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A STUDY OF THE DEVELOPMENT OF MOUSE EMBRYONIC TISSUE TRANSPLANTED INTO THE CHORIO-ALLANTOIC MEMBRANE OF THE CHICKEN EMBRYO, WITH SPECIAL REFERENCE TO THE DEVELOPMENT OF THE EMBRYONIC HEART

By

Ivonne Lastra

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INTRODUCTION

The normal embryonic development of the mouse has been studied and described by many investigators. G. H. Snell in the book called Biology of the Laboratory Mouse, prepared by the staff of the Roscoe B. Jackson Memorial Laboratory (1941), summed up the pertinent information.

The investigations of the early development of the mouse, which closely parallels that of the rat, were made on preserved embryos, on dissections of fresh material, and on microscopic sections of embryos at various stages of formation.

Many contributions to this problem deal chiefly with the question of accurately determining the time at which the different organs appear.

The present work is directed towards the study of the growth of embryonic tissue of the mouse in a foreign environment, that of the chorio-allantoic membrane of the chicken embryo. Hiraiwa (1927) transplanting thirds of rat embryos to the chorio-allantois, and Nicholas and Rudnick (1933) working with whole rat embryos or portions of embryos, have shown that mammalian embryonic tissue can grow in an avian environment and secure nourishment from such a quite distantly related species. They observed that rat tissue , growing in the chorio-allantois, still maintain, in general, its mammalian characteristics. Our series of experiments, in which either whole mouse embryos or embryonic hearts were grafted to the chorio-allantoic membrane, corroborate the results of the previously mentioned investigators who worked with rat tissues.

In order to present an intensive discussion of some phase of embryonic development, these experiments have been mainly centered on the development of the heart. However, in those cases where whole

embryos were transplanted observations were made on the differentiation of various other organs.

The development of the heart in the rat has been thoroughly studied by Burlingame and Long (1939). These authors reported the appearance of the cardiac rudiment in the embryo at 9½ days of gestation. Since the period of gestation of the mouse is shorter than that of the rat, the rate of development of the mouse is more rapid. The 7-day old mouse embryo presents the same level of development as does the 9½-day rat embryo. As our main interest was in cardiac development, no attempt was made to transplant embryos of earlier stages.

LITERATURE

For reasons of clarity, the review of the literature has been divided into sections covering a survey of the developmental embryology of the mammalian heart, and a survey of the literature dealing with the technique of tissue transplantation to the chorio-allantoic membrane of the chicken embryo.

The mammalian heart

Fundamental investigations on the development of the mammalian heart were carried out by Hensen (1876), His (1885), Hertwig (1910), and other early workers in the field of developmental embryology. Hensen (1876) noted the formation of a bilateral cardiac anlage within the right and left mesodermic layers at the cephalic portion of the mammalian embryonic disc. Since this observation, divergent theories have been advanced concerning the embryological development of the mammalian heart.

The origin of the intra-embryonic blood vessels and the endothelium of the heart has presented a controversial problem. According to His (1885), the early intra-embryonic blood vessels are formed by a proliferation of cells from the pre-existing endothelium of blood vessels in the extra-embryonic area. His claimed that the cell cords arising by this proliferation, travel through the space between the splanchnic mesoderm and endoderm until they reach the intra-embryonic area. When they reach this area, the cell cords anastamose and canalize, finally fusing in a longitudinal direction. The theories advanced by Lewis (1903) and Bremer (1912) are similar to His's explanation for the formation of the intra-embryonic blood vessels in the mammalian embryo.

On the other hand, Rabl (1887) and Ruckert and Mollier (1906) were of the opinion that the intra-embryonic blood vessels originated *in loco*. Experimental evidence appears to support this view. It was found that endothelium formed on both sides of the embryo even though one side was mechanically isolated from the extra-embryonic region.

Among the workers that agree to the formation *in loco* of the intra-embryonic blood vessels, there is a difference of opinion as to which germ layer gives rise to the angioblasts. Martin (1902), Parker (1915) and others, considered the possibility that the endothelium arises from the endoderm. Wang (1917), working with the ferret embryo, was of the opinion that the endoderm gives rise to the blood cells but that the endothelium is mesodermal in origin. However, Yoshinaga (1921) and David (1927) presented evidence which seems to indicate that all of the angioblasts are derived from the mesoderm.

Another problem subjected to a great deal of controversy has centered around the formation of the two lateral myocardial and endocardial tubes, their secondary fusion into a single heart, and the formation and lateral union of the two pericardial cavities.

Bonnet (1889) in the sheep, Strahl and Carius (1889) in the guinea pig, Yoshinaga (1921) also with the guinea pig, David (1927) in the human embryo and Burlingame and Long (1939) in the rat, found that the coelom in the U-shaped region of the mesoderm at the cephalic portion of the embryonic shield, appears as small vesicles, or primary coelomic spaces that later coalesce to form single right and left cavities. These cavities which will later join to each other through the base of the inverted U-shaped area, diminish in size caudadward and end blindly in the region of the first or second somite.

Burlingame and Long (1939) claimed that the pericardial cavities communicate with the exo-coelom on each side through one pore which later becomes a wide passage. Davis (1927) and Yoshinaga (1921) observed that at first each vesicle is lined by a single layer of cuboidal cells, but soon after, during the fusion of the vesicles to form a single coelomic cavity, the splanchnic layer on each side thickens and exhibits irregular areas. This thickened splanchnic mesoderm constitutes what is known as the cardiogenic plate. These sheets of angioblasts, which constitute the primordial endocardial elements, later become canalized to form the right and left endocardial tubes. Wang (1917) claimed that the first angioblasts delaminate from the splanchnic mesoderm of the cardiac region, even before the anlage of the pericardial cavity has appeared.

Each endocardial tube is covered on its dorsal and lateral surfaces by the epimyocardium, which has been formed by the infolding and arching of the cardiogenic plate. According to Yoshinaga (1921), these epimyocardial tubes are first defined at the region of the hind brain and then progress craniad until their foremost extremities communicate with each other at the base of the horseshoe-shaped pericardial coelom. On the dorsal surface of this fused portion of the epimyocardial tubes, the splanchnic mesoderm is turned back onto the dorsal surface of the pericardium forming the dorsal mesocardium on both sides. Between the two mesocardial walls there is an irregular space called the intermesocardial space through which the endothelial sprouts come out to give rise to the truncus arterious and part of the ventral aorta as well as the aortic arches.

Some workers claim that the heart of the mammal, like that of

other vertebrates, is provided with a ventral mesocardium during early development. However, Robinson (1903), Arey (1937), Lang (1917) and Parker (1915) agree on the absence of the ventral mesocardium in mammals due to the early formation of the pericardial cavity and the lack of fusion of the pleural pericardial wall in the region of the heart.

According to Arey (1937), Yoshinaga (1921) and Davis (1927), constrictions demarcating the future regions of the heart appear in the epimyocardial wall prior to the fusion of the bilateral hearts into a single one. According to Yoshinaga (1921), the first constrictions to appear are the atrial-ventricular sulci, which are asymmetrical, the sulcus on the right side being slightly caudad and the deeper of the two.

After the myocardial tubes have fused on the ventricular area, the sino-atrial constrictions appear. These constrictions are also asymmetrical, the one on the left being situated slightly cephalad to the shallower right one. The bulbus cordis differentiates from the dorsal wall of the future right ventricle and is delimited from that region by the bulbo-ventricular constriction which is deep on the left cranial side, but shallow on the right caudal side.

Burlingame and Long (1939) in their excellent work on the development of the heart in the rat, noted several ways in which myocardial and endocardial development in this animal differs from that in other mammals. They found that the molding of the heart starts only after the two bilateral cardiogenic anlage have united in the midline. During this period of embryogenesis, which extends from two to five somites, there is great activity of an ameboid character rather than mitosis of the cardiac cells.

The endocardium, developing also as a bilateral anlage, forms a thin, solid plate which is widest in the middle region. Vesicles appear in this endocardial sheet which will fuse together and give rise to the endothelial tubes of the heart, part of the ventral aorta, the ventral portion of the aortic arches, and part of the vitelline veins. When circulation begins during the eight somite stage, the atrial, ventricular, and bulbar regions are defined. However, there is little similarity between the shape of the myocardial and endocardial tubes. At this point the endocardium of the atrium is primarily composed of the endothelium lining of the horns of the sinus venosus. The atrium is connected to the ventricle through the atrio-ventricular canal, which is situated to the left of the median line. As the atrium expands in a lateral direction, the left atrial chamber becomes the terminus of the atrio-ventricular canal.

Burlingame and Long (1939) further noted that the myocardium of the ventricle becomes thick and trabeculate, and the endocardium forms folds over the myocardial surface. As yet, there is no actual longitudinal division of the heart although there is a slight constriction on the surface of the ventricle dividing it into two portions which seem to correspond to the future left and right ventricles. The bulbus is continuous with the right portion of the ventricle and is not trabeculate. The flat and spiral endothelium of the bulbus continues into the truncus arteriosus where it comes in contact with the myocardium.

At the 28 somite stage, the actual division of the heart into a four chambered organ begins with the formation of the first inter-auricular septum. Septum I, as the first inter-auricular septum is designated, grows from the atrial wall towards the atrio-ventricular canal. As it

grows posteriorly, it becomes thin and eventually ruptures at its point of origin near the atrial wall.

At first, the horns of the sinus venosus open symmetrically and almost independently from each other into the atrium. Later, through the elongation of the left horn and its union with the right one, the sinus venosus open through a single orifice into what has become the right atrium. The margins of the orifice of the sinus venosus become elongated to form the cusps of the sinus valve; the right one continuing with the atrial wall and forming the septum spurium; the left one fusing with the base of the septum I or first inter-auricular septum.

Later, the left anterior vena cava, representing the left horn of the sinus, becomes constricted from the common opening by the elevation of the dorsal ridge of the post-cava into the atrium. The dorsal ridge of the post-cava extends towards the atrial wall and the first inter-auricular septum. The left vena cava is connected directly to the right atrium by an orifice covered by the lower projection of the right cusp.

Burlingame and Long (1939) found in their dissections and preparations that what was considered by other workers to be septum II of the atrium is merely a fold of the atrial wall caused by the great expansion of the atrium in all directions.

Septum I is gradually fused to the atrial wall and, therefore, the foramen ovale, or first inter-auricular foramen, is slowly obliterated. The divisions of the atrio-ventricular canal, the ventricle, and the bulbus occur simultaneously with the atrial division. These divisions are related to the four endocardial cushions found in the atrio-ventricular canal. The two large middle cushions approach each other and fuse, dividing the canal into right and left parts. At the same time that septum I

meets and fuses with the middle cushions, the inter-ventricular septum, originating from the ventricular wall, approaches and fuses with the posterior cushion to the right of the median line.

The longitudinal division of the bulbus cordis begins in the truncus arteriosus. According to Burlingame and Long (1939) the division of the truncus has not been completely clarified. However, it has been observed that two conspicuous, spiral cushions unite to form the aorto-pulmonary septum. One of the cushions of the truncus becomes continuous with the inter-ventricular septum, the other connects to the right endocardial cushion, or the ventricular wall near it. At the junction of the bulbus with the ventricle the bulbar cushions enlarge and become conacave, forming thin-walled pockets, the semilunar walls of the ventricle. An inter-ventricular foramen remains and is finally closed by the advance of the inter-ventricular septum across the anterior endocardial cushion, as well as by the final division of the bulbus. As a result of these changes, direct connections are established between the right ventricle and the pulmonary artery, and between the left ventricle and the aorta.

According to Arey (1937), the atrio-ventricular valves originate when the endocardial cushions fuse to divide the atrio-ventricular canal into two canals. The margins of the canals are thickened, the folds of endocardium appear and later become invaded by muscle. These flaps of tissue are attached to the muscular trabeculae of the ventricle, three on the right side, and two on the left side.

Bremer (1928) states that there are two mechanical forces governing the torsion and division of the heart. These forces are the continual growth of the heart within a confined space, and the centrifugal

force of the blood as it flows around sharp curves.

Goss (1937, 1938, and 1940) working with hanging drop cultures of rat embryos found that the heart starts contracting when the embryo has from two to four somites. The cells which first start contraction are observed to be located in the lateral walls of the ventricular portion of the left heart near the atrio-ventricular junction. No contraction was observed in the right heart until two hours later. An independence of rhythm of the two sides was obvious. By the time that all of the myocardial cells were contracting, a wave or peristaltic action could be noticed. The atrial portions in the meantime were inactive. Extensive morphological development occurred with increased activity. There was an increase in size of the pericardial cavity and of both myocardial and endocardial tubes. After the two lateral ventricles become united into a single sacular ventricle, the independent activity of the right side is suppressed. The left side then becomes the pacemaker of the entire ventricle. The wave of contraction in the united ventricle travels from the atrio-ventricular junction of the left side to the right side. The circulation has not yet started.

It was also observed that the contractility of the cells of the heart primordia starts before the appearance of specialised cytological structures such as fibrillae and cross striations. The same absence of cytological differentiation at the beginning of pulsation was found by Copenhaver (1937) in his work with Amblystoma. He found that the earliest contractions in the heart arise in the ventricular region, but later the atrium initiates the beat and dominates the rhythm. He also found that when the heart is transplanted between *A. punctatum* and *A. trigrinum*, either to normal or heterotropic positions, the pulsation

rate of the donor is always retained. The striations in the transplant also differentiate at the donor rate.

Chorio-allantoic grafts

The method of chorio-allantoic grafts, since its introduction in the experimental field of biology, has been widely adopted and used by many investigators in attacking a very broad area of problems in development. It is an excellent isolation method because it utilizes a very richly vascularized area, the chorio-allantois, which is closely apposed to the shell membrane of the avian egg and, therefore, is easily accessible to the investigator.

The technique was originated by Murphy and Rous (1912) in their work with neoplastic tissue. Minoura (1921) used the technique in his work with the reproductive organs. Hoadley (1926) used the chorio-allantois in studying developmental potencies from early blastoderms of the chicken. Willier (1924 and 1930) studied the endocrine glands of the chicken embryo, especially the thyroids and the adrenals, by transplanting their primordia or their presumptive area of development to the embryonic membrane of the chick. Willier and Rawls (1929 and 1931) dealing with the developmental relations of the heart and liver, found that in chorio-allantoic grafts the appearance of the liver is always dependent in its origin on the heart rudiment. He concluded that the endoderm originally in contact with the heart rudiment has the capacity to differentiate into liver. Rudnick (1933) studied the differentiation of the respiratory tract and lungs in chorio-allantoic grafts of parts of chick embryos, and found that the respiratory material in presomite blastoderms is polarized in an anteroposterior direction.

Hiraiwa and Willier (1927) and Hiraiwa (1927 and 1930) introduced the technique for the study of rat embryos. Hiraiwa divided rat embryos into anterior, middle, and posterior thirds and grafted these isolated portions of embryos to the chorio-allantois of eight or nine day old chicken embryos. He found that some mesodermal derivatives, i.e., bone and cartilage, were the predominant tissues in grafts of all three levels. The nephric elements appeared to differentiate well but were very early attacked by the host. Ectodermal derivatives such as skin and nasal sacs had a great potency for independent differentiation, but the rest of the ectodermal elements had little or no capacity for differentiating independently. The endodermal elements had practically no capacity for isolated development, gut being the only endodermal structure that appeared in the grafts, and only very rarely so.

Nicholas and Rudnick (1933) published a very comprehensive work on the developmental reactions of whole or portions of young rat embryos when transplanted to the chorio-allantois of the chick. Their results were quite interesting, although in part contradictory to Hiraiwa's work of 1927 and 1930. On the whole, they found a very marked overproliferation of nervous tissue which they attributed to the tissue's release from mechanical restraint. They found no comparable overproliferation of mesodermal derivatives except in the case of the erythrocytes, whose production is enhanced. On the other hand, the mesoderm of somites and lateral plate shows a tendency to dedifferentiate. In regards to the heart, Nicholas and Rudnick found that in transplants of 8½-day embryos, the heart developed as a single chamber with no distinct masculature. They observed that in transplants of 9-day embryos the masculature might start independent development. If

the transplanted embryo was a 9½-day one, the endocardium might show extensive proliferation. In older embryo transplants, the hearts might show definite regional development, and septa and valves would appear. They, like Hiraiwa, observed that the endodermal derivatives had the least capacity for differentiation in the chorio-allantoic medium.

MATERIALS AND METHODS

The estrus cycle of mice was determined by means of vaginal smears using the technique described by Snell (1941), following the classification of stages given by Allen (1921). The smears were obtained by inserting the point of a fine medicine dropper containing a few drops of water, in the vagina of the animal. The water was pressed out of the pipette, immediately sucked in again, and transferred to a slide. A drop of methylene blue was added and the solution examined under the microscope. A set of permanent smears, stained with hematoxylin and eosin, was made for future reference.

The technique of chorio-allantois grafting, as devised by Willier (1924), was employed in these experiments. The host eggs were incubated from 9 to 11 days before the day of the operation. At the end of that period they were candled, and the site of junction of two large chorio-allantoic vessels marked on the shell. All the instruments and glassware had been previously sterilized, and sterile or semi-sterile conditions were maintained throughout the procedures. The area around the shell marking was swabbed with 70% alcohol and iodine, and a window about one-half sq. cm. was sawed in the shell, and removed, taking care not to break through the shell membrane. The square piece of shell was replaced on the opening and the egg placed in the incubator until the mouse tissue was prepared for transplantation.

The pregnant mouse was given an intra-peritoneal injection of about .04 to .06 cc. of nembutal. The operation was carried out according to Nicholas (1925). The hair on the dorso-lumbar surface of the animal was removed and the skin painted with iodine. An incision in the

skin was made slightly lateral to the long epaxial muscles. The lips of the incision were retracted, thus exposing the underlying muscle layers. A second incision was made, cutting through the muscles and the peritoneum. This latter cut was made slightly lateral to the first one to prevent herniation. By retracting the wound, the ovary and the horn of the uterus were brought into view. The ovarian and uterine vessels, and the proximal and distal ends of the horn of the uterus were clamped off with mosquito haemostats. Next, the uterus was opened, and the embryos excised within their deciduas. The embryos were placed in a syracuse watch glass containing sterile, physiological salt solution, and kept in an incubator at a temperature of 37°C. until needed.

In those cases where the embryos in the other uterine horn of the operated mouse were later to be used for comparison or for further grafting material, the uterus was placed back in position inside the body cavity, and the muscle and skin wounds sutured. The animal was then removed to a clean cage for recuperation. Those animals that were not going to be used any further were killed and discarded.

Figures 1 to 12 show the different age groups of the donors in these experiments. An embryo of each age group was placed in fresh, warm, sterile solution, sketched with the aid of the camera lucida and then, fixed immediately.

The other embryos were dissected for transplantation under a binocular miscroscope. The dissection was carried out in a syracuse dish of fresh, warm saline solution. The heart was located and isolated as completely as possible from the surrounding tissues. Complete isolation was not always accomplished, especially when dealing with young embryos.

Mesoderm and respiratory tissue often remained around the heart particle. One of the prepared host eggs was taken from the incubator, and the piece of shell covering the window was removed. Warm physiological saline solution was pipetted over the shell membrane before it was disturbed so as to allow the chorio-allantois, which closely apposes the membrane, to separate from it. A cut was made through the shell membrane. The particle to be grafted was removed from the watch glass with a micro-pipette if very small, or with a sterile needle if of a larger size, and transferred to the junction point of the two large vessels of the chorio-allantois. A sterile cover slip was placed on top of the window and sealed to the shell by means of paraffin. The host was then placed back in the incubator and the graft was allowed to grow from five to seven days before it was removed for histological examination. The transplants were not grown for a longer period of time in order to avoid the danger of losing them due to the breakdown of the chorio-allantoic membrane in the 18th or 19th day of the chicken embryonic life.

Transplants were made in a complete, continuous series from seven to eighteen-day embryos. The seven and eight-day embryos, being very small, were freed from their membranes, except the amnion and the proximal portion of the yolk sac, and transplated without further dissection. The older embryos were dissected, their hearts being isolated from other tissues as completely as possible, and grafted to the chorio-allantois. Thus, twelve groups of transplants were made: Group I to Group XII. Group I consisted of 7-day whole embryo transplants. These embryos were the youngest ones used, as the heart rudiments first appear at this age. Group XII contained the heart transplants

from the 18-day embryos, the oldest embryos used in these experiments.

The transplants with the surrounding chorio-allantois were fixed in either Carnoy's fluid (Huber, 1915) or modified Zenker's fluid (Fekete, Bartholomew, and Snell, 1940) after being recovered from the host.

As previously mentioned, some of the donors had been kept alive in order to use the embryos in the undisturbed uterine horn for comparison of the developmental capacities of the normal heart to those of the transplanted hearts. If that was the case, these animals were operated upon immediately after the transplants were recovered, and the embryos in the remaining uterine horn excised and fixed. This was possible only if the embryos removed during the first operation were less than eleven or twelve days old. It was found that in those instances where pregnancy was more advanced than twelve days, excision of the embryos in one of the uterine horns resulted in the degeneration and reabsorption of the embryos in the untouched horn very soon after the operation. For comparison of transplants from embryos older than twelve days, control embryos of similar ages were obtained from other animals in the same stages of pregnancy. The possible differences in development were relatively unimportant, since there are always slight variations in developmental rates, even between litter mates (Allen and McDowall, 1940). The tissues were dehydrated and embedded in paraffin by the usual procedures (Guyer, 1936), sectioned at 5 mu., and stained with alum haematoxylin or Mallory's triple stain. Congo Red (Huber, 1915; Fekete, Bartholomew and Snell, 1940) was used as a counterstain with the alum haematoxylin.

No systematic cytological survey was conducted in these experiments. However, sections from most of the transplant series were stained with iron haematoxylin for detecting the possible presence of fibrillae and striations.

OBSERVATIONS

A. Points of General Interest

Membrane. The chorio-allantois of the chicken embryo is a membrane of a double composition being originated by the fusion of the serosa or chorion with the outermost wall of the allantoic sac. It is, therefore, composed of an outer ectodermic layer, one-cell thick, a middle region of mesoderm forming rather loose mesenchyme, and an innermost one-cell endodermic layer.

Incorporation. The successful grafts incorporated into the chorio-allantois within 48 hours after they were transplanted to the membrane. In some cases, the incorporation was complete, the donor tissues being completely surrounded by host mesenchyme. In other instances, the transplants remained upon the chorio-allantois and were connected to the host tissue only at one or two points. There is a gradational series between these two extremes. Table I depicts the number of grafts made and the percentage of incorporation in each transplant group.

Reactions to Grafts

1. Stratification and cornification: After transplantation, there could always be noticed a stratification and cornification of the ectoderm adjacent to the graft. The same effects, but to a lesser degree, were often observed on the endoderm, which also was inclined to become folded. Throughout the sections, spherical structures of stratified epithelium with a single or double core were frequently seen. Huxley and Murray (1924) also reported the presence of these structures that they denominated as epithelial pearls.

2. Clots: Blood clots were often seen surrounding the grafted material. Sometimes too large a clot interfered with the successful incorporation of the transplants into the membrane.

3. Infiltration: Many times an accumulation of lymphocytes, erythrocytes and small, round cells was seen in or around the transplants. The infiltration of blood cells in grafts has also been observed by Minoura (1921), Loeb (1921), Willier (1924), and others. Nicholas (1933) reported the presence, in his transplants, of small, round, dark staining cells, which he interpreted as being products of degeneration.

B. Grafts

Group I. This group consisted of 42 transplants of 7-day complete embryos. As previously stated, the embryonic membranes were removed, except the amnion and the proximal portion of the yolk sac endoderm. Since the embryos in this group were quite small, no attempt was made to dissect out the heart rudiments. Sixteen of these grafts incorporated in the chorio-allantois were recovered.

In most cases, the transplants showed a core of necrotic tissue surrounded by dense mesenchyme and were highly infiltrated by lynphocytes and small, round cells. The cellular infiltration into the grafts made the identification of tissues or structures almost impossible. Often patches of stratified epithelium, sometimes vacuolated, and masses of smooth muscle fibers were observed to be present.

One graft, left on the host for seven days, exhibited considerable necrosis and degeneration. It contained a conglomeration of tubules lined with dark staining cuboidal cells, which closely resembled nephric tubules. However, their significance could not be ascertained since the rest of the graft gave no light as to their origin.

In one transplant, which was grown for five days, it was evident that progressive differentiation of some tissues had occurred. This transplant was observed to be deeply embedded in the chicken embryonic membrane. The degree of degeneration and infiltration was moderate, and some of the donor tissues were found to be well preserved. There was evidence of mitotic activity and growth. The nervous tissue was clearly defined, forming a series of closely related vesicles. As

successive sections were examined, a region was encountered in which distorted, characteristic neural vesicles were noted, surrounded by very loose tissue, probably chondroblastic in nature (Plate I). Latero-ventrally to the neural region, a tube, lined by simple columnar epithelium, was identified as gut. Lateral to the gut vesicle, a blood-filled liver mass was observed. The tissues present in this transplant showed differentiation comparable to that attained by like tissues found in 10-day embryos. However, no evidence of heart was found in this or other grafts of this group. The embryonic membranes did not appear either in these transplants.

Group II. Of nine 8-day embryos transplanted under similar conditions as the preceding group, only four grafts were recovered. All of them presented varying degrees of the same conditions of degeneration, necrosis, and cellular infiltration observed in Group I, making the identification of specific tissues very difficult. The most prominent constituents of the transplants were seen to be mesenchyme and red blood cells. The blood cells could have been derived either from the host or the donor, as mouse erythrocytes, at this early stage of development are still nucleated and resemble very much the chicken red cells.

One transplant of this group grown for six days showed a clearer picture than the rest. Nervous epithelium, cartilage, and some glandular epithelium resembling the gut mucosa were observed. A large necrotic area occupied the region near these structures. The nervous epithelium was developed to about a ten-day level. This was the first instance in which well defined cartilage appeared in the grafts. The cartilage, when compared to the series of normal embryos which were

prepared for controls, approached the condition of cartilage differentiation found in 12-day embryos. The gut epithelium was somewhat folded and columnar in character, containing several goblet-like cells. It was observed to be invested by a layer of dense mesenchyme. The gut epithelium seemed to be the best differentiated tissue in the transplant, although the surrounding mesenchyme did not exhibit any specialization at this stage. As far as the endodermal epithelium was concerned, it seemed to have gone to a level higher than the 13-day embryo stage. This graft furnishes a good example in which the independent developmental capacities of different tissues can be seen. Cardiac tissue was not seen in any of the 8-day whole embryo transplants, although the possibility remains that it had degenerated before the grafts were removed from the chorio-allantois.

In this group, there was no evidence of the maintenance of the embryonic membranes in the grafts.

Group III. In this group, twenty-three hearts of 9-day embryos and one 9-day whole embryo were transplanted, from which eleven heart grafts and the whole embryo transplant were recuperated. Most of the heart transplants showed heavy cellular infiltration, many of which presented degeneration and necrosis. Two transplants had such a vast amount of cytoplasmic vacuolation that the cellular pattern had been completely disrupted, and histological examination was inconclusive.

In all of the grafts except the two vacuolated ones, heart tissue could be observed although, most of the time, with little degree of organization. The hearts existed in a very attenuated condition, often being represented by bundles of cardiac fibers closely packed together and exhibiting no lumen (Plate II).

In two cases the hearts showed better organization, consisting of single chambered organs, with well defined epimyocardium and endocardium, and with projections of the walls extending into the blood-filled cavity. The hearts in all cases did not seem to have progressed histologically beyond the 9 or 10-day embryo level, and morphologically they showed no development whatsoever.

The 9-day whole embryo (Plate III) was transplanted without its membranes and allowed to grow for six days. It furnished a very striking case, some structures having attained a high level of development in the absence of other structures. It had undergone degeneration as shown by its highly vacuolated tissues, and it was infiltrated by very small polymorphonuclear cells of doubtful significance. Its periphery was occupied by extremely large, irregular cells, with conspicuous nuclei containing dark chromatin granules. There seemed to have been an overproliferation of heart and liver, these two structures attaining considerable size. The heart showed good development, with both epimyocardium and endocardium distinct. The heart complied to no regular outline, showing several blood-filled cavities communicating to one another without any definite arrangement. Histologically, it compared to the heart of a 13-day embryo. The liver showed extensive development, central veins and sinusoids being conspicuous. Along the sinusoids, certain stellate cells were seen which resembled the Kupffer cells of the adult. Near the liver respiratory tract tubules were found. Bundles of striated muscle could be seen throughout the transplant.

No evidence of nervous tissue, gut, cartilage, nephros, or skin was found during the thorough examination of the graft. There was abundant degenerating material in the graft, whose identity could not be ascertained.

Group IV. The hearts of twelve 10-day embryos were transplanted. Six of the hearts of 50% of these grafts were recovered.

In spite of the cellular infiltration, heart tissue could be identified in all cases. In many of these transplants, the hearts were very attenuated, without lumen or regional differentiation. In two cases the heart was seen to be divided into chambers with blood-filled cavities. The myocardium was well organized, and it was seen to be trabeculate in the ventricular region of the heart.

In another graft, the heart consisted of a small, single chamber, whose cavity was filled with round, dark cells, evidently degeneration products. The endothelium, obscured in large part by the great amount of these small, round cells, was apparent in some areas. Throughout the transplant necrotic spots were observed.

A transplant, grown for seven days in the chorio-allantoic membrane, showed development of the heart and some other structures (Plate IV). Gut was seen to be represented by a tube thrown into soft folds and lined by columnar epithelial cells. Mesonephros could be identified to one side of the gut. It consisted of a mass of tubules lined with cuboidal cells having regular and conspicuous nuclei. In following the serial sections, a region was reached in which a typical Bowman's capsule was observed. The cup-like structure was lined by very flat, pavement cells and the tubule connecting to it showed the transition from the flat epithelium seen at the capsule to the cuboidal cells characteristic of the proximal convoluted tubules. This tubule went for some distance and then ended blindly near a tube, whose histological features indicated it to be a collecting tube. Cartilage was also noticed in this graft.

In all cases, the differentiation of the heart had not progressed beyond the 10-day embryo stage. Thus, the level of differentiation present in the heart at the moment of transplantation had been maintained in the graft during its stay on the host. On the other hand, other tissues such as cartilage, gut, and mesonephros had been able to carry on differentiation to a stage as advanced as that of a 13 or 14-day embryo.

Group V. The donor material in this case was obtained from 11-day embryos. Eighteen such embryos were dissected and their hearts transplanted to the chicken embryonic membrane. Eight of these grafts were recovered and studied.

Some of these transplants had a large amount of necrosis and degenerating materials. In others, although showing the common cellular infiltration, the tissues were maintained in rather good condition and their identification was possible.

Two grafts showed the heart to be attenuated, consisting mainly of bundles of characteristic cardiac material without lumen or regular organization. One of these transplants showed the heart to consist of two bundles connecting to each other by a strand of cardiac fibers.

In several cases, the hearts showed characteristic bilobed regions or chambers, indicating the beginnings of their longitudinal division. In no case was the division completed. No valves were clearly distinct. The ventricular region was always characterized by its thick muscular walls, the walls of the atrial region being much thinner. It seemed that the tissues were kept at the same developmental grade that they were when transplanted. Some grafts in which the septa were seen to be more advanced than in others, seemed to have undergone some differentiation

in the new environment. This variation in differentiation might be interpreted as due to a different rate of incorporation to the host tissue, or to inherent variations in the donors.

A tube with stratified epithelium walls was followed for some distance in one of the grafts. It was thought to be oesophageal in nature because of its histological character.

Group VI. The hearts of twenty-two 12-day embryos were grafted and, after a period ranging from five to seven days, ten of these transplants were recovered.

The heart tissue, as in the preceding group, was always well differentiated. In four cases, there was considerable degeneration, large necrotic spots being surrounded by heart tissue. Identification of the different regions of the hearts was impossible as only a thin layer of cardiac fibers was seen, the central portion of the hearts being necrotic.

In one instance, it was found that the ectodermal surface of the membrane was covered by a large blood clot. The heart, left for seven days in the host, was well embedded in the host connective tissue and it was seen to consist of several chambers that did not show much difference from each other. The right atrium could be told by the entrance of two veins in it: a large bilobed one, the sinus; and a smaller one, the hepatic or later post-cava. Near the atrial region, cartilage and tubules lined with pseudostratified ciliated epithelium evidently belonging to the respiratory tract were identified.

In another case, the heart tissue, still well defined, was seen compressed, in the middle of a very large blood clot which occupied most of the space.

Cartilage and respiratory tract tubules were abundant in all the grafts of this group.

No great advance over the preceding group was observed in these transplants.

Group VII. This group consisted of six heart transplants from 13-day donors. Four of these grafts were recovered.

This group, as a whole, gave very good results, showing no necrosis, and slight apparent degeneration and cellular infiltration.

One of the transplants furnished a very striking example. It was allowed to grow for three days and removed, when it was noticed that the host had been dead for several hours. The transplant was resting on the surface of the chorio-allantois and had established contact with it only through one point (Plate V). Notwithstanding all these contrary factors, it was noticed, upon histological examination, that the tissues were in excellent condition, with no necrosis or infiltration and all the heart regions could be ascertained. The heart had not progressed, morphologically speaking, farther than the stage when it was transplanted, but it was perfectly preserved. The endocardial cushions were very conspicuous, separating the atrio-ventricular canal into a right and a left portion. The inter-ventricular septum was seen advancing towards the endocardial cushions. The first inter-auricular septum was completed and the beginning of the first inter-auricular foramen was seen near the atrial wall. There was no evidence of the second auricular septum. The left auriculo-ventricular valve was distinct. No definite valve was seen at the margins of the right atrio-ventricular canal. The entrance of the veins was not marked. The bulbus and truncus showed the beginnings of the aorto-pulmonary septum that divides the pulmonary arch

connecting to the right ventricle from the systemic branch communicating to the left ventricle. Near the right auricle, tubules of the respiratory system, lined with columnar cells, were observed.

Histologically, the grafted tissue seemed to have progressed beyond the 13-day stage (Plate V). The cardiac tissue in this transplant showed cellular differentiation (Plate VI) comparable to that in 16 or even 17-day embryos, and showed considerable advance over the 13-day normal heart (Plate VII).

The remaining grafts presented good development, although no other case as clear-cut as the one described was found.

Group VIII. In this series, ten hearts of 14-day donors were grafted, of which only four were recovered.

Compared to the preceding group, these transplants did poorly. In all cases, the tissue was undergoing degeneration and though trabeculate cardiac tissue was identified, no regions or partitions were definite.

Group IX. Of the fourteen hearts from 15-day donors transplanted, ten were recovered.

All the transplants showed good development and their tissues had been maintained in excellent condition. No apparent degeneration and necrotic spots were seen and in only two transplants was infiltration extensive.

The hearts, in both their histological and morphological features, had the general outlines of hearts of 15-day embryos (Plate VIII). Thus the tissues had been maintained at the same developmental level that they were when transplanted, and no progressive differentiation had occurred.

In several grafts, atrial and ventricular regions were distinct and typical and when septa were seen, they were very advanced or completely

formed. Although the endocardial cushions were noticeable, no values were observed.

In all instances, respiratory tract structures were present. In one case, dorsal and lateral to the heart, a complete gradation from trachea, to bronchiole, to respiratory bronchiole, to alveoli was present. Cartilage was seen supporting these structures.

Two transplants showed well developed heart tissue, but septa and valves were absent from it.

Group X. This group was composed of twelve hearts, transplants taken from 16-day donors, ten of which were recovered.

Septa were observed in all but two transplants. These two transplants had undergone considerable degeneration, having numerous necrotic spots. Their central portion was completely covered with round cells. Their periphery, nevertheless, was well preserved, and characteristic well developed heart tissue could be seen there. In one of these grafts the epicardium formed a layer which clearly delimited the heart from the surrounding host tissue. In the other one there was no clear separation between the heart and the host connective tissue. If viewed under high magnification, these two tissues were seen to intermingle forming a transitional zone of mixed fibers.

One of these grafts showed a very organized heart with septa and valves. Near its atrial portion there was a conglomeration of respiratory tract tubules, its epithelium ranging from the very flat pavement cells characteristic of the alveoli, to the pseudostratified ciliated columnar cells lining the trachea and bronchi. Cartilage rings surrounded the larger tubules. Lateral to the heart, an area occupied by

hepatic tissue could be seen. The surface of the membrane was covered by a large blood clot. The positional relations of these three organs were normal, and their properties were characteristic of 16-day embryos. Cartilage and respiratory structures were noticed in most of the transplants of this group.

Two of these transplants had been in the incubator at 37°C., when due to an accident, the temperature of the incubator was raised to about 65°C. When this was noticed, several hours later, the eggs were removed from the incubator, and as the hosts were dead due to the excessive heat, the grafts were recovered and processed to observe the effects of the very high temperature on them. Upon examination, they were seen to compare favorably with the other transplants, the hearts being well developed and the cells looking normal.

Group XI. Nine hearts obtained from 17-day embryos were grafted, and eight of these were recuperated.

All the transplants showed good development and tissues in excellent condition.

Only one graft showed considerable degeneration, and it was embedded in a coagulum of blood cells, the degeneration was abscribed to the mechanical pressure exerted by the clot and to its lack of nutrition due to its isolation from the host circulation.

A transplant of this group, left in the host for five days, was seen to be contracting rhythmically when removed from the membrane (Figure I). The rate of beating was not recorded as it was impossible to do so while still in the membrane, due to its position, and it was thought that the rhythm would be altered upon excision. Upon removal from the host, this heart, still active, was placed on a sterile watch

glass containing warm physiological solution, sketched, and placed in an incubator at a temperature of 37°C. It was examined at intervals of ten minutes and at the end of two hours, it was still contracting strongly. It was then placed in the fixative, as it was feared that the tissues would be damaged if left any longer in the saline solution.

When examined histologically, this heart was seen to be well formed, septa and valves being recognizable. All of its cavities were very much distended with blood, which also formed a clot around the transplant. The blood was shown to be host blood as its red cells were nucleated, and it compared exactly with the blood found on the vascular channels of the chorio-allantoic membrane whereas mammalian red blood cells of this age are enucleated. This indicated that the heart had made a connection with the host circulatory system and apparently was pumping its blood.

Another transplant, left for seven days in the chorio-allantois, presented a condition similar to what is known as fibrillation of the heart, i.e. different portions of the heart beating with independent rhythms. This heart was also distended by blood and showed as good development as the previous one.

Two transplants were surrounded by a capsule of dense connective fiber and apparently the pressure had caused the heart walls to collapse, the cavities being obliterated.

Most of these grafts presented bronchioles and other respiratory tubules, the one showing fibrillation containing them as specially well defined structures (Plate IX).

Group XII. This group of transplants, the last one, consisted of five hearts from 18-day embryos, the oldest embryos used in the experiment. All of the grafts were recovered.

The tissues were in very good condition, septa, valves and musculature being characteristic of that age for which they were grafted. One of these grafts was beating when removed from the membrane, and it kept active for some time after its isolation from the chorio-allantois. Cartilage and respiratory passages were common in all of these grafts.

DISCUSSION

The difficulties which a graft encounters in being incorporated into the host tissue, here the chorio-allantoic membrane of the chicken embryo, are not limited to transplants between different species, as would be the case in our study where mammalian tissue is used as donor. The same difficulties have been reported by Huxley and Murray (1924), and Willier (1924), who transplanted chicken embryonic tissue to the chorio-allantoic membrane. Thus, it is clear that these reactions cannot be abscribed exclusively to species tissue antagonism. There is, however, a higher degree of antagonism between mammalian grafts and the chick membrane. This might be due to the eroding capacity of the mammalian embryonic tissue, so clearly evidenced by its erosion of the mucosa during uterine implantation.

The stratification and cornification of the ectodermic layer is more noticeable than that of the endoderm, probably because this latter layer is farther removed from the region of operational trauma and, therefore, less disturbed by it.

Clots are frequently seen throughout this series of transplants. They are formed either by an extravasation of blood from the host blood vessels surrounding the grafts or by the ejection of the blood from the transplanted heart as it pulsates.

In most cases, the grafts are observed to present a very marked infiltration of vascular elements, mainly erythrocytes and lymphocytes. In transplants of young embryos, the origin of the invading erythrocytes cannot be ascertained as both the donor and the host red blood cells are nucleated and are indistinguishable from each other. From the evidence obtained in grafts from older embryos which have enucleated

red blood corpuscles, it is seen that most of the infiltrating cells are furnished by the host. Besides this vascular infiltration, numerous small, round, dark-staining cells are often found in and around the grafted materials. These cells have also been reported by Nicholas and Rudnick (1933), who are of the opinion that these cells are products of degeneration, since they are always found near necrotic materials and show staining reactions comparable to those of the degenerating areas. The fact that in these experiments these small cellular elements have been found under the same conditions depicted by Nicholas lends weight to this view. The successful incorporation of grafts into the chorio-allantoic membrane of the chicken embryo depends on two main factors, both resting within the transplanted particle.

The first factor influencing incorporation is the size of the transplant. In this experiment, a direct proportion of successful incorporation with size of the graft was obtained. The low percentage of incorporation of the smaller, younger grafts was not caused by an inability of isolated development at those stages, since in the few cases where incorporation was accomplished, independent differentiation of certain structures was achieved. The failure to incorporate seems to have been caused, rather, by the small and therefore ineffective size of the transplants. It is apparent that to initiate membrane reactions and subsequently incorporation, the grafted material must exert a certain amount of pressure against the membrane. The small-sized grafted particles do not weigh enough to exert the minimum effective pressure against the chorio-allantois and therefore, do not stimulate incorporation.

The other factor affecting incorporation of the grafts is the increased resistance, with the age of the transplant, to the mechanical

constraint established by the chorio-allantois.

Nicholas (1933) has found in his transplants that there is a maximum size limit for successful incorporation. The grafts exceeding this size limit frequently showed the formation of clots around them. The blood clots would interfere with the vascularization of the transplants and prevent their growth. In the series of transplants discussed in this paper, grafts from older, larger embryos have often showed the formation of large blood clots. However, in most instances, the tissues have fared well, and there has been a relatively consistent increase in the percentage of incorporation with the increase in age and size of the grafted particles.

This might be explained by the fact that the younger transplanted tissues are delicate and flexible, and when confronted by the pressure exerted against them by the chorio-allantois, they are easily collapsed and destroyed. As tissues grow older they become more firmly established in a set pattern. However, what they lose in plasticity, they gain in rigidity and resistance, so that the larger transplants are better equipped, physically and physiologically speaking, to cope with the antagonizing forces of the new environment.

Evidence for the importance of this factor is furnished by the higher percentage of incorporation obtained from the heart grafts of 9, 10, 11, and 12-day embryos. The hearts of these young embryos are very small; their size approximates the size of the 7 and 8-day whole embryos. The better incorporation of these particles might be due to the circumstances in which the grafted tissues were found at the moment of transplantation. That is, the tissues at transplantation were established well enough, both from a physical and from a physiological point

of view, to insure a better chance in their adaptation to the new environmental conditions.

In the case of the 9-day whole embryo transplant, both factors, the effective size of the graft and a certain degree of maturity of the tissues, have interacted to yield such a striking example of incorporation and development.

The mamalian tissue shows great powers of adaptability since it is able to survive and differentiate in such an altered environment as the chicken chorioallantois, which differs from the normal uterine environment in both temperature and mechanics.

It has been noticed, nevertheless, that in transplantation, the normal relative differentiation of tissues is disturbed, and some tissues show an accelerated differentiation or an accelerated growth and even overproliferation, while others merely maintain their original conditions or might even undergo dedifferentiation. This has been demonstrated by the findings in the 7, 8, and 9-day whole embryo transplants.

The ectodermal derivatives found in the whole embryo transplants are nervous tissue and skin stratified epithelium. Both tissues show progressive differentiation in these grafts. In some regions, the rapid and abundant overproliferation of the nervous tissue has forced it to form a series of closely related vesicles. This is the result of the extensive growth of the nerve tissue in a restricted space. Although the nervous tissue differentiates and grows in the abnormal environment, the altered mechanical conditions of the new environment probably cause the aberrant organization that this tissue presents as it goes in the formation of vesicles. The skin epithelium is found

usually in its early stages of development. Skin glands or other specialized skin structures have not been observed.

In 7-day embryo transplants, the nervous tissue proliferates and differentiates at a more rapid rate than the other tissues present. Histologically, it has progressed to a 10-day embryo level, and it shows great mitotic activity. In 8-day whole embryo grafts, the overproliferating activity of the nervous tissue has subsided. In 8 and 9-day whole embryo transplants, the endodermal derivatives are the most active elements. They reach a level of differentiation much higher than the nerve tissue ever did in the 7-day embryo grafts. The extremely well differentiated gut epithelium found in an 8-day embryo graft has attained a degree of development higher than that of a 13-day normal embryo. The liver found in the 9-day whole embryo graft, is also very well differentiated.

Mesodermal derivatives vary in their independent differentiational capacities. Well-defined cartilage first appeared in the 8-day embryo grafts. It differentiates in the grafts sometimes up to the 12-embryo level. Various stages of the formation of smooth muscle fibers from mesenchyme have been observed throughout the whole embryo transplants, and strands and bundles of differentiated muscle have also been found in the grafts. Heart tissue has not been identified in the 7 and 8-day whole embryo transplants, but when found in the 9-day embryo graft, it showed considerable differentiation and overproliferation. Again, here overproliferation results in an aberrant organization since the heart consisted of several blood-filled cavities with no regular arrangement. The presence of nephric material in the whole embryo grafts is undetermined. Structures bearing

resemblance to nephric tubules are seen in a 7-day whole embryo graft, but their identity has not been established, since the rest of the graft gives no further evidence as to their nature. On the other hand, a large amount of donor mesenchyme can be observed in all the whole embryo grafts. This has been interpreted to represent the differentiation or regressive differentiation of other mesodermal elements, since the quantity of mesenchyme is abnormally large for these embryo stages, and no other mesodermal derivatives are found in the grafts.

The early activity of the neural tissue, followed later by endodermal, and still later by mesodermal structures, seems to follow the sequence of normal ontogency. In mouse embryogenesis the ectodermal and endodermal germ layers are established before the mesoderm rudiments appear. The variation in independent differentiational capacities of tissues might be determined by their inherent specific properties such as metabolic activity at the time of transplantation. The absence of some structures and tissues from the grafts does not necessarily signify their inability to differentiate in the new environment. They might have reached a peak in development and then degenerate before the transplants were recovered from the chorio-allantois, since a large amount of necrotic and degenerating tissues, whose identity cannot be ascertained, are present in the grafts. However, the absence of tissues from the grafts might indicate that the capacity of such tissues to survive for long periods of time under the new environmental conditions is not great.

Whenever more than one structure appears in a graft, their relative anatomical positions are normal, although the tissue itself might show an altered form.

The hearts, as a rule, are seen to maintain their original differentiation levels throughout the grafts series. In some cases, there is

a variation in morphological differentiation among the grafts of a transplant group. Although this might signify that some transplants in a group have undergone more differentiation than others, it is more probably explained by the fact already mentioned that there is a variation in levels of development among embryos of the same age, or even of the same litter. Thus, the differences in development were present in the transplantation.

There are only two grafts in which differentiation has been seen to have progressed beyond the original level at the moment of transplantation. The first case is the one encountered in the 9-day whole embryo transplant. This heart presents a cellular differentiation comparable to that of a 13-day normal embryonic heart. It has also undergone extensive overproliferation and accordingly, its size has abnormally increased. Its morphology is altered, following no regular outline. In this instance, the six days growth period in the chorioallantoic membrane has been equivalent to about four days of normal uterine growth as far as the differentiation of the organ is concerned.

The other example of independent differentiation of the heart is furnished by one of the 13-day heart transplants. Histologically, this heart is comparable to that of a 17-day normal embryo. This is striking, for there is no evidence of growth in this heart, and its morphology has remained static since transplantation.

Grafting apparently not only disturbs the organisms causing their various tissues to differentiate independently and with altered rates, as shown by the whole embryo transplants, but it can also disrupt the correlation normally found between histological and morphological differentiation of an organ so that they proceed independently, as seen in the thirteen-day heart graft. This is the only manifestation of such a

phenomenon in this experiment. Usually the morphogenesis and histogenesis of grafted material is seen to proceed at a coordinated rate.

The frequent presence of tubules and cartilage of the respiratory tract and the occasional presence of liver in the heart grafts can be explained by the already stated failure of complete isolation of the hearts to be transplanted from their surrounding tissues. These tissues, whenever present, seem to have maintained their initial level of development. When heart, respiratory tissue and liver appear in the same graft, they always show their normal positional relation. Although in this series of transplants the preparation of slides for the detection of cytological differentiation has been sporadic, in all such cases, the presence of both fibrillae and striations seem to be indicated. According to Goss (1940), these specialized structures appear in the heart cells at the beginning of circulation, some hours after simple contractile action is noticed in the cardiac elements. Apparently, the elaboration of these structures is not related to the establishment of contractile activity of the heart cells but to the functional adaptation of this action. This indicates that whenever heart appeared in our grafts, these hearts were already functional when isolated from the embryos.

Three heart transplants have been found to be pulsating when recovered from the chorio-allantois. The cavities of these contracting hearts are seen to be greatly distended by blood, which is of host origin. These hearts seem to have become integrated and functional within the membrane circulation. As to the effectiveness of their pumping action there is no criteria. It is possible that they just provided another channel for the host blood, and that their contractions were not forcible enough to affect the host circulation.

SUMMARY

A study on the growth and differentiation of mouse embryonic tissue, especially the heart, was carried out by transplantation of tissue, at various stages of development, to the chorio-allantoic membrane of the chicken embryo.

Whole 7, 8, and 9-day embryos, and the isolated hearts of 9 to 18-day embryos were grafted to the chorio-allantois and allowed to grow from five to seven days.

The percentage of successful incorporation of the grafts in the membrane increased with increasing size and age of the transplanted tissues.

After removal from the membrane, the transplants were processed for histological examination.

Some of the whole embryo transplants of each age group were observed to have undergone growth and differentiation on the new environment. The nervous tissue showed differentiation and overproliferation in the 7-day whole embryo grafts. Another ectodermal derivative that appeared often on the 7-day embryo grafts was stratified squamous epithelium. The endodermal derivatives appearing on the 7-day whole embryo transplants were gut and liver, both having differentiated to about the 10-day embryo level. The mesodermal structures appearing in this group of transplants were muscle fibers, precartilage,* nephros* and a large amount of mesenchyme. No cardiac tissue was identified in these grafts.

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*The nature of these tissues could not definitely be ascertained as the rest of the graft gave no light as to their origin.

In 8-day whole embryo transplants, the overproliferating capacity of nervous tissue had subsided, although nervous tissue showed differentiation in these grafts. The tissue to show the best differentiation in the 8-day embryo grafts was gut epithelium. Cartilage, a mesodermal derivative, had undergone very good differentiation in grafts of this age group. The prevalent constituents of these transplants were mesenchyme and blood cells of undetermined origin. Heart tissue was not observed in this group of grafts.

In the 9-day whole embryo transplant the heart and liver had differentiated to high degree and had overproliferated. Muscular tissue and respiratory tubules also appeared in this graft. No evidence of nervous tissue, or other germ layers derivatives, was found in the 9-day whole embryo transplant.

The heart transplants, obtained from 9 to 18-day donors, generally maintained their original stage of development. There were two exceptions to this trend. The first one was the case of the 9-day whole embryo transplant in which the heart had undergone differentiation and overproliferation. The other case was presented by a 13-day embryo heart transplant in which the morphology of the heart had remained static while high histological differentiation had been taking place.

Two 17 and one 18-day heart transplant were actively contracting when removed from the chorio-allantois. Upon examination, they showed to be distended with host blood, apparently having been integrated with the host circulation.

Respiratory tract tubules and liver appeared frequently in the heart grafts. Mesonephros was present in a 10-day heart transplant. Whenever more than one structure appeared in the whole embryo or heart grafts they maintained normal anatomical relations to each other.

CONCLUSIONS

1. The incorporation of grafts into the chorio-allantoic membrane of the chicken embryo depends on whether the transplant exerts an effective pressure on the membrane to stimulate membrane reaction and incorporation, and on the ability of the grafted tissue to cope with the new environment.
2. The mammalian tissue shows great powers of adaptation to a new and different environment.
3. Grafting disturbs the normal relative differentiation of tissues in an organism. Some tissues show an accelerated differentiation and growth, while others maintain their original developmental levels, or even dedifferentiate.
4. The ectodermal derivatives, especially nervous tissue, undergo the earliest differentiation and proliferation in the whole embryo grafts.
5. The endodermal and mesodermal elements differentiate later than the ectodermal ones.
6. Gut and liver present the greatest degree of differentiation in the whole embryo transplants.
7. Some mesodermal derivatives, i.e. cartilage and muscle, show good differentiation; heart appears late but is found to undergo high differentiation and a great amount of overproliferation; other mesodermal elements dedifferentiate into mesenchyme.
8. The sequence of differentiation and growth of germ layer derivatives in the transplants seems to follow the sequence of normal ontogeny, where there is an early activity of nerve tissue, followed by the elaboration of endodermal and mesodermal structures.

9. The absence of some tissues and structures from the grafts does not mean that this structure did not differentiate, as they might have degenerated before the transplants were recovered. It might indicate, however, that these tissues are more susceptible to the adverse conditions of the new environment.
10. Isolated hearts do not seem to have a very great capacity for independent differentiation, but they were increasingly able, with age, to survive in the chorio-allantois.
11. From the evidence presented by one of the heart transplants, it seems that grafting may, at times, also disrupt the balance between morphological and histological differentiation, so that they proceed at independent rates.
12. Some of the heart grafts have become integrated with the circulation of the chorio-allantois and seem to circulate the host blood.

TABLE I

Donor Age (Days' Old)	Total No. of Grafts	Incorporated Grafts	Percentage of Incorporation
* 7	42	16	38.0%
* 8	9	3	32.2%
** 9	24	12	50.0%
10	24	12	50.0%
11	18	8	44.4%
12	22	10	45.4%
13	6	4	66.6%
14	10	4	40.0%
15	14	10	71.4%
16	12	10	83.3%
17	9	8***	88.0%
18	5	5****	100.0%
TOTAL	191	109	57.0%

* Whole embryo transplants

** One whole embryo transplant and 23 heart transplants

*** Two transplants beating

**** One transplant beating

The rest are all heart transplants

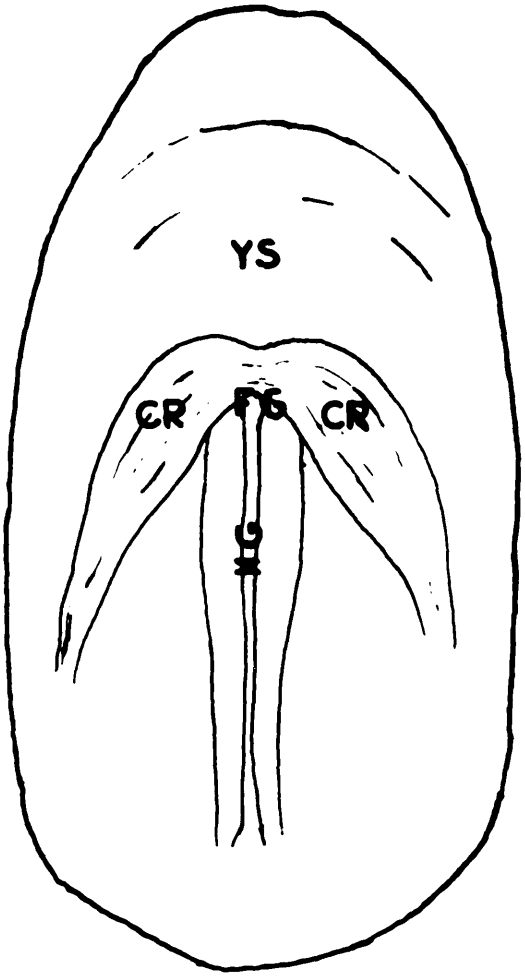

FIGURE I (165 X)

Seven-day mouse embryo. NG - neural groove. CR - cardiac rudiment. FG - foregut invagination. YS - proximal portion of yolk sac endoderm.

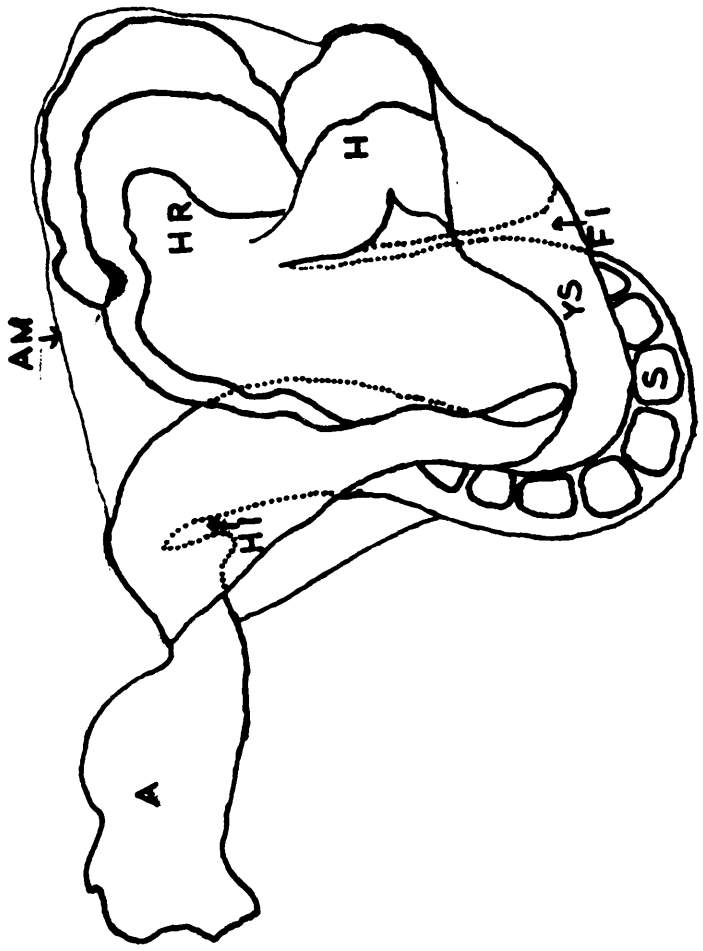

FIGURE II (78 X)

Eight-day mouse embryo. AM - amnion. A - allantois. HR - head fold. H - heart. FI - foregut invagination. HI - hindgut invagination. S - somite. YS - proximal portion of yolk sac.

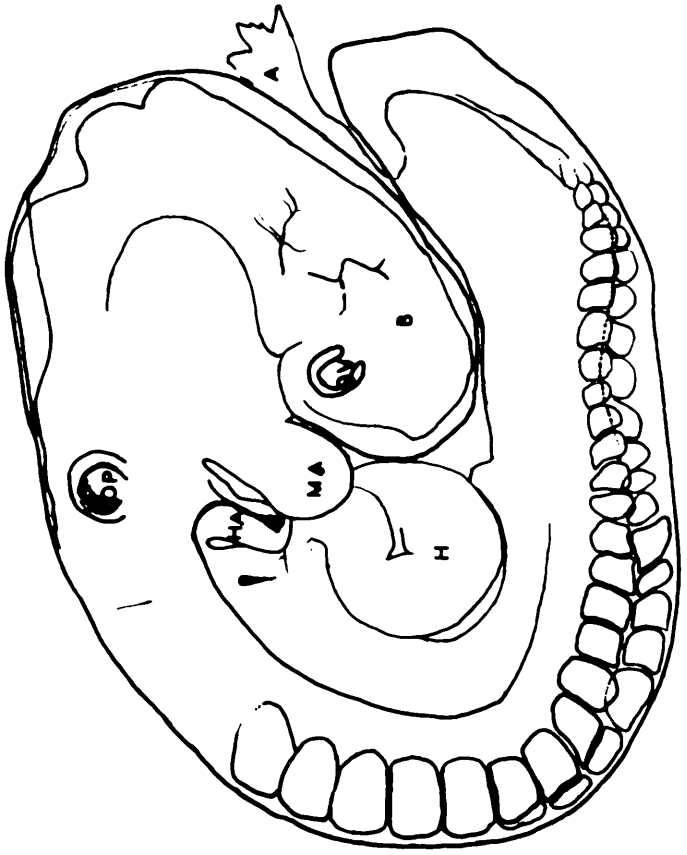

FIGURE III (46 X)

Nine-day mouse embryo. H - heart. B - brain. A -allantois. OV - optic vesicle. OP - otic vesicle. MA - mandibular arch. HA - hyoid arch.

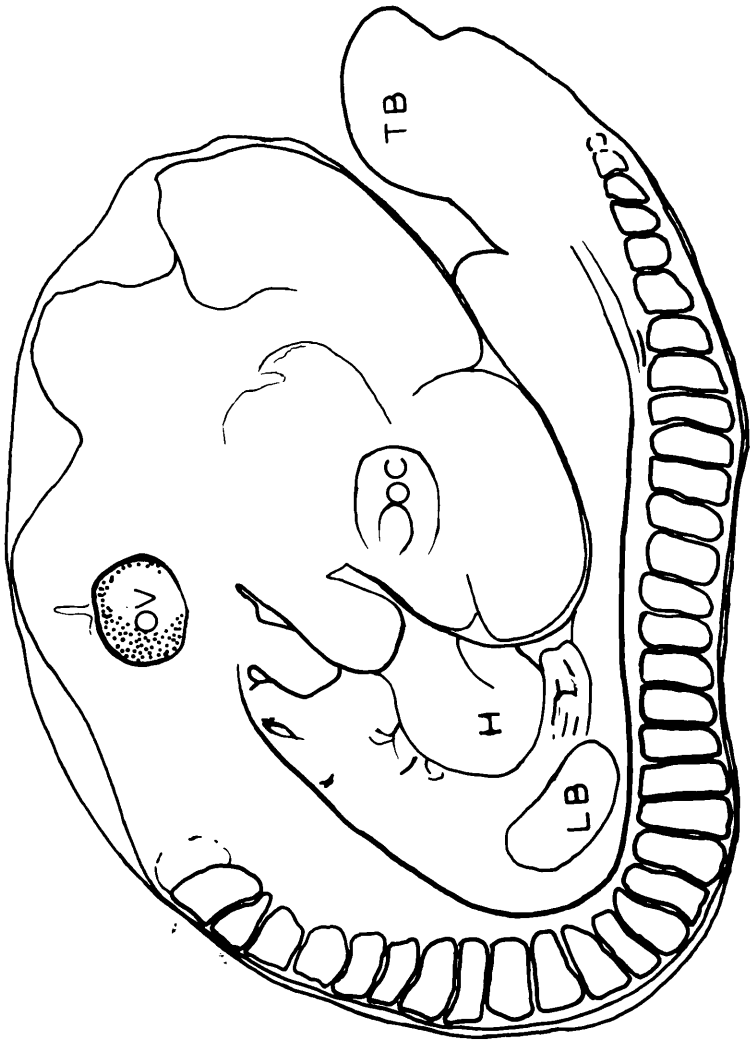

FIGURE IV (39 X)

Ten-day mouse embryo. H - heart. LB - anterior limb bud. TB - tail bud. OC - optic cup. OV - otic vesicle.

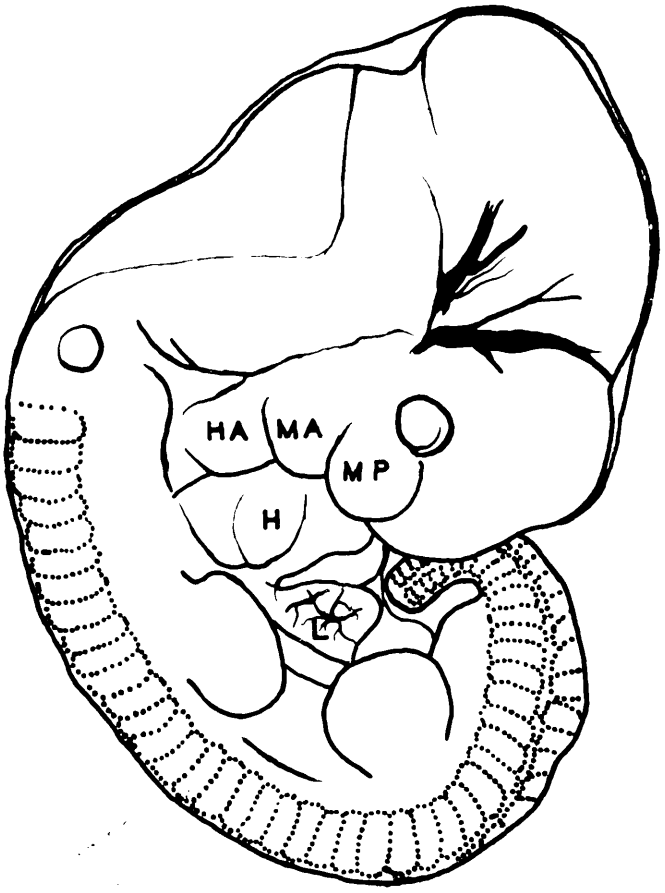

FIGURE V (20 X)

Eleven-day mouse embryo. H - heart. HA - Hyoid arch. MA - mandibular arch. MP - maxillary process.

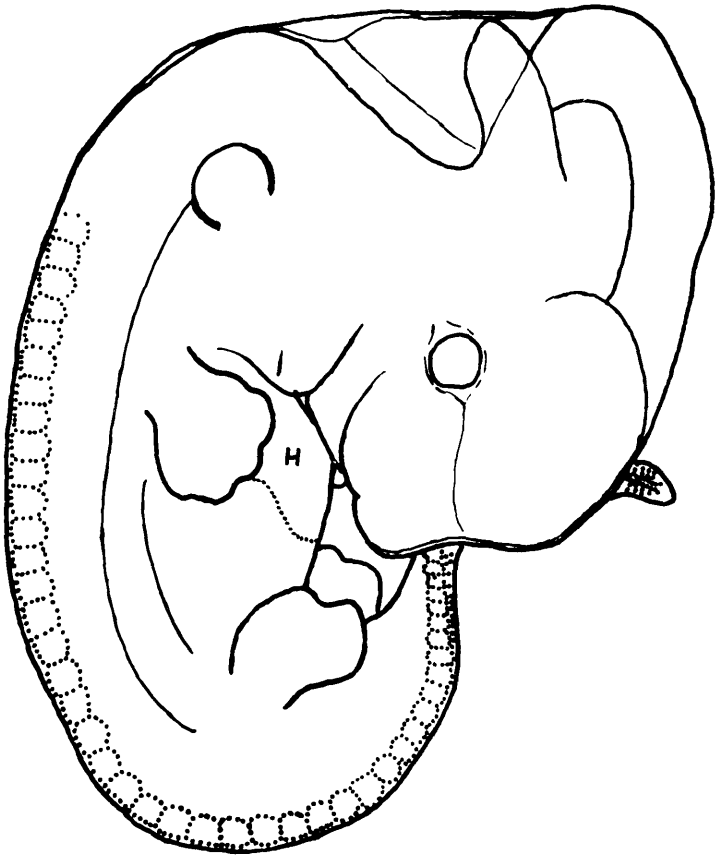

FIGURE VI (15 X)

Twelve-day mouse embryo. H - heart.

FIGURE VII (12 X)

Thirteen-day mouse embryo

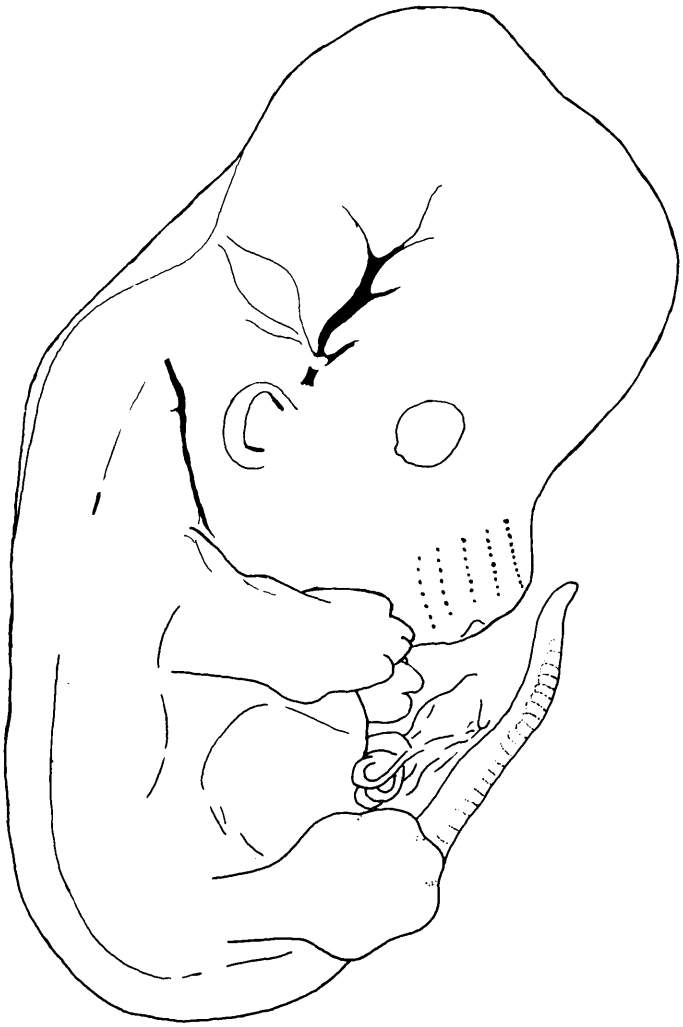

FIGURE VIII (10 X)

Fourteen-day mouse embryo

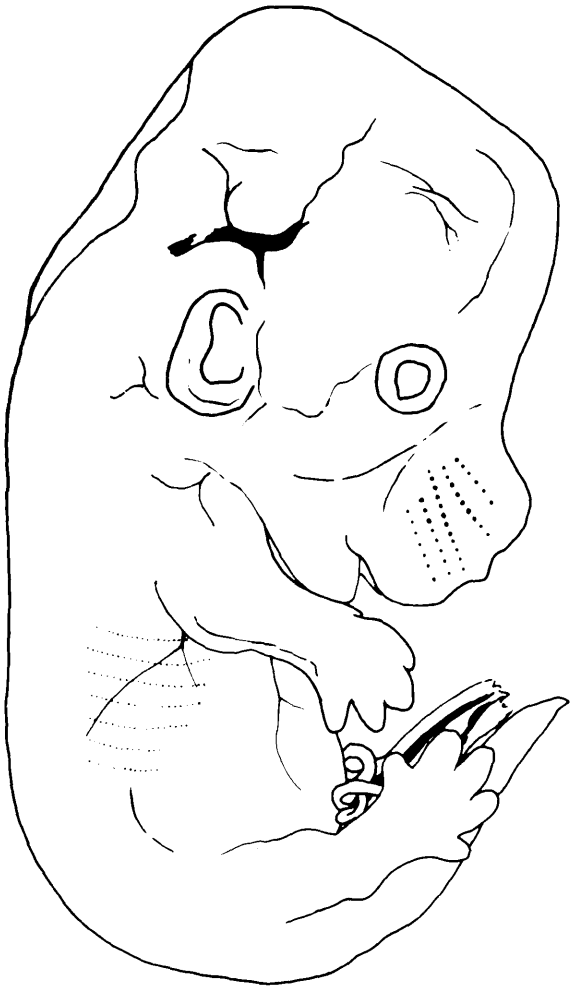

FIGURE IX (9 X)

Fifteen-day mouse embryo

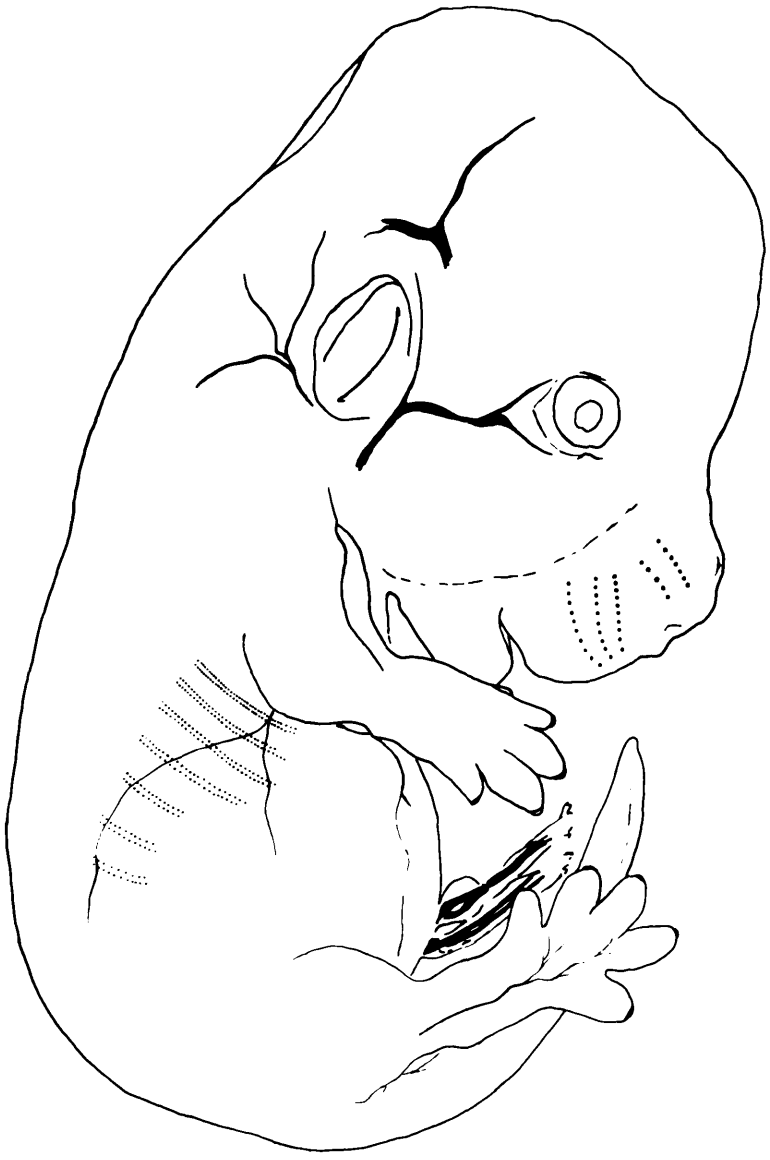

FIGURE X (8 X)

Sixteen-day mouse embryo

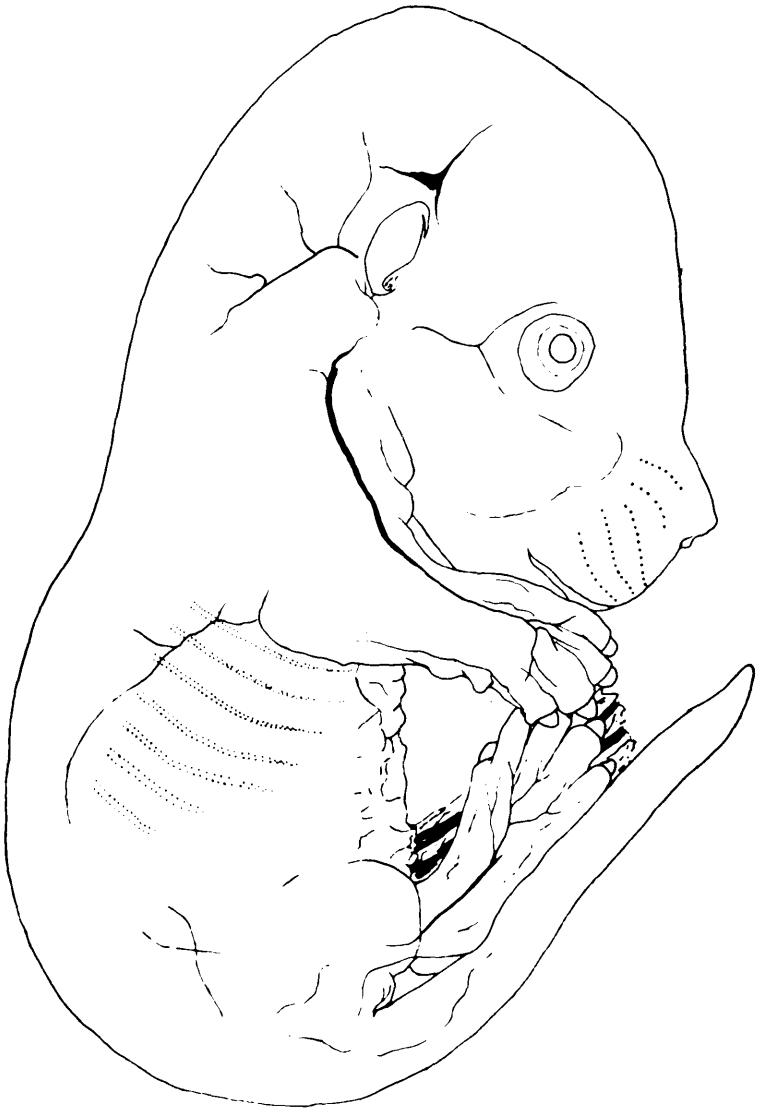

FIGURE XI (7 X)

Seventeen-day mouse embryo

FIGURE XII (6 X)

Eighteen-day mouse embryo

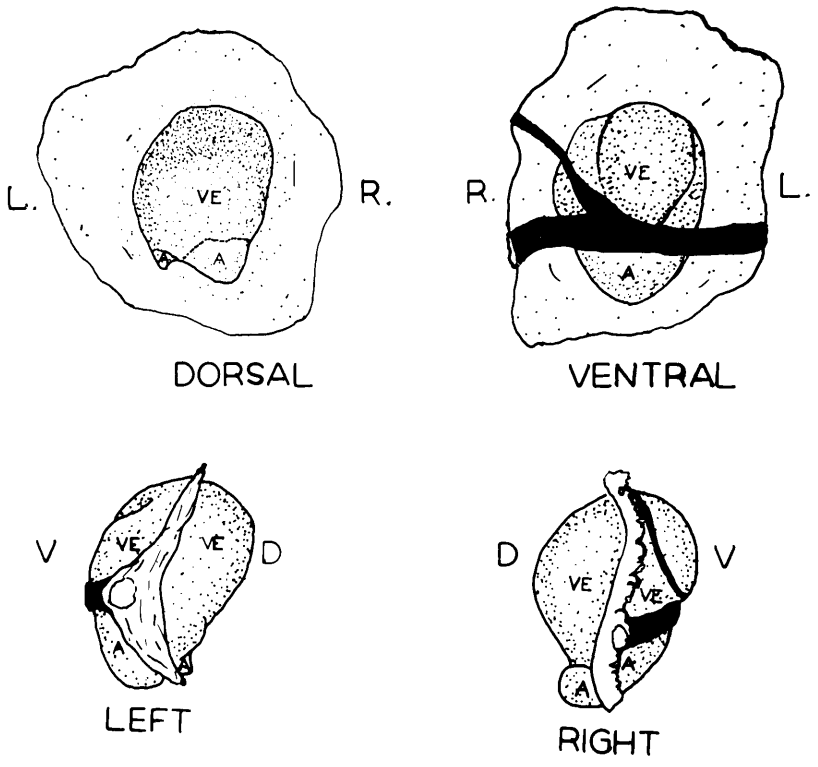

FIGURE XIII

Sketch of contracting 17-day heart transplant, grown in the chorio-allantois for five days. L - left side. R - right side. V - ventral surface. D - dorsal surface. VE - ventricle. A - auricle.

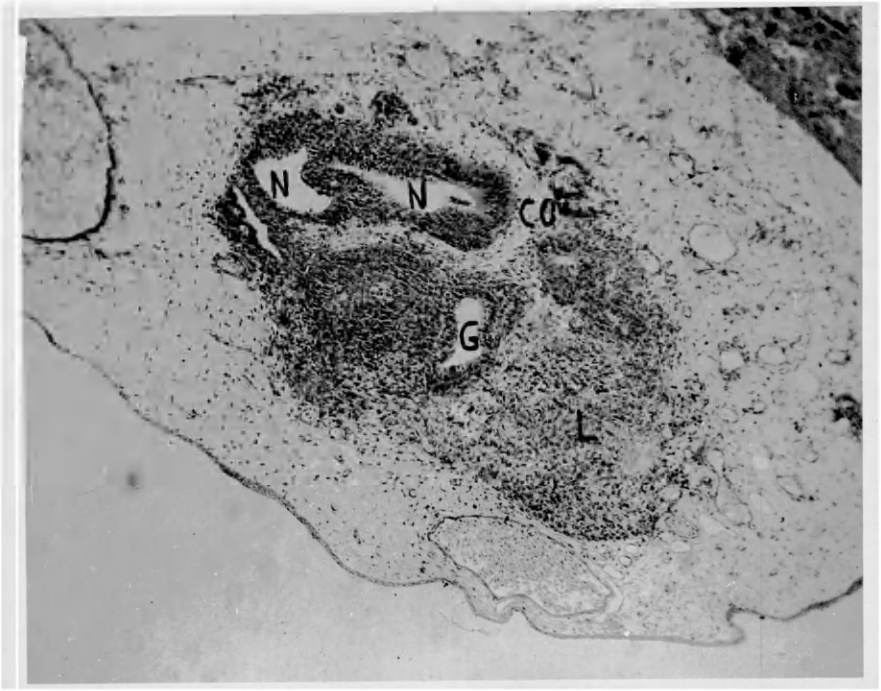

PLATE I (90 X)

Section from a 7-day whole embryo transplant grown in the chorio-allantois for five days. It shows dorsal neural vesicles (N), loose connective tissue which might be chondrioblastic in nature (CO), gut (G), and a blood-filled liver mass (L).

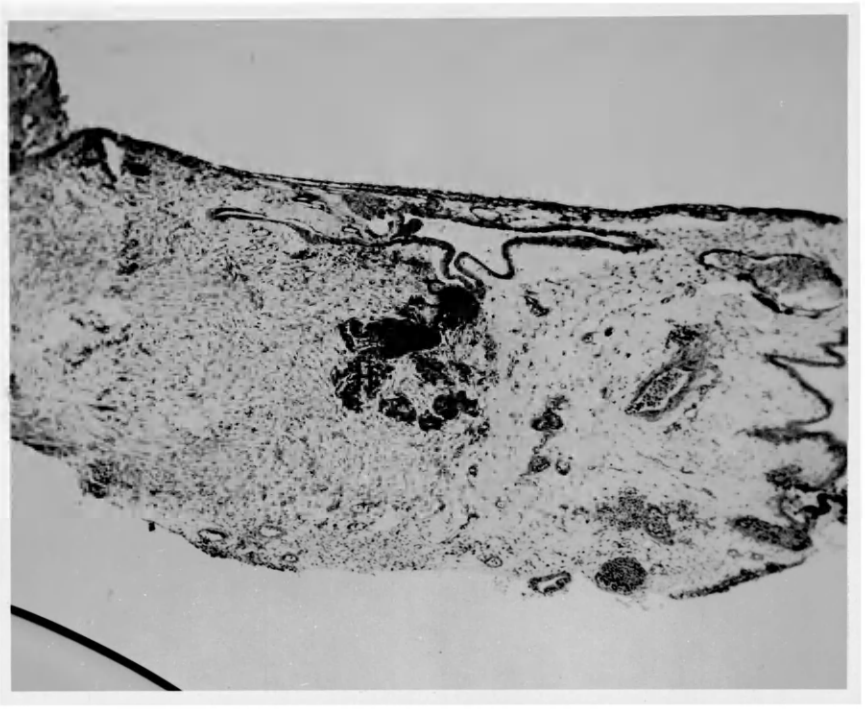

PLATE II (90X)

Photomicrograph of a section of a 9-day embryo heart transplant after six days in the chorio-allantoic membrane. The heart (H), is shown as a group of bundles of cardiac fibers closely packed together and exhibiting no lumen. A necrotic spot (S) is seen dorsal to the heart bundles.

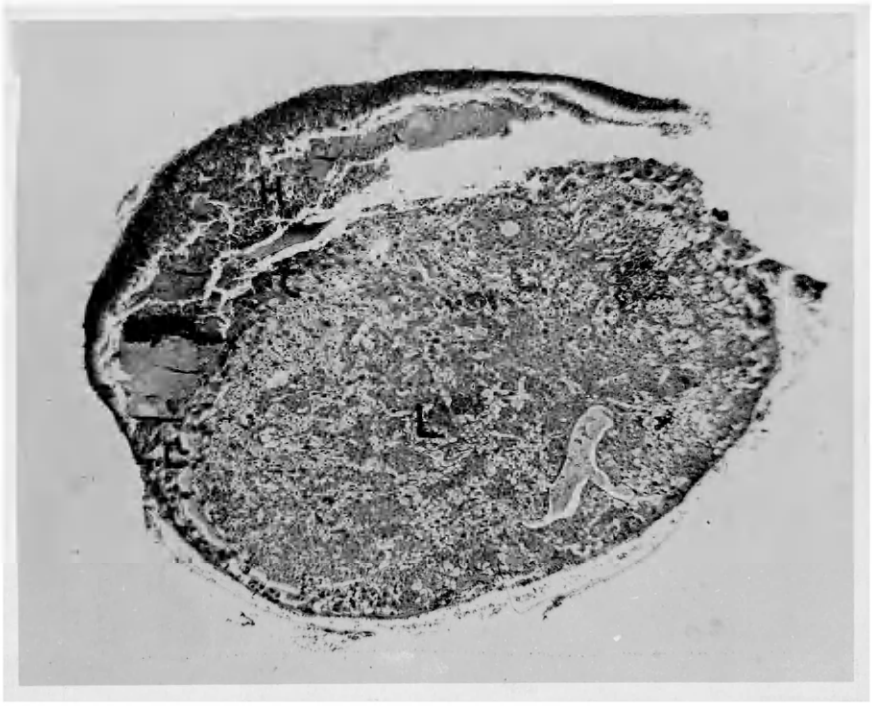

PLATE III

Section through a 9-day whole embryo transplant grown in the host for six days. The liver (L) and heart (H) show overproliferation. The tissue is highly vacuolated. Giant cells (C) are seen along the periphery of the transplant and between the liver and the heart.

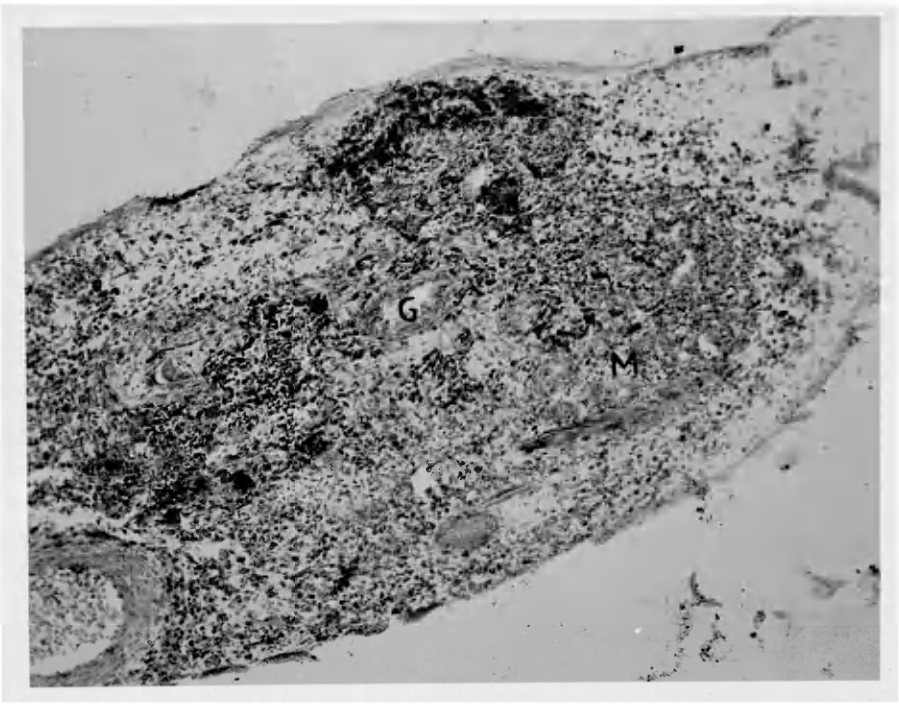

PLATE IV (125 X)

Section from a 10-day heart transplant grown for seven days in the chorio-allantois. Apparently, the heart was not completely isolated from surrounding tissues as is shown by the presence in the transplant of other structures such as gut (G), which appears as a folded tube linted with columnar cells, and a mass of mesonephric ducts (M).

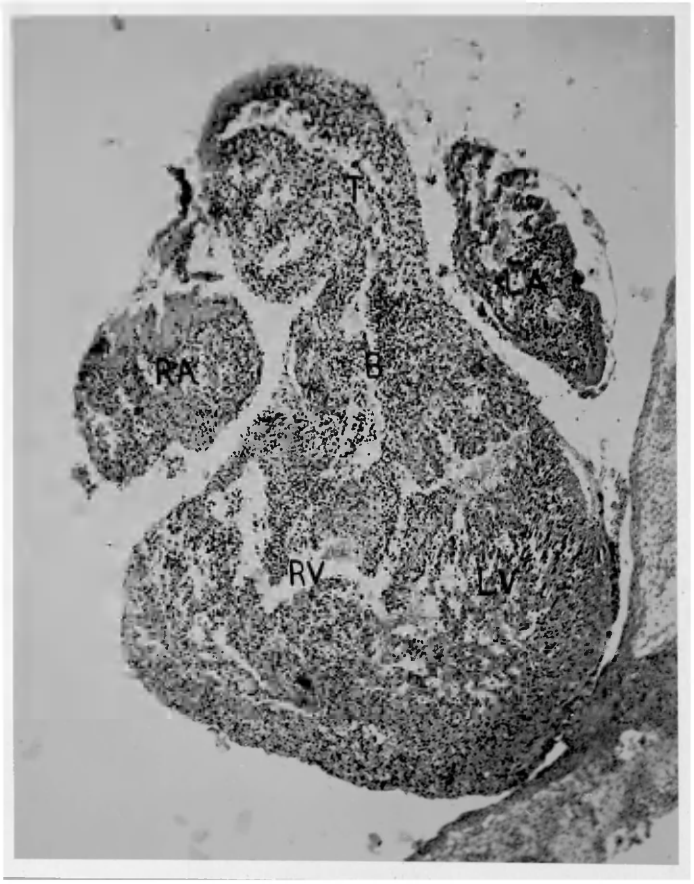

PLATE V (90 X)

Section from a 13-day heart transplant grown in the chorio-allantois for five days. The host died several hours before the transplant was recovered. The point of contact of the graft to the chicken embryonic membrane is shown at the lower right portion of the photomicrograph. The right (RA) and left (LA) auricles, the right (RV) and left (LV) ventricles, and the bulbus (B) and truncus (T) arteriosus are clearly seen.

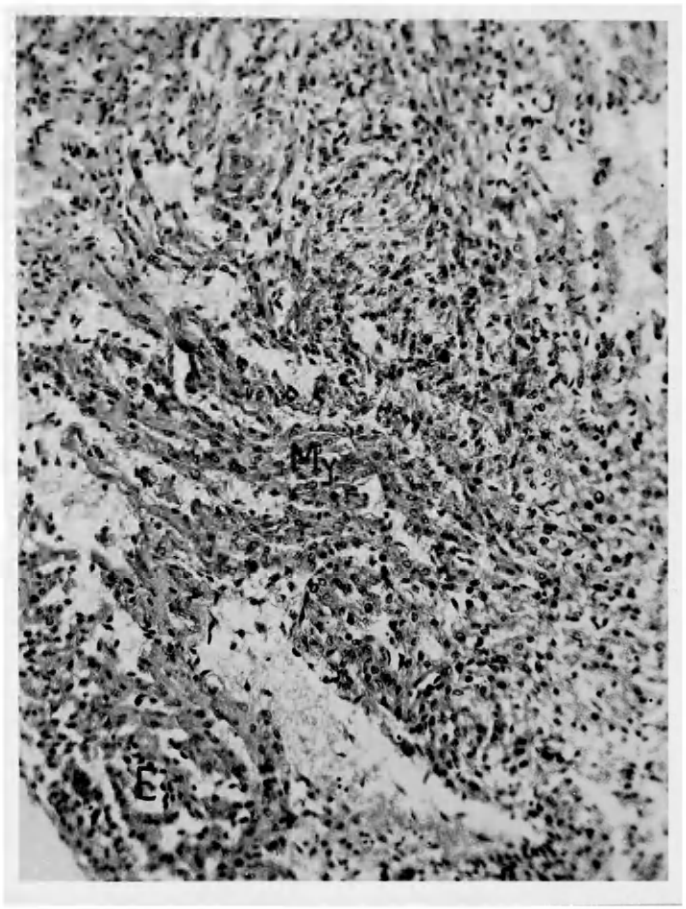

PLATE VI (200 X)

Section through the ventricle of a 13-day heart transplant grown in the chorio-allantois for three days, showing very well differentiated cardiac tissue. The epicardium (E) and myocardium (My) are very highly developed. If compared with Plate VII the highest differentiation of the tissue in this transplant can be noticed in the elongation of the nuclei and fibers, and the more fibrillar nature of the cellular cytoplasm.

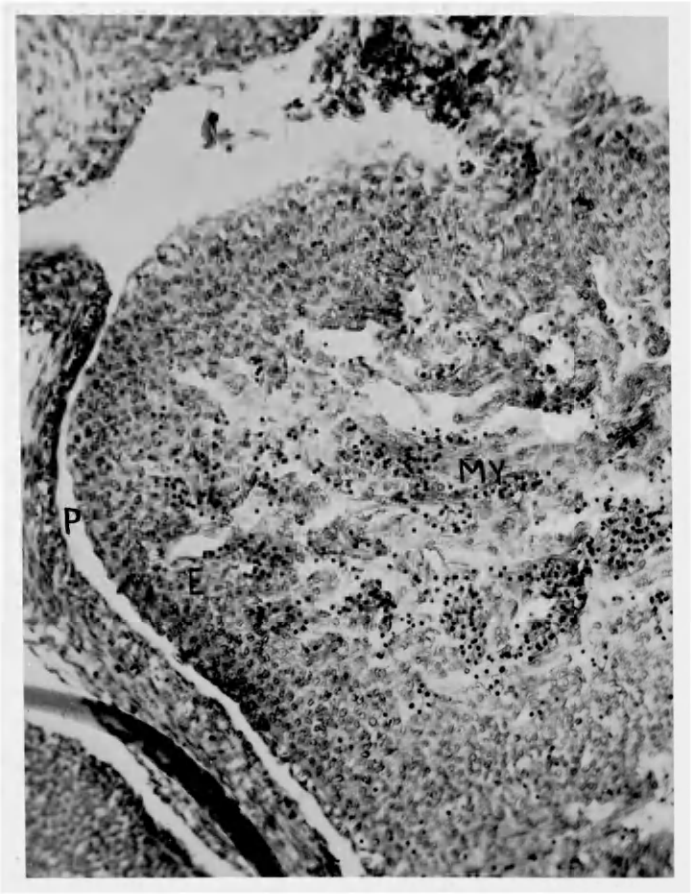

PLATE VII (200 X)

Section through the ventricle of a normal 13-day mouse heart showing the characteristic differentiation of the cardiac tissue at that stage of development. The pericardial cavity (P) surrounds the organ, which depicts well defined epicardium (E) and myocardium (MY).

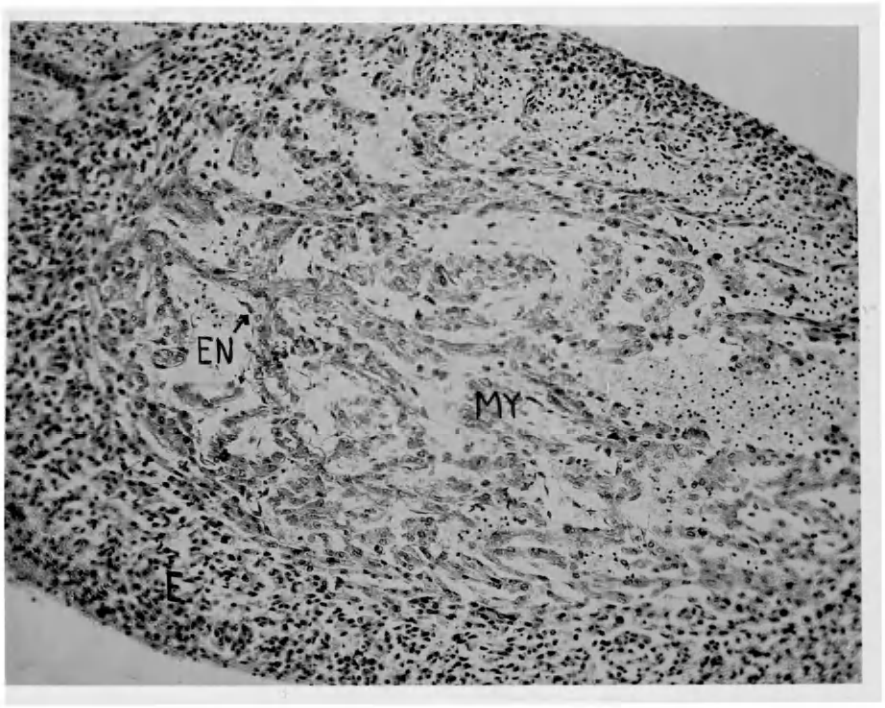

PLATE VIII (160 X)

Section from a 15-day heart transplant, left in the chorio-allantois for five days, showing development comparable to that shown by the heart of a normal 15-day embryo. The epicardium (E), myocardium (MY), and endocardium (EN) are very sharply defined.

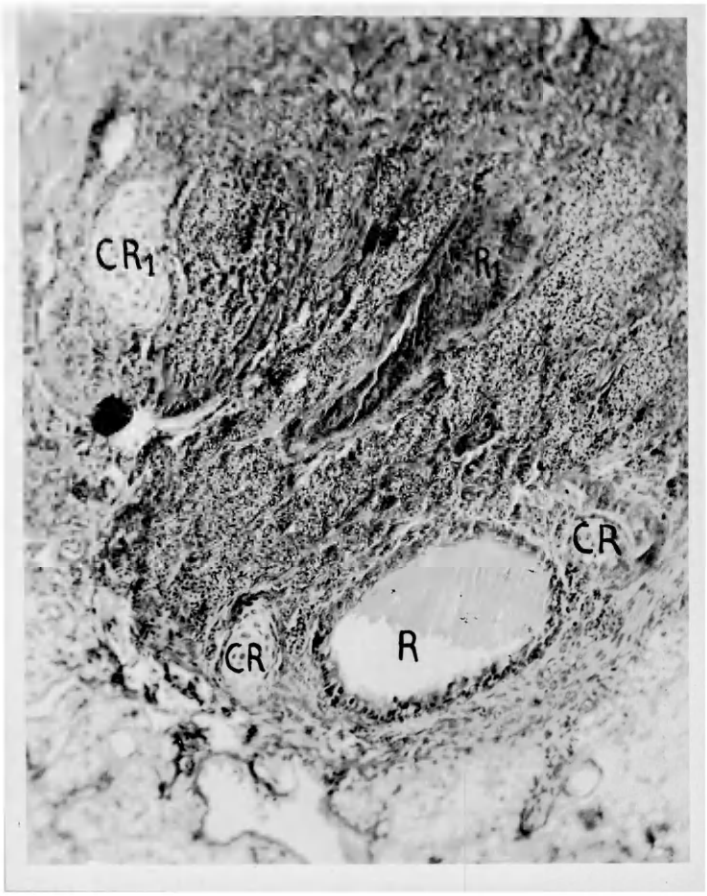

PLATE IX (160 X)

Section through a 17-day heart transplant, grown in the chorio-allantois for seven days, which showed fibrillation. The respiratory tube (R) is lined by columnar ciliated cells, and is surrounded by cartilage rings (CR). Another respiratory tube (R_1) was cut tangentially through the walls. A cartilage ring (CR_1) is seen on the upper left area of the photomicrograph.

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