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The Effect of the Destruction of the Germinal Crescent on the Origin of the Germ Cells and the Development of the Gonads in the Domestic Fowl

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A THESIS

THE EFFECT OF THE DESTRUCTION OF THE GERMINAL CRESCENT
ON THE ORIGIN OF THE GERM CELLS AND THE DEVELOPMENT OF
THE GONADS IN THE DOMESTIC FOWL.

IX Plates (17 figures)

PRESENTED BY ARTHUR JOHN SVEJDA B.S.M. TO THE FACULTY OF
THE LOYOLA UNIVERSITY GRADUATE SCHOOL IN REQUIREMENT FOR
THE DEGREE OF MASTER OF SCIENCE.

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1937

THE EFFECT OF THE DESTRUCTION OF THE GERMINAL CRESCENT ON
THE ORIGIN OF THE GERM CELLS AND THE DEVELOPMENT OF THE
GONADS IN THE DOMESTIC FOWL.

INTRODUCTION

The site of origin of the definitive sex cells of the domestic fowl is as yet not definitely known. Much work of an observational nature has been done and several theories have staunch supporters.

A logical method of experimental approach is to remove one of the possible sources of definitive sex cells by destruction of the so-called germinal crescent during its period of proliferation of migratory entodermal cells. This has been attempted by several investigators with unsatisfactory results because their operated specimens died too early to show the necessary developmental changes.

The present author has developed a method for overcoming this difficulty to a large extent. The results also offer a possible explanation for the high mortality of the operated specimens.

Acknowledgement is made of Dr. J. M. Essenberg's valuable assistance in times of difficulty, of Mr. O. I. Warren's advice on the technique of preparing microscopic sections, and of Miss M. E. Bakehouse's aid in photography.

LITERATURE

Waldeyer presented the problem of the embryonal origin of the definitive germ cells in 1870 in his work on the domestic fowl, "Eierstock und Ei". He surmised that the definitive germ cells arose from the modified peritoneal covering of the embryonal gonad, but he did not claim to have seen this transformation.

Nussbaum ('80 and '01) formulated the theory that "sexual cells do not come from any cells that have given up their embryonal character or have gone into building any part of the body, nor do sexual cells ever go into body formation".

At the close of the 19th century two opposed theories were held regarding the origin of the definitive germ cells:

(1) The germinal epithelium theory, in which early segregation of the germ cells was denied and germ cells were believed to arise from the modified peritoneal cells covering the gonad.

(2) The early segregation theory, in which the definitive germ cells were supposed to be set apart early in embryonal development, later to migrate into and become part of the gonad.

Later investigators supported one or the other of these two hypotheses. Semon in 1887 claimed to have seen a number of the germinal epithelium cells in the modified coelomic epithelium undergoing transition stages in the formation of primordial germ cells. He was the first to publish claims of having seen this transformation take place.

d'Hollander in 1904 described the production of oogonia from the germinal epithelium in a 10 day old chick embryo. He concluded that the germinal epithelium gave rise to buds which grew down into the mesenchymal ovarian stroma and by a process of differentiation, the epithelial cells gave rise to the oogonia and follicular cells.

Rubaschkin in 1907 and von Berenberg-Gossler in 1912, both working with chick embryos, were able to find primordial germ cells in the entoderm and splanchnic mesoderm lateral to the coelomic angle in embryos having 22 to 25 pairs of somites.

The present status of the problem is based upon the findings of Swift ('14, '15, '16), who supports Nussbaum, and Firket ('14, '20) who upholds the "germinal epithelium theory".

Swift claims that the "Entodermal Wandering Cells" of Dantschakeff ('08) which arise in the crescent area of 16 to 24 hour embryos are primordial germ cells. He traces these via the blood stream and their own amoeboid movements into the root of the developing mesentery in the region of the gonads. Although he did not actually see them enter the gonad he surmised as much.

Goldsmith ('28) in his work corroborates the conclusions of Swift.

Firket ('14, '20) admits the existence of the Swift Cells (primordial germ cells) but maintains that these degenerate upon entering the gonad, and concludes that the definitive cells arise from the modified coelomic epithelium.

In some work as yet unpublished, Essenberg and Garwacki observed

the embryo, i.e. the genital ridge region posterior to the 22nd somite.

The evidence presented in the literature warrants a study of the effects of crescent destruction upon the development of germ cells and gonads in embryos sufficient in development to have advanced differentiation of the gonads, i.e., at least six days incubation.

MATERIALS AND METHODS

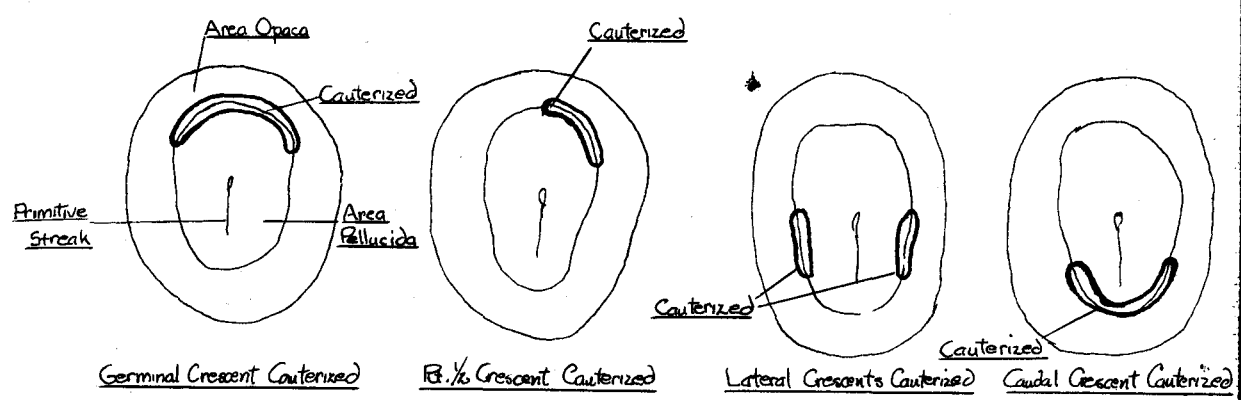
The destruction of the germinal crescent was the basis of the approach. Improvements in technique and sources of fertile eggs were made as the work progressed. These will be described under three groups of experiments.

First Group of Experiments

Fertile eggs of white and brown leghorn hens shipped from Rhon Johnson, Judson, Indiana were used. These were at least four days old before incubation was started.

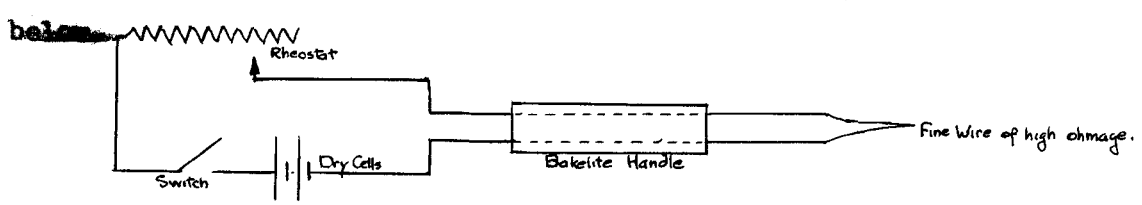
The eggs were incubated in an electrically heated and controlled incubator at a temperature of 102°F., with a relative humidity of 65%, from 18 to 44 hours. After a hole approximately $\frac{1}{2}$ cm. square was cut into the shell and shell membranes, the germinal crescent was destroyed with an electrically heated cautery. Controls were used in which areas other than the crescent were cauterized. The purpose of these was to determine whether the germinal crescent cells were vital for the growth of the embryo.

All factors except location of cauterization, were constant. Some eggs were merely opened but not cauterized. The controls were cauterized upon the right half of the germinal crescent; lateral areas where the areas pellucida and opaca merge; and posteriorly at the junction of the areas opaca and pellucida. Areas are shown in diagrams below:-



After the operation was completed the window was replaced by the original shell and sealed with melted paraffin. The eggs were returned to the incubator and the progress of development followed by daily "candling". At the end of six days incubation the eggs were opened and the embryos were fixed in Bouin's solution. Paraffin sections 8u thick were cut and stained with Acid-Hematoxylin-Eosin.

The instruments used consisted of a specially sharpened hack saw blade (for cutting the shell), electric lamp, convex lens for focusing the light upon the embryo, 8X hand lens for locating the embryonic structures, and a special cauterizer. The last mentioned is diagramed

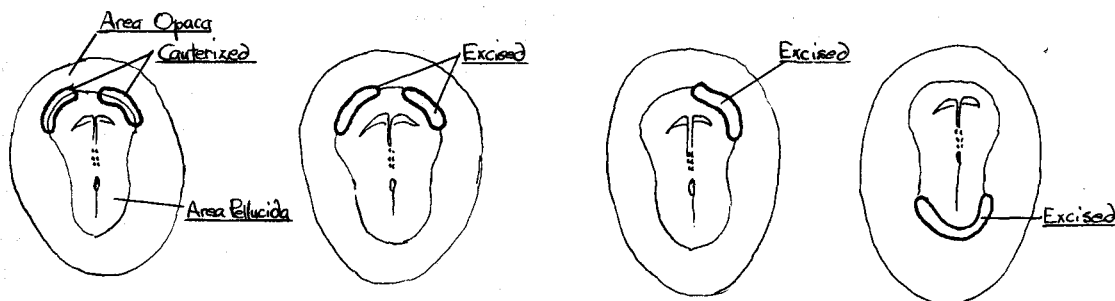


The results were unsatisfactory. The deformity of the operated embryos was excessive and the mortality very high, none survived to the age of six days.

Second Group of Experiments

White Leghorn eggs, cooled 24 to 48 hours, were used. These were obtained from John Benck, Blue Island, Illinois.

In this group of experiments it was desired to determine whether the cauterization or excision of the crescent wrought destruction upon the embryo through interference with the developing anterior vitelline veins. Therefore, the area of the germinal crescent which normally carries these structures was left intact while the right and left lateral wings of the germinal crescent were destroyed by either cauterization or excision with a fine pair of sterile scissors. Controls that left all or part of the migratory entodermal cells intact were made as follows; the right half of the germinal crescent was excised; the caudal portion of the junction of the areas pellucida and opaca and corresponding in extent to the germinal crescent was excised; some eggs were merely opened and resealed. The areas operated are diagramed below:-



A Spencer binocular dissecting microscope was used during the operation. In order to observe the progress of development of the embryo, the hole cut into the egg was sealed with a cover glass and melted paraffin.

The results did not solve the problem. The embryos died too early and presented great deformity.

Third Group of Experiments

A dozen pure-bred white leghorn hens and two cocks were housed on the roof of the medical building. Their diet consisted of grain and "chicken starter" daily, raw meat and "greens", each, once a week. Limestone was furnished for shell building. Their eggs, being collected every half hour, were placed without cooling in the incubator for development of 18 to 50 hours.

The results of the first two groups of experiments clearly showed that the degree of injury was too severe to allow the embryo to develop and that the vitality of the chicks was not sufficient to overcome the injury. Therefore, an attempt was made to localized the lesion while retaining the desired amount of destruction.

Cauterization was used and was tried with three intensities. The degree of cauterization was designated as light, medium, or heavy. In the light cauterization the hot cautery was delicately and quickly passed over the area of the germinal crescent so that the vitelline membrane and the blastoderm were visibly charred and coagulated. In the medium cauterization the blastoderm was charred through to the yolk, the hot cautery

being slowly applied and immediately removed when this had taken place. In the heavy cauterization the cautery was left in the lesion for a second so that destruction was caused to areas adjacent to the crescent. The temperature of the cautery was the least in the light cauterization, higher in the medium, (adjusted by the rheostat) and highest in the heavy.

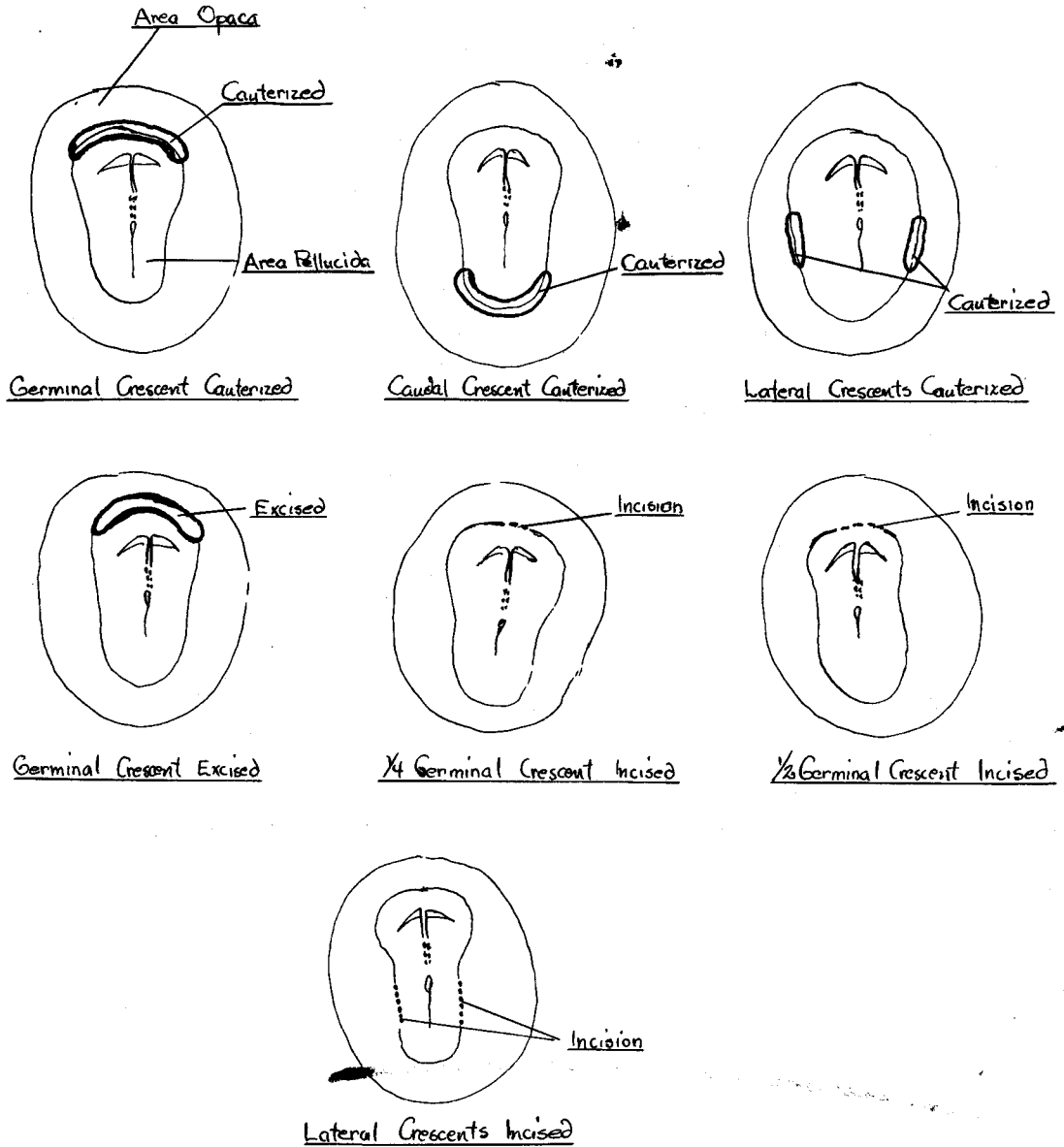
Then a variety of operations were tried in which the germinal crescent with its migratory entodermal cells was left intact but in which an interference with the developing vitelline circulation was produced by cauterization. These operations involved light, medium, and heavy cauterization of the caudal region corresponding to the germinal crescent; light and medium cauterization of the lateral areas of junction of the areas pellucida and opaca.

Finally some excision and incision experiments were undertaken. These eliminated the heat factor of the cautery. The following experiments were performed: excision of the entire germinal crescent with scissors; incision with scissors into $\frac{1}{2}$ to $\frac{1}{3}$ the extent of the germinal crescent, and incision into the lateral margins of the area pellucida. The incision into the crescent did not remove or destroy the migratory entodermal cells but did interrupt the future pathway of the anterior vitelline veins, hence this factor could be studied directly.

Some eggs were opened and sealed without operation.

The operated eggs were examined several times daily and fixed in Bouin's solution when life had apparently ceased. The criteria of death were the color and the heart action of the embryo. In some cases living

embryos were killed and fixed. Some whole mounts stained with Borax-Carmine were prepared. The areas operated are diagrammed below:-



RESULTS

First Group of Experiments

Three hundred and fifty two embryos were severely cauterized on the germinal crescent during the proliferation stage (18 to 24 hours incubation time). Examination of these embryos at the end of the six day period of incubation revealed that none had developed normally and that most were dead. Those that showed any signs of having survived the operation (36 in number) were small deformed masses of blister-like appearance. The vitelline circulation was deficient in structure and consisted mainly of collections of blood-islands; a small amount of blood being present in the entire embryonic structure. It was estimated that none had lived more than 3 to 5 days.

Those operations other than cauterization of the entire germinal crescent during the proliferation stage were performed to determine whether the absence of the migratory entodermal cells was responsible for the early death of the operated embryos.

Nineteen embryos were cauterized upon the entire germinal crescent region during 25 to 44 hours incubation, i.e., after the active proliferation of the migratory entodermal cells. At this age, the migratory entodermal cells have left the germinal crescent and are in the process of migration. The cells are not affected in this operation and the results must be attributed to other causes. Seventeen of these embryos did not live beyond 3 to 4½ days. One operated at 32 hours incubation and one at

Eighteen eggs were opened at 18 hours and resealed without operation. Twelve had normal living chicks at 6 days, 6 had dead and degenerated embryos. A mortality of 55% resulted from the mechanical disturbance of entering the shell.

Twelve were cauterized on the caudal margin of the area pellucida at 26 to 44 hours. Of these one was alive and seemingly normal at the end of six days incubation. This operation had no direct effect upon the germinal crescent or upon its cells. It gave some indication as to the ability of the embryos to withstand injury with increased age. The results were comparable to those operated in the earlier stages.

For the purpose of microscopic study 57 of the embryos cauterized upon the whole germinal crescent at 18 hours incubation time and fixed at the end of 6 days incubation time were sectioned. These specimens were taken at random from the large number operated. Two series showed gonads, four showed a germinal epithelium, and thirty-one series proved worthless because of too extensive malformation. Whenever a gonad could be demonstrated it was found to contain definitive sex cells and "transitional cell types". Those specimens containing a germinal epithelium showed it to be rudimentary in character. It is one cell layer in thickness and composed of cells suffering from the degeneration exhibited by the entire embryo.

In one series a gonad structure is attached to a mesentery. (Figure 1.) Definitive germ cells appear in the gonad. These cells are large with a round to elliptical nucleus. The nucleus contains sharply defined

chromatin masses. The cytoplasm stains lightly basophilic and is lighter in intensity than the other cells so that once recognized these cells are easily distinguished from others. (Figure 2.)

The other series showing gonads is markedly degenerate in its entirety. The gonads are small and loosely structured. The germinal epithelium contains transitional type cells. The gonad contains definitive germ cells. (Figures 3 and 4.)

Results of Second Group of Experiments

In a series of excision experiments, 18 embryos of 18 to 24 hours incubation time were operated for the removal of the right and left lateral wings of the germinal crescent. The heat effects of the cautery were eliminated and the most cephalic portion of the crescent was left intact for the purpose of providing some migratory entodermal cells and a path for the anterior vitelline veins. All were dead by $2\frac{1}{2}$ to 4 days of incubation time. The embryos appeared as indefinite masses of tissue. The vitelline vessels did not enter through the intact portion of the germinal crescent and those that had formed elsewhere were thin and deficient in structure. Six embryos were operated on this manner at ages ranging from 32 to 40 hours incubation time. None lived more than $3\frac{1}{2}$ to 4 days.

Cauterization was employed in a modification of the same operation. Twenty-eight embryos were cauterized in the lateral wings of the germinal crescent during the 18 to 25 hour stage. None survived the 4th day. Five were operated at ages ranging from 26 to 32 hours. All died before the

4th day. The anterior vitelline veins did not enter the uninjured part of the germinal crescent but attempted to form laterally to the operated area.

Four embryos of 18 hours incubation had their right half germinal crescent excised. None reached the 4th day.

Seven embryos ranging in age from 18 to 38 hours were excised in the tail region of the embryo at the junction of the areas pellucida and opaca. The size of this area was made to correspond to the germinal crescent in extent. All died on or before the 3rd day of incubation.

Concurrently a series of controls were run in which the shell was opened and sealed with a glass window. Forty-three were opened during 18 to 24 hours, two at 26 to 48 hours. Thirty-five died on or before the 3rd day and appeared similar to those suffering from the effects of cauterization. Eight survived from 4 to 18 days.

Third Group of Experiments

The use of eggs not older than one hour after laying and not cooled appreciably from the incubating temperature in conjunction with "light" cauterization of the entire germinal crescent enabled the operated chicks to survive for varying lengths of time from a few hours to hatching.

Sixty-five embryos were cauterized "lightly" upon the entire germinal crescent during the proliferation stage of the migratory entodermal cells, i.e., 18 to 24 hours incubation. Five died a few hours after the operation, 12 on the second day of incubation, 17 on the third day, 5 on

the fourth day, 4 on the sixth, 4 on the seventh, one each on the tenth, eleventh, twelfth, thirteenth, fifteenth, seventeenth days, and on three days after hatching. Nine were fixed in Bouin's solution while alive 6 to 24 hours after the operation.

The purpose of the last experiment was to determine the injury to the proliferating germinal crescent by "light" cauterization. Although the area passed over by the cautery was visibly coagulated and remained avascular, serial cross sections of this area were studied in order to determine the actual injury. The operation was performed at 18 hours and the chick was fixed at 40 hours incubation. The lesion penetrates the vitelline membrane and blastoderm down to the yolk and consists of an area devoid of the blastoderm and bordered by an abnormal proliferation of cells. These form a compact mass which appears to arise from the three germ layers. The cells are large and stain lightly. They have round to elliptical nuclei. Typical migratory entodermal cells were not seen. (Figures 5 and 6.)

A 4 day specimen in which the germinal crescent had been destroyed by "light" cauterization shows a well developed germinal epithelium containing stages of transitional cell types and several large precocious sex cells. These precocious cells possess a large to elliptical nucleus and faintly acidophilic cytoplasm. Degeneration is noted in the form of vacuolization and shrunken margins. (Figure 7.)

In another specimen that lived 6 days, we find gonads, the left of which is larger than the right. The germinal epithelium is fairly organ-

ized. It contains transitional cell types and degenerating precocious sex cells. The interior of the gonad is composed of transitional cell types, and degenerating precocious cells. (Figures 8 and 9.) Another chick, a 7 day specimen, having equally sized gonads presents a similar picture. (Figure 10.) Still another, a 10 day chick, shows an ovary containing primitive eggs. (Figures 11, 12, and 13.)

Continuing the study to determine the severity of injury necessary to destroy the germinal crescent without causing early death of the entire embryonic structure, 28 embryos were cauterized with "medium" intensity in the entire crescent during the proliferating stage (18 to 24 hours). The injury was much more severe and extensive than in the "light" cauterization. The crescent with some adjacent area was destroyed. Nine died shortly as a result of the procedure, 5 died on the second day of incubation, 10 on the third, 2 on the fourth, one on each of the fifth, and ninth days. Two of the 3 day specimens, one of the four day, and the five day chick were sectioned. The nine day specimen was lost. These specimens all suffered extreme degeneration, and only the five day specimen showed a rudimentary germinal epithelium of degenerated cells.

Nineteen embryos were cauterized "heavily" upon the entire germinal crescent during the 18 to 24 hour stage of incubation. The injury was very severe and involved the areas adjacent to the crescent. Four died shortly after the operation, two on the second day of incubation, eleven on the third day, one on the fourth, one on the sixth. Eight of these were sectioned for microscopic study.

Six of the 3 day embryos were sectioned. Three were retarded in development so that no germinal epithelium had as yet developed. They had the structure of embryos of about 55 hours incubation. Two were degenerate to the extent that structures could not be definitely identified. One showed a germinal epithelium composed of various sized cells. The entire embryo was composed of degenerated cells and the cells of the germinal epithelium were no exception.

The four day embryo showed a germinal epithelium composed of cells of various sizes (transitional stages). (Figure 14.) The six day embryo was so abnormally constructed that no structures could be positively identified.

In order to determine whether a lack of migratory entodermal cells was responsible for the high mortality of the operated chicks, a variety of control experiments were performed. Embryos older than the 18 to 24 hour proliferation stage when the primordial cells are supposed to have already left the crescent were used in some instances. In other cases operations in areas other than the crescent were made.

Thirteen embryos were cauterized "lightly" upon the entire germinal crescent at ages ranging from 26 to 48 hours. In these cases the migratory entodermal cells escaped injury, but the vitelline circulation was interfered with. Four died the same day, one on the second day of incubation, three on the third, one on the fourth, one on the sixth, one on the seventh, one on the tenth, one on the fourteenth. It was noted that when an embryo sufficiently advanced to have the anterior vitelline

veins formed was cauterized, a serious hemorrhage took place in this area. Those that survived for any length of time established efficient collateral circulation around the injured area. (Figures 15 and 16.)

Twelve embryos were treated similarly with "medium" intensity. They ranged from 26 to 48 hours in age. Here the results were very similar. Three died the same day, four died on the third day of incubation, one on the fourth day, one on the sixth, one on the tenth, one on the twelfth, two on the fifteenth day.

Twenty-one embryos of ages ranging from 26 to 44 hours were cauterized "heavily" upon the entire germinal crescent. Two died shortly, four on the second day of incubation, eleven on the third, two on the fifth, one on the sixth, one on the 15th. It was noted that for no apparent reason some occasional embryo could withstand the severe injury and proceed to advanced development. This could not be attributed to experimental error since "heavy" cauterisation always definitely produced severe injury.

Nineteen embryos ranging in age from 18 to 48 hours in incubation ages were "lightly" cauterized upon the caudal aspect of the embryos at the line of junction of the areas opaca and pellucida. The extent was of approximately the size of the germinal crescent. This operation could have no immediate effect upon the migratory entodermal cells. It interfered with the posterior aspect of the vitelline circulation. Yet the embryos failed to develop normally and showed high mortality. (Figure 17.) Five died on the same day, six on the second day of incubation, five on

the third day, one on each of the fourth, fifth, and sixth days. Those operated having the development of the posterior vitelline veins, suffered hemorrhage in this area.

Twenty-three embryos ranging in age from 18 to 50 hours were treated in a similar manner with "medium" intensity. Six died on the same day, four on the second day of incubation, ten on the third day, two on the fourth, one on the eighteenth.

Seventeen chicks were cauterized on the caudal aspect as before but with "heavy" intensity at ages ranging from 18 to 44 hours. Nine died soon after the operation, three on the second day, three on the third, two on the fourth.

Sixteen embryos of ages from 18 to 40 hours incubation were cauterized "lightly" at the lateral margins of the area pellucida where the vitelline arteries and veins later make their course. These blood channels are the largest and most important of the vitelline circulation. The results were as might be expected. None survived longer than $3\frac{1}{2}$ to 4 days. The chicks showed the same lack of nutrition as those suffering the effects of heavy cauterization of the crescent.

Twelve chicks were treated in a similar manner with "medium" cauterization. Their ages at the time of operation were from 18 to 29 hours. All except one died on or before the third day of incubation, one died on the fourth day.

Excision experiments that eliminated the heat of the cautery were made to determine the effect of the heat factor. Nine embryos of 18 to

28 hours incubation had their entire crescent area removed. Four died the same day, 5 on the second day, 2 on the third day. The heat factor appears of little significance from these results.

If the line of the germinal crescent were simply incised instead of bodily removed we would have the crescent cells left in the organism and the heat of cauterization eliminated. We would have just the mechanical interruption of the future vascular pathways. The most anterior portion of the germinal crescent was incised with fine scissors from $\frac{1}{4}$ to $\frac{1}{2}$ of the extent of the whole crescent. Ten chicks 18 to 24 hours old were incised to $\frac{1}{4}$ the extent of the crescent. Three died on the second day, two on the third, one on the fourth, one on the sixth, one on the ninth, one on the twelfth and one on the eighteenth. Eight chicks 18 to 24 hours old were incised to $\frac{1}{2}$ the extent of the crescent. Two died on the second day, three on the third, two on the fourth, one on the seventh.

Four embryos were incised at the lateral margins of the area pellucida at 29 hours. The interference with the developing vitelline veins was too great to be overcome. All were dead on or before the third day.

Concurrently with all the above procedures eggs were simply opened and sealed with a glass window. Forty-five were thus treated at ages ranging from 18 to 46 hours incubation. Four hatched into normal chicks, the rest survived anywhere from one to twenty days incubation before dying.

DISCUSSION

Previous workers with "crescent castrated" chicks (Reagan '16, Dantschakoff '51, Goldsmith '35) described material of 3 to 5 days incubation. This is too young to allow the differentiation of sex cells, especially, since general development of the chick is retarded by experimental manipulation.

Our experiments were directed toward growing "crescent castrated" chicks of at least six days incubation. This length of time would allow differentiation of the sex cells.

Earlier workers observed that cauterization or excision of the entire germinal crescent produced sterile gonads, in some cases a lack of posterior development in the prospective gonad region, and a detrimental affect upon the formation of the vitelline circulation. These effects were attributed as most likely due to a lack of migratory entodermal cells, while the operative injury as the causative agent was not given the consideration it deserved.

In our work it was found that whenever a gonad could be demonstrated it contained definitive sex cells. Those specimens that had failed to produce demonstrable gonads and definitive sex cells failed not only in this respect, but also in the formation of other organs to a proportionate degree. There was no demonstrable selective action on the gonads. The area vasculosa appeared to suffer the most. The anemic appearance of the area vasculosa and the stunted development of the

vitelline plexuses was characteristic of all the degenerated specimens. The faulty nutrition resulting thereby accounts for the degeneration. In contrast, those embryos that overcame the shock of the operation showed vigorous development of the vitelline vessels and very little avascularization of the vitelline area.

The location of the injury within the scope of our experiments was not the deciding factor in the survival of the chick. The germinal crescent was not any more vulnerable than the areas lateral or caudal to the embryo, in fact, the lateral areas were the most sensitive. The method of injury was not important. The intensity and extent of injury decided the fate of the specimen. Embryos operated at advanced ages showed somewhat greater viability after operation than the younger ones did. In some cases the mere sealing and opening of the shell with no other operation was sufficient to cause early death. These observations led to the conclusion that a lack of migratory entodermal cells could hardly be responsible for the great mortality of "crescent castrated" chicks. Tables 1 and 2 show the chances of the embryos to live six days under the various conditions of the experiments.

Those embryos that preceded to advanced stages of development (6 days or more) overcame the effects of the operation in the same manner regardless of the operation performed. The developing vitelline plexuses swung around the site of the injury so that very little of the vitelline area was left avascular.

It was found that eggs of the third group of experiments withstood

injury to a much greater extent without fatal consequences than those used in the first two groups. This appears to be due to the uninterrupted incubation of the third group so that the highest possible vitality was preserved. General resistance of the embryo to the shock of the operation and manipulation was therefore a very important factor in the survival of the operated specimen.

S U M M A R Y

1. Nine hundred and fifty two embryos were operated in various procedures, and of these, 101 were sectioned.
2. Male and female gonads containing germinal epithelium, transitional cell stages, and definitive germ cells were obtained in chicks having their entire germinal crescent cauterized during the migratory entodermal cell proliferation stage (18 to 24 hours incubation).
3. Whenever a gonad could be demonstrated, it was found to contain definitive sex cells.
4. Those that failed to produce demonstrable gonads failed in the formation of every organ to a proportionate degree.
5. The operative factors responsible for the early death of the experimental embryos were the extent and intensity of the injury.
6. The experiments performed tend toward the conclusion that the abnormality produced by cauterisation or other injury to the crescent is due to interference with the developing vitelline circulation.

T A B L E 1

Chick Embryos Surviving Six or More Days of Incubation

Group I Experiments

Cauterization of cephalic crescent at 18-24 hrs.	None survived
Cauterization of cephalic crescent at 26-48 hrs.	1 out of every 19
Cauterization of Rt. $\frac{1}{2}$ cephalic crescent at 18- 24 hrs.	1 out of every 19
Cauterization of caudal crescent at 18-24 hrs.	1 out of every 9
Cauterization of caudal crescent at 26-48 hrs.	1 out of every 12
Cauterization of lateral crescents at 18-24 hrs.	None survived
Controls at 18-48 hrs.	2 out of every 3

Group II Experiments

Excision of parts of cephalic crescent at 18-24 hrs.	} None survived
Cauterization of parts of cephalic crescent at 18- 24 hrs.	
Cauterization of parts of cephalic crescent at 26- 48 hrs.	
Excision of Rt. $\frac{1}{2}$ cephalic crescent at 18-24 hrs.	
Excision of caudal crescent at 18-36 hrs.	
Controls	1 out of every 9

TABLE 1

Group III Experiments

Light cauterization of cephalic crescent at 18-24 hrs.	1:5	} 1 out of every 5
Light cauterization of cephalic crescent at 26-48 hrs.	1:5	
Medium cauterization of cephalic crescent at 18-24 hrs.	1:25	} 1 out of every 7
Medium cauterization of cephalic crescent at 26-48 hrs.	1:5	
Heavy cauterization of cephalic crescent at 18-24 hrs.	1:15	} 1 out of every 10
Heavy cauterization of cephalic crescent at 26-48 hrs.	1:7	
Light cauterization of caudal crescent at 18-24 hrs.		1 out of every 19
Medium cauterization of caudal crescent at 18-48 hrs.		1 out of every 25
Heavy cauterization of caudal crescent at 18-48 hrs.		None survived
Light cauterization of lateral crescents at 18-24 hrs.		None survived
Medium cauterization of lateral crescents at 18-24		
	hrs.	None survived
Incision into $\frac{1}{2}$ extent of cephalic crescent at 18-		
	24 hrs.	2 out of every 5
Incision into $\frac{1}{3}$ extent of cephalic crescent at 18-		
	24 hrs.	1 out of every 8
Incision into lateral crescents		None survived
Controls		4 out of every 5



PLATE I

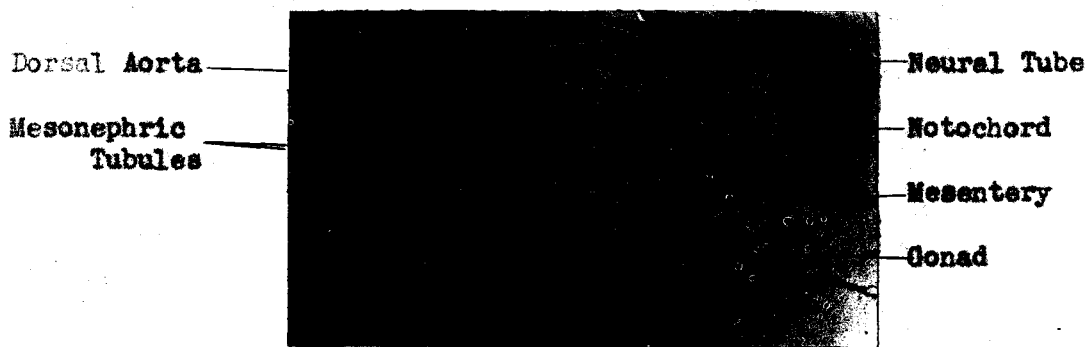


Figure 1. Section through the gonad region of an embryo that was cauterized on the entire germinal crescent at 18 hours incubation and fixed at the end of 6 days incubation. X25

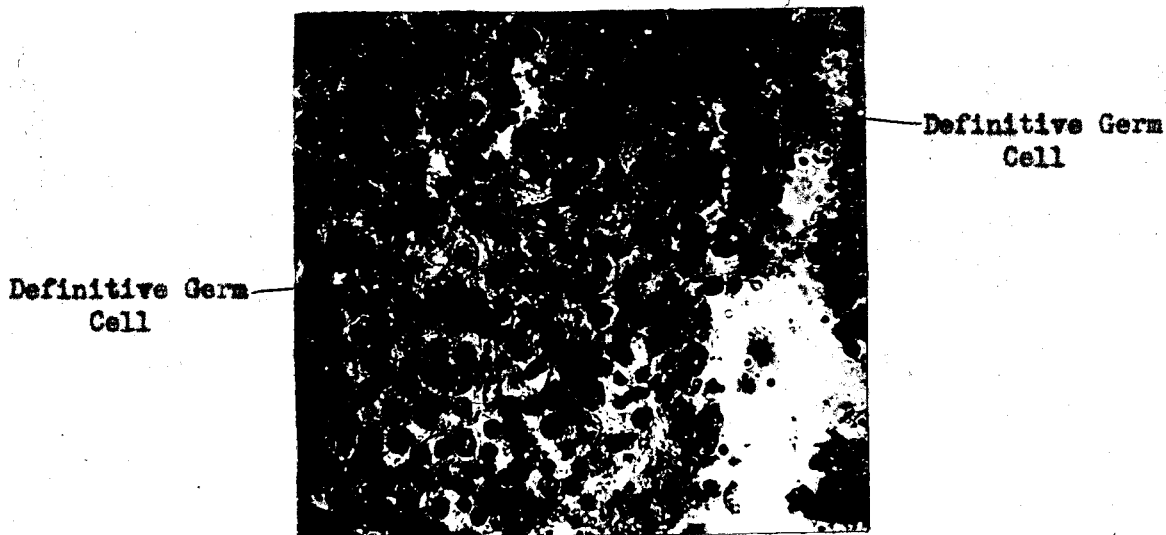


Figure 2. High power micro-photograph of the gonad structure in figure 1. X1336

PLATE II

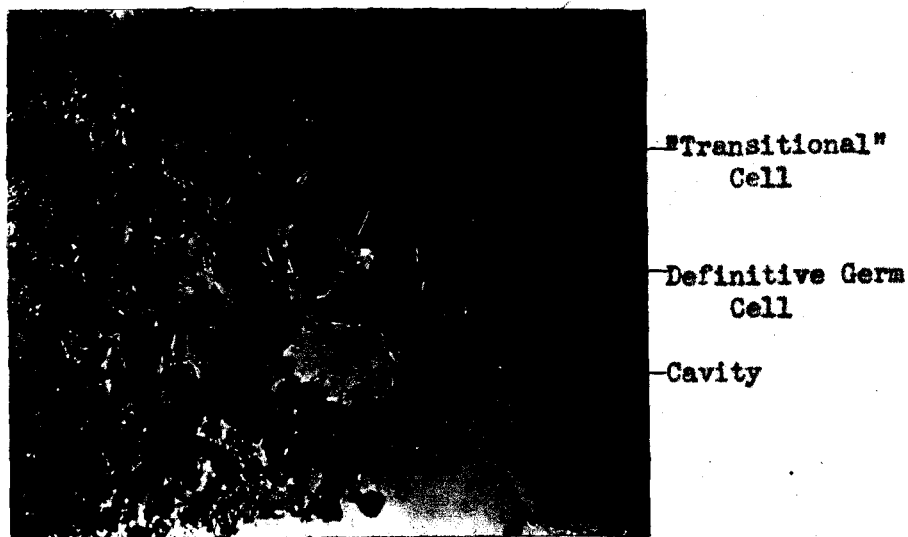


Figure 3. Section through the gonad of an embryo that was cauterized on the entire germinal crescent at 18 hours incubation and fixed at the end of 6 days incubation. X1588

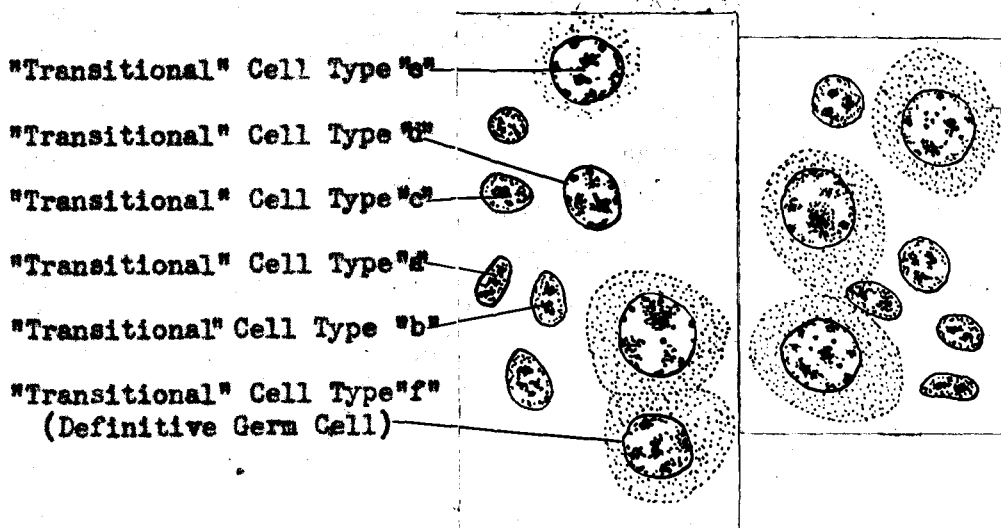


Figure 4. Camera lucida studies of cell types found in the gonad of figure 3. X2550

PLATE III



Figure 5. Section through lesion produced by "light" cauterization. This embryo was cauterized "lightly" on the entire germlinal crescent at 18 hours incubation and fixed at 40 hours incubation. X25

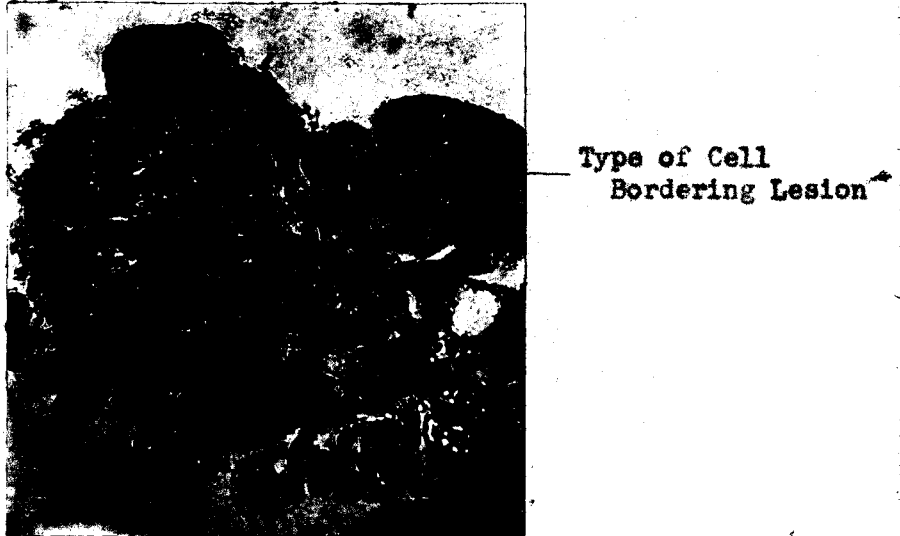


Figure 6. High magnification of the area indicated in figure 5. X1536

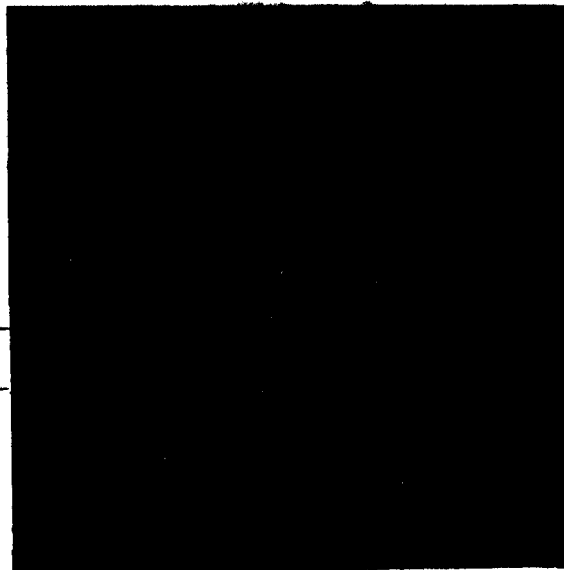
PLATE IV



Precocious Germ Cell

"Transitional" Type Cells

Figure 7. Section through the germinal epithelium of a 4 day chick embryo in which the entire germinal crescent was "lightly" cauterized at 22 hours of incubation. X1836



Neural Tube

Notochord

Dorsal Aorta

Mesonephros

Right Gonad

Liver

Mesonephros

Left Gonad

Figure 8. Section through the gonad region of a 6 day chick embryo in which the entire germinal crescent was "lightly" cauterized at 24 hours of incubation. X25

PLATE V

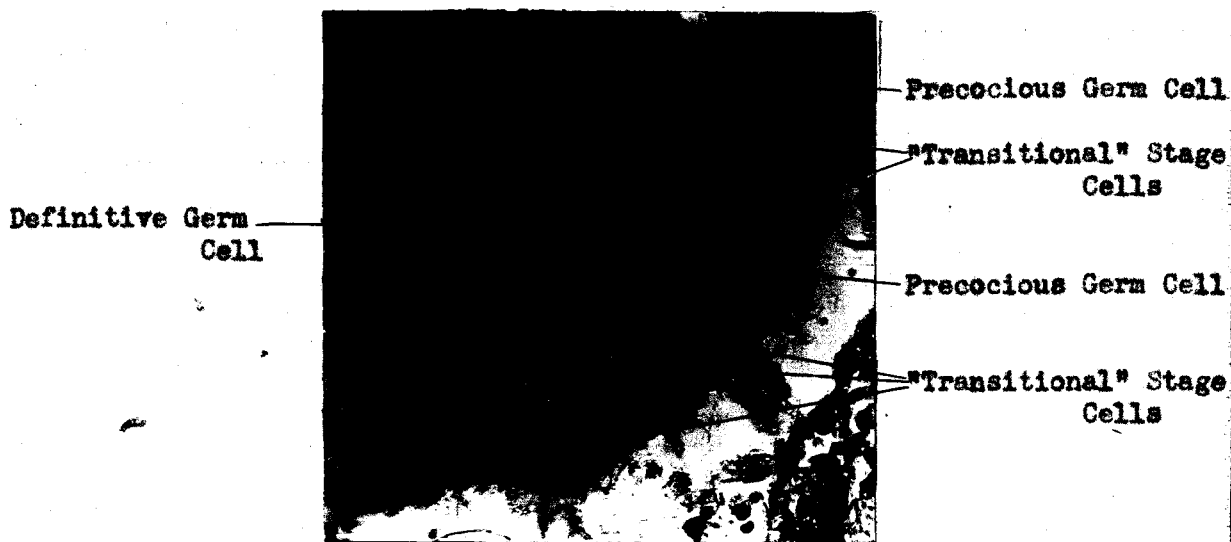


Figure 9. High magnification of the left gonad shown in figure 8. X1556



Figure 10. Section through the gonad of a 7 day chick embryo in which the entire gerainal crescent was "lightly" cauterized at 24 hours of incubation. X1556

PLATE VI

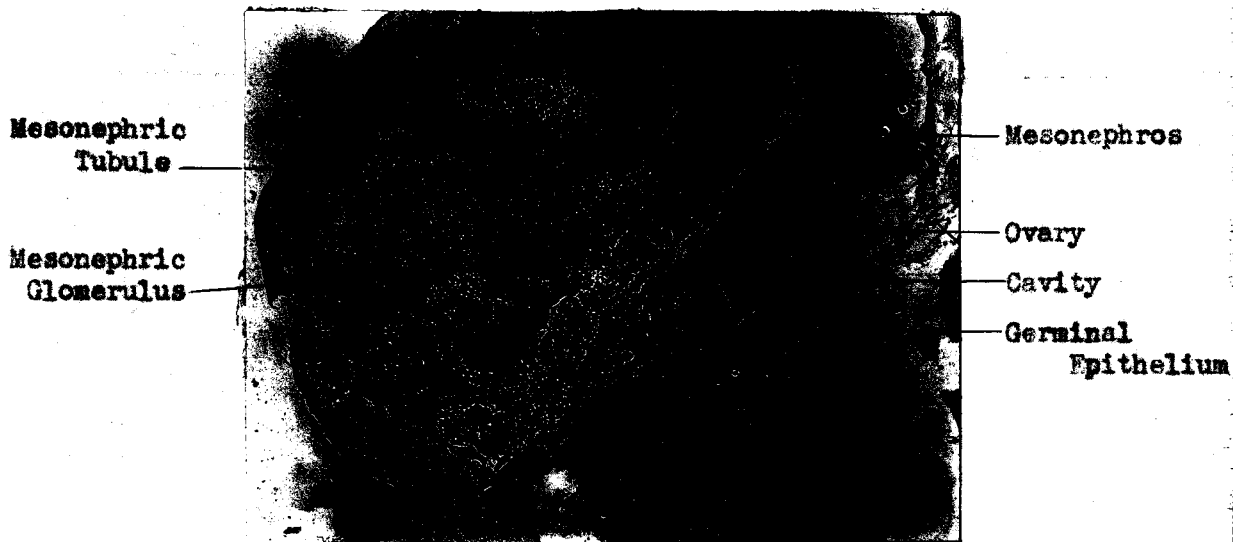


Figure 11. Section through the ovary of a 10 day chick embryo in which the entire germinal crescent was "lightly" cauterized at 18 hours of incubation. X40

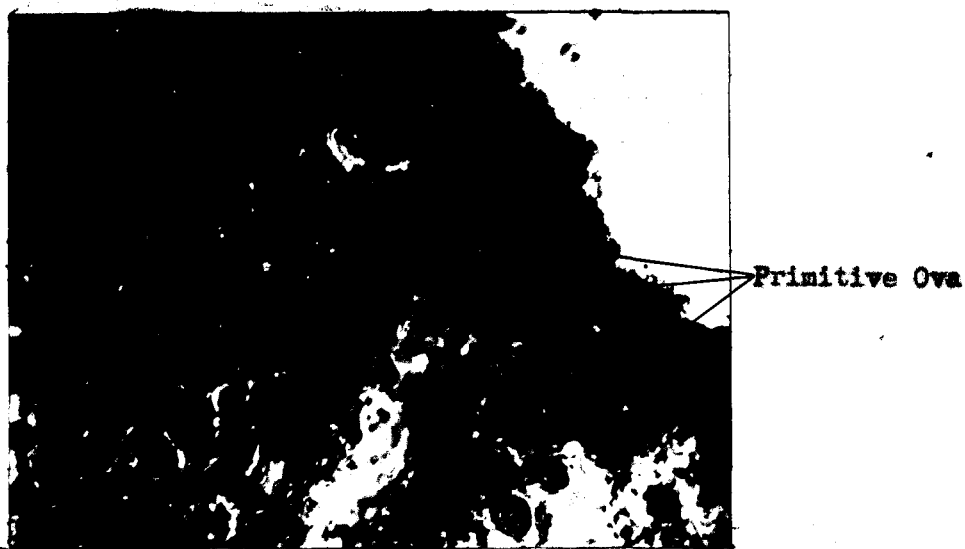


Figure 12. High magnification of ovary shown in figure 11. X1952

PLATE VII

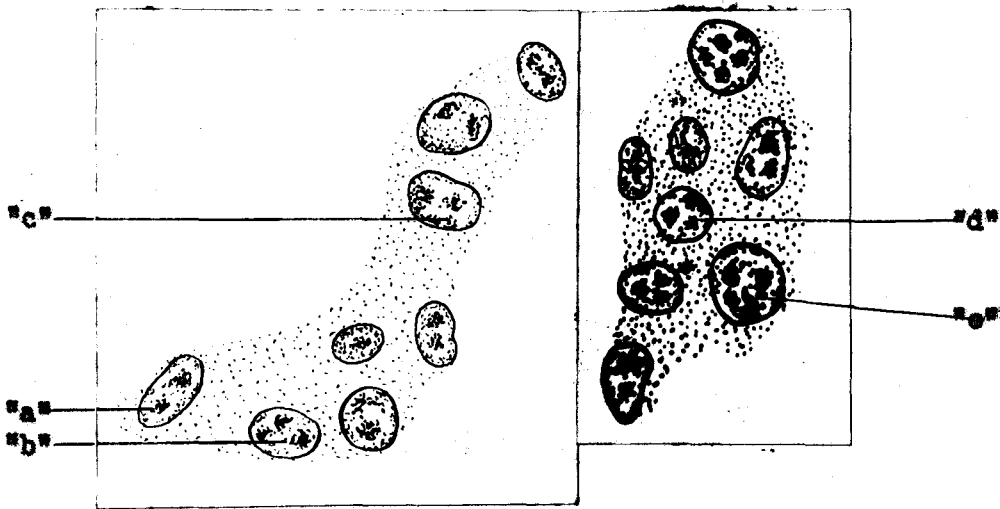


Figure 13. Camera lucida studies of "Transitional" cell types found in the ovary of figure 11. X2550

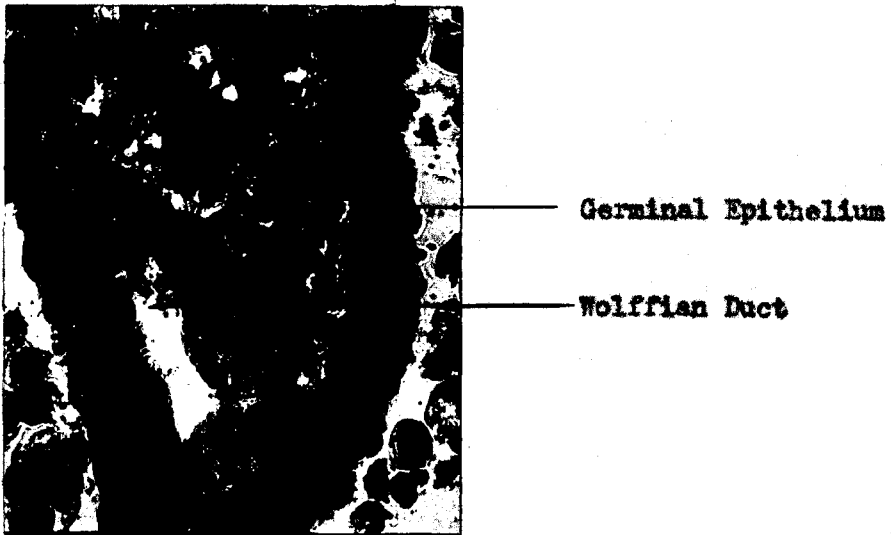


Figure 14. Section through the germinal epithelium of a 4 day chick embryo in which the entire germinal crescent was "heavily" cauterized at 24 hours incubation time. X1556

PLATE VIII

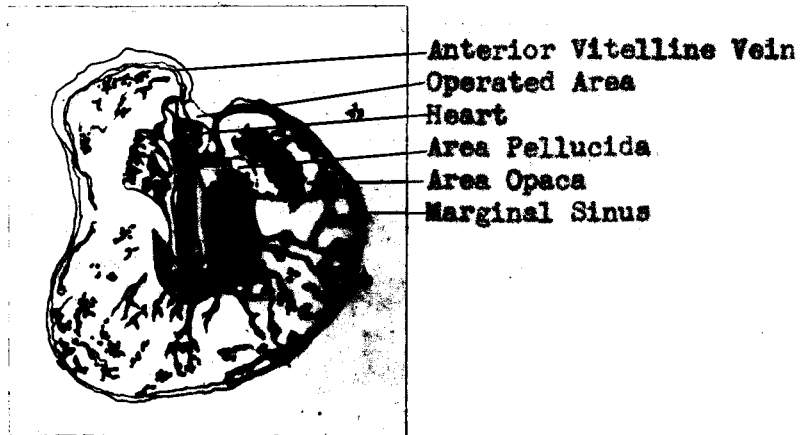


Figure 15. Reproduction of whole mount of 90 hour chick embryo. The germinal crescent was cauterized with "medium" intensity at 18 hours of incubation. This embryo showed the retarded development and failing vitelline circulation caused by destruction of the germinal crescent. X4

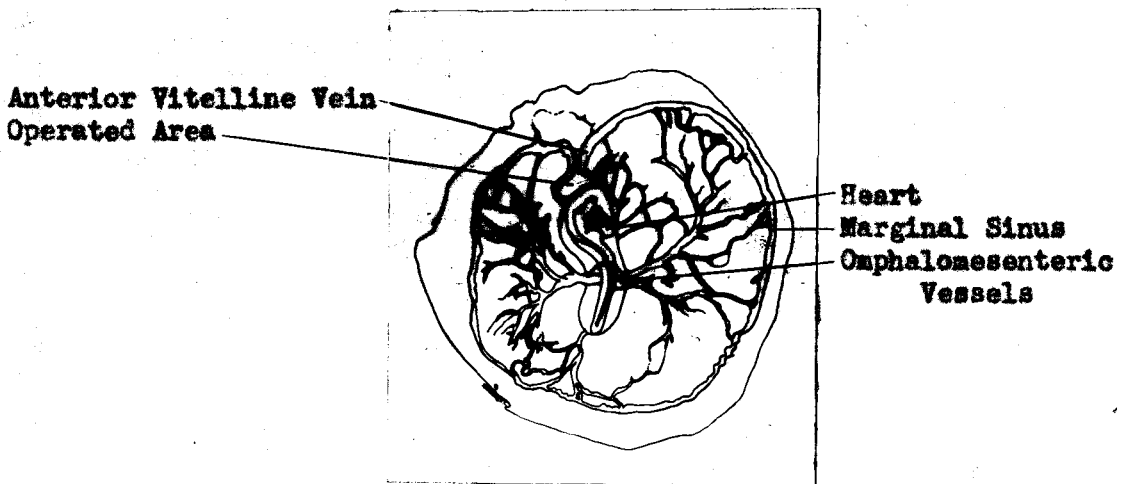


Figure 16. Reproduction of whole mount of 50 hour chick embryo. The germinal crescent was "lightly" cauterized at 18 hours of incubation. This embryo established efficient vitelline circulation and proceeded to develop in a fairly normal manner. X4

PLATE IX

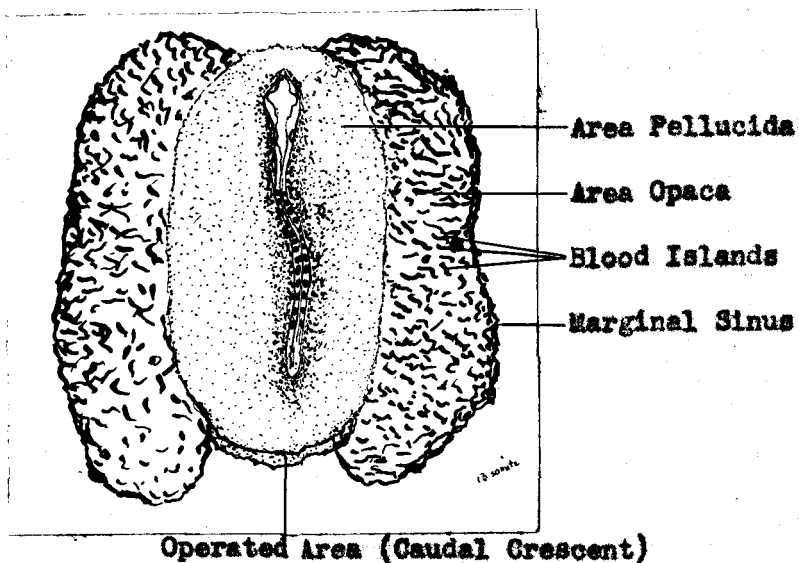


Figure 17. Reproduction of life-sketch of 48 hour chick embryo in which the caudal aspect of the junction of the areas pellucida and opaca was "lightly" cauterized. Development was retarded and the vitelline circulation was affected in a manner similar to that produced by cauterisation of the germinal crescent. X6

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