Roy. Soc. (Lond) B, 190:187-197.

Fort, A.T. 1970. Gonadal control of sexual organogenesis. Sthrn. Med. J. 63:620-622.

Fried, W., W. Chamberlain, W.H. Knospe, S. Huseini, and F.E. Trobaugh Jr. 1973. Studies on the defective hematopoietic microenvironment of Sl/Sl^d mice. Brit. J. Hematol. 24:643:650.

Fukudu, T. 1976. Ultrastructure of primordial germ cells in human embryo. Virch. Archiv (B) Cell Path. 20: 85-89.

Gier, H.T. and G.B. Marion. 1970. Development of the mammalian testis. In The Testis. ed. A.D. Jolmson, W.R. Gomes and H.L. Vandemark. Vol. I. Academic Press, N.Y.

Gomori, G. 1951. Alkaline phosphatase of cell nuclei. J. Lab. Clin. Med. 37:526-531.

Gondos, B. 1973. Germ Cell degeneration and intercellular bridges in the human fetal ovary. Z. Zellfarsch Mikrask Anat. 138:23-30.

Goodfellow, S.A., S.J. Strong and J.S. Stewart. 1965. Bouine freemartins and true hermaphroditism. Lancet I: 1040-1041.

Hamburgh, Max. 1971. Theories of Differentiation. American Elsevier, N.Y. pp. 360-389.

Hamilton, W.J., J.D. Boyd, and H.W. Mossman. 1972. Human Embryology. 4th ed. by W.J. Hamilton and H.W. Mossman. Williams and Wilkins Co., Baltimore, pp 398-404.

Hampton, S.H. 1973. Germ cell chimerism in male mormusets. Am. J. Phys. Anthropol. 38:265-268.

Hardisty, M.W. 1967. The number of vertebrate primordial germ cells. Biol. Rev. 42:265-287.

Hemsworth, B. and H. Jackson. 1963. Effect of busulphan on the developing ovary of the rat. J. Reprod. Fertil. 6:299-333.

Henderson, S.A. and R.G. Edwards. 1968. Chiasma frequency and maternal age in mammals. Nature 218:22-28.

Hsu, Yu-Chih. 1973. Differentiation in vitro of mouse embryos to the stage of early somite. Dev. Biol. 33:403-411.

Huckins, C. and Clermont, Y. 1968. Evolution of gonocytes in the rat testis during late embryonice and early post-natal life. Arch. Anat. 51:341-354.

Ioannou, J.M. 1964. Oogenesis in the guinea pig. J. Embryol. exp. Morph. 12:673-691.

Jean, Claude. 1971. Developpement volumetrique des gonades du foetus de souris. C.R. Soc. Biol. (Paris). 165:2341-2345.

Jean, Claude et Michel Berger. 1972. Developpement ponderal et histologique du testicule de souris de la naissance a un an. C.R. Soc. Biol. (Paris) 166:1685-1690.

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

Jones, E.C. and P.L. Krohn. 1959. Influence of ant. pituitary on the aging process in the ovary. Nature:183:1155-1158.

Jones, E.C. and P.L. Krohn. 1960. The relationship between age, numbers of oocytes and fertility in virgin and multiparous mice. J. Endocrinol 21:469-495.

Josso, N. 1971. Interspecific character of the mullerian inhibiting substance: of the human fetal testes, ovary and adrenal on the fetal rat mullerian duct in organ culture. J. Clin. Endocr. 32:404-409.

Josso, N. 1972a. Evolution of the Mullerian-inhibiting activity of the human testes. Effect of fetal, perinatal and post-natal human testicular tissue on the Mullerian duct of the fetal rat in organ culture. Biol. Neonate 20:368-379.

Josso, N. 1972b. Permeability of membranes to the Mullerian-inhibiting substance synthesized by the human fetal testis in vitro: A clue to its biochemical nature. J. Clin. Endocrinol. metab. 34:265-270.

Josso, Nathalie. 1974. Mullerian-inhibiting activity of human fetal testicular tissue deprived of germ cells by in vitro irradiation. Pepiat. Res. 8:755-758.

Jost, A. 1953. Problems of fetal endocrinology: The gonadal and hypophyseal hormones. Rec. Progr. Hormone Res. 8:379-418.

Jost, A. 1955. Modalities in the action of gonadal and gonadal stimulating hormones. Mem. Soc. Endocrinol. 4:237-248.

Jost, A. 1970. Hormonal factors in the sex differentiation of the mammalian foetus. Phil. Trans. Roy. Soc. Lond. (B)259:119-130.

Jost, A. 1972a. A new look at the mechanisms controlling sex differentiation in mammals. John Hopkins Med. J. 130:38-53.

Jost, A. 1972b. Freemartins in cattle: the first steps of sexual organogenesis. J. Reprod. Fertil. 29:349-379.

Jost, A. and J. Prepin. 1966. Donnees sur la migration des cellules germinales primordiales du foetus de veau. Arch. Anat. Microsc. Morph. exp. 55:166-186.

Jost, Alfred, Bernard Vigier, Jacques Prepin and Jean Pierre Perchellet. 1973. Studies on sex differentiation in mammals. Rec. Progr. Hormone Res. 29:1-41.

Kaplan, M. M. 1972. Alkaline phosphatase, Gastroenterology 62:452-468.

Kennelly, J.J., and R.H. Foote. 1966. Oocytogenesis in rabbits. The role of neogenesis in the formation of the definitive ova and the stability of oocyte DNA measured with tritiated thymidine. Amer. J. Anat. 118:573-589.

Mayer, T.C. 1970. A Comparison of Pigment Cell development in albino, Steel and dominant-spotting mutant mouse embryos. Develop. Biol. 23,297-309.

Mayer, Thomas C. 1973. The migratory pathway of neural crest cells into the skin of mouse embryos. Dev. Biol. 34:39-46.

Mayer, T.C. 1975. Tissue environmental influences on development of melanoblasts in Steel mice. Extracellular Matrix influences on Gene Expression. ed. H.C. Slankin and R.C. Gruelich. Academic Press (N.Y.) 1975. pp.555

Mayer, Thomas C. and M.C. Green. 1968. An experimental analysis of the pigment defect caused by mutations at the W and Sl loci of mice. Dev. Biol. 18:62-75.

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

McCoshen, J.A. and D. J. McCallion. 1975. A study of the primordial germ cells during their migratory phase in Steel mutant mice. Experientia 31:589-590.

McCulloch, E.A., L. Siminovitch, J.E.Till, E.S. Russell and S.E. Bernstein (1965). The cellular basics of the genetically determined hematopoietic defect in anemic mice of genotype Sl/Sl^{d}. Blood, 26:399-410.

McKay, D.G., A.T. Hertig, E. Adams, and S. Danziger. 1953. Histochemical observations on the germ cells of human embryos. Anat. Rec. 117:201-220.

McLaren, Anne. 1963. The distribution of eggs and embryos between sides in the mouse. J. Endocrinol 27:157-181.

McLaren, Anne, 1972. Germ cell differentiation in artifical chimaeras of mice. In "The Genetics of the Spermatozoon", eds. Beatty, R.A. and S.Glueckoalm-Waelsh. Bogtrykkerict Forum, Copenhagen. pp 313-324.

McLaren, Anne. 1975. The independence of germ-cell genotype from somatic influence in chimaeric mice. Genet. Res. (Camb.) 25:83-87.

McLaren, A., A. Chandley and Alfano S. Kofman. 1972. A study of meiotic germ cells in the gonads of fetal mouse chimaeras. J. Emb. exp. Morph. 27:515-524.

Merchant, H. 1975. Rat gonadal and ovarian organogenesis with and without germ cells. An ultrastructural study. Dev. Biol. 44:1-21.

Mintz, B. 1957. Interaction between two allelie series modifying primordial germ cell development in the mouse embryo. Anat. Rec. 128:591.

Mintz, B. 1959. Continuity of the female germ cell line from embryo to adult. Arch. Anat. Microscop. Morph. exp. 48 (suppl):155-172.

Mintz, B. 1960. Embryological phases of mammalian gametogenesis. J. Cell (comp). Physiol. 56(1):31-45.

Mintz, B. and E. Russell. 1957. Gene-induced embryological modifications of primordial germ cells in the mouse. J. Exp. Zool. 134(2):207.

Mittwoch, U. 1969. Do genes determine sex? Nature (Land), 221:446-448.

Mittwoch, U. 1970. How does the Y-chromosome affect gonadal differentiation? Phil. Trans. Roy. Soc. Lond. B,259: 113-117.

Mittwoch, Ursula. 1971. Sex determination in birds and mammals. Nature 231:432-434.

Mittwoch, Ursula. 1973. Genetics of Sex Differentiation Academic Press, N.Y., pp 163-200.

Mittwoch, Ursula. 1974. Cellular regulation in sexual development. Helv. Paediatr. Acta Suppl. 34:13-18.

Mittwoch, U. and J.D. Delhanty. 1969. Growth of differentiating testes and ovaries. Nature, 224:1323-1325.

Mittwoch, U., T.L. Narayanan and J.D. Delhanty. 1971. Gonadal growth in chick embryos. Nature (New Biol), 231: 197-200.

Mittwoch, U. and D. Kirk. 1975. Superior growth af the right gonad in human foetuses. Nature, 257(5529): 791-792.

Nebel, B.R., Amarose, A.P. and Hackett, E.M. 1961. Calendar of gametogenic development in the prepubertal male mouse. Science 134:832-833.

Neumann, F., W. Elger and H. Steinbeck. 1970. Anti-androgens and reproductive development. Phil. Trans. Roy. Soc. Lond. B, 259:179-184.

Nieuwkoop, P.D. 1949. The present state of the problem of the "Keimbahn" in the vertebrates. Experientia 8: 308-316.

Oakberg, Eugene F. 1956. A description of spermiogenesis in the mouse and its use in analysis of the cycle of the seminiferous epithelium and germ cell renewal. Am. J. Anat. 99:391-413.

Ohno, S. 1967. Sex Chromosomes and Sex Linked Genes. Part III, On so-called sex determining factors and the act of sex determination. Springer-Verlag, New York.

Ohno, S. 1969. The problem of the bovine freemartin. J. Reprod. Fert. (Suppl.) 7:53-61.

Ohno, S., J. Trujillo, C. Stenius, L.C. Christian, and R. Teplitz. 1962. Possible germ cell chimeras among newborn dizygotic twin calves (Bos Taurus). Cytogenetics 1:258-265.

Ohno, S. and A. Gropp. 1965. Embryological basis for germ cell chimerism in mammals. Cytogenetics 4:251-261.

Odzenzski, Waclow. 1967. Observations on the origin of primordial germ cells in the mouse. Zool. Polonica. 17(4):367

Odzenzski, W. 1969. Fate of primordial germ cells in the transplanted hind gut of mouse embryos. J. E. exp. M. 22:505-510.

Pearse, A.G.E., 1968. Histochemistry: Theoretical and Applied. Vol. I. Little, Brown and Company, Boston.

Pedersen, Torben, 1969. Follicle growth in the immature mouse ovary. Acta Endocrinol. 62:117-132.

Peters, Hannah. 1969. The development of the mouse ovary from birth to maturity. Acta Endocrinol. 62:98-116.

Peters, H. 1970. Migration of gonocytes into the mammalian gonad and their differentiation. Phil. Trans. Roy. Soc. London B 259:91-101.

Peters, H., E. Levy and M. Crone. 1962. Deox ribonucleic acid synthesis in oocytes of mouse embryos. Nature 195: 915-196.

Peters, H. and M. Crone. 1967. DNA synthesis in oocytes of mammals. Arch. Anat. microsc. Morph. exp. 56 (Suppl 3 - 4): 160-170.

Peters, Hannah. 1976. Intrauterine gonadal development Fert. Steril. 27(5):493-500.

Peters. H., and M. Crone. 1967. DNA synthesis in oocytes of mammals. Archs. Anat. microsc. Morph. exp. 56 (Suppl 3-4):160-170.

Picon, R. 1970. Modifications, chez le rat, au cours du developpement du testicule, de son action inhibitrice sur les canaux de Muller in vitro. C.R. Acad. Sci. 271:2370-2373.

Picon, R. 1971. Etude comparee de l'action inhibitrice des testicules de lapin et de rat sur les cannaux de Muller de ces ceux especes in vitro. C.R. Acad. Sci. 272:98-101.

Rogulska, T. 1969. Migration of the chick primordial germ cells for the intracoelomically transplanted germinal crescent into the genital ridge. Experientia 25:631.

Rogulska, T., W. Odzenzski, and H. Kamar. 1971. Behaviour of mouse primordial germ cells in the chick embryo. J. Embryol. exptl. Morph. 25:155-164.

Roosen-Runge, E.C. 1973. Germinal cell loss in normal metaxoan spermatogenesis. J. Reprod. Fert. 35:339-348.

Rugh, Roberts. 1968. The Mouse: its Reproduction and Development. Burgess Publ. Co., Minneapolis pp. 282-295.

Russell, E.S. and S. E. Bernstein. 1966. Blood and blood formation. In: Biology of the Laboratory Mouse. Ed. E.L. Green, 2nd ed. pp. 351-372. McGraw-Hill, N.Y.

Sapsford, C.S. 1962. Changes in the cells of the sex cords and seminiferous tubules during the development of the testes of the rat and mouse. Austral. J. Zool. 10: 178-192.

Sarvella, P.A. and L.B. Russell, 1956. Steel, a new dominant gene in the house mouse. J. Heredity. 47: 123-128.

Satoh, N. 1974. Multiplication and sex differentiation of germ cells in the chickembryo. J. Faculty of Science Univ. Tokyo. IV, 13(2):183-192.

Schaepman-van Geuns, E.J. 1972. De oorsprong van de oerkiem-cellen. Ned. T. Geneest. 116(39):1764-1765..

Short, R.V. 1972. "Germ cell sex". in: The Genetics of the Spermatozoon. eds., Beatty, R.A. and S. Gluecksolm-waelsch. Bogtrykkeriet Forum, Copenhagen. pp. 325-345.

Simon, D. 1960. Contribution a l'etude de la circulation et de transport des gonocytes primaires dans le blastodermes d'oisseau cultives in vitro. Arch. Anat. Morph. exp. 49:93-176.

Spemann, H. and H. Mangold. 1924. Induction of embryonic primordia by implantation of organizers from a different species. IN: Foundations of Experimental Embryology. eds., Willier, B.H. and J.M. Oppenheimer. Prentice Hall, N.J.

Spiegelman, M. and Dorthea Bennett. 1973. A light and E. M. study of primordial germ cells in the early mouse embryo J. Embryol. exp. Morph. 30(1), 97-118.

Stevens, L.C. 1967. Origin of testicular teratomas from primordial germ cells in mice. J. Nat. Cancer Inst. 38:549-552.

Stewart, J.S.S. 1965. The clinical investigation of inter-sexuality. Ann. R. Coll. Surg. Engl. 37:374-387.

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

Tarkowski, A. 1970. Germ cells in natural and experimental chimaeras in mammals. Phil. Trans. Roy. Soc. London B 259:107-111.

Teplitz, R.L., Y.S. Moon and P.K. Bassur. 1967. Further studies of chimerism in heterosexual cattle twins. Chromosoma. 22:202-209.

Theiler, K. 1972. The House Mouse. Springer-Verlag, N.Y.

Torrey, W.T. 1944. The development of the urogenital system of the albino rat. II. The gonads. Amer. J. Anat. 76:375-397.

Vickers, Anne D., 1967. Amniotic sex chromatin and foetal sexing in the mouse. J. Reprod. Fert. 14:503-505.

Wallace, M.E. 1971. An unprecedented number of mutants in a colony of wild mice. Environ. Pollut. 1:175-184.

Weibel, E.R., G.S. Kistler, and W.F. Scherle. 1966. Practical stereological methods for morphometric cytology. J. Cell Biol. 30:23-30.

Willer, B.H. 1939. Experimentally produced sterile gonads and the problem of the origin of the germ cells in the chick embryo. Anat. Rec. 70(1): Suppl 1:89-111.

Willis, R.A. 1962. Teratomas, In: The Borderland of Embryology and Pathology. Butterworth, Lond.

Witschi, E. 1948. Migration of the germ cells of human embryos from the yolk sac to the primitive gonadal folds. Carneg. Inst. Constributions to Embryology 209:67-80.

Witschi, E. 1951. Embryogenesis of the adrenal and reproductive glands. Rec. Progr. Hormone Res. 6:1-27.

Witschi, E. 1956. Development of Vertebrates. W.B. Saunders Co., Phil.

Wolff, E. and K. Haffen. 1952. Sur une methode de culture d'arganes embryonnaires in vitro. Texas Rep. Biol. Med. 10:463-472.

Wolf, N.S. 1974. Dissecting the hematopoietic microenvironment. Cell Tissue Kinet. 7:89-98.

Youngl ai, E.V. and D.H.K. Chui. 1973. Testicular function in sterile Steel mice. Biol. of Reprod. 9:317-323.

Zamboni, L. and H. Merchant. 1973. The fine morphology of the mouse primordial germ cells in extra-gonadal locations. Am. J. Anat. 137:299-336.