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THE EFFECT OF P-32 IRRADIATION ON THE ECTODERMAL AND MESODERMAL COMPONENTS OF THE DOWN FEATHER IN THE CHICK

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THE EFFECT OF P-32 IRRADIATION ON THE ECTODERMAL AND MESODERMAL COMPONENTS OF THE DOWN FEATHER IN THE CHICK

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CHAPTER I

INTRODUCTION

The down feather germ in the chicken embryo is a rapidly developing organ, making its appearance about the seventh day of incubation when most of the other organs have already become established, and attaining its definitive form by the fourteenth day of incubation (Davies, 1889). It has been reported that rapidly differentiating structures are especially sensitive to irradiation (Russell, 1950; Schneller, 1951), but there is nothing known about the response of chick embryos to irradiation in the period when the various components of down feather germs are becoming organized. This study is an investigation of the response of the down feather germ to irradiation by P-32 applied during the period of rapid differentiation. The down feather germ is an excellent organ for experimental research, in that it is readily accessible and its development and organization can be followed throughout its life history.

CHAPTER II

SURVEY OF THE LITERATURE

The use of radioactive agents as tools of research in the field of experimental embryology has been recorded in the literature for many years. Two outstanding motives seem to have guided investigators in this field: the desire to study the nature of alterations produced in living cells by irradiation, and the desire to produce the abnormal in an effort to better understand the normal (Butler, 1936). The bulk of the information published prior to 1936 has been compiled in two volumes edited by Duggar (1936).

The exact nature of the changes produced by irradiation in cells or tissues is still not known. The visible effects, however, are well known from the cytological and histological standpoint (Bloom, 1948). The changes brought about by irradiation of biological material are various and there are conflicting conclusions in the literature about radiosensitivity of cells, tissues, organs, organ systems, or the whole animal. It appears that no one change can be attributed to irradiation alone because of the large number of variables in biological material.

With regard to the effect of irradiation on embryonic development, the law of Bergonie and Tribondeau (1906) states that the sensitivity of cells varies directly with the reproductive capacity and

inversely with their degree of differentiation. This assumption that the sensitivity of an embryo should decrease with age was not borne out in the case of the chick. It was found by Strangeways and Fell (1927) that the younger embryos were less sensitive to x-rays than were the older ones. They concluded that the irradiation effect was secondary, due to certain physiological changes in older embryos which were not present in the younger embryos because of the lower level of organization. Except for the chick, there is a gradual increase in resistance to x-ray and radium as development of an organism progresses (Butler, 1936).

Bloom (1948) stated that x-ray, beta ray, fast or slow neutrons, and gamma rays produce the same biological effect when the irradiation is applied in comparable doses. One exception was noted, the response of bone marrow of the metaphysis, in which the greater effectiveness was from x-ray. This effect was thought to be from secondary rays, resulting from the x-rays striking the bone spicules, rather than from the primary rays. He also noted that the effect of internal irradiation was not distinguishable from external irradiation. Damage to an area selectively absorbing an isotope was greater than to any other part of the animal, but if localized external irradiation from another source was administered to another specimen, in the same area, the damage in the two animals was not distinguishable.

According to Butler (1936), the most commonly reported effect of irradiation is general retardation of development. Warren and Dixon (1949), in their studies of the effect of P-32 on chick embryos and de-

veloping chicks, stated that irradiation caused an overall growth retardation, resulting in small but well-proportioned birds. Dixon (1952) reported retardation of growth to be an overall effect, with no one tissue or system more affected than another, and that there were no deformities or malformations attributable to irradiation by P-32.

S. L. Warren (1936) stated that organs and organ systems exhibited differential sensitivity to irradiation. Butler (1930) found by non-lethal x-irradiation of 48 hour chick embryos that the cells of the neural tube showed a greater sensitivity than did other cell types. The most obvious result of irradiation was the complete disappearance of mitotic figures within a very few minutes. He noted that all of the mitotic divisions in actual progress at the time of irradiation completed the process but that the irradiation prevented the initiation of any further mitosis. The cells later regained to a limited extent their mitotic abilities. The lethal irradiation produced a complete cessation of mitosis, the nuclei pycnosed, and finally the cells died and disintegrated.

Bagg (1922), in his studies on the developmental disturbances produced in rat embryos by injecting the mothers with radium salts, found that developmental arrests of the nervous, gonadal, and vascular systems were characteristic. He also reported considerable hemorrhaging into the subcutaneous connective tissue of the embryos. He suggested that the irradiation interfered with the formation of vascular endothelium.

Brunst (1950) exposed the developing limbs of larvae of Siredon

mexicanum to x-ray and found a complete suppression of growth caused by complete arrest of cell proliferation. However, differentiation of the limb tissues took place. Rugh (1949) reported an inhibition of mitotic activity in irradiated salamander larvae. He noted severe damage to the central nervous system. In other studies (1950) he found the same results in irradiated frog embryos.

The degenerative changes in cells caused by irradiation are well known and have been reported in the literature for several decades. These effects were summed up by Bloom (1948). They include nuclear and cytoplasmic vacuolation, dissolution of cell nuclei, clumping of nuclear chromatin, stickiness of chromosomes in mitotic activity, pycnosis of nuclei, edema of tissues, and hemorrhagic response in tissues.

There is an abundance of literature concerned with radiosensitivity and radioresistance of organs and organ systems of animals. S. L. Warren (1936) has ranked the tissues and organs of animals according to their sensitivity to total body irradiation. Heading his list of sensitive tissues are bone marrow cells, intestinal crypt epithelium, lymphocytes, and lymphoid structures. The most resistant cells listed are adult thyroid, adult pituitary, and brain and nerve cells.

Stinson (1953) found that the femora of 12 and 13 day old chick embryos, irradiated on the 10th day of incubation by P-32, were differentially retarded, the lengths being normal but the diameters smaller.

The development and morphogenesis of the down feather germ in the chicken have been described by Davies (1889), Watterson (1942), and Goff (1949). Holmes (1935) described the feather tracts in the chicken,

pointing out that the conical primordia arise in a definite sequence and in definite tracts, each containing a definite number of feather-forming loci.

CHAPTER III

MATERIALS AND METHODS

The P-32 used in this study was procured from Oak Ridge National Laboratory as H_3PO_4 in weak HCl. The isotope was standardized by the National Bureau of Standards method, using a radium D and E beta ray standard. The stock solution was neutralized by the addition of NaOH. For the purpose of a visual check on the placement of the isotope in the amniotic cavity the solution was colored with a physiologically inert dye, 0.1% Evans blue. This use of color was also helpful in detecting possible contamination of laboratory equipment.

White Leghorn eggs from a commercial source were used. A total of 470 eggs was incubated at 37.5° ± .3° C. at a relative humidity of approximately 62.8%. The eggs were rotated three times daily until the time of operation, after which no eggs were turned. Three hundred and forty-two embryos survived until sacrifice. Of these, 225 were untreated controls; 99 were sham-injected controls; and 18 were experimental embryos. There was a 92% survival of untreated controls, 54% of sham-injected controls, and 45% of experimentals.

The following is the operative procedure for the experimental and sham-injected control embryos. The position of the embryo in each egg was determined by candling and marked by pencil on the shell. This

area was sterilized by swabbing with 70% alcohol. A window approximately an inch in diameter was cut in the shell by means of an abrasive wheel mounted on a flexible drive shaft. The small piece of shell was gently lifted out without injury to the chorio-allantoic and shell membranes. The shell membrane was peeled away, exposing the chorio-allantoic membrane. The injection needle was positioned into an opening torn in the chorio-allantois; the amnion was grasped with forceps and pulled over the needle and an aliquot of 0.1 ml. of the isotope was injected into the amniotic cavity. This procedure, plus the visual check by means of the colored solution, made certain that irradiation was applied uniformly to all embryos. The window was then sealed with a glass cover slip and melted paraffin, and the egg was allowed to continue incubation.

Injections were made on the tenth day of incubation, and irradiation was allowed for four days and two hours. The initial dose was 519 microcuries. With adjustment made for half-life of the isotope, the dose averaged 485 microcuries for the period. The approximate total activity was 6.3×10^{12} disintegrations per embryo. The solution used for the sham-injected controls was handled in exactly the same manner as that of the experimentals except that ordinary phosphorus was used.

Two classes of control embryos were employed. One which was left untouched was designated as untreated control embryos; the other was injected with ordinary phosphorus and was designated as sham-injected control embryos. Sacrifice of experimental embryos was made at 14 days and 2 hours incubation. Sacrifice of control embryos was begun at 10 days incubation and continued at half-day intervals throughout the

period of irradiation of the experimental embryos. This wide range of ages in the control embryos was needed for comparison with experimental embryos which might show varying degrees of retardation.

At sacrifice of all embryos the cover glass was removed and gross examination of the embryo was made through the window. The living embryos were then removed from their shells and severed from their extra-embryonic membranes. Further observations were made and fixation was accomplished with Orth's fluid for twenty-four hour periods. Embryos were injected intraperitoneally with the fixative to assure complete fixation. After chromation the embryos were washed in running tap water and then stored in 10% formalin. The fixation technique was taken from R. D. Lillie (1948). At the time of fixation the wings were removed and flattened.

The second down feather germ, numbering from the proximal end of the primary region on the left wing of the embryo, was used for the sake of consistency in comparing the three classes of embryos. These feather germs were removed from the fixed wings, placed in a water mount, and their lengths measured from the base of the follicle to the tip, using a calibrated ocular micrometer.

The feather germs were then dehydrated and embedded in paraffin. Infiltration was accomplished by the use of a vacuum oven (Faulkner, 1953). Serial cross sections were cut at seven micra, and the tissues were stained by hematoxylin and eosin. Microscopic observations of the feather germ components were then made.

For making cell counts, the number of sections obtained from

the feather germ was counted. Cell counts were made from a representative number of these sections. In shorter germs the cells in every second section were counted, while in the longer germs counts were made less frequently, for example, every fifth or every tenth section, depending on the number of sections obtained from the germ. The nuclei of the cells of the various components were counted and these numbers were used as the total number of cells for a given section. The total number of cells for a given component of the entire germ was obtained arithmetically.

CHAPTER IV

I. GENERAL OBSERVATIONS

The irradiated germs appeared as curled, flattened, and wrinkled structures. Upon inspection it was found that some germs had hemorrhaged internally, although this condition was not found consistently. It was calculated that 27% of the experimentals showed hemorrhaging, as opposed to 3% of the untreated controls and 1% of the sham-injected controls. Yet with respect to all these characteristics, no two experimental germs appeared to be affected in the same manner.

In order to determine the existence of specific sensitivity, it was necessary to establish the developmental progress of each embryo. The criteria of Hamburger and Hamilton (1951) were employed. However, it was not possible to assess the developmental achievements of the experimental embryos by their method because within a given embryo the characters used in staging were not all in the same stage of development, even though the experimental embryos were all 14 days and 2 hours old. It was necessary to find some other basis for comparison and a logical choice seemed to be the length of the wing. Not only is wing length a character closely associated with the primary feather germs, but the wing was a well established structure at the time of the experimental period of this investigation and therefore was less likely

to be specifically affected by irradiation. In order to validate this choice of wing length as the basis for comparison, the lengths of the wings of the control embryos were compared to their chronological ages. Using a wide field microscope, wing lengths were measured from the crotch of the alula to the distal tip of the wing, and these measurements versus the chronological ages were plotted on a graph (Fig. 3). It was found that developmental progress was directly correlated with chronological age. For example, untreated controls which were 14.30 days old had a range in wing lengths from 7.3 to 8.0 mm. and sham-injected controls 14.30 days old had a range from 7.0 to 7.9 mm. in length. In most cases wing lengths within a given age group varied less than one millimeter.

On the other hand, the experimental material, with a wing length range from 5.7 to 7.5 mm., showed no predictable relationship between age and length of wing. For example, an experimental embryo 14.00 days old had a wing length of 5.7 mm. and another 14.08 days old had a wing length of 7.5 mm., a difference of 1.8 mm. In controls, the group of embryos with this range in wing lengths would include embryos from 11.50 to 14.50 days of incubation.

To make comparisons between experimental and control groups, control embryos with wing lengths comparable tc the experimentals were grouped (Table 1). This includes an age range for the sham-injected control group of 11.92 to 14.38 days, and 11.46 to 14.55 days for the untreated control group, as compared with the 13.97 to 14.13 days of the experimental group.

In order to determine whether an observed difference between the chronological ages of embryos with comparable wing lengths is of such a magnitude that it cannot be attributed to chance, a statistical analysis of the data was made. The "t" test of significance was used. The results showed a significant difference at the 5% level for the comparison between the experimental and untreated control and 1% between experimental and sham-injected control groups. Controls with wing lengths comparable to experimentals were, on the average, about a day younger (Table 1). This indicates that the lengths of the wings of the experimental embryos have been retarded in relation to their age.

When comparing the wing lengths and feather germ lengths, an almost direct relationship was found between the length of the wing and the length of the feather germ in both of the control groups (Fig. 1). Correlation in the experimental group, however, appeared erratic. For instance, a wing 7.1 mm. long had a second primary 9.84 mm. long, while one 7.5 mm. long had a feather germ length of 4.85 mm. The experimental embryos were segregated into groups differing in their response to irradiation. Segregation was made on the basis of wing length, even though it was demonstrated that it was retarded. Theoretically that character is less likely to be specifically affected by irradiation. Wing length groups in the control embryos tend to represent age groups as shown by comparisons to other staging criteria.

The data were analyzed, as above, using wing lengths and feather germ lengths from the three classes of embryos. The differences were significant between the experimental and sham-injected control groups and the experimental and untreated control groups at the 5% level. The

results of comparison between sham-injected control and untreated control groups were not significant (Table 1).

To give a graphic representation of the relationship of the feather germ length to the length of the wing, the percentage of wing length (length of germ divided by length of wing multiplied by 100) was plotted in each wing length (age) group (Fig. 2). It can be seen that in all but one case the feather germ lengths were much shorter in the experimentals than in the controls with identical wing lengths.

On the basis of the observations presented here it is inferred that the down feather germ is specifically sensitive to irradiation in that the feather germ, as a result of irradiation, is differentially retarded in growth as compared to the length of the wing.

The general observations revealed that the germs had not developed normally but shed little light on the actual process involved in retardation. Therefore, the problem was further pursued from the microscopic level.

Fig. 1.--Comparisons between lengths of wings and lengths of feather germs in the three classes of embryos.

CC Untreated Controls
C Sham-injected Controls
E Experimental

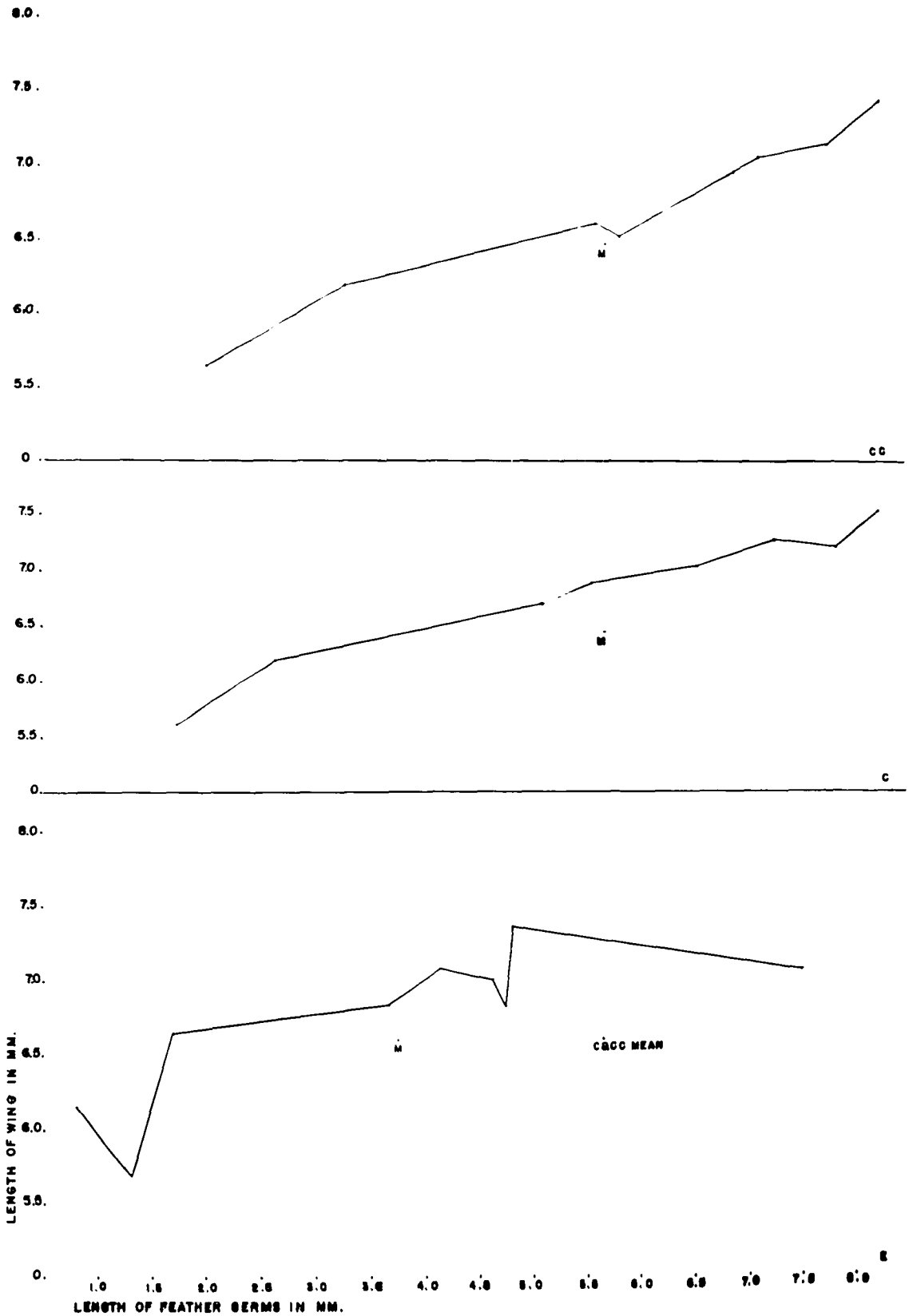
8.0.
7.5.
7.0.
6.5.
6.0.
5.5.
0.
M
CC
7.5.
7.0.
6.5.
6.0.
5.5.
0.
M
C
8.0.
7.5.
7.0.
6.5.
6.0.
5.5.
0.
M
C&CC MEAN
LENGTH OF WING IN MM.
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
LENGTH OF FEATHER GERMS IN MM.

FIGURE 1

Fig. 2.--Percentage relationship of feather germ length to wing length.

● Untreated Controls
□ Sham-injected Controls
○ Experimentals

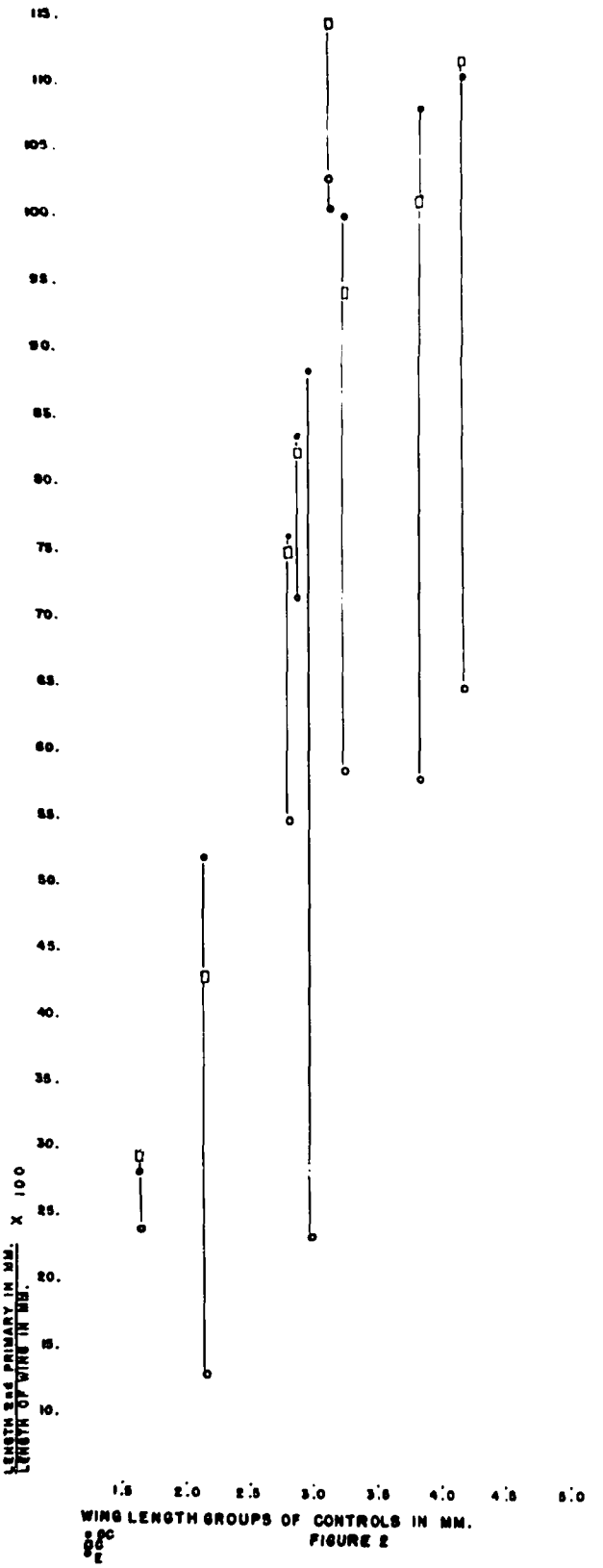
LENGTH 3rd PRIMARY IN MM.
LENGTH OF WING IN MM.
X 100
WING LENGTH GROUPS OF CONTROLS IN MM.
SC
DC
E

FIGURE 2

Fig. 3.--Relationship of wing length to chronological age in control embryos.

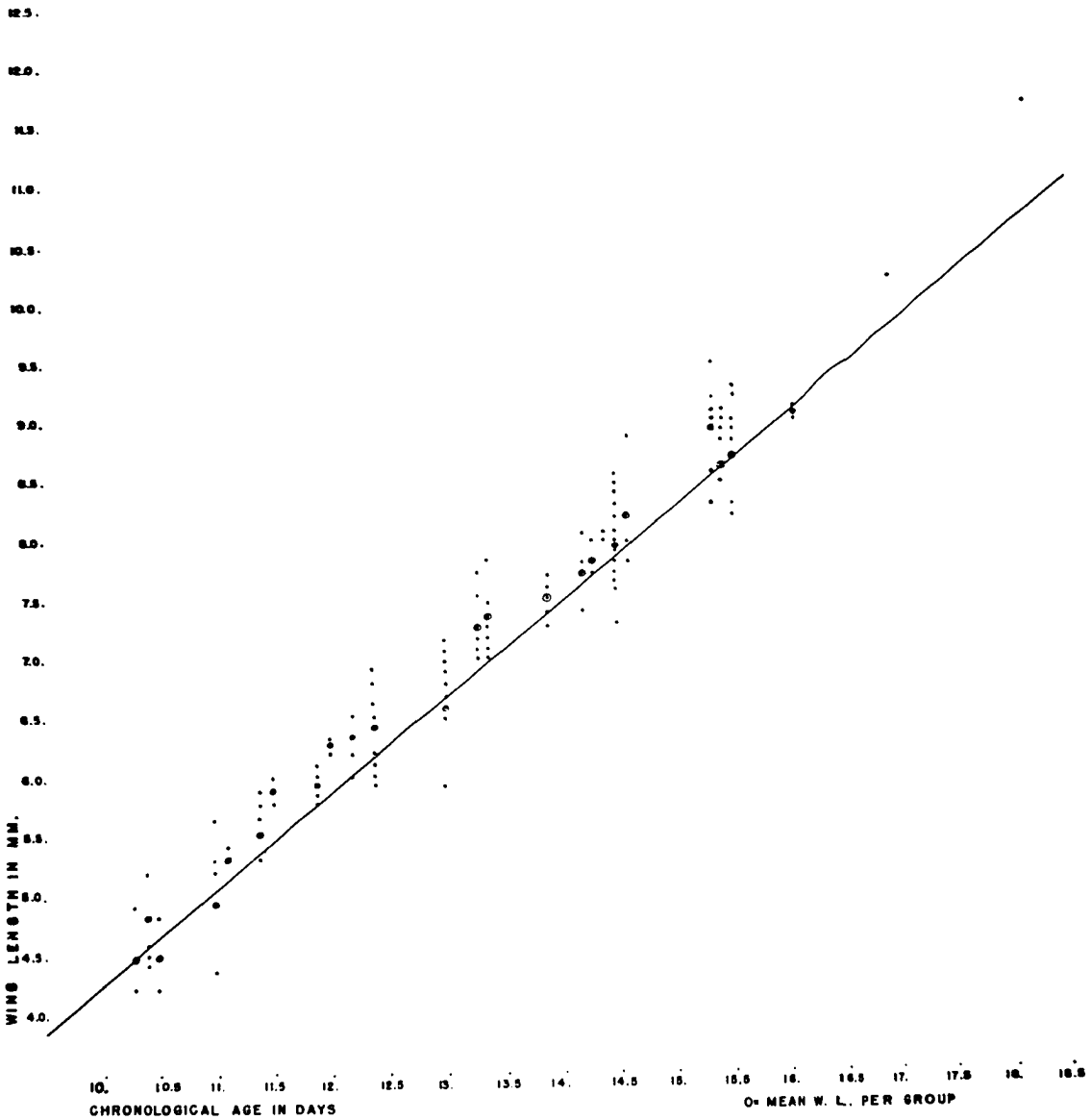
12.5.
12.0.
11.5.
11.0.
10.5.
10.0.
9.5.
9.0.
8.5.
8.0.
7.5.
7.0.
6.5.
6.0.
5.5.
5.0.
4.5.
4.0.
WING LENGTH IN MM.
10.
10.5
11.
11.5
12.
12.5
13.
13.5
14.
14.5
15.
15.5
16.
16.5
17.
17.5
18.
18.5
CHRONOLOGICAL AGE IN DAYS
O= MEAN W. L. PER GROUP

FIGURE 3

TABLE 1

QUANTITATIVE DATA REFERRING TO STATISTICAL TREATMENT

Emb. Class	No. Used	Chron. Age Range in Days	Mean Age
E	17	13.97 - 14.13	14.04
C	46	11.92 - 14.38	13.15
CC	62	11.46 - 14.55	12.99

Emb. Class	Wing Length Range in mm.	F. Germ Lgth. Range in mm.	F. Germ Mean Lgth.
E	5.7 - 7.5	0.75 - 9.84	4.37
C	5.7 - 7.5	1.60 - 10.08	5.58
CC	5.7 - 7.5	1.32 - 9.46	5.64

Emb. Class	Chron. Age "t" Value	Value of "t" 5%	Value of "t" 1%	Sign. Level	Std. Dev. Mean Age
E & CC	3.35	2.36	3.49	5%	.305
E & C	4.68	2.36	3.49	1%	.190
C & CC	0.46	2.36	3.49	None	.320

Emb. Class	Lgth. F. G. "t" Value	Value of "t" 5%	Value of "t" 1%	Sign. Level	Std. Dev. Mean F. G. L.
E & CC	2.54	2.36	3.49	5%	.500
E & C	3.39	2.36	3.49	5%	.360
C & CC	1.38	2.36	3.49	None	.150

E = Experimental group
C = Sham-injected control group
CC = Untreated control group

CHAPTER IV

II. MICROSCOPIC OBSERVATIONS

The down feather germs were examined microscopically to study the response of cells and the formation of the structures within the germs.

In his account of the origin of the down feather germ, Davies (1889) stated that the germ arises as a small hillock from two germ layers, an outer layer of ectoderm and an inner core of mesoderm. Shortly after the germ bud emerges, and as it continues to elongate, the adjacent skin surrounds the germ to form the follicle. The germ is completely formed at about the fourteenth day of incubation, at which time the elements begin to cornify.

The feather germs used in this study were viewed in polarized light and in no case was keratinization encountered.

Mesodermal Component

The mesodermal component, or pulp, was found to consist of loose mesenchymal tissue with capillaries, the main channels of which extend through it baso-apically. According to Davies (1889), no evidences of cell division are found in the pulp of the projecting germ itself. Cellular richness is greatest near the base and rapidly di-

minishes in an apical direction, with a corresponding increase of intercellular substances, and then remains practically uniform from just above the distal tips of the barb-vane ridges to the apex. Pulp cells are added at the base. As the germ becomes older and more completely developed, the pulp begins to regress from the apex toward the base. This allows a flattening of the apex and a reduction in diameter of the germ. Pulp regression begins at the time when proliferation of barb-vanes approaches completion (Goff, 1949).

The pulp cavities of the experimental germs consistently had fewer and smaller cells than did controls of comparable length, the reduction being almost half. For example:

	Length	Pulp Cell Count
Untreated Controls	1.37 mm.	18,981
	4.90 mm.	74,864
Sham-injected Controls	1.43 mm.	21,246
	4.98 mm.	82,780
Experimentals	1.51 mm.	9,535
	4.62 mm.	40,092

While many of the cells of the pulp appeared to be normal, others showed the cytopathological effects of irradiation (Fig. 11). Some appeared to have vacuolated cytoplasm; others had clumped chromatin, and still others had both. There were also nuclear distortions such as pycnosis and crenation. The blood capillaries in the shorter experimental germs appeared to be distended with the endothelium still intact. This was probably due to a loss of support from the pulp tissue. In the longer germs, where the pulp of the distal half of the germ was

reduced, only two or three pulp cells per section were present along with a small amount of debris, and the capillaries, still intact, had only a few blood cells in them.

Although hemorrhaging was noted in several of the germs, in no instance was there an embryo which showed hemorrhaging of all its germs, and there was no reason to suspect that the embryos did not have good circulation at the time of sacrifice. In the basal portion of these hemorrhaged germs, no endothelium could be recognized, and the blood appeared to have tunneled through the pulp, with blood cells being confined to position by the surrounding pulp cells. Distally, where the pulp cells were fewer, the blood cells spread out, pushing the pulp cells into a thin layer around the periphery of the pulp cavity. Still further distally, the number of blood cells decreased, as did the number of pulp cells, until the pulp cavity was completely devoid of cells at the apical end. There was considerable cellular debris dispersed among the blood cells in experimental germs. The same general picture of hemorrhaging was found in the control germs.

Ectodermal Component

The ectoderm gives rise to three distinct layers: a cylinder cell layer which borders the pulp and marks the dermoepidermal boundary; an intermediate portion from which the barb-vanes and barbules arise; and the sheath, which covers the outside of the germ. The cells of the cylinder layer are clearly distinguishable from the intermediate cells, being cylindrical in shape as opposed to the rounded shape of the intermediate cells (Watterson, 1942). After the barb-vanes are completed,

the cylinder layer becomes stratified in the distal portion of the germ but remains as a single layer in the basal portion. These layers of cells eventually split to form two layers in embryos much older than those of this study.

Cytopathological effects of irradiation were found in the cylinder cell layer of the experimental germs. The layer was very thin and compact and the cells were much reduced in size. This layer was found to be intact from the apex to the base of the germs. No germ had reached the developmental stage of stratification.

In normal down feather germs, according to Davies (1889), longitudinal ridges, which later become the barb-vanes and their associated barbules, begin to appear on the inner surface of the intermediate layer at about the eleventh day of incubation. These barb-vane ridges organize in an apicobasal direction. There are usually ten to fifteen ridges in a completed germ, ten or eleven of which are formed directly, while others are formed by splitting of one or more of the original ridges. Elongation of the germ as a whole is by the addition of cells to the basal region, whereas development of the barb-vanes and barbules is by rearrangement and increase in size of the cells already present. This is not to be construed to mean that specialization is not occurring at the same time.

In control germs, the barb-vane ridges, which are made up of the barbule plate cells and the barb-vane cells, were well formed and clearly demarcated (Fig. 5). These germs were selected by length to compare with the experimental germs. Cell counts of entire barb-vane

ridges in control germs showed a proportional increase in relation to the length of the germ. The barbule plate cells were lined up in orderly columns extending obliquely from the barb-vane (Fig. 6), and cell count results were of the same proportions as an entire barb-vane ridge. This same trend was observed in the sham-injected controls (Table 2).

In no case was there an experimental germ which did not exhibit some degree of organization. Multiple effects of irradiation were noted although all germs were not affected in the same manner. The intermediate layers of the shorter experimental germs were not as completely organized as were those of somewhat greater length. For example, a cell count of a barb-vane ridge in a 0.75 mm. germ was 356, and no differentiation between barb-vane cells and barbule cells could be seen. Another experimental germ 1.36 mm. long had a barb-vane ridge cell count average of 1110 per ridge, with a barbule cell count of 915 (Table 2). The cells were clearly visible, but the barbule cells were not lined up in columns. When these germs were compared with germs of control embryos of the same wing length, it was obvious that retardation had occurred in the experimentals. The control germs used for comparison with these experimental germs were 2.5 days younger chronologically. When these germs were compared with control germs of similar length, it was again obvious that developmental retardation had occurred in the experimentals. The cells of these experimental germs, as compared with the cells of control germs of comparable length, appeared shrunken and there was a considerable reduction in cell number (Figs. 5, 10, and 12).

In studying the barbule-barb-vane relationship it was noted that the barbule cells within the experimental down feathers were more affected than were the barb-vane cells when experimental germs were compared with controls of similar length. In control germs, there were approximately eight times as many barbule cells as there were barb-vane cells, whereas in comparable experimental germs there were approximately four and one-half times as many barbule cells as barb-vane cells. For example:

	Length	Ave. No. Barbule Cells	Ave. No. Barb-Vane Cells	Ratio
Control	1.38 mm.	2928	396	7.5:1
Experimental	1.36 mm.	915	195	4.7:1
Control	3.59 mm.	8800	1070	8.0:1
Experimental	3.62 mm.	2388	520	4.6:1

This is an indication of differential effect within the experimental down feather germs. The usual cytopathological effects of irradiation were evident. There was little or no cellular debris present and no mitotic figures were observed.

Experimental germs somewhat longer than those described above appeared to be more highly organized in that the barb-vane ridges extended further distally. Cell counts were considerably reduced as compared with controls of similar germ lengths (Table 2). The control germs used here for comparison came from embryos chronologically 2 days younger than the experimentals. Cytopathological effects of irradiation were noted and mitotic figures were found in the basal region, or that portion of the germ extending beneath the surface of the skin.

In some of the experimental germs it was found that the develop-

ment and organization of the barb-vane ridges were more extensive than in others. In general appearance, some of the germs were as well organized as were longer controls (Fig. 11). For example, an experimental germ 4.62 mm. long appeared to be as well organized as a control germ 6.78 mm. long. The barb-vane ridges were well shaped and extended as far distally, in proportion to length, as did those of the control. The cylinder cell layer was distinct; the barb-vane cells were in position, and the barbule cells were lined up in columns. Since the experimental germ showed what appears to be normal organization, but a marked reduction in the numbers of cells present (Table 2), it is clearly evident that histogenesis has proceeded out of proportion to elongation of the germ.

An experimental germ still longer (9.84 mm.) than those mentioned above appeared to be somewhat less affected as regards histogenesis and elongation but showed the greatest cellular effect. The diameter of the germ was considerably reduced and regression of the pulp had taken place for a short distance in the distal region; the barb-vane ridges were very compact and reduced in diameter, and there was considerable cellular debris. Cell counts could not be made because of these conditions. Proximally from this region the barbule cells were found lined up in columns. The usual cytopathological effects of irradiation were found in the various regions of the germ.

As compared with control germs there were fewer cells within the intermediate layers of experimental germs as a result of reduced mitosis; there were fewer cells due to cell deaths, and existing cells

were smaller. It would seem that all of these factors were effective in causing the retarded condition of the barb-vane ridges and subsequently the germ as a whole. The process of organization within the intermediate layer was retarded but not arrested.

The sheath of the feather germ is a somewhat rigid band of flattened cells (Fig. 6). By examining the sheaths of all three classes of embryos of this study, and taking measurements in the apical region, in the mid region, and in the basal region, it was found in all three classes that shorter, less organized germs had relatively thick sheaths at the apical end which became progressively thinner toward the basal region. The longer, more organized germs had relatively thin sheaths at the apical end which became progressively thicker toward the basal region. When germs of comparable lengths from the three groups were compared, it was found that the controls were more highly organized and had a larger diameter. In the experimentals and sham-injected controls a larger percentage of the total diameter of these regions was made up of sheath than in the case of the untreated controls. When comparing germs of comparable lengths it was found that the sheath toward the apical end of an experimental germ 1.36 mm. long made up 24% of the total diameter, and of a sham-injected control 1.43 mm. long, 17% of the total, while in an untreated control 1.38 mm. long, the sheath was 11% of the total. In the mid region of these same germs the sheath made up 8% in the experimental, 6% in the sham-injected control, and 4% in the untreated control. In the basal region, the figures are 6% in the experimental, 4% in the sham-injected control, and 2% in the untreated

control.

There was, however, no significant reduction in the number of cells in the thinner sheaths. For example:

	Length	Sheath Cell Count
Untreated Controls	1.38 mm.	1506
	4.90 mm.	5651
Sham-injected Controls	1.55 mm.	1558
	4.98 mm.	5793
Experimentals	1.36 mm.	1071
	5.42 mm.	4108

This finding of no reduction in the number of cells is not consistent with the findings in the other epidermal layers of experimental germs. It is suggested that these cells are more resistant to irradiation.

The question arises whether the greater thickening of the sheath of the experimental embryos could be due to possible loss of fluid from the operative procedure. If there is a generalized loss of fluid from the body of the embryo, it is conceivable that a proportionate amount would be lost from the feather germs. With a loss of fluid from the feather germs, the turgor within the pulp cavity would be lessened, allowing the sheath to contract and become thicker.

By checking the weights of embryos of the same chronological age, it was found that the experimentals weighed approximately 45% less than the untreated controls and approximately 33% less than sham-injected controls, whereas the sham-injected controls weighed approximately 12% less than the untreated controls. Also by checking the weights of embryos from the same wing length group, for example the 7.5 mm. group,

it was found that the experimentals weighed approximately 17% less than the untreated controls and approximately 9% less than the sham-injected controls. The sham-injected controls weighed approximately 7% less than the untreated controls.

These figures, which show that the difference in weight between the irradiated embryos and each of the two control groups is so much greater than the difference between the sham-injected and the untreated control groups, indicate that the operation was not completely responsible for this loss of weight. Therefore, the thickening of the sheath must be another manifestation of irradiation effect. This may be accounted for by the effect of the irradiation on the pulp which caused a decrease in its volume, thus allowing the sheath to contract and become thicker.

Although mitoses in the sheath of the control groups were rare, mitotic figures were found in the basal region. No mitotic figures were found in the sheaths of the experimental germs and the effect of irradiation on the cells of the basal area was conspicuous. There were, in the basal portion, some cells which appeared to have been in the process of mitosis but did not complete it, or else resting cells had become resistant enough to initiate the process (Fig. 4). The cells of the sheath exhibited the usual cytopathological effects of irradiation. Many of the cells appeared to be normal and there were no evidences of cellular debris. There were evidences of cell shrinkage and many of the cells were edematous. In several cases edematous cells located on the surface gave the appearance of small blisters (Figs. 10 and 12).

Germs which exhibited the greatest effect of irradiation in the pulp likewise showed the greatest effect in the ectodermal component. This may indicate that irradiation has affected the vascular system in such a way that the blood which was to serve the cells did not reach its destination, and for this reason the pulp did not develop, the barb-vane ridges did not organize properly, and the cells became greatly reduced in size. Mitoses were found near the basal portion of the pulp but with no greater frequency than in the ectodermal component. Cell deaths, as indicated by cellular debris, contributed to the overall condition of the components and the germ as a whole.

TABLE 2

AVERAGE NUMBERS OF CELLS IN BARB-VANE RIDGES FOR EMBRYOS OF COMPARABLE FEATHER GERM LENGTHS

Feather Germ Length in mm.		Barbule Plate Cells	Barb-Vane Cells	Barb-Vane Ridges
CC	1.38	2,928	396	3,324
	3.55	10,556	1,300	11,856
	5.28	15,174	2,484	17,658
	6.78	19,600	3,425	23,025
C	1.43	2,968	360	3,328
	3.59	8,800	1,070	9,870
	5.08	12,806	2,052	14,858
	6.91	20,200	3,575	23,775
E	0.75	- - -	- - -	356
	1.36	915	195	1,110
	3.62	2,388	520	2,908
	4.62	3,240	930	4,170

CC = Untreated controls
C = Sham-injected controls
E = Experimentals

CHAPTER V

DISCUSSION

When the down feather germs of the chick are irradiated by P-32 on the tenth through the fourteenth days of incubation, the resulting effect is differential retardation of development. This is in agreement with the findings of other investigators that certain tissues of the bodies of animals are more sensitive to irradiation than others (S. L. Warren, 1936, Butler, 1930, Bagg, 1922, and Stinson, 1953).

The fact that the ectoderm was most affected in germs whose pulp was also most affected may be due to some effect of irradiation on the vascular system not visible within the germ. Intact vessels with blood cells in them were observed, but this fact does not prove that the tissues which were to be served by these vessels were being served. Goff (1949) described the flow of blood into and out of the down feather germs. He noted the presence of a dense connective tissue in the region of the incurrent and excurrent vessels which may be the regulatory mechanism for the flow of blood into and within the germs. He also noted, while observing the living circulation, that when the germs on the wing are disturbed, or when the germs come into contact with the atmosphere, circulation within the germ stops. But when the wing is flooded with warm saline solution, circulation resumes in some of the

germs. F. R. Lillie (1940) reported a similar mechanism in regenerating feathers and stated that quite likely a turgor is built up in the pulp tissue which may also help to regulate flow. If the regulatory mechanism of blood flow into and within the germ was affected by irradiation in such a manner as to slow the flow to the degree that materials were insufficient to serve the surrounding tissues, the resulting effect on the pulp would in turn produce an effect on the ectodermal component.

With a considerable reduction of pulp tissue, intercellular transfer of materials would be slowed down. If the materials being transferred were food for the cells but were not reaching them, the cells would be reduced in size, or would cease dividing, or would die. If these materials were inducers of organization, the result would be retarded or altered organization. Cairns and Saunders (1954) demonstrated that the mesoderm has a specific inductive action on the overlying ectoderm during early embryonic development, producing regionally distinctive ectodermal derivatives in the chick. If histogenesis in the ectodermal component of the feather germ were dependent upon the inductive ability of the underlying mesoderm and irradiation interfered in some manner with the inductive ability, the resulting effect on the ectoderm would again be retarded or altered organization.

Bagg (1922) reported a hemorrhagic effect of irradiation and suggested that irradiation interfered with the formation of vascular endothelium. Hemorrhage was also found in this investigation but the incidence was not great and endothelial cells were clearly visible in non-hemorrhagic irradiated germs. It is not indicated from this study

that irradiation interfered with the formation of vascular endothelium to any extreme degree. A possible explanation of the hemorrhagic process involves the control mechanism located near the incurrent and excurrent vessels of the down feather germ. If the incurrent vessels allow blood to enter but the control mechanism does not allow it to exit, it is reasonable that blood pressure could force more blood into the vessels, thus trapping more in the germ than is normally there. This increased volume of blood could cause a vascular breakdown. This interpretation cannot be proved conclusively from the information in this study.

It would be difficult to conceive that the effects noted in this investigation would not be manifested in the hatched chick, even though Warren and Dixon (1949) observed no differential effects in the hatched chicken after embryonic irradiation, and Dixon (1952) reiterated, in his review of irradiation studies on the chick, that retardation of growth appears to be an overall effect with no one tissue or system more affected than another, and no abnormalities or malformations attributable to irradiation. The higher level and rate of irradiation in the present study, or the fact that Warren and Dixon did not observe the down feather germ, could possibly be responsible for the disparity in results between this and the above investigation.

The evidences of effect of irradiation presented in this study were of a conservative nature. As nearly as possible, controls and experimentals of morphological equivalence were compared rather than those of chronological equivalence. Had chronological equivalents been used the effects would have appeared much more severe.

CHAPTER VI

SUMMARY AND CONCLUSIONS

1. Chick embryos were irradiated with approximately 500 microcuries of P-32 on the tenth through the fourteenth day of incubation, producing specific effects on the down feather germs; these effects were determined by gross and microscopic observations and comparisons between irradiated and control embryos.

2. The wings of the experimental embryos were developmentally retarded in regard to length. Controls of similar wing length were about a day younger than the experimentals.

3. Macroscopic and microscopic observations show that the lengths of the feather germs were differentially retarded or specifically sensitive to irradiation with respect to the lengths of the wings.

4. Cytopathology of the irradiated feather germs showed edema, shrinkage, vacuolated cytoplasm, and vesicular nuclei in both mesodermal and ectodermal components.

5. Microscopic observations revealed a marked decrease in cell numbers in the experimental germs. This was attributed to retarded mitosis and cell death.

6. Although cell proliferation was reduced in the experimental germs, histogenesis within the components proceeded and in some cases

proceeded out of proportion to elongation of the germ.

7. P-32 in the dosage administered here did not interfere with the development of vascular endothelium in the germ.

8. Hemorrhagic effect did not appear to be a direct effect of irradiation since the incidence was not great and hemorrhage occurred in the controls.

9. It is indicated that interference with blood flow has taken place somewhere not visible within the germ itself. The severe effect on the pulp and ectodermal component could have been brought about by deprivation of food and oxygen.

10. In those germs where the pulp was most affected the barb-vane ridges were most affected. The cells were reduced in number as well as in size as compared with controls.

11. An overall reduction in size is indicated by the average loss of weight of the experimental embryos as compared with controls of the same incubation period.

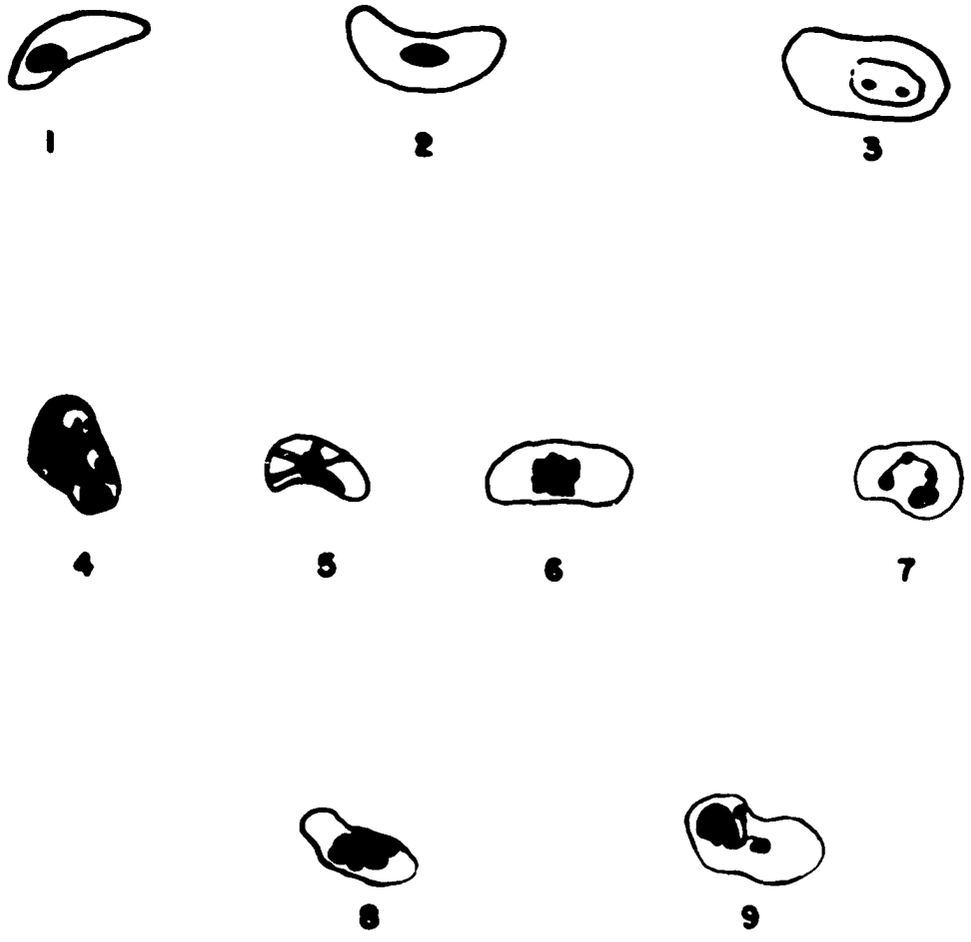

FIGURE 4. NORMAL AND IRRADIATED SHEATH CELLS

1–3. NORMAL SHEATH CELLS FROM APEX, MID REGION, AND BASE RESPECTIVELY.

4–9. IRRADIATED SHEATH CELLS.

4. BASAL REGION. VACUOLATED CYTOPLASM AND CLUMPED CHROMATIN
5. BASAL REGION. VACUOLATED CYTOPLASM.
6. BASAL REGION. CRENATED NUCLEUS.
7. BASAL REGION. INCOMPLETE MITOSIS.
8. BASAL REGION. INCOMPLETE MITOSIS.
9. BASAL REGION. PYCNOSIS AND FRAGMENTATION OF NUCLEUS.

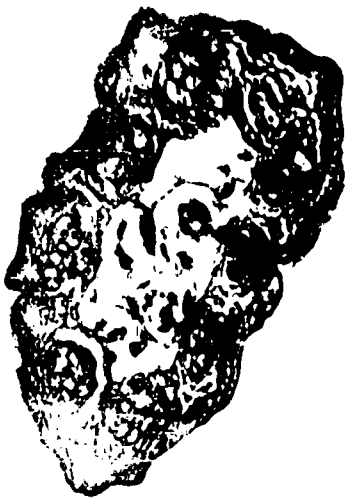

Fig. 5.--Cross section of apical region of control germ. x400. Shows relative abundance of pulp cells with blood vessels present. The ends of the barb-vane ridges are formed.

Fig. 6.--Cross section of mid region of control germ. x200. Shows barbule cells lined up in orderly columns. The pulp is relatively abundant and blood vessels are present.

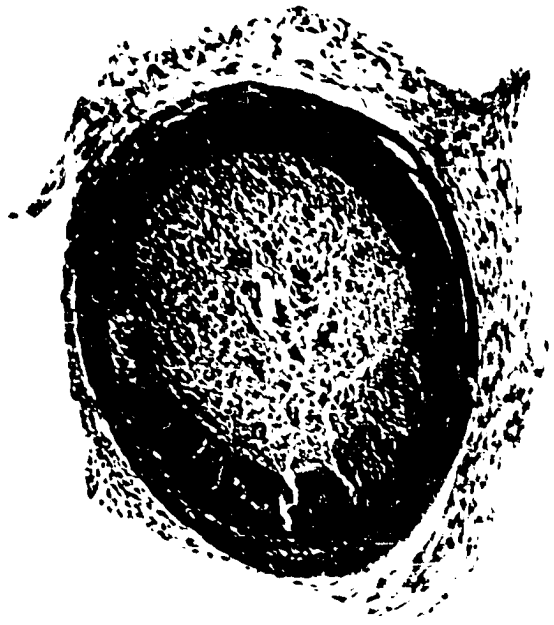

Fig. 7.--Cross section of basal region of control germ. x200. Shows cellular richness of the pulp. Three barb-vane ridges are forming. Follicular cells are surrounding the germ.

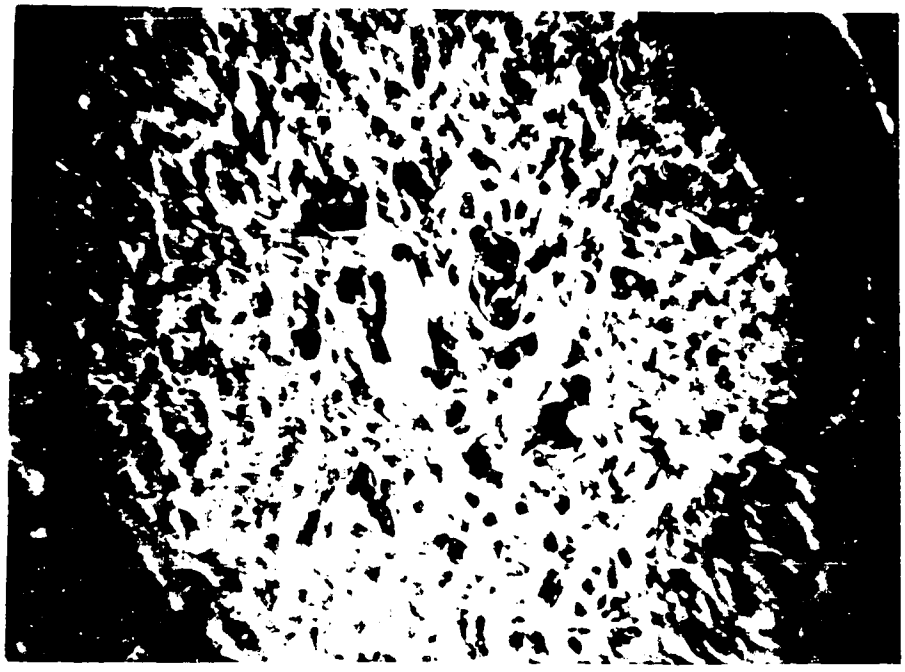

Fig. 8.--Cross section of basal region of control germ. x400. Shows cellular detail of the pulp cells and blood vessels.

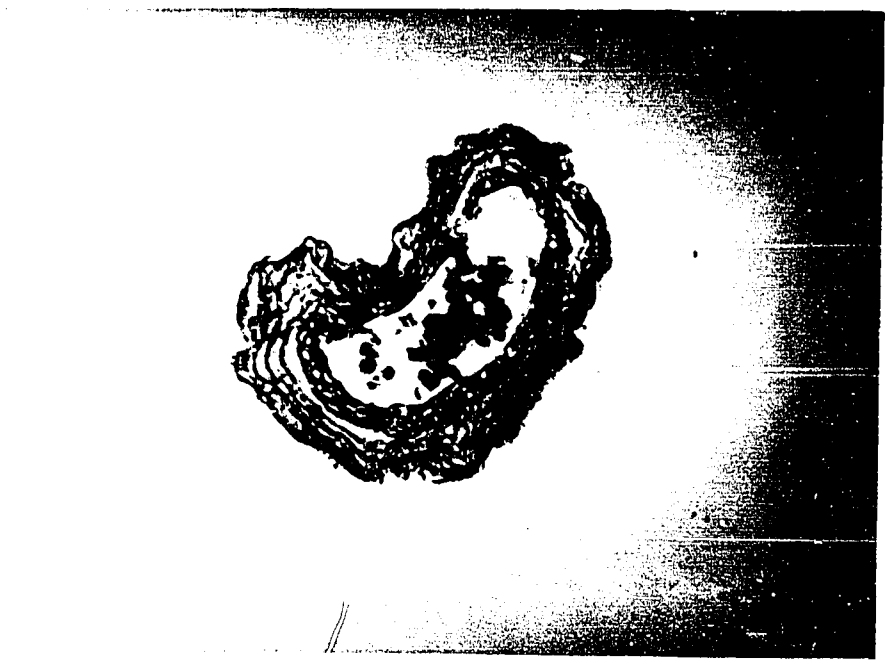

Fig. 9.--Cross section of apical region of irradiated germ. x400. Shows relatively empty pulp cavity with a few blood cells present and considerable amount of cellular debris. No barb-vane ridges are evident. The inner ectodermal portion is compressed toward pulp periphery. The sheath is very thick.

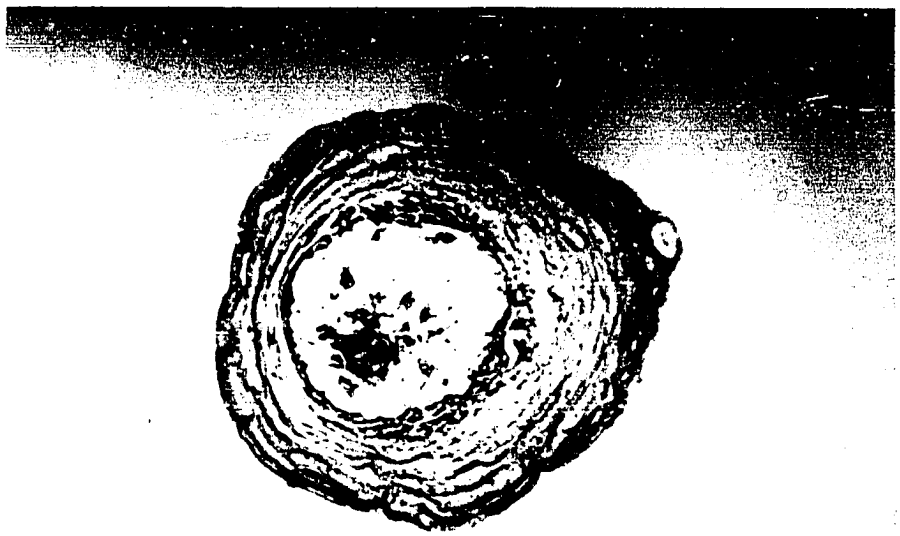

Fig. 10.--Cross section of mid region of irradiated germ. x400. Shows extremely thick sheath with "blister-like" cell on periphery of the germ. The barb-vane ridge area is compressed at the periphery of the pulp cavity. Pulp cells are sparse but blood vessels are still intact.

Fig. 11.--Cross section of mid region of irradiated germ. x400. By gross appearance germ looks normal. Cytopathological effects can be noted. Observe upper left portion, and middle left portion of barb-vane ridges.

Fig. 12.--Cross section of basal region of irradiated germ. x200. Note hemorrhaging in the pulp region, compression of the barb-vane region and the thickness of the sheath. Cells on periphery appear to form "blister."

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