# THE DEVELOPMENT OF THE EXTERNAL FORM OF THE GUINEA-PIG (CAVIA COBAYA) BETWEEN THE AGES OF 11 DAYS AND 20 DAYS OF GESTATION

by

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# THE DEVELOPMENT OF THE EXTERNAL FORM OF THE GUINEA-PIG (CAVIA COBAYA) BETWEEN THE AGES OF 11 DAYS AND 20 DAYS OF GESTATION

## INTRODUCTION

Much research has been done on the embryology of the guinea-pig but there remain many phases that have not been worked out.

### Purpose

It has been our purpose to work out the development of the structures that determine the external form, as well as to supplement the works on the growth in weight and length of embryos of the guinea-pig between the ages of 11 days and 20 days of gestation. As our tables show we have obtained many embryos of known copulation ages at intervals of every few hours throughout the day, thereby being able to study closely the slight but important changes occurring.

### Review of Literature

Earlier investigators, Reichert (1848), Bischoff (1852), Hensen (1866), Duval (1892), and Von Spee (1901)

contributed chiefly to the knowledge of the development of the very young blastocyst and the formation of the fetal membranes. Recently Maclaren (1926), Wilson (1928), and Hill and Sansom (1929) combined the contributions of these earlier workers with their own investigations and thus brought the development of the blastocyst up to about the 10th day. Draper (1920) and Ibsen (1928) worked out the growth of the guinea-pig embryos as to weight and length. Ibsen used only those embryos 20 days and older at intervals of five days. Draper observed some few cases of the stages as early as 10 days, but neither Draper nor Ibsen studied the structural development. Adloff (1904), Gruber (1906), Loeb (1906), Widakowich (1907), Ganzer (1908), Lohle (1913), Rabl (1913), Huber (1918), Dowd (1929), and others have contributed to the information concerning the development of tissues, organs, glands, etc.

## MATERIAL AND METHODS

All of the animals used for this research were obtained through the cooperation and courtesy of the Animal Husbandry department. The majority of the females which were discards from the Genetics research, were secured when about a month old.

## Breeding Conditions

Conditions which might give rise to variations in the development of the embryos were eliminated as nearly as possible by keeping the breeding conditions uniform. The animals were kept in metal cages in the basement of a limestone building so there was little range of temperature. A balanced ration of rolled oats mixture, alfalfa hay, either sprouted oats or fresh green plants, and water was regularly provided the animals each day. Since the feeding was uniform and adequate any differences in degree of development could hardly be interpreted on the basis of nutrition.

With a few exceptions, only young virgin females were used. These animals when secured were weighed, recorded, ear-tagged, and kept in cages separate from the males. At a regular time each day each female showing an open vagina was removed to a cage with a male and closely observed. If copulation occurred the vagina was examined for the presence of a vaginal plug. In each case the record was made on the female's card and in the general record book, as for instance (C6♀ x A1♂) 8:00 A. M., December 2, 1929; vaginal plug. Thus a double check was kept which eliminated chanc-

es of error. This was desirable because the age of each litter used was in every case the exact copulation age calculated from the time of copulation to the time of killing the female.

As a result of these careful observations only a few non-pregnant females were sacrificed. A total of 94 females was killed. Of the 68 that showed a vaginal plug after copulation 61 were found to be pregnant. Of the four showing no vaginal plug two were pregnant and two were non-pregnant. Twenty-two cases were uncertain and from that number only nine were pregnant.

## Procedure

Before killing a female for dissection all supplies were in readiness so that no delays need occur in removing the embryos. The female was again weighed and then killed. The use of illuminating gas was found to be the most satisfactory way to kill the animal.

The entire uterus with its contents was left in place in the animal until a drawing had been made showing the relative size and location of each embryo in the uterus. Embryos in the right uterine horn were indicated as R 1, R 2, R 3, etc., from the union of the horns distally to the Fal-

lopian tubes. In a similar manner in the left horn the embryos were accordingly designated as L 1, L 2, etc.

By cutting the uterine wall carefully along the antimesometrial side it was possible to remove each embryonic blastocyst intact along with the deciduate maternal tissue. Before further dissecting the blastocyst it was carefully weighed, on an analytical balance, sensitive to 1/1000 gram. Embryos of 16 days and older were removed from the blastocyst, weighed, measured by means of a vernier caliper, and then fixed in Bouin's fixative solution. Lastly, the remaining fluid and the membranes were weighed separately. In the case of embryos younger than 15 days the total weight of the blastocyst with the deciduate tissue was so small that it was found to be impractical to weigh the embryo, fluids, and membranes separately. Some 15 day embryos were weighed apart from the rest of the blastocyst but the chance for error on such small weights is as great as the significance of the weight.

## DATA AND OBSERVATIONS

In the accompanying table (page 37) the majority of the litters obtained are recorded as to the age of the litter, the location of each blastocyst in the uterus, the weight and length of the embryos, and the weights of the

membranes and fluids.

## Location in the Uterus

A total of 213 embryos was obtained from 71 litters. Reference to the table shows that there is a rather uniform distribution in the two horns of the uterus. The combinations most frequently found are "two right - one left" and "one right - two left."

| Number of Litters | Individuals in Right Horn | Individuals in Left Horn |
|---|---|---|
| 1 | 1 | 0 |
| 1 | 0 | 4 |
| 1 | 5 | 1 |
| 2 | 1 | 3 |
| 2 | 3 | 1 |
| 4 | 2 | 0 |
| 5 | 3 | 0 |
| 6 | 0 | 3 |
| 7 | 2 | 2 |
| 9 | 1 | 1 |
| 13 | 1 | 2 |
| 20 | 2 | 1 |
| Total 71 | 113 | 100 |

Table 1. The distribution of the blastocysts within the uterus, showing the number of litters in which each combination occurred.

## Size of the Ova

There is a gradual increase in the weight of the total blastocyst and deciduate maternal tissue. The total weight of each was not taken in every case for occasionally the membranes were broken, allowing the amnionic fluids to escape. Since the fluids have a high specific gravity the loss of these would affect greatly the weight of the total.

| Age | Average Weight | Range of Weights | |
|---|---|---|---|
| | | Lightest | Heaviest |
| 20 days | 1.685 gm. | 1.460 gm. | 1.935 gm. |
| 19 " | 1.325 " | 1.015 " | 1.625 " |
| 18 " | 1.017 " | 0.809 " | 1.200 " |
| 17 " | 0.904 " | 0.695 " | 1.060 " |
| 16 " | 0.662 " | 0.300 " (resorbed?) | 0.890 " |
| 15 " | 0.614 " | 0.340 " | 0.800 " |
| 14 " | 0.477 " | 0.200 " | 0.735 " |
| 13 " | 0.375 " | 0.252 " | 0.650 " |
| 12 " | 0.389 " | 0.340 " | 0.455 " |
| 11 " | 0.197 " | 0.147 " | 0.250 " |

Table 2. The weight of the smallest ovum and the heaviest ovum for each age, also the average weight of all ova secured for each age. These figures include the weight of the deciduate maternal tissue.

It will be noted from our data that in no instance does the weight of the heaviest ovum at any one age closely approach the weight of the heaviest ovum of the succeed-

ing day. However, the weight of the lightest ovum at any one age may fall within the range of weights of the preceding day. The above data show that the average weight of the 13 day ovum is less than that of the 12th day. No conclusions may be drawn from this for the number of cases recorded for those days was small. On the whole, though, there is an increase in weight from day to day so that in the 10 day period which we have studied the weight has increased itself almost 10 times.

Accompanying this gradual increase in the total weight of the blastocyst and deciduate membranes combined, there is a corresponding increase in the weight and length of the embryo alone. In the following table it is to be noted that the average weight of the embryo alone is 0.0116 gram at 15 days as contrasted with the average weight of 0.1385 gram at 20 days. There is a comparatively wide range between the minimum and maximum weight and length of a given age but this wide range does not alter the increase in the average.

Likewise there is the lengthening of the embryo from 3.73 millimeters at 15 days to 9.1 millimeter at 20 days. This, as we shall see later, is not the true increase in length for the shape of the embryo is undergoing such rap-

id changes that a true measurement of the length on the same basis can hardly be made.

| Age | Weight in Grams | | | Length in Millimeters | | |
|---|---|---|---|---|---|---|
| | Ave. | Min. | Max. | Ave. | Min. | Max. |
| 20 days | 0.1385 | 0.080 | 0.200 | 9.1 | 8.0 | 10.2 |
| 19 " | 0.1183 | 0.060 | 0.245 | 8.25 | 6.9 | 9.7 |
| 18 " | 0.0649 | 0.014 | 0.095 | 6.37 | 5.7 | 8.1 |
| 17 " | 0.0541 | 0.025 | 0.107 | 5.38 | 4.2 | 6.7 |
| 16 " | 0.0487 | 0.010 | 0.075 | 4.41 | 4.0 | 4.9 |
| 15 " | 0.0116 | 0.005 | 0.015 | 3.73 | 1.9 | 5.4 |

Table 3. The weight and length of the embryos for each given age, including the average, the smallest, and the largest of the group for each age.

## Variations in Development

It is evident that there is some variation in the weight of the total blastocyst or the embryo alone, for any given age. These variations may be due to one or more causes. An actual difference in development may occur for the same copulation age for it is generally accepted that not always the same period of time elapses between copulation and fertilization. At this early stage an hour's difference in fertilization age may result in a great difference in the size as well as the appearance of the embryo. Also, some of the variation can undoubtedly be accounted for by mechanical differences, that is, varying amounts of

fluids adhering to the materials weighed or varying amounts of membranes remaining attached to the embryo. Embryos at this stage are not at all firm so that when the length is taken a slightly different degree of curvature may present itself with each embryo. Thus, slightly differing lengths may be obtained from embryos that are approximately the same actual length. But in spite of these variations, it is self evident that there is a continual, more or less gradual, development of the ovum throughout the period.

## The Fetal Membranes

In the youngest ova with the decidua that we have removed from the uterus the placental side presents an appearance differing little from the free end. In the very early 11 day ovum there may still be present the blood clot marking the fusion of the decidua capsularis over the implanting ovum. In that case the placental pole may be distinguished as being at the end opposite the blood clot. Usually at the 11 day stage the blastocyst with its membranes is a slightly oblong vesicle with the long axis running through the placental pole. Examination of the blastocyst alone shows that it is an elongated closed cylinder (figure 1) lying in the implantation cavity. At the one

end, the placental pole, the thickened ectoplacental trophoblast has formed scattered villi which may be separated easily from the maternal tissue. The incomplete "inverted" yolk sac is continuous with the ectoplacental trophoblast. Within the yolk sac at one end, the obplacental pole, is the ectodermal amnio-embryonal mass, which by this time has arranged itself in the form of a hollow sphere of cells one layer thick. That portion of the sphere which is toward the placental pole will give rise to the ectoderm of the amnion; the remainder is destined to give rise to the ectodermal parts of the embryo.

By the 13th day (figure 2) the blastocyst can be separated from the decidua only with difficulty for the villi have continued to form at the placental pole until the ovum is becoming rather well implanted. The blastocyst is tending to widen and flatten (figure 3). The amnio-embryonal area at 13 days is well organized into two portions, the amnion and the ectoderm of the embryonic disc. Toward the end of the 13th day the anlage of the allantois appears as a spongy mesodermal mass at the posterior end of the embryonic disc.

The allantois enlarges and becomes more spongy so that by the 15th and 16th days it is almost as large as the em-

bryo itself. By the 17th or 18th day it has assumed a more stalked appearance and later it fuses with the placental area to form the allantoic placenta. Lying close beside the allantoic stalk are the umbilical vein and umbilical arteries carrying blood from and to the placenta. Although the allantois attains such an extended degree of development, it is practically devoid of endoderm. The endodermal diverticulum is so short that there is little or no endoderm out of the body (figure 17).

The yolk sac of the guinea-pig is peculiar in that it forms not a closed vesicle as in most other mammals, but an "inverted" vesicle, presenting the endodermal surface to the cavity of the uterus, and at the edge distally from the embryonic disc it is fused with the ectoplacental trophoblast (figure 19). However, it nowhere forms a villous attachment. The circulation of the yolk sac is carried on through the vitelline veins and arteries. There is no crossing over of blood vessels from the yolk sac to the placenta and vice versa, for in the full term fetal membranes the yolk sac may be stripped from the placenta with little or no resulting hemorrhage.

A thinning of the decidua capsularis and a thickening of the decidua basalis enables one to distinguish quickly

and easily the placental area. By the 19th or 20th day the embryonic placenta can be separated from the maternal placenta.

Thus, in the 10 day period which we have studied all the fetal membranes have become well established and have become functional.

## Early Stages of Differentiation

As before stated, in the blastocyst of the 11 day stage not only the primary germ layers are established, but the yolk sac and ectoplacental trophoblast are rather well defined and the ectodermal amnio-embryonal mass has split into a hollow sphere of cells (figure 1). A section through the 11 day blastocyst (figure 18) shows the mesodermal cells pushing outward along the endoderm of the yolk sac; the mesoderm already lies in a thin layer between the ectoderm and endoderm in the region of the embryonic disc.

Externally at 13 days, the amnio-embryonal area is seen to consist of the embryonic disc and the amnionic portion. Early in the 13th day (figure 2) the embryonic disc appears as a dome shaped plate of cells consisting of all three germ layers, of which the endoderm lies externally, a part of the "inverted" yolk sac. Within the cavity be-

tween the embryonic disc and the placental portion is the amnion lying rather close to the dorsal surface of the embryonic disc.

During the 13th day (figure 3) a rapid proliferation and dorsalward growth of mesodermal cells occurs at the posterior end of the embryonic disc marking the anlage of the allantois.

## The 14 Day Embryo

The study of the embryo itself really begins with the embryo of the 14th day (figure 4), for at the 14th day the embryonic disc has thickened and formed definite limits. Along the median line, in the posterior half of the embryonic disc is a narrow strip, the primitive streak. This appears lighter and thinner than the surrounding area, due to the presence of the primitive groove in the primitive streak. Marking the anterior end of the primitive streak is a somewhat thickened area, Hensen's node. Laterally the germ layers, especially the ectoderm, are thickening. However, this thickening does not extend quite to the edge of the embryonic disc, thus the area has a wing like appearance. Anterior to Hensen's node and lying between and ventral to the medullary plates is the prominent club shaped head process (figure 19).

Further development during the 14th day (figure 5) gives the embryo a different appearance. The head process no longer occupies such a prominent position, in fact it is not noticeable at all in the whole mount. The primitive streak has shortened and the primitive groove has almost disappeared. Hensen's node appears as hardly more than merely the end of the primitive streak. In the anterior and lateral portions of the disc the ectoderm is thickening decidedly to form the broad flat medullary plates.

These medullary plates at a slightly further development (figure 6) increase in length much more than in width, and as they do so the medullary groove is formed between them. A very shallow crescentic groove, the head fold, is beginning to raise the head from the embryonic disc. Ventrally this groove produces an internal fold in the endoderm as well as the other germ layers; this internal fold of the endoderm is the first appearance of the fore gut. Medially throughout most of the length of the embryonic disc a longitudinal split occurs in the heretofore continuous plate of mesoderm. By a proliferation of the mesodermal cells at either side of this median slit the segmental plates have been formed. From these plates then in turn the first pair of mesodermic somites arises a little posterior to midway in the embryonic disc. The number of so-

mites increases to as high as six pairs by the end of the 14th day (figure 7). Lateral to the mesodermic somites are the lateral plates. At the edge of the head fold the vitelline veins are forming in the splanchnic mesoderm. The head fold is deepening and extending farther posteriorly and in so doing it allows the head, consisting chiefly of the medullary plates and fore gut, to curl up dorsally into the amnionic cavity. The medullary plates have continued to thicken, especially anteriorly, and are beginning to be elevated as a pair of folds in the region just anterior to the first mesodermic somite.

## The 15 Day Embryo

Early in the 15th day (figure 8) probably the most striking thing about the embryo is the broad flat medullary plates which are so greatly thickened. Anterior to the region of the vitelline veins (figure 20) the plates are so flat that there is hardly an indication of the folding except at the most anterior "ear like" parts, which are really the extreme edges of the plates beginning to fold. From the region of the vitelline veins and proceeding posteriorly (figure 21) there is a deep groove between the medullary folds, especially in the anterior somite region. However,

the plates have not yet approached each other closely enough to fuse.

The folding and differentiation of the splanchnic mesoderm that had resulted in the vitelline veins at the 14th day have continued, and by the 15th day have given rise to the heart. At the beginning of the 15th day the two bulb like myocardial folds are approaching each other preparatory to fusing into a single heart tube.

These folds fuse a little later (figure 20) to form the single heart tube which grows so rapidly that it begins to twist almost immediately. Simultaneously the folding of the medullary plates has been extended forward. As the folds have become further extended they have also begun to fuse together, forming the medullary or neural tube in the region of the most anterior somites. The medullary folds terminate very abruptly at the anterior end, indicating a late closure of the anterior neuropore. The lateral plates have become much more distinct and show the location of the lateral folds. The number of somites continues to increase, but a difference of two or three somites does not in itself effect much change in appearance now.

The fusing together of the medullary plates proceeds anteriorly and posteriorly, converting the folds and groove into the medullary tube, except for the extreme anterior

end and the portion in the region of and posterior to the last two or three somites (figure 9). Meanwhile the walls of the neural tube have been growing more rapidly in some areas than in others, giving the neural tube an irregular bulging form. These bulges may be identified as the primary vesicles of the brain. Upon either side of the anterior vesicle, sometime before the medullary plates fuse dorsally, an evagination of the wall begins, forming the optic vesicles.

The heart tube in the late 15 day development has twisted to the right about as much as in the 36-hour chick embryo. When the heart first appeared it was in the mid brain region but now it has descended to a position ventral to the hind brain. The first pair of branchial clefts, although not prominent, is distinguishable. By a continued folding together of the ventral body wall the anterior part of the embryo is raised from the yolk sac.

## The 16 Day Embryo

The 16th day brings about the complete closure of the body wall ventrally, except for the region of the umbilicus (figure 12). This separation of the embryo from the extra-embryonic membranes has been accomplished by the pinching together of the head fold, the lateral limiting sulci, and

the tail fold. In the splanchnopleure the folds unite to form the alimentary canal, which is now in contact with the yolk sac only through the yolk stalk.

The ventralward bending of the head commencing late in the 15th day has increased to form the cephalic flexure. Due to the closing of the body wall the embryo tends to lie on one side and assumes a curvature throughout its length.

The flexure and curvature of the neural tube correspond to those of the body already described. Continued differentiation of the walls of the neural tube leads to the more definite establishment of the primary vesicles of the brain. The outer wall of the optic vesicle which had begun as a lateral evagination from the prosencephalon late in the 15th day invaginates to form the double walled secondary vesicle, the optic cup. Ventrally the wall of the cup is incomplete, leaving the opening known as the choroid fissure. An invagination of the thickened ectoderm external to the optic cup forms a thick walled pit, the lens vesicle. The auditory vesicle, the otocyst, appears as a pair of thickenings and invaginations of the ectoderm on the dorso-lateral sides of the head a little anterior to the first somite. These pits deepen and the lips of the pits begin to fold together at the 23 somite stage. At about this

same time the ectoderm in front of the optic vesicles begins to invaginate to form a pair of pits, the olfactory pits.

Three or four branchial clefts may be present, but there is little indication of the maxillary and mandibular processes, hence the oral fossa is large and open.

Further increase in the number of somites now ranging from 16 to 23 pairs decreases the length of the segmental plates.

## The 17 Day Embryo

The somites now range in number from 23 pairs to 29 pairs, and the segmental plates have shortened more (figure 13). Four branchial clefts may still be present but the third and fourth are no longer outstanding. In many 17 day embryos the fourth pair of clefts has been closed by the fusing together of the fourth and fifth pairs of arches. The first pair of arches begins to thicken, especially along the anterior border where the maxillary and mandibular processes are appearing. These processes are the primordia of the upper and lower jaws.

The cephalic flexure differs in appearance from the flexure at 16 days only in that the angle is a little more acute. The general curvature of the body has increased slightly. The only change in the neural tube is the fur-

ther thinning of the roof plate of the rhombencephalon. The complete separation of the otocyst from the outside ectoderm is effected during the 17th day.

## The 18 Day Embryo

The curvature of the embryo is becoming more marked in the region of the first somites and in the region immediately anterior to the tail (figure 14). These regions of increased curvature are the locations of the cervical or nape flexure and the sacral flexure. It is not until well toward the end of the 18th day that the sacral and nape flexures become well established.

In addition to the flexures of the neural tube that are induced by the body flexures there is the pontine flexure which occurs in the neural tube alone. This third brain flexure makes its appearance as a ventral bending of the floor of the anterior portion of the hind brain or rhombencephalon. Continued differentiation of the neural tube initiates the development of the secondary vesicles. A forward and dorsalward expansion of the anterior end of the fore brain gives rise to the telencephalon, of which the most prominent parts are the primordia of the cerebral hemispheres. The remainder of the prosencephalon is the diencephalon. Very little change has occurred in the mesenceph-

alon. Hardly any expansion occurs in the region between the hind brain and the mid brain; this region is now known as the isthmus. From the rhombencephalon arise two divisions, the metencephalon and the myelencephalon. The latter is characterized by the extremely thin roof.

The eye is assuming a more rounded appearance; the lens vesicle is separating from the outside ectoderm and is becoming a spherical body; the choroid fissure, although still present at the beginning of the 18th day, has almost disappeared by the end of that day.

Complete closure of the mouth of the otocyst converts the vesicle into a closed sac. A conical elevation of the external side of the most dorsal portion of the otocyst gives rise to the endolymphatic duct.

The anterior limb buds arise as a result of the rapid proliferation of the mesenchymal cells in the region of the sixth to 11th somites. Beginning proliferation of the mesenchyme and a slight swelling posteriorly indicates the hind limb buds, but as yet they are no more than slight ridges.

## The 19 Day Embryo

The sacral and nape flexures which had become well defined by the end of the 18th day have reached their great-

est development at 19 days (figure 15). No new structures in direct association with the neural tube originate during the 19th day. The optic vesicle is rapidly becoming much smaller in comparison to the size of the fore brain. The choroid fissure is no longer visible. Pigment is appearing in the iris and retinal layer of the vesicle.

The heart, last referred to in the 15 day embryo, has migrated posteriorly to lie in the body cavity in the region of the fore limb buds. Rapid elongation of the heart tube between the anterior and posterior fixed ends has produced a folding. During the 19th and 20th days some portions of the tube expand more than others and thus there are established the divisions of the heart, of which only the atrial and ventricular portions are discernible externally. The ventricular portion lies somewhat ventral to and posterior to the atrial portion.

The anterior limb buds which were mere swellings in the 18 day embryo have become well rounded outgrowths. The hind limb buds are located in the region of the 28th to 33rd somites.

At the 19th day the last somites have been formed; the final number is 49 pairs. From each anterior somite there have arisen the sclerotome and myotome, even before the last few somites have been formed.

## The 20 Day Embryo

Except for the few most posterior ones, all the somites have differentiated into the myotome or muscle plate, and the sclerotome which will give rise to the vertebrae (figure 16). Development of the neural tube during the 20th day produces no new structures but rather there is a further definition of the secondary vesicles already established. The eye, which is becoming relatively smaller in comparison to the size of the fore brain, shows much pigmentation. The limb buds are beginning to elongate to the extent that the distal end is free from the surrounding tissue. The heart presents an appearance similar to that at 19 days.

Two pairs of branchial clefts remain unclosed. The maxillary and mandibular processes from the first pair of arches are enlarging and are growing ventralward, but as yet they have not united so as to form the face. Hence, the oral fossa is still wide and open. The olfactory pits are still rather widely separated.

During the period from the 16th day to the 20th day the umbilical cord has been constricting until by the 20th day its connection with the body covers only a small area.

## DISCUSSION

These foregoing data as to weight and length parallel those by Draper (1920). However, Draper took his data from a fewer number of cases and he recorded embryo blastocysts of only every second or third day. He gives the weight of three ova at 11 days as 0.425 gram (average 0.142 gram per each) as contrasted with our figure of 0.197 gram. Likewise the weight he records at 20 days is not as heavy as we have found. On the other hand, his weights given for 14 days, 15 days, and 17 days are heavier than we have secured. But since the weights dealt with were so small and since the cases recorded were so few, the difference between our data and those of Draper is of no significance. Moreover, Draper records only a very few cases falling within the range of ages of the embryos we have observed as to weight and length so that a comparison with his results can hardly be made. Ibsen (1928) records data from embryos only 20 days of age and older so no comparison can be made between these data and his.

The establishing of the germ layers and the development of the blastocyst up to about the 10th day have been worked out by earlier investigators. Recent investigations and interpretations by Maclaren (1926), Wilson (1928), and Hill

and Sansom (1929) on the earlier phases of development, in addition to the contribution by Duval (1892) covering the fetal membranes, correspond to and substantiate our investigations and conclusions.

Reference to the plates I, II, and III shows that in the 10 day period studied the embryonic tissue has developed from the blastocyst, showing only the germ layers, to the embryo closely approaching the degree of development of a fetus. Development of the structures such as the eye, ear, limb buds, and heart is similar to the development of those same structures in other mammals. On the other hand, the presence of the "inverted" yolk sac, the extensive development of the allantois almost devoid of endoderm and the extremely flat medullary plates anteriorly up to the 15th day, are quite characteristic of the guinea-pig. The development of these structures in the other rodents is similar but does not parallel exactly the development in the guinea-pig.

## SUMMARY

1. The guinea-pig blastocyst presents the "inversion" of the germ layers.

2. The allantois arises on the 13th day as a mesodermal outgrowth, and by the 18th or 19th day it fuses with the

ectoplacental trophoblast to form the allantoic placenta.

3. The primitive streak appears late in the 12th or early in the 13th day and has completely differentiated by the end of the 14th day.

4. The medullary plates arise on the 14th day as broad flat plates; the plates fuse together first in the hind brain region on the 15th day; the neural tube differentiates into the primary vesicles late on the 15th day, and differentiates into the secondary vesicles on the 18th day.

5. The first branchial arch is formed at 15 days, at 16 days the maximum number is present, and at 20 days three arches are still present.

6. The otocyst appears at 16 days, closes off at 17 days, and shows the endolymphatic duct at 18 days.

7. The optic vesicle evaginates at 15 days; the choroid fissure disappears at 18 days; pigmentation begins at 19 days.

8. The first pair of somites is formed at 14 days and the final number of 41 pairs is formed at 19 days.

9. The body folds off from the yolk sac except for the umbilical cord at 16 days.

10. The limb buds arise at 18 days.

11. The cephalic flexure is found at 15 days and the

sacral and nape flexures at 18 days.

12. The weight increases from 0.0116 gram at 15 days to 0.1385 gram at 20 days; the length increases from 3.73 millimeters at 15 days to 9.1 millimeters at 20 days.

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| Time Elapsing between Copulation and Removal | Litter Number | Individuals in Litter | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Right Horn of Uterus | | | | | | | | | | | | Left Horn of Uterus | | | | | | | | | |
| | | R 3 | | | | R 2 | | | | R 1 | | | | L 1 | | | | L 2 | | | | L 3 | | | |
| Days / Hours | | Weight of Embryo | Length of Embryo | Weight of Membranes | Weight of Blastocyst | Weight of Embryo | Length of Embryo | Weight of Membranes | Weight of Blastocyst | Weight of Embryo | Length of Embryo | Weight of Membranes | Weight of Blastocyst | Weight of Embryo | Length of Embryo | Weight of Membranes | Weight of Blastocyst | Weight of Embryo | Length of Embryo | Weight of Membranes | Weight of Blastocyst | Weight of Embryo | Length of Embryo | Weight of Membranes | Weight of Blastocyst |
| 1-6 | D-8 | .200 | 9.9 | .84 | 1.63 | .15 | 9.3 | .905 | | .185 | 8.6 | 1.005 | 1.685 | | | | | | | | | | | | |
| | E-6 | | | | | .135 | 9.4 | .875 | 1.460 | .16 | 10.2 | .925 | 1.755 | .165 | 9 | .900 | 1.695 | | | | | | | | |
| | E-12 | | | | | | | | | .08 | 8. | .765 | | | | | | | | | | | | | |
| 20 | C-18 | .10 | 8.8 | .87 | 1.635 | .11 | 9.7 | 1.02 | 1.935 | .10 | 8.3 | .92 | | | | | | | | | | | | | |
| 19-24 | D-7 | | | | | .10 | 7.3 | .77 | 1.22 | .06 | 7.6 | .69 | 1.100 | .11 | 7.5 | .78 | 1.28 | .09 | 7.3 | .64 | 1.06 | | | | |
| | B-16 | .11 | 9.1 | .59 | | .245 | 9.7 | .620 | 1.275 | .155 | 9 | .630 | 1.190 | | | | | | | | | | | | |
| 7-12 | D-4 | | | | | .115 | 9.1 | .73 | 1.395 | .11 | 8.6 | .645 | 1.270 | .13 | 9.1 | .77 | | | | | | | | | |
| | D-12 | | | | | | | | | | | | | .115 | 8. | .78 | 1.495 | .150 | 8.9 | .755 | | .14 | 8.7 | .755 | 1.625 |
| 1-6 | D-2 | | | | | | | | | .12 | 8.2 | .745 | 1.280 | | | | (.550) | .105 | 7.6 | .725 | 1.240 | .97 | 6.9 | .69 | |
| | E-5 | | | | | | | | | | | | | .08 | 8. | .88 | | .080 | 7.8 | .86 | 1.42 | .10 | 9.1 | .875 | 1.530 |
| | B-1 | | | | | .090 | 7.1 | .715 | 1.190 | .080 | 6.9 | .71 | 1.040 | .11 | 7.6 | .915 | 1.420 | | | | | | | | |
| | C-2 | | | | | .160 | 9.4 | .74 | 1.35 | .145 | 8.2 | .63 | 1.08 | .11 | 8.3 | .660 | 1.015 | | | | | | | | |
| 19 | E-2 | | | | | | | | | | | | | .13 | 9.4 | .76 | 1.34 | .14 | 8.7 | .795 | 1.460 | .125 | 8.8 | .695 | 1.300 |
| 19-24 | D-11 | | | | | | | | | | | | | .055 | 8.1 | .70 | .820 | .075 | 6.5 | .705 | 1.120 | .060 | 6.1 | .57 | .935 |
| 7-12 | C-20 | | | | | .075 | 6.3 | .755 | 1.115 | .065 | 6.9 | .605 | .970 | .070 | 5.8 | .675 | | | | | | | | | |
| 1-6 | D-1 | | | | | .075 | 5.9 | .685 | 1.070 | .065 | 6.3 | .665 | 1.010 | .06 | 6. | .65 | 1.03 | | | | | | | | |
| | A-7 | | | | | .014 | 5.7 | .200 | .8095 | .045 | 6. | .7 | .965 | | 6. | | | | | | | | | | |
| 18 | E-8 | | | | | .095 | 7. | .775 | 1.160 | .090 | 6.6 | .82 | 1.200 | | | | | | | | | | | | |
| 7-12 | E-7 | | | | | .06 | 5.7 | .61 | .86 | .04 | 6. | .625 | .655 | .04 | 5.2 | .64 | .845 | | | | | | | | |
| | C-8 | | | | | | | | | .107 | 6.1 | .643 | 1.000 | .059 | 6.7 | .651 | | (resorbed) | | | | | | | |
| 1-6 | A-15 | | | | | | | | | .025 | 6.2 | .80 | .990 | .025 | 6.7 | .81 | 1.045 | (resorbed) | | | | | | | |
| | A-25 | | | | | .090 | 6.2 | .65 | .940 | .070 | 6. | .73 | 1.050 | .085 | 5.7 | .62 | .865 | | | | | | | | |
| | C-17 | | | | | .050 | 4.9 | .680 | .910 | .035 | 4.7 | .55 | .760 | .045 | 5. | .445 | | | | | | | | | |
| | D-5 | | | | | | | | | .045 | 5.1 | .55 | | .045 | 4.6 | .47 | | .035 | 4.3 | .57 | | | | | |
| | E-1 | | | | | .055 | 5.2 | .79 | 1.020 | .045 | 4.9 | .79 | 1.055 | .065 | 5.3 | .785 | 1.060 | | | | | | | | |
| 17 | A-21 | | | | | .06 | 4.5 | .52 | .74 | .064 | 5. | .506 | .695 | .055 | 5.4 | .51 | .785 | | | | | | | | |
| 1-6 | D-13 | | | | | | | | | .035 | 4.1 | .525 | .650 | .040 | 4.1 | .545 | .780 | .060 | 4.3 | .575 | ..790 | | | | |
| | C-4 | | | | | | | | | (resorbed?) | | | .30 | .055 | 4.2 | .55 | .700 | .070 | 4.6 | .585 | .745 | | | | |
| | C-1 | | | | | | | | | | | | | | 4.6 | .505 | .625 | | 4. | | | | | | .57 |
| | E-10 | | | | | .075 | 4.9 | .665 | .890 | .060 | 4.3 | .695 | .890 | .055 | 4.2 | .620 | .800 | .035 | 4.6 | | .665 | | | | |
| | E-16 | | | | | | | | | | | | .360 | | | | .425 | | | | | | | | |
| 16 | D-6 | | | | | | | | | | | | | .040 | 4.9 | .555 | .735 | .010 | 4.6 | .545 | .650 | .050 | 3.5 | .520 | .685 |
| 7-8 | G-13 | | | | | | | | | | | | .57 | | | | .575 | | | | .66 | | | | |
| 1-6 | F-9 | | | | | | | | .565 | | 3. | | .345 | | | | .465 | | | | | | | | |
| | C-6 | | | | | | | | .475 | | 1.9 | | .575 | | 3.2 | | .414 | | | | | | | | |
| | E-15 | | | | | | | | | .015 | 4.5 | .555 | .650 | .005 | 4.4 | .575 | .655 | .015 | 5.4 | .650 | .775 | | | | |
| | E-3 | | | .745 | | | | | .650 | | | | .655 | | | | .582 | | | | (R 4.59) | | | | (R 5 .57) |
| | G-9 | | | | | | | | | | | | .64 | | | | .655 | | | | .565 | | | | |
| | G-8 | | | | | | | | .62 | | | .61 | .74 | | | | .662 | | | | .65 | | | | |
| 15 | H-11 | | | | | | | | .675 | | | | .80 | | | | .655 | | | | | | | | |
| 19-24 | H-5 | | | | | | | | | | | | .735 | | | | .69 | | | | | | | | |
| | H-10 | | | | | | | | | | | | .359 | | | | .424 | | | | | | | | |
| | D-3 | | | | | | | | .310 | | | | .200 | | | | .235 | | | | .275 | | | | |
| 7-12 | E-18 | | | | | | | | | | | | .57 | | | | .615 | | | | .635 | | | | .615 |
| | G-14 | | | | | | | | .375 | | | | .465 | | | | .447 | | | | .480 | | | | |
| 1-6 | H-2 | | | | | | | | | | | (L 4 .540) | | | | | .495 | | | | .475 | | | | .524 |
| 4 | H-12 | | | | | | | | | | | | .455 | | | | .58 | | | | | | | | |
| 19-24 | G-10 | | | | | | | | | | | | .650 | | | | .560 | | | | | | | | |
| 1-6 | B-13 | | | .350 | | | | | .380 | | | | .360 | | | | | | | | | | | | |
| | H-6 | | | | | | | | | | | | .252 | | | | .335 | | | | .305 | | | | |
| | H-1 | | | | | | | | | | | | .312 | | | | .342 | | | | .412 | | | | |
| 13 | I-10 | | | | | | | | .302 | | | | .322 | | | | .335 | | | | | | | | |
| | I-6 | | | | | | | | .340 | | | | .455 | | | | .350 | | | | | | | | |
| 12 | A-17 | | | | | | | | .390 | | | | .410 | | | | | | | | | | | | |
| | H-7 | | | | | | | | | | | | .149 | | | | .160 | | | | | | | | |
| 11 | B-8 | | | | | | | | .205 | | | | .180 | | | | .240 | | | | .250 | | | | |

## PLATE I

The outlines for the drawings were made with the aid of a microprojector apparatus so the proportions of the drawings are an exact reproduction of the proportions of the embryos drawn.

Fig. 1. The 11 day blastocyst (X 10). E, ectodermal amnio-embryonal mass; Y, yolk sac; V, villi; T, ectoplacental trophoblast.

Fig. 2. Early 13 day blastocyst (X 10). D, embryonic disc; A, amnion.

Fig. 3. Late 13 day blastocyst (X 10). L, allantois; B, extra-embryonic blood vessels.

Fig. 4. Early 14 day embryonic disc with the adjacent yolk sac (X 10). H, head process; N, Hensen's node; P, primitive streak.

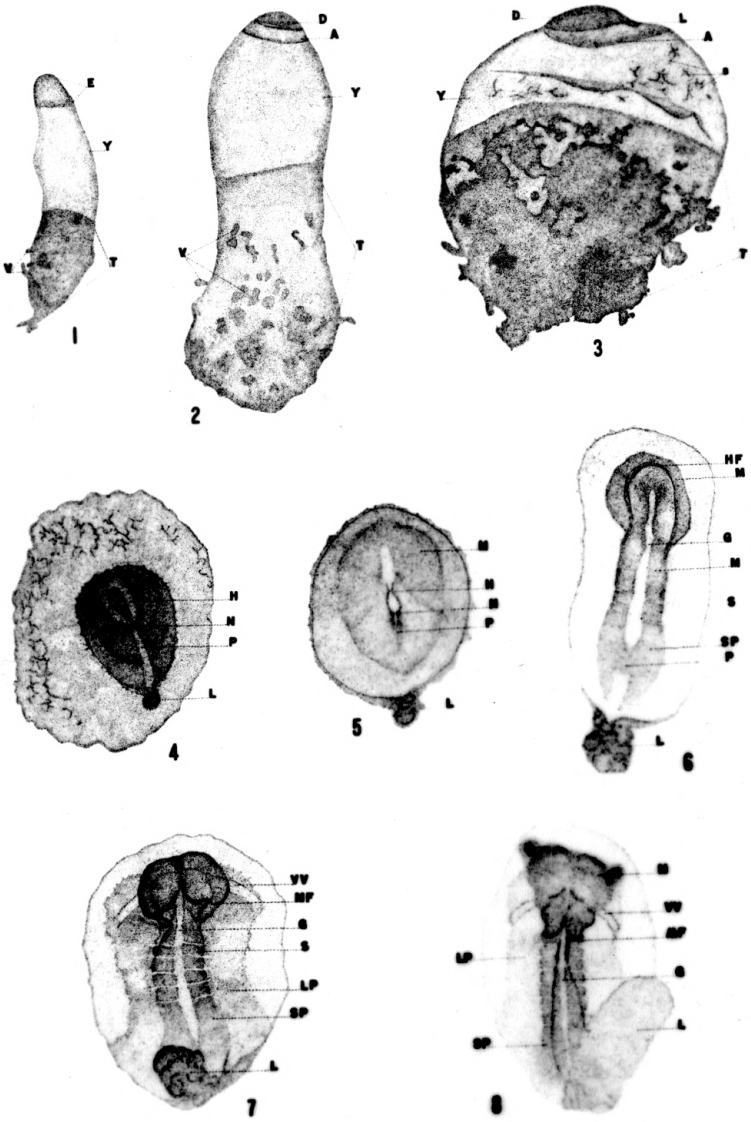
Fig. 5. The 14 day embryonic disc showing a little further development (X 15). M, medullary plate.

Fig. 6. Ventral view of a 14 day embryonic disc (X 15). HF, head fold; G, medullary groove; S, mesoblastic somite; SP, segmental plate.

Fig. 7. Dorsal view of a slightly curled 14 day embryo showing further development (X 15). VV, vitelline vein; MF, medullary fold; LP, lateral plate.

Fig. 8. Early 15 day embryo (X 15).

# PLATE I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## PLATE II

Fig. 9. Embryo of 15 days showing the folding of the medullary plates (X 7). MF, medullary folds; C, heart; S, somite; LP, lateral plate; SP, segmental plate; L, allantois.

Fig. 10. Dorsal view of late 15 day embryo (X 10). MC, mesencephalon; HC, rhombencephalon; VV, vitelline vein.

Fig. 11. Lateral view of 15 day embryo (X 8). FC, prosencephalon; OV, optic vesicle; GA, branchial arch; LF, lateral fold.

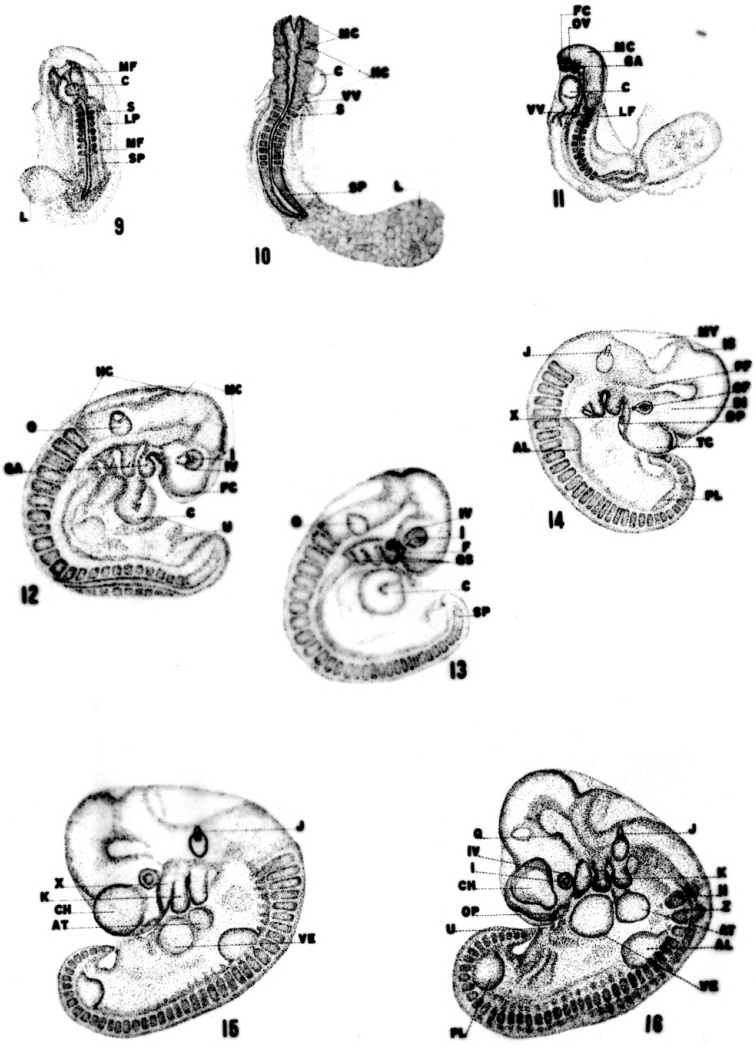
Fig. 12. The 16 day embryo (X 10). O, otocyst; I, lens vesicle; IV, optic cup; U, umbilical cord.

Fig. 13. The 17 day embryo (X 6). F, oral fossa; GS, branchial clefts.

Fig. 14. The 18 day embryo (X 4). AL, fore limb bud; PL, hind limb bud; MY, myelencephalon; IS, isthmus; PF, pontine flexure; J, endolymphatic duct; CF, choroid fissure; DI, diencephalon; OP, olfactory pit; TC, telencephalon; X, maxillary process.

Fig. 15. The 19 day embryo (X 4). VE, ventricular portion of heart; AT, atrial portion of heart; K, mandibular process; CH, cerebral hemisphere.

Fig. 16. The 20 day embryo (X 4). II, myotome; Z, sclerotome; Q, infundibulum.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## PLATE III

Fig. 17. Longitudinal section through a 15 day embryo showing the allantois. L, mesodermal portion of the allantois; LD, endodermal diverticulum of allantois; Y, yolk sac; HG, hind gut; A, amnion.

Fig. 18. Transverse section through the 11 day blastocyst in the uterine lumen. UM, uterine mucosa; D, embryonic disc.

Fig. 19. Transverse section through a 14 day blastocyst. T, ectoplacental trophoblast; H, head process.

Fig. 20. Transverse section through the heart region of a 15 day embryo. M, medullary plate; C, heart; PH, pharynx; NC, notochord.

Fig. 21. Transverse section through the somite region of a 15 day embryo. G, medullary groove; LP, lateral plate; MF, medullary fold; S, somite.

# PLATE III