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I hereby recommend that the thesis prepared under my supervision by Gordon Oliver Potts

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A STUDY OF RAT TOOTH PRIMORDIA TRANSPLANTED INTO THE ANTERIOR CHAMBER OF THE EYE

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by

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TABLE OF CONTENTS

1. Introduction

Two general methods have usually been employed in the study of growth and differentiation of teeth. They are: (1) observation of developing teeth at various stages of growth; (2) transplantation of whole or partial tooth primordia into heterotopic positions or to nutritive media outside of the body.

Investigators in numerous fields of developmental studies have used the transplant method extensively in studying the processes of growth and differentiation. A relatively small amount of work has been done, however, in which the soft tissue elements of teeth germs have been transplanted.

1. REVIEW OF THE LITERATURE.

In 1874, Legros and Margitot removed dental follicles from young pups immediately after death and transplanted them subcutaneously into guinea pigs or older dogs. The heterotransplants were all lost through suppuration or resorption as were most of the homeotransplants. Seven out of twenty-six homeotransplants survived and underwent further development. No reference was made to the extent of growth. These investigators were, however, the first to show that pulp-odontoblast tissue is able to survive and produce new dentin when transplanted.

The investigations of Huggins, McCarroll, and Dahlberg, 1934, consisted of the autotransplantation of tooth germ elements to the connective tissue of the abdominal wall of young pups. Young pups between the ages of 3 and 6 weeks were used as donors in all operations. The authors made three significant observations.

They were:

(1) Odontoblast-pulp transplants showed a slight dentin formation after twenty-six days.

(2) The enamel epithelium, isolated from other dental tissues, showed no tendency to form new enamel when it was transplanted. In addition, there was a total disappearance of ameloblasts.

(3) Entire tooth primordia were transplanted and growth maintained for twenty-five days. No actual growth measurements were made. There was a preservation of the cylindrical character of the ameloblasts and the formation of new enamel in normal relation to these cells and to newly formed dentin. The influence of mesordermic connective tissue derivatives on the form and function of epithelium was demonstrated in this manner.

Hahn, 1941, stated that the enamel epithelium, in whole or in part, is necessary for odontoblast formation and for establishing the pattern of the future tooth. The dental papilla seemingly organizes under the influence of the enamel epithelium. This epithelium is not essential, however, for the production of dentin after the odontoblasts have been formed or for maintaining the form of the dentin once a layer of calcified tissue has been laid down. Thus, an interrelationship between supporting tissues and epithelium is shown to exist.

Willis, 1935, perfected a technique for intracerebral implantation of embryonic tissue in rats. Minced tissue from embryos of various ages was introduced into the cerebral hemisphere. No specific results were obtained due to the diversity of the tissues that were implanted. However, the brain appears to be highly favorable site for such implants, in which cartilage, bone, teeth, salivary gland tissue, and skeletal muscle grow readily and differentiate well.

Glasstone, 1936, in her investigation used the molar tooth germs of eighteen to twenty-one day rat embryos cultured in vitro. Hanging drop preparations were used throughout the investigation. Her observations showed that odontoblast formation could be watched but that degeneration started after fourteen to twenty days in vitro. A further finding was that twenty days of dentin formation in vitro was of the same magnitude as four days in vivo. She proved that embryonic dental tissue was differentiated normally when isolated from its blood supply, nervous system attachment, and surrounding bone, and cultivated in vitro.

Further work by Glasstone, 1938, demonstrated cusp differentiation in the molar teeth of the rat. These in vitro cultures corresponded to normal differentiation from the fifteenth day of fetal life to parturition. The tissue culture experiments show that the development of the cusps is a self-differentiating process.

According to Glasstone, 1952, halved molar teeth primordia from twenty to twenty-one day rabbit embryos developed their normal whole tooth pattern of cusps when cultured in vitro. At twenty-two days, when odontoblasts and dentin appear, this capacity for regulative development is largely lost.

Hahn, 1941, transplanted various portions of the soft tissues of the developing canine teeth of young female dogs into the ovaries of the same animals. The unerupted maxillary canines of female dogs, approximately six weeks old, were used in all experiments. He found that odontoblast-pulp tissues retained their form and produced normal dentin after fifteen days transplantation. Isolated enamel epithelium failed to maintain its cellular pattern and formed epithelium-lined cysts. The transplanted tissues showed no greater capacities for differentiation in the ovary than in other sites of transplantation previously used.

In 1941, Nukolls cultivated molar teeth primordia from one day old rats in vitro. The author seemed to be interested in technique alone as no specific references were made to degree of development.

Losee, 1943, grew twenty to twenty-one day embryonic upper rat first molars in vitro using the depression slide. He used a hollow-ground slide, the hollowed out portion of which served as a dish. The transplants were placed in a medium of embryonic chick extract and rabbit blood plasma. The chamber was sealed by placing a cover slip over the depression and paraffining the edges to the slide. As soon as the slide was prepared it was placed in an incubator at 38° c. The culture was transplanted to a fresh medium every forty-eight hours. The tooth germ was thus kept alive for seventy-eight days in vitro. However, no description was given of the degree of differentiation as compared to a tooth growing in vivo. An improved in vitro technique appears to be his main contribution.

More recently Shapiro and MacLean, 1945, transplanted developing canine or incisor teeth, of 1 to 5 month old kittens, in place of permanent canine or premolar teeth removed from older animals and, in a few cases, of young animals approximately the same age as the donors. They took intra-oral roentgenograms at frequent intervals after the operation. These indicated that the transplant, if intact, became established in the alveolar socket of the host and continued its development.

As far as can be determined, the anterior chamber of the eye, up to the present time, has not been used as a site for transplanting developing dental tissue. The eye, as a site for implantation, is extremely favorable, the major advantage being the ease of observation of the transplant at various intervals in the course of growth and other changes.

Albrink and Wallace, 1951, presented evidence which indicates that the anterior chamber is conducive to the growth of transplanted embryonic tissue. They found that a medium composed of a mixture of beef aqueous humor and 20% desiccated embryo extract results in extensive proliferation of chick fibroblasts which is superior to that obtained in media prepared from desiccated horse serum, embryo extract and saline solution. The latter are components of widely used tissue culture media. The authors state that the most important difference between aqueous humor and serum media lies in the low protein (and low antibody) content and high ascorbate values of the former. The importance of the low protein content is uncertain, but the correspondingly low antibody level may reduce inhibitory influences. The authors suggest that the much higher levels of protein in serum may be the explanation for the toxic effects of some sera. They observed that the concentration of ascorbate in aqueous humor is about twelve times that of plasma. The specific vitamin C levels required in fibroblast cultures are unknown, but large amounts of ascorbate are found in both embryonic and tumor tissues.

The same authors report that the efficacy of aqueous humor as a tissue culture nutrient suggests that the success of intra-ocular transplantation depends on the ability of the fluid to maintain cells for relatively long periods, or at least until vascularization occurs, permitting continued growth.

In the light of the above mentioned investigations, it was of interest to the author to determine the capacity of the aqueous humor to support embryonic tooth primordia.

II. Materials and Methods:

Tooth primordia of fetal rats were transplanted into the anterior chambers of the eyes of mature male albino rats. Albino rats were selected as recipients since it was found that their non-pigmented irides permitted excellent visibility.

The age of the tooth germs to be transplanted was determined by accurately timing the age of the embryos from which the germs were to be taken. Daily vaginal smears of the mothers were studied routinely. After at least two normal and regular estrous cycles had occurred, males were introduced into the cages. The finding of spermatozoa in the vaginal tracts indicated that fertile copulation had occurred. The day that spermatozoa were found was considered to be the first day of pregnancy. The pregnant animals were sacrificed according to the age of the transplant desired. The body cavity was opened under sterile conditions and the embryo rats removed aseptically as needed. All instruments were sterilized previous to their use in the operation. A scapel was employed to sever the mandible and body from the top portion of the head. All further manipulation was done under a binocular dissecting microscope. The severed head was then placed on a dissecting plate (previously prepared black paraffin in a watch glass) and held in place by inserting sterile pins on each side in the buccal fold. These pins also held the buccal tissue away from the field of operation. The mucous membrane was peeled back so as to expose the bony surface. An incision was made in the soft premaxillary bone and the buccal portion removed. The right incisor germ was exposed in this manner. A scapel was then slipped under the developing tooth to elevate it so that it could be removed. It was then placed in a watch glass containing sterile mammalian saline solution. The left incisor primordium in each case was placed in 10% neutral formalin and retained as a control. A twenty-gauge Quincke needle was attached to a two cc. hypodermic syringe preliminary to the operation. For each implantation the needle was loaded by applying its bevelled point to the small end of the incisor and withdrawing the plunger sufficiently to suck the primordium and some saline solution into the end of the needle. The syringe was removed and a stilette inserted in the needle. It was moved slowly toward the tip bringing the primordium into position near the bevelled point. Air bubbles and saline solution were also expressed from the needle in this manner. The recipient animals were placed under light ether narcosis just prior to the transplantation. The rat was held loosely and the lids of the right eye forced apart with the thumb and index finger. Slight pressure with the fingers caused the eye to protude. The eyeball was maintained in this position by the use of a small curved hemostat. The bevelled tip of the needle was then introduced through the cornea into the anterior chamber of the eye. The stilette was gently pushed downward causing the tooth primordium to flow from the needle. In order to prevent the extrusion and escape of the transplant through the incision, all pressure exerted on the eyeball by the hemostat was released before the needle was removed. The needle was then slowly withdrawn from the corneal incision. A blunt nosed scapel was employed to insure the retention of the transplant in the eye during removal of the needle. There was very little bleeding and but little loss of aqueous humor. However, any aqueous humor that exuded during the operation appeared to be replaced within 24 hours. The transplants were then, under gentle pressure, orientated in the anterior chamber, usually in a transverse position. In general, they were located in the lowest part of the angle between the iris and the cornea. The eye was then washed thoroughly with sterile saline. After washing, a small quantity of sulfanilamide was sprinkled on the surface of the eyeball to prevent infection. This proved to be a very successful method for making intraocular transplants.

At autopsy the eyes were removed and placed in 10% neutral formalin. In order to facilitate sectioning of the eyes, the lens was removed through an opening in the back of the eye after fixation. The eyes containing teeth were decalcified in 5% nitric acid after fixation. The tissues were dehydrated through a series of alcohols, cleared in carbon tetrachloride, and embedded in paraffin. Serial sections were prepared whenever possible and stained with hematoxylin and eosin.

1. Normal Animals with Intraocular Transplants.

To provide a standard of comparison for the experimental results, serial sections were made of decalcified heads of young rats fixed at daily intervals from birth to seven days, and then weekly up to seven weeks. The age of the tooth primordia used in all of the following experiments refers to days of fetal life.

Exp. A - Six adult male rats, 3-5 months old, were given intraocular transplants of 21 day incisor primordia. Weekly observations were made under a dissecting microscope on the progress of these transplants. Four of the animals were sacrificed eight months after the date of transplantation and the eyes containing the transplants removed for sectioning. The fifth animal was sacrificed ninety weeks after the date of transplantation. The sixth animal was still alive 21 months after receiving the transplant.

Exp. B - Four adult male rats, 3-5 months old, were given intraocular transplants of 20 day incisor primordia. Their progress was followed weekly by observation through a dissecting microscope. Three of the animals were sacrificed nine months after the date of transplantation. The eyes containing the transplants were removed and sectioned. The fourth animal was sacrificed thirteen months after the date of transplantation.

Exp. C - Three adult male rats, four months old, were given intraocular transplants of 19 day incisor transplants. They were observed weekly, sacrificed after nine months, and the tissues sectioned as above.

Exp. D - Six adult male rats, five months old, were given intraocular transplants of 18 day incisor primordia. They were observed weekly as in the previous three experiments. In none of these did the transplants grow, therefore, their eyes were not removed for sectioning.

Exp. E - Six adult male rats, four months old, were given intraocular transplants of 17 day incisor primordia. Weekly observations were made but only one transplant was successful. The eye was removed from this animal and sectioned nine months after the date of transplantation.

2. Experimental Animals with Intraocular Transplants.

Parke-Davis' desiccated sheep thyroid in powder form was used in all feeding experiments. The dosage varied from .25 - .5 gm. daily, depending upon the experiment. In order to insure complete consumption of a definite amount of thyroid material the animals were fed by means of a modified stomach tube. The equipment consisted of a twenty-gauge Quincke needle attached to a 5 cc. syringe. The needle was bent so that it could be easily inserted through the mouth and down the esophagus. The powder was weighed and mixed with enough water to make a free-flowing liquid suspension. The material was drawn up into the syringe, the needle introduced into the esophagus, and the plunger inserted slowly. In this manner, the efficiency of feeding was increased, and an accurate dosage assured.

Injections of sodium fluoride were given in order that the rate of dentin growth might be measured, (Schour and Smith, 1934). This method of vital staining consisted of giving intraperitoneal injections of .1 cc of a 2.5% solution of sodium-fluoride. A distinct stratification appeared in the dentin forming the calcifying at the time of sodium fluoride administration.

Exp. F - To determine the weekly growth rate of tooth primordia transplanted into the anterior chamber of the eye.

Each of six rats, containing intraocular transplants of several months duration, was given injections of .1 cc of 2.5% sodium fluoride once a week for three successive weeks. These injections were given over the three weeks just prior to sacrifice. In seven other animals, the rate of dentin growth was determined by measuring the width of the dentin up to the time of the first injection and dividing the figure obtained by the number of weeks intervening since the date of transplantation. The treatment of the individual animals is summarized in Table I.

Exp. G - Effect of feeding desiccated thyroid gland on the growth rate of tooth primordia transplanted into the anterior chamber of the eye.

Each of six rats, containing intraocular transplants of seven months duration, was fed .5 gm. of desiccated thyroid gland daily for four weeks prior to autopsy. Concurrently, weekly injections of .1 cc of a 2.5% solution of sodium fluoride were given so that the growth rate could be observed. The transplants were eight months old when the animals were sacrificed. The tissues were then prepared for histological study.

3. Tooth eruption of new-born rats.

Exp. H - Effect of injecting thyroxin into new-born rats on the eruption date of their incisors.

Two litters comprising twelve new-born rats were used. Each of the twelve was given injections of .1 mg. of thyroxin daily for seven days. Examinations were made frequently so as to ascertain the time of eruption. The thyroxin experiment was run in order to obtain first-hand data to compare with the action of feeding desiccated thyroid gland.

Exp. I - Effect of feeding desiccated thyroid gland to new-born rats on the eruption date of their incisors.

Three litters comprising seventeen new-born rats were used. Each of the seventeen was fed .25 gm. of desiccated thyroid gland daily for six days. The animals were examined frequently in order to determine the time of eruption of the incisor teeth.

III. Results

1. Normal animals with intraocular transplants.

The control primordia were sectioned so as to have a record of the initial appearance of the transplant for the sake of comparison. A twenty-one day normal primordium is shown in Figure 1.

Exp. A - The total growth of twenty-one-day-old transplants is in excess of any of the other groups. The most highly developed transplant, shown in Figure 2, is from a twenty-one-day primordium which grew in the anterior chamber of the eye for twenty-one months. The thickness of the dentin was the criterion used to indicate growth in all the experiments. The thickness of the intraocular transplant, at its greatest width, was compared with a similar point measured on serial sections of normal teeth prepared previously. The width of the five most advanced twenty-one-day-old transplants ranges between 44 and 266 micra. The widest area of dentin formation in a seven-week-old normal incisor was found to be 170 micra. An intraocular transplant having a dentin width of 55 micra as compared with a three-week normal incisor of the same thickness, is shown in Figures 3 and 4. A similar comparison is made in Figures 5 and 6 between an intraocular transplant having a dentin width of 266 micra and a seven-week-old normal incisor having a dentin width of 170 micra. The seven-week-old normal incisor was the oldest normal incisor available for comparison with the experimental transplants. Evidently then, in the most successful transplants, the growth approaches that of an adult animal.

Exp. B - The growth of the twenty-day-old transplants approaches that attained by twenty-one-day transplants. The dentin width of the four twenty-day transplants ranges between 39 and 99 micra. Figure 7 shows a well developed twenty-day transplant that was dissected out of the eye after thirteen months and photographed in toto.

Exp. C - All three of the nineteen-day transplants survived, but developed less extensively than the twenty-and twenty-one-day transplants. The dentin width of the most highly developed nineteen-day transplant is 50 micra.

Exp. D - Negative results were obtained in all the eighteen-day transplants. Resorption had evidently occurred soon after transplantation.

Exp. E - Only one of the seventeen-day transplants was found to survive. However, there was very little development in the single transplant that became established, as compared to twenty-and twenty-one-day transplants. The infantile state of development in this transplant is shown in Figure 8. It reached the stage that is shown in the photomicrograph about three months after the date of transplantation. No further development was observed from that date until the time of autopsy.

All the transplants, regardless of age, remained in the anterior chamber of the eye after the transplantations were made. The lens was found to regress in many of the eyes. It was almost entirely missing in three. The anterior chamber of the eye was found to occupy a larger area of the intraocular cavity after extensive growth of the transplant. Thus, the posterior chamber became smaller and the lens exhibited a regression in size. The transplants became attached in the angle between the iris and the cornea. The point of attachment was not necessarily the bottom area of the anterior chamber. In nearly all cases, however, the attachment was near the periphery of the anterior chamber. Within forty-eight hours after the transplantation, nearly all the primordia were attached in such a manner that their position did not change. A blood clot appeared on the base of each primordium the day after the transplant was made. The clot persisted from five to seven days in most cases. A meridional artery in the sclera seems to enlarge and within eight to ten days terminates in a fan-shaped series of vessels which course along the sclerocorneal junction adjacent to the attached transplant. The prominent blood clot disappears with the development of this permanent blood supply. The cornea was not invaded by blood vessels although some did course along the inner surface of the cornea in reaching the transplant. The majority of vessels were in close proximity to the iris or to the angle between the iris and cornea. They were found to be embedded in the iris and sclerocorneal junction. Even though the blood supply to the transplant was well developed, it was always confined to the general vicinity of the transplant. Some of the transplants had blood vessels coursing over most of the surface in their early phases of growth. However, the vessels became localized around the basal region of the transplant as growth continued. Two typical attachments are shown in Figures 9 and 10. No extensive enlargement of the eye was noted in any of the transplants.

A complete lack of pigment was noted in all intraocular growths. This is contrary to the condition found in normal incisors.

2. Experimental animals with intraocular transplants.

Exp. F - Table I indicates the average weekly extent of growth and other pertinent information for thirteen transplants. The results indicate that the average growth varies from a low of .77 micra to a maximum of 2.9 micra per week. In addition, teeth of rats given injections of sodium fluoride at seven day intervals indicate weekly dentin growth of approximately 2.5 micra. Figure 11 illustrates the latter.

Exp. G - Feeding of desiccated thyroid gland to animals containing intraocular transplants stimulated the growth of dentin. The normal growth of dentin in transplants was found not to exceed 3. micra per week (the entire range was from .77 to 2.9 micra per week). The average growth of six thyroid-treated transplants was found to be 5.3 micra per week. This would indicate an increased growth of about 2.5 micra or nearly a doubling of the dentin deposition per week. These results are illustrated in Table II. Figure 12 shows several stratification rings in the dentin of a tooth growing under influence of thyroid hormone and injected with sodium fluoride.

3. Tooth eruption of new-born rats.

Exp. H - It was observed that the incisor teeth of new-born rats erupted precociously when they were given daily injections of .1 mg. of

Rat #	Age of Transplant (Day of fetal life)	Total number of weeks between date of transplantation and Naf. Inj.	Total number of Naf. Injections	Total width of dentin up to Naf. Inj. (in micra)	Total width of dentin (in micra)	Average growth per week (in micra)
29	17	36	1	28		.77
27	19	34	1	44		1.3
32	19	38	1	50		1.3
44	20	21	3		39	1.6
35	20	36	1	66		1.8
16	20	29	3		77	2.4
33	20	37	1	99		2.7
23	20	36	1	99		2.8
48	21	15	3		44	2.4
39	21	19	3		55	2.5
34	21	36	3		111	2.8
49	21	90	1	266		2.8
37	21	37	3		116	2.9

Table I. - Average weekly dentin growth of various aged intraocular transplants.

Rat #	Age of Trans-plant (day of fetal life)	Total number of NaF. Inj.	Average growth per week (in micra)
22	20	3	5.5
31	20	3	4.5
36	20	3	5.8
38	21	3	5.
45	21	3	5.8
46	21	3	5.3

Table II. Average Weekly Dentin Growth of Seven-month-old Intraocular Transplants Growing for the Last Four Weeks Under the Influence of Thyroid Hormone (desiccated thyroid gland).

Accelerated Incisor Eruption Induced by Thyroxin Injection			
Litter	Number of Animals	Number of Animals Surviving 7 Injections of .1 mg.	Time of Eruption after Birth in Hours
1	6	5	72 - 78
2	6	4	80 - 84
Accelerated Incisor Eruption Induced by Feeding Desiccated Thyroid Gland			
Litter	Number of animals	Number of Animals Surviving 6 Feedings of .25 gm.	Time of Eruption after Birth in Hours
1	5	4	66 - 70
2	6	6	68 - 72
3	6	5	66 - 72

Table III. Comparison of Acceleration of Incisor Eruption Induced by Feeding Desiccated Thyroid Gland and Injecting Thyroxin.

thyroxin. The incisors were found to erupt between 72 and 84 hours after birth. Table III indicates the eruption date and other pertinent information.

Exp. I - The eruption of the incisor teeth in new-born rats was accelerated by feeding .25 gm. desiccated thyroid powder daily. Eruption was found to occur between 66 and 72 hours after birth. These results are illustrated in Table III. A comparison indicates that desiccated thyroid gland is slightly more effective than thyroxin as regards acceleration of eruption of incisors.

IV. Discussion

The superiority of the eye as a site for the study of tooth growth must be noted in comparison to other heterotopic sites. No definite measurements of the growth of transplants in other sites have been made. Glasstone, 1936, was the only previous investigator to indicate a relationship between the growth of transplants and normal teeth. She compared the growth of the most successful in vitro transplants with that of a normal four-day-old molar. The distinction that most of the previous investigators have made is that dentin, or some other structure, is either formed or is not formed. Thus, they have been mainly concerned with whether various portions of the embryonic dental tissue will survive when transplanted rather than with the extent to which they develop. Their problem involved a study of the relationships of the various parts of tooth primordia to one another. Willis, 1935, in perfecting a technique for intracerebral implantation of embryonic tissue, merely states that the brain appears to be a highly favorable site for the transplantation of bone, teeth, etc. The work of Shapiro and MacLean, 1945, is of importance as it shows that developing primordia continue their development when transplanted in place of premolar teeth in older animals. However, their work is essentially a study of the tooth in its normal position with all of its natural advantages. The success of the technique of transplantation, as described above, as well as the ease of continued observation are both factors which contribute to the superiority of the anterior chamber over other transplantation sites. The observable maximum dentin growth of 266 micra, over a period of ninety days, demonstrates a definite continuation of growth.

According to the findings of Albrink and Wallace, 1951, the two factors contributing to the success of the aqueous humor as a tissue culture medium are: (1) its low protein (low antibody) content; (2) its high ascorbate values. Their suggestion of a relationship between the large amounts of ascorbate in the aqueous humor, as a correspondingly high content in both embryonic and tumorous tissue is an important factor. This may possibly reflect higher needs of vitamin C for optimal growth by embryonic and tumorous tissues. The low protein content and high ascorbate values probably contribute to the maintenance of the transplant until vascularization occurs.

The development of a blood supply to intraocular tooth transplants parallels that observed by Fawcett and his coworkers, 1947, when ova were transplanted into the anterior chamber. However, they state that the cornea gradually becomes edematous and is invaded by blood vessels.

Furthermore, they find that due to their changes, which make the cornea somewhat opaque, a study of these ova by direct observation ceases to be profitable beyond the tenth or eleventh day. The corneal invasion by blood vessels was not encountered in any of the tooth transplants. Therefore, constant observation was possible throughout the entire period of growth.

There is a limitation of growth in the intraocular transplants as opposed to normal teeth growing in the jaw. This was shown by the fact that transplants, as old as twenty-one months, had not penetrated the cornea. Mechanical limitation does not seem to be a factor in intraocular transplants.

Runner, 1947, reports that implanted embryos grow and the eyeball frequently ruptures due to the intraocular pressure caused by expansion of the graft. In addition, he states that implants in those eyes which do not rupture deepen in color and begin to decrease in size on about the tenth day. Subsequently the aqueous humor becomes clear, the graft becomes smaller and darker in color; eventually being reduced to a scar on about the twentieth day. Duke-Elder, 1934, states that since the distensibility of the envelope of the eye is restricted the intraocular pressure may be varied by altering the volume occupied by its contents. In addition, the structure of the eye is such that a dilatation of the capillaries brings about a large increase in pressure. An intraocular transplant would intensify the pressure on this basis. The change in volume brought about by the introduction of a transplant, and the increased blood supply, would cause an increased pressure according to the above factors. This is but one explanation of the cause of increased intraocular pressure. Browning, 1949, agrees that mechanical factors probably do not limit growth. He demonstrated that transplants of ileum or stomach easily rupture the cornea, and those of limb buds readily distend it. Furthermore, he states that the rupture of the cornea is not followed by any appreciable further growth of the transplant. Fawcett and his coworkers, 1947, state that developing ova sometimes erode through the corneal incision giving a semblance of the eye having ruptured as a result of the growth of the blastocyst. Their histological examinations reveal that the fetal tissue seldom occupies more than two-thirds of the space available in the anterior chamber. They, therefore, suggest that the enlargement may be due to increased production of aqueous humor or to interference with its absorption. They advance the idea that extravasted blood in the anterior chamber may interfere to some extent with the absorption of aqueous humor via the canal of Schlemm, and that there may be an increased production of fluid as a result of abnormal permeability of the blood vessels of the eye.

At no time did the intraocular tooth transplants distend the eye to any great extent or occupy the total space available. The tooth transplants did not bring about corneal rupture. The eye became distended only when infected. Greene, 1947, made some interesting observations in the course of transplanting various organs and tumorous tissues into the mouse eye. He observed that embryonic ovaries, kidney, spleen, etc. usually reach their maximum size in from two to three weeks, and no further increase in size occurs. Some transplants show no signs of regression and appear able to persist throughout life. Tumor growth, however, is rapid in the anterior chamber and after several weeks causes the cornea to rupture and the tumor protrudes as a fungating mass. The tumor soon becomes infected and death follows. His observations on the early attainment of maximum growth are contrary to the observations reported here on intraocular tooth transplants. The tooth primordia appear to grow steadily over several months and an intense growth over a short period does not occur.

Browning, 1949, transplanted thirteen to sixteen day old embryonic parts into the anterior chamber of the rat eye and noted that growth of these parts ceases with the completion of their organization and differentiation. He states that the transplanted organs increase to a size comparable with that normally attained at birth and further growth does not occur thereafter. Tissues of new-born animals usually persist after transfer but show no increase in size. The development of tooth primordia does not fit into the above pattern of development. The results in this paper indicate that definite and continued, but limited growth is attained. Figure 2 illustrates the most advanced transplant obtained in these experiments. However, limitation of growth is a factor that is common to all intraocular transplants. Browning, 1949, suggests that the lack of expression of full growth potentialities by an intraocular transplant may be due to a lack of stimulatory factors elaborated by the embryo as a whole. This is demonstrated by the more extensive growth in tissue cultures where embryonic tissue extract is involved. The limitation of growth in intraocular transplants is a problem that merits further investigation.

The fact that 17 and 18 day old tooth transplants do not grow as well as 19, 20, and 21 day old transplants has two possible explanations. First, there may be a lack of organization at this time of fetal life. Second, it is very difficult to remove tooth germs in the earlier stages of development. The use of smaller instruments to dissect out the incisor primordia would enhance their chances of survival and development.

A brief review of the main features of development associated with the various days is informative.

14 day.	Slight thickening of the oral epithelium.
15 day.	A distinct thickening and ingrowth of oral epithelium.
16 day.	The dental ridge has formed and the beginning of a flask-shaped enamel organ is present.
17 day.	The dental papilla is capped by an enamel organ.
18 day.	A transitory stage.
19 day.	Both odontoblasts and ameloblasts are differentiated.
21 day.	No dentin or enamel has been laid down as yet.

It is evident that previous to the nineteenth day, tooth primordia are still in a stage of formation. At that time, it is easy to disturb the normal relationships of the various parts in removing primordia.

The lack of pigmentation in the intraocular transplants is a significant observation. According to Addison and Appleton, 1926, the enamel is divided into two layers; a thin outer and a thicker inner layer. The outer layer in its superficial part contains the yellow or orange pigment which gives color to the enamel. They state that the pigment covers the anterior two-thirds of the enamel of the upper incisor. Furthermore, they report that the pigmented layer makes up about a fourth of the width of the enamel layer in the tooth of a five month old animal. Farris and Griffith, 1949, state that the pigmentation is first seen in the rat incisor at three weeks of age. The yellow-orange color deepens with age. Furthermore, they state that the significance of normal pigmentation is still obscure. However, changes in pigmentation are often the earliest demonstrable responses to various experimental procedures. Hodge, Luce-Clausen, and Brown, 1939, observed the effect of radiation on the pigmentation of normal incisors. They noted that all measurements of degree of pigmentation in incisor teeth of rats confined to dark quarters indicated less pigment than in normal rats. No explanation of this phenomenon is given. M. C. Smith, 1933, observed an absence or diminution of pigmentation in the incisors of anemic rats. She suggests that the iron content of the pigment merits investigation. A limited blood supply is characteristic of all the intraocular transplants. This limitation of blood supply may be an important factor in the lack of pigmentation.

Sodium fluoride proved effective in measuring dentin growth in intraocular transplants. It was found that an increase in amount of dentin could be measured at a minimum of seven day intervals. The extremely limited growth of the transplants prohibited measurements at shorter intervals. Schour and Smith, 1934, determined the rate of dentin deposition in the normal rat incisor. They gave adult rats several injections of 0.3 cc. of a 2.5 solution of sodium fluoride forty-eight hours apart and then killed the animals forty-eight hours after the last injection. They observed that the dentin showed a stratification, while the odontoblasts and pulp appeared to remain normal. They observed a pair of layers in the dentin for each injection of sodium fluoride. They state that each pair consists of:

A. A light layer which represents a primary and immediate reaction to the injection. This layer is imperfect in formation and calcification.

B. A dark layer which immediately follows the light one and represents a recovery reaction. This layer is normal in formation and normal or excessive in calcification.

The width of each pair is approximately 32 u. Thus the daily depositional rate is indicated as being 16 u. per day.

The fact that enamel grows at the same rate as dentin was ascertained by the same investigators. In addition, they noted that four weeks after the first injection, the incisor began to lose its orange pigment and luster. Rings, lacking pigment, on the outer enamel surface corresponded to a given number of injections. They state that sodium fluoride exerts a direct local action on the enamel and dentin calcifying at the time of injection. They suggest that when the sodium fluoride, which is highly soluble, enters the blood stream, the fluorine molecule unites with calcium and forms calcium fluoride. The latter may be taken up by the cells concerned with the formation and calcification of enamel and dentin and injure them by acting in a way similar to that of a foreign body.

It was observed that the incisor teeth of new-born rats erupted precociously when daily injections of .1 mg. of thyroxin were administered. The incisors were found to erupt between 72 and 84 hours after birth.

This is in agreement with the findings of Karnofsky and Cronkite, 1939. They observed that the injection of thyroxin caused the eruption of the incisors between 70 and 80 hours after birth. According to Farris and Griffith, 1949, the eruption of the incisor teeth normally occurs between eight and ten days after the birth of the animal. The eruption in control animals was found to be in agreement with their findings. Desiccated thyroid gland proved to be slightly more effective in producing premature eruption of the incisor teeth of new-born rats than did thyroxin. The technique of administration, however, may be an important factor. It is possible that some of the injected thyroxin is lost through the very thin skin of the new-born rat. It is also suggested that desiccated thyroid, as a whole, may differ from thyroxin in stimulating growth in addition to increasing the metabolic rate. Desiccated thyroid also accelerates dentin growth in intraocular tooth transplants.

Previously Hoskins, 1927, had noted a two day acceleration in the eruption of the incisor teeth. She injected .1 mg. of thyroxin every second or third day, beginning usually when the rats were two to three days old and continuing to the fifteenth day. She used acetyl thyroxin, which according to Kendall, 1929, is not a metabolic stimulant. She believes that thyroxin contains elements which affect the rate of differentiation apart from the rate of energy production. The acceleration of tooth eruption was attributed to an effect other than stimulation of metabolic rate.

A stimulation of growth potential in teeth was also indicated by Rowntree, 1934. He found that rats injected with thymus extract (Hanson) showed general precocious development and accelerated tooth eruption following daily injections of successive generations of animals. The incisors, which normally erupt on the eighth to tenth day were found to be erupted at birth in the eighth generation of thymus-treated animals. This observation has not been confirmed by others.

The original purpose of making intraocular transplants was to enable the author to observe directly the processes involved in growth of teeth. In the course of this investigation the various observations reported here were brought to light.

A few problems suggested by this work should be mentioned:

1. The factors that limit the expression of full growth potentialities of intraocular transplants warrant further investigation.

2. A further study of the factors effecting pigmentation in intraocular transplants may contribute to a better understanding of the process of pigment deposition in normal rat incisors.

3. A comparison of the rate of acceleration in tooth eruption brought about by administration of desiccated thyroid should be made with other metabolism stimulating drugs.

V. Summary.

1. Tooth germs of embryo rats grow, but only to a limited extent, when transplanted into the anterior chamber of the eyes of adult male recipients.

2. The anterior chamber is superior to other transplantation sites even though growth of tooth germs is limited.

3. Intraocular transplants of tooth germs of 17-18-day-old em-

bryos appear not to grow as well as those of 19, 20, and 21 day embryos.

4. Pigment normally present on the anterior face of incisor teeth fails to appear in intraocular transplants.

5. Feeding of desiccated thyroid gland is effective in accelerating growth of teeth of normal young as well as that of embryonic teeth transplanted to the eye.

LIST OF ILLUSTRATIONS

D, Dentin; O, Odontoblasts; P, Pulp; A, Ameloblasts; N, Dentin Stratification after NaF Injections.

FIG. 1

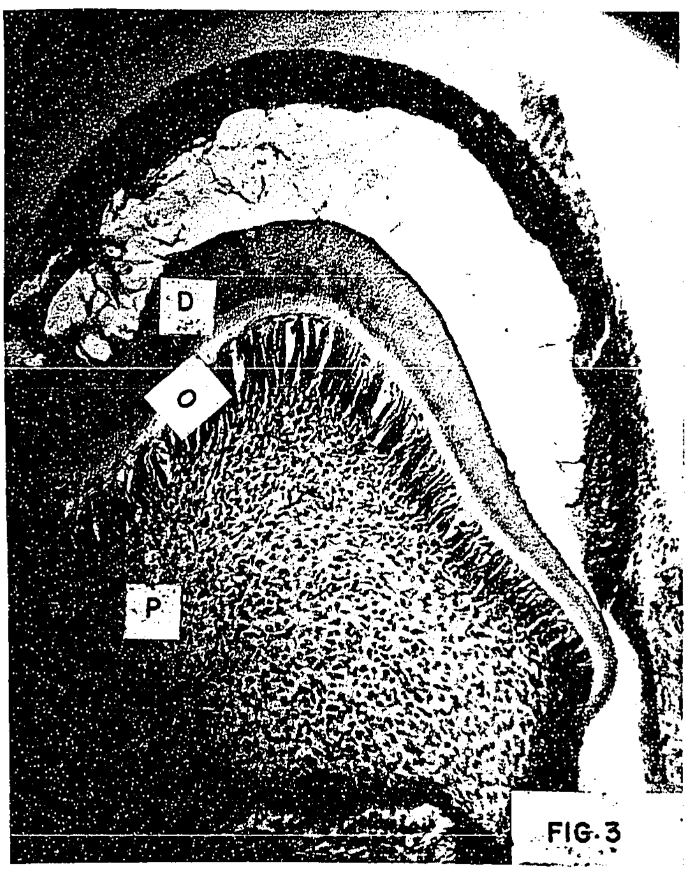

FIG. 3

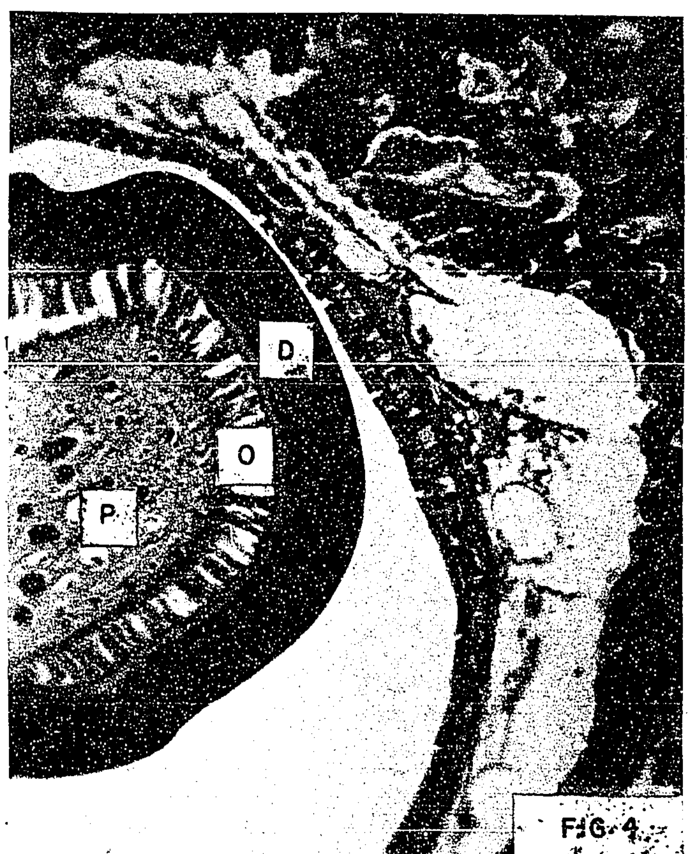

FIG. 4

FIG. 2

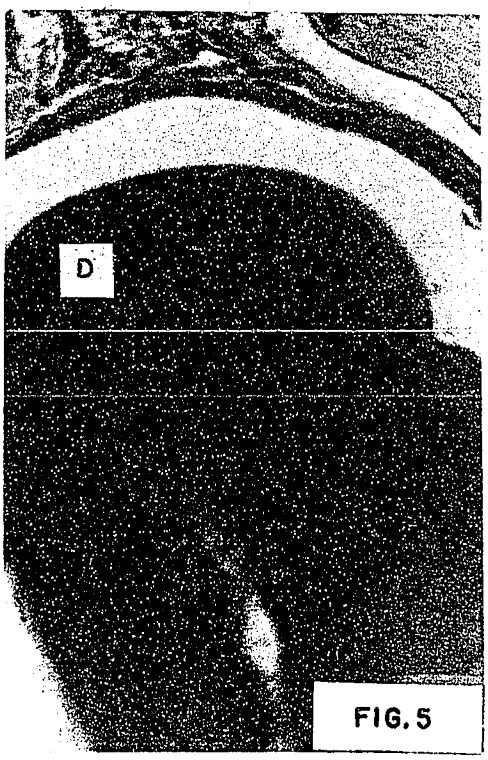

FIG. 5

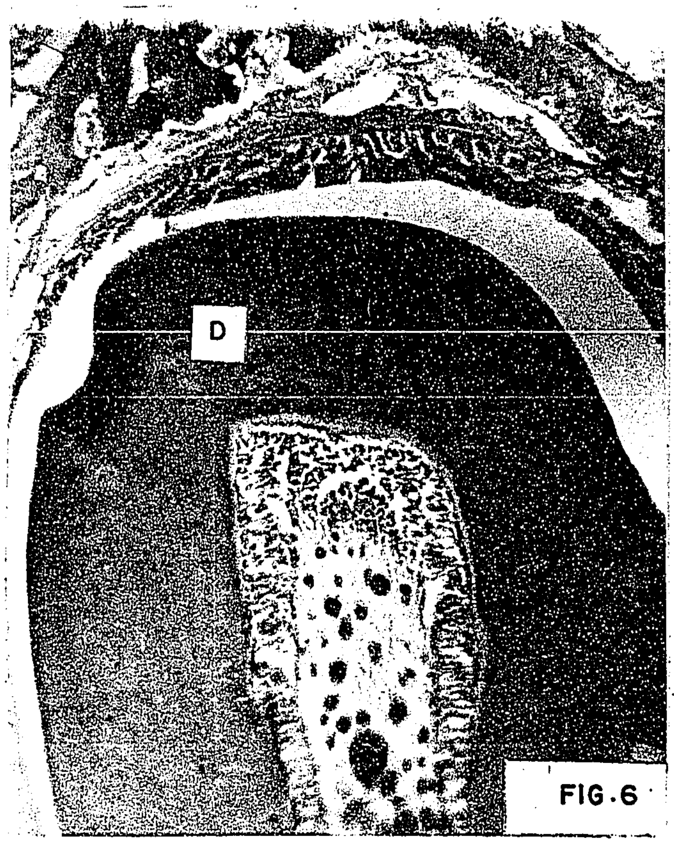

FIG. 6

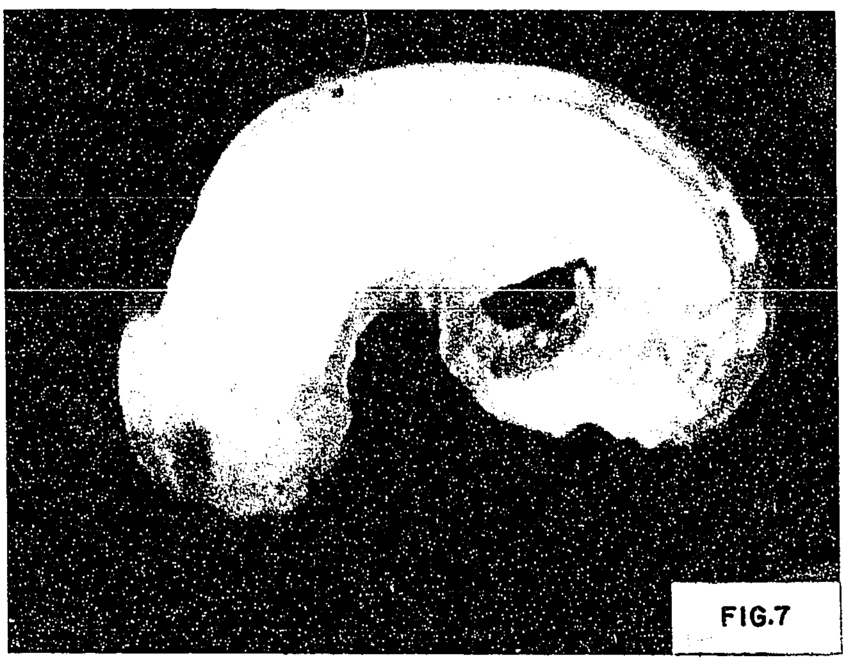

FIG. 7

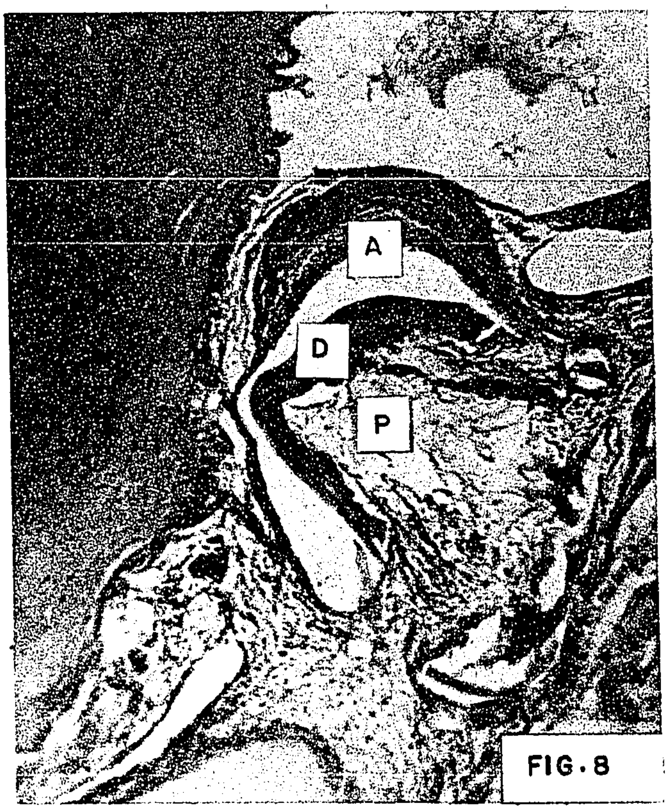

FIG.8

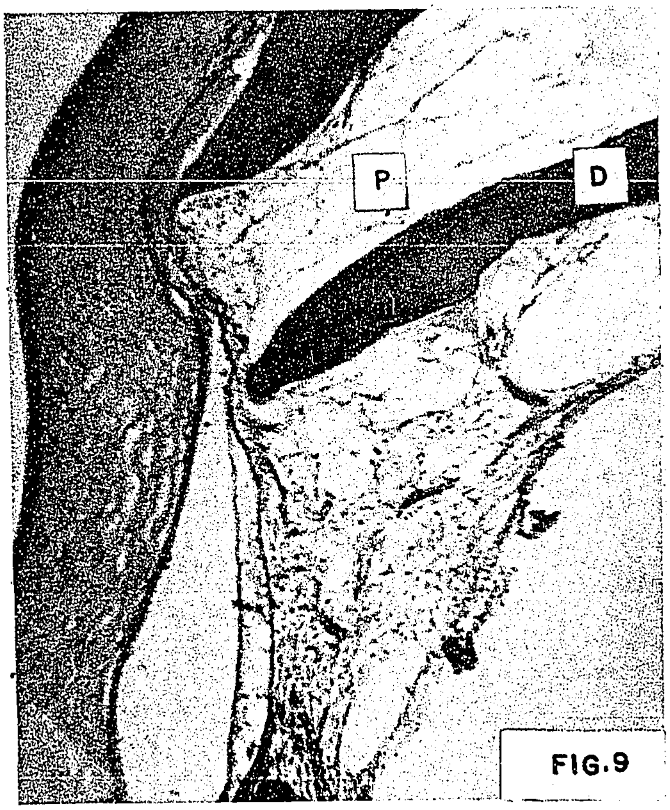

FIG.9

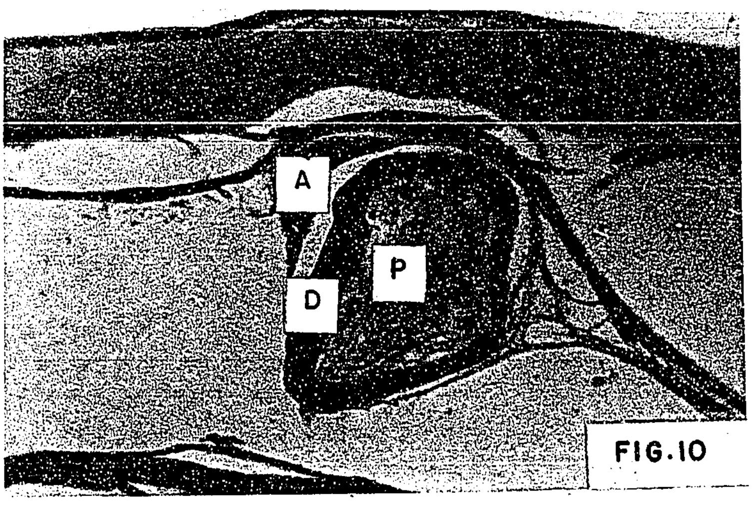

FIG.10

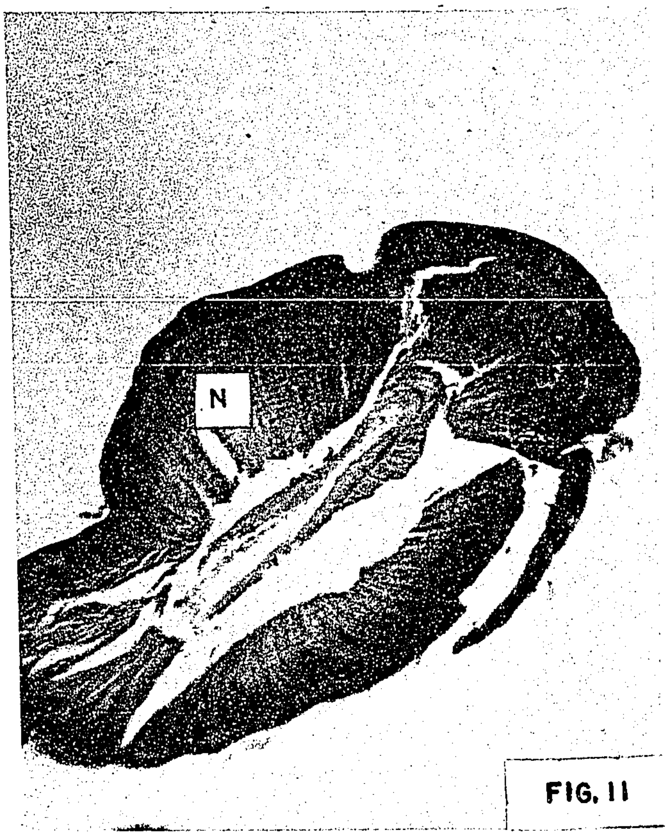

FIG. 11

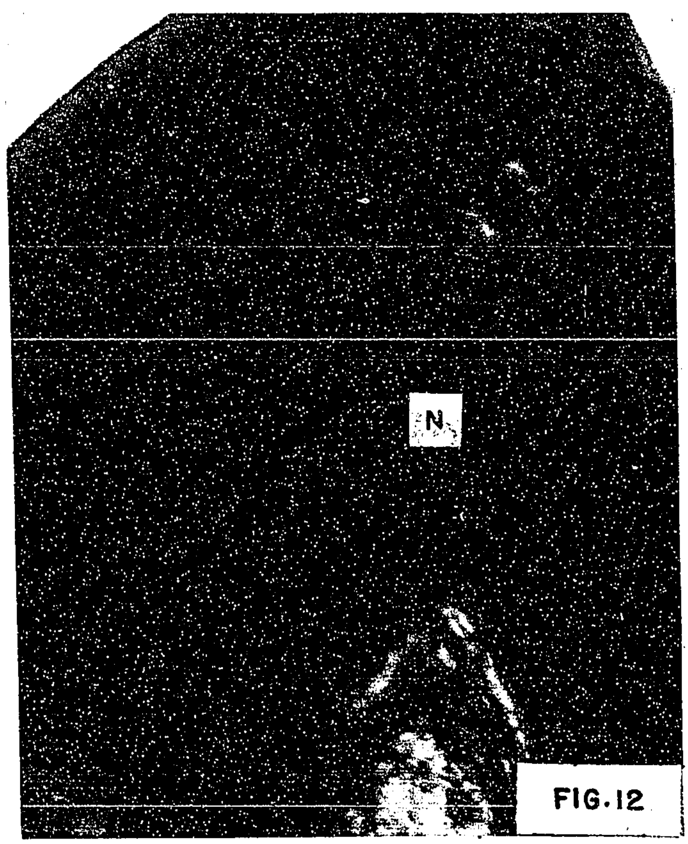

FIG. 12

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