THE UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

STUDIES ON THE SURVIVAL, GROWTH, AND DIFFERENTIATION OF EMBRYONIC TISSUE HOMOTRANSPLANTS

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the

degree of

DOCTOR OF PHILOSOPHY

BY

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Oklahoma City, Oklahoma

1957

STUDIES ON THE SURVIVAL, GROWTH, AND DIFFERENTIATION

OF EMBRYONIC TISSUE HOMOTRANSPLANTS

APPROVED BY

DISSERTATION COMMITTEE

ACKNOWLEDGMENT

The author takes this opportunity to acknowledge the encouragement, help, and assistance of Professors H. C. Hopps, Walter Joel, G. Townley Price, and A. M. Shideler, and other members of the Department of Pathology of the University of Oklahoma School of Medicine. Appreciation is also extended to Professors J. F. Lhotka, Department of Anatomy, and J. W. H. Smith, Department of Physiology, for the interest they have shown.

Acknowledgment of indebtedness is also made to the American Cancer Society for partial support of this study through the Institutional Cancer Research Grant.

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CHAPTER I

INTRODUCTION

Very early in the nineteenth century discerning observers, Lobstien (1829), Rokitansky (1852), Remak (1854) and others, according to Ewing (1942), noted the similarity of neoplastic and embryonal tissues. Durante (1874), according to Ewing (1942), enunciated the principle that all tumors of a neoplastic nature developed from groups of embryonal cells. However, the formulation of the doctrine by Cohnheim (1877), that tumors arise not from normal cells but from isolated embryonal cells or groups of resting embryonal cells received the greater attention and stimulated many workers throughout the world to attempt to produce tumors from embryonic tissue. In the intervening years a very great amount of work has been done by capable investigators to test its basic concepts. Many of these labors were concerned with attempts to produce teratomas experimentally in animals such as arise spontaneously in man.

Many of the early transplantation experiments were varied and conflicting results were obtained. However, the homologous transplantation of embryonic tissue was an effort in which all investigators were successful and in a variety of animal species these investigators

produced tumors closely resembling, grossly and histologically, the spontaneous teratomas of man.

The more recent experimental studies have been concerned, not so much with attempts to produce experimental teratomas but with efforts to contribute more to an understanding of the early organization, differentiation and growth of embryonic tissues. A basic understanding of the phenomena involved is essential to a clearer insight into the forces that govern the organization, differentiation and growth of normal and abnormal tissues. These investigations were undertaken with the intent to inquire into the behavior of embryonic tissue homotransplants under various influences and conditions.

CHAPTER II

REVIEW OF LITERATURE

The early experiments dealing with the production of tumors by the transplantation of embryonic tissue were reviewed and summarized by Jentzer (1908) in his publication, Etude Experimentale des Teratomes par la Greffe D'Embryons, Conserves Extra Corpus.

Zahn (1878), was the first to publish results of work on tissue grafts in living organisms. Into these organisms he grafted the cartilage of adult animals and embryos. The grafts of adult cartilage were uniformly unsuccessful while those of the embryonic cartilage always succeeded, producing tumors which attained the size of a pea.

Leopold (1881), according to Jentzer (1908), grafted various tissues of rabbit embryos, 2.5 to 8.0 cm. in length, into the abdominal cavity and into the anterior chamber of the eye of rabbits. These grafts not only survived but continued to grow. The only grafts that failed to grow were those taken from embryos which were at term. He then grafted into the peritoneal cavity an entire embryo still enveloped within its membranes. The grafted fetus, 2.5 cm. in length, was transformed into a mass of proliferating cartilaginous tissue. A second fetus, 5.5 cm. in length, was completely ossified at the end of ten weeks. Leopold compared the ossification of the fetal grafts with that which occurs in

abdominal pregnancy.

In a second paper, Leopold (1883), according to Jentzer (1908), again working with rabbits, grafted fragments of embryos into the abdominal cavity, subcutaneous tissues, jugular vein, and anterior chamber of the eye. Some of the tumors produced attained a size as great as 1.5 cm. in diameter. One tumor produced in the anterior chamber of the eye survived for 205 days and was estimated to be 300 times the size of the original graft. The tumors produced were composed predominantly of skin, bone, and cartilage. Leopold described some of the tumors produced as being enchondromas. He concluded from his experiments that he had been able to obtain, on several occasions, durable tumors, especially enchondromas.

A series of experiments begun in 1897, by Fere, et al., provided additional knowledge. Working with chick embryos of various ages, he found that grafts of young embryos into adult cocks and hens gave rise to neoformations at the end of 2-4 days, while older embryos were immediately resorbed. He advised the use of embryos ranging from 24-48 hours of age. His first group of animals included two cocks and two hens. Eleven grafts were made in each fowl. The cocks proved refractory. The hens, however, developed a total of seven tumors, three of which were progressive and the remainder gave rise only to transient tumefaction.

Fere modified later experiments by severing the nerve supply to the region in which the graft was made. He used three hens in this experiment. One hen produced two tumors which developed after a latent period of seven to eight weeks. One of these was not thought to be a tumor of embryonic tissue origin.

Fere concluded that embryonic elements are capable of development while enclosed in normal tissue. In his opinion this confirmed the teratological theory of tumors and their embryonic origin. However, it is to be noted that all of these tumors were resorbed at the end of two months, never attaining a diameter of more than 15-18 mm. He postulated that in the development of experimental teratoid tumors 1) there exists a species and racial specificity, as well as predisposition of certain individuals to develop these growths; 2) the site of inoculation plays a role (Fere was unable to obtain any positive results in the peritoneum); 3) the severing of nerves to the area had no effect upon the development, nor upon the survival of the tumors; 4) a deficiency diet did not influence the spontaneous resorption of the grafts; and 5) with very young animals, grafts developed more slowly, but resorption was more rapid. Only when using adult animals did the author succeed in obtaining persistent tumors.

Berch, Hirschfeld and Garten (1899), according to Jentzer (1908), worked with rabbits and chickens. The embryonic material used was teased to more or less isolate the cellular elements, with the purpose of facilitating contact between the cells and the tissue at the site of inoculation, thus encouraging proliferation. For their experiments the liver was selected as the transplantation site of preference because of its glycogen content - a substance common to embryonic and tumor cells. The macerated tissues were injected with a Pravaz syringe. Cartilaginous tumors were formed, developing from the grafts which, at the time of their transfer, contained mesenchymal but no cartilaginous elements. In two cases the authors noted the formation of foci of

lymphatic tissue in the liver in addition to the cartilaginous nodules. In one case they also observed cartilaginous nodules in the lungs. These were not considered metastases but possibly the result of the injection of the material into a hepatic vessel. This area of cartilaginous tissue was proliferating at a time when the hepatic area was being resorbed, suggesting that the lung might be a more favorable site for the development of embryonic tissue.

The authors concluded that:

1. They had produced by this means a differentiation of embryonic tissue.

2. The neoformation induced was not of the nature of a true tumor since proof of continued proliferation of cellular elements and unrestrained growth were lacking.

3. It was always necessary to consider two factors (a) the ability of the cells to proliferate, and (b) the host reaction. In the latter conclusion they did not indicate whether the augmentation of (a) or the retardation of (b) was the more important.

Fere and Luther published a paper on the survival of tumors produced by transplantation of embryonic tissue. They cited the experiments performed on a cock, which received a total of 155 subcutaneous embryonic tissue transplants over a period extending from 1896-1899. Upon the death of the animal in 1899 they recovered 36 tumors, composed of multiple tissues. Three tumors had previously been removed in 1896. Seventeen transplants placed in the peritoneum, as well as the remainder of the 155 transplants, failed to develop. Twenty-eight per cent of the transplants, therefore, provided material for study. This particular

experimental fowl apparently offered a very favorable medium with which to work, for other cocks with multiple grafts had not given such favorable results. To illustrate, in one cock only eight per cent of the transplants developed. Of thirty-six tumors some persisted for as long as three years and seven months. Histological examination of the tumors showed that they closely resembled spontaneous teratomata. The types of tissue identified included elastic fibers, connective tissue, blood vessels, both smooth and striated muscle, cartilaginous plaques of various forms, cystic formations of different types; sometimes resembling the intestinal type with muscular walls and different glandular and epithelial structures, sometimes the respiratory type, and finally epidermal and dermal tissue types. There was also evidence of calcareous degeneration, some hemosiderin, and occasionally lymphatic tissue.

In 1901 Fere and Petit observed a tendency toward a "sclerocystic" type of degeneration in most of their grafts. The greater number of tumors produced, however, were composed of fibrous connective tissue with cystic cavities, enclosing material which they failed to identify. In several instances they demonstrated structures common to experimental teratomas and spontaneous tumors. They cited, among other developments, pathological mitoses, karyolysis, necrosis and giant cells with three or four nuclei.

Wilms (1904), according to Jentzer (1908), grafted macerated chicken embryos five to seven days old. He observed that in repeating grafts in a cock, eight days apart, a take always developed quickly. One of the tumors attained a size of 6.0 x 3.5 cm. in eight weeks. It contained well differentiated tissues, e.g., skin with feathers,

cartilage, pigmented epithelium, bone, and bone marrow.

Petrow (1906), according to Jentzer (1908), ground an embryo of a guinea pig in physiological saline and injected it into the testicle of an adult of the same species. In five or six days a nodule formed which in four weeks attained a size equal to the original volume of the testicle. Thirty-seven days after inoculation it was removed by castration. In addition to skin, hair, cartilage, sebaceous glands, smooth muscle, and small endodermal cysts, the author claimed to have found an embryonic nervous system within the tumor. He emphasized the necessity of making a fine suspension of the tissues used for inoculation and recommended the testicle as the organ in which transplant survival and development was most likely to be successful.

Von Hipple (1906), according to Jentzer (1908), suspended the tissues of the head of a twelve day rabbit embryo in saline and injected this preparation into the anterior eye chamber of an adult rabbit. A violent inflammatory reaction resulted with the development of a cataract and a very hard spherical tumor. Histological examination of the tumor revealed it to be a teratoid tumor with tissue components of the three embryonal germ layers.

Del Konte (1907), according to Jentzer (1908), inserted tissues of canine embryos into the brain of adult dogs. Negative results were obtained with transplants of thymus, esophagus, kidney, liver, and hairy skin. Tumors developed from transplants of cartilage, hairless skin, and hypothalamus. All of the tumors produced were very small.

Askanazy (1907) published the results of his studies on white rats and rabbits. He employed homogenates of embryos in preference to

fragments of embryos, having found that the latter preparations presented no special advantages. Basing his conclusions on several dozen experiments, Askanazy concluded that teratoid tumor development was most easily produced in white rats. He also demonstrated that the use of older embryos was not cause for lessened success and used embryos one to four cm. in length. The use of young embryos demonstrated that in the resulting teratoid tumors certain tissues, such as bone, which were not present in the transplanted material later became prominent. He found that resorption of teratoid tissue was most variable but that almost always the tumors were eventually resorbed. Askanazy could not affirm any analogy between these growths and human tumors; however, he cited the case of a dermoid ovarian cyst containing hair in which the characteristic wall was lacking. The resorption of human tumors is not unusual. Concerning the persistence of these induced tumors, he noted that in the limited number of his experiments some of the tumors produced were not diminished in size and were progressing after seven or eight months. One rat, at the time of this statement, was bearing a tumor 27 months old. Askanazy became interested in the continued growth potential of this induced tumor tissue and in a group of serial inoculations of an experimental tumor through three rats, he failed to notice any increased growth activity. On the contrary, the tumors became successively smaller. This experiment demonstrated for the first time that experimental tumors could be serially transplanted.

Askanazy emphasized the influence of pregnancy on the development of a tumor in a test animal and noted that in one pregnant rat a tumor attained the size of a small orange. He also cited work performed

by one of his students, Rasumoff, who studied the influence of certain physical and chemical factors on the development of experimental tumors. Rasumoff felt that cold acted favorably upon tumor development. Other agents seemed to have a negative effect on the production of the tumors.

Petrow (1908) published his results of further experiments on guinea pigs, rabbits, mice, and dogs. After having removed young embryos, he prepared a homogenate of the embryos in saline and injected this into the kidney, spleen, and ovary of the animal from which the embryos were removed, or into the same organ or the testicle of other animals. He worked principally with guinea pigs, choosing the kidney as the inoculation site of choice. Of eighty-seven experimental animals forty-five developed tumors. Some of these had been observed for eleven months and the majority of the tumors were 3.5 to 4.0 cm. in diameter. He performed his experiments by serially transplanting teratoid tumors into additional guinea pigs in a manner similar to that of Askanazy. He grafted twelve tumors subcutaneously in test animals and followed their development for sixty-five days. All of these tumors were resorbed with one exception. This was a tumor produced in the testicle, removed by castration, and transplanted into the subcutaneous tissue of the same animal.

Jentzer (1908) presented to the Faculty of Medicine, University of Geneva, in partial fulfillment for the degree of Doctor of Medicine, the results of an original investigation concerning the ability of embryonic rat tissue, kept outside the mother's body (extra corpus) for varying periods of time, to develop into living transplants. He confined his experiments to white rats. Employing aseptic procedures he removed

ten rat embryos, 1 cm. long, from a pregnant female. The embryos, still within their fetal membranes, were placed immediately in sterile tubes, plugged with gauze and kept iced. The embryos were then homogenized and transplanted successively, at different intervals, in the following sequence:

1st embryo ½ hour after removal

2nd embryo 1 hour after removal

3rd embryo 3 hours after removal

4th embryo 16 hours after removal

5th embryo 24 days after removal

6th embryo 3 days after removal

7th embryo 4 days after removal

8th embryo 5 days after removal

9th embryo 6 days after removal

10th embryo 7 days after removal

The host animals were sacrificed and the tumors removed thirty days after transplantation. Each tumor was examined grossly and microscopically. Based on the results of his experiments Jentzer made the following observations.

He emphasized that successful results were obtained in all transplants. In each case he demonstrated a new growth visible to the naked eye, measuring between 9 mm. and 2 cm. in diameter. The tumors were composed of various elements characteristic of teratoid tumors. He felt that he had thoroughly confirmed the theory of Askanazy that rats, at least in their subcutaneous tissues, exhibit a characteristic species predilection for the success of these grafts, and for the proliferation

of embryonic tissues. He further stated that it must not be forgotten that he subjected the embryos to a particular condition that would appear to be detrimental to the results he obtained. He also felt that he could dismiss the suggestion that had he worked with the testicle, or some other organ, he might have had even greater success than he had in transplanting into the subcutaneous tissues.

Jentzer's results show that it is possible to preserve embryonic tissues extra corpus for a period of days without their losing the ability to act as transplants and to produce teratoid tumors. Even after seven days preservation on ice the embryo transplants gave rise to tumors as perfect in composition as those produced by the immediate transplantation of embryonic tissue. He points out that after being held on ice for six days before transplantation, one of the resulting tumors contained tissues of three embryonic layers, something not always found after an immediate transplantation. He also cites an instance of a tumor produced by a transplant in a pregnant rat which contained even more complexly differentiated tissue. He felt that this indicated some relationship between the pregnancy of the animal host on the one hand and the absence of necrosis and the differentiation of the tissues on the other. This observation demonstrated the favorable influence of pregnancy on the survival and proliferation of embryonic tissue transplants. This, he stated, confirmed the theory of Askanazy.

The possible modification of tissues of a tumor produced by a transplant held extra corpus would not lessen the chance of a successful transplant, according to Jentzer, since in every case he had succeeded in producing an experimental tumor. The relationship between the size of

these tumors and the size of the embryo transplanted is proportional to that seen in the case of the immediate transplants. He was not able to prove to his satisfaction that the technique used, produced any modification of growth potential or exaggerated proliferation. Jentzer states that the procedure was not in itself sufficient to create a condition comparable to that of the germ cells that were, at that time, considered the site of origin of malignant teratomas.

Jentzer stated that all tumors persisted for the observation period of one month. He did not believe that a resorption was probable, since in all tumors he demonstrated the presence of nuclei in a state of intense proliferation.

Askanazy (1908) published the results of experiments which were the continuation of the extra corpus experiments of Jentzer. Using the same procedure as Jentzer, Askanazy was able to produce experimental teratoid tumors from embryonic tissue held extra corpus for fourteen days.

Askanazy (1909,1911) carried out experiments concerning the survival of experimentally produced teratoid tumors. At the time of the latter of these publications, tumors which had survived for periods of one to two years were under observation.

Faldino (1924a,1924b), working with rabbits, transplanted various embryonic tissues into the anterior chamber of the eye. His results showed very good growth and differentiation.

Bucciante (1931), using growth in vitro as the test of viability, demonstrated that chick embryonic tissue kept in Ringer's solution at 5 and 10° C. survived for 21 days. Cooling down to 0° C. lengthened the

survival time but temperatures below 0° C. shortened this time. He showed that skin survived the longest and hematogenous elements of the spleen and hepatic cells survived for three days.

Nicholas (1934) reported a series of investigations consisting of a survey of the capacity of the embryo at different developmental stages to undergo development under conditions different from the normal. The results obtained by Nicholas show that none of the glandular structures within the abdominal cavity serve successfully as loci for grafted embryos with the exception of the testes and the kidney. The mammary gland of the male is just as effective in supporting development of embryonic grafts as is that of the pregnant female. The female endocrine secretions do not influence graft development.

Waterman (1934) reported that the hepatic, pancreatic, and lung rudiments of the rabbit embryo have a moderate capacity for independent differentiation into identifiable tissue in both omental and kidney grafts.

Waterman (1939), using transplants of young rabbit embryos 13 and 15 days old, stored for varying lengths of time at 5° C. produced tumors which had grown and differentiated. Using the omentum of adult rabbits as the site of transplantation, he found that with the exception of liver, pancreas, kidney, and nervous tissue, two to three days storage had little effect upon the quality and quantity of the differentiations obtained. Many tissues and organ rudiments survived eight days exposure while a longer period of storage finds chiefly epidermis, hair, bone, and cartilage appearing in the tumor. The size of the tumors became appreciably smaller with longer exposure.

Greene (1943) reported the results of a series of homologous and heterologous embryonic transplantation experiments. He found that rabbit embryos, either in whole or in part, could be readily transplanted to the anterior chamber of the eye and testicle of other rabbits or guinea pigs. The resulting growths resembled teratomas and could be transplanted serially. He obtained similar results with human and mouse embryonic material transplanted to the eyes of rabbits and guinea pigs. Organs transplanted in both homologous and heterologous species underwent differentiation and resembled mature structures. A variety of embryonic human organs including heart, lung, brain, stomach, intestine, and kidney were successfully transplanted, but all attempts to transfer liver or organs of internal secretion failed.

Browning (1949), using embryonic mouse tissue, extended the work of Greene. The results of his experiments were virtually the same and confirmed those which were obtained by Greene.

Crouse (1956) transplanted 11 to 20 day old rudiments of gut, kidney, gonad, and limb bud from rat embryos into the cerebral hemisphere of 4 to 12 day old rats. The results obtained prove the brain to be a very favorable site for transplantation. The lack of infiltrations into the graft or other cellular reactions commonly seen at other transplantation sites were absent.

In a well written review article Rawles (1952) summarizes as follows: "It may be said that the application of specific methods of isolation and transplantation to the solution of special problems has contributed importantly to our knowledge of the early organization of the embryo and the progressive differentiation and growth of many of its

parts. An understanding of the developmental capacities of normal tissues and their reaction, under as wide a variety of environmental conditions as it is possible to impose, is essential to an interpretation of normal as well as abnormal tissue growth."

CHAPTER III

STATEMENT OF PROBLEM

This project was undertaken to study the influence of a variety of factors upon the survival, growth, and differentiation of embryonic tissue homotransplants. Some of the factors to be tested in this study have been partially tested by previous workers in the field and other factors which have not been investigated will be tested.

The peritoneal cavity, subcutaneous tissue, and testes are the three most often used sites of transplantation of embryonic tissue. The value of each of these body regions as a site for transplantation has often been mentioned in the literature. The first part of this study is a determination of sites offering the most favorable conditions for the growth and differentiation of embryonic tissues. The second part of this study inquires into the possibility of modifying the proliferative capacity of the new growths by conditioning the host animal prior to transplantation. The use of the term conditioning implies an oriented change induced by controlled factors (Hauschka, 1953).

The third and final part of this study concerns the maximal time that embryonic rat tissue will survive when kept extra corpus. Askanazy (1908) has shown that embryonic tissue kept extra corpus will remain viable for a period of fourteen days and upon transplantation

into an adult rat will produce teratoid tumors. As a part of this study, the maximal survival time extra corpus of various tissues will be determined.

CHAPTER IV

MATERIALS AND METHODS

Animals utilized in these studies were albino rats of the Holtzman strain. The animals were within three ages. (1) Immature group, 45 to 65 days of age; (2) Discarded breeders, all over 14 months of age, and (3) Breeding stock, 5 to 12 months of age.

Embryos, 1.0 to 2.0 cm. in length, to be used as transplants, were removed intact from gravid females. If the embryos were not to be used immediately they were placed in sterile test tubes containing a few ml. of Earle's solution (Difco) to prevent dehydration. The tubes were then plugged with sterile cotton and refrigerated at 2-4^{o} C. until the embryos were needed.

All transplanted embryonic material in this study was in the form of a homogenate. The embryos, removed from their fetal membranes, were homogenized in a Ten-Brock ground glass homogenizer and transplanted into the host animal using a tuberculin syringe and 20 gauge hypodermic needle.

To determine which of the three most often used transplantation sites offered the most favorable conditions for transplantation, the following procedure was employed: Twenty-one animals, discarded breeder males, were divided into seven groups of three each. Each animal

received a transplant of 0.5 ml of homogenate in each of three sites: a) subcutaneous tissue of the right axilla, b) right lower quadrant of the peritoneal cavity, and c) the right testis. The first group received transplants of embryonic tissue homogenate within 30 minutes after the removal of the embryos from the pregnant females. At 48 hours intervals thereafter, transplants of embryos kept extra corpus were made into the remaining six groups. Thus, the transplants received by each group were kept extra corpus for specific lengths of time, ranging from less than 30 minutes in the first group to 12 days in Group 7.

One hundred sixty immature rats 45-60 days of age, 160 discarded breeder rats and 16 pregnant (8-10 days) rats 5-12 months of age were used in carrying out the experiments in the second part of this study. They were divided into eight groups. Each group contained 42 animals, 20 (12 males, 8 females) immature rats, 20 (12 males, 8 females) discarded breeder rats and 2 pregnant rats.

In the second part of this study each rat received 0.2 ml. of homogenate. Prior to receiving transplants of embryonic tissue, groups of animals were conditioned in the following manner:

a) Eight animals (2 male + 2 female immature rats, 2 male + 2 female discarded breeders) were conditioned with 30 mg. of cortisone acetate (Sharp & Dohme) per kg. of body weight per day for two days before receiving the transplants and for 10 days thereafter. Hawes, 1951.)

b) Eight animals (2 male + 2 female immature rats, 2 male + 2 female discarded breeders) were conditioned with an intraperitoneal injection of 2 ml. of a 0.5% solution of trypan

blue two days before receiving the transplant. (Ludford, 1931.)

c) Eight animals (2 male + 2 female immature rats, 2 male + 2 female discarded breeders) were conditioned by castration two days before receiving the transplants.

d) Eight animals (4 male immature rats, 2 male discarded breeders) were conditioned by castration with subsequent daily parenteral administration of 10 ugm. of estradiole benzoate (Schering) in 0.05 ml. of sesame oil for two days before receiving the transplant and for 10 days thereafter. (Hellbaum and Campbell, 1956.)

e) Two animals were conditioned by pregnancy 8 to 10 days before receiving the transplants. These animals were permitted to deliver their litters and nurse them until the termination of the experiment.

f) Eight animals (2 male + 2 female immature rats, 2 male + 2 female discarded breeders) were used as unconditioned controls.

Each animal received one transplant of homogenate into the subcutaneous tissues of the right axilla. The first group received transplants of tissue immediately after the removal of the embryos from pregnant rats. Each succeeding group received transplants of embryos kept extra corpus at 24 hour intervals. All animals were sacrificed by decapitation at the end of 30 days. The tumors were removed and weighed on a torsion balance then fixed in 10% buffered formalin.

Extensive histological studies were done to determine the

morphological characteristics of all tumors produced. Serial sections were made of selected tumors with one of every ten sections being mounted. The sections were cut at 8 u thickness. Sections of the remaining tumors were cut at 1-2 mm. levels and these embedded. Two slides were made from each block. In this manner it was felt that representative sections would be obtained from all parts of the tumors. Special stains, Azan stain for connective tissue, Periodic acid, Schiff for neutral mucopolysaccharides, combined-Schiff and Hale for acid-mucopolysaccharides and Gomori reticulum silver stain, were utilized to better identify the types of tissues found in the tumors.

Eight embryos removed at random from those to be used as the homotransplants were fixed immediately after removal from the uterus and later sectioned as tissue controls.

CHAPTER V

RESULTS AND DISCUSSION

Part I

The results obtained in the first part of this study (Table I) show clearly that there is little difference between the testis and subcutaneous tissues of the rat as an optimal site for the transplantation of embryonic tissue. The grafts proved ninety percent successful, although the embryos were kept *extra corpus* for 0 to 12 days. It is also shown by the small number (6) of transplants recovered that the peritoneal cavity, although it will sustain embryonic tissue, is a poor site for the successful transplantation of this material.

The gross and histological characteristics (to be discussed in Part III) of the tumors from the three tested sites were essentially similar. The only difference noted was one of size. The tumors of the testes were slightly larger than those from the subcutaneous tissues. The average weight of the tumors recovered from the testis was 0.115 gm., while those recovered from the subcutaneous tissue averaged 0.108 gm. The tumors recovered from the peritoneum were about one-half the size of those recovered from the testes.

These findings are in agreement with those of Askanazy (1907) and Jentzer (1908). The subcutaneous tissues and testes of the albino

TABLE I

NUMBER OF TUMORS RECOVERED FROM SITES OF EMBRYONIC TISSUE TRANSPLANTATION

Animal Group*	Subcutaneous Tissue	Testis	Intra-peritoneal
0	3	3	2
II	3	3	2
IV	3	2	1
VI	3	3	0
VIII	2	2	1
X	1	3	0
XII	2	2	0
Total tumors recovered	17	18	6

* Numbers in this column also indicate the time (days) the embryonic tissue was kept extra corpus before transplantation.

rat do offer optimal conditions for the successful transplantation of embryonic tissue. This is not intended to imply that other sites utilized by other workers, i.e., the anterior chamber of the eye, Faldino (1924), subcapsular tissues of the kidney, Nicholas (1934), omentum, Waterman (1939), and brain, Crouse (1956), are not as suitable for successful transplantation of embryonic tissue. Subcutaneous tissues and testis, do have some advantage over the other above mentioned sites in that they are accessible and transplants can be conveniently accomplished without a surgical approach.

With these advantages in mind and the need of a site common to both male and female, it was decided to utilize the subcutaneous tissues as the site of transplantation in all the succeeding experiments.

Part II

The results of the second part of this study are shown in Tables II through IX. In Tables II through VI the animals are separated according to the imposed or natural condition of the host group (group numbers also indicating the number of days the embryos were kept extra corpus), sex, and age of host animals. Tables VII and VIII are composite summaries of Tables II through VI, according to condition, sex, and age of host animals. Table IX is the total of all animals according to sex and hormonal status.

In attempting to modify the proliferative capacity of the transplants by conditioning the host animals, the most striking feature is that of the great variation with which the individual animals acted as hosts for the transplants. This is partially shown in Tables II

TABLE II

CONTROL ANIMALS RECEIVING EMBRYONIC TISSUE HOMOTRANSPLANTS (ARRANGED IN GROUPS, AGE, SEX, AND DAYS EMBRYOS WERE KEPT EXTRA CORPUS)

	IMMATURE RATS			
	MALES		FEMALES	
Group*	Transplants/ Tumors Recovered	Gm. Total Weight of Tumors	Transplants/ Tumors Recovered	Gm. Total Weight of Tumors
0	2/2	0.90	2/2	0.94
I	2/1	0.48	2/0	0.00
II	2/0	0.00	2/0	0.00
III	2/2	0.30	2/0	0.00
V	2/2	0.12	2/0	0.00
VI	2/1	0.08	2/0	0.00
VII	2/2	0.14	2/2	0.06
VIII	2/0	0.00	2/0	0.00
TOTAL	16/10	2.02	16/4	1.00
	DISCARDED BREEDERS			
0	2/2	0.78	2/2	0.69
I	2/2	0.65	2/2	0.28
II	2/2	0.36	2/2	0.10
III	2/2	0.14	2/2	0.04
V	2/2	0.03	2/1	0.01
VI	2/0	0.00	2/0	0.00
VII	2/0	0.00	2/0	0.00
VIII	2/0	0.00	2/0	0.00
TOTAL	16/10	1.96	16/9	1.02

*The group number also indicates number of days embryos were kept extra corpus.

TABLE III

CORTISONE CONDITIONED ANIMALS RECEIVING EMBRYONIC TISSUE HOMOTRANSPLANTS (ARRANGED ACCORDING TO GROUPS, SEX, AGE, AND DAYS EMBRYOS WERE KEPT EXTRA CORPUS)

IMMATURE RATS

Group	MALES		FEMALES	
	Transplants/ Tumors Recovered	Gm. Total Weight of Tumors	Transplants/ Tumors Recovered	Gm. Total Weight of Tumors
0	2/2	1.44	2/2	1.09
I	2/1	0.35	2/1	0.14
II	2/2	0.23	2/1	0.19
III	2/1	0.31	2/1	0.16
V	2/2	0.21	2/0	0.00
VI	2/2	0.08	2/0	0.00
VII	2/2	0.53	2/2	0.03
VIII	2/1	0.02	2/2	0.03
TOTALS	16/13	3.17	16/9	1.64
		DISCARDED BREEDERS		
0	2/2	0.74	2/2	0.88
I	2/2	0.85	2/2	0.64
II	2/2	0.66	2/2	0.36
III	2/1	0.10	2/2	0.03
V	2/1	0.04	2/1	0.01
VI	2/1	0.08	2/0	0.00
VII	2/0	0.00	2/0	0.00
VIII	2/1	0.02	2/0	0.00
TOTAL	16/10	2.49	16/9	1.92

TABLE IV

TRYPAN BLUE CONDITIONED ANIMALS RECEIVING EMBRYONIC TISSUE HOMOTRANSPLANTS (ARRANGED ACCORDING TO GROUPS, SEX, AGE, AND DAYS EMBRYOS WERE KEPT EXTRA CORPUS)

IMMATURE RATS

Group	MALES		FEMALES	
	Transplants/ Tumors Recovered	Gm. Total Weight of Tumors	Transplants/ Tumors Recovered	Gm. Total Weight of Tumors
0	2/2	0.87	2/2	0.69
I	2/1	0.43	2/1	0.15
II	2/2	0.21	2/1	0.20
III	2/1	0.42	2/1	0.51
V	2/2	0.12	2/0	0.00
VI	2/2	0.07	2/0	0.00
VII	2/1	0.43	2/2	0.16
VIII	2/0	0.00	2/0	0.00
TOTAL	16/11	2.55	16/7	1.71
	DISCARDED BREEDERS			
0	2/2	0.58	2/2	0.57
I	2/2	0.49	2/2	0.40
II	2/2	0.34	2/2	0.19
III	2/2	0.40	2/1	0.01
V	2/1	0.02	2/1	0.01
VI	2/0	0.00	2/0	0.00
VII	2/0	0.00	2/0	0.00
VIII	2/0	0.00	2/0	0.00
TOTAL	16/9	1.83	16/8	1.18

TABLE V

ANIMALS CONDITIONED BY CASTRATION RECEIVING EMBRYONIC TISSUE HOMOTRANSPLANTS (ARRANGED ACCORDING TO GROUPS, SEX, AGE, AND DAYS EMBRYOS WERE KEPT EXTRA CORPUS)

	IMMATURE RATS			
	MALES		FEMALES	
Group	Transplants/ Tumors Recovered	Gm. Total Weight of Tumors	Transplants/ Tumors Recovered	Gm. Total Weight of Tumors
0	2/2	0.94	2/2	0.75
I	2/2	0.62	2/1	0.15
II	2/1	0.06	2/1	0.10
III	2/1	0.02	2/2	0.19
V	2/2	0.03	2/0	0.00
VI	2/0	0.00	2/0	0.00
VII	2/2	0.13	2/1	0.11
VIII	2/0	0.00	2/1	0.01
TOTAL	16/10	1.80	16/8	1.31
	DISCARDED BREEDERS			
0	2/2	0.71	2/2	0.76
I	2/2	0.66	2/2	0.46
II	2/2	0.20	2/2	0.07
III	2/1	0.09	2/1	0.01
V	2/0	0.00	2/0	0.00
VI	2/0	0.00	2/0	0.00
VII	2/0	0.00	2/0	0.00
VIII	2/0	0.00	2/0	0.00
TOTAL	16/7	1.66	16/7	1.30

TABLE VI

ANIMALS CONDITIONED WITH ESTRADIOLE BENZOATE AND PREGNANCY RECEIVING EMBRYONIC TISSUE HOMOTRANSPLANTS (ARRANGED ACCORDING TO GROUPS, SEX, AGE, AND DAYS EMBRYOS WERE KEPT EXTRA CORPUS)

	ESTRADIOLE BENZOATE			
	IMMATURE MALES		DISCARDED BREEDER MALES	
Group	Transplants/ Tumors Recovered	Gm. Total Weight of Tumors	Transplants/ Tumors Recovered	Gm. Total Weight of Tumors
0	4/1	0.39	4/3	0.98
I	4/0	0.00	4/4	0.41
II	4/0	0.00	4/3	0.02
III	4/0	0.00	4/1	0.01
V	4/0	0.00	4/1	0.01
VI	4/0	0.00	4/0	0.00
VII	4/2	0.07	4/0	0.00
VIII	4/0	0.00	4/0	0.00
TOTAL	32/3	0.46	32/12	1.43
	PREGNANCY			
0	4/2	0.54		
I	4/4	0.44		
II	4/4	0.17		
III	4/1	0.02		
V	4/0	0.00		
VI	4/0	0.00		
VII	4/0	0.00		
VIII	4/0	0.00		
TOTAL	32/11	1.17		

TABLE VII

RESULTS OF EMBRYONIC TISSUE HOMOTRANSPLANTS INTO PRE-CONDITIONED IMMATURE RATS (45 TO 60 DAYS OLD)

Condition	Transplants/ Tumors Recovered	Gm. total Weight of Tumors	Gm. Average Weight per Tumor	Gm. Average Weight per Transplant
MALES				
Controls	16/10	2.02	0.20	0.12
Cortisone Acetate	16/13	3.17	0.24	0.20
Trypan Blue	16/11	2.55	0.23	0.16
Castration	16/10	1.80	0.18	0.11
TOTAL	64/44	9.54	0.21	0.15
Estradiole Benzoate	32/3	0.46	0.15	0.01
FEMALES				
Controls	16/4	1.00	0.25	0.06
Cortisone Acetate	16/9	1.64	0.12	0.10
Trypan Blue	16/7	1.71	0.24	0.11
Castration	16/8	1.31	0.16	0.08
TOTAL	64/28	5.66	0.19	0.09
Pregnancy	16/11	1.17	0.10	0.07

TABLE VIII

RESULTS OF EMBRYONIC TISSUE HOMOTRANSPLANTS INTO PRE-CONDITIONED DISCARDED BREEDER RATS (ALL OVER 14 MONTHS OF AGE)

Condition	Transplants/ Tumors Recovered	Gm. Total Weight of Tumors	Gm. Average Weight per Tumor	Gm. Average Weight per Transplant
MALES				
Controls	16/10	1.96	0.20	0.12
Cortisone Acetate	16/10	2.49	0.25	0.15
Trypan Blue	16/9	1.83	0.20	0.11
Castration	16/7	1.66	0.24	0.10
TOTAL	64/36	7.94	0.22	0.12
Estradiole Benzoate	32/12	1.43	0.02	0.04
FEMALES				
Controls	16/9	1.02	0.11	0.06
Cortisone Acetate	16/9	1.92	0.21	0.12
Trypan Blue	16/8	1.18	0.15	0.07
Castration	16/7	1.13	0.16	0.08
TOTAL	64/33	5.25	0.18	0.08

TABLE IX

TOTALS ON ALL ANIMALS ACCORDING TO SEX AND HORMONAL STATUS

Groups	Transplants/ Tumors Recovered	Gm. Total Weight of Tumors	Gm. Average Weight per Tumor	Gm. Average Weight per Transplant
Males	128/80	17.48	0.218	0.135
Females	128/61	10.91	0.180	0.085
Estradiole Benzoate*	64/15	1.89	0.126	0.029
Pregnancy*	16/11	1.17	0.106	0.073

*These groups are not included in the totals for the males and females.

through VI. An explanation for this individual variation is not apparent. Snell (1953) states, "Now we can point with confidence to a complex variety of environmental and genetic agencies that influence the degree of their (transplants) development in the host, though it need hardly be said that the unknown agencies still outnumber the known."

The results of the effect of the age of the host upon the transplants are summarized in the totals of Table VII (immature rats) and Table VIII (discarded breeders). When these tables are compared they give an equivocal answer but closer inspection of the data, as provided in Tables II through VI, shows that the older animals are more refractory to transplants of embryonic tissue kept extra corpus longer than five days. The data indicates that the younger immature animals, being less refractory to transplanted tissue kept extra corpus, are the more favorable hosts.

The effect of the sex of the host animal on the success of transplantations has received very little attention, previously. Sex as a condition for successful transplantation was first proposed by Fere (1894). In his study, using two hens and two cocks, the cocks proved refractory to transplants. To Fere, this, at least in the chicken, showed the female to be a more favorable host animal.

Nicholas (1934) in a series of experiments showed that the male mammary gland was as good a site as that of the pregnant female. He also stated that female endocrines do not influence transplant development.

The findings (Table IX) in the rat show the male to be a more favorable host animal than the female. It can be seen that transplants into the male were generally more successful than those into the female.

Not only were the number of successful transplants greater in the male but the size of the tumors recovered averaged 0.038 gm. heavier.

Closely paralleling the findings in the difference of the sex of the host animal are the results obtained by altering the hormonal status of the host animal (Tables VII, VIII, and IX). It is shown that alteration of the sex hormonal status of both males and females alters the success of transplantation and the size of the tumors recovered.

The effects of altering the hormonal status of the male host animal may be seen in two conditioned groups. These groups are the castrated males and the males which received parenteral estradiole benzoate. Castration of the male slightly decreases the number of successful transplants and also decreases the size of the tumors produced. The total weight of the tumors recovered from the castrated males was 0.52 gm. less than those recovered from the control males.

Males administered estradiole benzoate, parenterally, show a marked decrease in the number of successful transplants and a marked decrease in the size of the tumors recovered as compared to those of the control animals. These animals also proved less favorable as host animals in comparison to the castrated males.

The effect of the alteration of the hormonal status, castration and pregnancy, of the female hosts is presented in Tables VII, VIII, and IX.

The castration of the female host increased the number of successful transplants and also the total weight of tumors recovered as compared to those of the controls.

The effects of pregnancy on the host animal are not as clearly

defined as the effects of castration. The number of successful transplants recovered from the pregnant host animals was proportionally greater in number and size than those recovered from the control females. A comparison of the condition of pregnancy with that of castration of females shows that pregnancy increased the number of successful transplants but the total tumor weight and the average size of the tumors was decreased.

Thus, the results obtained in these experiments lead one to conclude that sex hormones play an important role in the growth and differentiation of embryonic tissue transplants. The androgens of the male appear to have a stimulating effect and the estrogens of the female an inhibitory effect in this development.

The fact that the results obtained with the rats conditioned by pregnancy are not easily fitted into the same pattern as shown by the other animals indicates that hormonal factors, other than the estrogen titer of the host, also exert an influence upon the transplants.

These findings of the influence of sex and hormonal status do not substantiate the theory of Askanazy (1907) and Jentzer (1908) that a state of pregnancy is beneficial to the success of transplants. They are not in agreement with the statement of Nicholas (1934) that female endocrine agents do not influence graft development.

The reason for the inhibitory effect of estrogens on tissue growth is not clear. Leathem and Granitsas (1957) showed that estradiole benzoate reduced the growth rate and size of transplants of the Walker rat sarcoma. In their experiments the estrogen reduced the food intake and caused a subnormal retention of nitrogen. Neither of these effects

could be counteracted by concomitant androgen.

The use of cortisone has been widely employed to modify host resistance. Its use has appealed more to the clinician than to the laboratory investigator as a possible practical means of promoting the takes of homologous skin grafts. (Toolan, 1953.)

In this study, cortisone acetate, administered parenterally, has definitely increased the number of successful transplants and has also increased the size of the tumors (Tables III, VII, and VIII). These increases were evident in both males and females. The percentage increase was greater in the immature rats than in the adult discarded breeders.

Sugiura, et al., (1950), and Higgens and Bennett (1952), reported that cortisone and ACTH inhibited growth of established tumors. Others, Foley and Silverstein (1951), Howes (1951) and Foley (1952), reported that transplantable tumors of mice that would ordinarily regress, or do poorly, showed progressive growth in homologous hosts treated with cortisone. These findings indicate that the response to the transplant is dependent upon the tumor or the tissue transplanted.

The effect of cortisone acetate in promoting the success of transplants has been difficult to evaluate. Many factors operating in the action of this hormone have been proposed to account for the desensitization of the host animal to transplants. Thus, it is difficult to evaluate how the cortisone affects either the natural or immune resistance of the host.

It is perhaps a combination of several factors, a delay of inflammatory response, Michael and Whorton (1951), Spain, et al., (1952),

the relative impermeability of blood vessels, Ebert (1951), the inhibition of hyaluronidase, Osboe-Hansen (1952), and an alteration of connective tissue substrate, Layton (1951), which enhances the success of the homologous transplants.

Another of the well known but comparatively little used methods of desensitizing host animals to homologous transplants has been the use of vital stains. The most often used of these are trypan blue and vital red.

In this study the use of trypan blue did influence the number of successful transplants (7% increase) and the total weight of tumors recovered was increased (1.27 gm.) over the total weight of the tumors recovered from the controls.

Ludford (1931a and b) postulated the theory that dyes and colloids overload the reticulo-endothelial system which he believed was associated with the host's aggressive reaction to the transplants. Andervont (1932,1936) also proposed that the mechanism of action of trypan blue was by lowering the resistance of the host animal to such an extent as to permit tumors, that would ordinarily regress, to grow progressively larger.

Thus, the second part of this study can be answered affirmatively. It is possible to alter the proliferative energy of new growth produced by the homotransplantation of embryonic tissue by conditioning the host. With the results obtained it can be definitely stated that conditioning the host animals with cortisone acetate (males and females), trypan blue (males and females) and castration (females) increases the number of successful transplants and size of tumors recovered. Conditioning agents

which decrease the number of successful transplants and size of tumors recovered were found to be parenteral estradiole benzoate (males), castration (males) and possibly pregnancy.

Part III

In performing the third part of this study, the findings discussed in Part II were utilized. It has been shown (Tables VII and VIII) that immature male host animals conditioned with cortisone acetate offered the most favorable conditions for successful transplantation. Thus, the last series of experiments was limited to 60 of these animals.

The results shown in Figure I are the results of these experiments. Lymphatic tissue survives for a period of 16 days, while bone and cartilage survive a maximum of 20 days extra corpus.

Grossly the tumors produced from the transplanted embryonic tissues were of a variety of sizes and shapes. The largest tumors were developed from tissues kept extra corpus for periods of less than 3 days. Tumors produced from tissues maintained for longer periods of time were proportionally smaller.

The tumors contained numerous cystic cavities filled with clear yellow fluid or pasty granular material. In contrast to the findings of Waterman (1939) large cyst formation was more common in tumors produced from tissues kept extra corpus for the shorter period of time.

Microscopically the tumors closely resemble those described by Jentzer (1908), Askanazy (1911) and Waterman (1939). (Figures 2 through 26)

In the substance of the tumors of Group 0 there is a highly

THE EXTRA CORPUS SURVIVAL TIME OF TISSUES AS DETERMINED BY THEIR APPEARANCE IN RECOVERED TUMORS

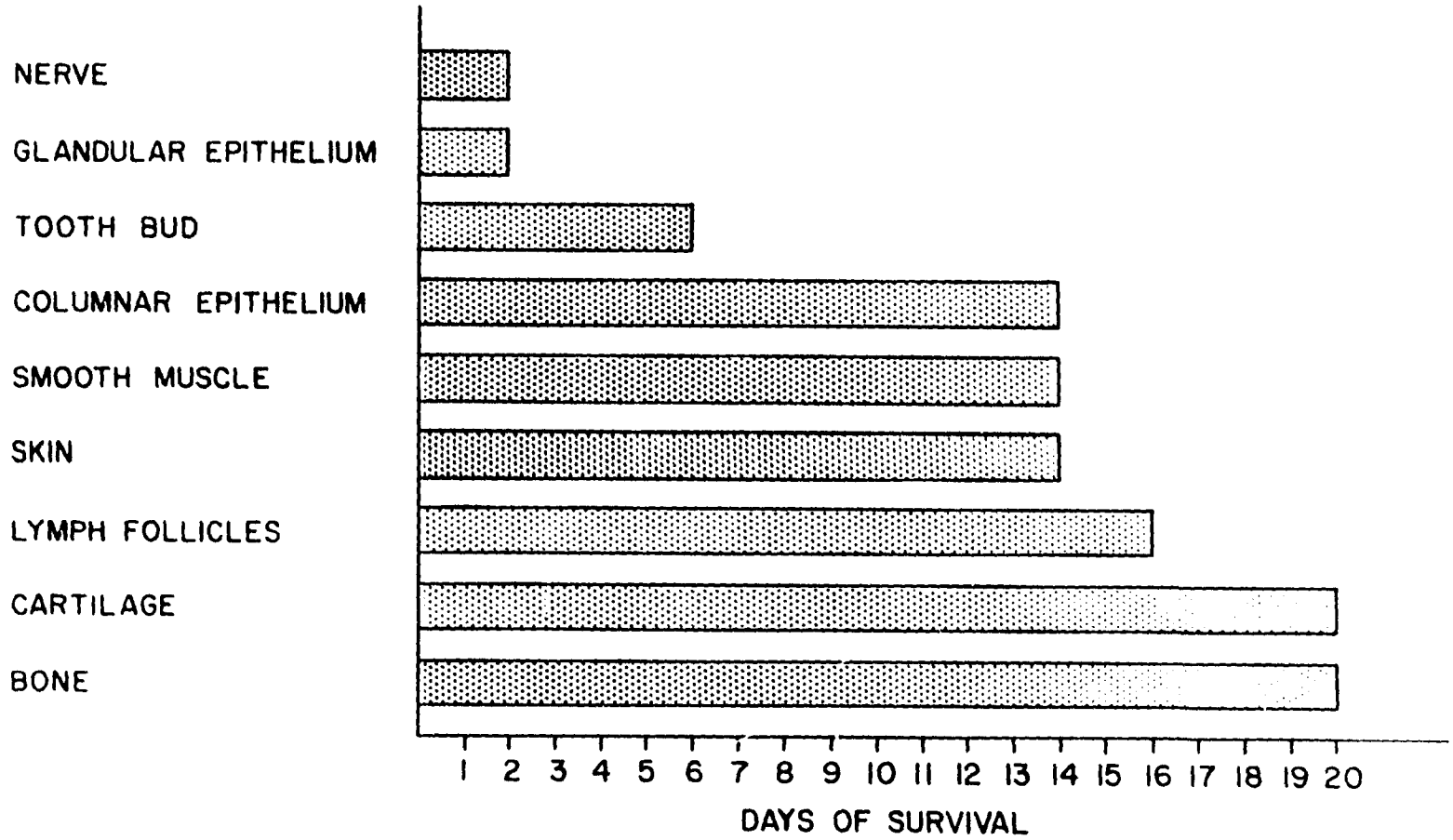

FIG. 1

cellular fibrous connective tissue. Frequently seen within this stroma are numerous lymphocytes that in some areas are assembled or arranged to form lymph follicles. Numerous small blood vessels permeate the tumor. Contiguous to the tumor and about its periphery, adipose tissue, mammary gland tissue, and striated muscle is observed. These clearly are tissues of the host and do not represent portions of the tumor. Large cyst formation appears to be the most prominent feature of this group of tumors. These may be described as being of four major types.

I. These cysts are lined with stratified squamous epithelium containing all of the epidermal layers. The walls present prominent papillae, hair follicles, and glandular structures resembling sebaceous glands. Desquamated epithelium, fragments of hair and a homogenous eosinophilic staining material fill the space within the cysts.

II. Other cysts are lined by stratified squamous epithelium but contain no papillae, hair follicles, or sebaceous glands. The cavities contain but small amounts of desquamated epithelium.

III. A third type of cyst is lined with columnar epithelium thrown into numerous folds resembling villi. Intermixed with the columnar epithelium are numerous mucus containing goblet cells. The cavities contain a homogenous eosinophilic material.

IV. In the fourth type, cysts are also lined with columnar epithelium but in some areas the cells are more cuboidal than columnar. Some of these cells are ciliated. The cavities of these cysts contain granular basophilic material and numerous polymorphonuclear leukocytes.

Cartilaginous tissue is also a prominent feature of these tumors. It appears as elongated or rounded islets surrounded by

perichondrium. The cells within the lacunae are large and well differentiated and in some areas are massed together indicating proliferation.

The transition of cartilage to bone is seen in many areas. Epiphysial lines where bony trabeculae are replacing cartilage are quite distinct. The bony trabeculae can be seen to enclose cellular elements characteristic of bone marrow.

Throughout the tumors numerous other tissue elements are observed. A tooth bud in an advanced stage of development is a prominent feature in several sections of one of these tumors. Clearly distinguishable are the enamel primordium, ganablasts, dentin, dentin tubules, and ondontoblasts.

Tissues of central nervous system derivation are not well defined. When present, there are masses of tissue closely resembling glial elements. Within these are large nucleated cells which were identified as pyramidal cells. More clearly distinguishable are ganglion cells surrounded by satellite cells. Also clearly distinguishable within the muscular layers of what resembles intestine are large cells which appear to be elements of myenteric plexuses.

Smooth muscle is associated with the cysts formed by the differentiating gastrointestinal elements.

In several sections spherical follicles composed of uniformly large cuboidal cells are present. These contain a polychromatic material. It is most likely that this tissue represents poorly differentiated thyroid elements.

A second type of glandular tissue is seen in several sections. This tissue is composed of low cuboidal epithelium with large centrally

placed nuclei arranged to form acini. This resembles salivary gland tissue.

There is no microscopic difference between the tumors of the first three groups. The only additional element seen in the tumors of Groups I and II is the appearance of multinucleated giant cells.

Tumors of Groups III through VI show a decrease in the number and size of cysts, although cyst formation remains a prominent feature. Missing from these are the ganglion cells and glial elements present in the tumors of groups 0, I and II. Elements of the myenteric plexus are present in the tumors of Group III and IV. The glandular tissues found in the previously described tumors are also absent in these tumors. There is a relative increase in the amount of bone and cartilage present.

Tumors of Groups VII through XII show fibrous connective tissue, lymphoid elements, bone, and cartilage constitute the major portion of the tumors. Epithelial elements of skin, gastrointestinal tract and respiratory tract are present in most sections, although the cysts formed by these elements are considerably smaller than those which dominated the picture in tumors of the earlier groups. An absolute increase in the amount of necrotic tissue, hemosiderin, and numerous multinucleated giant cells is present in these tumors.

The prominent feature of the very small tumors of Group XIV is the predominance of connective tissue, lymphoid elements, bone, and cartilage. Necrosis, pigmentation and multinucleated giant cells are more abundant than in the previous groups. Two small islands of epithelium are present in one tumor. One is of gastrointestinal tract and the other of epidermal origin with a few well defined hair follicles.

In tumors of Group XVI epithelial elements are entirely absent. The greater part of the tumors consists of necrotic tissue, hemosiderin, and connective tissue. There appears to be a marked decrease in the amount of bone and cartilage.

One tumor was recovered from Group XVIII and one from Group XX. They are essentially similar and consist of necrotic tissue with much hemosiderin and many giant cells. In several areas very small islands of still living bone and cartilage remain. There are also occasional areas of dystrophic calcification within the necrotic masses.

The results of the third part of this study add to the accumulating evidence in support of two concepts. 1. The developmental direction and fate of embryonic parts are determined at a very early stage of embryonic development, as has been reported by Strangeway and Fell (1926), Needham (1931, 1942), and Browning (1949). 2. Many mammalian embryonic tissues can survive subnormal temperatures for long periods of time, as previously reported by Jentzer (1908), Askanazy (1908), Bucciante (1931), Nicholas (1934), Alvarez (1937), and Waterman (1939).

In substantiating the first concept, it was shown that poorly differentiated embryonic tissues, taken at a very early stage of development (9 to 10 days), homogenized and broken into numerous small groups of cells, subjected to conditions detrimental to their existence and transplanted into an adult animal, go on to develop and become structures quite similar to those ordinarily attained in the course of normal intrauterine development.

Substantiating the second concept, the following was shown: (a) Nervous tissue and some glandular tissues (thyroid and salivary

gland) do survive *extra corpus* at 2-4° C. for two days. (b) Columnar epithelium of the respiratory and gastrointestinal tracts, epidermal elements, and smooth muscle survive for 14 days *extra corpus*. (c) Lymphatic tissue survives for 16 days and (d) bone and cartilage survive for 20 days *extra corpus*.

CHAPTER VI

SUMMARY AND CONCLUSIONS

A total of 417 rats were used as host animals in this study on the survival, growth, and differentiation of embryonic tissue homotransplants. The animals were of three different ages and of both sexes.

The study was divided into three parts:

I. To determine the site which would offer the most favorable conditions (environment) for successful transplantation.

II. To attempt to modify the proliferative capacity of transplants by conditioning the host animals, and

III. To determine the maximal time that embryonic rat tissue will survive extra corpus.

Part I. The three most commonly used sites for the transplantation of embryonic and tumor tissues (subcutaneous tissue, testes and peritoneal cavity) were tested. The peritoneal cavity proved to be a poor site for the transplantation of embryonic tissue. The results show that there is little difference between subcutaneous tissue and testes as the preferred site for transplantation. The subcutaneous tissue and testes have an advantage over other sites in that they are accessible and the transplants can be conveniently accomplished without a surgical approach.

Part II. Three hundred thirty-six animals were divided into groups according to age and sex to determine if it is possible to modify the proliferative capacity of new growths. The groups were conditioned by the parenteral administration of cortisone acetate, trypan blue, estradiole benzoate and by castration and pregnancy before and after receiving transplants kept extra corpus for varying lengths of time. Control animals were included in each group.

From the results obtained, it was concluded that:

1. There is a great variation in the way that individual animals act as hosts for transplants.

2. The sex of the host animal plays an important role in the successful transplantation of embryonic tissue in that the male host is more suitable for the growth and differentiation of the transplants.

3. The hormonal state of the host is closely related to sex as a condition for successful transplantation. The androgens of the male increase the proliferative capacity of embryonic tissue transplants, while the estrogen, estradiole benzoate, decreases the proliferative capacity. The high estrogen titer of pregnancy is partially counterbalanced by other hormonal factors.

4. The proliferative capacity of embryonic tissue transplants may be greatly increased by the desensitization of the host animal. Cortisone acetate and trypan blue are two agents that will desensitize the host animal. The sensitivity of host animals to transplants is also reflected in the age of the host. The more mature animal with well developed immune responses is a less favorable host animal than the immature one.

Part III. Immature rats conditioned by parenterally administered cortisone acetate served as hosts for transplants kept extra corpus at 2-4° C. for 22 days. It was shown that tissues, bone and cartilage, kept extra corpus for 20 days remained viable and upon transplantation grew and differentiated. Other tissues were found to survive for shorter periods of time.

Thus, embryonic tissues taken at a very early stage of development and transplanted into adult animals develop and become structures similar to those seen in the course of normal intrauterine development. The death of animal tissues does not occur with the death of an animal as a whole but takes place at varying intervals after somatic death.

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APPENDIX

Figure 2

Normal embryo. A cross section of the thorax of a 1.9 mm. embryo shows poorly differentiated cartilage, spinal cord, skin, aorta, lungs, and mesenchyme.

Figure 3

Normal embryo. A sagittal section of vertebral column from a 1.9 mm. embryo, shows undifferentiated bone, cartilage, and connective primordia.

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Group VII tumor. An epiphysial line demonstrates linear arranged cartilage cells merging into trabecular bone.

Figure 7

Normal embryo. The lung bud of a 1.5 mm. embryo demonstrates poorly differentiated alveolar epithelium and branching characteristic of an embryo at this stage of development.

Figure 6

Figure 7

Figure 8

Group IV tumor. This clearly shows a highly differentiated bronchus surrounded by cartilaginous plates.

Figure 9

Group IV tumor. A greater magnification of Figure 8 clearly demonstrates the ciliated columnar epithelium of the bronchus.

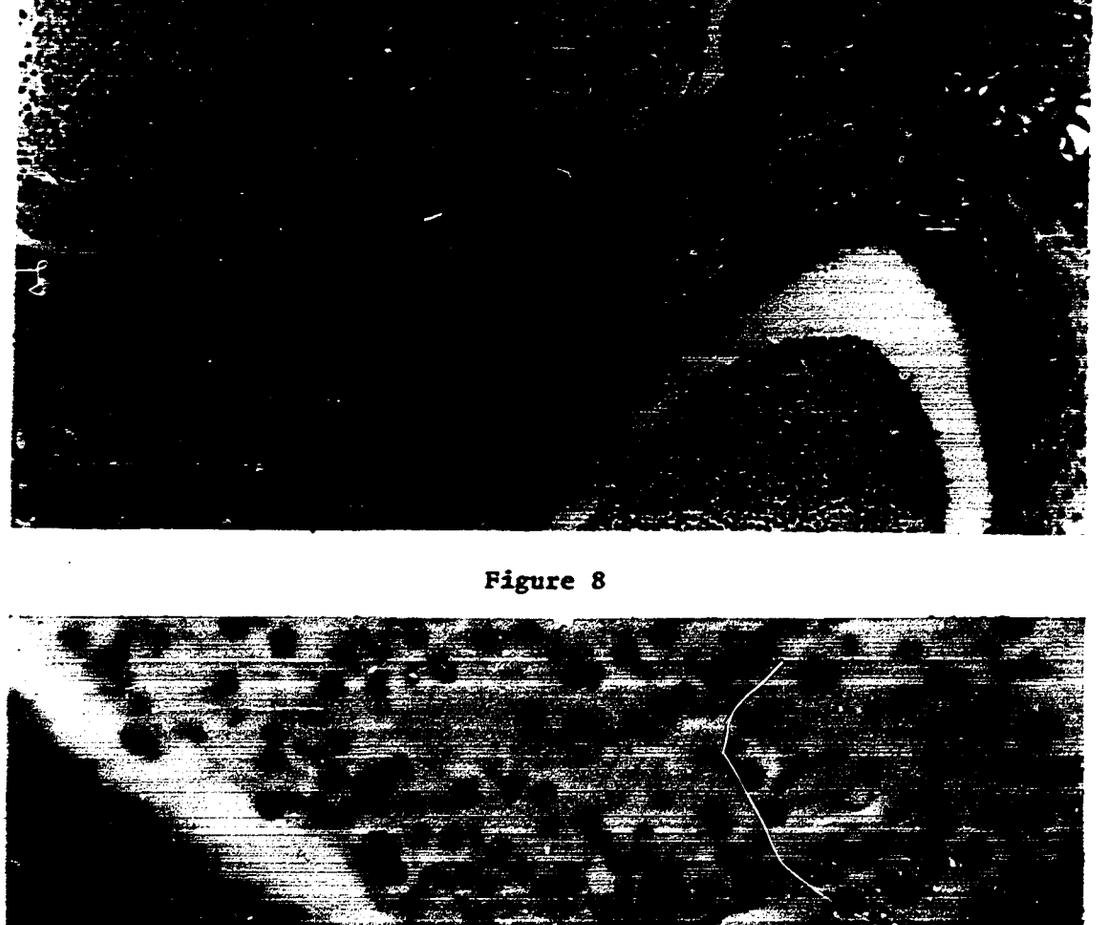

Figure 8

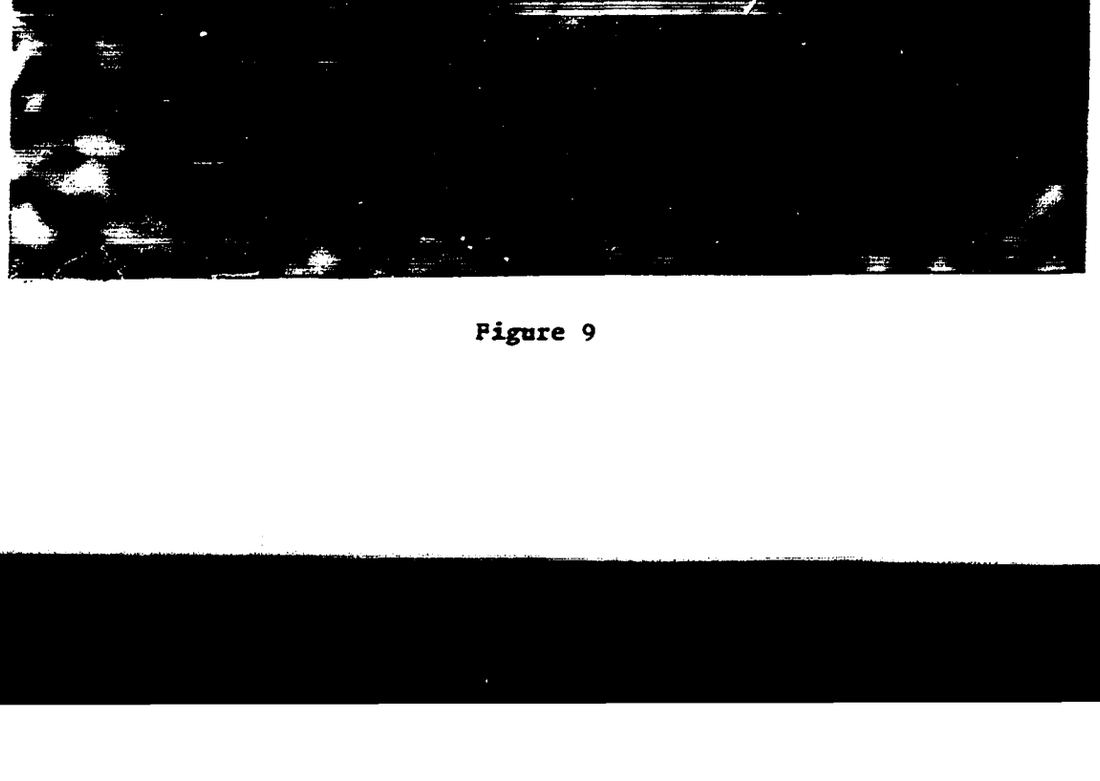

Figure 9

Figure 10

Normal embryo. A section of gastrointestinal tract from a 1.9 mm. embryo shows poor differentiation of the columnar epithelium and muscular wall at this atage of development.

Figure 11

Group VI tumor. This includes gastrointestinal tract in which columnar epithelium, goblet cells, villi, and muscularis are clearly evident.

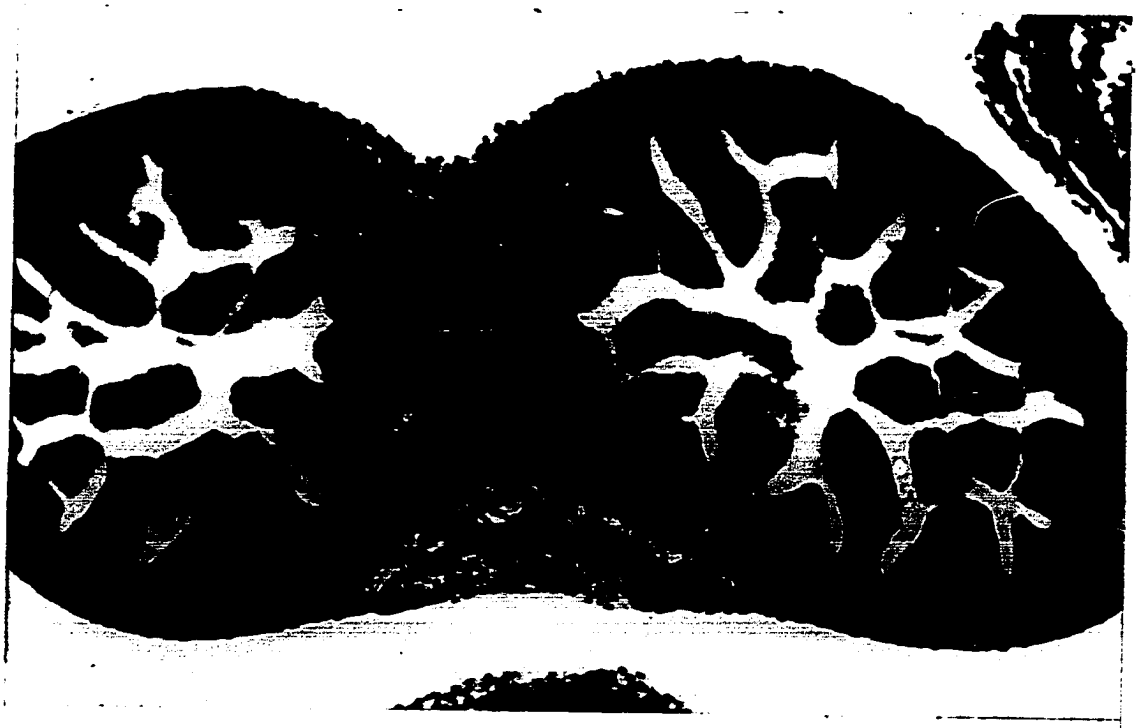

Figure 10

Figure 11

Figure 12

Group XIV tumor. Gastrointestinal tract in the tumor shows well differentiated columnar epithelium, goblet cells, crypt and gland formation and muscularis.

Figure 13

Group X tumor. Organoid gastrointestinal, osseous and cartilaginous structures are evident. The relation of these structures is quite different from that in normal animals and is typical of teratoid tumors.

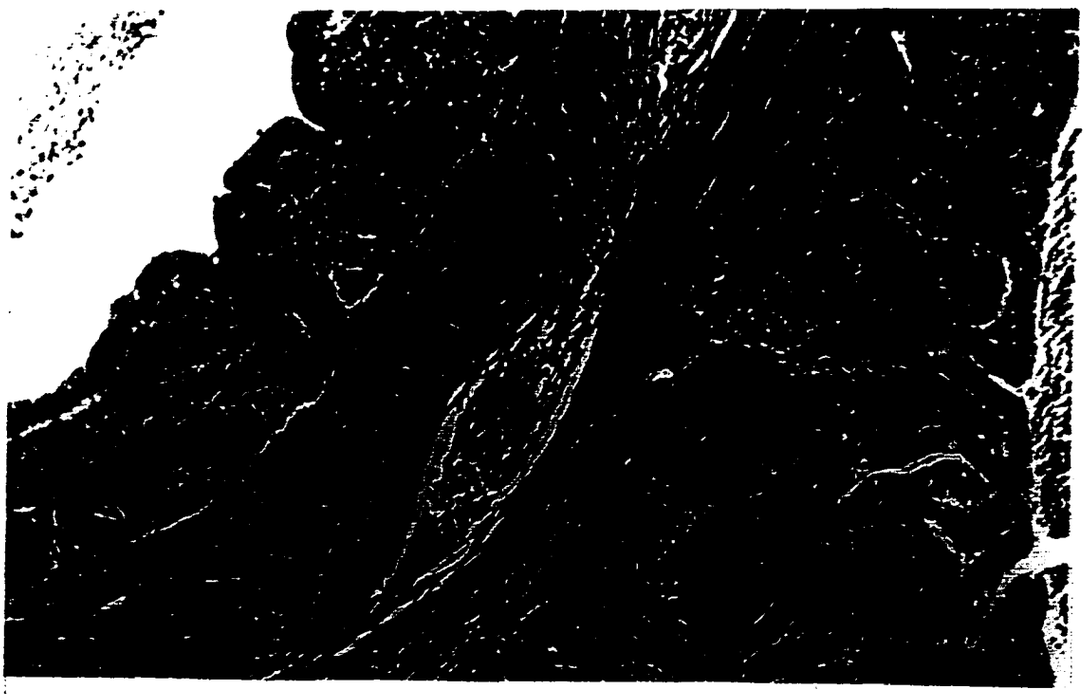

Figure 12

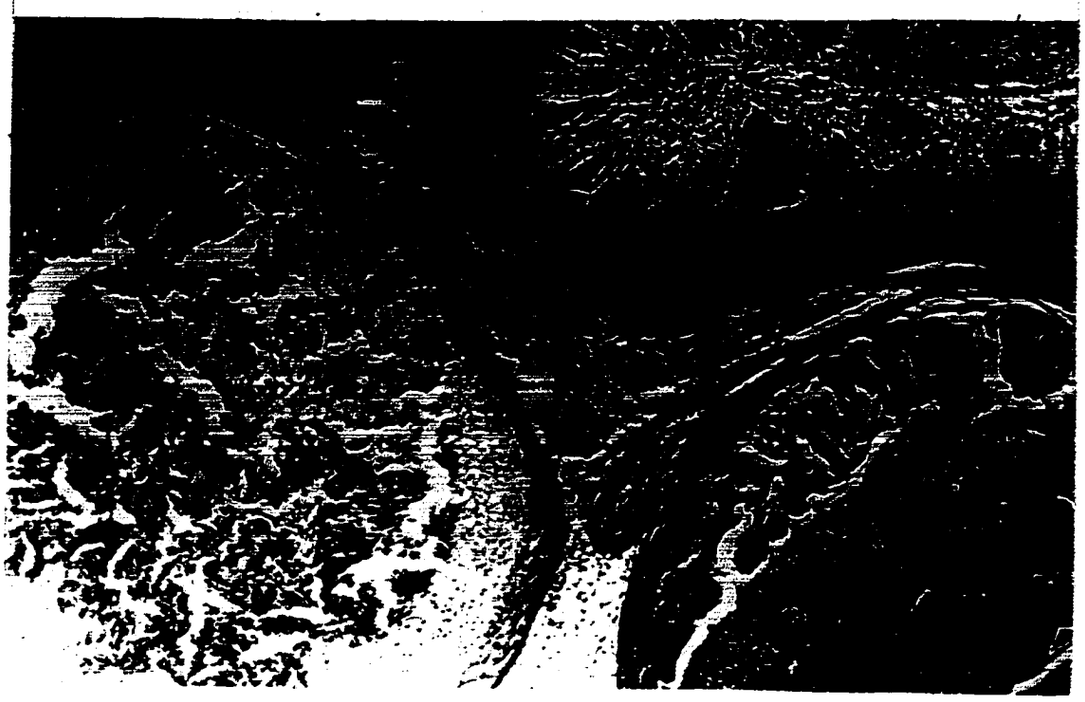

Figure 13

Figure 14

Group II tumor. Mimicking of normal gastrointestinal tract is well demonstrated by mucosa and muscularis. Myenteric plexuses are well formed.

Figure 15

Group II tumor. A greater magnification of Figure 14 shows more clearly the myenteric plexuses.

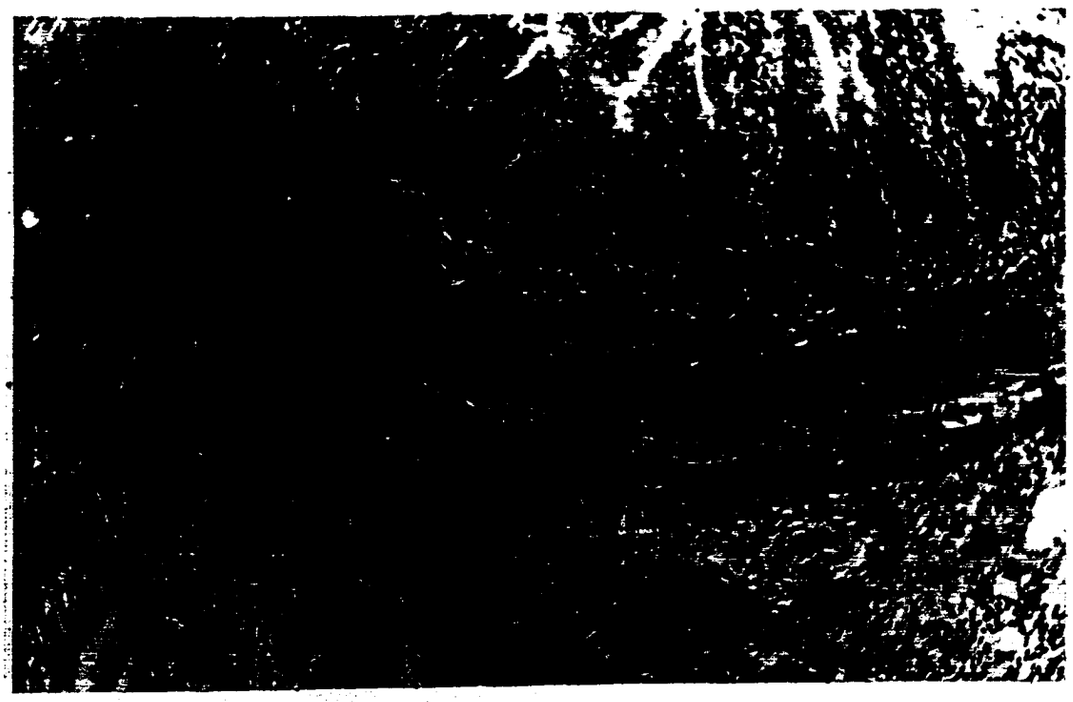

Figure 14

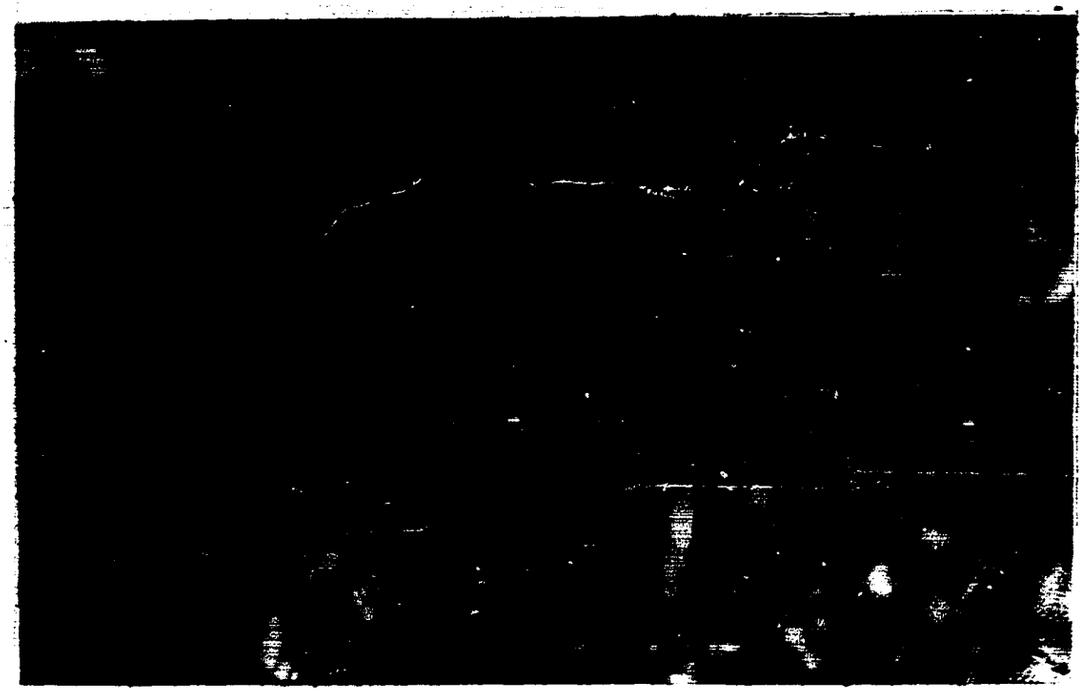

Figure 15

Figure 16

Normal embryo. A section of epidermis from a 1.5 mm. embryo demonstrates the poor differentiation of stratified squamous epithelium, dermis, and hair follicles at this stage of development.

Figure 17

Group XII tumor. A cyst formed by hairless stratified squamous epithelium shows distinct cellular layers and sloughed keratinized material in the cavity of the cyst.

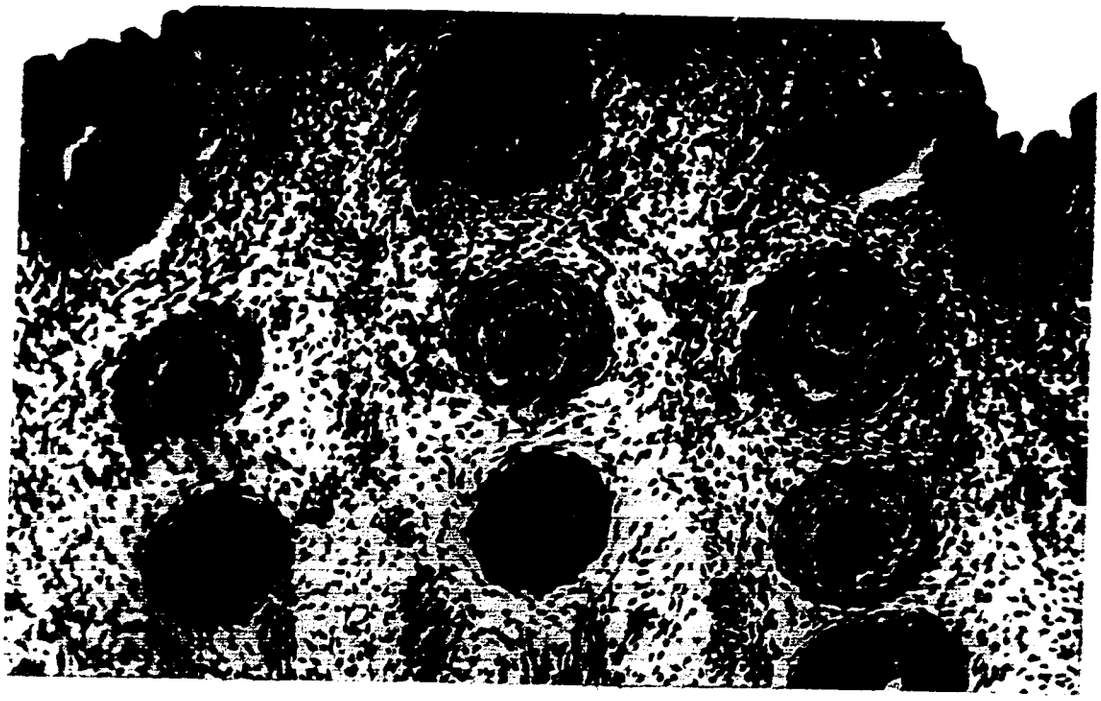

Figure 16

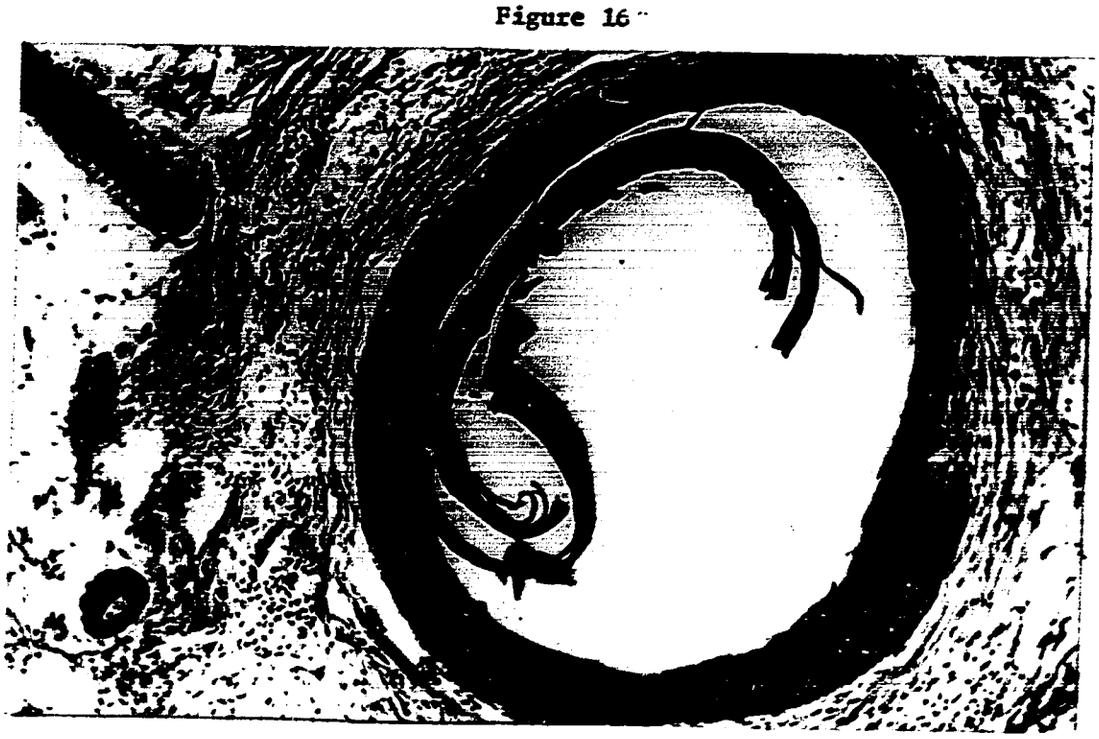

Figure 17

Figure 18

Group 0 tumor. This section of skin demonstrates well differentiated hair follicles. This was a common finding in most tumors.

Figure 19

Group VI tumor. This is a clear demonstration of glandular duct formation. The ducts are lined by a low columnar epithelium.

Figure 18

Figure 19

Figure 20

Group II tumor. This section of serous type glandular tissue clearly demonstrates acinus formation.

Figure 21

Group II tumor. A greater magnification of Figure 20 shows the cellular detail of the acini more clearly.

Figure 20

Figure 21

Figure 22

Group 0 tumor. This section of mucinous type glandular tissue shows the mucus containing cells arranged in acini.

Figure 23

Group 0 tumor. A greater magnification of Figure 22 shows more clearly the cellular detail of the mucus producing cells.

Figure 22

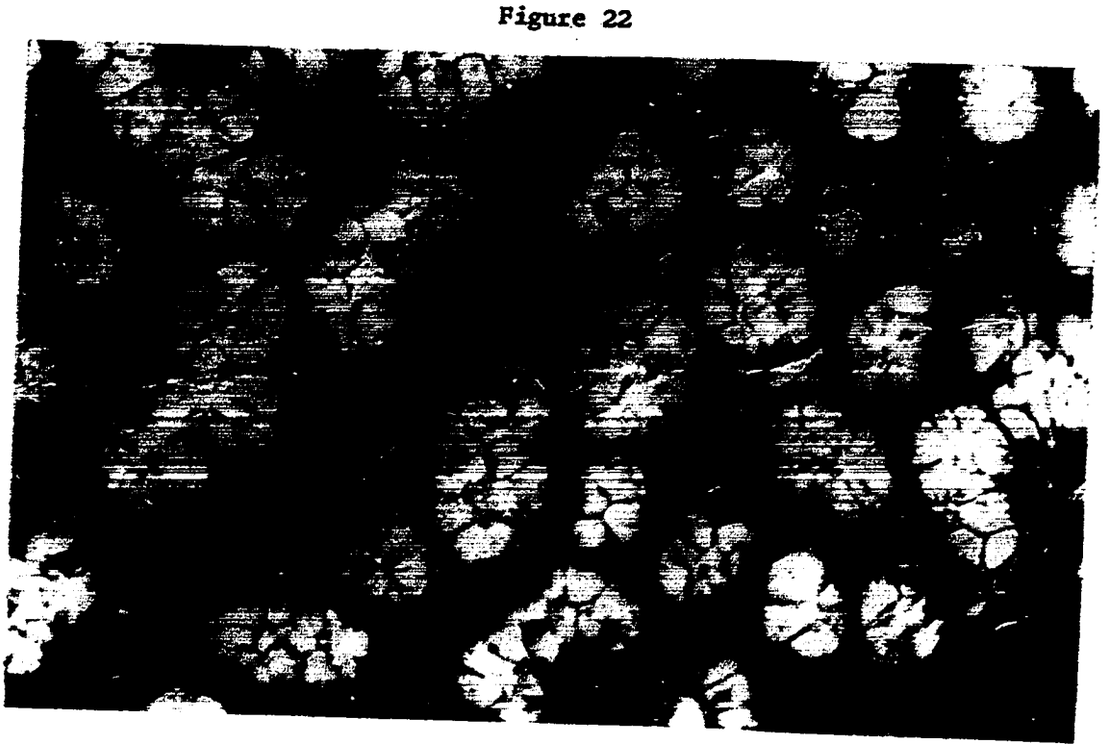

Figure 23

Figure 24

Group II tumor. Elements of thyroid gland show definite colloid containing follicles.

Figure 25

Group II tumor. Ganglion cells with accompanying satellite cells demonstrate the presence of nervous tissue in tumors of this as well as earlier groups.

Figure 24

Figure 25

Figure 26

Group VI tumor. A tooth bud shows the high degree of differentiation attained by structures found in these tumors.

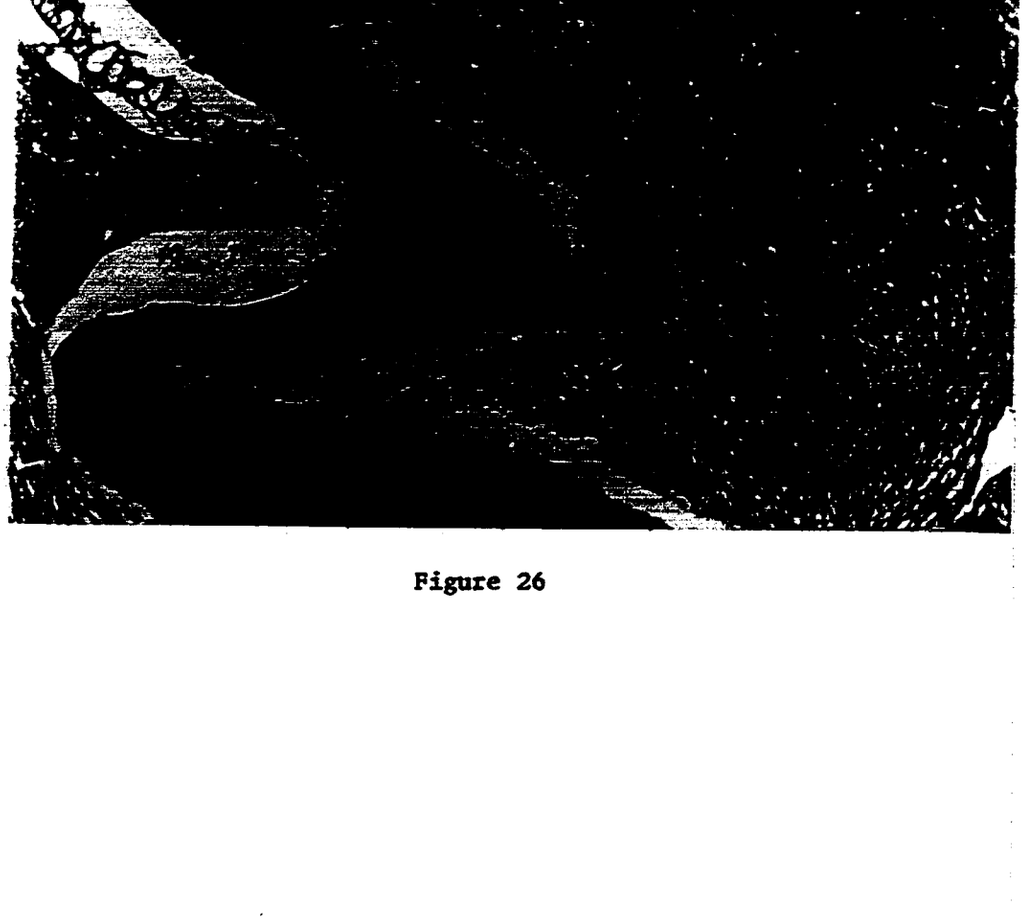

Figure 26