The Biological Effects of X-Rays: I. A Critical Analysis of the Methods of Timing Insemination. II. The Development of the Albino Rat from the Ninth to the Fourteenth Day of Gestation, Correlated with Certain X-Ray Results

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THE BIOLOGICAL EFFECTS OF X-RAYS.

I. A CRITICAL ANALYSIS OF THE METHODS OF TIMING INSEMINATION. II. THE DEVELOPMENT OF THE ALBINO RAT FROM THE NINTH TO THE FOURTEENTH DAY OF GESTATION, CORRELATED WITH CERTAIN X-RAY RESULTS.

A THESIS SUBMITTED TO THE FACULTY OF LOYOLA UNIVERSITY GRADUATE SCHOOL IN CANDIDACY FOR THE DEGREE OF MASTER OF SCIENCE DEPARTMENT OF ANATOMY

BY
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INTRODUCTION

It is generally believed that at certain periods in the life cycle of a cell, the physiological processes of the cell are easily influenced by environmental changes, whether such changes be in the nature of stimulants or depressants. In this respect, x-rays have been shown to have a profound effect upon cells at certain periods of their metabolism (Packard, '33). Just as critical periods exist in the life cycle of cells, so critical periods are thought to exist for the organism as a whole. Thus by the use of x-rays, Job, Lieboldt and Fitzmaurice
2. ('35) demonstrated the presence of critical periods in the development of the albino rat. They report that irradiation within a specific range of dosage on certain days of gestation (critical periods) is apt to cause maldevelopment of several structures of the albino rat embryos.

The knowledge of the existence of critical periods in development served as a nucleus for the evolution of this thesis. Interest centered itself mainly about the determination of the stages of development of the embryo at the periods which were found to be critical for the formation of certain embryonic structures. Because the time in gestation at which the results could be effected was so limited in duration, and because the time and the dosage of x-rays could be so well correlated with the type of defect produced, it immediately became apparent that there was need of accurate knowledge of the age of embryos used in the experimental procedures. Thus the aims of this research are: to determine, if possible, an accurate means of establishing the age of embryos of the albino rat; to make a study of the stages of development at the time of the critical periods; to describe the stage of development of the embryo on the various days throughout the period of organogenesis; then, to correlate these findings with the anomalies of development resulting from irradiation which were reported by Job and his coworkers ('35).
I take this opportunity to express my indebtedness to Dr. T. T. Job, Professor of Anatomy at Loyola University School of Medicine for his suggestions which stimulated interest in this research; also for his helpful advice and expert criticism throughout the period of this investigation. To, Mr. O. I. Warren I express my thanks for his instruction and aid with regard to the technical phases of preparation, sectioning and staining of the tissues.

A CRITICAL ANALYSIS OF THE METHODS OF TIMING INSEMINATION IN THE ALBINO RAT

Many authors in the performance of experimental work, utilizing albino rat embryos, have been faced with the problem of determining the exact age of embryos. Some workers have established the approximate age of their material by the size and weight, while others have used the degree of development as an indication of the age. However, where the embryos are subjected to experimental procedures in utero, other methods must be relied upon for this information.

Before entering upon a discussion of the methods employed for establishing the age of embryos, it should be pointed out that the exact length of gestation lies between 21.5 and 22
days and is the period intervening between copulation and the occurrence of parturition. The time of casting of litter can be recorded within the limits of a few hours, but difficulty is encountered in ascertaining the time of copulation. Timing insemination in mated rats presents many difficulties, and for this reason a number of methods have been used, no one of which is without some bad features. The information may be obtained indirectly from the knowledge of the stage of oestrus cycle of the female, as determined by external examination of the genitalia or from examination of the contents of the vaginal fluid; or directly, either by observation of the act of copulation or by discovery of evidences left after copulation such as blood about the vaginal orifice, vaginal plug or spermatzoa in the genital tract.

One method which has been used in the past in this department and which yielded fairly good results was to place the time of insemination at the 24th hour after casting. This is based on the findings of Kirkham and Burr ('13) who report that females ovulate regularly 20 to 48 hours following parturition during the months of April to October but that they are apt to show irregular and prolonged cycles during the rest of the year, ovulation not occurring until as much as three weeks after parturition. Long and Evans ('22) have since then investigated the time of ovulation and find
that it takes place between the 16th and the 24th hour post-partum. The experiences in this laboratory confirm the tendency to irregularity in mating during certain seasons, for it has been difficult to carry out a regular mating schedule during the winter months of the year. It is interesting to note that Huber in his report on the development of the albino rat states that he obtained good results by mating females on the 30th day after the birth of a litter, but that if they showed no response to the male he would again attempt a mating after two days. This, of course, is a trial and error method of mating, and is mentioned only to be condemned.

It has been known for a number of years that there are external evidences of "heat" in the female rat. Workers who are familiar with the breeding habits and the cyclic changes in the appearance of the vagina are able to select and mate animals at the proper stage of the cycle, i.e., when the characteristic swelling of the radiating folds are seen. (Greeman and Duhring) These animals were found to mate at once. It is obvious that a great deal of experience in handling rats is required in order to utilize the external signs as a basis for mating, and is doubtless bound to be accompanied with poor results when used by the novice. More recently with the discovery of the cyclic changes in the vaginal fluid contents of albino rats, there has been
shown to be a correlation between the above condition and the cornified cell stage (of oestrus) in the vaginal smear technique (Long and Evans, '22). It is interesting to note in passing that Allen ('22), in working with the oestrus cycle in the mouse, came to the conclusion that external signs were of no value, for only about half of the females showed well-marked changes, and a few showed external signs of "heat" during the metoestrus and the dioestrus.

A thorough investigation was made of the vaginal smear technique described by Long and Evans, ('22), a method which has since its development been employed by a number of workers who report uniformly satisfactory results. It may be stated that the method avoids the need of interpretation of external signs or guessing at the stage of the cycle, for by making a microscopic examination of the vaginal fluid, one is able to state with accuracy, whether the animal may be mated with any possibility of success. To review briefly the principle of the smear technique, one need only refer to the summary by Long and Evans, ('22) of their work. They state: "An orderly sequence of changes of the contents of the vagina are shown each with a different and characteristic histological makeup. The stages in the cycle may with equal propriety be named from the cell contents of the vaginal smear. We could designate them as the stage of sudden appearance of
masses of uniform sized, nucleated, epithelial cells, dehisced from the surface; the stage of a few cornified cells; the stage of extremely abundant cornified cells; and finally, in the dioestrus pause, the stage of leucocytes with scanty epithelial cells." Since there is this cyclic change in the contents of the vaginal fluid, a change which can be followed by systematic micro-examination, the method adapts itself readily to an experimental problem requiring knowledge of the age of embryos, for copulations are reported to occur typically in the transition from stage I to stage II of the smear, and in the early part of stage II. (Long and Evans, '22, Defrise, '33).

Another phase which offers some difficulties and which deserves consideration is the determination of successful coitus, for copulation does necessarily imply insemination. Several authors have pointed out that the first copulations are unsuccessful (Long and Evans, '22, Greenman and Duhring, '22), while Hartman and Ball ('30) state that the rat copulates as many as 5 to 20 times before ejaculating. What means have we of knowing whether insemination has taken place? The vaginal plug, or vaginal sign, may be looked for, or the vaginal tract examined for spermatozoa; the first two being easily observed, the latter involving greater expenditure of time and effort. It is said that the vaginal plug always left by the male rat following a successful effort is
a positive diagnosis of insemination (Long and Evans, '22, Greenman and Duhring, '22). The copulation sign is described by Chandler ('32) as the appearance of blood around the vaginal orifice. He reports a high percentage of females casting close to 21.5 days if the sign is observed. There are two reports in the literature where examinations were made for spermatozoa. Mc Donald and Long ('34) examine the vaginal smear the morning after mating for the presence of the male secretions, and if found, the time of coitus is then taken as midnight. Gilchrist and Pincus ('32) make frequent examinations of the mated animals and place the time of insemination within a range of accuracy of 15 minutes.

The discussion up to this point has concerned itself with the determination of "heat" in the females and of successful coitus. It is apparent from the foregoing discussion that there is no one method of timing insemination which is wholly satisfactory. The approach to this goal which is most likely to give the best results is to mate animals of proper ages, which show the stage of the vaginal cycle characteristic of "heat", and then to observe these animals further for evidences of insemination.

There are, however, several facts which must be considered at this point because of their bearing upon the moment of conception and further development of the ovum. What is the relation of ovulation to oestrous? Does oestrus
always appear at the same time in the cycle? Do all embryos grow at the same rate? In answering these questions it will become apparent to one that here are three variables which must be always considered when stating the age of albino rat embryos. The relation of ovulation to oestrus has a bearing upon the time of conception because it does not occur at a uniform period of time. Gilchrist and Pincus ('32) found no ova before 8.5 hours following copulation and suggest that fertilization may presumably occur shortly after ovulation. Recently it has been discovered by Hartman and Ball ('30) that the rat sperm reaches the upper end of the uterus 100 seconds after insemination. It was suggested by Long and Evans in 1922 that it is probable that the sperm awaits the arrival of the ovum for a period of time and that fertilization occurs immediately upon release of ova. The careful researches of Long and Evans ('23) indicate that ovulation does not necessarily occur at a uniform period of time, but is related to the general progress of the oestrus changes as a whole. Although oestrus is found to occur typically during the last part of stage I and during most of stage II, instances of long duration of oestrus, and either its early or late occurrence, are not unusual. Considerable variation in the occurrence of maturation is demonstrated by their data. The evidence shows that in the great majority of instances, ovulation does occur 24 hours following the appearance of the
first cornified cells in the vaginal fluid, but also may occur at any time during the period from the 18th to the 30th hour after stage II. Because of this potential variation in the relation of oestrus to the appearance of stage II of the vaginal smear, age differences could arise, and could involve a period over 24 hours in length. Finally there is the question as to the growth rate of individual embryos. From illustrations in Huber's paper on the development of the albino rat it is apparent that slight differences in the degree of development may occur in embryos removed from the same uterus, whereas embryos of the same insemination age, but removed from different uteri, may show age differences exceeding 24 hours. Furthermore, it has been reported that gestation is prolonged if the mother is lactating (King, '13). If this is true it might be expected that the litter being carried by the mother would show delayed development when measured in terms of the day of gestation.

The data presented show the difficulty of stating accurately the age of the albino rat in utero. Although most embryos dated from the hour of occurrence of copulation will show approximately equal development, it must be remembered that with the best technique available, embryos may still show age differences amounting to 24 hours or more. The variables which must be considered when stating the age of embryos can be summarized as follows:
1. The time of insemination may be ascertained by a variety of techniques, preferably by mating on the basis of the vaginal smear, followed by observation of evidences of successful copulation. The information so obtained may be accurate within a range of 12 hours and may be narrowed down within 15 minutes if examinations are made for sperm in the vaginal fluid.

2. Oestrus does not necessarily bear a constant relation to the changes in vaginal content.

3. Ovulation most frequently occurs 24 hours after the appearance of cornified cells in the vaginal smear, but may appear within a range from the 18th to 24th hours.

4. Differences in growth rate can be seen in embryos of the same age, particularly if from different litters.

THE DEVELOPMENT OF THE ALBINO RAT FROM THE NINTH TO THE FOURTEENTH DAY OF GESTATION

MATERIALS AND METHODS

Selection of animals. After reviewing the various methods employed in determining insemination in the albino rat, it was decided to mate animals on the basis of the vaginal smear technique of Long and Evans ('22). Females over 90 days of age were mated with males of corresponding age, at a time when the microscopic examination of the stained vaginal fluid revealed the presence of the cornified epithelial plates charac-
Vaginal smears were made in the late afternoon. These were obtained by gently swabbing the vagina with a toothpick which had a small amount of cotton wound around its tip. The material which was withdrawn was applied to glass slides and allowed to dry. The Wright staining technique was employed with the express purpose of differentiating the leucocytes which appear at times during the cycle, but it served admirably in staining the contents of all stages of the oestrus cycle.

In an attempt to eliminate as many variables as possible, only those animals showing the cornified epithelial plates in the smear were mated. The task of selecting females for mating on the basis of the vaginal fluid content, proved to be a task of no small proportion. The group which was being examined daily, at one time numbered 20 animals. By accurate count it was found that 302 vaginal smears were examined to find 27 animals in the proper stage of oestrus, an average of 11 smears per mating. This work was done in the fall and winter session.

Mating. A total of 46 matings were made to obtain sufficient material for completion of the problem. Observations revealed typical mating approaches on the part of the male in almost every instance and attempts at copulation many times. Thus it seemed that the females were being selected for mating at the proper period in their cycle. The vaginal plug was ob-
served on several occasions and the vaginal sign once.

During the first few matings, the males were kept in the mating cages throughout the period of gestation. However, a new procedure was soon resorted to when following a series of laparotomies on the ninth and tenth days of gestation it was found that the nodules of most uteri were much smaller than expected for that particular day of gestation.

In the second series of matings animals were selected in the same manner as previously and were mated in the late afternoon, but the males were removed from the cages the following morning. Thus the maximum amount of time permitted for insemination was about 15 hours (5 p.m. to 8 a.m.). The age of the embryos was calculated from midnight of that period. This method yielded but few pregnancies, but these at least were timed with fair accuracy.

Removal of the embryos All operations were performed in the morning. Considerable difficulty was encountered in removing the embryos from the uterine horns. In the first series of operations the animals were opened by a midventral incision and the embryos were removed directly from the uterine horns and were fixed, sectioned, and stained. The results were poor, for in all but two instances the embryos were lost or destroyed in the process. In the second series, the basal and the ovarian extremities of one horn of the uterus were ligated and the intervening structure excised.
The abdominal incision was repaired and the animal returned to its cage. The animal was reopened within 3 days in order to remove the embryos remaining in the other uterine horn.

Because of their small size and incomplete membrane formation, no attempt was made to free the ninth and tenth day embryos from the uterine wall. These embryos were prepared by transferring the section of the uterus with the nodule of the embryo directly to the fixation fluid. For the eleventh, twelfth, thirteenth and fourteenth day embryos the uterine horn, with its contents which had been excised, was transferred to saline solution and then under a dissection microscope a nodule was carefully slit open and the decidua capsularis torn with a sharp needle allowing the embryo with its yolk sac to flow out. The yolk sac was incised, the yolk stalk cut, and the embryo itself transferred by the use of a large-mouthed eye-dropper to the fixation fluid.

**Fixation and orientation of the embryos** Bouin's picric-formol fixative fluid proved satisfactory and was used for the preparation of all embryos. The tissues were dehydrated, embedded in paraffin, sectioned at 12μ thickness and stained routinely with hematoxylin and eosin. The ninth and tenth day embryos were sectioned in a definite manner with relation to the plane of the mesometrium. Series cut at right angles to the long axis of the uterus, resulted in longitudinal sections of the embryo, while series cut parallel to the mesometrium,
14. gives sections in the transverse plane. Orientation of the embryos in utero is possible only up to the eleventh day at which time torsion begins to take place. Embryos obtained on the twelfth to the fourteenth days of gestation were cut in the sagittal and transverse planes for serial study. Borax-carmine stained whole mounts were prepared from twelfth, thirteenth and fourteenth day material.

Number and age of embryos  Twenty-four embryos were prepared for this study. The ages range from the ninth to the fourteenth day of gestation. In all instances, the embryos for any one day were obtained from at least two litters, to offset the possibility of an error being made in timing the duration of gestation up to the time of removal of the embryos from the uterus, and also to serve as confirmation of the stage of development found in other embryos of like age.

DATA ON THE STAGES OF DEVELOPMENT

Huber('15) described the development of the albino rat from the pronuclear stage to the end of the ninth day of gestation; Widakowich('11) covered the same period, the tenth day especially well, and the eleventh day in part. Robinson's findings('92) were of special value in studying the formation of the membranes, the ectoplacental cavity and the extraembryonic coelom. Descriptions concerning the development of some of the individual organs are to be found in the litera-
ture, but as yet a comprehensive picture of the period of organogenesis, extending from the tenth to the fourteenth day has not appeared. An attempt has been made in this work to cover that gap.

NINTH DAY OF GESTATION

Six specimens obtained on the ninth day of gestation were prepared for study. These were obtained from two litters. An attempt was made to section the embryos in the horizontal and the longitudinal planes. Favorable series, however, were not obtained in all instances. Examinations made of the serial sections showed all embryos at approximately the same stage of development, and corresponding closely to the descriptions made by Huber of specimens which he obtained on the ninth day of gestation. The structures which he described in detail were easily identified in the prepared sections.

Figure 1. is a photomicrograph of a longitudinal section through a typical specimen. The parts are labeled to correspond with the terminology employed by Huber(15) and Widakowich(11). The structures which are present at this time are the embryonic ectoderm, entoderm, mesoderm and the primitive streak. The primitive streak can be identified in some specimens as a mass of closely packed, irregular, linearly arranged ectodermal cells on one wall of the antimesometrial portion of the proamniotic cavity. The mesoderm is not present in all embryos, but when present it appears as a spreading sheet of irregular, lightly stained cells placed
between the ectoderm and the entoderm. The primary embryonic ectoderm consists of regular, darkly stained, columnar cells lining the antimesometrial portion of the proamniotic cavity. One ovum, slightly further advanced than the others obtained on this day, shows a constricting band pushing into the proamniotic cavity in the region of the junction of the extra-embryonic and embryonic ectoderm. These are the amniotic folds described by Widakowich ('11). They presage the formation of the amniotic cavity which forms during the early part of the tenth day.

**TENTH DAY**

There are 5 embryos in this group. They were obtained from two different animals. The specimens from one litter show slight differences in the degree of development, and are more advanced than one other specimen obtained from a second litter. On examination the embryos were found to correspond closely to the reconstructions, and the illustrations of tenth day embryos made by Widakowich ('11). Rapid progress has been made during the period from the ninth to the tenth day. The amnion and allantois have made their appearance in association with the amniotic, pleuroperitoneal (extra-embryonic coelom), and ectoplacental cavities. These changes are accompanied by a marked increase in the vascularity of the surrounding maternal tissue and vascularization of the ectoplacental cone.
Figure 2. is a photomicrograph of a longitudinal section through a tenth day embryo. The parts have been labeled using the terms employed by Widakowich (11). The entoderm, medullary fold, primitive streak, mesoderm, allantois, and amnion are easily identified.

The ectoderm cephalic to the primitive streak has proliferated to form the medullary plate. Some specimens show the medullary folds and groove, while more advanced specimens show increased bulgings of the ectoderm in the cephalic region, the beginning of the prosencephalon. The site of the mesencephalon and the rhombencephalon is indicated by shallow transverse grooves of the surface of the ectoderm. One embryo shows the head fold and the invagination of the entoderm below it, forming the foregut. This invaginated pocket is lined with cells which are columnar in shape in contrast to the more flattened type of entodermal cells elsewhere in the embryonic area.

ELEVENTH DAY

Because of the difficulty encountered in removing the embryo from its amniotic sac, and the consequent injury to the tissues, only two eleventh day embryos are available for study. Although obtained from two different litters, the developmental stages seen in the two embryos are almost identical.

Body form The embryos during the tenth day show a
convexity toward the future ventral surface. Beginning the latter part of the tenth day and during the eleventh day, however, the embryo grows rapidly and the cephalic region assumes the dorsal convexity typically found in other mammal embryos. Horizontal sections through the embryo at this time show the neural tube cut in three regions since the process of flexion is incomplete. (figure 3.). Torsion takes place at the same time, thus accommodating the rapidly growing embryo in its allotted space without impingement upon the ectoplacental cone.

**Nervous system**  The primitive cerebral vesicles, prosencephalon, mesencephalon, and rhombencephalon, are well marked off from each other. The neural tube is still unfused in its cephalic extremity.

**Gut**  The intestinal tract is still incomplete since the embryo is still in the process of establishing its dorsal convexity. The foregut is much larger than it is in tenth day specimens, and is identified as an entodermal-lined cavity lying dorsal to the heart, continuous caudally with the yolk sac cavity. Cephalically the cavity is broadened in a lateral direction forming the pharynx. One specimen shows shallow outpocketings in the lateral wall. These mark the point of origin of the pharyngeal pouches. A very short posterior gut is also present in this specimen.

**Heart**  The heart is in a very primitive state of develop-
19. Examinations reveal an S-shaped tube which is continuous cephalically with the ventral aortae and caudally with the common cardinal veins.

TWELFTH DAY

Five embryos were obtained on the twelfth day of gestation, three different litters being used to obtain the material. Serial sections were made in both the sagittal and the transverse planes. One whole mount was prepared.

Body form In contrast to the partial dorsal convexity seen in the eleventh day embryos, the body of the twelfth day specimen has become completely flexed, assuming the typical embryonic form. (Figure 4.) Both sets of limb buds are in evidence, the hind ones, however, being rudimentary. The body folds are unfused. The pharyngeal arches are established, the first two being especially well shown in the whole mount.

Nervous system Except for a small area caudally, the neural tube is entirely closed. The brain shows further differentiation, the various subdivisions of the prosencephalon, mesencephalon and rhombencephalon having made their appearance. The otic vesicle has formed and lies in close proximity to the first pharyngeal pouch. The eye has made its appearance, being represented by the optic vesicle and stalk. Indentation of the vesicle is shown in the Figure 5. a photomicrograph of a section through the optic region. The beginning of the lens placode is indicated by the thickening of the
surface epithelium superficial to the optic cup.

Gut Four pharyngeal pouches open laterally from the pharynx and are associated with the formation of the branchial arches. The thyroid anlage is pushing down from the pharyngeal floor. From the posterior end of the pharynx a tubular outgrowth can be traced in a caudal direction, ventral to the esophagus, to its termination in two small masses, the lung buds. The liver diverticulum is present. The gut terminates caudally in the cloaca.

Heart The heart has advanced from the single tubed structure seen on the previous day to a two-chambered stage. Parts which contribute to the formation of the four chambered heart are coming into evidence.

Mesonephros The mesonephric tubules are in the process of formation.

THIRTEENTH DAY

Body form The body folds are still unfused. The presence of the tail is noted and the limb buds are found to be somewhat larger than they are on the previous day. Face formation is beginning. The nasal pits are present and the first branchial arch is separated into its maxillary and mandibular processes.

Nervous system The nervous and pigment layers of the retina have differentiated from the original single layer of the optic cup. The lens vesicle has become separated from the
21.
surface epithelium and is now fitted down into the optic cup. 
(Figure 7.)

Gut The primordia of the pharyngeal derivatives and the 
dorsal and ventral pancreas are making their appearance. Dif­
ferentiation of the simple tube into the stomach and the in­
testine is noted. A loop of bowel is herniated into the body 
stalk.

Heart The heart is almost completely divided into four 
chambers, the interventricular septum being incomplete in its 
cephalic portion.

Urinary system The mesonephric duct, connecting with the 
mesonephric tubules, can be traced along the dorsal body wall 
to its termination into the cloaca.

**FOURTEENTH DAY**

Further differentiation is present in all systems of the 
embryo. Outstanding changes that are evident concern the pro­
cess of face formation and the appearance of the metanephros. 
(Figure 8.)

**SUMMARY OF THE FINDINGS**

**Ninth day of gestation (Huber, '15)**

1. Establishment of the primitive streak
2. Appearance of the mesoderm toward the latter 
   part of the day.

**Tenth day of gestation (Widakowich, '11)**

1. Formation of the amniotic folds and cavity
2. Formation of the extraembryonic and ectoplacental cavities.
3. Appearance of the allantois.
4. Formation of the medullary plate, medullary folds and groove, with beginning of the brain and its divisions.
5. Head fold noted, associated with the beginning of the foregut.

Eleventh day (Widakowich, '11, and Robinson, '82, in part)
1. The body is assuming its typical dorsal convexity. Torsion is apparent at the same time.
2. The neural folds are widely open.
3. The three primary divisions of the brain appear. The telencephalic vesicles are pushing out from the prosencephalon.
4. The foregut is well established and is continuous behind the heart with the yolk sac cavity.
5. The pharyngeal pouches are just beginning to push out from pharynx.
6. The heart is in the form of an S-shaped tube.

Twelfth day
1. The body is found to be completely convex to its dorsal aspect.
2. The pharyngeal arches are established.
3. Both sets of limb buds are present, the hind
ones, however, being rudimentary.

4. Except for a small area posteriorly, the neural tube is closed.

5. Both the otic and optic vesicles are present.

6. Primordia of the hypophysis, lung, liver, and mesonephros have appeared.

7. The heart is in the two-chambered stage, but shows evidence of parts which contribute to the formation of the four-chambered heart.

**Thirteenth day**

1. The optic lens has formed.

2. The body folds are unfused.

3. Primordia of the pancreas, thyroid, pharyngeal pouch derivatives are present.

4. The maxillary and the mandibular processes can be identified.

**Fourteenth day**

1. Face formation is taking place.

2. Primordium of the metanephros appears.

**DISCUSSION**

Critical periods in the development of the albino rat were shown to exist by Job, Lieboldt and Fitzmaurice ('35). In the first part of their experimental work, each of a series of pregnant animals was exposed to a single dose of
x-rays, at some time between the 6th and 18th day of gestation. Gross anomalies of development were found to result from such treatment. From further experiments, wherein the time of application and the dosage of x-rays was delimited, it became apparent that there was a specific period and a dosage which was optimum for production of abnormal development of several structures of the embryo. These abnormalities were found to involve the brain, eye, and jaw. A hydrocephalic condition was produced by irradiation on the ninth day of gestation; a defective development of the eye on the tenth day; a malformation of the jaw on the eleventh day. The eye defect also appeared in some animals exposed on the ninth and eleventh days. Job and his coworkers were of the opinion that at these periods the anlagen of the susceptible structures were present and that the stage of development was such that further growth was influenced by the effects of the x-rays. They pointed out, however, that "if there were a latent period, then the time of irradiation must precede the critical period by the length of the latent period."

The demonstration of critical periods in development by Job and his coworkers served as a background for this research. This problem was undertaken with the demonstration of the relation of the critical periods to the actual development of the embryo as one of its aims. With this idea in mind, careful examinations were made of ninth, tenth, and
eleventh day specimens, which had been prepared for serial study. The investigation resulted in the establishment of findings which were of great interest, for it revealed that at the critical period, in no instance has the primordium of the susceptible structure made its appearance. The morphology of the embryos on the various days of gestation from the ninth to the fourteenth has already been described, but it is well to review here certain essential features of development, to state the relation of these features to the critical periods, and to point out the stages at which the susceptible primordia can be definitely demonstrated. The first organ to be considered is the brain. On the ninth day, the critical day for that structure, no indication of the cerebral anlage is evident, the only parts which have formed are the three primary embryonic layers and the primitive streak (Figure 1.) One must look to tenth day embryos to identify the proliferation of the ectodermal cells forming the medullary plate. (Figure 2.) Thus, there is a period about 24 hours in length intervening between the critical period and the appearance of the brain anlage. Similar findings were established with regard to the optic enlage. Embryos removed on the tenth day, the critical day for the eye, show a stage of development wherein medullary plate, medullary folds, beginning of brain, and foregut can be identified (Figure 2.). At this stage no indications of the optic primordia are demonstrated, in fact
not until the twelfth day, two days later than the critical period, can the optic vesicle and lens placode be identified (Figure 5.). Finally we come to the consideration of the relation of the critical period for the jaw and the time of origin of pharyngeal arches. On the eleventh day, the critical day for the jaw, the embryo is beginning to assume a dorsal convexity and the structures which can be identified are the primary divisions of the brain, the foregut, heart and pharynx (Figure 3.). In one eleventh day specimen, the pharynx shows shallow outpocketings, but is without evidences of the pharyngeal arch formation. The period intervening between the critical period of the jaw and the appearance of the anlage is somewhat less than 24 hours, because the arches are well formed in embryos removed on the twelfth day of gestation (Figure 4.).

It is apparent from the foregoing facts that the administration of an optimum dosage of roentgen rays at a definite period in embryonic development results in the modification of the progress of a structure, the primordium of which appears subsequent to the time of irradiation. Interpretation of this finding is difficult for with x-rays we are dealing with an experimental agent whose exact mode of action is not definitely known. It is believed that clarification of this point may be had by a series of experiments which would have as a goal the following points: First, Can changes
be demonstrated immediately after irradiation? Second, if no changes are apparent at that time, can any alteration in normal structure be detected before the appearance of the anlage? Third, are the effects of irradiation demonstrable at the time of proliferation of the anlage? Fourth, what changes take place in the development of the susceptible irradiated primordia? Since experiments of this nature have not been performed, it is necessary to rely upon the literature for knowledge concerning the manner in which x-rays act.

Warren ('28), in a review of the effects of x-rays on tissues, states: "great confusion exists in the study of the effects due to the lack of suitable standardizing devices, the absence of an easily established unit of dosage, and the failure of various investigators to consider the variable introduced by the different wave lengths with their variation in penetration and absorption. Radiation of body tissues causes an injury to the cell structures and life processes, though the amount of injury produced is proportional to the amount of radiation administered a a particular wave length, and varies with the tissue irradiated, and to a certain extent with the species studied". Hinrich ('25) summarizes much which is known of the effects of x-rays in the following:

1) the chromatin is more susceptible than the rest of the cell; 2) the dividing cell is more susceptible than the resting cell; 3) embryonic, rapidly differentiating tissues
are more susceptible than adult tissues; 4) a weak dosage may accelerate, while a strong dose delays cell activity; 5) the more intense the irradiation and the earlier applied, the more marked are the defects; 6) the effect is more pronounced in systems where the development is most precocious such as in the nervous and vascular systems. L. Loeb (1922) reports: "Two factors which determine the sensitiveness of the organ, tissue or cells are, 1) the intensity of their proliferative activity, and 2) the difference in the degree of general sensitiveness which do not necessarily run parallel to the proliferative activity." Baldwin (1919) indicated that susceptibility involves both the chromatin and the cytoplasm, and that a chemical change is brought about by the x-ray energy.

One phase of x-ray work which is particularly confusing is the problem of the latent period. The term refers to the period of time following irradiation during which changes in the normal structures cannot be detected. Frequent references are made to this period in x-ray literature and many authors can be found who agree to the conception of such a period (Baldwin '19, Colwell, Gladstone and Wakely '22 and '26, L. Loeb '22, Hinrich '25, Laurens '28, Warren '28, Packard '33, Spiedel '35). The rather recent analysis by Packard ('33) of the biological effects of irradiation may be quoted at this point because of its pertinent nature. "-----physical and
chemical changes occur within the cells at the time of ir-
radiation resulting in the primary effects of an increase in
the hydrogen ion concentration of the protoplasm, increase in
cell membrane permeability and a change in respiratory rate". 
Packard believes that "all changes follow a latent period 
which may be short if our methods for detection of minute 
structural, chemical and biological changes were sufficiently 
accurate". It is apparent that no unanimity of thought exists 
as to the exact meaning of the latent period.

The knowledge of the time of action of x-rays obviously 
has a bearing upon the interpretation of the finding which 
was previously established, namely, that irradiation of albino 
rat embryos which results in abnormal development must precede 
the appearance of the primordia of the susceptible structures by 
a period of time which is somewhat less than 24 hours for the 
jaw, about 24 hours for the brain and approximately 48 hours for 
the eye. Since one cannot state definitely whether the x-rays 
have an effect upon tissues immediately at the time of irradia-
tion or only after the length of the latent period, two poss-
ible interpretations of the above findings may be considered: 
First, the time of appearance of the anlage is the period 
most easily affected by changes in the environment, so it is 
then that roentgen rays may be effective in producing ab-
normal development; Second, roentgen rays have a selective 
action upon the brain, eyes and anterior part of the head at a
period prior to the time of formation of the primordium.

That the appearance of the anlage is the period of sus-
ceptibility is supported by Stockard('21). He reports that
when an important organ is entering its initial stage of r
rapid proliferation or budding, a serious interruption of the
developmental processes is accompanied by serious consequences
to the organ, while only few or no ill effects are felt by the
embryo in general. It may be that the changes effected
by the x-rays do not occur at once, but are delayed and make
themselves felt at some subsequent period, a period which
coincides with the beginning of the proliferation of the pri-
mordium. The time intervening between the time of irradia-
tion and the appearance of the primordium might be the
latent period.

We must now consider the second possibility, i.e., that
roentgen rays have a selective action upon the tissues of the
brain, eyes, and anterior part of the head at a period prior to
the formation of the primordium. The two contentions which
must be supported for this explanation of the relation of the
critical period (demonstrated by irradiation) and the time of
appearance of the anlagen might well be put in the form of
questions. Are certain organs or areas of the embryo more
easily upset by environmental changes? Can the development
of certain structures of the embryo be affected before they
31. Are present as primordia? Similar types of malformations have been produced in embryos by the application of a variety of agents, such as, lowering of the temperature, toxic substances, ultraviolet light, radium and x-ray. Hyman ('19), after reviewing the literature on the experimental production of teratological development in teleosts, finds that the fore-brain, head in general, sense organs especially, heart and circulatory system and tail are predominately affected. Bellamy ('22) showed that the regions which differentiate earliest and grow most rapidly are the most susceptible to conditions which affect developmental or functional processes. Ultraviolet rays were utilized by Hinrich ('25), while Stockard ('21) reduced the temperature and thereby decreasing developmental rate, yet both report defective formation of the forepart of the head, eye and brain. Many authors have irradiated developing embryos and have found defective development following the treatment. The literature indicates that there is an almost constant selective action of roentgen rays, radium and ultraviolet light for the tissues of the brain, eye and anterior part of the head (Gilman and Baetjer '04, Bardeen '11, L. Loeb '22, Bagg '22, Hinrich '25, Gladstone, Colwell and Wakely '22 and '26, Hanson '25, DeNoble and Lams '25, Essenber, Loyola University School of Medicine, Department of Anatomy, (unpublished paper) Job, Leiboldt and Fitzmaurice '35). In these experiments a variety of animals were used, including the tele-
ost, amphibia, chick, rat and guinea pig. In many species malformations resulted when the effective agent was employed at an early stage in embryo formation, while in the case of the chick, similar results were obtained by Essenberg (unpublished work) when the embryos were exposed to the roentgen rays after the establishment of the primordia of the various systems. The experimental data which has been cited would seem to indicate that there is such a thing as tissue selectivity and that in embryos the tissues of the brain, eye, and jaw are predominately affected by various experimental agents, including roentgen rays.

Faulty development by interference with normal growth at an early stage in embryonic formation has been reported by Stockard and Hinrich. In the development of the fundulus eggs Stockard ('21) found the critical moment of origin of: 1) the primary embryonic axis, 2) the eyes and their associated structures, 3) the mouth and the branchial system, 4) the primary brain vesicles, 5) the inner ear, 6) the liver and pancreas. In these structures suppression of development or modified growth were found to occur as the result of retardation of the developmental rate by lowering the temperature or reducing the rate of oxidation, at certain definite points of progress between the early cleavage stages and the formation of the embryonic shield and embryonic line. Hinrich ('25) found that, "the development of the heart, circulatory system, eyes, and the anterior part of the brain is subject to modification at a period when there is no distinguish-
able primordium conceived of as a morphological precursor of these structures". From the data presented it appears convincing that there is justification in the belief that certain regions or parts of the embryo are predominately affected by changes in the environment and that this differential susceptibility of certain tissues may be present even before the time of appearance of the anlagen.

In teratological work frequent references have been made to the susceptibility of the cardio-vascular system. It would appear probable that rat embryos irradiated on the tenth and eleventh days would show some cardio-vascular defects. None such have been reported by Job and his coworkers, but the probable explanation is that interference with normal circulation so incapacitates the embryos that they become resorbed and do not go on to full term. Other defects which may have appeared but which were not reported by them are faulty development of the ear, hypophysis, lungs and liver. If the x-rays have their effect upon these structures after the latent period, then the critical period would precede the time of appearance of the anlage by the length of the latent period, but, if the action of x-rays is immediate upon the susceptible structures then the critical period would coincide with the formation of the anlage. Were the explanation to lie, however, in the peculiar susceptibility of the individual organ or the cells which are to give rise to it, then only by careful investigation of various dosages and different periods will the critical periods for these structures
SUMMARY AND CONCLUSIONS

1. An analysis has been made of the various techniques employed in establishing the age of albino rat embryos, with the purpose of securing, if possible, an accurate method. The investigation revealed that with the present means at our disposal, using the most careful technique, embryos dated from the same hour of copulation usually will show approximately the same stage of development, but may show age differences amounting to 24 hours or more. It was decided that the method which was most likely to give the most accurate information was to mate females at the proper stage of their cycle, the stage being determined by the vaginal smear technique developed by Long and Evans.

2. The stage of development of the albino rat embryos on certain critical days, the ninth, tenth, and eleventh, has been described.

3. Descriptions of the morphology of the embryo from the tenth to the fourteenth day of gestation, the period of organogenesis have been given.

4. The results which were obtained by Job and his coworkers when irradiating pregnant rats at certain times in gestation were correlated with the knowledge gained in this investigation of the embryology of the albino rat. This study demonstrated that at the periods when the x-rays were effective in
in producing abnormal development of the brain, eye and jaw, the primordia of these structures have not made their appearance. The exact mode of action of x-rays is not fully understood, but it is believed that the primary action of roentgen rays is probably upon developmental processes of the embryo at the time of irradiation and prior to the time of appearance of the anlagen of the susceptible structures. Further investigation may reveal critical periods for other structures such as the ear, hypophysis, lung and liver.
36.

BIBLIOGRAPHY


Essenberg, J.M. Unpublished research. Department of Anatomy, Loyola University School of Medicine.


37.

pp. 222-224.


Huber, G.C. 1915 The development of the albino rat, mus. norvegicus. I. From the pronuclear stage to the stage of the mesoderm anlage; end of the first to the end of the ninth day. Memoirs of the Wistar Institute of Anatomy and Biology, pp. 1-114.


Packard, C. 1933 The biological effects of roentgen rays and radium. The Science of Radiology.


FIGURES

Figure 1. Ninth day embryo. Sagittal section
Figure 2. Tenth day embryo. Sagittal section
Figure 3. Eleventh day embryo. Horizontal section
Figure 3a. Schematic representation of an eleventh day embryo.
Figure 4. Twelfth day embryo. Sagittal section
Figure 5. Section at the level of optic vesicle. Twelfth day.
Figure 6. Thirteenth day embryo. Sagittal section.
Figure 7. Section at the level of eye. Thirteenth day.
Figure 8. Fourteenth day embryo. Sagittal section.

KEY TO THE FIGURES

a. amnion
al. allantois
a.c. amniotic cavity
e.c. ectoplacental cavity
e.co. ectoplacental conus
e.e.e. extraembryonic ectoderm
en. entoderm
ex.c. extraembryonic coelom
d. diencephalon
h. heart
in. infundibulum
li. liver
m.f. medullary folds
40.

mes. mesencephalon
met. metencephalon
m.t. mesonephric tubules
my. myelencephalon
n.c. nasal cavity
n.t. neural tube
p.c. procamniotic cavity
p.e.e. primary embryonic ectoderm
p.s. primitive streak
pr. prosencephalon
o.v. optic vesicle
ot.v. otic vesicle
rh. rhombencephalon
st. stomach
t. telencephalon