EXPERIMENTAL MURINE TERATOMAS

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ABSTRACT

Early in the development of the embryo, cells lose their ability to differentiate into any adult type of cell and become determined. A cell which has achieved a certain degree of differentiation can ultimately reproduce to form only a limited number of cell types along the original line of differentiation; for example, a primitive mesenchymal cell can give rise to only connective tissues such as muscle or bone.

Apart from the primordial germ cells (those cells which later give rise to oocytes in the female and spermatocytes in the male), all cells have begun to specialize, with a consequent narrowing of their potential, very early in gestation.

Teratomas are neoplasms which contain many kinds of tissues which are foreign to the organ in which they arise. There is controversy over whether these tumours arise from the growth of cells which have escaped early developmental controls, or whether they arise from the proliferation of the only multipotential cell in the body, the germ cell. Rather than following their normal path of differentiation to oocytes or spermatocytes, they might fulfil their potential to form almost any kind of tissue. Thus tumours composed of skin, bone, muscle, nervous tissue, and glands

are found in adult testes and ovaries.

Since the germ cells arise in the embryonic yolk sac and travel up the umbilical cord to the developing gonads, there is a theory that these multipotential cells may occasionally become lost and much later, often in adulthood, begin their proliferation in sites along the midline: at the base of the spine, in the chest and throat.

The experiments in this thesis explore an animal model in which teratomas can be produced by the grafting of 7½-day mouse embryos to the gonads of adult hosts. According to the literature the tumour which develops as a result of this operation is identical to those arising spontaneously in a highly inbred strain of mouse. Those experimental tumours which contain areas of undifferentiated tissues can be transplanted to other hosts, sometimes retaining their embryonic nature for many generations.

Whole embryos develop as teratomas in up to 80% of grafts to male hosts. The embryos can be cut in such a way that one half contains extra-embryonic material (that which eventually forms the placenta) and also the yolk sac containing the germ cells, and the other half which contains only somatic tissue.

Grafts of the somatic parts result in teratomas in 74% of the testes, a rate similar to tumours from whole embryos, whereas grafts of the extra-embryonic parts containing germ cells resulted in only 1 small teratoma out

of 35 grafts.

In this experimental model, it has been demonstrated that the primordial germ cells of the embryo are not responsible for the development of the neoplasms, but rather it is the somatic cells which are giving rise to these tumours. One must ask whether the controls which govern normal differentiation may become faulty and allow somatic cells to retain or regain their pluripotential nature making it possible, when conditions are right, to proliferate to form the "monstrous" tumour, teratoma.

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INTRODUCTION

"A teratoma is a true tumour or neoplasm composed of multiple tissues of kinds foreign to the part in which it arises" (Willis, 1962a). The meaning of the word teratoma is descriptive of its bizarre nature, literally monstrous tumour. Usually it contains a chaotic mixture of representatives of all three germ layers. Some common components of teratomas are skin, cartilage, glands and nervous tissue, all of which are "foreign" to the gonads--a favourite site of this tumour.

In man teratomas can be found anywhere along the midline or in the gonads, but the main sites, in order of frequency, are ovaries, testes, anterior mediastinum, retroperitoneum, presacral and coccygeal region (Willis, 1962a).

There have been reports of teratomas in species other than man. Mammals in which gonadal teratomas have been described are the horse (Willis and Rudduck, 1943), in which they are occasionally found in testes removed in routine gelding operations, the guinea pig (Vink, 1970), and the mouse (Jackson and Brues, 1941; Stevens, 1954; and Stevens and Varnum, 1974).

Non-mammalian creatures in which the tumour has been reported are the toothcarp (Stolk, 1959) and fowl

reviewed by Guthrie, 1964, and Bresler, 1959). Even the plant kingdom is afflicted with teratoma, the crown gall disease (Braun, 1965).

Except for experimental breeding the incidence of teratomas in mammals is extremely low. For many species of mammals there have never been any reports of the tumour. The dog, for example, has been well investigated, yet testicular teratomas have not been described in this mammal (Stevens, 1967), and only 3 ovarian teratomas have been reported (Willis, 1962a). Meier et al (1969) describe the only case known in rabbits. Man is probably the species most prone to the development of this neoplasm.

It is difficult to obtain figures on the incidence of teratomas because of differences in reporting. Some registries divide these neoplasms into several groups, from the less differentiated "embryonal" tumours to the more highly differentiated teratomas (Miller and Seljelid, 1971) to reflect differences in the life history of a neoplasm, from the embryonic to the more mature stages. Others list seminoma as a separate entity, and the remaining tumour types come under the heading of teratoma (Collins and Pugh, 1964).

Although ovarian teratomas are more common than those in all other locations (Willis, 1962a), they are usually well differentiated and benign. In a 20-year review of 305 cases (Caruso et al, 1971), only 3% were considered

malignant and more than 90% of the total were found in women over age 20. The average age in Caruso's study was 32. There have been many such studies, but none gives the incidence of ovarian teratomas in the general population.

The second most common site for teratomas is the testis, and because testicular carcinoma is a leading cause of cancer deaths in young men (Mostofi, 1973), there are many more statistical records in the literature than for its female counterpart. The incidence rates are reviewed by Krain (1973) who compares data from the California Tumor Registry with those of other states and countries. There is a bimodal distribution of testicular cancer rates (all histological descriptions including seminoma) with peaks at ages 25-34 and 75-84 years. The incidence rates of testicular tumours per 100,000 males vary greatly: 1.4 (Israel), 2.1 (England and Wales), 2.6 (California and Connecticut), 5.3 (Denmark), and 6.3 (Copenhagen). In Norway the average incidence is 3.5 per 100,000 per year (Miller and Seljelid, 1971).

The mortality rate for testicular tumours in American children ages 0 to 14 who have not reached puberty is less than 1 per million per year (Li and Fraumeni, 1972). There is an age peak in infancy in which 51% of the deaths in the 0 to 5 age group resulted from embryonal carcinoma. In contrast to the highly malignant testicular teratomas in adults, most teratomas of the testis in infants and young

children are benign (Abell and Holtz, 1963).

Apart from the gonads, the most common site of teratomas is the anterior mediastinum. Tumours in this location are usually not detected until adult life and are generally malignant (Martini et al, 1974). Whether teratomas in the anterior mediastinum arise de novo or as the result of metastases from the testis is an open question. Martini et al (1974) suggest that because 8 of 35 patients in their study were female, these tumours originated in the mediastinum. They do not say whether the gonads were inspected for scarring.

All other midline teratomas are generally present at birth or are discovered soon afterwards (Partlow and Taybi, 1971). These tumours are located in the retroperitoneum, presacral and coccygeal regions, and they are usually benign (Willis, 1962b).

In addition to these more common locations, teratomas have been found in some unusual sites. There have been recent reports of these rare tumours in the face (Gifford and MacCollum, 1972), eye (Barber et al, 1974), tongue (Bras et al, 1969), heart (Cabañas, 1973), kidney (Dehner, 1973), uterine tube (Mazzarella et al, 1972), and placenta (Joseph and Vogt, 1973).

There have been many conflicting hypotheses on the etiology of teratomas, and even today researchers are not in

agreement. Because of the variation in morphology and distribution of this tumour, it is difficult to postulate an explanation of its origin.

Some investigators divide teratomas into two groups--gonadal teratomas and their metastases, and extra-gonadal teratomas--making it possible to put forward a different theory for each group. Usually fetus in fetu and conjoined twins are excluded from both groups, although Potter (1961), and others, would include as teratomas these abortive attempts at twinning. She feels there is a natural progression from normal twins to conjoined symmetrical twins, asymmetrical twins, parasitic fetuses, fetal inclusions and finally to teratomas.

Those who exclude fetus in fetu do so because these tissues have no powers of progressive neoplastic growth, whereas teratomas may contain mixtures of embryonic and differentiating tissue at all stages of maturity. Others exclude fetus in fetu and twinning from teratomas by the criterion of a vertebral axis. Teratomas lack a spinal axis and possess no organs or systems (Pai and Rae, 1966).

It is this progression which led early workers to suggest that teratomas, particularly those located near the base of the spine, were the result of abortive attempts at twinning. Certainly these sacrococcygeal tumours originate during embryonic life, as they are almost always detected at birth (Vaez-Zadeh et al, 1972).

A theory based on Spemann's work in developmental biology was advanced by Askanzy in 1907 (reviewed by Pierce and Dixon, 1959). He believed that either a single cell or a group of cells could remain undifferentiated and dormant for years, and then commence growing and differentiating. This hypothesis is not in disagreement with an earlier theory of Bonnet that teratomas originated from blastomeres misplaced during the cleavage period.

In 1926 Budde hypothesized an origin from foci of plastic pluripotent embryonic tissue which have escaped from organizing influences during the primitive streak stage of development (reviewed by Stevens, 1967). The evidence for this theory is the location of teratomas along the midline where disturbances in the cells leaving the primitive streak might be expected. This theory has had the support of many researchers (Willis, 1962a; Collins and Pugh, 1964) and is still widely held.

The most recent theory on the origin of teratomas has developed from our increased knowledge of embryological development and some of the newer techniques of studying the growing fetus. Using 23 serially sectioned human embryos, Witschi (1948) first described the migration of primordial germ cells from the yolk sac endoderm where they are first seen, up the stalk of the yolk sac into the hindgut and finally into the developing gonads. This work was later confirmed by other investigators using histological

techniques (Pinkerton, 1961 - human; Mintz and Russell, 1957 - mouse).

The theory that gonadal teratomas are derived from germ cells has become very popular (Dixon and Moore, 1953; Stevens, 1962, 1968). Non-gonadal teratomas, according to this theory, are derived from ectopic primordial germ cells (Friedman, 1959). Mintz (1960), working with the mouse, occasionally noticed germ cells straying from their usual pathway.

Arguments for a somewhat different germ cell theory have been reviewed recently by Ashley (1973). He suggests that gonadal tumours arise by parthenogenesis from haploid cells, probably by the fusion of two such cells. All extragonadal teratomas are explained as incomplete conjoined twins.

Evidence for parthenogenesis in man is the finding of chromatin positive cells in testicular teratomas of adults (Ashley and Theiss, 1958; Dayan, 1963; Koch, 1970; Myers, 1959; Taylor, 1965; Theiss et al, 1960). These workers suggest that this female sex chromatin pattern supports the hypothesis that testicular teratomas are the result of conjugation of haploid cells. On the other hand, Atkins (1973) found fluorescent Y bodies in all 12 malignant testicular teratomas in his study indicating the presence of a Y chromosome. Few karyotypes of testicular teratomas have been made because of the difficulty in obtaining

satisfactory preparations from solid tumours. In an analysis of 3 testicular teratomas, Rigby (1968) found all the diploid cells were male 46, XY, although modal chromosome numbers ranged from 52 to 58. Similar results were obtained by Martineau (1969) in a study of 8 testicular teratomas.

Recently an inbred strain of mouse has been discovered in which about half the females have ovarian teratocarcinomas (Stevens and Varnum, 1974). The authors have shown that in the LT strain teratomas originate from eggs that develop parthenogenetically within the ovary. The eggs developed in stages similar to those of normal mouse embryos until they reached the stage of 6- to 7-day embryos, at which time they became disorganized.

An animal model for the study of testicular teratomas has been developed by Stevens at the Jackson Laboratories in Bar Harbor, Maine. These tumours are very rare in mice, except for inbred strain 129 in which teratomas arise in 10% of male mice. Manipulation of the genome through breeding experiments has produced this highly susceptible strain (Stevens and Little, 1954; Stevens and Mackensen, 1961). Incorporation of the Steel-J (Sl^J) locus onto the 129 background doubled the incidence from 5 to 10% in second and later litters (Stevens, 1966). More recently Stevens (1973b) has described a new subline, 129/terSv, in which

about 30% of the males develop spontaneous congenital testicular teratomas.

The incidence of tumours is the same in fetal mice as in older mice, and Stevens (1959, 1962) suggests that testicular teratomas arise within the tubules of mice during embryonic life and that they may be derived from the neoplastic transformation of primordial germ cells.

Unlike human testicular teratomas, these murine teratomas rarely metastasize (Stevens, 1973b), although transplants occasionally develop into rapidly growing tumours, consisting of undifferentiated cells, which can be converted to ascitic form (Pierce and Dixon, 1959). That these undifferentiated cells are pluripotent and capable of producing most of the somatic tissues of the body was clearly shown in an in vivo cloning technique of Kleinsmith and Pierce (1964). They dissociated cells of small embryoid bodies taken from a transplanted teratoma of strain 129 and implanted single cells directly into mice. Tumours developed which contained, in addition to the original embryonal carcinoma type cell, as many as 14 well-differentiated tissues.

Testicular teratomas have been produced by the grafting of 12- to 13-day male genital ridges to adult testes (Stevens, 1966, 1967, 1970a). Teratomas developed within the fetal testis in up to 82% of the grafts to strain 129

and A/He hosts. Genital ridges of hybrid male embryos developed teratomatous foci in 97% of grafts (Stevens, 1970b). Only histocompatible grafts were made, and these were recovered after 12 days.

Ridges from female mice developed into ovaries without teratomas (Dunn and Stevens, 1970). Some strains are not susceptible to teratocarcinogenesis; Stevens grafted 122 genital ridges from C3H fetuses to adult testes, and all developed into ovaries or testes without teratomas (Dunn and Stevens, 1970).

Genital ridges grafted to nonscrotal sites such as the liver, kidney, and spleen developed teratomas in a small percentage of cases (Stevens, 1970a). Although a few teratomas were produced in grafts of male genital ridges to the ovarian fat pad, no grafts to the ovary itself were made.

Grafts of tubal eggs to ectopic sites usually result in growths of only extra-embryonic tissues (Kirby, 1963). According to Billington (1965), morulae grafted to the testis never gave rise to embryonic shield derivatives; both donor and host mice were from a randomly breeding colony. However, Stevens (1968) grafted 2-cell and 4- to 8-cell mouse embryos to adult testes of strain 129 and found embryonic derivatives in 7% of the testes with 2-cell grafts and 8% of testes with 4- to 8-cell grafts. No embryonic

derivatives were seen in similar grafts to other strains (A/HeJ, C57BL). (All of the grafting experiments reported by Stevens were to histocompatible hosts.) Some of the grafts which were left 30 to 60 days contained undifferentiated embryonic cells and immature tissues. They looked grossly like teratomas and were regrafted to other hosts, behaving as spontaneous transplantable tumours with a mixture of undifferentiated and mature cells for five transplant generations (165 days).

Testicular teratomas have also been produced by the grafting of older embryos (Stevens, 1970c). Of 647 3-day blastocysts grafted to strain 129 and 129 x A/He hosts, 99 were recovered 39 to 60 days later. Of these, 36 contained undifferentiated cells. Three of these grafts were transplanted for many generations retaining some undifferentiated tissues.

Six-day embryos were grafted to strains 129, A/He and their F_1 hybrid (Stevens, 1970c). Of those recovered between 30 and 60 days later (115 out of 190), 112 contained embryonic derivatives and 4 were transplantable. One growth derived from a 6-day blastocyst metastasized to the left renal lymph node and formed a large mass of neural tissue. This tumour was transplanted for more than 40 generations and still contained undifferentiated embryonic cells.

According to Stevens (1973) transplantable teratomas

derived from 6-day embryos are indistinguishable from those derived from spontaneous testicular teratomas. Evans (1972) has reported the isolation of pluripotent stem cells from solid tumours derived from the implantation of 3-day embryos into the adult testis. These embryo-derived tumours, which had been maintained by solid subcutaneous transplantation for seven generations, were minced and trypsinized, and cloning was performed with the resulting single cell suspension. On inoculation into the mouse flank, tumours developed which contained at least ten types of tissue as well as the original pluripotential stem cell.

Some strains are resistant to teratocarcinogenesis. Dunn and Stevens (1970) grafted 120 6-day C3H embryos to adult testes, and only 6 gave rise to transplantable teratomas.

Karyotypes of 8 transplantable teratomas derived from early mouse embryos have been made (Dunn and Stevens, 1970). Half of the tumours had a male karyotype and half were female. Spontaneous testicular teratomas arising in strain 129 have a normal male karyotype and a modal number of 40 chromosomes (Stevens and Bunker, 1964).

Other workers have produced teratomas in mice by the grafting of early embryos. Solter et al (1970) grafted 7½-day C3H/H egg cylinders to the kidney of male mice of the same strain. Teratomas developed in 21 mice within 2 to 8 months after grafting. Six of the largest tumours were

successfully transplanted under the femoral fascia of syngeneic mice (Damjanov et al, 1971) and examined by electron microscopy. They contained undifferentiated cells typical of embryonal carcinoma occurring in the human testis. Small teratomas were not transplantable and were composed of only differentiated adult tissues.

On reviewing the literature of spontaneous and experimental teratomas, several questions come to mind:

Is the testis an immunologically privileged site?

Can teratomas be produced in the ovary?

What role does the host tissue play in the experimental production of testicular teratomas?

Are donor or host spermatogonia capable of teratocarcinogenesis?

Do the germ cells contribute to the production of these tumours?

The experiments reported in this thesis were designed to explore these areas and determine some of the

factors involved in the experimental production of gonadal teratomas in mice. Experiments have been divided into three sections:

Section A — Production of teratomas by the grafting of whole embryos to testes and ovaries of allogeneic mice

Section B — Experiments determining the contribution of testicular tissues in teratoma growth

Section C — Experiments designed to determine germ cell participation in growth of teratomas at the embryonic stage investigated

Each section will have a brief introduction and a discussion.

S E C T I O N A

Production of teratomas by the grafting of whole embryos to the testes and ovaries of mice

Experiment I The grafting of C3H/F X ICR embryos to the testes of ICR, C3H/F and C3H/F X ICR hosts

Experiment II The grafting of C3H/F X ICR embryos to the ovaries of C3H/F and C3H/F X ICR hosts

Introduction

The strains in which teratomas have been readily produced by the grafting of mouse embryos are 129, A/He and their hybrid (Stevens, 1970c). The greatest percentage of growths was obtained when the hosts were hybrid.

In the following experiments F_1 hybrids and both parent strains were used as hosts. The hybrid animals were the result of matings between C3H/F females and ICR males, two strains which have not previously been tested. C3H/F is an inbred strain with a brown coat colour, while ICR is a randomly bred white mouse.

All of the grafts of 6- and 7-day embryos in the literature were histocompatible. Stevens (1973a) states "grafts of embryonic cells are rapidly rejected when histocompatibility differences exist." There has been a report of allogeneic grafts to the kidney in the rat (Škreb et al, 1971), but it was inspected after only 15 days.

The testis is thought to be "immunologically privileged" giving some protection against rejection of allogeneic grafts (Billingham and Silvers, 1971; Patrick et al, 1972). In order to determine whether teratomas could be produced in testes of incompatible hosts, hybrid embryos were grafted to the testes of the two parent strains: C3H/F and ICR.

There have been no reports of teratomas having been produced by the grafting of embryonic tissue to the ovary. Because it is the commonest location of teratomas in man, the ovary was tested as a graft site for experimental teratomas.

Materials and Methods

Experiment I

ICR and C3H/F mice were obtained from Health Research Laboratory in Buffalo. Hybrid animals were produced by mating C3H/F females with ICR males. (Throughout this thesis "hybrid" will mean the F_1 generation of the cross between C3H/F females and ICR males.)

Embryos to be used as grafts were obtained by superovulating C3H/F females with follicle stimulating hormone (Gestyl, Organon) and chorionic gonadotropin (CG) (Follutein, E. R. Squibb and Sons). These females were then placed in cages with an ICR male for a two-hour period in the late afternoon, 7 to 8 hours after the CG injection. The following morning was designated as day ½ if a vaginal plug was seen. Seven days later the uterus was removed and placed in Hank's balanced salt solution (Grand Island Biological Company). There were usually between 12 and 20 implantation sites in each uterus. Sterile 23 gauge needles were used to free the embryo from the decidua and to remove the trophoblast and distal endoderm (Plate 1, Figures 2-4).

Hosts were anesthetised with ether (Mallinckrodt) and testes drawn out through a midline incision in the skin and abdominal wall. A siliconized micropipette (made from

capillary tubes) containing a single embryo in Hank's solution was inserted in each gonad and the embryo expelled. The skin was closed with clamps.

Strains used as hosts in this experiment were ICR, C3H/F and their hybrid, all between 7 and 16 weeks of age. In Series A the right testis was used as a control and given an injection of the balanced salt solution. Embryos were grafted to both testes in Series B.

All testes were removed one month after implantation and fixed in Davidson's solution (20% formalin, 35% alcohol, 10% glacial acetic acid, 35% distilled water) for 24 hours. After fixation the gonads were cut in halves or thirds and put through graded alcohols (70% to absolute), cleared in terpineol (Sargent-Welch) and embedded in paraffin (Paraplast, Sherwood Medical Industries, melting point 56°-57°C). The tissue was step-sectioned at 10 μ and stained with hematoxylin (Ehrlich's Original Formula, BDH Chemicals) and eosin (BDH Chemicals). Mounting medium was DEPEX (George T. Gurr).

Experiment II

Embryos grafted to ovaries were obtained by the procedure described in the previous experiment. Hosts were anesthetised with ether and a single incision made in the skin and dorsal body wall over either the right or left ovary. A single embryo was expelled into the gonad and the skin clamped.

One ovary in each host was treated using equal numbers of left and right ovaries. The remaining ovary was left intact (and used as a control) in order to avoid making a second incision and prolonging the time under anesthesia.

A month later all ovaries were removed and fixed in Davidson's solution. The next day the gonads were cut in half and processed for histological examination using the method described in the first experiment. All ovaries were serially sectioned.

PLATE 1

Figure 1 Embryo of 7½ days' gestation situated in the decidua which has been cut in half

x 20

Figure 2 Embryo after removal from the decidua, trophoblastic tissue and distal endoderm intact

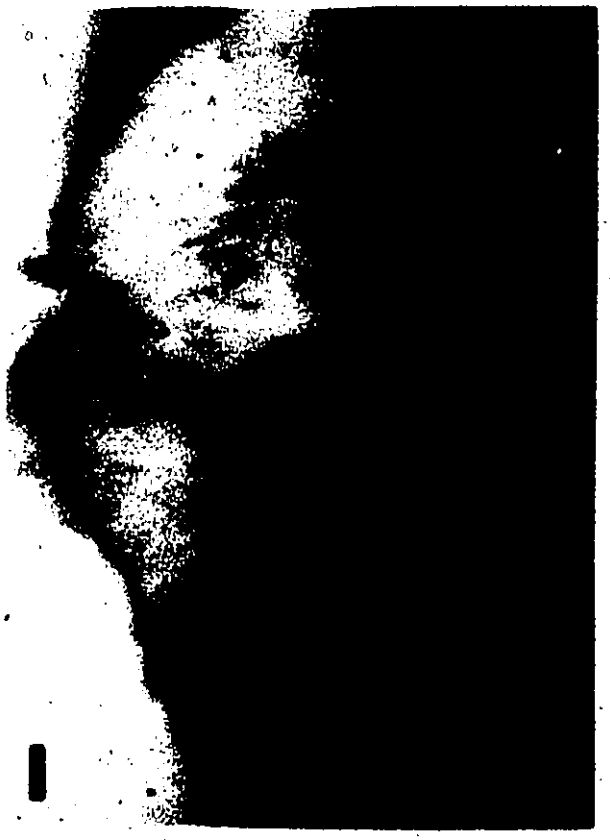
x 80

Figure 3 A section of 7½-day embryo, as above, with intact endoderm and trophoblast

x 80

Figure 4 Embryonic cone, 7½ days, after removal of trophoblast and distal endoderm

x 80



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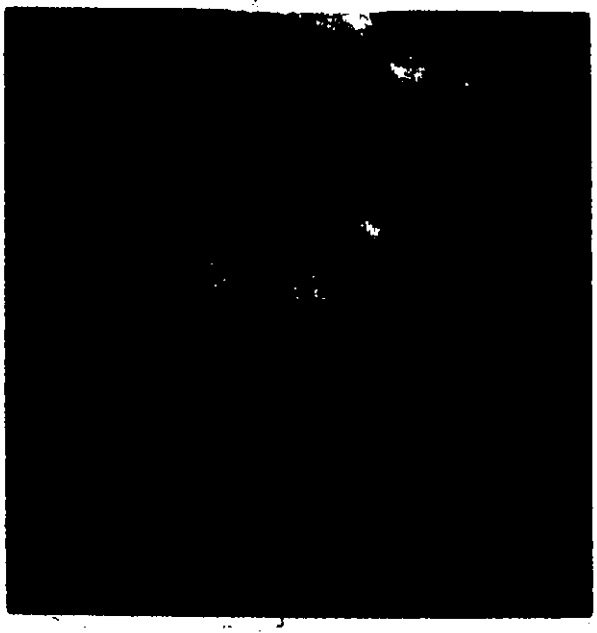

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PLATE I

Results

Experiment I

The results of grafting embryos to adult testes are presented in Tables 1 to 6. The growths observed were classified as teratomas when tissues were found which are not normally present in the gonads, according to the definition of Willis (1962a). These tissues commonly included cartilage, bone, nervous tissue and glands (Plate 2).

When only connective tissue, small lymphocytes, hemorrhage or necrosis were present, the appearance was classified as "reaction" (Plate 3).

Because the whole gonads were not serially sectioned, it is possible that some small growths were missed. However, this seems unlikely because teratomas (80%) or "reaction" (14%) were found in 94% of grafts to hybrid testes (Table 5), providing evidence of the high precision in placing donor embryos.

The frequency of teratomas was much higher when both the donor and recipient were hybrid than when grafts to ICR and C3H/F mice were made. Hybrid embryos developed into teratomas in 78% of hybrid host testes, 50% of C3H/F testes, and 40% of ICR testes (both Series A and B). The right testis seemed slightly more receptive than the left with teratomas growing in the right testis only in a total of 23 hosts and in the left testis only in a total of 12 hosts.

Sizes of the growths varied greatly, affecting not only the proportion of displacement of normal testicular structures, but the total volume of the testis. The greatest diameter of the testis was measured through a cross section using an eyepiece micrometer. These measurements are summarized in Table 6. The hybrid testes containing teratomas showed a marked deviation from the normal, in particular on the right side. The median size of this group was 6.8 mm and ranged up to 13.5 mm.

The occurrence of small lymphocytes in association with the teratomas was recorded. They were present in 80 to 90% of teratomas in all hosts of Series A and B. There was no apparent difference in frequency of occurrence or intensity of lymphocyte infiltration between either C3H/F or ICR hosts and the hybrid hosts.

PLATE 2

Figure 1 Testicular teratoma in ICR host containing cornified epithelium, columnar epithelium, hair, muscle, bone, cartilage and fat

x 25

Figure 2 A view of the rectangle in Figure 1 at a higher magnification

x 100

Figure 3 Testicular teratoma in C3H/F host containing connective tissue and cartilage.

x 100

Figure 4 Testicular teratoma in hybrid host composed of undifferentiated mesenchymal tissue, epithelium, nests of cartilage, and bone with marrow

x 100

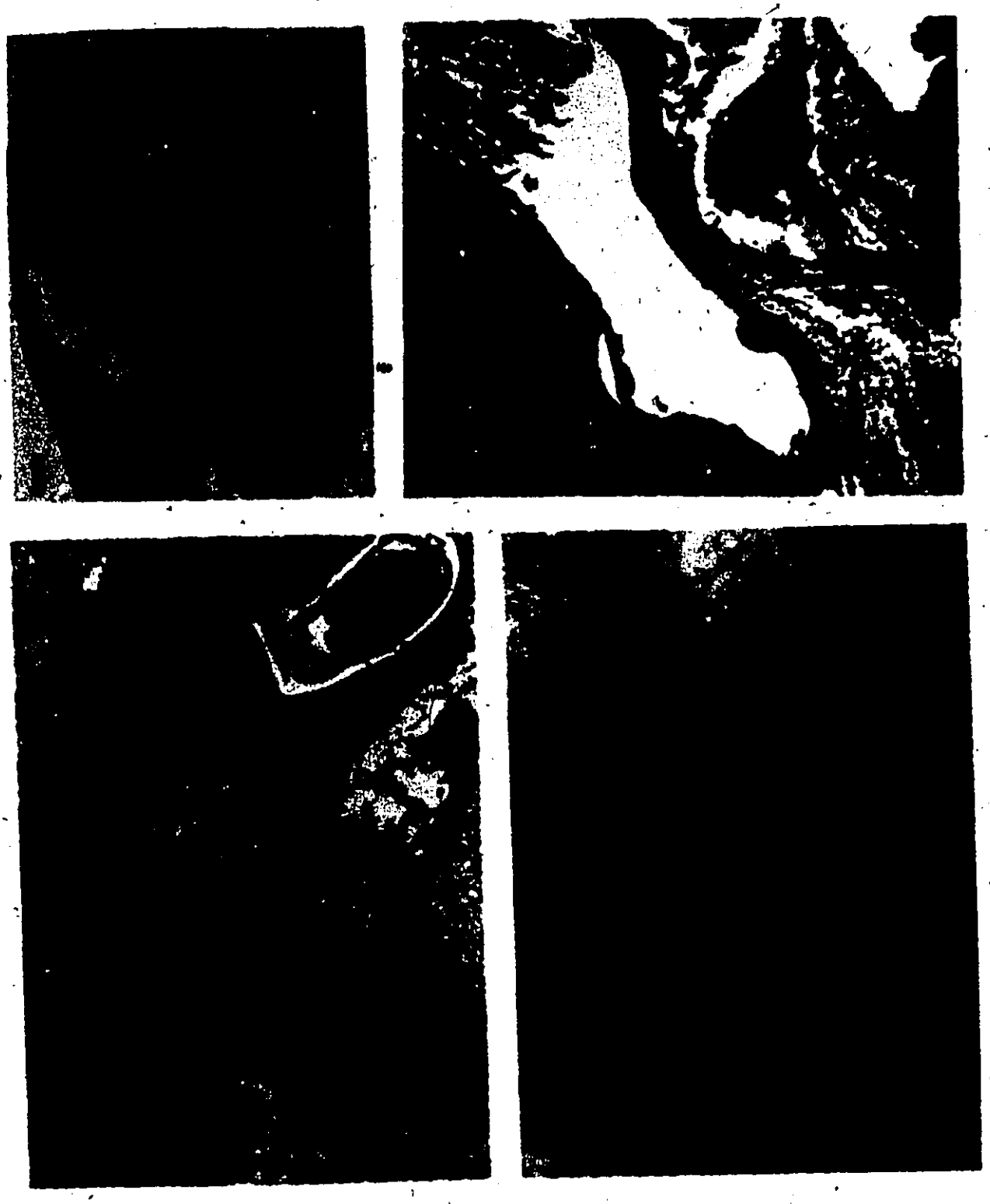


PLATE 2

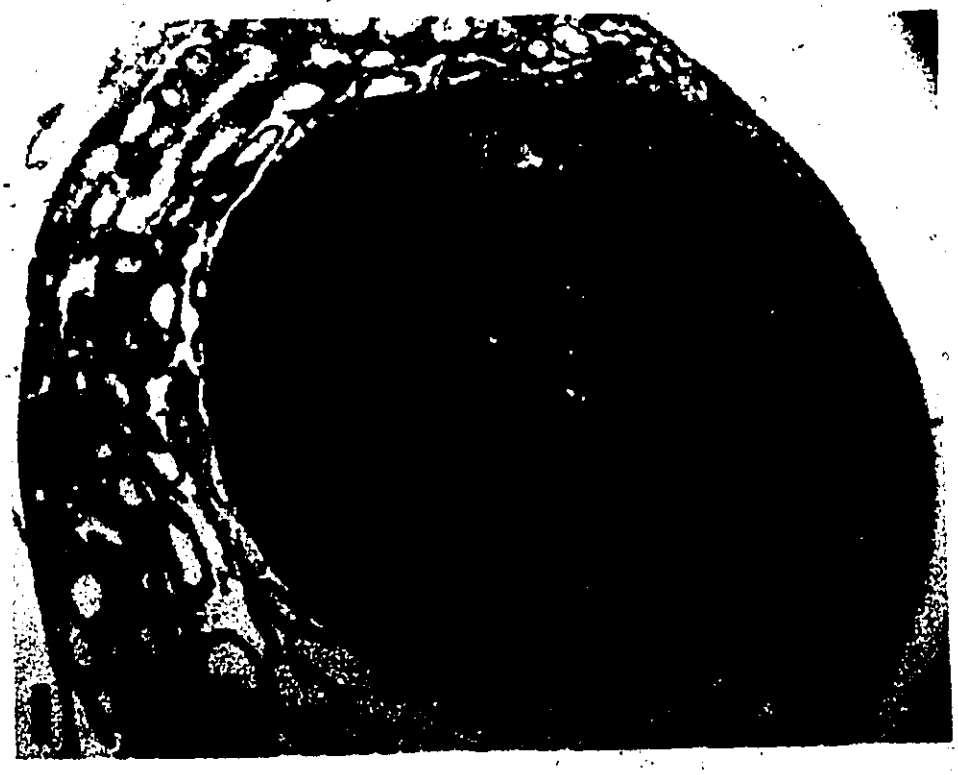
PLATE 3

Figure 1 Reaction in ICR host with a large hemorrhage containing cellular debris

x 25

Figure 2 Reaction in C3H/F testis consisting of connective tissue with lymphocyte infiltration

x 25



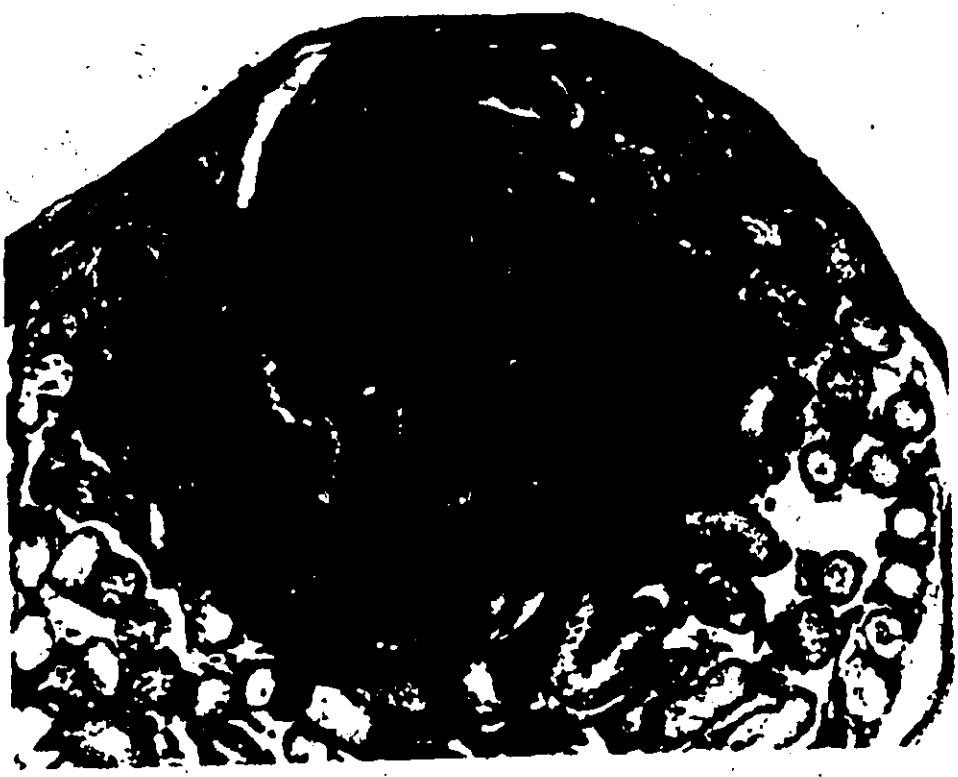


PLATE 3

TABLE 1

Frequency of teratomas arising from grafts of 7½-day embryos (C3H/F X ICR) to the left testis only - Series A

Host strain	ICR	C3H/F	Hybrid
No. of hosts	15	24	14
Teratoma	5 (33%)	16 (67%)	10 (72%)
Reaction	6 (40%)	5 (21%)	2 (14%)
No tumour	4 (27%)	3 (12%)	2 (14%)

TABLE 2

Frequency of teratomas arising from grafts of 7¼-day embryos (C3H/F X ICR) to both testes of strain ICR - Series B

	ICR	
No. of hosts	52	
	Left	Right
Teratoma	20 (39%)	23 (44%)
Reaction	25 (48%)	20 (39%)
No Tumour	7 (13%)	9 (17%)
Teratoma in one testis only	5	8
No. of hosts with at least one teratoma	28 (54%)	

TABLE 3

Frequency of teratomas arising from grafts of 7½-day embryos (C3H/F X ICR) to both testes of strain C3H/F - Series B

	C3H/F	
No. of hosts	32	
	Left	Right
Teratoma	11 (35%)	15 (47%)
Reaction	17 (53%)	13 (41%)
No tumour	4 (12%)	3 (12%)
Teratoma in one testis only	5	9
No. of hosts with at least one teratoma	20 (62%)	

TABLE 4

Frequency of teratomas arising from grafts of 7½-day embryos (C3H/F X ICR) to both testes of hybrids (C3H/F X ICR)

Series B

	Hybrid	
No. of hosts	25	
	Left	Right
Teratoma	18 (72%)	22 (88%)
Reaction	4 (16%)	3 (12%)
No tumour	3 (12%)	0
Teratoma in one testis only	2	6
No. of hosts with at least one teratoma	24 (96%)	

TABLE 5

Summary of Tables 2, 3 and 4

(Frequency of teratomas arising from grafts of 7½-day embryos (C3H/F X ICR) to both testes of strains ICR, C3H/F and their F_1 hybrid - Series B)

	ICR	C3H/F	Hybrid
No. of grafts (to both left and right testes)	104	64	50
Teratoma	43 (41%)	26 (41%)	40 (80%)
Reaction	45 (43%)	30 (47%)	7 (14%)
No tumour	16 (16%)	8 (12%)	3 (6%)
No. of hosts with at least one teratoma	28 (54%)	20 (62%)	24 (96%)

TABLE 6

Diameter of testes in millimeters

	ICR	C3H/F	Hybrid
Mean diameter of control testes	5.0	4.0	4.8

Series A

Mean diameter of testes with teratomas	4.8	4.7	5.8
Range	4.2 - 5.2	3.6 - 6.2	3.6 - 7.3

Series B

Mean diameter of testes with teratomas			
Left	5.1	4.1	5.8
Range	3.7 - 8.0	3.6 - 4.7	3.6 - 12.6
Right	5.3	4.2	7.3
Range	4.2 - 6.5	3.6 - 5.0	4.2 - 13.5

Experiment II

Teratomas developed in the ovaries of only 4 C3H/F females used and in 5 of the hybrids, 14% and 12½% respectively (Table 7). Since 2 of the latter were located in the fat pad, it is possible that many of the embryo implants were not successfully performed, particularly in the young hybrids whose small ovaries were more difficult to penetrate with a micropipette.

The remaining 3 growths in the hybrids were in the right ovary and contained a large variety of well differentiated tissues - cartilage, bone, muscle, nervous tissue, and skin with hair follicles and sebaceous glands. Two of these were large, 5.5 mm and 6.5 mm in diameter (Plate 4).

Of the 4 C3H/F tumours, 2 were located in the left ovary and 2 in the right. All were 1.4 mm in diameter and contained cartilage. Other tissues present were bone, muscle and small lymphocytes.

PLATE 4

Figure 1 Cystic ovarian teratoma in C3H/F X ICR host containing many epithelial cysts, nervous tissue, and undifferentiated mesenchymal tissue

x 25

Figure 2 A higher magnification of the rectangle in Figure 1 showing connective and nervous tissue and a blood-filled cyst

x 100

PLATE 4

TABLE 7

Frequency of teratomas arising from grafts of 7½-day embryos (C3H/F X ICR) to ovaries - one side only

	C3H/F	*Hybrid
No. of hosts	29	40
Teratoma	4 (14%)	5 (12½%)
Reaction	9 (31%)	5 (12½%)
No tumour	16 (55%)	30 (75%)

Discussion

It has been shown that both malignant and benign tumours can be grown from embryos transplanted to extra-uterine sites of an adult host (Stevens, 1968, 1970c; Solter et al, 1970). The malignant tumours, called teratocarcinomas by these workers, attain a large size during the first two months after implantation of the embryo and histologically contain undifferentiated cells. Although they rarely metastasize, these teratocarcinomas continue to grow and eventually kill the host.

Smaller embryo-derived tumours usually stop growing by six weeks after grafting and are composed predominantly of mature somatic tissues. These benign tumours are called teratomas, although the two terms are used interchangeably. The growths produced in this study are of the benign type.

One of the factors which control the growth and development of this embryonic tumour is a genetic predisposition. In previous investigations of experimental teratomas derived from embryonic grafts, only a few strains have been tested for their susceptibility, and the most successful results were in strain 129 or sublines known for their high incidence of spontaneous tumours. Stevens (1968; 1970c) reported teratomas derived from grafts of 3- and 6-day embryos in testes of strains 129, A/He and their hybrid, but was unable to induce tumours in C3H using

this method (1970c). Solter et al (1970) were able to produce teratomas in the kidney of C3H/H mice using 7½-day embryos. Experiment I has shown that C3H/F, ICR, and their F_1 hybrid are also suitable for this experimental model.

Only syngeneic embryonic grafts were used in the studies reported in the literature. The grafting of hybrid embryos to ICR and C3H/F hosts demonstrates that teratomas can be produced in testes even when histocompatibility differences exist between the donor and host. Histocompatibility antigens in the mouse have been demonstrated as early as 3½ days' gestation (Vandeputte and Sobis, 1972). Skin grafting experiments done in our laboratory confirm the incompatibility of these strains (Dins Gooden, personal communication). Using C3H/F males as recipients, 11 isografts remained healthy for more than 6 weeks. Allografts (C3H/F X ICR) to the same recipients were all rejected between 9 and 16 days. When skin grafts were made to ICR males, all of the isografts were rejected between 11 and 40 days. Hybrid allografts (C3H/F X ICR) to the same mice were also rejected, but more quickly (9 to 15 days).

Although allogeneic embryonic grafts have not been reported in mouse testes, there has been one such report in the rat (Skreb et al, 1971). These grafts were between an inbred and randomly bred strain, but were inspected after only 15 days. The grafts in Experiment I were all left in place for 30 days, allowing time for immunological rejection.

The numbers of teratomas resulting from grafts of hybrid embryos to both testes (Series B) are summarized in Table 5. Teratomas were found in 41% of both ICR and C3H/F testes and in 80% of hybrid testes. The numbers of hosts with at least one teratoma were 28/52 (ICR), 20/32 (C3H/F), and 24/25 (C3H/F X ICR), a difference which is highly significant statistically ($P = 0.999$). These results show that with the strains used the testicular site gives considerable protection against immunological rejection.

On the other hand, Stevens (1973) and Damjanov and Solter (1974b) state that when there are antigenic differences between strains, the grafts are completely rejected. This observation has been partially confirmed in our laboratory using strain C57BL as hosts and strain C3H/H as donors. Growths composed mainly of cartilage developed from 2 out of 16 grafts (Dins Gooden, personal communication). Certainly some immunologic phenomenon is taking place in the interaction between the host and the tumour; Damjanov and Solter (1974a) report that the spleen increases in weight in all animals bearing grafted embryos, and that in animals with teratocarcinomas the spleens were heavier than in those animals with small non-proliferating teratomas.

Sites other than the testis which have been described as "immunologically privileged" are the anterior chamber of the eye and the brain (Barrett, 1974). Because they lack

lymphatic drainage, foreign antigens administered to these two sites cannot be transported to lymph nodes where sensitization takes place. This cannot be the explanation for the limited privilege exhibited by the testis, however, since it contains large lymphatic sinusoids (Fawcett et al, 1969). It has been suggested that because of the distance between the testes and their draining lymph nodes, the immune reaction is limited at this site (Billingham and Silvers, 1971).

Patrick et al (1972) suggest that the local action of testicular steroid hormones may prolong graft survival. There is some data to suggest that teratomas may be susceptible to endocrine influences. Animals are prone to induction of germinal cell tumours only during their breeding season (in the fowl, Guthrie, 1964) or when exposed to exogenous hormones (in the mouse, Bresler, 1959). Direct studies of hormonal influences upon human tumours are inconclusive; Kallén and Rohl (1962) using 6 "embryomatous tumours (embryonic carcinoma and teratocarcinoma) in tissue culture reported that in 5 cases the tumours could be influenced with androsterone, 2 being arrested and 3 stimulated; Pierce et al (1959) using Pitt 61, a human embryonal carcinoma of the testis, have shown that it secretes a chorionic gonadotropin-like hormone in 20% of heterologous hosts, its growth rate being stimulated by

the administration of chorionic gonadotropin to the hosts but not by the administration of follicle stimulating hormone.

Another factor which may prolong graft survival in the testis is the lowered temperature in the scrotum. Stevens (1970a) has concluded that, when compared with other sites, this difference in temperature provides ideal conditions for the development of tumours in grafts of genital ridges to the testis.

In strain 129 spontaneous testicular teratomas occur three times more frequently in the left testis than in the right (Stevens, 1954, 1962). This asymmetry has also been reported in the new subline 129/terSv (Stevens, 1973b) with 13% having teratomas in the left testis, 7% in the right, and 12% in both. In Experiment I the right testis appears more receptive, not only in numbers of tumours (Tables 2, 3, 4) but in their size (Table 6). The difference in numbers of teratomas in the left and right testes is not statistically significant ($P = .944$).[1] In Series B there is no significant difference between sizes of left and right testes with tumours in either C3H/F or ICR hosts. However, the right hybrid testes containing teratomas are significantly larger than the left ($P = .975$).[2] In man, testicular teratomas are found more frequently in the right than the left testis (Meier et al, 1970).

1 X^2 test

2 unpaired t test

Although the numbers of teratomas which developed in the ovaries were small, there was no obvious difference between their survival in C3H/F and hybrid hosts as there was in males. Because of the technical difficulties involved in this operation, no statistical significance can be assigned to these results.

The low frequency of successful grafts in ovaries is probably due to the difficulty of placing and maintaining embryos in these small organs, which unlike testes, lack a defined capsule. However, the good growth of teratomas in 12½% of the allogeneic ovaries at 30 days is evidence that these organs too are "immunologically privileged."

Another factor which may influence isograft, as well as allograft, rejection is the Eichwald-Silsmer phenomenon (1955). These workers have demonstrated that an antigen which originates on a gene in the Y chromosome may be a histocompatibility factor. In Experiment II, one half of the donor embryos were female and one half, male. This "male" antigen may have been responsible for some of the rejections. An ascitic form of a spontaneous testicular teratoma does not grow in females, although it grows well in males (Isa, 1974) suggesting that there is a histocompatibility factor in sex difference.

The immunological privilege exhibited by the ovaries in Experiment II cannot be attributed to their lack of

lymphatic drainage. The ovaries are close to their draining lymph nodes and have plentiful lymphatics in several mammals studied (Morris and Sass, 1966). It has been suggested that the local action of estrogen may prolong graft survival (Hulka, 1971).

Histologically, the teratomas produced in these experiments were similar to those described by other workers. Many well differentiated adult tissues were found as described, but liver tissue and teeth were not seen. Although undifferentiated cells were present, these did not predominate as they sometimes do in highly malignant tumours produced by using blastocysts and 6-day embryos as grafts (Stevens, 1970c). By 7½ days' gestation the embryonic cells may be already committed to follow their normal path of differentiation, although genetic and environmental factors are important. Damjanov et al (1971) were able to produce transplantable teratomas containing undifferentiated embryonic cells by grafting 7½-day embryos under the kidney capsule of C3H/H mice; when the experiment was repeated using C57BL isogeneic grafts (1974b), no teratocarcinomas were produced.

The experiments in Section A have demonstrated that strains C3H/F, ICR, and their F_1 hybrid are favourable hosts

for the production of embryo-derived teratomas, and that the antigenic differences between C3H/F and the randomly bred strain ICR are not sufficient to provide rejection of the tumour in the testis or ovary at 30 days, even though skin grafts between hybrid and parent strains are promptly rejected.

S E C T I O N B

Experiments determining the contribution of testicular tissues in teratoma growth

Experiment III — Testes of ICR mice are treated with copper wire and testosterone

Experiment IV — Injections of embryonic cell-free filtrate into testes of hybrid mice

Experiment V — The grafting of embryos bearing T6 marker to the testes of histocompatible mice

Experiment VI — The grafting of adult spermatogonia to the testes of ICR and hybrid mice

Introduction

Because of the hypothesis that gonadal teratomas arise from the abnormal histogenesis of primordial germ cells, several researchers have attempted to induce carcinogenesis in the testis. The intra-testicular inoculation of zinc chloride, sulphate, or nitrate, and copper sulphate has resulted in testicular teratomas in fowl (reviewed by Guthrie, 1964). These experimental tumours arise only in the spring during the period of increased spermatogenesis. Spontaneous testicular teratomas are very rare in birds.

There is only one report in which this work has been reproduced in mammals. Bresler (1959) was able to induce teratomas in two animals by injecting an emulsion of peach oil and copper sulphate in a 1.5% solution of formalin into the right testicle of more than 100 white mice. Injections of methyl-testosterone-propionate were also given in order to increase spermatogenesis. Stevens (1967) was not able to reproduce Bresler's experiments, even though he used strain 129 mice known for their high incidence of spontaneous teratomas.

Experiment III is an attempt to induce teratomas in a white mouse, randomly bred ICR, with an implant of copper wire in the testis and injections of male sex hormone.

Formalin was not used because it is highly toxic. In Bresler's experiment a large percentage of treated mice died within a few days.

There was the possibility, in the experiments of Section A, that embryos grafted to the gonads might have induced the germ cells of the host to differentiate into some of the tissues seen in the tumours. Experiment IV uses a cell-free suspension of embryonic organelles and fluids to see whether it could initiate differentiation of host spermatogonia.

Experiment V was designed to give additional evidence that it is the embryonic tissue which is responsible for the growths which develop in testes. It is possible to identify the donor tissue by karyotypic analysis of the tumour if a chromosome marker is used. For this experiment CBA mice homozygous for an easily identified translocation known as T6 were mated to provide the donor tissues. Hosts were histocompatible but with a normal karyotype.

It has been suggested that teratogenesis may be initiated in adults by the rupture of the seminiferous tubules, perhaps through injury, and the subsequent release of germ cells into the interstitial area; a different environment could alter the normal controlling mechanism

for differentiation of spermatogonia to spermatocytes. To explore this possibility adult spermatogonia were injected into the testes of both ICR and Hybrid mice in Experiment VI.

Materials and Methods

Experiment III

Thirteen ICR mice were used in this experiment. A small piece of copper wire 7 mm in length was folded and inserted, through a small tear in the tunica, into one testis of each animal. One week later 0.05 ml testosterone propionate (100 mg per ml, E. L. Stickley & Company) was injected into the leg muscle. Every second week thereafter the same dosage of testosterone was administered, a total of 15 injections over 7 months.

Not all the animals survived the whole treatment period, four dying after 2, 4, 5 and 6 months. The internal organs were examined at the time the testes were removed. After one day of fixation (Davidson's), the organs containing copper were cut in half, the copper removed, and the tissue processed and embedded in paraffin. Sections were cut at 10μ and stained with hematoxylin and eosin.

Experiment IV

Embryos of 7½ days were obtained from matings between C3H/P females and ICR males (from the stocks used in the preceeding experiments). After the trophoblast and distal endoderm were removed, 11 embryos were placed in 10 ml of sterile 0.9 Gm% saline, homogenized in a Sorvall blender for

one half minute, about 5 seconds at high speed, and then centrifuged for 15 minutes at 2200 rpm.

The supernatant was injected into both testes of 4 hybrid adults through a 0.45μ Millipore filter. Smears were made of both the supernatant and homogenate. These slides were placed in 95% alcohol fixative and then stained with methylene blue (Fisher Scientific Company). No nuclei or whole cells could be seen after careful scanning.

Animals used in this experiment were killed two months later. Their testes were removed and processed for histological examination. Sections were made at 10μ and stained with hematoxylin and eosin.

Experiment V

Mice for this experiment were obtained from Jackson Laboratories, Bar Harbor, Maine. Six or more embryos of 7½ days were obtained from each mating between CBA/H-T6 adults, both carrying the homozygous translocation, known as T6, which is easily identified in metaphase spreads (Plate 5, Figure 1). The embryos were grafted to both testes of 40 histocompatible adults (CBA/CaJ) with normal chromosomes (Plate 5, Figure 2). The technique used in preceeding experiments was followed.

Testes were removed one month later. On gross inspection, 27 testes contained large tumours and were cut in two; one half was fixed for histological processing, the

other half was used for chromosome analysis. Those testes which appeared normal were also preserved and later embedded in paraffin.

Chromosome preparations were made directly from the solid tumours, using a technique modified from that of Bunker (1965). Care was taken to avoid contaminating one tumour with another.

Pieces of tumour were placed in Hank's BS solution and minced with scissors. One or two drops of Colcemid (1 mg/ml, Grand Island Biological Co.) were added. Three hours later the tissue was transferred to the barrel of a syringe with a Swinney adapter and forced through the fine wire mesh filter. The single cells recovered were then spun at 800 rpm for 5 minutes (154.6 G) and the Hank's solution removed. After a 15-minute incubation in 1.12% hypotonic sodium citrate at 37°C, the cells were spun again and resuspended in 3 ml aceto-alcohol fixative (1 part glacial acetic acid to 3 parts methanol). This solution was removed one hour later after spinning. Enough fresh fixative was added to make a hazy suspension.

Two or three drops of this suspension were allowed to fall from a pipette an inch above a slide wet with ice cold water and held horizontally. The fixative was ignited by touching the edge of the slide to a flame. After the fixative extinguished itself, the slide was stained in undiluted Giemsa (BDH Chemicals) for one half hour and then

rinsed in water. The slides were air dried overnight, dipped in toluene, and coverslipped with DEPEX. Several slides were made from each of 27 tumours.

All of the good metaphase spreads were photographed; after dividing the chromosomes into small groups on the photograph, they were counted and the marker chromosomes circled. Counts were checked by re-examining the spread under the microscope.

Experiment VI

Spermatogonia were obtained from the testes of four hybrid mice (C3H/F X ICR). A glass rod was used to tease cells from the tubules into 5 ml of Hank's BS solution. This suspension was then drawn into a micropipette and expelled into both testes of three adult hybrids using the technique described earlier.

The same procedure was followed using ICR males for donor spermatogonia and as hosts (four animals). Hybrid hosts were 9 months of age and ICR hosts 3 months.

Five months later the testes were removed and processed for histological study as described in earlier experiments.

PLATE 5

Figure 1 Mouse metaphase cell with homozygous T6 translocation. The marker chromosomes are circled

x 800

Figure 2 Normal mouse metaphase spread with 40 chromosomes

x 800

PLATE 5

Results

Experiment III

No teratomas were found in the testes which had held a piece of copper wire for seven months. All but one, however, were a smaller size than normal (control mean, 5.0 mm); the largest diameter was 5.3 mm.

Cross sections were all similar to that shown in Figure 1, Plate 6, in which there was a central area with no tissue (the wire had been removed), surrounded by connective tissue with small lymphocytes and seminiferous tubules in various stages of degeneration.

Other organs examined histologically were normal as well as those which were inspected at the time the testes were removed.

Experiment IV

The testes of animals which had been treated with an embryonic cell-free filtrate all appeared normal when examined under the microscope. In some specimens there seemed to be a thickening of connective tissue just under the tunica albuginea.

Other internal organs appeared normal on gross examination.

Experiment V

Only those tumours which produced at least 15 good metaphase spreads are reported here. The results are summarized in Table 8. Approximately half the cells had the normal number of chromosomes (40 in the mouse), all of which contained 2 T6 marker chromosomes (Plate 5, Figure 1).

The number of spreads with 40 chromosomes varied from one tumour to another (6% in No. T6-38 to 77% in No. T6-11). A third of the cells contained fewer than the normal, while 18% had extra chromosomes. There were 9 cells with about 80 chromosomes (Plate 7, Figure 1a, b, d) and one example of endoreduplication.

Most of the testes from which these preparations were made were larger than normal (mean 4.2 mm). In cross section their measurements ranged from 4.1 to 11.0 mm. The largest tumour (No. T6-38) had the fewest normal spreads, 1 out of 17.

Tissue types could not be correlated with the chromosome analysis. Most tumours had many well differentiated tissues (Plate 7, Figures 2, 3). Very few seminiferous tubules remained in the gonads, and those present had degenerated into remnants.

TABLE 8

Chromosome analysis of eight tumours bearing T6 markers produced by grafting 7½-day CBA/H-T6 embryos to the testes of CBA/CaJ hosts

Tumour No.	Total Spreads	No. of cells		
		< 40	40	> 40
T6-7	26	10 (38%)	12 (46%)	4 (16%)
T6-9	29	12 (41%)	15 (52%)	2 (7%)
T6-10	24	8 (33%)	14 (58%)	2 (9%)
T6-11	35	7 (20%)	27 (77%)	1 (3%)
T6-33	24	9 (37%)	10 (42%)	5 (21%)
T6-34	15	3 (20%)	7 (47%)	5 (33%)
T6-36	20	3 (15%)	7 (35%)	10 (50%)
T6-38	17	11 (65%)	1 (6%)	5 (29%)
TOTALS	190	63 (33%)	93 (49%)	34 (18%)

Experiment VI

The results of injecting adult spermatogonia into host testes for several months are negative. No teratomas were present although there was some necrosis, tubule degeneration not unlike that found in aged mice, and some lymphocyte infiltration (Plate 6, Figures 2, 3).

PLATE 6

Figure 1 Testis of ICR host after removal of folded copper wire. There is much necrosis and degeneration of tubules

x 20

Figure 2 Testis of hybrid host 5 months after injection of adult spermatogonia and spermatocytes. There are few spermatocytes developing in the tubules and the interstitial areas are much larger than normal

x 20

Figure 3 Testis of ICR host 5 months after injection of adult spermatogonia and spermatocytes showing area of connective tissue and tubule breakdown

x 80

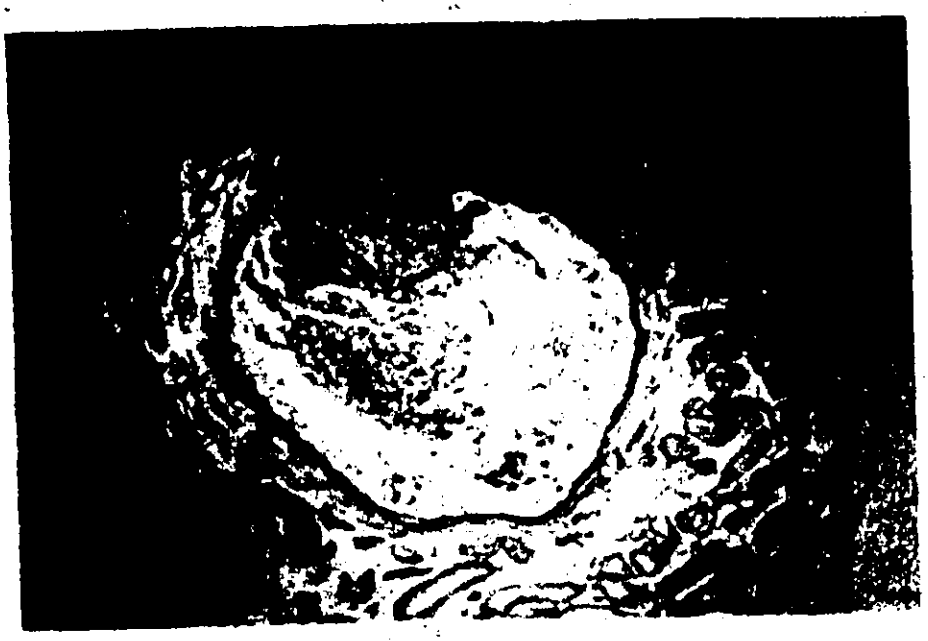


PLATE 6

PLATE 7

Figure 1 Metaphase cells in CBA mouse testes with teratomas

x 800

a 81 chromosomes, No. T6-34

b 80 chromosomes, No. T6-36

c 122 chromosomes, No. T6-36

d 80 chromosomes, No. T6-36

Figure 2 Section of tumour, T6-34, from which the metaphase spread in Figure 1a was taken

x 80

Figure 3 Section of tumour, T6-36, from which the spreads in Figure 1b, c, and d were taken. This tumour was composed predominantly of nervous tissue and connective tissue. There is a tubule remnant in the lower right

x 80

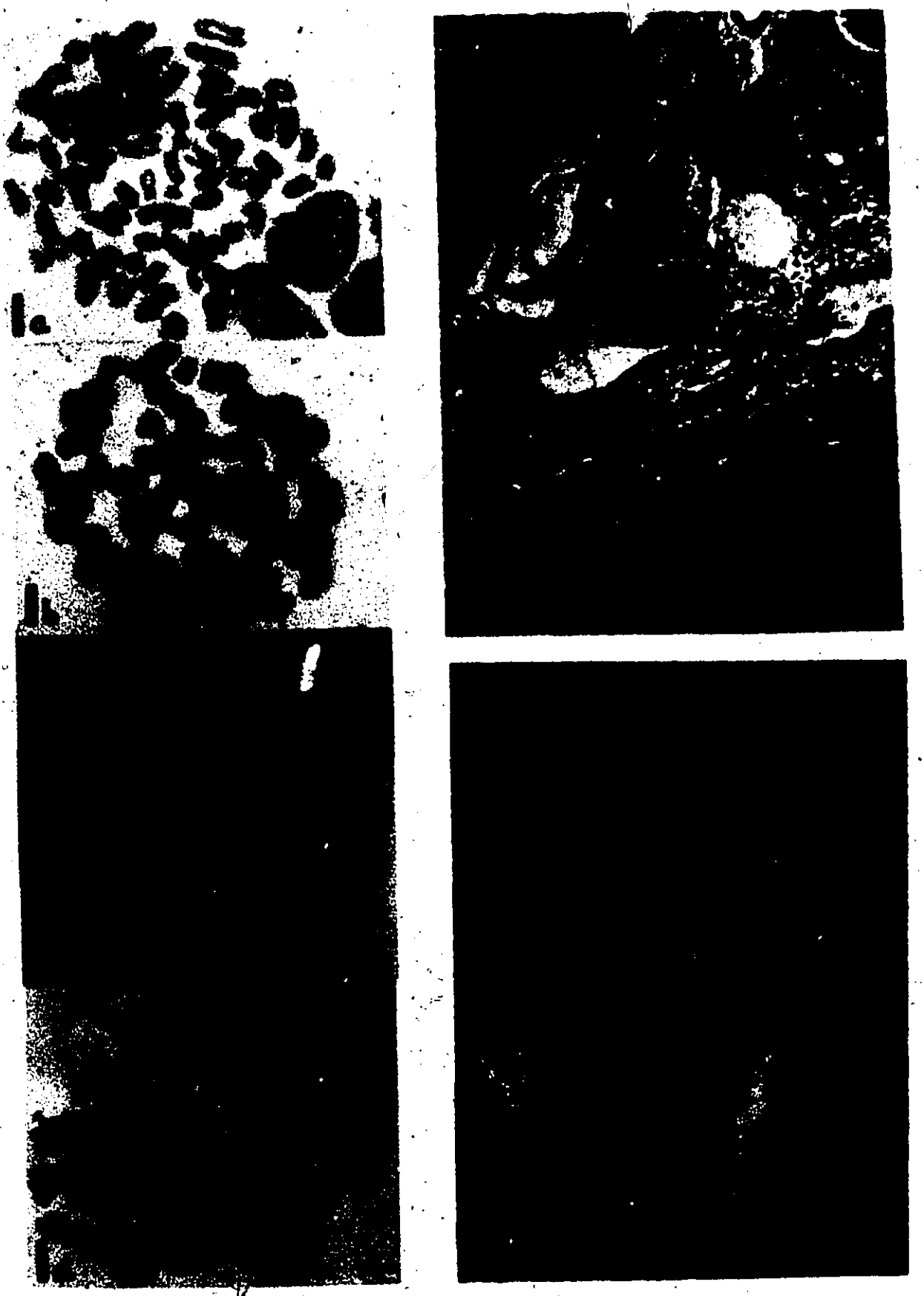


PLATE 7

Discussion

Although experiments in which teratomas have been induced in birds by injections of zinc or copper salts are convincing (Guthrie, 1964), they are not conclusive evidence for the origin of teratomas from germ cells in mammals. The fowl gonad is a very labile organ in which sex reversal is easily accomplished (Erickson and Pincus, 1966).

The work of Bresler (1959) in which teratomas were induced using copper salt in formalin has not been repeated with success, although attempts have been made using a white mouse (Stevens, 1967). The strain Stevens used was likely different from that used by Bresler.

Experiment III is another unsuccessful effort to bring about a neoplastic change in the germ cells of a white mouse, the randomly bred strain ICR. It was not a duplication of Bresler's experiment, in that copper wire was used rather than copper salts in formalin. Bresler used formalin to delay absorption of the copper salt with the result that many mice died. Perhaps the formalin, although highly toxic, is a tumour inducing agent.

It is known that copper is absorbed slowly, and therefore copper wire was used in Experiment III along with injections of testosterone. The germ cells, nevertheless, appear to be resistant to teratogenesis in the adult mouse.

It has been assumed, and probably correctly, that it is the embryonic grafts which give rise to the teratomas found in the gonads. However, the possibility existed that the embryos might have induced the host germ cells to differentiate into some of the tissues seen in the tumours. Therefore a supernatant composed of embryonic cell organelles and fluids was used in Experiment IV to see whether it could initiate differentiation of host spermatogonia. The results indicate that this supernatant is an ineffective agent for tumour induction. Whatever inducing factors are present in the 7½-day embryo, at the concentration used they do not bring about a change in the differentiation of spermatogonia. It is possible, however, that the injections were made into the interstitial spaces and the supernatant was prevented from entering the tubules by the blood-testis barrier. It has been demonstrated in the monkey (Dym, 1973) and in other mammals that occluding junctions between adjacent Sertoli cells prevent vascularly injected electron-opaque marker lanthanum nitrate from reaching the tubule lumen.

The results of Experiment V, in which the tumours displayed the T6 marker chromosome, have also shown that these growths are derived solely from the embryonic donor tissue. It has been demonstrated as well that teratomas can be easily induced in the CBA strain. Tumours were

observed in more than 60% of the gonads receiving grafts.

On tabulating the results of Experiment V it was surprising to find that only half of the cells had a count of 40 chromosomes (Table 8). Dr. Charles Ford (personal communication) has made a chromosomal analysis of 607 mouse embryos aged 8 to 11 days, and found only 13 (2.1%) with abnormal karyotypes.

There is a discrepancy in the results of this experiment when compared with those of Stevens and Bunker (1964) who analysed primary spontaneous testicular teratomas in strain 129 mouse. In their study 8.3% of cells had 38 or fewer chromosomes, 87.6% had counts between 39 and 41, and 2.6% had 42 or more chromosomes. The respective percentages in Experiment V are 22.5%, 63%, and 9.5%. Personal techniques could account for these differences in counts. On the other hand, the disruption in the normal environment of the embryonic cells may bring about faulty cell division.

Although the vast literature bearing on the relation of aneuploidy to malignancy will not be discussed here, there is more and more evidence to associate these two phenomena (Arias-Bernal and Jones, 1968). In a recent review, Atkin (1973b) states that malignant teratomas in man commonly have modes in the 50 to 69 range. The fact that spontaneous testicular teratomas in mice have near diploid modes may account for the low degree of malignancy

in these tumours. Tissue types usually correlate with the degree of malignancy in both man and mouse; well differentiated adult tissues are most often benign, while those tumours with undifferentiated embryonic tissues are likely malignant.

Experiment VI was performed to test whether extratubular adult germ cells might have potential for tumour formation; spermatogonia and maturing spermatocytes were placed in the interstitial area of adult hosts. The results indicate that the environment in which they were placed could not induce the donor cells to alter their differentiation.

There has been a report of sperm penetrating somatic cells in an in vitro experiment (Bendich et al, 1974). This situation, in which spermatogonia and spermatocytes were placed in hosts' testes, may have been suitable for autofertilization. If teratomas had developed, it would have added convincing evidence to Ashley's theory (1973) on the origin of teratomas from the fusion of two haploid gametes.

S E C T I O N C.

Experiments designed to determine germ cell participation in growth of teratomas at the embryonic stage investigated

Experiment VII	Embryological studies to demonstrate the first appearance of primordial germ cells
Experiment VIII	The grafting of C3H/F X ICR embryonic shields to hybrid (C3H/F X ICR) testes
Experiment IX	The grafting of C3H/F X ICR extra-embryonic cones to testes of hybrid (C3H/F X ICR) mice
Experiment X	Early stages in the growth of C3H/F X ICR extra-embryonic cones in the testis

Introduction

When 12½-day male genital ridges are grafted to the testis of adult mice, teratomas develop within the tubules of the fetal testis in a very high percentage of grafts. Mount and Stevens (1971) have studied the histogenesis of these tumours and found multiple adjacent foci which they believe arise from a single source, a primordial germ cell which has undergone a neoplastic change. At 12 days of fetal life there are over 5000 germ cells in the genital ridge.

If the germ cells in the 12½-day fetus are susceptible to neoplastic transformation, it would not be surprising if the primordial germ cells in earlier embryos were responsible for the growths observed when whole embryos are grafted to adult testes.

In the mouse, primordial germ cells are first identified at 8½ days' gestation in the yolk sac endoderm at the base of the allantois. Because of their extra-embryonic origin, they can be separated from the embryonic tissue. The experiments in Section C were done to determine which tissues give rise to teratomatous growths. Do the primordial germ cells located in the extra-embryonic portion give rise to these tumours? Or are they the result of abnormal cellular relationships which develop from the embryonic body of the shield portion?

Embryological studies (Experiment VII) were done to demonstrate the first appearance of primordial germ cells in the mouse. This work confirms the reports of other researchers on the origin and migration of these cells from the yolk sac endoderm to the genital ridges of mice (Chiquoine, 1954; Mintz, 1960; Spiegelman and Bennett, 1973). It also illustrates the changes taking place in the mouse embryo between 7½ and 8½ days' gestation.

After determining the position of the egg cylinder where the germ cells arise, it was possible to separate this portion from the embryonic shield. In Experiment VIII the embryonic portion is grafted to testes of hybrid hosts, and in Experiment IX the extra-embryonic portion of the cone (containing the germ cells) is grafted to the testes of another group of mice.

All of the gonads in the other embryonic graft experiments (I, II, V, VII and IX) were inspected one month after the initial operation. Odzdeński (1969) reported that germ cells from 8-day fetal mouse survive only a few days when implanted in the anterior chamber of the eye. Experiment X was designed, therefore, to study the early stages in the grafts of extra-embryonic cones.

Materials and Methods

Experiment VII

This experiment was designed to study the development of the yolk sac and the primordial germ cells between the ages of 7 and 9 gestational days. The mothers were C3H/F or hybrids and the fathers ICR. From 20 litters, 135 intact embryos were dissected at various intervals between the 7th and 9th days to give a spectrum of developmental stages.

The trophoblast was not removed in order that the yolk sac remain intact, and so that the embryos could be easily seen during their processing. Small embryos were transferred with a pipette and larger ones with a spatula. After 10 to 20 minutes' fixation in 95% alcohol the specimens were moved to absolute alcohol (5 minutes) and then to xylene for 20 minutes. Paraffin embedding was done at 55°C for 1/2 hour and the blocks refrigerated until serially sectioned at 10 μ.

Approximately half of the embryos, some from each litter, were stained with hematoxylin and eosin. The remaining littermates, representing all stages of development, were processed and tested for the alkaline phosphatase activity of their germ cells using the following procedure adapted from Pearse (1968):

Wax was dissolved from the slides in 3 changes of xylene; they were cleared in absolute acetone and 40% acetone and taken to cold water. Slides were then incubated for 10 to 15 minutes in a cold substrate of 15 mg sodium alpha-napthyl-acid-phosphate (Hartman-Leddon Company), coupled with an excess of azo dye, either fast red (Sigma Chemical Company) or fast blue (Hartman-Leddon Company) in 20 ml 0.05 M Tris buffer (Tham, Fisher Scientific Company), pH 10.4. Two ml of 0.1 N hydrochloric acid reduced the buffer solution to pH 9.4. Enzymatic activity is visualized as either red or blue precipitate depending on the dye used.

As a control for the technique young embryos were incubated in the same dishes as older embryos whose primordial germ cells contain the enzyme. Slides were rinsed in cold water and cover slipped with Hydramount (George T. Gurr).

Experiments VIII and IX

C3H/F female mice were mated with ICR males, and their embryos carefully dissected from the uterus seven days later. Mothers were not superovulated, and the number of sites in each uterus varied from 4 to 12. All trophoblast, distal endoderm and Reichert's membrane were removed, and each embryo was transferred with a pipette to a clean dish in a few drops of Hank's BS solution.

Using a new pair of sterile 23 gauge needles each time, the embryos were cut in two, separating the embryonic and extra-embryonic portions. The cut was made well into the embryonic shield, so that no extra-embryonic tissue was included in the shield portion, although some embryonic tissue remained with the extra-embryonic cone.

This procedure is illustrated in the photographs of Plate 10. The shield portion measured about 1/3 mm and the extra-embryonic part 2/3 mm in length.

These cut tissues were grafted to the testes of 36 hybrid mice using the technique employed with whole embryos in the previous experiments. This procedure was a little more difficult because the cut portions were more sticky than whole embryos; and because of their small size, it was not always possible to see whether the graft was successful.

If both testes were treated in a host, a shield was grafted to each testis (or an extra-embryonic cone to each testis). None of the hosts received both an embryonic shield and an extra-embryonic cone.

One month later the gonads were removed. Those testes which, on gross examination, appeared to be tumourous were cut in two--one half to be used as a transplant, the other to be fixed and processed for histological examination.

The pieces of tissue used in transplants were minced with scissors in 1 ml Hanks's BS solution, drawn into a syringe and injected through a 23 gauge needle into the abdomen and leg muscle of adult hosts. Most were male hybrids, although 3 female hybrids and 4 ICR males were also used as transplant hosts. Altogether 13 transplants from shield testes and 9 from extra-embryonic cones were attempted.

Experiment X

The material and methods used in this experiment were similar to those of the previous experiment. Extra-embryonic cones from 7½-day hybrid embryos were grafted to both testes of ten animals. On each of the 3rd, 5th, 7th, 9th and 15th days the gonads were removed from 2 hosts, fixed in Davidson's solution, cut in half and processed for hematoxylin and eosin staining.

PLATE 8

Figure 1 Drawing of embryo, 7 days 10 hours
Extra-embryonic areas are stippled, and the embryonic tissue is solid
Cf. Figure 3 of Plate 10

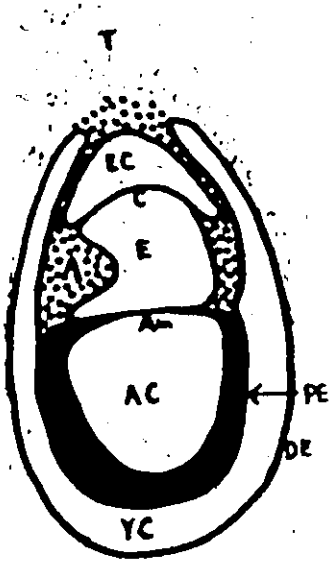
Figure 2 Drawing of embryo, 7 days 18 hours

Figure 3 Drawing of embryo, 8 days 0 hours
The ectoplacental cavity has disappeared

Figure 4 Drawing of embryo, 8 days 6 hours
Two somites have developed

Figure 5 Photograph of embryo in Figure 4 stained for alkaline phosphatase activity. The primordial germ cells are just beginning their migration up the hindgut.
x 80

Legend:
T - trophoblast
EC - ectoplacental cavity
C - chorion
E - Exocelome
A - Allantois
Am - amnion
AC - amniotic cavity
YC - yolk cavity
PE - proximal endoderm
DE - distal endoderm
F - foregut
H - hindgut
PGC - primordial germ cells



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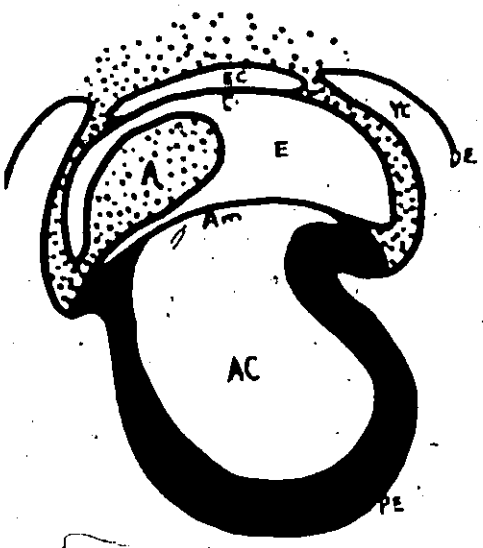

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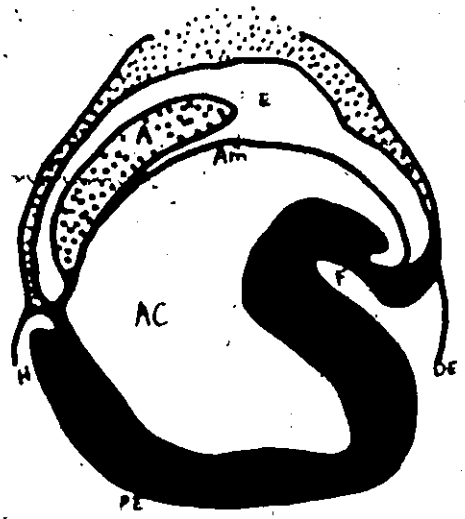

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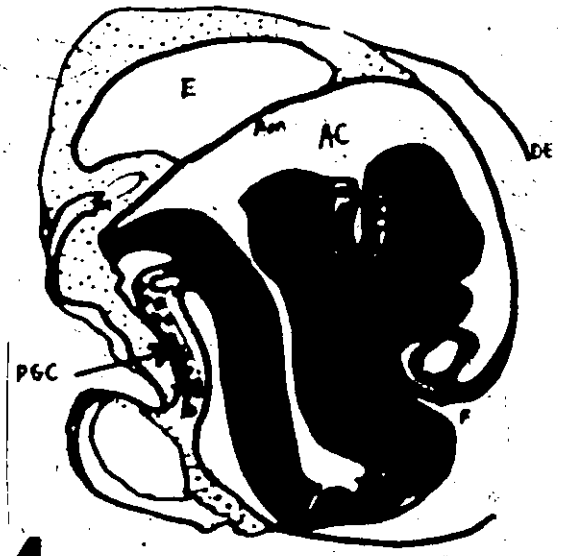


4

PLATE 8

PLATE 9

Figure 1 Photograph of embryo, 8 days 10 hours
Four somites have developed and the germ cells are stained for their alkaline phosphatase activity.
x 80

Figure 2 Photograph of rectangle in Figure 1
x 320

Figure 3 Photograph of embryo, 9 days 0 hours
Ten somites have developed and the embryo has turned
The germ cells are more numerous and have progressed farther along their migratory path
x 80

Figure 4 Photograph of rectangle in Figure 3
x 320

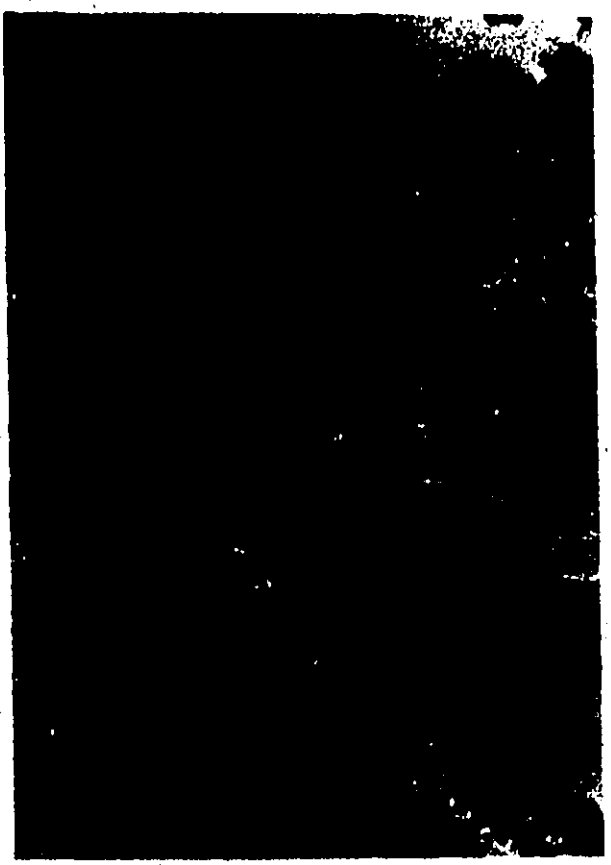


PLATE 9

PLATE 10

Figure 1 Embryo of 7½ days' gestation dissected from decidual tissue

x 80

Figure 2 The same embryo after removal of trophoblast and distal endoderm

x 80

Figure 3 A hematoxylin and eosin stained section of an embryo of 7½ days similar to that in Figure 1. An arrow indicates the point where the separation is made, as in Figures 4 and 5

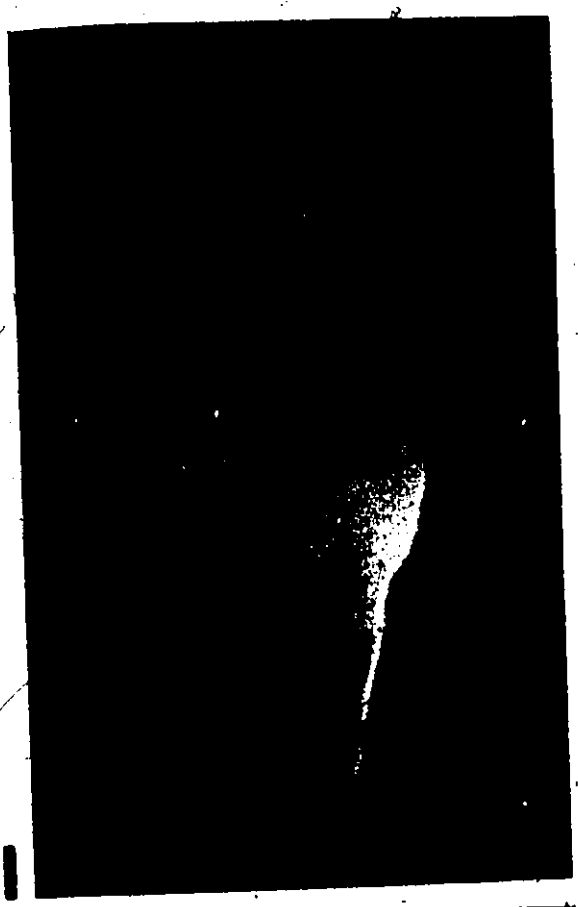
x 80

Figure 4 The separation of the embryo into two parts below the notch
The extra-embryonic portion (2/3 mm in length)

x 80

Figure 5 The embryonic shield portion (1/3 mm long)

x 80



1

2

3

4

5

PLATE 10

Results

Experiment VII

All of the 135 embryos were examined through the microscope, and photographs were taken of many of them. The changes taking place during this period are illustrated in the drawings and photographs of Plates 8 and 9.

The earliest stages represent the 7½-day embryo which was used in Experiments VIII to X. Blood islets develop in the wall of the yolk sac from 7½ days. It is in this area that primordial germ cells are first identified at 8½ days. They very quickly begin their mitotic activity and migration towards the gonads. The embryo develops rapidly between the ages of 8 and 9 days, forming somites, foregut and hindgut. Towards the end of this period the embryo turns right side out, and the hindgut, along which the germ cells travel is easily seen (Plate 9, Figures 3 and 4).

The results of grafting hybrid extra-embryonic cones and embryonic shields to testes are compared (Tables 9 and 10) with the grafting of whole embryos (from Experiment I).

Experiment VIII

Teratomas developed from 26 of 35 grafts (74%) of embryonic shields; this rate is very similar to the percentage found when whole embryos were used (80%).

Histologically these two groups of tumours were very similar, both containing a good variety of well differentiated tissues such as bone, muscle, cartilage, nervous tissue, epithelial tissues in both simple and stratified arrangements usually surrounding cysts, glands, hair follicles and black pigment. The largest of all teratomas was found in the shield group and is shown in Figures 1 to 4 of Plate 11.

Experiment IX

In sharp contrast are the results of grafting extra-embryonic cones to testes. Only 1 of 35 developed as a tumour (Plate 12, Figures 1 to 3). The testis measured 3.0 mm in diameter, and the tumour occupied approximately 1/4 of the section. Tissues contained in this small tumour were cartilage, smooth and striated muscle, fat, cysts surrounded by columnar epithelium with brush border, cuboidal epithelium, desquamated epithelial cysts, small glands of mucous secreting cells.

A reaction was found in 22 of 35 testes giving evidence of accurate placement of the grafts (Plate 12, Figure 4). Although 12 testes were classified as "normal" because there was no hemorrhaging or lymphocyte infiltration, many had tubules with few spermatogonia and spermatocytes surrounded by large intratubular spaces, not unlike those of older animals. These hosts, however, were

all young adults.

Testes receiving extra-embryonic cones as grafts were much smaller than those of untreated animals (mean 4.8 mm). Only 5 measured 4.8 mm. One was larger, 5.0 mm, and it contained a large hemorrhage 0.6 mm in diameter surrounded by necrotic cells (Table 10).

Experiment X

Extra-embryonic cones were grafted to both testes of 10 adult hosts. These grafts were recovered 3, 5, 7, 9 and 15 days later. The results are summarized in Table 11.

There were hemorrhagic areas in all but 4 of the testes; 2 testes at 3 days appeared normal, and 2 testes at 15 days had no hemorrhage, although there were areas of necrosis and scarring with degeneration of the tubules.

Generally, the size of the hemorrhage increased from 3 to 9 days (Plate 13, Figures 1 to 4). Very small areas of embryonic tissue were seen in 3 testes, at 3, 7, and 15 days (numbers 8, 9, and 17 of Table 11). These areas of embryonic tissue are illustrated in Figures 1 to 4 of Plate 14.

PLATE 11

Figure 1 C3H/F X ICR testis almost 3 cm in length containing teratoma derived from an embryonic shield

Figure 2 A section from the tumour shown in Figure 1 showing a variety of well differentiated tissues, epithelium, muscle, cartilage, black pigment, and nervous tissue

x 80

Figure 3 Another area of the same tumour with several small cysts surrounded by connective tissue

x 80

Figure 4 A higher magnification of the rectangle in Figure 3 showing pseudostratified epithelium, undifferentiated mesenchymal tissue and a spicule of bone

x 320

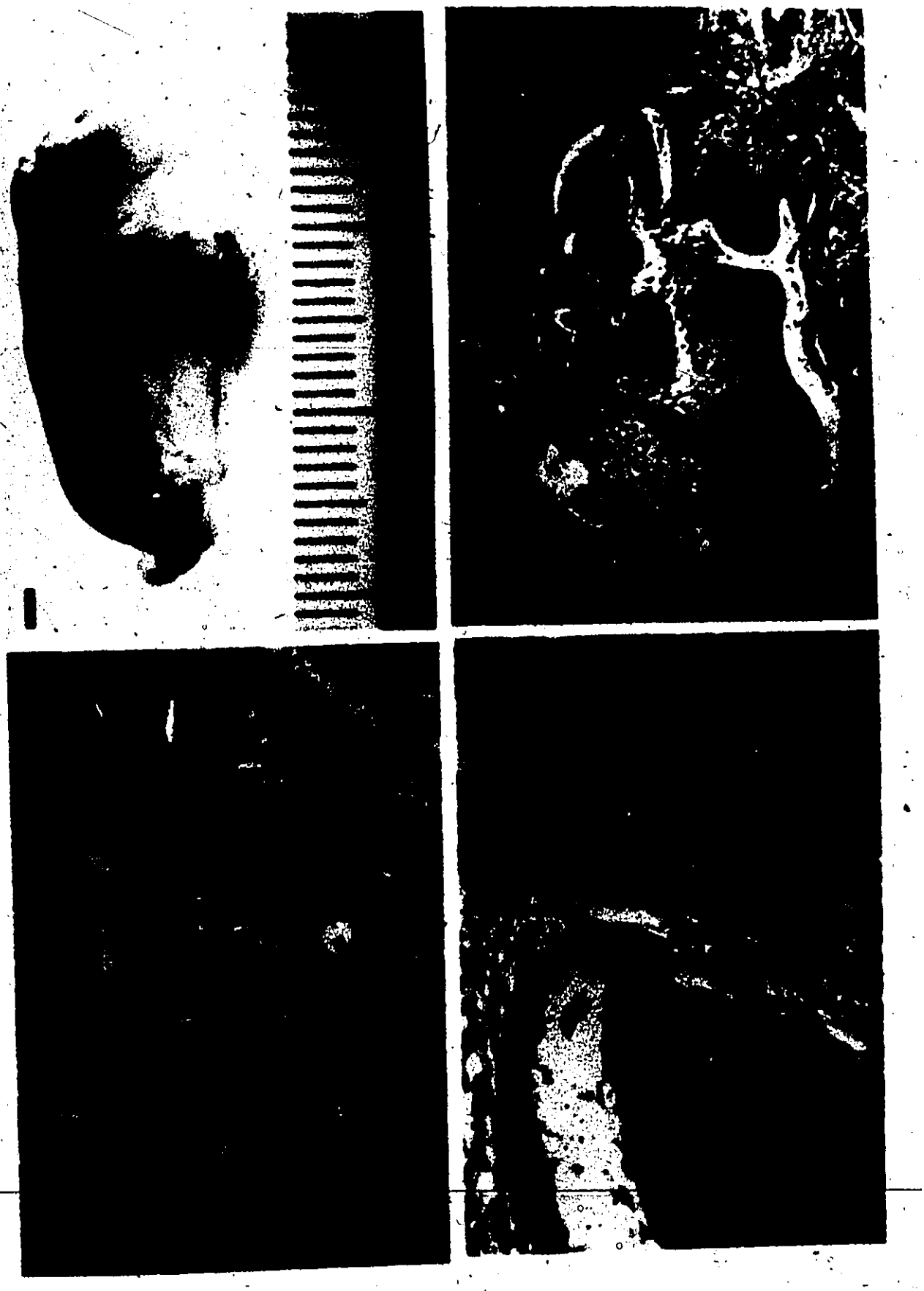


PLATE II

PLATE 12

Figure 1 The only tumour which developed from extra-embryonic cone portion of 7½-day embryo in a C3H/F X ICR testis, number C3L. This small teratoma contained many well differentiated tissues as described on Page 73

x 80

Figure 2 A higher magnification of the rectangle in Figure 1 showing glandular tissue of mucous secreting cells surrounded by mesenchymal tissue

x 320

Figure 3 Another view of the same tumour

x 320

Figure 4 A reaction from graft of extra-embryonic cone, with connective tissue and scarring surrounded by degenerating tubules

x 80

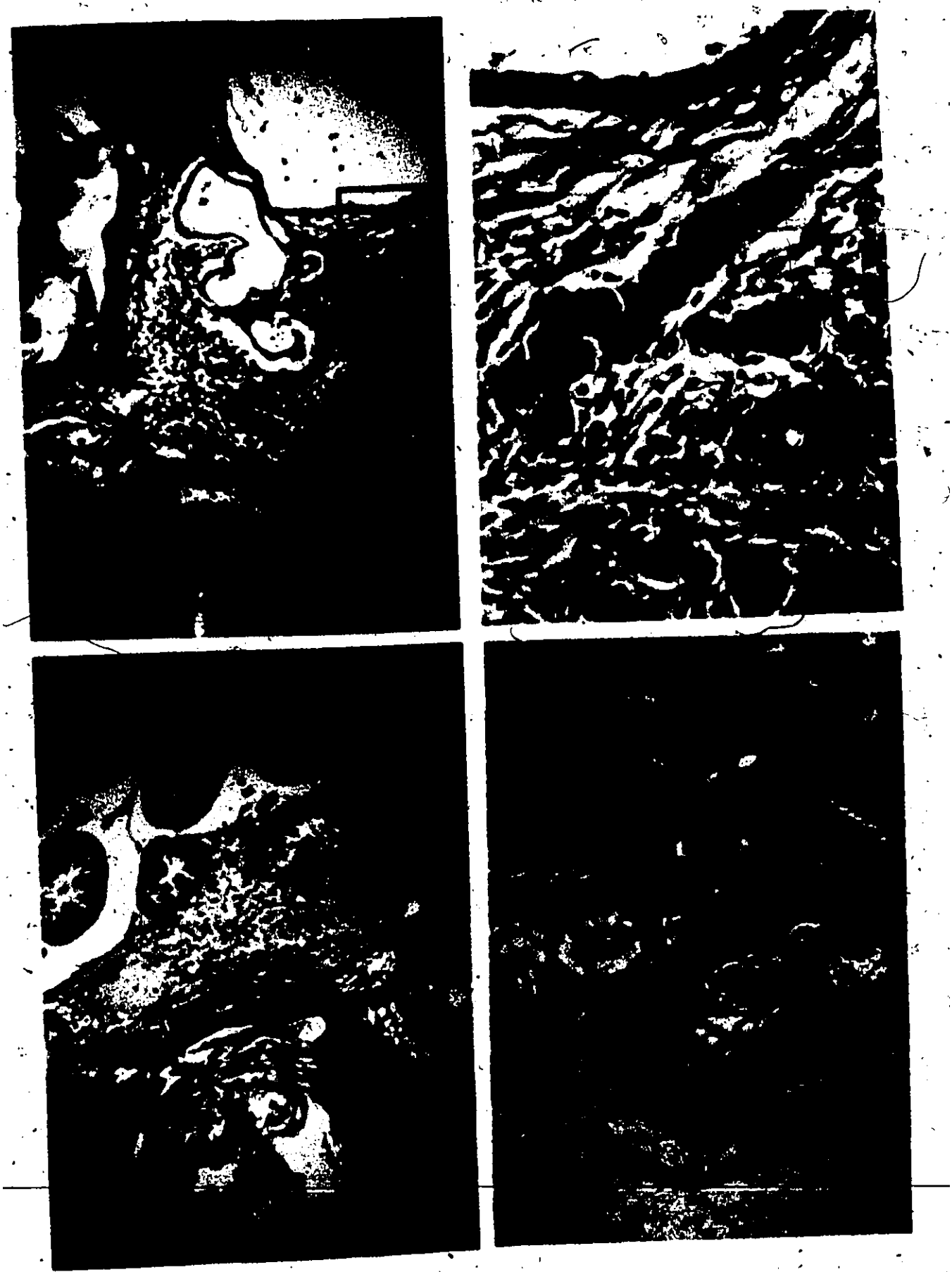


PLATE 12

TABLE 9

Frequency of teratomas arising from 7½-day C3H/F X ICR grafts to both testes of C3H/F X ICR hosts

	Whole Embryos	Embryonic Shields	Extra-embryonic Cones
Teratoma	40 (80%)	26 (74.3%)	1 (2.9%)
Reaction	7 (14%)	4 (11.4%)	22 (62.9%)
No tumour	3 (6%)	5 (14.3%)	12 (34.2%)
Total number of grafts	50	35	35

TABLE 10

Diameter of hybrid (C3H/F X ICR) testes in millimeters

	Left	Right
Mean of untreated hybrids	4.8	4.8
Testes containing teratomas from whole embryos, mean	5.8	7.3
Range	3.6 - 12.6	4.2 - 13.5
Testes containing teratomas from embryonic shields, mean	5.4	5.1
Range	3.4 - 12.5	4.3 - 7.0
Testes containing extra-embryonic cones (all 35), mean	4.0	4.1
Range	2.8 - 4.6	2.5 - 5.0
One teratoma, C3L	3.0	

PLATE 13

Figure 1 C3H/F X ICR testis 3 days after grafting of extra-embryonic cone with small hemorrhage

x 20

Figure 2 A higher magnification of the rectangle drawn in Figure 1 showing degeneration of the tubules surrounding the hemorrhage

x 80

Figure 3 C3H/F X ICR testis 7 days after grafting of extra-embryonic cone

x 20

Figure 4 C3H/F X ICR testis 9 days after grafting of extra-embryonic cone. The hemorrhage has displaced most of the testicular structures

x 20

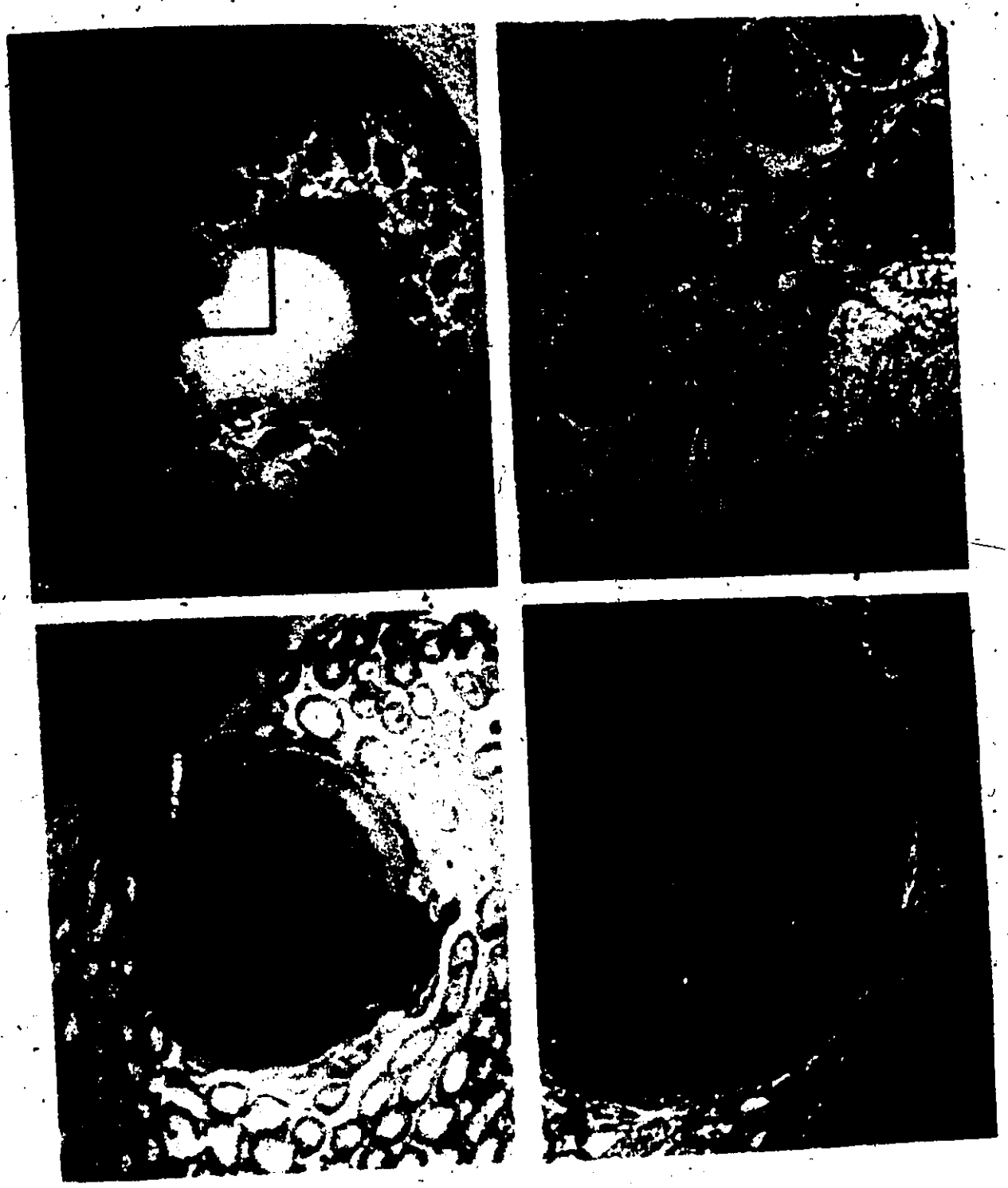


PLATE 13

PLATE 14

Figure 1 C3H/F X ICR testis 5 days after grafting of extra-embryonic cone containing a small area of embryonic tissue with many blood filled cystic areas

x 80

Figure 2 A higher magnification of the rectangle in Figure 1 showing tissues of ecto-dermal origin

x 320

Figure 3 C3H/F_1 X ICR testis 15 days after grafting of extra-embryonic cone containing a small area of embryonic connective tissue

x 80

Figure 4 A higher magnification of the rectangle in Figure 3 showing a small cyst with low columnar cells

x 320

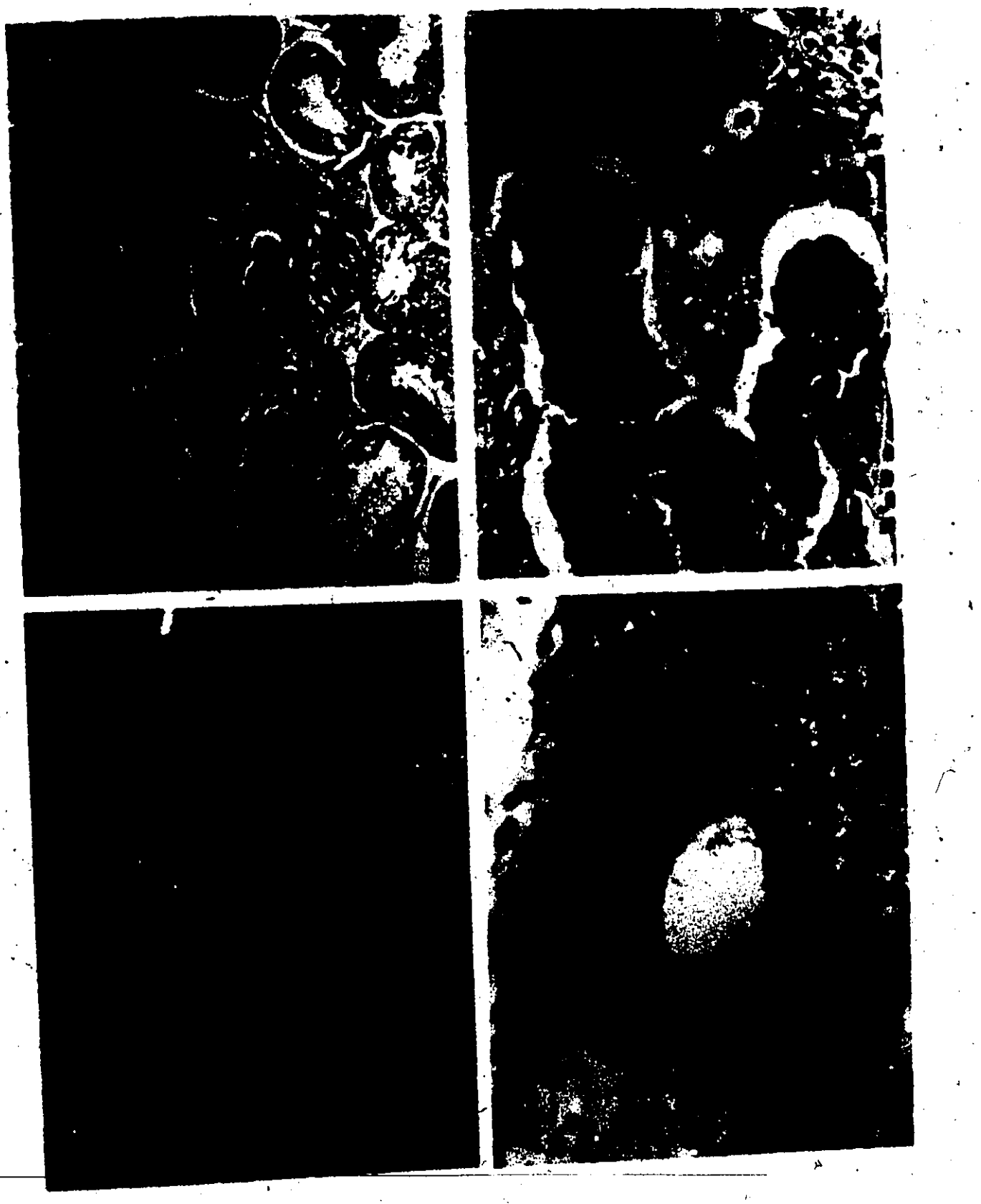


PLATE 14

TABLE 11

Early stages in the development of extra-embryonic cones (C3H/F X ICR) grafted to adult testes (C3H/F X ICR)

Number of Days	No.	Diameter of testis in mm	Size of hemorrhage in mm
3	1	4.2	1.6
	2	4.8	–
	3	4.0	0.8
	4	4.6	–
5	5	4.8	0.8
	6	4.2	0.8
	7	5.0	2.0
	8	4.3	0.5
7	9	4.5	1.5
	10	5.0	2.5
	11	4.5	2.5
	12	5.0	2.5
9	13	5.0	3.2
	14	4.5	4.0
	15	4.2	1.3
	16	4.5	4.0
15	17	4.0	3.0
	18	4.0	–
	19	3.7	–
	20	4.1	2.0

Discussion

The two major theories which explain the origin of teratomas appear to conflict with each other. Do teratomas originate from germ cells or from embryonic pluripotent cells which have escaped the influence of embryonic organizers? If one adheres to the germ cell theory, are the extra-gonadal tumours derived from primordial germ cells which have strayed from their migratory pathway?

Stevens (1970c) argues that since spontaneous testicular teratomas in mice (and also those induced in fetal testes by the grafting of 12½-day ridges) originate within the seminiferous tubules of fetal mice, they must develop from either the supporting cells or the germ cells. It follows that because sterile mice, who lack primordial germ cells, do not develop teratomas as do their normal littermates (Stevens, 1967a), it is the germ cells which give rise to the tumours. First the germ cells produce clusters of disorganized undifferentiated cells which in turn differentiate into the chaotic mixture of tissues found in teratomas.

How can the development of teratomas from grafts of early mouse embryos be explained? Do they arise from the disorganization of the grafted embryonic tissue, or is it the primordial germ cells which eventually give rise to the tumour?

The experiments in Section C were designed to answer these questions. By separating the mouse cylinder in two parts in such a way that one part contains the extra-embryonic membranes, the primordial germ cells, and some somatic tissue, and the other part only somatic tissues, it can be determined what role is played by the primordial germ cells. The only time this separation can be made is at 7½ days' gestation. Earlier embryos are too small to allow one to distinquish the various parts of the embryo after removal of the trophoblastic material, and by 8½ days the primordial germ cells begin their migration.

Although the primordial germ cells cannot be identified at 7½ days' gestation, the embryonic studies conducted in Experiment VII have confirmed the observations of other researchers who have identified primordial germ cells by histochemical methods at 8½ days. From the extra-embryonic site where they are first seen at 8½ days, they migrate up the hindgut and arrive in the gonads at 11 days (Chiquoine, 1954; Mintz, 1960; Spiegelman and Bennett, 1973).

Embryos used in the grafting experiments were all of 7½ days' gestation. By this time the amnion has extended across the proamniotic cavity separating the amniotic cavity (and the embryonic portion of the egg cylinder) from the exocelome (the extra-embryonic portion). Also by this time the primitive streak, which started at the posterior junction of the two portions has progressed around the shield

and has proliferated mesoderm laterally and caudally (Theiler, 1972; Snell and Stevens, 1966; Rugh, 1968).

There is a notch in the endodermal layer of cells at the level of the amnion and just anterior to the point where the foregut develops. This notch is easily seen under the dissecting microscope, making it possible to separate the extra-embryonic portion from the embryonic shield. This separation, however, was made well below the notch in order to be certain that there were no germ cells in the shield portion (Plate 10, Figures 4 and 5).

The good growth of tumours from the embryonic shield (74%) is evidence that these growths are the result of proliferation of embryonic cells and that they are not derived from germ cells (Table 9). There is no statistical difference between tumours resulting from grafts of whole embryos, 40 out of 50, and those resulting from grafts of embryonic shields, 26 out of 35 ($P = 0.57$).[1]

The one small growth (Plate 12) resulting from implants of extra-embryonic cone may have also developed from the small amount of embryonic tissue contained in these grafts. In fact, it was rather surprising that only one teratoma developed from these grafts. The difference in the numbers of growths derived from embryonic shields (26 out of 35) and from extra-embryonic cones (1 out of 35) is highly significant statistically ($P = 0.9999$).[1]

1 x^2 test

Many of the testes which had been grafted with extra-embryonic cones had large areas of Hemorrhage. It is not clear why extra-embryonic but not embryonic tissues would elicit this reaction. Stevens (1954, 1959) reported that fetal and postnatal teratomas arising spontaneously in strain 129 mice could be easily identified because of the hemorrhaging seen on gross inspection of the testes.

The testes containing growths derived from embryonic shields were somewhat smaller than those derived from whole embryos (Table 10). Using an unpaired t-test, the difference is significant ($P = 0.99$). This difference is particularly evident in the right testis. The mean diameter of all right testes containing growths from embryonic shields was 5.1 mm compared with 7.3 mm in the right testes containing grafts of whole embryos. The much smaller size of the shield grafts could account for this difference in the sizes of growths.

In Experiment I of Section A the right testis appeared more receptive to the production of teratomas when whole embryos were used as grafts. Tumours developed in 18 out of 25 left testes (72%) and 22 out of 25 right testes (88%) of hybrid hosts (Table 4). Similar percentages were obtained when grafts of embryonic shields were made to hybrid host testes: 10 out of 17 (59%) on the left side and 16 out of 18 (89%) on the right. These differences, however, are not significant ($P = 0.90$), using X^2 test.

Although the techniques used by other workers were employed, none of the transplants from shield-derived tumours grew. Only 4 out of 112 of Stevens' (1970c) 6-day grafts were transplantable after a recovery period of 30 to 60 days. Those tumours which were transplantable for many generations had large areas of undifferentiated embryonic cells.

Teratomas developing from 7½-day embryos have been transplantable (Damjanov, 1971). These embryos were implanted under the kidney capsule and left in place for 2 to 8 months. These workers were able to transplant only the largest tumours composed of much undifferentiated embryonic tissue. None of the smaller teratomas composed of differentiated adult tissues were transplantable.

The growths derived from embryonic shields in Experiment VIII were left in place only one month, and since they were composed predominantly of differentiated tissues, it is not unexpected that these growths were not transplantable. Because the extra-embryonic grafts developed poorly, it is doubtful that, when embryonic grafts of this stage develop as transplantable tumours, the germ cells are the precursers of the malignant stem cells. Ożdżeński (1969) has shown that germ cells in 8-day mice quickly deteriorate when the hindgut is transplanted to the anterior chamber of the eye.

The final experiment (X) is a study of the early development of the extra-embryonic grafts. Even as early as three days after the operation, the host has reacted to the graft and no embryonic tissue could be seen. However, very small areas of embryonic tissue were observed in 3 grafts (days 5, 7 and 15). There was an area of hemorrhage in most of the testes, the size of which increased with the number of days from implantation.

The experiments of Section C have demonstrated that the tumours obtained by the grafting of 7½-day embryos are derived solely from somatic tissues. These results lend considerable support to the theory that spontaneous teratomas are derived from undifferentiated embryonic cells which have escaped the influence of primary organizers, particularly in extra-gonadal locations. The theory that extra-gonadal teratomas are derived from germ cells which have strayed from their migratory pathway is still plausible. Should these cells survive, they would not be subject to the same controlling mechanisms had they arrived in the gonads.

Congenital tumours of the gonads also arise from clusters of disorganized undifferentiated cells, which are likely derived from primordial germ cells. This process has been carefully studied and documented in the mouse ovary (Stevens and Varnum, 1974) and in the testis (Mount and

Stevens, 1971).

There is a strong genetic predisposition to spontaneous gonadal teratomas in mice. It is possible that the germ cells begin to proliferate because the controlling influences in the ovary or testis have failed, allowing the germ cells to continue their developmental potential--as do the pluripotential cells in extra-gonadal locations when their controlling influences fail.

SUMMARY

The results of the experiments reported in this thesis suggest some answers to the questions posed at the end of the introduction:

Is it possible to produce teratomas in the gonads of strains which are not genetically susceptible to spontaneous teratomas?

The only strain of mouse in which spontaneous testicular teratomas arise is strain 129. Teratomas have been produced by the grafting of genital ridges or embryos in only a very few strains. The studies reported here have demonstrated that strains ICR, C3H/F, their F_1 hybrid, and CBA are also susceptible to the development of teratomas by the grafting of embryos to the testis.

Is the testis an immunologically privileged site?

Teratomas developed in more than half the testes of ICR and C3H/F mice from grafts of C3H/F X ICR embryos, even though skin grafts between these strains are rapidly rejected.

Can teratomas be produced in the ovary?

Teratomas were produced in one eighth of the ovaries grafted with embryos. This low frequency is likely due to the difficulty of placing and maintaining embryos in these small organs.

What role does the host tissue play in the experimental production of testicular teratomas?

Two experiments have demonstrated that it is the embryonic tissue which develops as the tumour. The injections of embryonic cell-free filtrate were unable to induce host spermatogonia to become neoplastic; the experiment using embryos bearing T6 chromosome marker have shown clearly that these tumours are composed of the grafted embryonic tissues.

Are donor or host spermatogonia capable of teratocarcinogenesis?

Injections of testosterone and placement of copper wire in the testes of mice were not able to induce a neoplastic change in spermatogonia. Donor spermatogonia and spermatocytes injected into testes displayed no further development.

Do the germ cells contribute to the production of these tumours?

The experiments of the final section have demonstrated that the embryonic shield, which lacks primordial germ cells, is the portion of the graft which results in a teratoma. The tumours obtained by the grafting of 7½-day embryos are derived solely from somatic tissues with no contribution from the germ cells.

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