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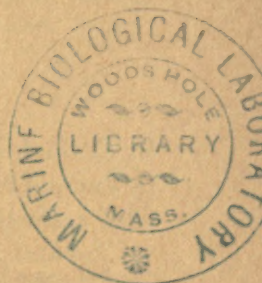
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## THE EMBRYOLOGY OF THE OPOSSUM

EDWARD McCRADY, JR.

*The Estingham B. Morris Biological Farm of The Wistar Institute*

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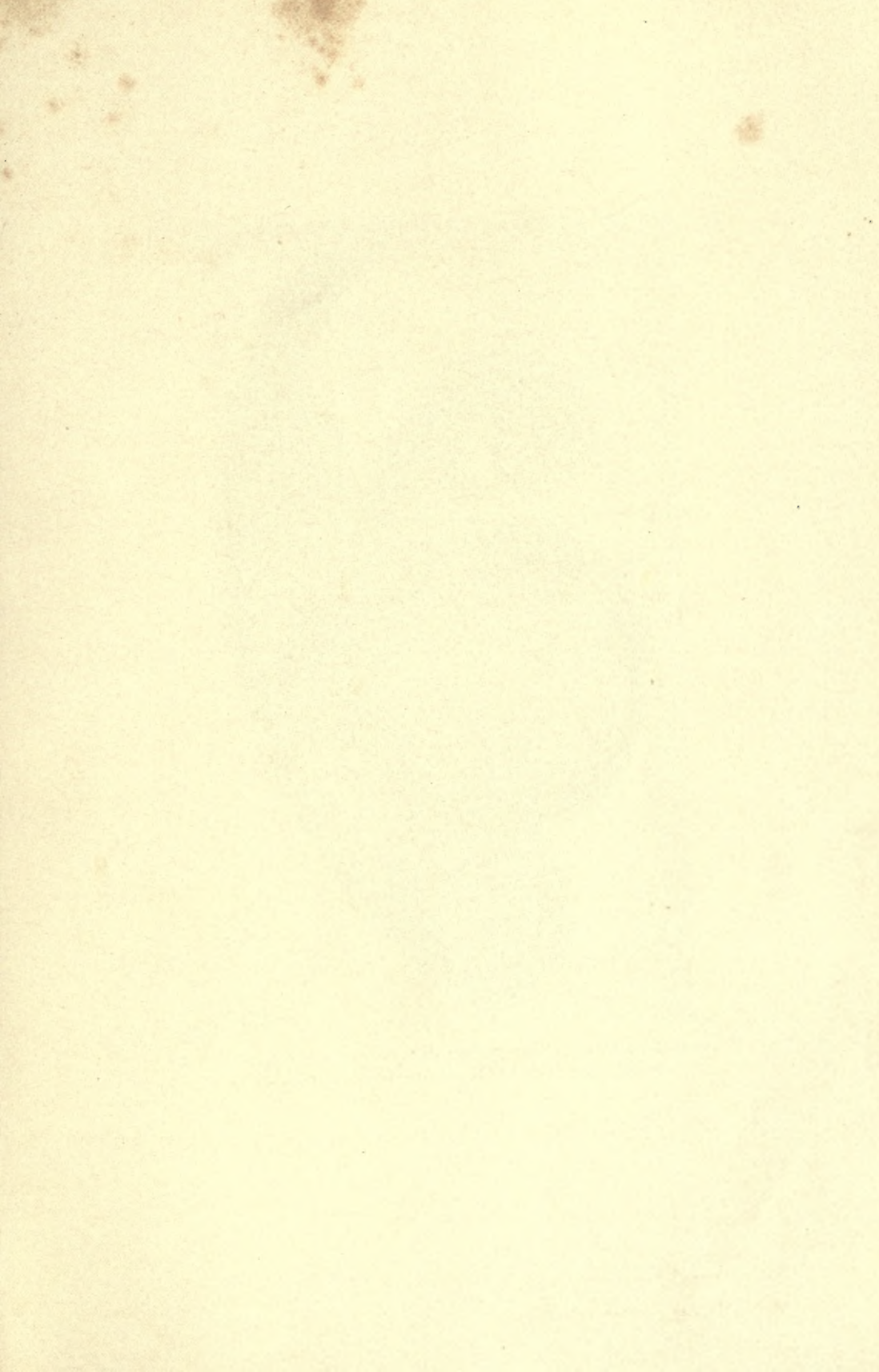
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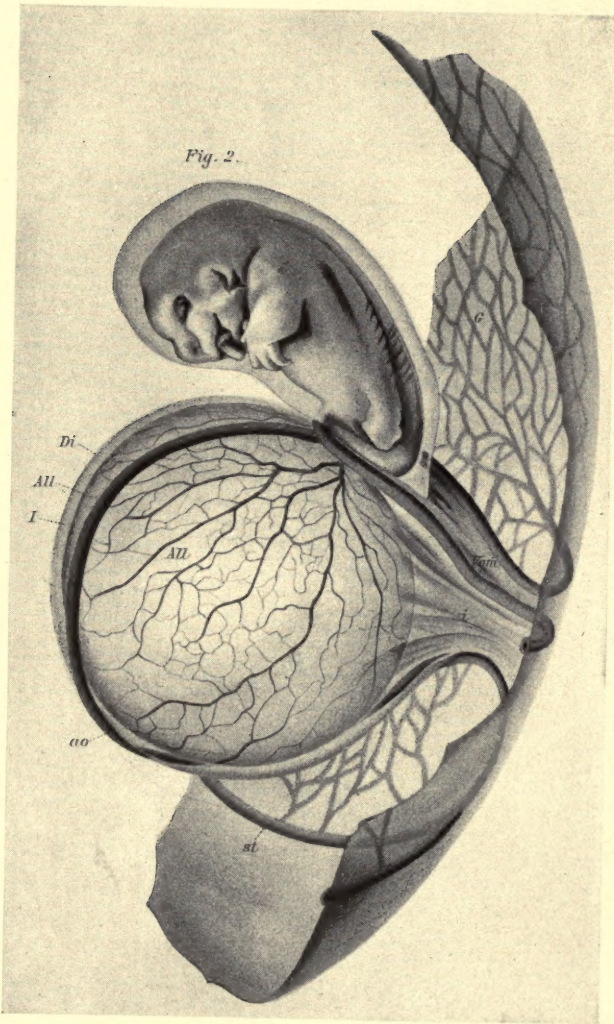


Fig. 2.

Opossum embryo of the twelfth day showing chorion, amnion allantois and body stalk. Taken from Selenka, 1887.



THE EMBRYOLOGY OF THE OPOSSUM

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EDWARD McCRADY, JR.

*The Effingham B. Morris Biological Farm of The Wistar Institute*

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SIXTY-SIX TEXT FIGURES AND THREE PLATES

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THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY

Philadelphia







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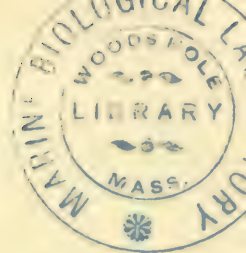


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#### ACKNOWLEDGMENTS

To begin with, I cannot be too enthusiastic in expressing my indebtedness to Dr. Carl Gottfried Hartman. His studies on the opossum have been more extensive than those of any previous investigator. That they have been liberally drawn upon in the following pages will be apparent from the frequent appearance of his name in the text. Not only has my account of cleavage, germ-layer formation, and the rate of development, been based primarily upon his researches, but even my studies of the later stages which he did not examine microscopically, have been made, for the most part, upon material which he collected and left at The Wistar Institute.

I have come to differ with Doctor Hartman's interpretations in several respects which are discussed in the appropriate parts of the text; but these differences, which, incidentally, do not involve any differences in actual observations within the stages he studied, came to light only after I had had the advantage of his fundamental researches and the opportunity to study a large series of later stages of which he had no sections, and which threw new light on the significance of the earlier material.

To Dr. C. H. Heuser, also formerly of The Wistar Institute, I am indebted for the negatives of most of the photographs of external views of the embryos, and for the use of figure 60. In the few cases in which I had to make the negatives of external views myself, the difficulties I encountered impressed me very strongly with my indebtedness to his exceptional skill.

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## INTRODUCTION

The Virginia opossum (*Didelphys virginiana* Kerr)<sup>1</sup> is very abundant and easily caught in almost every part of the United States from about the latitude of Pennsylvania south. Being a marsupial, and an extremely primitive one, it would have been studied very thoroughly and used very widely for laboratory purposes long ago, if it had been more amenable to domestication.

The first serious attempt to breed this animal in captivity was that of Dr. Emil Selenka, who imported a large number of them to Erlangen, Germany, some time just prior to 1887. He had the good fortune to obtain several litters of eggs and embryos before the colony went sterile and the animals died. He tried the same experiment also with the South American opossum, *Didelphys cancrivorous*, and with three Australian marsupials (*Hypsiprymnus penicillatus*, *Phalangista vulpina*, and *Phalangista orientalis*); but in these ventures he met with less success than he had had with the Virginia opossum.

Since his time several American zoölogists and embryologists have tried to establish breeding colonies of *Didelphys*, and all have met with failure until The Wistar Institute in 1930 decided to invest a considerable sum of money in a large-scale effort to solve the problem. After several years of disappointment the major difficulties seem at last to have been overcome, and for the convenience of those who may want to breed the opossum in their own laboratories I shall pass on what information we have gained.

The first essential is to prevent the animals from developing rickets. Though from the very inception of our colony all the

<sup>1</sup> Another spelling of the generic name often seen substitutes an 'i' for the 'y' in the last syllable. Etymologically this change makes the term mean 'two dolphins or porpoises.' I prefer the spelling which means 'two uteri.'

animals were given whole milk and eggs every day, they, nonetheless, developed acute rickets. The ones which were adult when brought into the colony occasionally bred once before going sterile. They rarely lived more than a year, and finally died in tetanic convulsions similar to those often associated with osteomalacia and rickets. The young born in the colony appeared normal during the first 80 days (i.e., the nursing period), but then rapidly developed some very characteristic abnormalities. Their legs grew less rapidly than their heads and trunks, and became disproportionately short and bowed. Their heads appeared disproportionately large, and their backs showed kyphosis. Their fur was scrawny, and their teeth abnormal, the canines frequently breaking off. When the thorax was opened, the ends of the ribs showed a typical rachitic rosary. Such animals never attained sexual maturity, and rarely lived as long as a year; though I have on record one case of a male which showed all the rachitic symptoms in an extreme degree, but which survived for 2 years and 3 months.

At first I found it difficult to believe that the animals could really have rickets when they were being fed eggs and milk every day, but as soon as I saw that there was no doubt about the fact that they actually did, I thought the solution of that difficulty would be easy—we would simply add cod liver oil to the diet. This was done for 1 year without noticeable, beneficial result. Not only were the diseased specimens not cured, but normal young introduced into the colony from outside became just as abnormal with cod liver oil as without.

After trying other vitamin D concentrates, and combinations of vitamin D and vitamin A, all to no avail, the next resource was to try forcing the animals to live in the open sunlight. This was decidedly contrary to their natural inclinations, as under normal conditions they are nocturnal. But even the enforced exposure to sunlight did them no good.

Finally, I began opening the stomachs of animals just captured, to discover what the wilds ones were eating which the captive ones were not. One such thing which I noticed was



bones. Under natural conditions they eat, among other things, small vertebrates (frogs, lizards, salamanders, small birds, etc.) entire—bones and all. In our colony they were getting plenty of meat (beef, beef heart, and liver) but no bones. So after that we sprinkled bone meal into the food pans every day, and that cured the rickets. This suggests a curious calcium and phosphorus metabolism which might be worthy of further study. As their parathyroid glands are also peculiar, there may be an interesting correlation here.

After the addition of bone meal to the diet the animals grew to full, normal adult stature and proportions, and did not die prematurely; but still they did not breed. Examination of the testes of the males raised in the colony showed plenty of normal-looking, mature spermatozoa; but the ovaries of the females were definitely atrophic, and contained few or no mature graafian follicles. Hartman ('23) had reported some evidence that this condition of the ovary might be associated with inadequate opportunity for exercise, so we tried enlarging the pens and introducing devices for climbing. The nest boxes in which the animals sleep during the day were hung on racks on the walls of the colony rooms, and the food pans were placed on the floor. The animals had to climb down to feed during the night, and with the return of daylight, if they wanted a dark hole in which to hide, they had to climb back up. This they invariably did, rather than sleep on the floor in open daylight, so a certain amount of exercise was forced upon them, but they took advantage of their opportunities to a greater extent than that, and at night I often found them chasing each other up and down the tree branches which led to the nest boxes.

After that change the females began to ovulate, and we got our first litters from animals which had been raised in captivity. In the spring and summer of 1935 we had 250 young born in the colony. This year (1937) we have some third-generation, captive-born young; so we feel that at least our major problems must have been solved.

My first recommendations, therefore, for the breeding of opossums are that the breeding pens should have at least 50 square feet of floor space, and that the daily diet should include bone meal. The principal foods are meat, milk, and eggs. Vegetables and fruits are eaten, but less avidly. Wild opossums also eat beetles, grubs, and viscera, but apparently these are not necessary constituents of their diet.

#### I. PRELIMINARY DATA

##### *The ovarian egg. The testicular sperm. Mating*

*The ovarian egg.* Aside from the exceptional frequency of polyovular follicles and polynuclear ova (Hartman, '36), the general histology of the ovary of the opossum is not significantly different from that of other mammals. Certainly most, and perhaps all, of these atypical ova and follicles degenerate within the ovary, and so a detailed discussion of them is not necessary in connection with the normal embryology of the opossum.

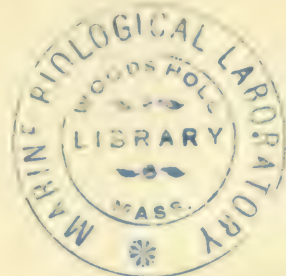
Just before ovulation the normal egg (Hartman, '16, '19) is slightly elliptical, the average of the longest and shortest diameters being 0.165 mm. and 0.135 mm., respectively (fig. 1). This is larger than the tubal ovum. The cytoplasm includes a considerable quantity of yolk in the form of granules and fat droplets. These are not concentrated at either pole of the egg, but are principally confined to a rather diffuse spherical layer a little beneath the surface of the ovum (fig. 1B, y.g.). The cytoplasm both medial and superficial to this layer is more homogeneous.

The first maturation division (fig. 1, A) takes place within the ovary. The spindle forms in the superficial cytoplasm usually near one of the ends of the slightly elongate oocyte. The haploid number of chromosomes is eleven, all of which are tetrads in the metaphase of this division. One of these tetrads represents the synapsed X-chromosomes. The first polar body (fig. 1 B, p.b.) is spindle shaped and almost devoid of cytoplasm. It is variable in size, but averages about 0.025 mm. in its longest diameter. A distinct zona pellucida,



## ABBREVIATIONS USED IN ALL FIGURES

a., atrium of heart	h., hypophysis
a.c., anterior semicircular canal	H.n., Hensen's node
a.c.a., anterior cerebral artery	i., infundibulum
a.c.v., anterior cardinal vein	id., idiozome
a.f., axial filament	j., jugular vein
a-g., anal musk gland	k., metanephros
a.g.o., anal gland orifice	l.b., lung bud
alb., albumen	l.c., lateral semicircular canal
all., allantois	l.c.v., lateral cephalic vein
a.l.r., anterior limb ridge	l.g., lateral groove of pharynx
am.c., amniotic cavity	l.p., lung pouch
amp., ampulla	l.r., lateral ridge of otocyst
an., anus	l.v.c., lateral vaginal canal
ao., aorta	m., mesonephros .
a.r., anterior ridge of otocyst	m.c.v., middle cephalic vein
at., atrium of otocyst	M.d., müllerian duct
b., bladder	met., metencephalon
b.a., branchial arch	m.g., medullary groove
b.p., branchial pouch	m.p., medullary plate
b.r., branchial ridge	m.t., mesonephric tubule
c., coelom	m.v.c., median vaginal cul-de-sac
c.a., conus arteriosus	n., notochord
can.r., canalis reuniens	n.c., neural crest cells
c.c., crus commune	n.p., notochordal plate
ce., centriole	nu., nucleus
cho., chondriosome	n.v.ch., non-vascular chorion
chr., chromosome	o., ovum
cl., glans clitoridis	o.a., ophthalmic artery
co., cochlea	o.p., otic placode
c.o., cumulus oöphorus	op.c., optic cup
c.r., centriolar ring	op.l., optic lobe
c.t., caudal tube	ot., otocyst
c.u., cervix uteri	ot.c., otic cup
d.a., dorsal aorta	ot.p., otic placode
d.m., dorsal mesocardium	ov., ovary
d.m.l., dorsal mesocardial line	o.v., omphalomesenteric vein
e.d., endolymphatic duct	p., perineum
end., endoderm	p.b., polar body
e.s., endolymphatic sac	p.b.c., posterior branchial complex
ex., exerescence from distal centriole	p.c., posterior semicircular canal
F., fallopian tube	p.g., primitive groove
f.g., fat globule	ph.b., pharyngeal bursa
f.l., follicular liquid	ph.m., pharyngeal membrane
fo., foregut	pl., placenta
g., ganglion	p.m., parietal mesoderm



pr., pronucleus  
 p.r., posterior ridge  
 p.s., peri-intimal space  
 p.t., pronephric tubule  
 R., Rathke's pouch  
 r., rectum  
 r.b., residual body  
 s., somite  
 sac., sacculus  
 s.e., seam of closure  
 s.ch., serous chorion  
 s.e., endolymphatic sac  
 s.f., spiral filament  
 s.g., stratum granulosum  
 s.m., shell membrane  
 sp., spinal cord  
 S.p., Seesel's pouch  
 s.r., sinus rhomboidalis  
 st., stomach  
 s.t., sinus terminalis  
 s.v., sinus venosus  
 t., tingierbare Körner

th., thymus  
 thy., thyroid  
 u., uterus  
 u.o., urogenital orifice  
 ur., ureter  
 u.s., urinogenital sinus  
 u.s.g., utriculosaccular groove  
 utric., utriculus  
 u.v., umbilical vein  
 v., ventricle  
 v.a., ventral aorta  
 v.c.f., vestibulocochlear fossa  
 v.ch., vascular chorion  
 v.c., ventral concavity  
 v.p., ventral pouch  
 W.d., wolffian duct  
 x., X-chromosome  
 y., Y-chromosome  
 y.b., yolk balls  
 y.g., yolk granules  
 y.s., yolk sac  
 z., zona pellucida

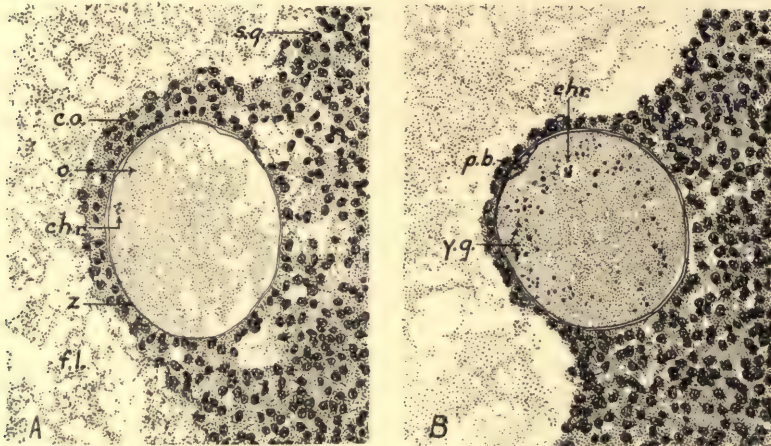


Fig. 1 Ovarian ova. A, specimen 16270<sup>2</sup> showing first maturation division. B, specimen 16274 showing first polar body and the sub-peripheral distribution of yolk granules.

<sup>2</sup> This is the catalogue number of this specimen in The Wistar Institute embryological collection. Throughout this book such numbers are recorded for the convenience of any investigators who may wish to examine the original material.



which is from 0.0006 to 0.004 mm. in thickness, fits closely about the egg cell and the first polar body and presses them tightly together, so that the latter makes an elongated depression on the surface of the egg. Beneath the zona pellucida a radially striated layer some 0.0012 mm. thick is visible in some preparations. Hartman ('16) noticed it, but considered its homology to the zona radiata of other forms uncertain.

*The testicular sperm.* The spermatogonium (Painter, '22) has twenty-two chromosomes (fig. 2, A), including ten pairs of autosomes, an X-chromosome, and a Y-chromosome. The last two, the sex chromosomes, are visibly represented during

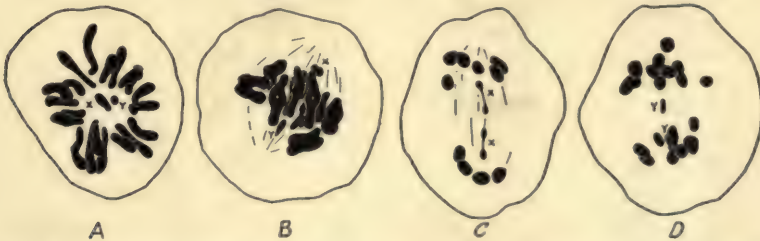


Fig. 2 Spermatogenesis (after Painter, '22). A, polar view of spermatogonium showing ten pairs of autosomes, an X-chromosome, and a Y-chromosome. B, equatorial view of first maturation division showing 12 chromosomes on account of precocious separation of X and Y elements. C, equatorial view of second maturation division showing X-chromosomes. Not all chromosomes are included in this figure. D, equatorial view of second maturation division showing Y-chromosomes. The true haploid number (11) may be counted at the upper pole.

the growth stage of the primary spermatocyte as the chromatin nucleolus (Painter, '24), which is the product of their synapsis. The X and Y components segregate (fig. 2, B) in the first maturation division, so that two kinds of secondary spermatocytes are formed in equal numbers. Both Jordan ('11) and Painter ('22), have recognized a distinct resting stage between the two maturation divisions. In the second maturation division the sex chromosomes divide equationally (fig. 2, C and D).

Spermiogenesis (fig. 3) has been described by Duesberg ('20). Fat droplets are always present in early spermatids.

These later disappear. The tail fiber grows out from two centrioles which are situated near the surface at the posterior of the cell (fig. 3, A). The nucleus migrates to a point opposite the centrioles (therefore at the anterior of the cell) and becomes condensed into a flattened egg-shaped mass in which no details are recognizable. The centrioles, which are connected by a fine fiber, follow the nucleus, and the proximal one becomes attached to it (fig. 3, B). The distal one, which

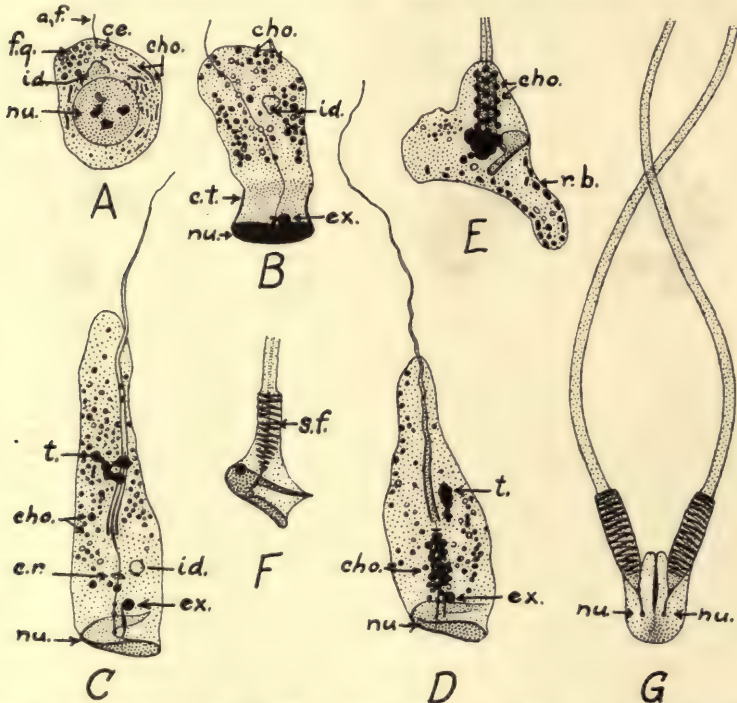


Fig. 3 Spermiogenesis (redrawn from Duesberg, '20). A, early spermatid. The idiozome has already broken and released the two centrioles which are now giving rise to the axial filament. Fat globules are numerous, and are always collected into one group. Chondriosomes are filamentous. B, chondriosomes have become granular. Caudal tube formed. Exerescence still attached to distal centriole. C, the ring has begun its migration. Exerescence attaches separately to swallow-tail nucleus. D, many chondriosomes have collected around axial filament. E, residual body about half formed. F, residual body has been cast off. Chondriosomes have formed spiral around axial filament. Spermatozoon is now free in semeniferous tubule. G, in epididymis the spermatozoa have paired by fusion in the head region along the heavier arm of the V-shaped nucleus. Middle piece now runs between legs of the V instead of at right angles to it.



is still attached to the axial filament, produces an exerescence which becomes quite large and conspicuous. This later degenerates, and its function is unknown. Next, the distal centriole flattens and divides into a proximal granule and a distal ring, the latter encircling the axial filament (fig. 3, C). By this time the nucleus has assumed a curious swallow-tail or V-shape, the proximal centriole being attached at the inside of the angle, and the axial filament extending at right angles to the plane of the V. The ring now moves distally along the axial filament, and as it does so many of the chondriosomes from the surrounding cytoplasm migrate to the axial filament and become attached to that part over which the ring has passed (fig. 3, D). The remaining chondriosomes and the remnants of the Golgi material and idiosome, together with most of the cytoplasm, collect in a residual body anterior to the nucleus (fig. 3, E), and are cast off. At this time the spermatozoon is discharged into the lumen of the seminiferous tubule. Here the chondriosomes which had become attached in the middle piece fuse into a spiral around the axial filament (fig. 3, F); the V-shaped nucleus turns through an angle of  $90^\circ$  so that the middle piece now runs between the legs of the V; and, finally, the spermatozoa fuse together in pairs (fig. 3, G), the line of junction being only in the head region along the heavier leg of the V. These conjugate sperms, as Wilson ('28, p. 305) calls them are a rare phenomenon, but not unique—outside of the marsupials they are found in a beetle, *Dytiscus* (Ballowitz, 1895), and in a gastropod, *Turritella* (Retzius, '06). The significance of the pairing is not known. Selenka (1887) pointed out that when the individuals of a pair separate later on they can no longer swim in a straight line, but describe circles. He thinks that most of the spermatozoa remain paired until they have reached the upper part of the oviduct, and then normally separate before fertilization.

*Mating.*<sup>3</sup> According to Hartman ('23 and '28), the breeding season in Texas begins with the first week of January.

<sup>3</sup> This word is here used only as a synonym for copulation, and does not imply permanent pairing. Any male will copulate with any female in oestrus.

Unmated females show a regular dioestrous cycle of about 28 days, as indicated by urinogenital sinus smears, beginning in January and ending in September. The last 3 months of the year constitute an anoestrus. Ovulation does not occur during lactation. Pregnancy lasts nearly 13 days, and lactation from 70 to 80 days, so that certainly two litters, and possibly three, may be borne by one female in a year. My own experience seems to indicate that the anoestrus may be somewhat longer in Pennsylvania during cold winters, and probably two litters is a maximum for this part of the country.

The graafian follicles usually burst during or very near the time of oestrus. Hartman ('28, p. 162) records one observation which he interprets as evidence that copulation sometimes takes place too late for fertilization (i.e., after the ova have become enclosed in too much albumen, or have begun to degenerate). If so, the implication is that in the opossum ovulation is not dependent upon any stimulus from coitus or related to oestrus in any invariable way. The cleavage stages or early vesicles occasionally found at abnormally long intervals after coitus may be interpreted as confirmatory evidence. Hartman's no. 298 is a case in point. The vesicles he found in the first operation 6 days after coitus belong to my stage 10 and are approximately 2 days late. In the second operation, 9½ days after coitus, he found vesicles of my stage 21. This represents a perfectly normal rate of development between the two operations, so the whole batch of eggs must have started development 2 days late. This means they would have entered the uterus uncleaved about 3 days postcoitum. On March 1, 1937, I obtained eleven uncleaved uterine ova (no. 137) in perfect condition from a uterus opened exactly 73 hours postcoitum. In this case the female had been isolated immediately after the observed copulation, so the possibility of a second copulation 2 days later is ruled out.



Also of interest in this connection is the evidence of a difference in fertility between the matings which occur during daylight hours and those which occur in the night or very early morning. Selenka said that he had never seen mating occur before 7 in the morning or after 11. Though I have seen one mating at 3 o'clock in the afternoon, it is true that it is very exceptional for matings to occur after 11 A.M. On the other hand, the 7 o'clock early limit is quite definitely wrong. By far the majority of all matings occur before that time, and I have some evidence that the matings which occur between 7 and 11 are not really normal.

Of the sixty-one matings which have been observed in my colony after 7 o'clock in the morning, only eleven were fertile. There is thus a ratio of five and one-half infertile matings to one fertile mating after 7 A.M.

Only occasionally have observations been continued through the whole 24 hours, so the total number of matings which occurred before 7 A.M. is not known for any large number of days. But the total number of adult females which were in the colony at any given time is known, and the normal length of the oestrous cycle (Hartman, '23) is known, as already mentioned. If it is assumed for the sake of a calculation that every adult female mated once each cycle except during the annual anoestrous period and the times when she was either pregnant or lactating, the maximum possible number of matings in my colony can be computed for any given length of time. For the year 1935 this number is 209. Since thirty-one matings were observed after 7 o'clock in the morning, there are left only 178 conceivably possible matings which might have occurred before seven. If the total number of litters born during 1935, minus those from matings which occurred after 7 o'clock, be multiplied by  $5\frac{1}{2}$ , the product is 203.5. This is the number of matings which must have occurred before 7, if the ratio of infertile matings is as high before 7 as it is afterward. But this number is 13% greater than 178, the maximum possible number of matings before 7 o'clock. In other words, it is impossible that the percentage

of fertility was as bad in matings before 7 as it was in those after 7. As the real number of matings was probably much less than the computed maximal possible number, the real percentage of fertility must be very much higher in the earlier matings.

A possible explanation is that ovulation and oestrus both usually occur in the early morning hours (i.e., before 7), and that when mating is delayed it usually means that oestrus is late with reference to ovulation, and correspondingly the sperm reach the ova too late for fertilization. Usually only unfertilized ova are found in females which have been seen to mate after 7.

All the ova from one ovary are discharged simultaneously, and both ovaries ovulate together. The average number of eggs from each ovary at each ovulation is eleven (Hartman, '19), though as many as twenty-two from each ovary have been recorded.

Mature spermatozoa may be found in testes at any time of the year, but maturation stages are more abundant at the beginning of the breeding season (Painter, '22). The male is probably capable of fertile coitus at any time, but is aroused to activity only by the 'heat' of the female.

During copulation the penis (fig. 4) passes directly into the urinogenital sinus (fig. 5). The glans of the penis is bifid, but the urethra opens between, not at the tips of, the prongs. However, a groove, which is very nearly converted into a tube by its overhanging edges, extends along the medial side of each prong for more than half of its length. It is possible that these grooves serve in place of a bifid urethra to carry the semen directly into the lateral vaginal canals.

These latter organs show a considerable muscular activity during oestrus (Hartman, '24). By alternate contraction of their proximal and distal portions they churn the liquid within them. When semen is introduced into this liquid two things happen. In the first place, some spermatozoa are delivered to the os uteri almost immediately by the currents set up by the churning. And in the second place, a coagulation



of the mixture of lateral vaginal canal fluid and semen begins within a very short time. The spermatozoa which do not escape quickly into the uterus are then imprisoned in a coagulated mass—the vaginal plug. Twenty-four hours later the vaginal plug has disappeared.

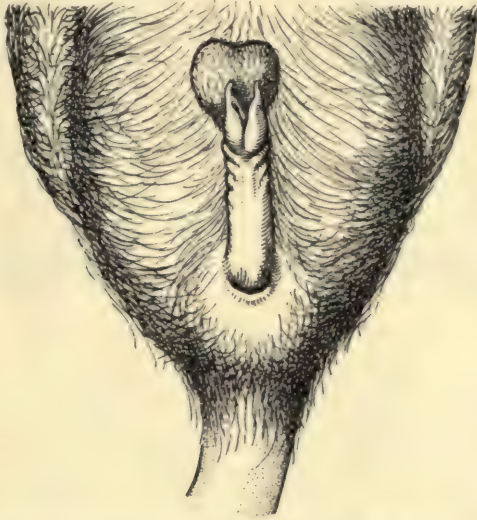


Fig. 4 Ventral view of male external genitalia. The penis is caudal to the scrotum, and its glans is bifurcate. During detumescence it retreats out of sight to a position similar to that of the clitoris.

Lewis ('25) studied the bacteria present in these canals at different stages of the oestrous cycle. During the anoestrus he found their number to be reduced to a minimum. During the prooestrus the numbers, both of individuals and of species, increase rapidly. The maximum is attained at oestrus. As all of the species identified, of which there are about a dozen, are well-known saprophytes, he concluded that they are responsible for the removal of the vaginal plug and for the general cleaning of the canals during the dioestrus and anoestrus.

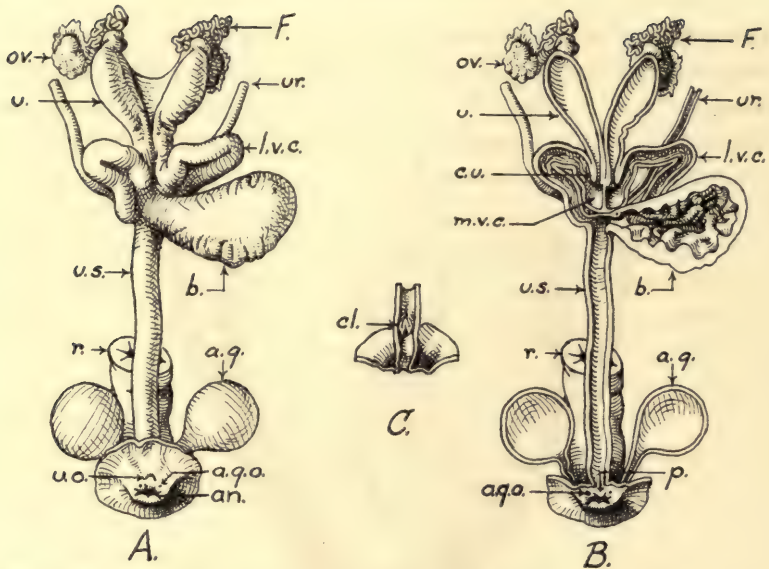


Fig. 5 Female urinogenital system. A, ventral view of adult organs in anoestrous condition. B, dissection of the same specimen. The uteri are seen to be completely double, each protruding into its own median vaginal cul-de-sac. From this median position the vagina runs anteriorly and laterally, and then turns back upon itself to empty into the urinogenital sinus immediately dorsal to the neck of the bladder. The ureter on each side passes between the arms of the lateral vaginal loop to enter the bladder through a prominent papilla on the dorsal side of the neck of this organ. The urinogenital sinus and the rectum open to the exterior separately, but very near each other. There is no cloaca. The anal musk glands, which are here shown fully distended, open exteriorly through two very small pores. C, the ventral wall of the urinogenital sinus shows a bifurcate glans clitoridis. The skin fold beyond this point is the prepucce.

## II. THE FIRST DAY

### Stage 1. Fertilization. The tubal ovum.

*Fertilization.* As the ovum can descend the oviduct in as short a time as 24 hours (Hartman, '28), and as it comes to be surrounded by an impenetrable layer of albumen and a thin shell membrane while doing so, and as unfertilized eggs begin to degenerate within 24 hours (S. C. Smith, '25), it is necessary for the spermatozoon to find the ovum at the extreme upper end of the oviduct, and soon after ovulation, if fertilization is to be accomplished. But ovulation does not bear a precise or consistent time relation to copulation. Some-



times, apparently, the ova are discharged more than 12 hours before copulation, and on these occasions the mating is infertile.

The sperm twins always separate before fertilization. This is indicated by the fact that the sperm found entrapped in the albumen of the egg are always single. The actual entrance of the spermatozoon into the ovum has not been observed in any marsupial.

In summary: Occasionally precocious ovulation results in sterile mating. Occasionally, perhaps, delayed ovulation results in a post-oestrous period. Most often ovulation occurs within a few hours before or after copulation, and fertilization by single spermatozoa takes place in the upper part of the fallopian tubes within a few hours after ovulation.

### *Stage 1*

*The tubal ovum.* From about the twelfth hour to the thirty-sixth hour after coitus one may expect normally to find the ova in the tubes. It is possible that immediately after dehiscence of the follicle the granulosa cells are adherent to the zona pellucida for a short time, but, if so, they are very soon shed and the ovum is left covered only by the zona. In some way (perhaps by a change in osmotic pressure in the surrounding fluid) the volume of the egg is slightly reduced at this time (Hartman, '19). Its new dimensions are 0.122 mm. for the longest diameter, and 0.104 mm. for the shortest (fig. 6).

After the formation of the first polar body in the ovary the chromosomes apparently do not enter a resting stage as they do at the corresponding stage of spermatogenesis (vide, p. 17), but immediately arrange themselves on the spindle for the second maturation division. This is the condition in the early tubal ovum (fig. 6). If fertilization does not take place, the second maturation division does not complete itself, though the X-diad, which is sometimes precocious, may divide. The other chromosomes remain in a metaphase distribution during the entire descent of the tubes (24 hours).

At the end of this time all chromosomes show signs of degeneration, and the egg undergoes irregular fragmentation without mitosis.

If fertilization has occurred, the second maturation division giving rise to the second polar body takes place. In the normal metaphase of this division there are at first eleven diads; but the X-diad often divides precociously, so that spindles are frequently found showing ten diads and two monads. This led Hartman ('19, p. 35) to the conclusion that the haploid number of chromosomes is twelve. Painter

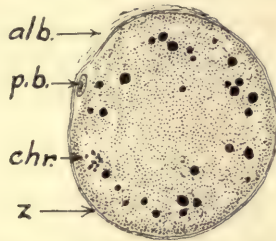


Fig. 6 Early tubal ovum (16266). Albumen is just beginning to be deposited on the zona. There is still only one polar body. The organization of the cytoplasm into a shallow, peripheral, homogeneous, darkly-staining zone; a sub-peripheral, irregular, yolk-laden zone; and a broad, homogeneous, lightly-staining, central zone; is well shown. The yolk granules are larger than they were in the ovarian ovum.

('22, p. 35) after a study of spermatogenesis, where a parallel precocious splitting of the sex chromosome often occurs (v, fig. 2, B), noted this mistake and corrected it.<sup>4</sup>

The two polar bodies are visible in many of the very early cleavage specimens. Hartman ('19) has seen what he interprets as polar bodies as late as the 34-celled stage; but usually one finds them difficult to recognize after the second cleavage on account of the phenomenon of yolk extrusion, which will be described below.

<sup>4</sup>To anyone interested in the source papers one further explanation is necessary. At the same time that Painter corrected Hartman's original mistake he inadvertently introduced another which makes his account somewhat puzzling. He consistently refers to the diads in Hartman's figures of second maturation divisions as tetrads, and to the monads as diads. I have pointed this out to him in conversation, and he agrees that it was a slip of the tongue.



Soon after the ovum enters the tube albumen begins to be deposited upon the zona in thin concentric sheets, which sometimes entrap numerous spermatozoa and occasionally a few epithelial cells from the oviduct. The stratification of the albumen seems to be due to the rolling of the egg during its descent of the oviduct. The final thickness of the albumen before resorption begins is some 0.25 mm., though there is considerable variability in this dimension.

Just before the egg passes into the uterus a shell membrane is secreted upon the surface of the albumen by the shell glands of the lower part of the oviduct. These glands have been seen by Hartman ('16) and Anderson ('28). The shell is about 0.001 mm. in thickness when the ovum reaches the uterus (Hartman, '16). The diameter of the egg including ovum, albumen, and shell is about 0.6 mm. The shell is leathery rather than brittle, and seems, as evidenced by staining reactions, to be composed of two quite different materials. If not overstained, a clear, yellowish, homogeneous, non-chromophilic matrix may be distinguished as the principle substance of the shell. Within this, and usually confined to its inner half, is a granular, intensely basophilic, less flexible deposit. When the albumen beneath the shell shrinks during dehydration the shell wrinkles, and the darker material frequently cracks up into many small pieces which remain embedded in the more flexible yellowish material. I mention this detail because I have no doubt that patches of this dark-staining, granular material were what Selenka (1887, plate XVII) mistook for remnants of the nuclei of granulosa cells.

Soon after the formation of the second polar body both pronuclei may be seen in the resting stage near the surface of the egg and surrounded by a relatively yolk-free region of cytoplasm. The yolk in other parts of the egg retains its typical sub-peripheral distribution, but the granules and droplets have fused into fewer elements which are of correspondingly larger size. The two pronuclei now migrate toward the center of the ovum carrying their yolk-free area of cytoplasm with them (fig. 7). As they pass within the limits of the

spherical yolk layer, this latter comes together again behind them. In this condition the ovum reaches the uterus.

### III. THE SECOND DAY

Stage 2. The first cleavage. The extrusion of yolk.

*The first cleavage.* During the first half of the second day after mating the fertilized ovum reaches the uterus in the

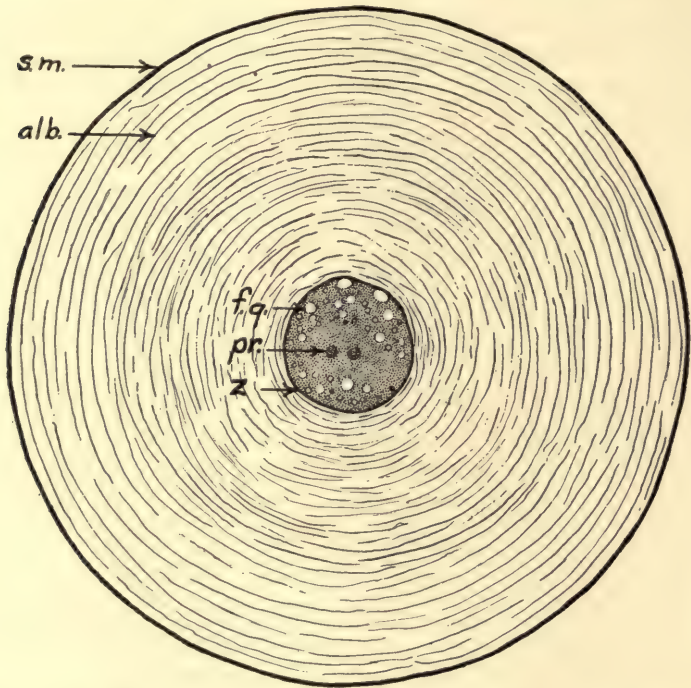


Fig. 7 Ovum with complete albumen layer and shell membrane ( $\times 150$ ). The pronuclei have migrated to the center of the ovum. In this figure the ovum proper is taken from specimen 16244; but the dimensions of the albumen and shell represent the average dimensions in fresh specimens. After dehydration the albumen shrinks considerably and the shell wrinkles (see fig. 10).

pronuclear stage. The fusion of the pronuclei and the formation of the spindle for the first cleavage occur at the center of the egg. I have studied two eggs which show this cleavage in process. The first is a late anaphase (no. 16265)



(fig. 8). The ovum has become very elongate and equatorially constricted. The chromosomes are grouped about two centrosomes at opposite poles, with the exception in each group of one chromosome which lags nearer the equator. This lagging chromosome is much nearer the equator in one case than in the other. Indeed, a closer inspection reveals that all the chromosomes on one side of the equator are at a slightly different stage of development from those on the other, those approaching one pole (the lower pole in fig. 8, B) being nearer the telophase condition than those approaching the other.



Fig. 8 Late anaphase of first cleavage from one of Hartman's specimens (16265). A, the entire ovum showing elongation and equatorial constriction of the cytosome, much deutoplasmic material in the cytoplasm, the beginning of yolk extrusion in the lower hemisphere, and the situation of the spindle a little above center. B, enlarged view of the spindle to show the difference in stage of vacuolation of the chromatin at the two poles.

Furthermore, the yolk is not equally divided between the two poles. A count of the fat droplets in camera lucida tracings of serial sections of this specimen reveals that about 60% of them are in that half of the egg which has the more advanced chromosomes. This is not a difference in concentration of yolk, but merely in volume of cytoplasm plus its inclusions at the two poles. The location of the spindle and the plane of division are off center so that the two daughter cells will be unequal in size. These early indications of a difference between the two poles of the egg are of interest as they foreshadow the fact that the cells at one pole of the prospective blastocyst will divide more rapidly than those at

the other, and that these cells are descended from the larger of the first two blastomeres.

The second specimen showing the first cleavage in process is a telophase (no. 16256). The equatorial constriction of the cytosome is not yet complete, but the wells of the nuclei have formed. The only indication of polarity recognizable at this stage is a difference in size of the two prospective daughter cells. With reference to the polarity indicated in these two specimens the plane of the first cleavage is equatorial.

*The extrusion of yolk.* A very extraordinary change has taken place in the cytoplasm while the nuclear events just described were in progress. It was observed in the last chapter that during the passage of the ovum down the tubes the yolk granules and fat droplets collect into fewer units of larger size. The next change which can be recognized takes place when the pronuclei are approaching each other in the center of the egg and before the dissolution of their membranes. At this time a few large fat droplets can be seen protruding from the surface of the egg, as if they were being slowly extruded. That they meet considerable resistance at this surface is witnessed by the degree of distortion they undergo. The globules below the surface are spherical, but those at the surface are invariably much flattened—more, probably, than the laws of capillarity would account for. The resistance responsible for the compression is in part due to a true cell membrane, but the zona pellucida is still in contact with the surface of the ovum and may participate in this resistance. The globules apparently burst through the surface of the ovum, and their contents are dissipated into a space which now arises for the first time between the zona and the egg proper. This perivitelline space first appears only in the equatorial region when the ovum elongates during the first cleavage.

But not only fat globules are extruded. A little later, as the first cleavage furrow sinks into the ovum, large balls of cytoplasm including numerous granules of yolk from the sub-peripheral zone are forced through the surface and deposited



in the perivitelline space (figs. 8 and 9). These balls contain no chromatin, of course. They vary considerably in size, the largest being sometimes 0.030 mm. in diameter. The extrusion of fragments of this size makes a drastic reduction in the volume of the ovum proper. Such extrusion seems to begin at the more advanced pole (the lower in all my figures), but I have not been able to discover any considerable difference in the eventual extent of this process at the two poles.

The probable explanation of the difference in size of the first two blastomeres is that the nucleus of one is formed earlier than the nucleus of the other. This means that the process of gelation associated with this mitotic pole is in advance of that associated with the other, and accordingly,

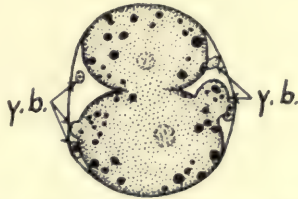


Fig. 9 Late telophase of first cleavage (16256) showing yolk extrusion.

comes to involve or appropriate a larger portion of the available cytoplasm. This difference in stage of the two poles is recognizable in specimen 16265, as has been pointed out in a preceding paragraph.

The first cleavage is completed about 30 hours after the ovum has entered the uterus in the pronuclear stage, and therefore about 54 hours post coitum.

#### IV. THE THIRD DAY

Stages 3 to 8. The second cleavage. The third cleavage. The fourth cleavage.

##### *Stages 3 and 4*

*The second cleavage.* The inequality in size of the first two blastomeres is about the same as the inequality in yolk distribution noted at the poles during the first cleavage. The

smaller blastomere averages about 0.082 mm.  $\times$  0.061 mm. in its longest and shortest diameters, respectively; whereas the larger averages 0.099 mm.  $\times$  0.070 mm. These measurements indicate a discrepancy of about 26% by volume, the larger blastomere containing 63%, the smaller 37% of the total volume of the egg. This information is useful in solving the question of cell lineage at the next stage.

The nuclear difference between the two poles of the egg reappears when the spindles form for the second division. The two eggs of The Wistar Institute collection which show these spindles (no. 16258 and no. 16275) are both anaphases, and in

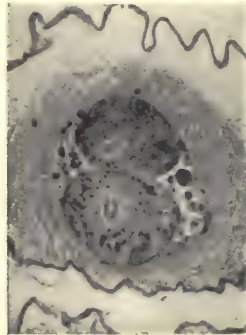


Fig. 10 Section of 2-celled ovum 16258. Photograph from Hartman ('19). Showing spindles for second cleavage, and yolk granules in the perivitelline space.

each case the chromosomes in the larger blastomere are further advanced than those in the smaller. Furthermore, the spindles are not parallel in the two blastomeres, but are approximately at right angles to each other. Both are also at right angles to the position of the original spindle in the first cleavage (fig. 10).

From this point on for the sake of brevity I shall call the larger blastomere of the 2-celled stage A and the smaller B. Before the cleavage furrow has appeared in B, A divides meridionally, so that a 3-celled stage (stage 3) is formed, composed of A<sub>1</sub>, A<sub>2</sub> and B. B then divides producing a 4-celled stage (stage 4) which, in accord with the difference

in spindle orientation mentioned above, is crossed like that of the Eutheria.<sup>5</sup>

The evidence for this account of the second cleavage is derived from a study of the volumes of the blastomeres in the 2-, 3-, and 4-celled stages, and from glass plate and wax plate reconstructions of the spindles in two 2-celled eggs. In all, six 2-celled eggs, two 3-celled eggs, and seventeen 4-celled eggs were studied.

It was mentioned above that in the 2-celled stage blastomere A contains about 63% of the total volume of the egg, and blastomere B about 37%, or roughly, 60% and 40%, respectively. In the 3-celled egg (stage 3) there is one large blastomere representing about 50%, and two smaller blastomeres representing about 28% and 22%, respectively, of the total egg volume. The actual volume of this large blastomere is about 229,859  $\mu^3$ , which is the same as the volume of blastomere B of the 2-celled stage; but this volume now represents 50% instead of 40% of the total egg volume. The explanation of this is that the total egg volume has been reduced by 10% through yolk elimination, and that this reduction has taken place almost exclusively in the dividing blastomere. The identification of this large blastomere of the 3-celled stage would be reversed if it were true that yolk elimination takes place in resting cells and not in dividing cells; but the evidence from the slides uniformly indicates that the opposite is true. Deutoplasmolysis, as Hill ('10) has named this process, makes its initial appearance when the pronuclei approach the center of the egg and the formation of the mitotic spindle begins. It reaches its climax during cytokinesis. It subsides during the resting stage. It seems reasonably certain, therefore, that the large blastomere of the 3-celled stage which has the same

<sup>5</sup> In the fairly closely related Australian marsupial *Dasyurus viverrinus* Hill ('10) found that this cleavage always results in a radial 4-celled stage instead of a crossed one. Selenka (1887) figured the 4-celled stage in the opossum as radial, but more recent studies on a large number of specimens by Hartman ('16, '19) and Smith ('25) show conclusively that in the opossum such radial 4-celled stages are always abnormal and are usually not cleavage stages at all, but fragmented, unfertilized eggs.



volume as blastomere B of the 2-celled stage is actually blastomere B still undivided, and that the two smaller cells are the descendants of blastomere A, which lost  $51,079 \mu^3$  of its volume by discharging that amount of yolk into the 'perivitelline' space during division.

The 4-celled stage (stage 4) is produced by the division of blastomere B. It divides meridionally, but at right angles to the plane of division of A; and, in doing so, it in turn discharges yolk while blastomeres  $A_1$  and  $A_2$  remain quiescent. In this manner there is a restoration of the original volume relations of the A and B poles of the egg. That is to say, in

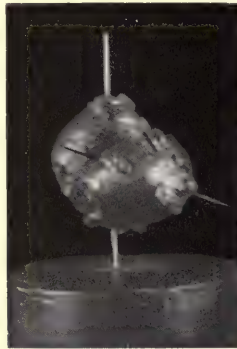


Fig. 11 Photograph of wax model of 16275, showing yolk granules protruding through the surface of the blastomeres. The needles run through the poles of the mitotic spindles and show their angle of inclination to each other.

the 4-celled stage the daughter cells of A taken together again represent about 60% of the total volume of the egg; and those of B about 40%.

It should be mentioned that Hartman ('19) believed that the spindles in A and B are parallel when they first form, and subsequently rotate during division. His opinion was based upon an egg (no. 306.2 of the Hartman series, now no. 16275 of The Wistar Institute collection) in which he considered the spindles to be parallel. I have made both a glass plate reconstruction and a wax plate reconstruction (fig. 11) of this egg, and both show this not to be the case. The

spindles are in reality inclined at an angle of about  $70^\circ$ . But one spindle is in an early anaphase (that in B), whereas, the other shows all the chromosomes already at the poles (that in A), and the plane of section is such as to make interpretation without reconstruction a little difficult. All of the spindle in B and one pole of that in A may be seen in the same three sections. The other pole of the more advanced spindle in the larger blastomere is several sections below, and might conceivably have been overlooked. At any rate, in reconstruction it is very clear that the spindles in this egg are much nearer to being at right angles to one another than they are to being parallel.

My own interpretation of this spindle orientation is that it is the mechanical result of some fairly simple pressure relations which need not involve any rotation of spindles or blastomeres. Since the spindle in A forms first, the process of gelation which is associated with the poles of the mitotic spindle is more advanced in this blastomere than in B. The denser spheres of protoplasm about the poles of A during its elongation exert a pressure upon the more liquid blastomere B just at the time when the spindle is beginning to form in the latter. As spheres of gelation arise and enlarge in B they can find room only in the direction where the least pressure is applied by the already elongated blastomere A. This means in a direction at right angles to the long axis of A, for both blastomeres are surrounded and mutually appressed by the zona pellucida.

Like the first cleavage, the second is slightly unequal, so that  $A_1$  is slightly larger than  $A_2$ , and  $B_1$  is slightly larger than  $B_2$ .

In summary: The larger of the first two blastomeres, A, divides first in a meridional plane, producing two slightly unequal daughter cells,  $A_1$  and  $A_2$ , and losing about 10% of the total egg volume by yolk elimination. B then divides, also in a meridional plane but at right angles to the plane of division of A, also unequally, and also losing volume by yolk elimination.

*Stages 5 and 6*

*The third cleavage.* At the end of the 4-celled stage (stage 4') the total mass of the blastomeres has been reduced by the elimination of deutoplasm to such an extent that they no longer fill the space within the zona pellucida, but are separated from one another and to some extent from the zona by yolk granules (fig. 12, B). After this there is little further yolk extrusion.

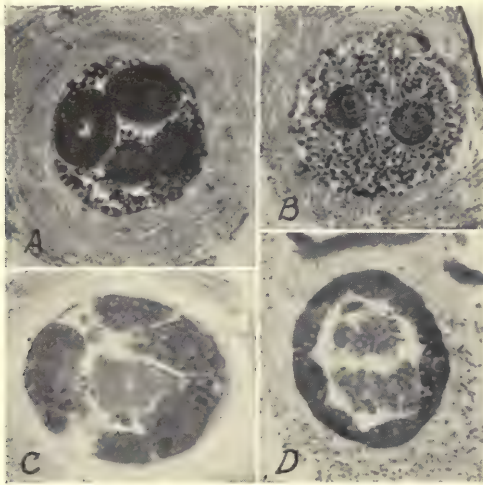


Fig. 12 Photographs from Hartman ('19). A, early 4-celled stage showing large blastomeres in contact with zona. B, late 4-celled stage showing blastomeres much reduced by yolk extrusion. C, 14-celled protoblastocyst with yolk showing liquefaction. D, 32-celled complete unilaminar blastocyst.

The larger cells at the A-pole of the egg continue to divide precociously. A 5-celled stage is formed by the division of  $A_1$ . This is followed quickly by a 6-celled stage after division of  $A_2$ . In stage 5 the two larger cells are  $B_1$  and  $B_2$  still undivided. The larger cells, therefore, are now at the B-pole instead of the A-pole of the egg. In the 8-celled stage (stage 6) the A-pole again contains the larger cells (on account of the division of  $B_1$  and  $B_2$ ), though the difference is less decided than it was originally.



*Stages 7 and 8*

*The fourth cleavage.* Eggs containing very nearly every number of blastomeres from one to thirty-two (a fully formed unilaminar blastocyst) were collected by Hartman and left here at The Wistar Institute. This collection has been the basis of a detailed study of cell volumes which I have made in an effort to discover the fate of the first two blastomeres. The identification of each individual cell can be followed as far as the 12-celled stage with accuracy.

At the end of the third cleavage (stage 6, no. 16232) there are four large cells and four smaller cells. The four large ones are at the A-pole. In the 10-celled ovum (16233a) there are six large cells and four small ones. This is because two of the large cells at the A-pole have divided producing the four smallest cells, and the remaining two at the A-pole plus the four at the B-pole are now classified as large by contrast. In the 12-celled ovum (stage 7, no. 16233b) there are four large and eight small cells. This is because the two remaining A-cells have divided, making eight small cells at the A-pole, and the four B-cells are still undivided. Hartman ('16, p. 32) figured wax models of the 10-celled and 12-celled ova here referred to.

Mitotic figures in the 12-celled specimen indicate which cells are prepared to divide next. The cells showing these figures are two of the large B-cells. I do not have a 14-celled egg, but according to these mitotic figures the 14-celled stage must be composed of two cells at the B-pole which belong to the fourth generation, and twelve smaller which belong to the fifth generation. The 16-celled stage (stage 8, no. 16229) seems to be produced, not by the division of the remaining two large cells, but by the fifth cleavage beginning at the opposite pole before the fourth cleavage has completed itself at the B-pole. This is inferred from the fact that two small cells are found at the A-pole, the two large cells can still be seen at the B-pole, and twelve intermediate cells are arranged between, of which twelve probably four belong to the B-group and

eight to the A-group. Since each of these cell divisions is slightly unequal, there is a fairly evenly graded series of cell sizes from large to small. I do not mean, of course, that they are lined up according to graded sizes; actually, the two B-cells which we saw in mitosis at the 12-celled stage form four small cells at the B-pole near the remaining two undivided and therefore large B-cells. The two smallest cells are at the opposite pole. The intermediate ones cannot be dependably distinguished into A and B groups. Furthermore, the last large cells at the B-pole soon divide, making it impossible immediately thereafter to be sure where the B-pole is.

There are three factors which together make the further tracing of cell lineage impossible: 1) the daughter cells from each division are slightly unequal; 2) all the cells become so small that the inherent margin of error in the wax plate method, introduced by the square corners of the individual wax sections, becomes proportionately too great for the method to be practical; 3) the cells begin to resorb yolk and to grow, so that daughter cells attain the size of their parent cells before they divide again. Hartman also found it impossible to recognize any polarity or to identify the individual blastomeres immediately after the 16-celled stage.

However, before two more cleavage waves have passed (i.e., in the 50- to 60-celled ova) a new polarity appears, which is permanent. It is naturally of interest to inquire whether the original polarity of the ovum is related to the secondary polarity of the blastocyst, and, if so, how. The evidence bearing upon this point will be discussed in chapter V.

#### V. THE FOURTH DAY

Stages 9 to 12. The unilaminar blastocyst. The endoderm mother-cells. The new polarity. The primitive endoderm. The medullary plate. The beginning of expansion of the vesicle.

##### *Stage 9*

*The unilaminar blastocyst.* No true morula is formed. It was pointed out in the last chapter that at the 4-celled stage

the blastomeres lose contact with each other as a consequence of the elimination of a considerable quantity of yolk (stage 4'). This extruded yolk lies between them and around them, and within the zona pellucida practically all the space not occupied by the blastomeres is filled with yolk granules (fig. 12, A). The blastomeres themselves have hardly become separated from each other before some of them begin to flatten out against the zona. Those which are not in contact with this membrane are spherical. Those which touch it flatten out against it. Even in the early cleavage stages (stages 5 and 6 and fig. 12, B) this leads to some semblance of blastocyst formation. By the 16-celled stage (stage 8) nearly all of the blastomeres are somewhat flattened against the zona, though the wall of the blastocyst is not yet complete and a few spherical cells which are not yet in contact with the zona may still be found. By the time there are thirty-two cells (stage 9 and fig. 12, D) all the gaps in the wall are usually filled, and the unilaminar blastocyst is complete.

This flattening out of individual cells against the zona pellucida before there is any such thing as a blastocyst wall, and when no morula stage has been formed, seems to be clear evidence that the accumulation of fluid in spaces between the cells of the morula by endosmosis is not the causative factor in blastocyst formation. It seems necessary to think of stereotropism as a more likely factor. And in this light, perhaps an important function of the zona is that of providing the proper stimulus for the tropism which leads to blastocyst formation. The yolk granules, of course, not exhibiting any such tropism, are gradually crowded away from the zona by the cells and thus passively come to occupy the cavity of the blastocyst.

So far the total volume of the egg proper has not increased. The zona pellucida has become thinner until it serves only to give a progressively diminishing distinctness to the inner border of the albumen. The albumen layer, if it has changed at all, is slightly thicker. The shell membrane, however, has definitely increased in thickness, measuring now between



0.003 mm. and 0.004 mm. All of the eggs roll or float about freely in the uterine fluid. Hartman's litter no. 314 (e.g., W.I.C. 16220), removed  $3\frac{1}{2}$  days after copulation, are type specimens for stage 9.

### *Stage 10*

*The endoderm mother-cells.* During the second half of the fourth day when the total number of cells in the blastocyst is between fifty and sixty some extraordinary events occur which were first noticed by Selenka (1887) and then more completely worked out by Hartman ('19).<sup>6</sup> Certain cells in

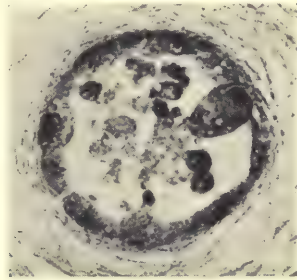


Fig. 13 Photograph of section through 16284, showing endoderm mother cell. From Hartman ('19).

one-half of the blastocyst which may be called the endoderm mother-cells (Urentodermzellen of Selenka) begin to enlarge enormously (stage 10 and fig. 13). When I first examined them I wondered whether they were just cells of an earlier generation which had remained large while their neighbors became reduced in size by continued division. But a study of the volumes of the cells of the cleavage specimens showed that these endoderm mother-cells are larger than any of the

<sup>6</sup> Hill ('10) denied the morphological significance which Selenka had attributed to the cells about to be described here, but at that time Hill had not seen any *Didelphys* preparations, and his opinions were based only upon his data from *Dasyurus*. Hartman ('16) following Hill's lead, made the same denial, but later ('19) he confirmed Selenka's interpretation and gave a very complete and perfect account of the origin of the endoderm.

cells in the immediately preceding stages, so that they necessarily have attained this size by growth, not by remaining undivided. For instance, in specimen 16218 there is a single endoderm mother-cell the longest diameter of which is  $60\ \mu$ , the shortest  $42\ \mu$ . There has not been a cell of this size in the egg since the two B-cells of the 6-celled stage divided. The average endoderm mother-cell in the early stages of endoderm formation is about  $46\ \mu \times 35\ \mu$  in its longest and shortest diameters respectively. This is about the size of the four large A-cells in the 8-celled stage. The last of these four is present in the 11-celled egg. From the 12-celled stage until the first endoderm mother-cell appears (50- to 60-celled stage) the cells become progressively smaller. Accordingly, it is clear that the large size of the endoderm mother-cells is due to a rather sudden and exceptional growth on their part.

Being hemmed in laterally by the surrounding cells and peripherally by the zona pellucida, which is still distinctly visible though thin, the endoderm mother-cell as it enlarges extends itself principally in a median direction. In other words, it juts conspicuously into the blastocoel. When it attains its maximal size it usually rolls out of the protoderm (the primitive pluripotential layer from which all the germ layers are derived) and into the blastocoel. Sometimes, however, it divides first; and occasionally two divisions occur before the base of the original cell loses contact with the cells remaining at the surface. This produces for a time a chain of cells projecting into the blastocoel. But in any case, the cells soon free themselves from the protoderm altogether. When free in the blastocoel these endoderm mother-cells are perfectly spherical. They show no consistent peculiarities except those of size and the habit of leaving the blastocyst wall. Some, but not all, stain a little darker than the other cells.

The gap left at the surface by the inward migration of this large cell is soon effaced. Selenka considered such a gap the homologue of the blastopore; and though he recognized that the gap itself is very transitory (1887, p. 114) he described

and figured a slight depression which, he says, for a long time marks the site of the blastopore. In a study of about 100 vesicles of the appropriate stages neither Hartman nor I have been able to find this.

*The new polarity.* As all of these cells arise in one-half of the blastocyst, a polarization of the vesicle is again recognizable. Accordingly, though it will involve a slight anticipation of details to be described more fully in the later sections of this chapter, this seems to be the logical point at which to discuss the possible relation of the polarity of the cleavage stages to the polarity of the late blastocysts.

Hartman's opinion on this question was that the larger of the first two blastomeres is probably the one which divides more slowly, giving rise to a pole of large and primitive cells which eventually become the 'embryonic area.' The endoderm mother-cells, he supposed, arose from this half of the vesicle before the so-called 'embryonic area' was sharply defined. Shortly thereafter, this area lagged so many generations behind the more rapidly dividing 'trophoblast' that it came to be sharply demarcated from the latter.

There are several difficulties with this interpretation. The first is that it does not account for and is not compatible with the loss of all visible polarity in the early unilaminar blastocyst, which Hartman described. If the larger of the first two blastomeres and its descendants are retarded in cleavage rate, they will always remain larger than the rapidly dividing descendants of the other blastomere which was smaller even to begin with.

This first objection, however, is obviously met by my own studies of cell volume which showed that (as in the pig, vide Heuser and Streeter, '29) the larger of the first two blastomeres is actually the first to divide, and that it, not the smaller one, gives rise to the pole of rapidly dividing cells. This very clearly accounts for the temporary loss of size distinctions among the cells in stage 9; for if the more rapidly dividing cells are larger to begin with, they must necessarily pass through a stage in which they are indistinguishable in



size before they eventually become noticeably smaller than the retarded cells at the opposite pole.

However, even with this correction the attempt to relate the early polarity to the later polarity meets further difficulties. If, for instance, the difference in rate of cell division at the two poles of the cleavage stages be considered to persist long enough to be responsible for the thickness of the 'embryonic area' in contrast to the thinness of the 'trophoblastic area,' this means that the cells of the 'embryonic area,' which have divided more slowly according to the theory, and belong to an earlier cell generation, must correspondingly be fewer in number. Hartman's own cell counts show this not to be the case.

Hartman's counts in four blastocysts of what I call stage 11 show an average of 93.5 cells in the 'embryonic area' and ninety-six cells in the 'trophoblastic area' ('19, p. 62), which is practically identical. The average number of endodermal cells in these same vesicles is thirty-three. If these come from the 'embryonic area' as he believes, then instead of fewer cells in this area there are at least 30% more than in the 'trophoblast.' Or if his theory be modified so as to have them come from the 'trophoblastic area,' their excess would not be enough to indicate a difference of even 1-cell generation. The only possible way to reconcile these figures with the theory of a persistent difference in rate of cell division at the two poles is to assume that the trophoblastic pole is constantly contributing cells to the embryonic pole (just the opposite of what Heuser and Streeter, '29, assume to be the case in the pig).

One other point which must be mentioned is that though so much depends upon this assumption, the cells of the 'embryonic area' have never really been shown to be larger than the cells of the 'trophoblast.' In cross sectional view they are definitely thicker (at least in some stages), which means that their lateromedial dimension is greater. On the other hand, in surface views of whole mounts they appear definitely smaller, as their two surface dimensions are less. This has been shown in the figures by Selenka, Hartman, and myself.

They are, then, larger, in one dimension and definitely smaller in two others, and their actual volumes have not been studied.

Personally, I feel that the source from which all this theorization springs—namely, the idea that the first two blastomeres represent in any significant way the ‘embryonic’ and ‘non-embryonic’ portions of the later vesicles—is unsound. In the next few pages I shall suggest a totally different interpretation of the polarization of the later blastocysts.

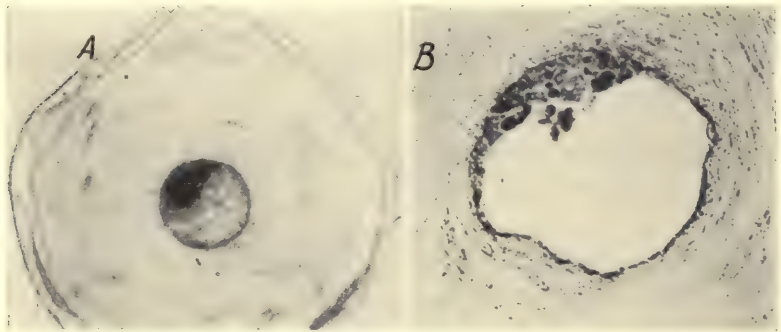


Fig. 14 Photographs of stage 11 (Hartman, '19). A, living ovum in Ringer's solution. B, section through primitive endodermal cells and medullary plate.

### *Stages 11 and 12*

*The primitive endoderm.* In the 1-celled stage the ovum measured about 0.11 mm. in diameter. No enlargement at all occurs until the endoderm mother-cells begin to form. The principal changes up to this time have consisted of the extrusion of most of the deutoplasm, the segmentation of the protoplasm proper, the orientation of the segments or cells along the zona leaving the yolk balls in the center, the liquefaction and resorption of most of the yolk. These are changes in the distribution and condition of materials contained in the original unicellular ovum.

When the endoderm begins to differentiate, changes which involve the acquisition and utilization of new materials are initiated. Such changes are reflected in the growth of the

blastocyst and the reduction of the albumen. Both of these processes are more conspicuous in the events of the fifth day, but their first stages can be seen in vesicles approximately 4 days old.

Among these vesicles, which are from 0.20 to 0.34 mm. in diameter, the endoderm mother-cells have undergone further differentiation. The spherical endoderm mother-cells, which were free in the blastocoel, soon come into contact with the external layer of protoderm. They then flatten out upon it just as the spherical blastomeres in early cleavage stages flatten out upon the zona pellucida, and doubtless as a result of the same reaction—stereotropism. When more or less flat and in contact with one another or with the protoderm they are called primitive endoderm cells (stages 11 and 12 and fig. 14). These cells multiply mitotically and soon accumulate to a thickness of two or three cells immediately under the protoderm, which at the same time thickens throughout the region that they touch.

*The medullary plate.* The most interesting and unexpected fact that I have come across in my study of the embryology of the opossum is that the dense plate of cuboidal superficial cells thus formed is a medullary plate (McCrary, '37). In the light of the embryology of all the lower vertebrates this is exactly what one should expect to find; but in the opossum this structure was observed 50 years ago by Selenka (1887) and has since then been seen in many other marsupials, and has been studied by many investigators, all of whom regarded it as an 'embryonic area.' In other words, it has always been supposed that all the embryonic ectoderm (epidermis as well as nervous system) is derived from this plate, and that the ectoderm outside this region gives rise to nothing but extra-embryonic membranes. As a matter of fact, none of the epidermis is derived from this plate, and the extra-embryonic ectoderm is not in any way demarcated from that which will produce body epidermis. The previous interpretation was based upon analogy with the supposed separation of embryonic and non-embryonic or trophoblastic cells in the



eutherian vesicle, and was possible, I believe, only in the absence of a complete series of sectioned specimens. In a study of external views it is easy to be deceived on this point, and, for my own part, I accepted the old interpretation without misgiving until I had studied sections of stage 22 (specimen 16139) which made the error obvious.

In the opossum there is no differentiation of embryonic and trophoblastic cells. The entire vesicle corresponds to the ovum of a fish, an amphibian, a reptile, or a bird, and no line can ever be drawn between the cells which are to be retained in the definitive body and those which are to be discarded.

As mentioned in the first part of this chapter the unilaminar blastocyst shows no polarity or visible differentiation of any sort. Probably most of the cells at this time are indifferent protodermal cells—just as Mangold ('24) has shown most of the cells of the amphibian egg are at the beginning of gastrulation. But a few have a peculiarity<sup>7</sup> which makes them later enlarge and migrate to the interior. These constitute the endoderm and probably also part of the axial mesoderm, the prechordal plate, which though embedded in the endoderm may be seen proliferating mesoderm in stage 22. The 'invaginated' material then induces the formation of a medullary plate in the hitherto pluripotential external material.

These facts are perfectly parallel to what is known to occur in the lower vertebrates, and they make the primitive mammalian blastocyst fit naturally into the phylogenetic series.

<sup>7</sup> I do not mean, of course to assert that the peculiarity which causes the endodermal mother-cells to enlarge and migrate into the blastocoel will never be traced back to some sort of cytoplasmic localization in the uncleaved ovum. Nor do I mean that there is any a priori objection to the idea that such material, if existent, may be consistently allotted to one of the first two blastomeres. All I mean is that this peculiarity which causes some cells to migrate internally and subsequently to induce a medullary plate would correspond to the primary organizer, not to the whole embryo, and that we do not yet know whether the plane of the first cleavage bears any constant relation to any such material or not. In the only mammal for which experimental evidence is at present available (the rat), Nicholas ('34) has shown that either of the first two blastomeres can produce a whole embryo.

*Stage 12*

*The beginning of expansion of the vesicle.* During the formation of the endoderm mother-cells and the primitive endoderm (stages 10 to 12) the vesicle enlarges from 0.17 mm. to 0.34 mm. This expansion, which is rather sudden, is probably due for the most part to stretching under the influence of fluid accumulating in the blastocoel. It results in a noticeable thinning out of all the cells of the vesicle, though those of the medullary plate still appear thicker in sectional view than any of the others.

The eggs of Hartman's litter no. 194' were removed from the uterus exactly 4 days after copulation, and form a good type series for stage 12.

## VI. THE FIFTH AND SIXTH DAYS

Stages 13 and 14. The definitive endoderm. The bilaminar blastocyst. The uterine glands.

*Stage 13*

*The definitive endoderm.* In the early part of the fifth day the primitive endodermal cells begin to migrate beyond the bounds of the medullary plate, and as they do so they become very much flatter and exhibit long pseudopod-like processes. The migration is almost certainly accomplished by amoeboid movement, though this cannot be established beyond question with evidence from fixed material alone. These new, extremely flattened, migrating cells are the definitive endoderm.

About the time the endoderm begins to migrate beyond the medullary plate two changes in the gross aspect of the vesicle become apparent: 1) it changes from a perfect sphere to an ellipsoid with the shortest diameter in the direction of the egg axis; and 2) it becomes eccentric in relation to the egg shell and albumen (stage 13 and fig. 15).

The explanation of the first of these two changes is not clear, but it is fairly certain that the second, the eccentricity of the vesicle, is due to the more rapid absorption of the

albumen at the animal pole than at the vegetal. This difference in rate of absorption is so decided that it causes the vesicle to migrate across the albumen layer until the medullary plate comes into contact with the shell membrane. This

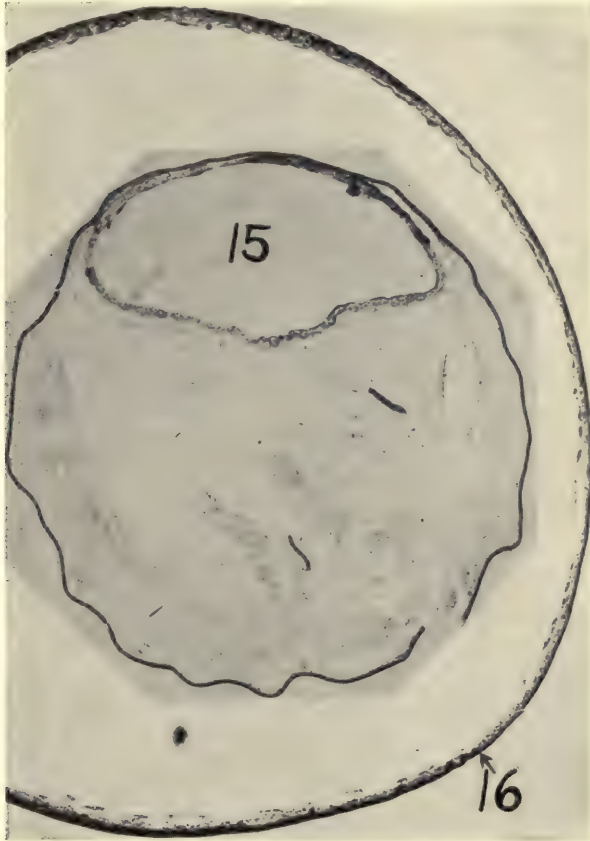


Fig. 15 Stage 13 ellipsoid blastocyst showing medullary plate in contact with shell membrane (Hartman, '19).

Fig. 16 Stage 14 bilaminar blastocyst (Hartman, '19).

contact is not attained until the end of the sixth day; and by this time the endoderm has extended itself completely around the inside of the vesicle.



During the rapid increase in diameter which accompanies the migration of the endoderm the walls of the vesicle become markedly thinner. This thinning affects all portions of the vesicle but in the medullary plate it seems less conspicuous. The migration of so much endoderm from beneath the medullary plate, however, reduces that region to a thickness of only two cell layers again (i.e., medullary plate and endoderm). The medullary plate remains distinctly thicker than any other portion of the vesicle.

The end of the fifth day is not characterized by any very distinctive feature. The vesicle has enlarged to about 0.50 mm., and has not yet reached the shell membrane. The vegetal pole is at the center of the albumen. The endoderm extends to the general neighborhood of the equator of the vesicle.

#### *Stage 14*

*The bilaminar blastocyst.* The end of the sixth day is marked both by the contact of the medullary plate with the shell membrane, and by the completion of the endodermal lining of the vesicle. The diameter of the vesicle at this time is about 0.75 mm. (stage 14 and fig. 16).

The cells of the different layers show the following distinguishing characteristics. The ectodermal cells outside the medullary plate are intermediate in thickness; they stain more lightly than any of the others; they contain practically no yolk granules; their nuclei are far apart (on account of the flatness of the cells); and their cytoplasm is rather easily broken down by fixing agents. The medullary plate cells are nearly columnar, so that their region is thicker than any other part of the vesicle and their nuclei are very close together; their cytoplasm is denser and more yolk laden than that of the rest of the ectoderm, and rather more basophilic. The endodermal cells are thinner than any others; their nuclei are larger in polar view than those of the ectoderm, and they stain very darkly.

As the animal pole at this time has absorbed all of the overlying albumen, it probably begins to derive nutriment from the so-called uterine milk—a secretion from the uterine glands to be described presently, which filters through the shell membrane. The vegetal pole is still covered by albumen, which it is absorbing slowly.

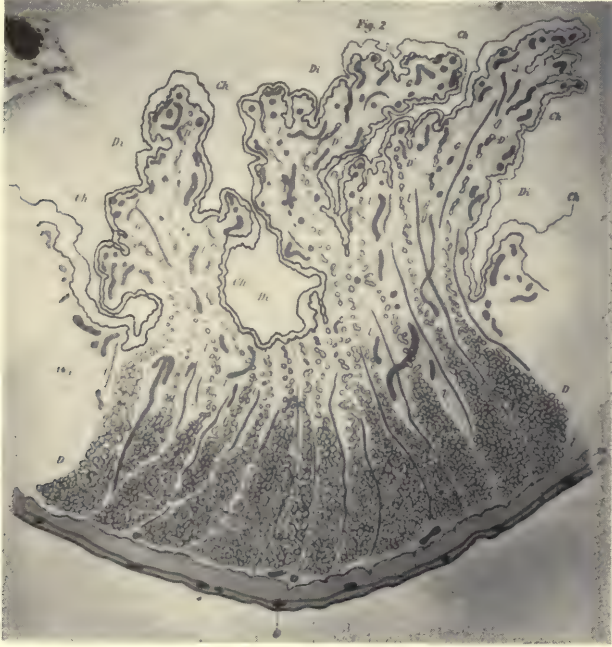


Fig. 17 Uterine glands (Selenka, 1887).

*The uterine glands.* Inasmuch as the uterine glands play no part in the development of the vesicles until the sixth day, I have postponed discussion of them until this point; but this arrangement necessitates a short digression now to summarize their previous history.

Selenka (1887) was the first to describe and figure the condition of these glands during pregnancy (fig. 17). Hartman ('23) traced their development throughout the oestrous cycle.

During the anoestrus (October, November, December) the uterine mucosa contains many long, tubular, fairly straight, simple, or double-branched glands. The walls of these glands are made up of low columnar or cuboidal cells with large nuclei that nearly fill the cell body. The lumen of the gland is small, and is not apparently ciliated. During the pro-oestrus (first week of January and the beginning of each oestrous cycle thereafter [vide, chapter 1]) the glands begin to lengthen and coil. The peripheral half of the thickness of the mucosa is the region where the coiling of the glands is most pronounced and conspicuous—so much so that the mucosa in sections appears to be made up of two layers. In the medial layer the glands are straighter and are separated by wide lymph spaces; in the distal layer they are so complexly coiled that they occupy most of the available space. At this time the nuclei, for the most part, migrate to the bases of the cells, and cilia become abundant in the lumen. During oestrus and ovulation the lumen of the gland enlarges, the number of mitoses increases, and the cilia become more prominent. During pregnancy the glands increase progressively in their complexity, even becoming considerably coiled in the neighborhood of the endometrial surface. All the nuclei are basal, and no cilia have been recognized.

The secretion of these glands is a clear, cell-free, lymph-like fluid which is delivered into the uterine cavity and absorbed by the vesicles. The animal pole, as we have just seen, is at the end of the sixth day the first part of the vesicle to exhaust the supply of the albumen above it, and must from this time forward depend upon this secretion for nourishment. The rest of the albumen supply disappears very shortly afterward and the entire vesicle is thereafter nourished only by the secretion of the uterine glands.



## VII. THE SEVENTH DAY

Stages 14 to 17. The late bilaminar blastocyst. The primitive streak. The mesodermal crescent.

*Stages 14 and 15*

*The late bilaminar blastocyst.* In the first half of the seventh day the blastocyst enlarges from a diameter of 0.75 mm. to one of 1.0 mm. During this enlargement all of the ectoderm gains in thickness, but the endoderm remains as thin as it became during the fifth and sixth days. The medullary plate lies in immediate contact with the shell membrane. Its cells are quite crowded and are high-cuboidal in shape. Their cytoplasm is granular, and very chromophilic. All of these peculiarities make it a well-marked structure, conspicuous in whole mounts or sections. The rest of the ectoderm is somewhat less sensitive to injury by fixing agents than it was when it was thinner. It is in close contact with the small amount of albumen left between it and the shell. This albumen disappears as the vesicle enlarges at its expense, and consequently the vesicle gradually regains its spherical shape. The nuclei of the endoderm still appear larger than those of the ectoderm (possibly because they are flatter), and still stain very deeply. Both layers are in close contact at all points.

It should be mentioned that occasionally some endodermal cells in a restricted region near the margin of the medullary plate become noticeably larger than the others. The significance of this change is not definitely known. Hartman ('19, p. 86) suggests that they mark the region above which the primitive streak is soon to arise. It seems possible that they represent the prochordal plate, a portion of which, the prochordal plate, will later be seen proliferating mesoderm.

During the second half of the seventh day the last traces of albumen disappear. The vesicle is again perfectly spherical. When the diameter is about 1.4 mm. the medullary plate in a small circular area becomes thinner, so that by transmitted light this region appears as a light spot near one

edge of the relatively opaque disc (stage 15). This light spot, which was first described by Hartman, is the area in which the first mesodermal cells appear.

### *Stage 16*

*The primitive streak.* When the vesicle is about 1.8 mm. in diameter Hartman discovered that certain cells in the peripheral half of a radial line through the center of the light spot mentioned above, drop down beneath the medullary plate and lie between the latter and the endoderm. These are the first mesodermal cells. When enough of them have collected in this region to form a slight local opacity they show up in transmitted light as a dark streak in the light spot at the point where the latter is nearest the edge of the medullary plate (stage 16). This is the primitive streak. It is the only part of the vesicle which is 3 cell layers thick.

It may seem strange on first thought to find the primitive streak arising within the medullary plate. The process of 'gastrulation,' or its equivalent, began back in the fourth day with the origin of the endoderm. The primitive streak stage, as is always the case, represents a late stage of gastrulation, and it is not surprising at a late stage to find the equivalent of the blastopore within the medullary plate. Such a situation is universal, and is, of course, the explanation of the neurenteric canal in those forms in which one occurs.

From this time (stage 16) right on through the end of the tenth day (stage 29) the primitive streak will be permanently recognizable in the posterior growing tip of the medullary plate—the sinus rhomboidalis. During stage 30, the first half of the eleventh day, the posterior neuropore will finally close over, and the primitive streak will be lost in the floor of the spinal cord of the tail.

The origin of the first mesodermal cells is the point at which Hartman's studies of the early embryology of the opossum came to an end. An exhaustive account of the origin of these cells, the formation of Hensen's node, and the spreading of the mesodermal crescent, is in preparation by Dr. C. H.

Heuser of the Carnegie Institution. His material, which is very complete, is that which was collected by himself and Doctor Hartman in 1918. They have kindly allowed me to take the figures for my stages 15 to 20 from some of their beautiful photographs. As Doctor Heuser's account of the next four stages will be much more detailed than I am able to give, I shall content myself here with listing the distinctive features which I have chosen as criteria for defining them.

### *Stage 17*

*The mesodermal crescent.* Hartman's litters 346 and 346' show the changes which occur between the middle and the end of the seventh day (interval of  $9\frac{3}{4}$  hours). The eggs of 346 represent late stage 14, and those of 346' are types for stage 17. The latter are 2.0 mm. blastocysts characterized by a strongly developed primitive streak from the posterior portion of which mesoderm streams laterally to form two symmetrical wings—the mesodermal crescent.

The remaining three neurula stages (i.e., stages with no sign of organogenesis except the medullary plate) belong to the eighth day, and are described in the next chapter.

## VIII. THE EIGHTH DAY

Stages 18 to 23. Hensen's node and the primitive groove. Elongation of the medullary plate. The mesodermal rim. The notochord and the medullary groove. The first somites and the parietal mesoderm. The coelomic cavities and the subcephalic fold.

### *Stage 18*

*Hensen's node and the primitive groove.* In the early part of the eighth day when the vesicle has attained a diameter of 2.2 mm. a distinct thickening of the external layer appears at the anterior end of the primitive streak. This is Hensen's node. At this point ectoderm and endoderm seem to fuse, and mesoderm may be seen proliferating



from this fusion in a lateral direction, as well as from the whole length of the primitive streak. The latter structure has developed two folds along its edges near the posterior tip so that it may now more appropriately be called the primitive groove. The mesoderm occupies only a small oval region around the primitive groove, and beyond this region the medullary plate is still in contact with the endoderm. Hartman's litter no. 338 represents this stage.

### *Stage 19*

*Elongation of the medullary plate.* Shortly after the primitive streak appears the posterior portion of the medullary plate including it begins to grow in a caudal direction. This process actually begins in stage 18, but it becomes conspicuous for the first time in stage 19. At this time the whole primitive streak has become converted into a groove, and its edges have begun to wrinkle as if they are growing too fast for the surrounding cells to keep up with them. This is particularly true at the posterior tip. The mesoderm now extends anterior to Hensen's node, but there remains a crescentic strip of the anterior portion of the medullary plate which is still in contact with the endoderm. It is interesting that at this time the mesoderm is continuous across the midline in front of Hensen's node.

### *Stage 20*

*The mesodermal rim.* Just before the mesoderm reaches the anterior extremity of the medullary plate it begins to extend beyond it posteriorly. The extreme edge of this spreading sheet forms a dark irregularly circular line—the mesodermal rim—along which the mesoderm is fused with and possibly arising from the endoderm. In stage 20 this line first becomes visible beyond the medullary plate in the posterior region. In slightly later specimens (e.g., 17179) it has migrated well beyond the medullary plate and completely surrounds it.

Being the border of what will later become the area vasculosa, the prospective site of origin of the sinus terminalis, and a region of fusion of mesoderm and endoderm, the mesodermal rim has much in common with the 'germ wall' of avian eggs. The absence of yolk in the opossum vesicle at this time, however, makes the question of their homology difficult to decide.

At this stage the primitive groove attains its greatest relative and absolute length, and simultaneously a clear spot appears at its anterior tip. The vesicle is now 3.2 mm. in diameter.

### *Stage 21*

*The notochord and the medullary groove.* The clear spot in stage 20 is due to the shortening of the primitive streak at its anterior end. When the vesicle is about 3.7 mm. in diameter (specimen 17179) this shortening at the anterior end is going on more rapidly than the growth at the posterior end, so that the primitive streak is decreasing in total length. The shortening process is correlated with the appearance of two new structures—the notochord and the medullary groove—for it seems to be due to a tangential split in the substance of Hensen's node. The lower or more medial cells are separated from the more superficial cells and become the notochord. This leaves only a thin sheet of medullary plate in the middle line above the notochord, which, correspondingly, when illuminated from below appears as a light streak. The medullary plate sinks in slightly along this thin line and thus forms a medullary groove which is anterior to the primitive groove.

During all this time the medullary plate is constantly increasing in length. Most of this increase seems to be due to the multiplication of cells at the posterior growing tip; but part of it may be due to migration of cells, for the plate is also narrowing in width at the same time that it is increasing in length and I see no indication of changes in cell shape which might account for this.

*Stage 22*

*The first somites and the parietal mesoderm.* In specimen 17180 (see stage 22 in the second normal stage plate) in which the medullary groove has become nearly twice as long as the primitive groove, two slight condensations of the mesoderm beneath the medullary plate on either side of the central mesoderm-free strip represent the first somites.

Selenka figured (somewhat diagrammatically) a similar specimen (1887, Taf. XX) with three somites. In it he recognized a longitudinal mesodermal thickening lateral to the medullary plate, which he called the parietal mesoderm. In the absence of a series of intermediate stages, however, he did not realize that the ectodermal thickening which he now correctly calls the 'Medullarplatte' is the same ectodermal thickening which in the bilaminar blastocyst he labeled 'Keimscheibe.'

This ectodermal plate, which first appears in stage 11 (latter part of the fourth day), is continuously and conspicuously recognizable from then on. Its visible distinction from the rest of the vesicle in all the early stages seems to be due principally to a greater density of its cytoplasm. In fixed but unstained specimens this dense plate is more opaque than the rest of the vesicle, so that it appears darker by transmitted light and lighter by reflected light. In stained specimens it is always more chromophilic—not particularly basophilic or acidophilic, but more retentive than the rest of the vesicle for almost all stains.

I emphasize this difference in density of cytoplasm and consequent staining reaction because the distinctive feature usually attributed to this plate (that of thickness) is rather inconsistent. At the time when the vesicle is swelling rapidly under the pressure of fluid accumulating in the blastocoel (stages 11 and 12) the less dense portion of the vesicle becomes enormously stretched and flattened, whereas the denser medullary plate remains comparatively thick and occupies a smaller proportion of the surface. Later on, the so-called



trophoblast thickens again, and many of Hartman's own figures ('19, pl. 22, figs. 1, 2, 4, 4A, 3B, 5, 6, 8, 9) show practically no differentiation between trophoblast and 'embryonic area' except that of staining reaction. In his figures 2 and 4 the trophoblast at the opposite pole is decidedly thicker than the 'embryonic area.' Selenka (Taf. XIX, fig. 6) shows the same thing. In still later stages (stages 20, 21, and thereafter) the trophoblast at the opposite pole becomes at least twice as thick as the medullary plate, but the medullary plate is thicker than the immediately adjoining non-medullary ectoderm and much more dense than the opposite trophoblast.

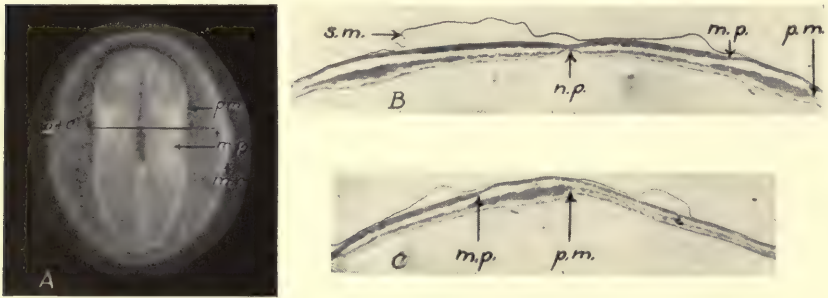


Fig. 18 Stage 22. A, photograph of whole mount 16139. B, photograph of section of same specimen showing medullary plate, notochordal plate, parietal mesoderm, and shell membrane. C, more lateral view of same section.

There is thus no stage after its first appearance when this ectodermal plate is not sharply demarcated in sectioned specimens by density, staining reaction, thickness, or all three, from the rest of the vesicle. In external views (see the second normal stage plate) it is easy to recognize until stage 22.

Figure 18 A is a photograph of specimen 16139 (stage 22) before it was sectioned. The long transverse line indicates the level at which the section represented in 18 B and 18 C was taken. The two arrows above the line show the extent of the section photographed in 18 B, and those below the line show the extent of 18 C. Figure 18 B shows clearly that the parietal mesoderm is lateral to the thick ectodermal plate.

Figure 18 C, a more lateral view of the same section, shows that the ectoderm covering the parietal mesoderm is continuous with and indistinguishable from the extra-embryonic ectoderm, which means that there is no such thing as an embryonic area in the sense in which this term is ordinarily used in mammalian embryology. The opaque light edge of the 'embryonic area' seen in the whole mount (fig. 18 A, p.m.) is the edge of the mesodermal thickening, not the ectodermal thickening. This could not be determined from an external view, but in sections it is obvious. After this stage it is easy to see even in external views that the ectodermal thickening is a medullary plate. The hearts, the limb ridge, and the whole body wall form lateral to it.

In describing his 3-somite specimen Selenka says (S. 125):

Was aber den peripherischen Endsaum den "Keimwulst" des Mesoderms betrifft, so zeigte sich hier nirgends die mindeste Andeutung einer Entstehung von Mesodermzellen in loco, noch eine Beteiligung des Ektoderms oder Entoderms; er markirte sich auf den Schnitten niemals als Wulst oder Anschwellung, und erst mit der Anlage des Sinus terminalis erscheint auch die peripherische Grenze des Mesoderms bezw. des Gefässblattes, scharf abgesetzt.

As pointed out in a previous paragraph the edge of the spreading sheet of mesoderm is always sharply defined from the very beginning, and the mesoderm definitely merges with the endoderm along this line. This line is the mesodermal rim, marked m.r. in figure 18 A. Whether any mesodermal cells are actually arising from the endoderm is difficult to determine, but at any rate endoderm and mesoderm are completely fused along this line, and many mitoses are found in the region of fusion.

### Stage 23

*The coelomic cavities and the subcephalic fold.* I have only one specimen upon which to base a description of stage 23. This specimen (17181) was a retarded member of a litter, the other members of which belonged to stage 24. Also it

was very badly handled before sectioning. During an incautiously rapid dehydration the vesicle collapsed so that the sections are wrinkled and in many cases broken. In all of these respects the material is unsatisfactory, but as no specimen of this stage has been described before, and as no other material is available at present, it seems worthwhile to include it tentatively until better material can be obtained.

Fortunately the drawing of the external view was made before the vesicle collapsed. This is shown as stage 23 in the second normal stage plate. It can be seen that there are four well-formed somites. They lie beneath the medullary plate which is now easily recognizable even in a whole mount. Lateral to the medullary plate the parietal mesoderm forms a dark shadow. Anteriorly (i.e., in the region cranial to the somites) the parietal mesoderm has split to form the two coelomic cavities, which at this time do not extend beyond the pericardial region. There are no heart tubes recognizable in this specimen though the splanchnopleure is very decidedly thickened throughout the pericardial region, and must have been prepared to fold into myocardial tubes very soon.

No sensory anlagen are recognizable. The ectoderm is beginning to fold under the anterior edge of the medullary plate (the subcephalic fold), but the mesoderm is still continuous across the midline in front of the embryo so that there is no pro-amnion.

In the posterior fifth of the medullary plate the primitive groove is well marked. Immediately caudal to it and outside the medullary plate a light mark represents the spot where the ectoderm folds in slightly to form the anal pit or proctodeum.

#### IX. THE NINTH DAY. Stages 24 to 26

##### *The first third of the ninth day*

Stage 24. The heart tubes and the first blood vessels. The first sensory anlagen. The nephrogenic ridge. The first branchial pouches. The hypophyseal plate and the pharyngeal membrane. The proamnion. Differentiation in the



medullary plate and the neural crest. Miscellaneous details. Comparative notes.

*The heart tubes and the first blood vessels.* In two embryos which by somite number and general appearance should belong to my stage 24 Selenka was unable to find any blood vessels, though he described the hearts with their endothelial tubes. As he was also unable to recognize sensory anlagen in this material, it must be assumed that fixation was bad, or that many of the sections were lost, or that some other such accident made the description of the internal anatomy of these specimens incomplete.

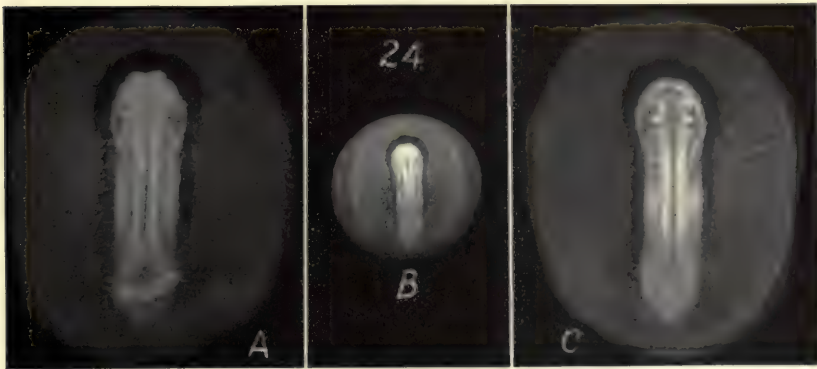


Fig. 19 Photographs of external views of stage 24. A, dorsal view of embryo and area vasculosa. B, dorsal view of whole vesicle. C, ventral view of embryo and area vasculosa.

In the six sectioned specimens in The Wistar Institute collection which belong to this stage there is a considerable vascular system. The hearts contain endothelial tubes which communicate anteriorly with a capillary plexus running through the mandibular mesenchyme, and posteriorly with a vitelline plexus from the yolk sac (fig. 20, totomount and section L). The mandibular plexus on each side connects medially with a dorsal aorta, also somewhat plexiform in early specimens, which runs caudally beneath the medullary plate. At the level of the first few somites the two aortae are

gradually diverging laterad to pass from beneath the medullary plate at about the level of the fourth somite. Beyond this point they give off a few small intersomitic twigs, from which branches pass to the limb ridge, and finally they break up into a complicated plexus leading ventrally and laterally into the area vasculosa.

In connection with the hearts a most unexpected detail is represented in the totomount drawing (fig. 20) between the levels indicated by the arrows D and E. Here may be seen a large vessel branching from what appears to be the heart tube medially toward a slight mesenchymal opacity at the edge of the medullary plate. This vessel turns out to be the primordium of the second aortic arch, and its point of junction with the lateral tube marks the anterior extremity of the heart tube proper. The mesenchymal condensation is the hyoid mesenchyme. In the late specimens of stage 24 the head region is rounding up so that the vessel, which ran medially at first, comes to run dorsally into the hyoid arch. At this time there is no corresponding dorsal twig from the aorta, but such a twig develops in stage 25. Then the capillary plexus and every stage in the differentiation of the arch can be followed in stages 26 and 27.

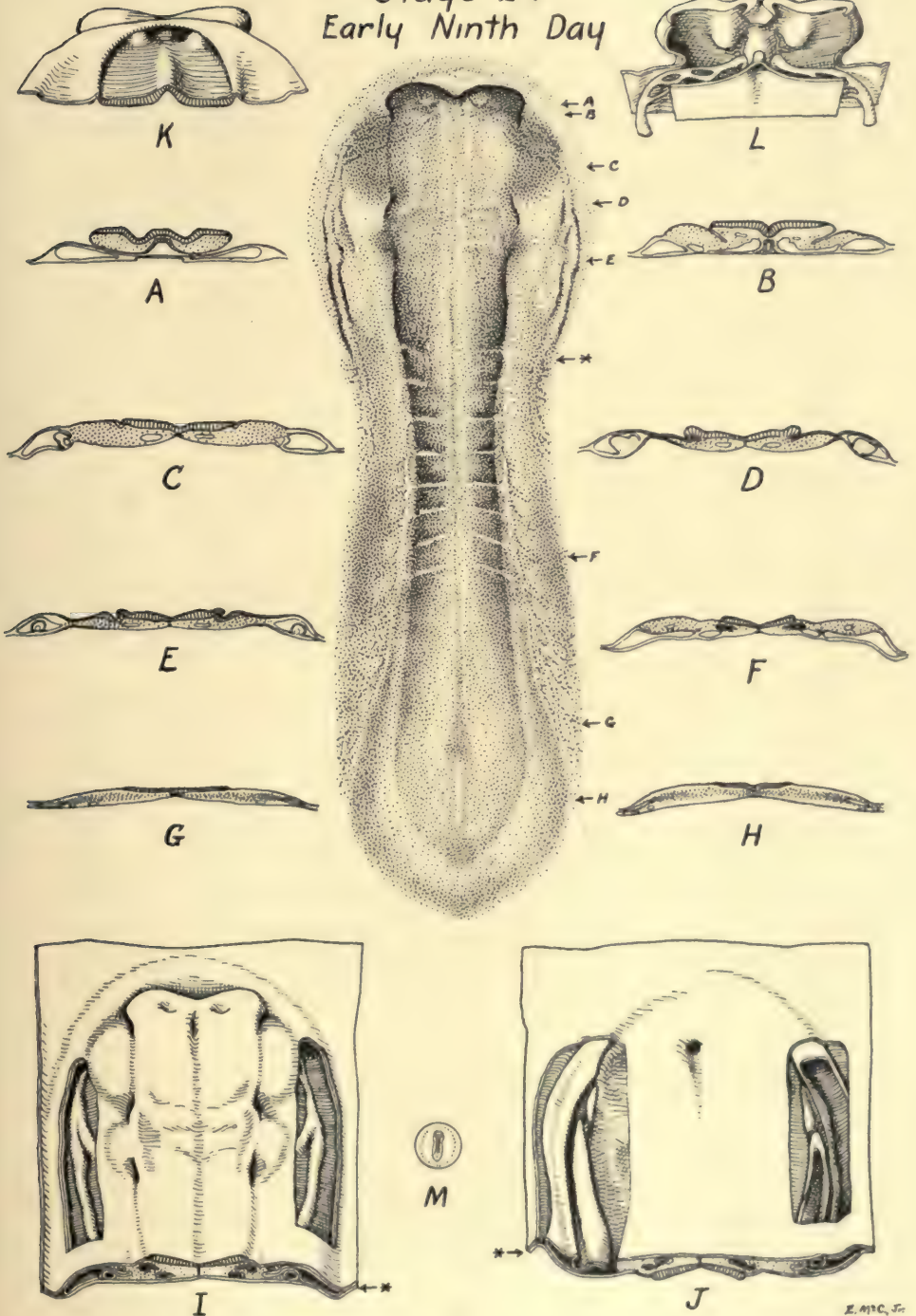
Four veins appear during stage 24. The first, the vitelline (or omphalomesenteric), is the vein of the yolk sac. This will be by far the most important vein in the embryo until just before birth. It grows out as a capillary plexus from the posterior end of the heart tube, which meets a similar plexus arising in the area vasculosa and growing toward the embryo.

The next two veins, the vena capitis medialis (or primary head vein) and the allantoic (or umbilical) arise simultaneously. Many small vessels which appear to be remnants of the same primitive capillary plexus from which the aortae

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Fig. 20 Totomount, sections, and reconstructions of stage 24. Totomount—Edinger apparatus drawing from 17176 with arrows to indicate levels of sectional views. The level marked with the asterisk is shown in sectional view in I and J. All sectional views and reconstructions are from 17153. M indicates the actual size of vesicle, embryo, and area vasculosa.

Stage 24  
Early Ninth Day



E. M. C. Jr.



arose, run together in the mesenchyme under the midbrain and grow back between the otic placode and the medullary plate. They pass the second branchial pouch (which will be described below) on its medial side (later dorsal) and end blindly except for scattered capillary communications with the dorsal aorta. These vessels constitute the anlagen of the primary head vein.

At the same time a similar plexus appears in the parietal mesenchyme dorsal to the point where the vitelline vein enters the body. From this plexus one channel eventually becomes enlarged. It lies (fig. 20, I) close against the parietal pericardium, which in turn is in contact with the visceral pericardium at this point, but no communication is attained until late stage 25. From this level it grows caudad until its posterior tip reaches the limb ridge (fig. 20, F). This vein, the umbilical, is thus in early stages exclusively a vessel of the anterior limb and the lateral body wall, and in the opossum this primary association is preserved until a relatively late stage of development (stage 30, see p. 165).

In the latest specimens of stage 24 (e.g., 17136, 12 somites) a continuous border vessel is formed around the area vasculosa—the sinus terminalis. It has innumerable communications with the yolk sac vessels and through them eventually with the vitelline and aortic plexuses from the embryo.

*The first sensory anlagen.* In stage 23 no sensory anlagen were found, though it should be remembered that my material for that stage is inadequate. In the earliest specimen of stage 24 which I have (17153) both the optic cups and the otic placodes are present, so that I cannot establish any priority between these two.

As far as my evidence goes the optic cups are paired and lateral from the beginning (fig. 20, totomount, A, I, K, L). At first they are exceedingly difficult to recognize in totomounts unless the lighting is carefully adjusted. But they show very clearly in the photograph (fig. 19, A) and I do not doubt that if Selenka's light had been satisfactory he would have found them in the specimen he figured in his

Tafel XXI, and they were very likely also present in his slightly younger specimen shown in Tafel XX. In sections, of course, there is not the slightest difficulty in identifying them (fig. 20, A).

The acoustic placode (fig. 21) is situated on the dorso-caudal surface of the hyoid swelling just anterior to the second branchial groove (v.i.). This section (17153, 13,4,12) passes through the anterior portion of the placode, which is flat, but figure 20, E, shows that posteriorly the placode wrinkles under the edge of the medullary plate.

For the discussion of the sensory ganglia see the section on the medullary plate and the neural crest.

*The nephrogenic ridge.* The pronephros in the opossum is no better developed than in other mammals, and no less



Fig. 21 Section through otic placodes and medullary plate at stage 24 (17153, 13, 4, 12).

well developed than in the lower amniotes. At the height of its development it is composed of only a few very small, solid tubule anlagen attached at one end to the coelomic epithelium and at the other end to a solid duct. As the tubules are solid no true nephrostomes appear, though in many cases a small indentation in the coelomic epithelium at the point of origin of the tubule may represent the vestige of one. The development of the pronephros, however, has acquired a renewed interest in the light of the theory recently proposed by Burlend ('31), which will be discussed below; and the details of this development in the opossum turn out to be significant in connection with this theory.

Of the six specimens representing stage 24 in The Wistar Institute collection, all but one show definite signs of nephrogenesis. The one exceptional embryo (17166) is abnormal in

several other respects (distended coelomic cavities, highly elevated embryonic region, excessively dense mesenchyme with retarded vascularization) and so probably should be omitted from this discussion.

The most anterior pronephric anlage is always found opposite the seventh somite. The intermediate mesoderm is unsegmented and, at this level, very sparse. In the earliest specimen (17153) a distinct condensation involving only a few cells is formed in the intermediate mesoderm immediately adjoining and continuous with the somatic layer of the lateral mesoderm. This condensation appears in individual sections as a knob projecting medially from the coelomic lining, but in series it shows itself to be for the most part a ridge. The ridge tends to be thick and round on its free edge (where it is broken away from the somites or dorsal mesoderm) and quite thin on its attached edge, which latter occasionally breaks through, leaving the thicker edge as a cord of cells running parallel to the coelomic epithelium. These breaks, however, are small, irregular, far apart, and quite unrelated to the segmentation of the dorsal mesoderm.

Beyond the last somite (the tenth) the intermediate mesoderm becomes much more abundant, is still markedly condensed, but not completely separated from the dorsal unsegmented mesoderm, and also not from the lateral mesoderm. This is the condition back to the neighborhood of the primitive streak where dorsal and intermediate mesoderm become indistinguishable.

There is thus a continuous condensation of the intermediate mesoderm from the level of the seventh somite to the posterior end of the body, which condensation shows a tendency to separate from the dorsal segmented mesoderm and remain as a ridge on the coelomic lining. In the next specimen to be described this nephrogenic ridge will be seen to be the anlage of the wolffian duct and of all the tubules of the pronephros and mesonephros.

Specimen 17139 represents the end of stage 24. Figure 22 shows a reconstruction of the nephrogenic ridge in this specimen from its anterior extremity to the end of the eleventh



somite. This includes all of the pronephric region and the beginning of the mesonephric.

The pronephric anlage extends from a point opposite the middle of the seventh somite to a point opposite the middle of the tenth. There are no separate tubules. The thick, rounded edge of the ridge, which represents the pronephric duct, is continuous antero-posteriorly, and is connected with the coelomic lining laterally by a thinner edge from which the tubules will be differentiated. What breaks there are in the tubular portion at this time bear no relation to the somites, but occur at both somitic and inter-somitic levels. The well-formed break near the beginning of the tenth somite admits the passage of a small artery from the dorsal aorta.



Fig. 22 Reconstruction of nephrogenic ridge in 17139. The somite levels are indicated above the reconstruction. The dotted ellipses near the right end of the figure indicate the extent of the lumina of the anlagen of the first four mesonephric tubules.

Near the beginning of the eleventh somite the thick portion of the ridge enlarges very markedly, the dorso-ventral diameter (that perpendicular to the plane of the paper) being particularly increased. This marks the beginning of the mesonephros. In section the more dorsal portion can be recognized as the anlage of the wolffian duct, which does not yet possess a lumen. The more ventral portion already shows some organization into tubules, the lumina of the first four of which are indicated in the reconstruction by dotted lines. But the tubule anlagen have not become detached from each other, or from the somatopleure, or from the wolffian duct. In fact, in the opossum the mesonephric tubules are not at any time separate from the wolffian duct. They are formed from the same anlage. Only the lumina of the duct and the tubules arise independently and later coalesce. The walls of

the duct and the walls of the tubules are continuous from the very beginning.

The important features of this development from a theoretical point of view are: that pronephric tubules, mesonephric tubules, and wolffian duct all arise from the same anlage; that this anlage is a longitudinal unsegmented structure which is continuous from its anterior end back to the region of the prospective cloaca; and that this anlage has for awhile the form of a ridge on the coelomic lining.

All of these features are in accord with Burlend's theory, which is, in part, that "the nephric system originated phylogenetically from a right and left 'primitive nephric groove' which became differentiated from the somatopleuric mesoderm lining the primitive splanchnocoel;" that "the groove became closed off from the splanchnocoel at intervals, and thus a duct . . . was evolved." This is to be contrasted with the familiar textbook account, which may be called the Brauer-Rabl theory, according to which the tubules arise first as separate segmental units. Their distal tips then grow together to form a duct (Sammelgang) which from the posterior end of the pronephric region sprouts independently (Endabschnitt) all the way back to the cloaca.

There is much evidence that the Brauer-Rabl theory is based upon some special cases (principally that of *Hypogeophis*) in which the intermediate mesoderm becomes segmented before nephrogenesis begins. Burlend was able to adduce strong contradictory evidence from cyclostomes, elasmobranchs, teleosts, ganoids, and dipnoi, all of which groups are more primitive than *Hypogeophis* and should accordingly be more significant. The pronephros in all the amniotes is so vestigial that not much hope has been entertained of securing interesting evidence from this source. The opossum evidence, supporting as it does the Burlend theory, is of particular interest, therefore, because it comes from the highest group of amniotes—the mammals.

One implication of the theory which is of general interest should be mentioned here. If the theory of the non-metameric

origin of the nephric system should prove to be the correct one, as the present writer believes it will, the last substantial evidence for either the annelid or arthropod theory of the evolution of the chordates will have been removed. For every other structure in the chordate body which exhibits any degree of metamerism can be shown to arise in the first instance as a non-metameric structure. The medullary plate, the neural crest, the mesoderm and its coelom, the notochord, the chondrostyle, and other structures to be mentioned below, all acquire whatever degree of metamerism they possess secondarily. Even the coelomic pouches of *Amphioxus* arise from a longitudinal groove in the enteron. All this seems to mean that the chordates arose from unsegmented ancestors and acquired a certain degree of metamerism independently during their own evolution.

*The first branchial pouches.* At stages 14 and 15 (second normal stage plate) ectoderm and endoderm are in immediate contact throughout the vesicle. At stage 16 the first mesodermal cells begin to separate them. By stage 20 the mesoderm completely underlies the medullary plate and is beginning to extend beyond it posteriorly. In stages 21, 22 and 23 a wide vascular area is formed and the sheet of mesoderm is uninterrupted throughout this region except in the midsagittal line where the notochordal plate in the endoderm is in immediate contact with the keel of the medullary plate (fig. 18, B). Now at stage 24 migration or resorption of mesoderm at six different points allows ectoderm and endoderm to make secondary contact with each other.

Four of the six points of secondary contact constitute the closing plates of the first two pairs of gill pouches (McCrary, '36). These are best illustrated in figure 31 where under the caption, first branchial pouches, stage 24, ectoderm and endoderm are seen to form a broad plate of contact medial to the hearts and lateral to the medullary plate. I do not know of any other animal in which branchial pouches have been recognized before the pharynx has acquired a floor, but that these are actually the branchial pouches is obvious enough from the



uninterrupted series at The Wistar Institute showing their transition to the more familiar condition.

In external views by transmitted light these thin closing plates appear as very light areas as shown at the level D in the tootomount and section in figure 20. In this particular series (17153) the second branchial pouches have not quite met the ectoderm (fig. 20, E). This illustrates the cephalocaudal progression in the formation of the pouches, which is shown in further detail in figure 31. More details of the origin and development of these pouches will be discussed in connection with the lung anlagen in stages 25, 26 and 27.

*The hypophyseal plate and the pharyngeal membrane.* As the subcephalic wrinkle, first observed in stage 23, pushes farther beneath the medullary plate it makes two contacts which are of permanent significance. The first is a contact of ectoderm with ectoderm, the ectoderm of the wrinkle with the infundibular depression in the medullary plate. The infundibular depression is seen from a dorsal view in figure 20, I, from a ventroposterior view in K, and from a ventroanterior view in L. In the last figure particularly, it is easy to see how the ectoderm in turning back upon itself in the midline will come to touch first the infundibular depression, and second, the minute endodermal diverticulum which is the first trace of the foregut. The plate which touches the infundibular depression will later form Rathke's pouch and the anterior lobe of the pituitary. That which touches the endoderm forms the pharyngeal membrane which separates the stomodeum or buccal pouch from the pharynx until stage 29 when it becomes resorbed.

*Proamnion.* The sixth point of secondary contact of ectoderm and endoderm mentioned above is the so-called proamnion—a crescentic region extending around the cranial part of the embryo as far back as the vitelline plexus. It appears in reflected light as a dark region (fig. 19, A) surrounding the head. This structure, which is identical with that seen in an 8½-day, 3.4-mm. rabbit, or in a 4-somite, 23-hour chick, and

which in these forms is transitory, is in the opossum permanent; and, as Selenka has shown, it contributes the major part of the definitive amnion.

*Differentiation in the medullary plate and the neural crest.* The medullary plate has elongated until it is about six and one-half times the length of the primitive groove, which is still clearly visible in its caudal tip (fig. 20, totomount and section H); and in the brain region the anlagen of the three primary divisions are now distinguishable.

The forebrain consists only of its diencephalic portion, and is the region under which the subcephalic fold extends in figure 20 (totomount and I). It includes the above-mentioned optic cups and infundibular groove, and it is broader than the midbrain region next to be described.

The midbrain is demarcated laterally by two very shallow grooves best seen in sections (vide fig. 20, C). Anteriorly and posteriorly it is demarcated by the flaring edges of the wider fore- and hind-brains. Selenka was unable to see any definite lateral border to the midbrain. This is another indication that most of his information about this stage came from his study of the whole embryo before sectioning, and that some of the sections were either lost or injured. The groove which bounds the midbrain laterally being shallow and being underlaid by the thick opaque mass of mandibular mesenchyme is almost impossible to see except in sections.

The hind brain extends from the point where the gasserian ganglion (v.i.) is proliferating, back to the somite region. In its anterior third it includes at least three recognizable neuromeres (fig. 20, I). It will later show six.

The cordal portion of the medullary plate is composed of a narrower cervical region and a broader thoracic region. The latter, which is posterior to the seventh somite, includes Hensen's node and the primitive groove.

The neural crest is proliferating most abundantly just anterior and just posterior to the first gill pouch. These proliferations are at the sites of the anlagen of the gasserian and acoustico-facial ganglia, respectively (vide fig. 21), but

several facts seem to indicate that only a few of these cells actually contribute to the ganglia. In the first place the cells thus derived are perfectly continuous with the mesenchyme of the whole adjoining branchial arch. It is not until stages 25 and 26 that the ganglionic rudiments become fairly distinct from the mesenchyme. In the second place, when the ganglia do become distinct they are composed of a relatively small number of cells—much smaller than the total number which must have been proliferated during stage 24. And finally, there is the fact that until this proliferation begins there is no recognizable mandibular or hyoid mesenchymatous mass. There is mesenchyme in the head region from stage 20 on, but only a thin and even layer. Simultaneously with the proliferation of neural crest in stage 24 the mesenchyme becomes so abundant anterior and posterior to the first branchial pouch that it shows up in transmitted light as two dark masses (fig. 20, totomount), which thereafter may be followed through later stages as the mandibular and hyoid arches.

It seems necessary to believe, therefore, that a considerable portion of the mesenchyme of these branchial arches is derived from mesectoderm proliferated at the sites of origin of the gasserian ganglion and the acoustico-facial complex. It should also be mentioned that the visible evidence in the opossum does not support the view that the otic placode contributes any cells to the acoustico-facial complex, but in the light of experimental evidence from *Amblystoma* this visible evidence must at least be held inconclusive.

Posterior to the second branchial pouch the neural crest is again fairly abundant at the site of origin of the glosso-pharyngeal ganglion and the third branchial arch. In fact, in figure 19 a third opacity is quite distinctly recognizable in all three photographs.

In the cordal region of 17153 (early stage 24) there is very little neural crest proliferation, but in later specimens as 17166 and 17139 it is well formed. A consistent difference between the neural crest in the cranial and spinal regions seems to be that the former arises from a broad region extending a



little way down the wall of the brain plate, whereas, the latter arises from a more restricted region at the very edge of the medullary plate.

*Miscellaneous details.* No nephrocoele ever develops in the opossum, so the myocoele is never connected with the splanchnocoele. Also the intermediate mesoderm does not become segmented. The somites separate first from each other (i.e., anteriorly and posteriorly), then from the intermediate mesoderm (i.e., laterally). They then have the form of flattened rectangular sacs enclosing a slit-like myocoele.

The paired coelomic rudiments (i.e., splanchnocoeles) elongate during stage 24 until they meet anteriorly and posteriorly, thus surrounding the embryo. The anterior loop is seen in figure 20, I, J and A. It extends beneath the subcephalic wrinkle as the sectional view shows. However, instead of expanding farther forward and thus eliminating the proamnion, as is the case in other animals, in the opossum as the head grows forward and the subcephalic wrinkle grows deeper, this anterior loop of coelom is brought beneath the body and in stage 25 it is simply incorporated into the definitive pericardium, there being no extra-embryonic coelom anterior to the embryo thereafter. This has an important effect upon the constitution of the amnion, which will be discussed in chapter X.

The posterior loop of coelom remains largely extra-embryonic. The anal plate dips in between it and the medullary plate. Subsequently, as the caudal part of the body rounds up, the proximal part of the extra-embryonic coelom becomes ventral, enlarges, and receives the allantoic diverticulum as that develops in stage 28 (fig. 37 and fig. 39, L). The extra-embryonic somatopleure goes into the formation of the caudal amniotic fold, and the splanchnopleure serves as the bridge along which the vitelline artery passes to the area vasculosa.

The notochord in stage 23 was a broad plate in the endoderm except at its most posterior end where it was separate from the endoderm and proliferating from Hensen's node.

In stage 24 its relation to the endoderm has not changed, but it is no longer a broad plate except at its anterior end where it merges into the prechordal plate, and its posterior end where it lifts above the endoderm and merges into Hensen's node. In early specimens of stage 24 it is throughout most of its middle portion only one or two cells in thickness, the cells being tall, columnar, and extending from the endoderm to the medullary plate. In the totemount (fig. 20) its width at various levels can readily be observed, the narrowest points in this specimen (17176) being at the levels of the hind-brain and again between the sixth and seventh segments. This extreme attenuation, however, is peculiar to early specimens. In later ones like 17166 and 17138 the notochord is again a thick plate in the endoderm.

A mesodermal condensation at the level F in figure 21 is the earliest primordium of the forelimb. I know of no other case in which the forelimb is begun at such an early stage.

*Comparative notes.* The stage 24 opossum embryo resembles a rabbit of the same age more than it does any other embryo. It is nearly identical with Minot's ('05) rabbit embryo no. 624 in the stage of development of the heart tubes, the aortae, the area vasculosa, the notochord, and the proamnion. It is further advanced in having more somites, having optic and otic anlagen, a forelimb blastema, branchial pouches, infundibular groove in contact with hypophyseal plate, and a pharyngeal membrane. It is less advanced in having no caudal amniotic fold. In this last respect it is intermediate between the mammalian type and the reptilian type as seen in *Lacerta agilis* (Keibel, '04). The early and rapid development of the first somites is, as will be seen in later chapters, to some extent peculiar to the opossum itself.

The chick of 4 to 6 somites (23 to 24 hours incubation) is similar in most respects, but has fewer somites and a medullary plate which is more advanced with respect to folding, though not with respect to differentiation.

*The second third of the ninth day*

Stage 25. First contact of the neural folds. Fusion of the hearts in the bulbar region. Origin of the sinus venosus. Origin of the pharyngeal floor. The lung anlagen. Miscellaneous details. Comparative notes.

*First contact of the neural folds.* When there are twelve or thirteen pairs of somites the neural folds come together and fuse in the region of the myelencephalon just caudal to the otocysts (fig. 23, A). Almost as soon as the edges fuse, they pinch off from the overlying ectoderm and form a tube

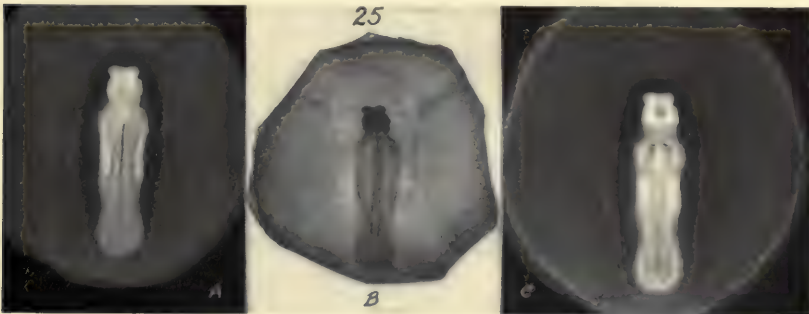


Fig. 23 Photographs of external views of stage 25. A, dorsal view of 16171 by reflected light. B, dorsal view of 16144 by transmitted light. C, ventral view of 16171 by reflected light.

which is laterally compressed (fig. 25, G and H). During the latter part of stage 25 the tube continues to close caudad through the first six or seven segments, that is, to about the anterior level of the limb ridge (fig. 23, B), but no progress at all is made craniad (fig. 25, A, B, C, D, E and F). In fact the brain plate will not begin to close over until the end of stage 27 (early tenth day, chapter X).

In the region of closure the neural crest seems to have ceased proliferating, and in the somite region it is aggregated segmentally. The gasserian ganglion and the acousticofacial complex (fig. 24) are rather well demarcated, but are still receiving proliferations from the brain plate. Neural crest



is also still proliferating from the edges of the posterior end of the neural plate.

*Fusion of the hearts in the bulbar region.* The process which leads to the fusion of the paired lateral hemicardia into the single median heart began when the pericardial sacs, which were completely separate in stage 23, grew forward around the head region and met in the midline in stage 24 (fig. 20, totomount, I and J). This region of first fusion of the pericardial sacs is anterior to both the aortic and cardiac primordia. The aortic plexuses lie for the most part outside the pericardia, but, as was shown in figure 20, J, the point of common origin of the first and second arches, and even a portion of each of these arches, is at first covered by

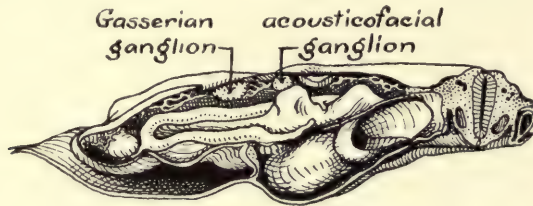
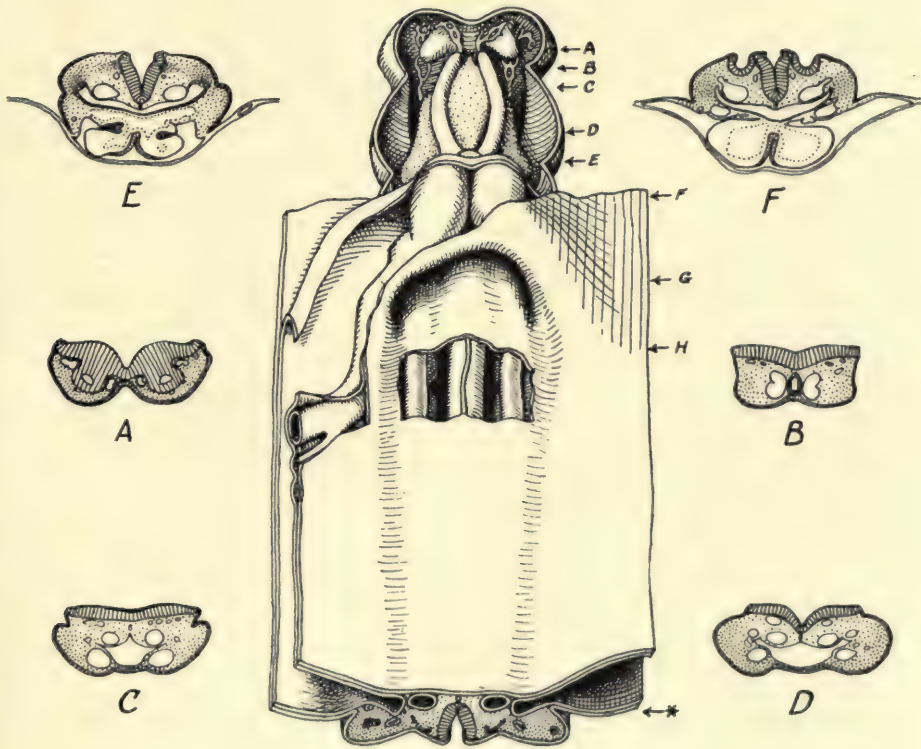


Fig. 24 Lateral view of reconstruction of stage 25 (17137).

myocardium. Accordingly, as the region of fusion of the pericardia progresses caudally the first portion of the endocardia to fuse is this bulbar portion. And the endothelial tubes which form the bases of the first aortic arches actually fuse before any part of the hearts proper. This is the condition in the last specimen of stage 24 (17139). In stage 25 (fig. 25, reconstruction and sections E and F) the fusion has not progressed beyond the bulbus cordis, but the ventricular portions of the tubes have been brought into close apposition. The reconstruction figure shows also that the atria are on either side of the anterior intestinal portal and are still widely separate.

The process of apposition and fusion of the pericardia and heart tubes in the opossum is somewhat peculiar in that it



Stage 25  
Middle Ninth Day

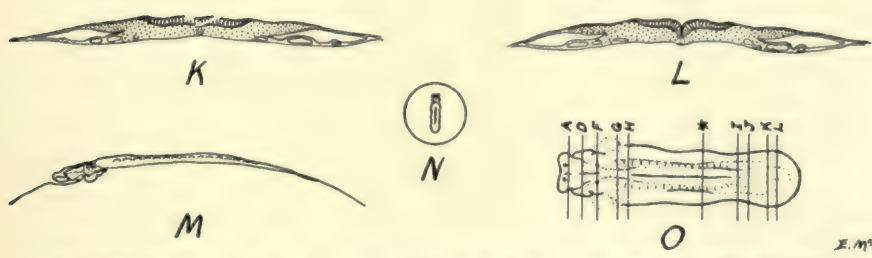


Fig. 25 Ventral view of reconstruction and sections of stage 25 (17136).

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is accomplished without the formation of a ventral mesocardium at any time. This is illustrated in figure 26. The chick sections, taken from Patten's Early Embryology of the Chick, are presented for comparison. It will be seen that in

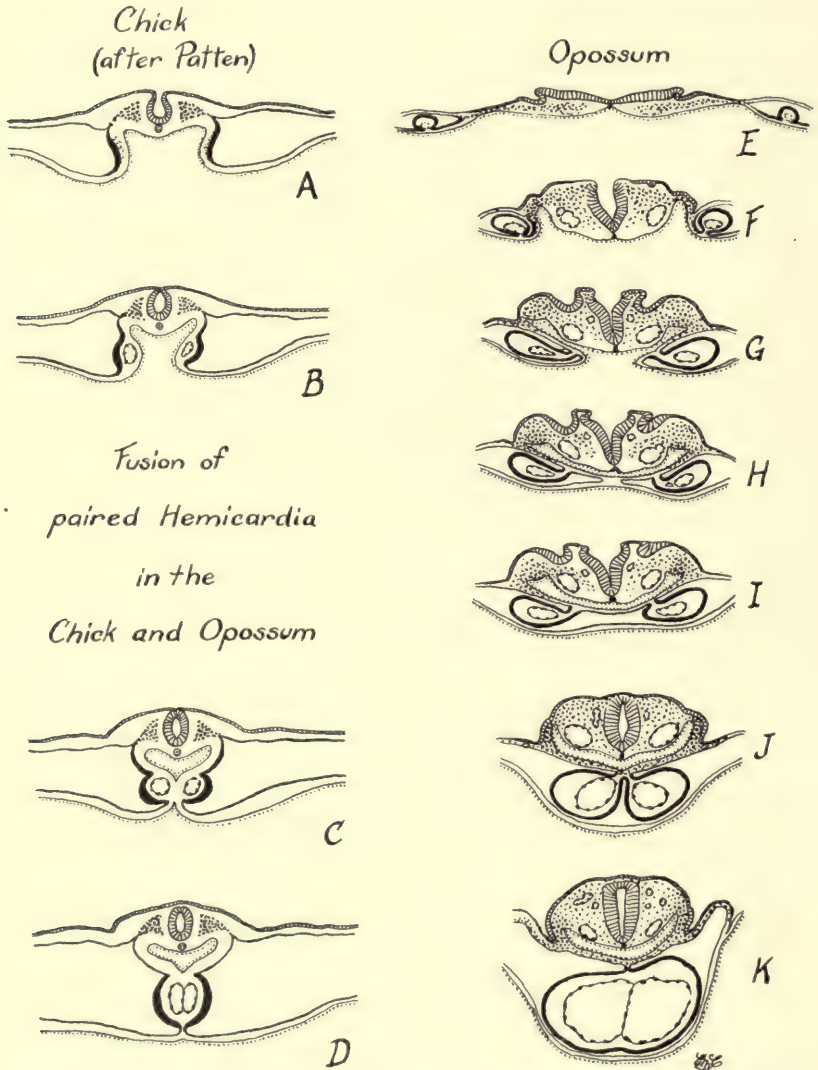


Fig. 26 Sectional views of opossum embryos of stages 24 to 26 and chick embryos (25 to 28 hours) to show that no ventral mesocardium ever forms in the opossum.



the chick the myocardial elements which approach each other near the midline are only half cylinders (B). As a result, when they fuse, mesenteries are formed both dorsal and ventral to the heart tube (C and D). In the opossum, on the other hand, each hemicardium is a nearly complete tube (E) long before the two approach each other. When they do draw near the midline (F, G, H, I) each is suspended by a single dorsal mesocardium. The two dorsal mesocardia fuse when the tubes meet (J and K), and no ventral mesocardium is formed.

*Origin of the sinus venosus.* In the last chapter it was mentioned that the allantoic vein arises just dorsal to the point where the vitelline vein enters the body. The two vessels are at first separated by a slight interval of coelom, but as they enlarge, the parietal layer of pericardium which underlies the allantoic vein, meets and fuses with the visceral layer which overlies the vitelline vein, and the vessels thus come into contact with one another (fig. 25, H). In late specimens of stage 25 their walls break down at the point of contact and they become confluent. Hereafter the original vitelline vein anterior to the point at which it receives the allantoic is the sinus venosus.

At the same time that this communication is established, capillary sprouts growing caudad from the vena capitis medialis, or primitive precardinal, reach this same level, and a few fuse with the allantoic vein near its entry into the sinus. The head vein thus gains access to the sinus. Some of the growing tips, however, pass this level (which is opposite the second somite) and continue caudad to the neighborhood of the fourth somite. These constitute the anlage of the post-cardinal vein, which, however, will not be clearly established until stage 26.

*Origin of the pharyngeal floor.* In the early specimens of stage 24, though two branchial pouches with closing plates had formed, there was no pharyngeal floor beneath them. Figure 20, section B and reconstructions L and J, shows the small thimble-shaped diverticulum which is the anterior extremity and first-formed portion of the foregut. The same

process which leads to the conerescence of the heart tubes leads to the formation of a pharyngeal floor as seen in figure 27. By the end of stage 24 this process has extended the floor beneath the first branchial pouches (fig. 31), but the second branchial pouches are still over the empty yolk sac. During stage 25 the anterior intestinal portal moves caudad beneath them as the ventricles of the hemicardia draw near each other. The further migration of the portal caudad and some parallel changes in the shape and differentiation of the pharynx will be discussed in chapter XII in connection with the lung pouches.

*Lung anlagen.* Though the more familiar steps in the development of the lungs come after stage 27, the earliest primordia are distinguishable in late specimens of stage 24 and in all specimens of stage 25 (McCrary, '36). These primordia need special comment as they are different from any that have hitherto been described.

At stage 24 the endoderm in the cervical region shows on its ventral surface three longitudinal depressions: a medial notochordal groove, and two lateral branchial furrows. Each branchial furrow deepens locally at two points so as to extend through the flat body wall to the ectoderm. The points of contact between endoderm and ectoderm thus established have already been pointed out as the closing plates of the first and second branchial pouches. Posterior to them the furrow becomes shallower until it gradually disappears near the anterior limit of the pronephric anlagen. No such furrow ever develops in the endoderm of the thoracic, lumbar, and sacral regions.

At the level where the sections show the anterior end of the allantoic vein protruding through the somatopleure toward the vitelline vein, the pleuro-pericardial cavity is inserted between the lateral half of the branchial furrow and the ectoderm, and the splanchnopleuric mesoderm which thus comes into contact with the endodermal wall of the groove is conspicuously thickened. This thickening which is a center of rapid proliferation, is the mesodermal blastema of the lung.

The processes of formation of the branchial furrow, its deepening and subsequent development of local outpocketings show a definite cephalocaudal progression. This is best illustrated in figure 31 in which the principal point to be noticed at present is the striking similarity between the section through the lung region in stage 25 and that through the second branchial pouch in stage 24+. These two sections on casual examination might be confused. The only important differences are: first, that in the case of the lung anlagen the endodermal pouch does not actually touch the ectoderm; second, that the mesothelium in contact with the lateral side of the lung pouch is markedly thicker than that touching the branchial pouch; and third, that the location of the lung section may be identified by the presence of the anterior end of the allantoic vein immediately dorsal to and more or less in contact with the vitelline vein.

The endodermal constituent of the lung is thus a local outpocketing from a longitudinal groove, which outpocketing in early stages is indetical with others from the same groove which become gill pouches. The lung pouch is simply the hindermost gill pouch. Further evidence for this interpretation will be discussed in chapter X when the rudiments here described will be followed to a more familiar stage.

*Miscellaneous details.* The origin of the branchial pouches and lungs as secondary, metamerie outpocketings from originally longitudinal, non-metameric grooves, is another illustration of the fact referred to above—that all metamerism in the vertebrates is secondary.

The notochord is in process of separation from the endoderm. From about the posterior end of the limb ridge back to Hensen's node it is a solid cord of cells quite free from the endoderm. But anteriorly it may be seen in all stages of pinching off. The originally flat plate wrinkles high in the midline so that its sides approach each other and include a narrow, vertical slit between them. When the sides unite ventrally, the notochord pinches off from the endoderm and sometimes shows a transitory notochordal canal; but where formed at all, this canal is quickly effaced.



The inner wall of the somites is breaking up and proliferating the sclerotome. The dermo-myotome remains as a square plate with its edges rounded inward.

The otic placodes have deepened into well-formed cups. The optic diverticula are becoming stalk-like.

The forelimb blastema has thickened into a very opaque plate.

*Comparative notes.* Rabbit embryos of  $8\frac{1}{2}$  to 9 days of age are at a comparable stage of development. In comparison with Minot's specimens 571, 573 and 621, the opossum at stage 25 is somewhat more advanced in differentiation of the hind-brain and cervical cord, the heart, the ear and the somites. And unless their anlagen have been overlooked in the rabbit, the opossum is also ahead with respect to the development of the lung pouches. The rabbit is ahead in having a distinct caudal amniotic fold.

The lizard of 1.5 mm. length (Peter, '04, N.T. 10) is at the same stage of development of the general body form, the central nervous system, the digestive tract, and the notochord. But the opossum is far ahead in the vascular and urinogenital systems, and in the eye and ear. The lizard is more advanced in the formation of a head amnion, but is like the opossum in still having no caudal amnion.

A 25-hour chick (7 somites) is like the opossum in central nervous system, digestive tract, vascular system, and in having no amnion of any sort. The opossum is much ahead of it in development of the urinogenital system.

Comment on the significance attributed to these comparisons is deferred to page 89.

#### *The last third of the ninth day*

Stage 26. Cephalic flexure. Beginning of amnion formation. Fusion of hearts in the ventricular region. Origin of the postcardinal vein. Miscellaneous details. Comparative notes. General comments on comparative data.

Toward the end of the ninth day the vesicles, which are still spherical and roll or float freely in the uterus (fig. 27, C), have attained a diameter of 9 or 10 mm. The embryo itself, however, is not more than about 6 mm. in greatest length, and it will not exceed this length until the end of the eleventh day. For between the ninth and the eleventh days several flexures occur, and the 'length' of the embryo undergoes such reduction and fluctuation that linear dimension loses all significance as a criterion of developmental stage. An embryo of 5.5 mm. greatest length may belong to stage 25, or to any of the



Fig. 27 Photographs of external views of stage 26. A, dorsal view of 16146. B, ventral view of 16145 after removal of non-vascular chorion. C, vesicles and part of uterine mucosa from Hartman's litter 291.

stages of the tenth day (27 to 29), or to the middle or late parts of the eleventh day (stage 31). After stage 31 crown-rump length becomes a useful datum for identification, and remains so until several weeks after birth when the head begins to assume a more axial alignment and it is necessary to shift to snout-anus measurements.

*Cephalic flexure.* The first of the flexures referred to is the so-called cephalic flexure, which is a right angle, ventral bend in the central nervous system at the level of the mesencephalon. It would be more proper to call this the mesencephalic flexure, as the pontine flexure, which occurs in stage

33, is equally 'cephalic;' however, the classical nomenclature here is probably too well established to be displaced.

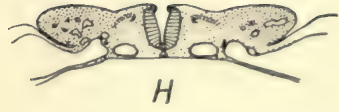
This flexure in the opossum occurs at a time when closure of the neural folds has not progressed anterior to the myelencephalon (fig. 28, reconstruction and sections A, B, C, D).

*Beginning of amnion formation.* It will be recalled that there has been no Rauber's layer in the opossum at any time. The cells from which the surface of the embryonic body is formed have been superficial from the beginning. Also the vesicle has not formed any association with the endometrium, but floats freely in the 'uterine milk.' Amnion formation, therefore, is not complicated by the presence of any extraneous material, or by any implantation process.

The homology of the unilaminar and bilaminar blastocysts with the blastula and gastrula of the lower vertebrates has already been commented upon. The entire external lining of the vesicle is ectodermal. The so-called trophoblast is merely the primitive ventral ectoderm. The internal lining of the vesicle is entirely endodermal, at first corresponding to the archenteron, and later to the enteron plus the yolk sac. When mesoderm forms at the animal pole, it separates ectoderm and endoderm. As it spreads, it extends this separation throughout one-third to one-half of the vesicle; but it never invades the ventral half. Along an elongate, elliptical line enclosing the animal pole the mesoderm splits tangentially to form the somatopleuric and splanchnopleuric layers with the coelomic space between them. Part of each of these layers is to be incorporated into the embryonic body and part to be discarded, but there is no visible distinction between the embryonic and accessory cells. Finally, the most dorsal mesoderm, which is within this ellipse, is again unsplit.

At stage 26, therefore, the original bilaminar vesicle remains unmodified in its ventral half to two-thirds. The next most dorsal portion including the layer of unsplit mesoderm is the area vasculosa or vascular portion of the yolk sac. The definitive embryonic body is developing approximately at the center of this vascular area, and the coelom is confined to a narrow elliptical strip in the immediate vicinity of the embryo.

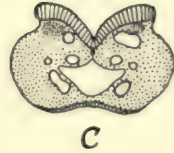




Stage 26  
Late Ninth Day



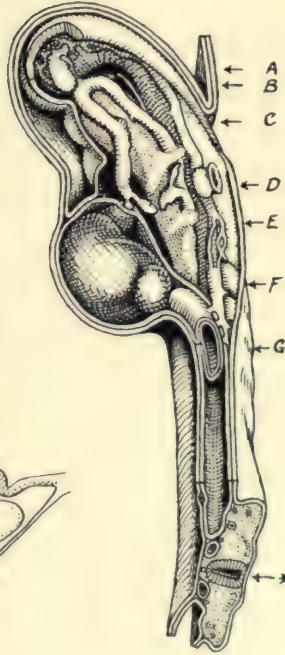
A



C



B



A

B

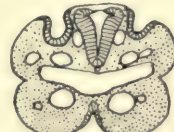
C

D

E

F

G



D



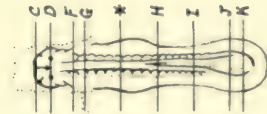
F



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I



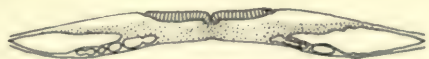
M



L



J



K

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Fig. 28 Lateral view of reconstruction of stage 26 (16146) and sections from 16145.

In the early part of the ninth day (stage 24) the embryonic head begins to grow forward beyond the coelomic ellipse so that it comes to overlie a portion of the area vasculosa, and simultaneously the mesoderm in this region (i.e., the floor of the subcephalic pocket) becomes resorbed. The portion of the yolk sac which is thus inside the area vasculosa but devoid of mesoderm (the proamnion) is the portion into which the head dips when the cephalic flexure forms. The layer of ectoderm and the layer of endoderm which thus come to enclose the head constitute the head fold of the amnion. Laterally and posteriorly the embryo is still surrounded by the coelomic ellipse, but anteriorly it reaches over the coelomic region and dives head first into a pocket in the yolk sac.

In the opossum, and generally in the Sauropsida, the cephalic flexure seems to play a mechanical part in making the head fold form before the tail fold, but this is possible only where amniogenesis begins at a late stage of development. In mammals the general tendency is for the amnion to form at an early stage, and 1) to dispense with folds altogether (as in man where it forms at a stage corresponding approximately to the opossum stage 14); or 2) to form from folds simultaneously on all sides (as in the pig where the time corresponds approximately to the opossum stage 21 or 22); or 3) to be slightly more advanced at the caudal fold (as in the rabbit where the time is very nearly the same as in the opossum). The opossum thus conforms to the reptilian or avian type in time of amnion formation and in precocity of the head fold, but probably these relationships are not very significant.

Selenka (loc. cit.) was the first to point out the unique constitution of the head fold of the amnion in the opossum. The fact that the proamnion, which is very transitory in other animals, is persistent in the opossum leads to a very complicated situation when all the amniotic folds meet during stage 29.

*Fusion of the hearts in the ventricular region.* In stage 25 the concrecence of the lateral body walls in the head region

resulted in the fusion of the heart tubes in their bulbar portions, and the formation of a pharyngeal floor as far caudad as the second branchial pouch. During stage 26 this same process carries the fusion of the hearts through the ventricular region, and the floor of the pharynx beneath the posterior branchial complex—a dorsolateral outpocketing of the pharynx from which the third, fourth and fifth branchial pouches will be derived (fig. 28, reconstruction). At the same time the heart twists slightly so that the bulb is pushed to the right and the ventricle to the left. The atria and sinuses of the hearts are still separate, and the floorless portion of the pharynx immediately between them is the portion in which the lung pouches are forming.

*Origin of the postcardinal vein.* The general manner of origin of the postcardinal has already been indicated in a previous section. It is part of a primary longitudinal venous complex which originates beneath the floor of the mesencephalon and grows caudad on the medial side of the sensory ganglia and the otocyst. As it reaches the level of the anterior end of the umbilical vein it forms capillary communications with the latter and thereby gains access to the sinus venosus; but other capillary sprouts from it continue caudad to the anterior level of the limb ridge—fourth somite. Here they invade the dorsal part of the limb. This seems to be their primary association in this part of the body, but as the nephric anlagen are developing from the seventh somite caudad, the postcardinal venules, still growing caudad, soon become associated with them also. In later stages the postcardinal retains its connection with the dorsal part of the forelimb and establishes a similar connection with the dorsal part of the hindlimb, but the association with the developing mesonephros becomes much the most important.

There is still one group of branches from the postcardinal plexus which have not been mentioned. At intersomitic levels capillary sprouts run dorsomedially between the somites and communicate with similar sprouts from the aortae. These are the precursors of the segmental veins.



*Miscellaneous details.* The first aortic arch is no longer plexiform, and its branches near the optic lobe have become organized into the ophthalmic artery. Between the first and second gill pouches plexiform branches from the ventral and dorsal aortae are uniting to form the second aortic arch (fig. 28, reconstruction and section D). Posterior to the level of the eleventh somite many small vitelline branches leave the aortae and pass into the area vasculosa (sections I, J, K). From this plexus the vitelline or superior mesenteric artery will later be derived. There are segmental branches from the aortae at all intersomitic levels.

The area vasculosa is rich in blood islands and includes a continuous plexus of small vessels connecting the sinus terminalis with both the vitelline veins and the vitelline arteries, so that dislodgement of the blood cells from the islands and continuous circulation will begin as soon as the heart starts beating.

The forelimb plate has thickened into a ridge which overhangs the more lateral somatopleure.

*Comparative notes.* The 9-day-old rabbit (e.g., Minot's specimens 619, 568, 570) is more advanced than the opossum of the same age in having its brain roofed over, and in having a caudal amniotic fold. It is less advanced in number of somites, in the stage of development of the optic and otic anlagen and of the pharyngeal pouches. In other respects it is very similar.

A 1.77-mm. lizard (*Lacerta agilis*—Peter's specimen no. 42) is slightly ahead of the stage 26 opossum in the development of the head amnion, and in having a rudiment of the allantois. It is very decidedly behind the opossum in somite number, in the development of the eye and ear, in the pharyngeal pouches, and most of all, in the vascular and urinogenital systems, which can hardly be said to have begun in the lizard.

A 33-hour chick (10 or 12 somites—Keibel's no. 19) is similar in the development of the optic anlagen, and in the vascular and urinogenital systems. It is more advanced in

the roofing of the brain, and it is less advanced in the differentiation of the head amnion, the pharynx, the somites and the otic anlagen.

*General comments on comparative data.* In the comparative notes I have tried to select a representative type for each of the principal groups of amniotes except the monotremes, for which last-mentioned the available data are insufficient. The lizard was chosen to represent the cold-blooded Sauropsida because it is considered the least specialized living reptile (Parker and Haswell, vol. 2, p. 338). The chick was chosen to represent the warm-blooded Sauropsida because it is the best known bird. The rabbit was selected for the placental mammal, not only because it is fairly unspecialized, but also because for the first  $10\frac{1}{2}$  days of gestation it has almost precisely the same developmental rate as the opossum.

The existence of the term Sauropsida implies that the reptiles and birds have more in common with each other than either has with the mammals. In general this is probably true, but some conspicuous exceptions were listed in the comparative notes in this chapter. The vascular and urinogenital systems appear in the lizard at a much later stage of development than they do in either the marsupial or placental mammal. In this case the other sauropsidan, the bird, accords with the mammals, not with the lizard. The obvious implication of these facts is that the early differentiation of these two systems is not a mammalian in contradistinction to a sauropsidan feature, but is an homoiothermal in contradistinction to a poikilothermal characteristic. Actually neither the bird nor the opossum can control its body temperature at birth, but both of them are 'incubated' until they can. This parental attention enables them to maintain a constant temperature and a high rate of metabolism. It seems very likely that the high rate of metabolism in the warm blooded animals makes necessary the early development of the circulatory and excretory mechanisms.

There are some features in which the opossum is different from all the other forms with which I have compared him.

For instance, the pharynx develops gill pouches and lung rudiments at an earlier stage than in any of these other animals. That the lung begins its development early is not surprising, for the lung must be put to use in the opossum at an extremely early stage of general body development. The chick begins to breathe during the eighteenth day of incubation, which corresponds to about the twenty-fourth day after mating in the opossum; the rabbit begins to breathe during the thirtieth day; but the opossum has been breathing since the thirteenth day! The opossum at this time corresponds in general development to a chick of about the seventh day of incubation, or a rabbit of about the fifteenth day of gestation.

The reason for the precocity of the branchial pouches is perhaps less obvious; but, if the theory that the lungs are really modified gill pouches be correct, then part of the explanation may be that the whole branchial apparatus begins development early because the lungs, which are part of it, must be used early. But, regardless of the origin of the lungs, much of the essential mechanism of sucking and breathing, e.g., the larynx, is derived from the branchial arches, which must, accordingly, be precocious.

Other unique features in the opossum are the precocious development of the myelencephalon, the cervical cord, the anterior somites (the opossum has about 13 somites before the neural folds make contact anywhere), and the ear primordium. These features, I believe, are all associated with the early birth of the marsupial. The myelencephalon is precocious because the nerves to the sucking mechanism (the tongue, etc.), the lungs, and the stomach, must be used at an early stage. The cervical cord must be rushed ahead to assist in the sucking mechanism, to provide a brachial plexus to control the forelimb (by means of which the opossum gets into the pouch), and to provide a phrenic nerve to move the diaphragm and make breathing possible. The anterior somites must be developed at a rapid rate to provide musculature for the forelimb.



But what about the early development of the ear? The opossum has no functional vestibular apparatus until about 41 days after birth, and cannot hear until about the fiftieth day. So there would seem to be no need for an early start in the differentiation of the otocyst.

I believe the explanation lies in the fact shown by a number of operative experiments on amphibians—that the otocyst is induced by the underlying tissues. The hyoid mesoderm, according to Harrison ('35), is the primary inductor, and the hind-brain a secondary inductor. If the inductor is precocious, the induced organ will be precocious. We have already seen that both of these inductors are precocious for other reasons. Paradoxical as it may sound, the ear develops early, not because the opossum will have to hear at an early stage, but because he will have to breathe, and suck, and use his stomach at an early stage.

#### X. THE TENTH DAY. Stages 27 to 29

##### *The first third of the tenth day*

Stage 27. External distinctive features. The cervical flexure. The lung buds. The tail fold of the amnion. Changes in the vascular system. Miscellaneous details.

*External distinctive features.* Stage 27 is distinguished externally from stage 26 by the cervical flexure and the tail fold of the amnion; from stage 28 by the fact that the anterior neuropore and the otocysts are still wide open.

Associated with the cephalic and cervical flexures are changes in the extent of the head fold of the amnion. In stage 26 the cephalic flexure had occurred in the region of the midbrain and the head had become enclosed in the pro-amnion back to the level of the otocysts. In stage 27 the cervical flexure occurs at the level of the cervical cord, the heart, and the first somites, and the head fold passes over the otocysts and back to this new level. The caudal fold, which will be described below, first appears in stage 27 and covers part or all of the sinus rhomboidalis.

*The cervical flexure.* The flexures which play such a conspicuous part in changing the external as well as the internal appearance of the embryo between stages 26 and 35 seem to be primarily associated with local spurts of growth in the central nervous system. The first flexure is the cephalic, which was described in connection with stage 26. This seems to be due to a lengthening of the mesencephalic plate which causes it to buckle and lift dorsally at the level at which the

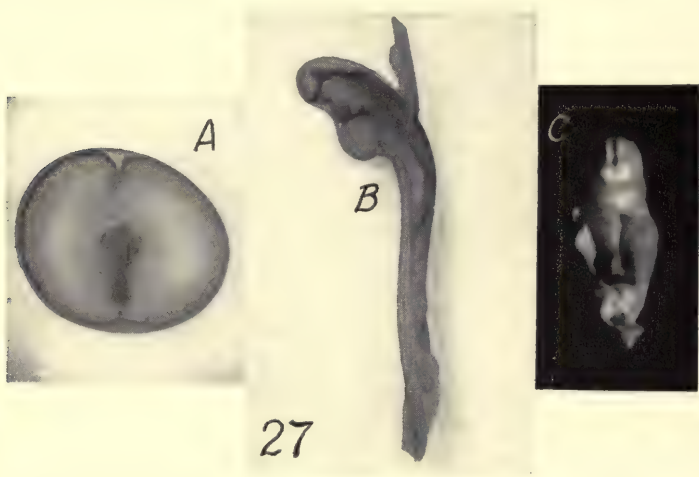


Fig. 29 Photographs and drawing of stage 27. A, ventral view of 16148 after removal of non-vascular chorion showing posterior intestinal portal, and open anterior neuropore. B, drawing of specimen 17141, showing caudal amniotic fold and open otocyst. C, photograph of caudo-ventral view of 17185 showing open anterior neuropore and anterior intestinal portal.

nuclei of the third and fourth cranial nerves and the nucleus ruber and the substantia nigra will later appear.

During stage 27 two new growth centers appear in, 1) the alar plates of the mesencephalon, and 2) the cervical cord. The growth of the alar plates (from which the corpora quadrigemina develop) causes the mesencephalic walls to rise dorsally and close into a tube before either the prosencephalon or the metencephalon have closed over. The growth of the cervical cord causes the central nervous system to bend and

lift away from the pharynx at the level of the lung buds (fig. 30, reconstruction and sections B and C). This is the cervical flexure. Both it and the cephalic flexure are conspicuously reflected in the external body form.

*The lung buds.* The earliest anlagen of the lungs were mentioned in chapter IX where they were pointed out in stages 24 and 25 as first, the mesothelial thickenings, and then, associated local endodermal outpocketings from paired longitudinal branchial furrows. In figure 31 these furrows can be followed to the point where the lung pouches are located at the dorso-lateral corners of the pharynx, which is just in process of acquiring a floor at their level (stage 27). Attention has already been called to the striking similarity between the lung pouches of stage 25 and the second branchial pouches of stage 24+.

Figure 32 shows photographs of models of the pharynx taken from early and late specimens of stage 27. The early specimen (A) shows a pharynx which is triangular in section at the lung level, with the apex placed ventrally between the venous sinuses, and the lungs situated at the dorso-lateral corners of the triangle. The roof of the pharynx is beginning to lift into a dorsal ridge which forms when the central nervous system and the notochord lift dorsally as just described in connection with the cervical flexure. The late specimen (B) shows the ventral apex obliterated (by the confluence of the atria of the hearts) and the dorsal ridge lifted so high that the lung buds are now at the ventro-lateral corners of a new triangle.

These changes are represented from the lateral view in figure 33. The branchial ridge illustrated here is, of course, the outside view of the branchial furrow previously referred to. The rise in the floor of the pharynx beneath the first branchial pouches in stage 25 is due to the fusion of the anterior end of the heart tubes into a single median bulbus cordis. The ridge labeled dorso-mesocardial line indicates the position of the mesocardium of the atrium, which does not meet its fellow of the opposite side in the midline until the end of stage 27.

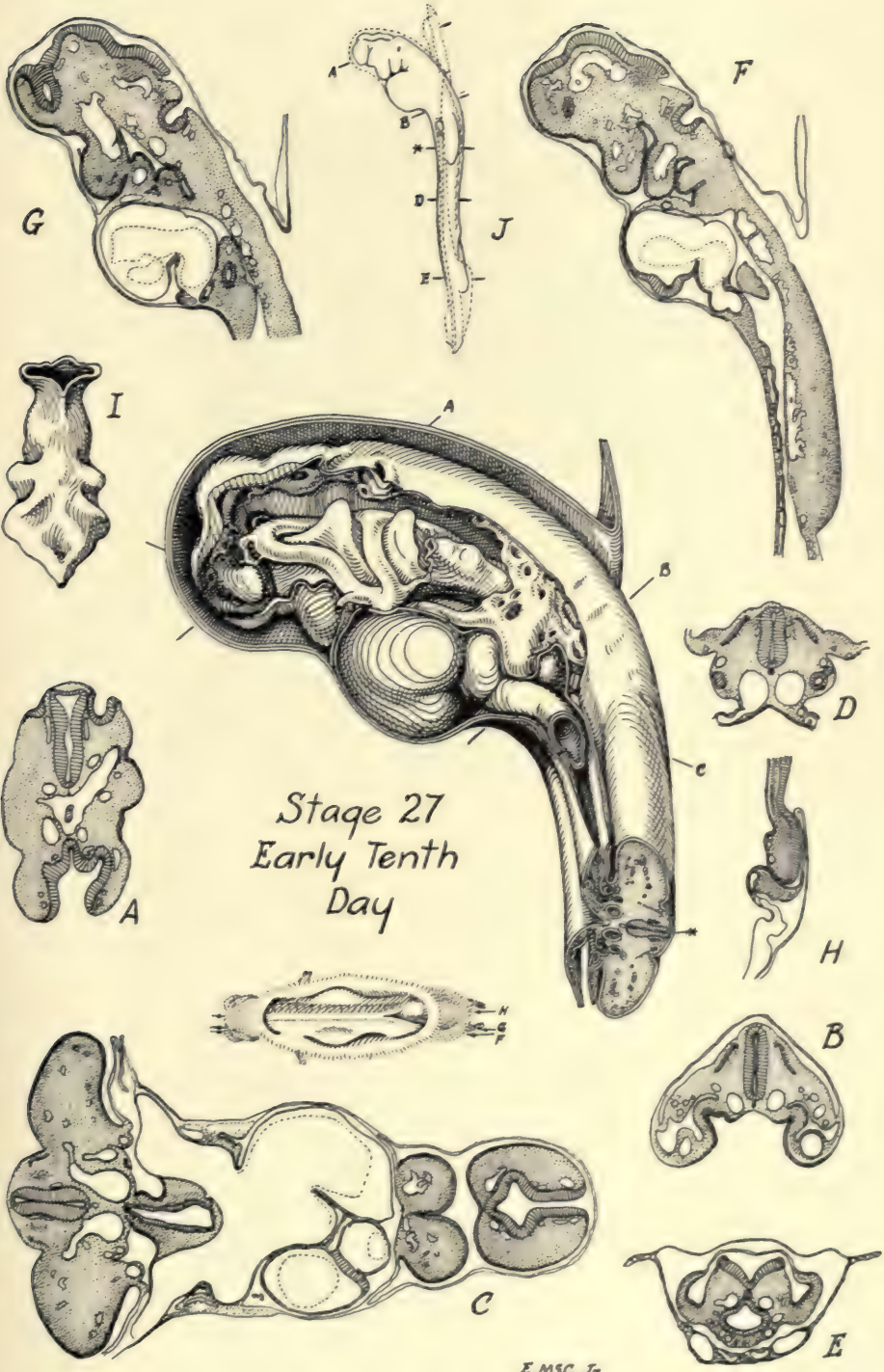


The branchial pouches arise as successive local out-pocketings of the branchial ridge from the anterior end caudad. In the earliest specimen here shown (stage 25) the first pouch is well developed and the second is distinctly recognizable. In stage 26 in addition to these two a new out-pocketing is seen, which is labeled posterior branchial complex, and which represents the fused anlagen of the third, fourth, and fifth gill pouches. As the third is separating from this complex in stage 27, a still more caudal pouch (l.p.) in this same ridge forms the anlage of the left lung. The line across it in the figure shows the location of the cut end of the model seen in figure 32, A.

At this stage the atria of the heart tubes are still separate and the deep ventral groove in the pharynx lies between them. On first sight this groove appears to be the laryngo-tracheal rudiment, but an examination of the late specimens of stage 27 shows this not to be the case. For when the atria come together in the midline (fig. 32, B, and fig. 33, stage 27+) this groove is completely eliminated and the floor of the pharynx is pushed up to the level of the lung pouches. Meanwhile, the roof of the pharynx, which was depressed by the central nervous system in stage 25 (see also fig. 31, second branchial pouches), has lifted dorsally as the central nervous system moved away from it in connection with the cervical flexure, so that now the lungs are at the ventro-lateral corners of a new triangle. In stage 28 the lung buds and the branchial ridges begin to pinch off from the more dorsal part of the pharynx from behind forward, so that in stage 29 a distinct trachea is formed from the fusion of the posterior portions of the original lateral branchial ridges. This is in keeping with the fact that the cartilages of the trachea, which will form in stage 34, are generally regarded to be of branchial origin.

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Fig. 30 Reconstructions and sections of stage 27. The central reconstruction is from 17141 as are also sections G, F and H. Sections A and B are from 17142, and sections C, D and E and reconstruction I are from 17154.



Stage 27  
Early Tenth  
Day

E. M. S. C., Jr.

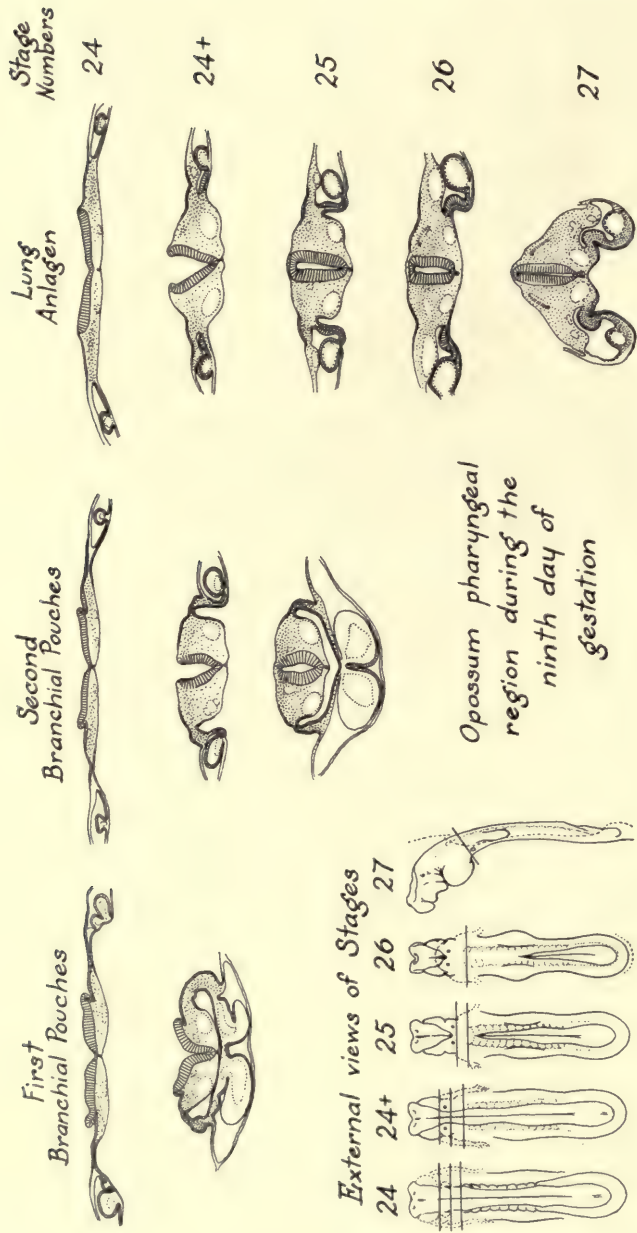


Fig. 31 Sections to show early stages of branchial pouches and lungs.



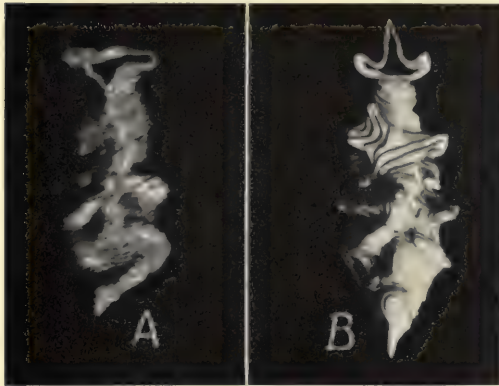


Fig. 32 Photographs of wax models of pharynx of early and late specimens of stage 27. A, early specimen (17154) showing lung pouches at dorsolateral corners of pharynx. B, late specimen (17142) showing lung pouches at ventrolateral corners of pharynx.

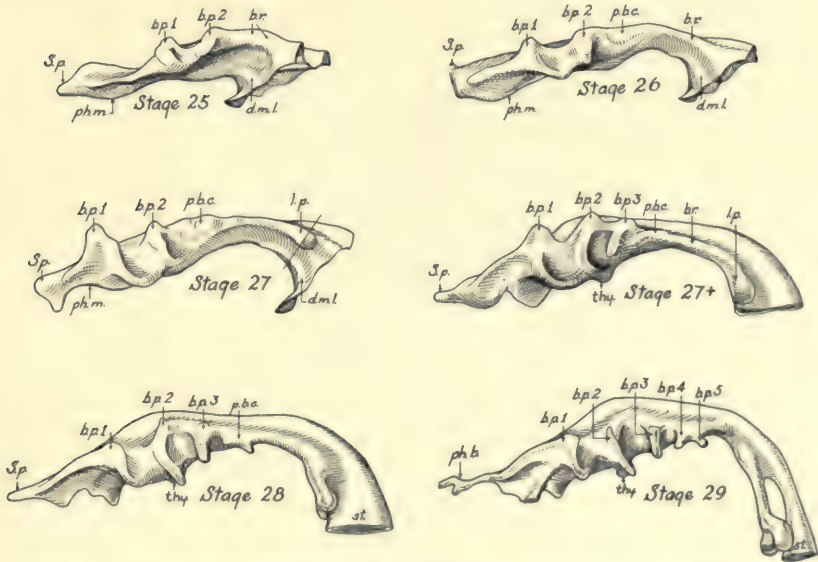


Fig. 33 Lateral reconstructions of the pharynx from stage 25 through stage 29 to show the development of the branchial pouches and lungs.

As in all other mammals, the pulmonary arteries arise (see stage 30 in the opossum) as outgrowths of the sixth aortic arches, or if you interpret it that way, as posterior extensions of the ventral aortae—at any rate, as *branchial* arteries. The nerves to the lungs, the vagi, are universally considered to represent a fusion of the posterior branchial nerves. All of these facts have led me to believe that the lungs are modified gill pouches.

According to Goodrich ('30) this theory dates back to Goette (1875). After a considerable period of neglect it has been revived and championed by Makuschok ('11, '12, '13) and others. Evidence (not always recognized as such) has accumulated from Amphibia, Reptilia, Aves, and Mammalia, i.e., all the pulmonate groups; but two objections have been urged. First and most important is Greil's opinion that the lung rudiments, though often paired and lateral in all these groups, are in all cases that he knows too ventral to be homologous with gill pouches. That objection seems best answered by the evidence from the opossum just presented, in which the lungs at the beginning are definitely dorso-lateral, not ventro-lateral, and are part of a conspicuous branchial ridge. The second objection is simply that the lung pouches never make contact with the ectoderm. This latter does not seem to be an important criterion for there are other cases in which gill pouches fail to make contact with the ectoderm, particularly the more posterior ones—for instance, the sixth in Triton (Makuschok, '11) and the fourth and fifth in the rat (Pischinger, '32).

Having seen, therefore, that the lungs themselves arise in the same manner and from the same source as the gill pouches, that their blood supply and nerve supply are branchial, that the cartilages of the trachea and bronchi as well as those of the larynx are of branchial origin, and that in the face of all this confirmatory evidence no important contradictory evidence has been found, there seems to be no escape from the conclusion that the lungs are modified gill pouches. Makuschok has shown the same to be true of the

swim bladder of fishes; so the long accepted theory of the homology of the lungs and the swim bladder is not contradicted by this interpretation—they are homologous, and they are both gill pouches.

*The tail fold of the amnion.* The anterior and the posterior amniotic folds in the opossum are of different constitution and never completely merge. The definitive amnion thus has two quite distinct portions, which Selenka (loc. cit.) recognized and accurately described.

After pointing out that the pro-amnion, which van Beneden and Julin first described in the rabbit and the bat, is a transitory structure which contributes nothing to the definitive amnion in all other known cases, he says:

Beim Opossum umhüllt das aus Ekto- und Entoderm bestehende Kopfamnion (wie ich das von Van Beneden und Julin als 'Proamnion' bezeichnete Gebilde nennen will) vier Tage nach Beginn der Furchung [stage 27] ungefähr des vordere Drittel des Embryonalkörpers, während das Rumpfamnion, welches aus Ekto- und Mesoderm zusammengesetzt ist, dessen hintere zwei Drittel umfasst. Am Ende des fünften Tages [stage 29] sind beide Falten fast gleich gross und am Ende des sechsten Tages [stage 32] ist der ganze Embryo ausschliesslich vom Kopfamnion (Ekto- und Entoderm) umkleidet, während das Rumpfamnion sich hinter den Schwanz zurückgezogen hat. Das Kopfamnion spielt also hier die Rolle eines Dauerorgans, hingegen ist das Rumpfamnion das transitorische Gebilde geworden.

The closure of the amniopore is illustrated in figure 34. The germ layer constitution of the amnion can be seen in the reconstruction of stage 29 (fig. 39). The final proportions of the head-amnion and trunk-amnion referred to by Selenka are shown in stage 33 (fig. 52).

Abnormalities are fairly common among opossum embryos. I have one specimen of stage 29 which has a head fold back to the limb buds as in stage 28, figure 34, but no tail fold at all. Another specimen has a tail fold like that of a normal stage 27, but no head fold at all. That these specimens represent real abnormalities, not merely extremes of normal variation, is



abundantly clear from other anatomical features. I am fairly confident that they would never have been born, but would have been completely resorbed during the next day or so of gestation.

*Changes in the vascular system.* The atria of the heart tubes meet and fuse during stage 27 and the heart begins to beat. From this time on corpuscles may be found in all the blood vessels of the body. For the sake of clarity, however, they have been omitted from the section tracings.

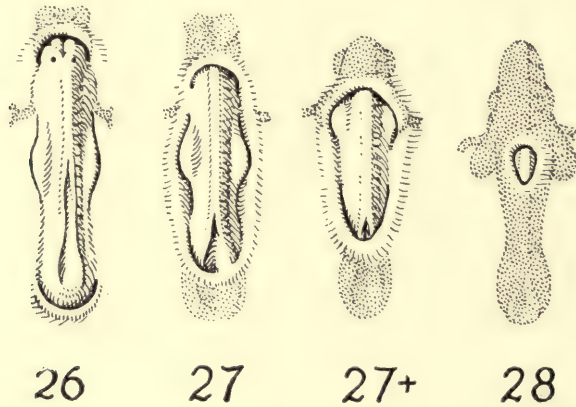


Fig. 34 Dorsal views of embryos showing closure of the amniopore.

In connection with the beginning of circulation of the blood it is interesting to note how far the vascular system has already developed. In early specimens of stage 24, as already described, there is on each side of the body a capillary plexus leading from the anterior end of the heart tube through the mandibular mesenchyme, then caudad to the end of the body and out into the area vasculosa. In later specimens of the same stage there has been a selection and enlargement of two main arterial vessels from this plexus, the dorsal aortae, and an elimination of most of the other original channels. By a similar process in stage 25 the mandibular capillary plexus is converted into two well-formed aortic arches. In stage 26 the capillary plexus in the hyoid mesenchyme shows a reduction in

the number of channels, and by stage 27 there are only the two well-organized second aortic arches at this level. The vitelline veins, the primary head vein, and the umbilical veins as far caudad as the anterior limb ridge, have also passed through similar capillary stages and by stage 27 have likewise become organized into single large channels. The point to be emphasized here is that all this has occurred before there has been any circulation of the blood.

The theory that all blood vessels are differentiated from primitive capillary plexuses seems first to have been proposed by Aeby in his 'Der Bau des menschlichen Körpers' (1868). In 1893 Thoma (*Untersuchungen über die Histogenese und Histomechanik des Gefäßsystems*) suggested that the enlargement of certain channels and the neglect and gradual elimination of others were due to the circulation of the blood—the hydrodynamic theory. When Evans ('09 b, from which the above two references are cited) established the soundness of the morphological facts, he did not concern himself with the question of causation. Thoma's theory of the mechanism is still the only one which has been proposed, and is generally accepted today. Arey, for instance ('30), says, "The selection of appropriate channels from the diffuse capillary bed results both from the action of heritable patterns and the hydrodynamic factors incident to the blood flow" and ". . . those capillaries from which the blood flow has been diverted, atrophy." I quote this passage because it should be emphasized that the mention of heritable patterns in no way extends or supplements Thoma's theory. Heredity, which determines all possible responses of the cells concerned, forms the basis of the process no matter what mechanical factors play a part in eliciting or provoking the special response which actually does occur. As heredity, then, in a general sense, is the basis of any and all possible explanations of the process, the only specific factor which has been suggested is the blood flow.

The experimental work of Oppel and Roux ('10) seems to have shown quite definitely that after the blood begins to

circulate, it can and does influence the size, course, and muscularity of vessels; but all of the development which occurs during the prefunctional period (Huxley's period of 'chemo-differentiation') remains totally unaccounted for. What I call attention to here is that, with one exception, every sort of change which occurs in the circulatory system at all occurs in the prefunctional as well as the functional stage. The one exception is the development of the muscular layer around the vessels. As this occurs only after function begins, there is no histological distinction between arteries and veins in stage 27.

The reconstruction of stage 27 (fig. 30) shows a number of new details in the vascular system which have not yet been mentioned. The capillary plexus of the third aortic arch is in process of forming. Capillary twigs from the vena capitis medialis can be seen extending laterally around the otocyst. The great venous plexus near the heart represents the proximal ends of the anterior and posterior cardinal veins, the duct of Cuvier, and the paracardinal plexus. The umbilical vein, which enters this plexus from behind and near its ventral end, has been cut off at its mouth to expose the lung bud.

More or less opposite the mouth of the umbilical a new vein can be seen coming from the ventral part of the head back across the dorsal wall of the pericardium to empty into this plexus from in front. This is the inferior jugular, which is not to be confused with the external jugular or the anterior jugular of mammals. This vein was first described by Grosser ('01) in the embryo of the bat, and later ('07) shown by him to be homologous with the inferior jugular of fishes, amphibians, and reptiles. He also found it in the cat, guinea pig, and human embryos. Lillie ('08) described it in the chick as the external jugular; and Lewis ('09) described it in the human embryo as the linguofacial. It is very well developed in the opossum and remains an independent vessel until stage 32, though meanwhile its mouth migrates up the duct of Cuvier to the internal jugular vein. At this time it also acquires connection with branches from the external jugular, and its flow is soon diverted into the latter channel



so that it loses its other connections and becomes a tributary of the external jugular.

The capillary plexus which originally connected the aortae with the sinus terminalis has in stage 27 become organized into two vitelline arteries which leave the aorta in the region of the midgut, run caudad beneath the extra-embryonic coelom and through the area vasculosa to the sinus. In advanced specimens the two have fused into one in their extra-embryonic portion.

*Miscellaneous details.* There is a recognizable hind-gut diverticulum or posterior intestinal portal in even the very early specimens of stage 27, but no allantois. Section H, figure 30, shows the posterior end of the body of a very early specimen in sagittal section. It can be seen that a deep and pointed proctodeum makes contact with an equally pointed hind-gut. The contact is, of course, the cloacal membrane.

Anterior to the hind-gut diverticulum another pocket in the endoderm can be seen. This represents the vestige of a notochordal canal. At this point there is no limiting membrane on the dorsal side of the endoderm or on the ventral side of the ectoderm, and endoderm, notochord, and Hensen's node are continuous. Up until stage 26 there had been a distinct primitive pit in Hensen's node, but in stage 27 this pit closes. Selenka mentioned not having seen a neurenteric canal, though he thought it likely that this was due to his not having the appropriate stage. No neurenteric canal is formed, but the endodermal pit which marks the site where it nearly formed remains distinctly visible through stage 28 (fig. 37).

In the earliest specimens of stage 27 (17141, 17142) the neural tube is closed anteriorly only through the myelencephalon—the metencephalon, mesencephalon, and prosencephalon being wide open (fig. 30, A). In specimen 17154 the mesencephalon is closed but the metencephalon and prosencephalon are still open (fig. 30, C). In the last six specimens the metencephalon has closed and only a small neuropore remains in the prosencephalon at the level of the optic stalks.

In stage 28 that also closes and its location at the time of closure is seen in the reconstruction (fig. 36). The posterior neuropore, however, is very wide in stage 27 (fig. 30, E), and does not close until stage 30.

The otic cups, though still open, are in late specimens somewhat constricted at the mouth.

The third gill pouch is now distinct from the posterior complex.

Comparative data reveal the same set of relations between the different organ systems as that described in connection with earlier stages. As the generalizations based upon the early stages apply equally well to the later ones, detailed comparisons with other species will be omitted until the discussion of the condition at birth (stage 35).

#### *The middle of the tenth day*

Stage 28. External distinctive features. The primary lumbar flexure. The mesonephric tubules. The liver diverticulum. The paracardinal plexus. The veins of the limb buds. The allantois. Miscellaneous details.

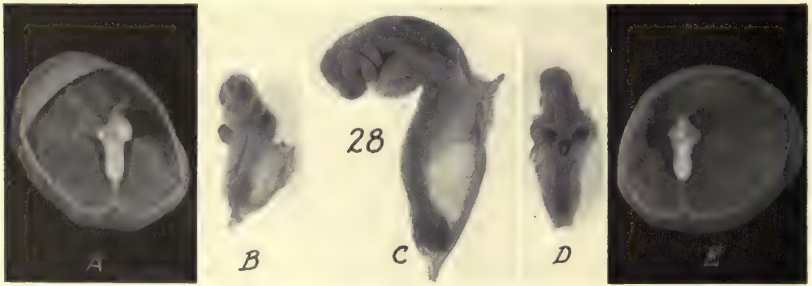


Fig. 35 Photographs of external views of stage 28. A, ventral view of 16149 after removal of non-vascular chorion. B, ventral view of 16154 after removal of all extra-embryonic membranes except anlage of the body stalk. C, lateral view of 16150. D, dorsal view to show amniopore of embryo shown in A. E, ventral view of 16150 before the removal of the area vasculosa.

*External distinctive features.* Stage 28 is distinguished externally from stage 27 by the closure of the otocysts and anterior neuropore (fig. 35, C and B), and by the fact that the

head fold of the amnion has covered the limb buds. It is distinguished from stage 29 by the presence of an amniopore (fig. 35, D), and the absence of a deep naso-oral groove (fig. 35, C).

Though no distinct naso-oral groove has formed in stage 28 the olfactory placode is well developed and noticeably depressed. The naso-lachrymal groove, which was quite conspicuous in stage 27, is becoming less so as the maxillary process fuses with the lateral part of the naso-frontal process.

The forelimb rudiment has grown out until its length is greater than the width of its base. It is now described as a limb bud instead of a limb ridge. En route to the area vasculosa the omphalomesenteric vein sweeps around the anterior or radial border of the limb bud.

Though not confined to stage 28, perhaps the most conspicuous external feature which has not heretofore been described is the primary lumbar flexure.

*The primary lumbar flexure.* When the embryonic body was relatively flat (early stages up to 26) its dorsal curvature conformed to that of the rest of the vesicle—that is, it was slightly convex. During stages 26 and 27 the anterior third of the body bent sharply in the ventral direction at two points (cephalic and cervical flexures) and thus thrust itself down into the yolk sac. During stage 27 the dorsal line from the tenth somite back is fairly straight, but there is often a slight concave flexure in the posterior third of the body. This is the beginning of the primary lumbar flexure.

During stage 28 this flexure becomes very marked. It extends from about the tenth to the twenty-sixth somite and is deepest at the level of the twentieth to the twenty-second. It occurs at the time of constriction of the amniopore. The amnion at this time may be compared to an open bag with a loop of string tied around the mouth, which loop is gradually being tightened so that the opening (the amniopore) is being closed and the bottom of the bag (which includes the flat thoracic and lumbar parts of the embryonic body) is being



folded into a deep concavity. When the amniopore is actually closed (during stage 29) the concave bending stops, and very shortly thereafter (stage 30) the flexure reverses itself. I mention this only as a simile, for it is difficult to imagine a force which might correspond to the closing of the string loop.

In the human embryo of the early part of the fifth week there is a similar concave curvature of the back. The amnion in that case has formed very much earlier and not by a folding process at all, which may be taken as evidence that there is no causal relation between the origin of the amnion and the formation of this flexure. To explain this case it has been suggested that the 'weight' of the yolk sac, which 'hangs' from this point, is responsible for pulling the back down. I don't know whether the yolk sac has any weight or not in the fluid in which it is immersed, also I don't know just what 'hangs' means when the site of implantation may be dorsal or ventral with equal frequency and is often lateral. But the final answer to the weight-of-the-yolk-sac theory comes from the opossum in which the yolk sac is the lining of the entire embryonic vesicle, and the embryo actually descends into and becomes completely surrounded by its own yolk sac. Nonetheless, the opossum embryo develops a good primary lumbar flexure, which obviously in this case cannot be caused by any weight of the yolk sac.

More likely than a connection with either the process of formation of the amnion or the weight of the yolk sac, is the possibility that, like the other flexures, this one is due to local differences in growth rates. If so, at the time of its formation growth in the thoracic and lumbar regions should be more rapid on the ventral than on the dorsal side of the body. There is good reason to think this is so, for the rapidly developing mesonephros is not only at exactly the right place, but in both man and the opossum it is at exactly this time developing tubules which are lengthening rapidly and being twisted into S-shapes for lack of space. This is also true of the 55-hour chick, which corresponds to a stage 28 opossum.

and which also has a well-developed primary lumbar flexure, though here the picture is somewhat complicated by the fact that the anterior part of the body, being unable to dip into the dense yolk, is forced to twist to one side. The rabbit and the lizard, which do not show the flexure, also do not have any mesonephric tubules developing at this time. In these cases the tubules form later on when there are other forces at work which compel the mesonephros to expand by projecting into the coelom instead of by flexing the back. The 'other forces' referred to will be discussed in connection with stage 30.

*The mesonephric tubules.* In the description of the nephrogenic ridge during late stage 24 the earliest anlagen of the mesonephric tubules were mentioned (p. 67 and fig. 22). The anlagen at that time were differentiating within a continuous longitudinal ridge. They consisted simply of a series of small, separate lumina along the center of the thickest part of the ridge. The cells around the lumina were radially disposed, but between adjacent lumina the cells showed no recognizable organization.

In stage 28 two important developments take place. First, the ridge pinches off from the coelomic epithelium altogether, thus becoming a nephrogenic cord. And second, the mesonephric tubule anlagen begin to separate from each other, increase rapidly in length, and become bent into double curves to accommodate themselves to the inadequate, available space. Though the adjacent tubule anlagen separate from one another (probably by the organization of the intermediate cells about the separate lumina), they do not separate from the most dorsal part of the original ridge. This once dorsal edge has now become lateral as a result of the rounding up of the body. Though it itself remains unsegmented and for the most part at stage 28 still uncanalized, it never loses continuity with the walls of the tubule anlagen which differentiate out of the same longitudinal anlage. It is, of course, the wolfian duct, which is thus continuous with all of the pronephric and mesonephric tubules from the very beginning.

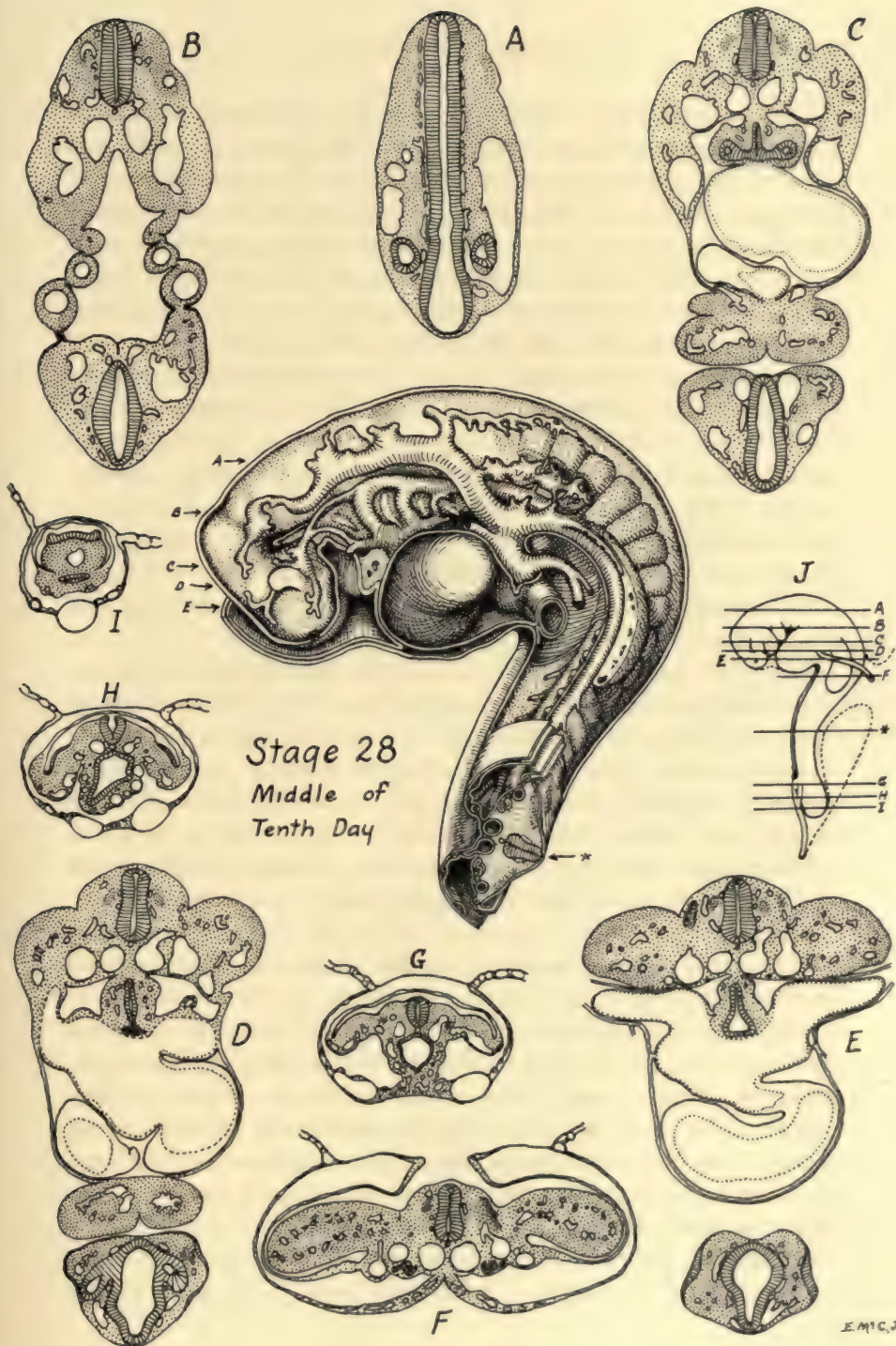
The changes just described occur in cephalocaudal progression, and are correspondingly most advanced in the anterior mesonephric segments. In stage 28 the tubule anlagen are S-shaped from the tenth somite (fig. 36, section F) back to about the eighteenth. From the eighteenth to approximately the twenty-fourth the tubules are separate from each other but are not long enough to be very much twisted, and the most posterior of them are more sac-like than tubular. Caudad from this point they merge into a continuous nephrogenic cord much like that described for the region of the eleventh somite in stage 24. It was a section through a tubule anlage in this cord that Selenka (pl. XXII, fig. 13) mistook for the wolffian duct. And finally, the cord merges into the undifferentiated mesoderm beyond the last somites. At the level of the notochordal canal (W.I.C. 16149, sections 913 to 918) this undifferentiated mesoderm merges with the endoderm which has no basement membrane.

The pronephros at this time is about as well developed as it will ever be. It consists of five or six very slender, solid 'tubules' which are connected with both the coelomic epithelium and the wolffian duct. They are situated between the seventh and tenth somites (fig. 36, section E), and occur at both somitic and intersomitic levels. In specimen 16149 on the left side there are about thirty-six nephric tubules in all, of which the first six are pronephric, and the last thirty mesonephric. This count includes the most posterior anlagen which are not separate from one another except in their lumina.

*The liver diverticulum.* A diverticulum from the foregut in the midventral line at the level at which the omphalomesenteric veins empty into the sinus venosus, is the first anlage of the liver. It is shown in cross section in figure 36, section D. During stage 28 it is a simple diverticulum without any liver cords branching from it. It is found also in a few of the late specimens of stage 27, that is, specimens in which the otocysts and anterior neuropore are still open.

Fig. 36 Lateral reconstruction and cross sections of stage 28. The reconstruction is from 16150, the sections are from 16149.





Stage 28  
Middle of  
Tenth Day

E.M.C.V.

*The paracardinal plexus.* Part of the originally extensive venous plexus from which the duct of Cuvier and the proximal ends of the pre- and post-cardinals differentiate persists in the angle between the cardinals until stage 32 when the jugular lymph sac begins to form in this region. This persistent portion is called the paracardinal plexus, and its fate will be discussed in detail in connection with stages 32 and 33. It is seen in the reconstruction (fig. 36) to have its principal connection with the precardinal, not very far from the cardinal anastomosis. It lies immediately beneath the dermo-myotomal plates, is constricted at the intersomitic levels, and communicates by small twigs (not shown in the figure) with the segmental veins. The first somite has disappeared, and only a trace of the second is left, but the sites of both may be identified by the segmental vessels and by the bulges in the paracardinal plexus, which is quite sinusoidal in the somitic regions at this time.

*The veins of the limb buds.* It was mentioned in connection with the origin of the umbilical vein in stage 24 that its first association is with the anterior limb bud, on the ventral or radial side. Also it was pointed out that the postcardinal in stage 26 established communication with the dorsal border of the limb ridge. Thus when the blood begins to circulate in stage 27 the rich venous plexus which ramifies through the limb drains dorsally into the postcardinal and ventrally into the umbilical.

At first the plexus seems altogether irregular, but by stage 28 it is beginning to show a more orderly arrangement of the vessels. The veins form a sort of subperipheral layer around the limb bud (fig. 36, D and E), the dorsal part of this layer draining by many small mouths into the postcardinal, the ventral part in the same way into the umbilical. The arterial supply from the dorsal aorta enters the medial part of the limb and communicates through capillaries with all parts of the subperipheral plexus.

Caudad of the anterior limb bud both the umbilical and the postcardinal veins extend all the way back to the end of the



body. Here on either side of the rhomboidal sinus they unite in a very rich plexus through the region in which the posterior limb ridge is forming (sections G and H).

*The allantois.* Section H also shows a ventral diverticulum from the hind-gut, which is the allantois. Its vascularization is very rich, as the figure shows, and is derived from the following sources. The arterial supply is from the dorsal aortae. In the region of the mid-gut the aortae give off innumerable mesenteric arterioles (shown in the reconstruction). Just before reaching the hind-gut two exceptionally

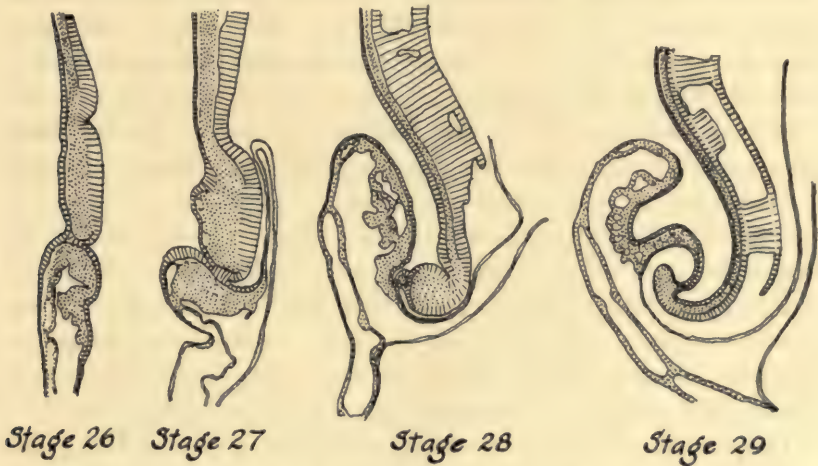


Fig. 37 Sagittal sections of posterior end of embryos from stage 26 through stage 29 to show cloacal membrane, caudal amniotic fold, extra-embryonic coelom, hindgut, allantois, and the absence of any neurenteric canal. The section for stage 26 is from 16146; for stage 27, 17141; for stage 28, 16150; and that for stage 29 is a sagittal reconstruction from the cross sections of 16154.

large branches, the vitelline arteries, descend into the walls of the yolk sac, run caudad beneath the body (G and H), and finally unite into one (I) which courses through the area vasculosa to the sinus terminalis (see also fig. 35, A and E). Posterior to these, more arterioles are given off, and those beyond the posterior intestinal portal run into the walls of the hindgut and thence into the allantois (H). The posterior wall of the allantois (prospective ventral wall) is not surrounded



by coelom, but is continuous with the ventral body wall, which is just in process of forming anterior to the cloacal membrane (fig. 37). At this point of continuity with the body wall the vessels of the allantois meet and empty into the umbilico-postcardinal plexus already described in connection with the posterior limb ridge. The developing allantois thus has a venous connection with the umbilical veins at its very inception, but not with the vitelline or omphalomesenteric veins. These latter vessels are at first almost exclusively vitelline, and what few, small, mesenteric branches there are, are too short to cross the wide mid-gut and so do not reach the allantoic region. The subintestinal vein, which forms later and acquires a connection with the allantois, will be described in connection with stage 32.

*Miscellaneous details.* The anterior neuropore completely closes during stage 28. The reconstruction in figure 36 shows the last vestige of it at the extreme anterior end of the prosencephalon. The posterior neuropore, however, is still in the form of a flat rhomboidal sinus (fig. 36, I). The first traces of a marginal layer outside the mantle layer in the central nervous system, indicate that axones and dendrites are beginning to grow out from the nerve cells. However, the cranial and spinal sensory ganglia are still unattached.

The vascular system shows many new features. The capillaries which surrounded the otocyst in stage 27 have now shifted the principal flow in the primary head vein to the lateral side of the otocyst (fig. 36, A). Posterior to the otocyst and anterior to the paracardinal plexus the head vein receives some rather large tributaries from the surface of the central nervous system. These are the posterior cerebral veins which play an important part in the formation of the venous sinuses of the dura mater. A similar set just anterior to the otocyst constitutes the middle cerebral group, and a third set from the mid-brain and forebrain is the anterior cerebral group. These three groups become more sharply demarcated from each other in later stages. The stub of a

small branch of the anterior cerebral shown in the reconstruction (fig. 36) running ventrally at the isthmus, is the transverse hypophyseal vein, which crosses the midline and unites the veins of the two sides.

The first aortic arch has become much more slender, but is still a single channel, not a plexus. The second aortic arch is now the largest; the third is quite large; the fourth is complete, but small; and the fifth, which in most mammals forms late or not at all, is in process of forming on time (fig. 36).

The first three gill pouches are well formed and in contact with ectoderm (fig. 36, B). The fourth and fifth are still fused (fig. 33).

The otocyst is detached from the ectoderm, and the ectoderm has thickened into a lens placode at the point of contact with the optic vesicle (fig. 36, D).

There is a slight thyroid depression in the middle of the floor of the pharynx between the second branchial pouches (fig. 33).

#### *The last third of the tenth day*

Stage 29. External distinctive features. The extra-embryonic membranes. The pharyngeal bursa. The dorsal pancreatic diverticulum. The thyroid anlage. Miscellaneous details. Comparative notes.

*External distinctive features.* Stage 29 is distinguished from stage 28 by the naso-oral groove, the tail bud, the club-like enlargement of the end of the anterior limb, the prominent posterior limb ridge, the closed or nearly closed amio-pore with some extra-embryonic coelom anterior to its level, and the fact that the omphalomesenteric veins pass behind instead of in front of the anterior limbs. It is distinguished from stage 30 by the absence of the secondary lumbar flexure, and by the following negative facts: the anterior limb club has not flattened into a paddle; the posterior limb ridge has not become pointed enough to be called a bud; and the allantois has not enlarged enough to be noticeable externally.

*The extra-embryonic membranes.* Though there will be changes in the proportions of the parts in later stages, there will be no changes in the constitution of the amnion, chorion, yolk sac, and body stalk after the closure of the amniopore in stage 29.

In the description of stage 26 (chapter IX) the history of the constitution of the blastocyst wall from the earliest stages up to the beginning of amnion formation was summarized. It was pointed out that the ventral two-thirds of the vesicle is never invaded by mesoderm, and so represents a persistent

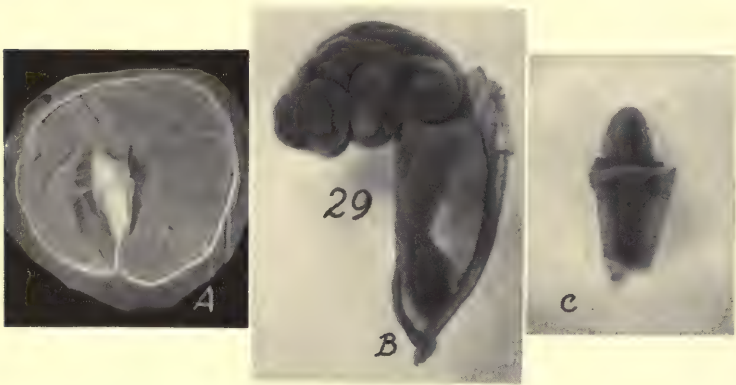


Fig. 38 Photographs of external views of stage 29. A, ventral view of 16153 after removal of non-vascular chorion. B, lateral view of 16153 with vascular chorion and head amnion also removed, but with trunk amnion intact. C, dorsal view of 16154 to show closing of amniopore.

portion of the original bilaminar vesicle—yolk sac endoderm covered by ectoderm. We shall call this the non-vascular yolk sac chorion (fig. 40, nv.ch.). In the dorsal third of the vesicle the mesoderm spreads between the ectoderm and the yolk sac endoderm, and provides a vascular supply for part of the yolk sac. This part is the area vasculosa or vascular yolk sac chorion (v.ch.), and the embryo proper is located approximately in the center of it, though slightly nearer its caudal edge. Finally, in a very restricted region within the embryonic body and immediately around its borders, the



mesoderm splits to form somatic and splanchnic layers with all the blood vessels outside the body in the lower of the two—the splanchnic layer (fig. 39, reconstruction). This leaves the surface of the vesicle non-vascular in the immediate vicinity of the embryo. I have already described how the mesoderm becomes resorbed in the proamniotic region, and how the head elongates, reaches over the coelomic loop and dips ventrally into the proamnion, thus forming the head fold of the amnion of only ectoderm and endoderm. When the caudal and lateral folds arise (stage 27) they are formed from the roof of the extra-embryonic coelom. Accordingly when they meet above the embryo and fuse, they form a non-vascular somatopleuric spot in the midst of the area vasculosa. This sheet of somatopleure underlain by extra-embryonic coelom, though of small area, is a permanent part of the outside wall of the vesicle and is of different constitution from the other two parts already described. This is the serosal chorion (fig. 40, s.ch., and fig. 39, reconstruction).

There are thus three parts of the definitive chorion in the opossum: 1) the non-vascular yolk sac chorion—ectoderm and endoderm; 2) the vascular yolk sac chorion—ectoderm, mesoderm, and endoderm; 3) the serosal chorion—ectoderm and somatic mesoderm. This last is the spot at which an allantoic placenta (such as is characteristic of all Eutheria) could, theoretically, form, if the allantois, which projects into the extra-embryonic coelom, should grow out caudally far enough to reach and fuse with the outside wall of the vesicle. But this does not occur in the opossum (see stage 33).

The principal peculiarities which give rise to this type of chorion are 1) the mesoderm does not spread more than one-third of the way around the vesicle; 2) the process of splitting into somatic and splanchnic layers with a coelom between them occurs in an exceedingly restricted region around the embryo proper; 3) as a consequence of the second, the yolk sac remains a permanent constituent of the outside wall of the vesicle throughout most of its area; and 4) the

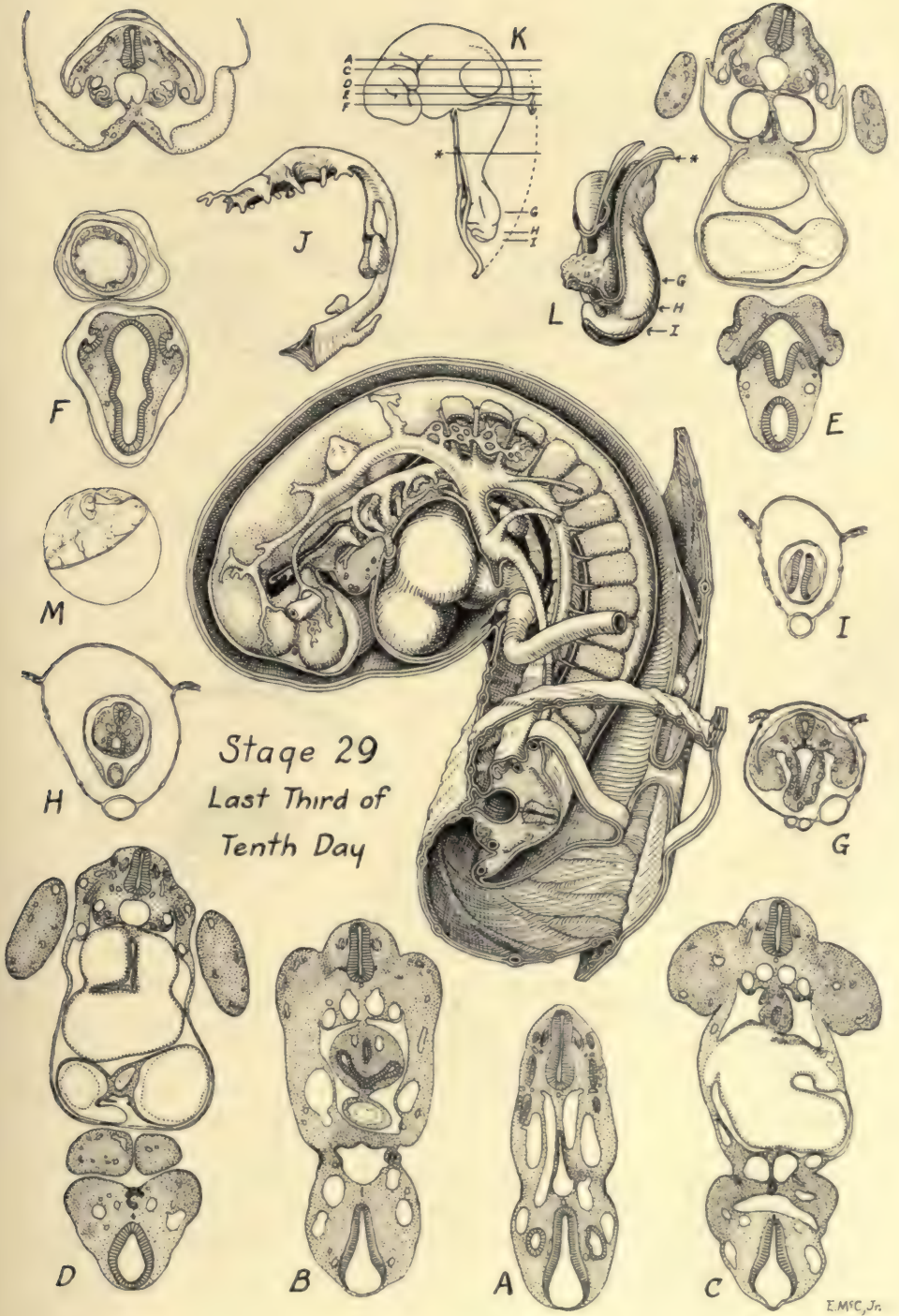
allantois, instead of extending out through the extra-embryonic coelom to fuse with the serosal chorion, remains in a pocket of the yolk sac.

Though this situation is remarkably different from that found in most placental mammals, there is at least one of the latter which is sufficiently like the opossum to be a useful subject for comparison. The extra-embryonic membranes of the 10-day rabbit (fig. 40, C) differ from those of the 10-day opossum (A) in only two important points: 1) the allantois is much further developed, and is fused with the serosal chorion; and 2) the extra-embryonic coelom extends all the way to the sinus terminalis, so that the yolk sac circulation is completely separated from the surface of the vesicle. With these exceptions the opossum and rabbit are strikingly similar—especially so in having the ventral two-thirds of the vesicle composed only of yolk sac endoderm and ectoderm, and the anterior third of the amnion composed only of proamnion.

In later development the two species diverge considerably. In the rabbit (D) the mesoderm invades the proamnion and splits, so that the yolk sac comes to be completely separated from the amnion by extra-embryonic coelom. At the same time the allantois extends its fusion with the chorion throughout the serosal region, so that the allantoic vessels very literally replace the vitelline vessels. In direct contrast to this, in the opossum it is the yolk sac which enlarges and displaces the extra-embryonic coelom, so that almost the entire amnion comes to be composed of proamnion, and the vitelline vessels remain permanently in the chorion. In this situation the allantois comes to be surrounded by the yolk sac, is prevented from reaching the chorion, and serves only as an urinary reservoir (see stage 33).

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Fig. 39 Reconstructions and sections of stage 29. The central reconstruction taken from 17162 shows, in addition to the embryo proper, the constitution of the amnion and area vasculosa. The sections are from 16154. The reconstruction L shows, on a slightly smaller scale, the portion of the body cut off in the central reconstruction. J shows the pharynx, lung buds, liver diverticulum, and dorsal pancreatic diverticulum. M shows the actual size of the vesicle.



Stage 29  
Last Third of  
Tenth Day

E.M.C., Jr.



The description thus far has been written with primary reference to the chorion, but it has necessarily included a sort of implicit description of the amnion. It has already been

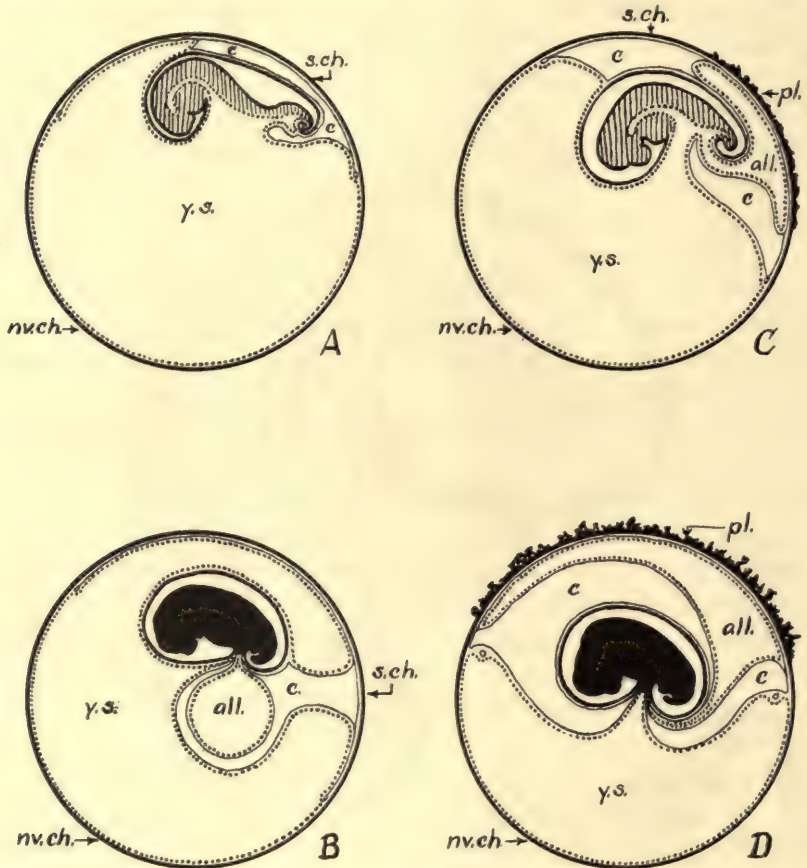


Fig. 40 Diagrammatic sections of vesicles to show the relations of extra-embryonic membranes in the opossum and the rabbit. The heavy line represents ectoderm; the light line mesoderm; the dotted line endoderm. A, opossum stage 29. B, opossum stage 33. C, rabbit at 10 days. D, rabbit at 13 days.

indicated, for instance, that the head fold is composed entirely of proamnion, and that the tail fold and lateral folds are formed of the somatopleuric, non-vascular roof of the extra-embryonic coelom.

Before the four folds meet, mesoderm invades the proamnion for a short distance ahead of the amniopore, so that the latter comes to be surrounded by extra-embryonic coelom. When the amniopore then closes (at the site indicated in the reconstruction, fig. 39) the partition or stalk which tends to form is quickly resorbed and amnion and chorion become separated from each other by extra-embryonic coelom throughout the region beneath the serosal chorion. However, as the mesoderm has invaded the proamnion for only a short distance anterior to the amniopore, its anterior extremity is marked by a two-layered membrane still connecting the amnion with the chorion. This membrane is composed of somatic mesoderm and yolk sac endoderm. If it were to break down, coelom and yolk sac would open directly into each other; but this never occurs. If traced laterally along this membrane, the somatic mesoderm will be found to meet the splanchnic mesoderm along a line marked conspicuously by the large omphalomesenteric vein coming in from the area vasculosa. As this vein always marks their line of junction it will be an interesting landmark by which to follow the later changes in proportion between the yolk sac and the extra-embryonic coelom and the corresponding parts of the amnion. Tracing the splanchnopleure, which forms the lateral wall of the extra-embryonic coelom, caudally from the omphalomesenteric vein, we find that it meets its fellow of the opposite side in the midline behind the tail bud; and that their line of junction is marked by the large vitelline artery which follows it out to the area vasculosa (fig. 39, H, I, K). There are thus three large vessels running between the embryonic body and the chorion and marking the corners of a roughly prismatic extra-embryonic coelom. The membranes which connect these three vessels to each other also connect the embryonic body to the chorion, and form a sort of prismatic, hollow, body stalk. These membranes are two-layered. The outside layer on all sides is yolk sac endoderm. The inside of the anterior face (i.e., the one between the two veins) is somatic mesoderm. The inside of the other two faces is

splanchnic mesoderm. The end of the hollow stalk is capped by a sheet of somatopleure—the serosal chorion. In stage 29 the two splanchnopleuric faces carry many small vitelline vessels (mostly arterioles from the dorsal aortae), but in later stages only the three large vessels survive.

Though I have written this description of the foetal membranes from my own observations, most of the facts had been worked out before, and accordingly a few words about the literature are necessary at this point.

Osborn's three papers on the foetal membranes of the opossum (1883 a, 1883 b, 1887) are so full of errors that it seems impossible to understand what sort of observations they could have been based upon. Even the third paper, which purported to correct the errors recognized in the first two, introduced an almost equally rich crop of new ones. It would be confusing and unnecessary to dwell on all these mistakes, but one of them which persisted through all three of his publications seems to have been borrowed from Chapman's paper on the foetal kangaroo (1881). This author, who examined grossly a single 14-day *Macropus* foetus, incorrectly described the sinus terminalis as marking the boundary between the chorion and the umbilical vesicle, not realizing that the umbilical vesicle or yolk sac forms the lining of the entire vesicle with the exception of the very small serosal spot mentioned above. While this error is not incomprehensible when based only upon gross observation, it is difficult to understand how Osborn could have failed to detect it in his microscopical slides. At any rate it was not corrected until the second half of Selenka's paper was published. Selenka's account of the development, anatomy, and significance of all the foetal membranes is remarkably accurate and complete.

In fact, I have come across only one error, and that a minor one, in his lengthy account. He described the membrane which forms the anterior wall of the body stalk between the two omphalomesenteric veins as including a double layer of ectoderm between its endodermal and mesodermal constituents (*loc. cit.*, p. 133). This double layer of ectoderm does



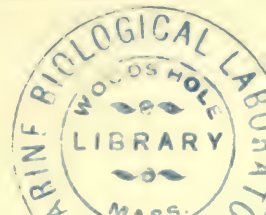
not exist. The membrane referred to is composed of endoderm and somatic mesoderm, as I have already mentioned. It is illustrated in the reconstruction in figure 39. Selenka doubtless inferred the presence of the ectodermal sheets from the composition of the edges of the amniopore in stage 28, but he forgot momentarily that during stage 29 the mesoderm and its coelom push around the cranial edge of the amniopore, and separate the ectoderm at the edge of the head fold from the endoderm. The nearly closed amniopore in my reconstruction (fig. 39) shows the two layers of ectoderm which he had in mind, but they are some distance caudal to the lamella, and the latter is composed only of endoderm and mesoderm.

With this trifling exception Selenka's account of all these membranes is extraordinarily good, and it was partly with a view to giving him credit for this achievement that I chose one of his beautiful figures for a frontispiece.

*The pharyngeal bursa.* The reconstruction of the pharynx and foregut in figure 39 J, shows a curiously gland-like extension of the extreme anterior tip. This structure is variable in shape, number of branches, and extent of lumen. It occurs only in stage 29, which represents roughly some 8 hours of development, is always connected with the anterior end of the notochord, and has no known function. Its principal interest lies in the fact that it appears so suddenly, attains such conspicuous size, and disappears so quickly.

Selenka described it as 'die Gaumendrüse' and then again as 'die Gaumentasche.' He noticed its relation to the end of the notochord, decided that it represents the anterior notochordal canal, and that it is really not glandular in spite of appearances.

Before Selenka the following authors had described the pharyngeal bursa in man: Mayer (1840), Lushka (1860), Dursy (1869), Kölliker (1879), Froriep (1882). Of these, only the last recognized the causative part played by the notochord in this connection. Others had supposed the bursa to represent either Seesel's pouch or Rathke's pouch. As



Seesel's pouch is just another name for the anterior end of the foregut, and as the pharyngeal bursa in the opossum is located at this point, the two may be said in a sense to coincide; but that this is nothing more than a coincidence, seems clear from the fact that in man the obviously homologous bursa lies some distance caudad of Seesel's pouch. Of course, Rathke's pouch is on the opposite side of the pharyngeal membrane and has no connection with either of them.

In the same year that Selenka wrote his description, Schwaback (1887) erroneously interpreted the bursa as "a crypt connected with the formation of the pharyngeal tonsil." Selenka was correct in attributing a causal role to the notochord, but was probably mistaken in supposing that the duct of the bursa represents the notochordal canal. A recent study of this structure in man was made by G. C. Huber ('12), to whose paper I am indebted for the references to the other work on man.

The pharyngeal bursa in the opossum differs from that in man in being formed as a result of a terminal instead of a sub-terminal contact of the notochord with the pharyngeal epithelium; but I do not doubt that it has the same morphological significance.

*The dorsal pancreatic diverticulum.* The dorsal anlage of the pancreas arises as a long, finger-like diverticulum from the middle of the roof of what turns out to be the duodenal portion of the foregut, though it would really be a prolepsis to speak of a duodenal portion at this time as there is no visible indication of the stomach as yet. The pancreatic diverticulum is slightly caudal to that of the liver as well as being dorsal instead of ventral. It is shown in figure 39, J. In the general reconstruction it is partly hidden behind the omphalomesenteric and umbilical veins.

It is interesting that in the opossum the pancreatic anlage is present before there is any recognizable stomach rudiment. In man and in the pig it does not appear until the gastric enlargement is quite distinct. This difference may be correlated with the fact that in the opossum the liver and pancreas are both needed for the digestion and utilization of milk

at a very early time, whereas the stomach is not needed except as a reservoir and has no glands at birth.

*The thyroid anlage.* The thyroid in stage 29 is represented by a median ventral pocket in the floor of the pharynx between the first and second branchial pouches, but more nearly in line with the second pair (fig. 33). This location is just cranial to the truncus arteriosus. There has been a slight depression at this point since late stage 27, but it becomes quite distinct in stage 29, and in late specimens is somewhat constricted at the neck.

*Miscellaneous details.* The pharyngeal membrane breaks down and is resorbed during stage 29. The trachea separates from the oesophagus by the fusion and resorption of the middle portion of the lateral walls from behind forward (fig. 33).

The cranial ganglia send processes into the central nervous system. The posterior neuropore is still open on the tail bud (fig. 39, I and L). The otocyst acquires an endolymphatic duct (fig. 63, e.d.) which grows out from the dorsomedial tip of a vertical ridge on the medial wall. This point of origin is medial to the seam of closure, which is still recognizable at s.c. The first suggestion of demarcation of the seventh (geniculate) ganglion from the acoustic is recognizable in their most ventral parts, but the separation is imperfect and irregular. Dendrites from the eighth ganglion reach the otocyst. The lens placode is depressed and is in close contact with the primary optic vesicle.

The first aortic arch breaks down, leaving that portion of the ventral aorta which connected it with the second arch as the external carotid artery supplying the mandibular arch. The fifth aortic arch is still incomplete. Posterior to the level of the fifth arch a plexus from the ventral aorta extends caudad along the side of the trachea. The vitelline veins approach each other very closely in the midline behind the pancreatic diverticulum on the dorsal side of the foregut. Here they establish capillary communication with each other. In late specimens a similar situation has developed ventrad of the gut and caudad of the liver bud.



*Comparative notes.* With the exception of the precocious organs already listed (forelimb, lungs, etc.) the stage 29 opossum resembles very closely a 10-day rabbit (Minot's no. 562), a 3-day chick (Patten, fig. 43), a 1.4-mm. lizard (Peter's no. 74). The pancreas and allantois were not discussed in chapter IX, and need special comment.

In comparison with the corresponding organs in placental mammals, the pancreas in the opossum is precocious and the allantois is retarded. In both of these respects the opossum is more like the lizard and the chick. But in the case of the allantois the opossum is the least advanced of all the animals studied.

The placental mammal develops his allantois early and constructs from it a mechanism by which he can take advantage of his mother's pancreas, and lungs. The lizard and the chick use the allantois for respiratory purposes, but need the pancreas to enable them to utilize some of the more complicated constituents of the yolk. It is not likely that the opossum uses his pancreas in the utilization of uterine milk, but he needs it at an early stage for another reason. Even if the allantois should grow out and fuse with the serosa, it would not find any richer source of oxygen than that already found by the well-developed yolk sac circulation. The yolk sac thus serves as the source of both food and oxygen; and the allantois, which becomes completely surrounded by it, serves only as a urinary reservoir.

Apparently the need of the opossum foetus which is least well served by this arrangement is that of oxygen. And it may be that the three most precocious features of opossum development—the development of the lungs, the development of the forelimbs, and the act of parturition—are all primarily adapted to fill that need. The act of parturition carries the foetus to a rich source of oxygen, which the precocious lungs can take advantage of. But this act also necessitates the finding of a new source of food. The precocious forelimbs, as will be seen later, carry him to the new source of food. The well-developed pancreas then makes the utilization of this new food possible.

## XI. THE ELEVENTH DAY. Stages 30 and 31

*The first half of the eleventh day*

Stage 30. External distinctive features. The secondary lumbar flexure. Bronchiolar buds. The pulmonary arteries. The liver cords. The septum transversum and the pleuro-pericardial membrane. Advances in the nervous system. Miscellaneous details.

*External distinctive features.* Stage 30 is distinguished from stage 29 by the secondary (convex) lumbar flexure, the

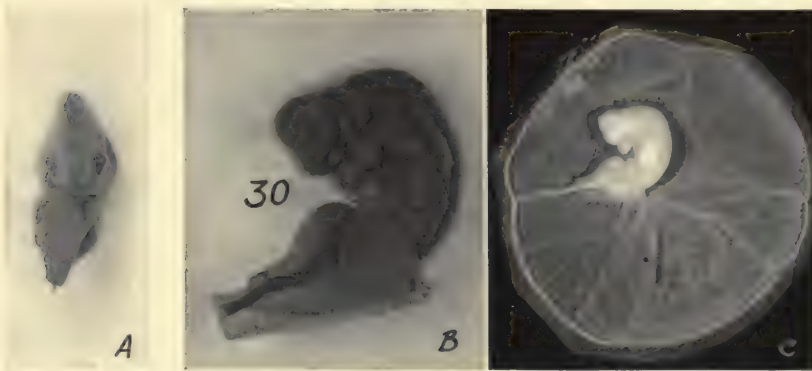


Fig. 41 Photographs of external views of stage 30. A, ventral view of 16158 after removal of all extra-embryonic membranes except the allantois and the portion of the yolk sac covering it. B, lateral view of 16160 with amnion still in place. C, lateral view of 16160 with only the non-vascular chorion removed.

flattening of the forelimb club into a paddle, and the protrusion of the allantois well beyond the body (fig. 42, I). Also for the first time there is a well-formed tail, and in most specimens the posterior neuropore has closed.

It is distinguished from stage 31 by the presence of the naso-oral groove and the absence of prominent digital ridges on the anterior limb paddle.

*The secondary lumbar flexure.* If the photographs of type specimens of stages 29 and 30 reproduced in the third normal stage plate be compared with each other, it will be seen that by far the most conspicuous difference is that caused by a

convex flexure in the lumbar region in stage 30. The heads, gill arches, and hearts of the two specimens are strikingly similar. The forelimbs differ principally in the degree of flattening. And yet the stages could never be confused. For in stage 30 the caudal end of the body has been flexed sharply in the ventral direction until it is brought up under the heart. The coiling is so extreme that the greatest linear dimension of many specimens at this time is as little as 4.0 mm. This length is less than that of any specimen since stage 23.

Linear dimension is, of course, in most animals a notably inaccurate clue to developmental stage, but this is perhaps in an exceptional degree true of the opossum. A 6-mm. opossum embryo may belong to the end of the ninth day (stage 26), the end of the tenth day (stage 29), or the end of the eleventh day (stage 31). And a 4-mm. opossum may be just acquiring its first somites (stages 22 and 23), or it may have the principal lobes of the lungs indicated (stage 30). For this reason, though average measurements are occasionally mentioned, they are given very little emphasis in this paper.

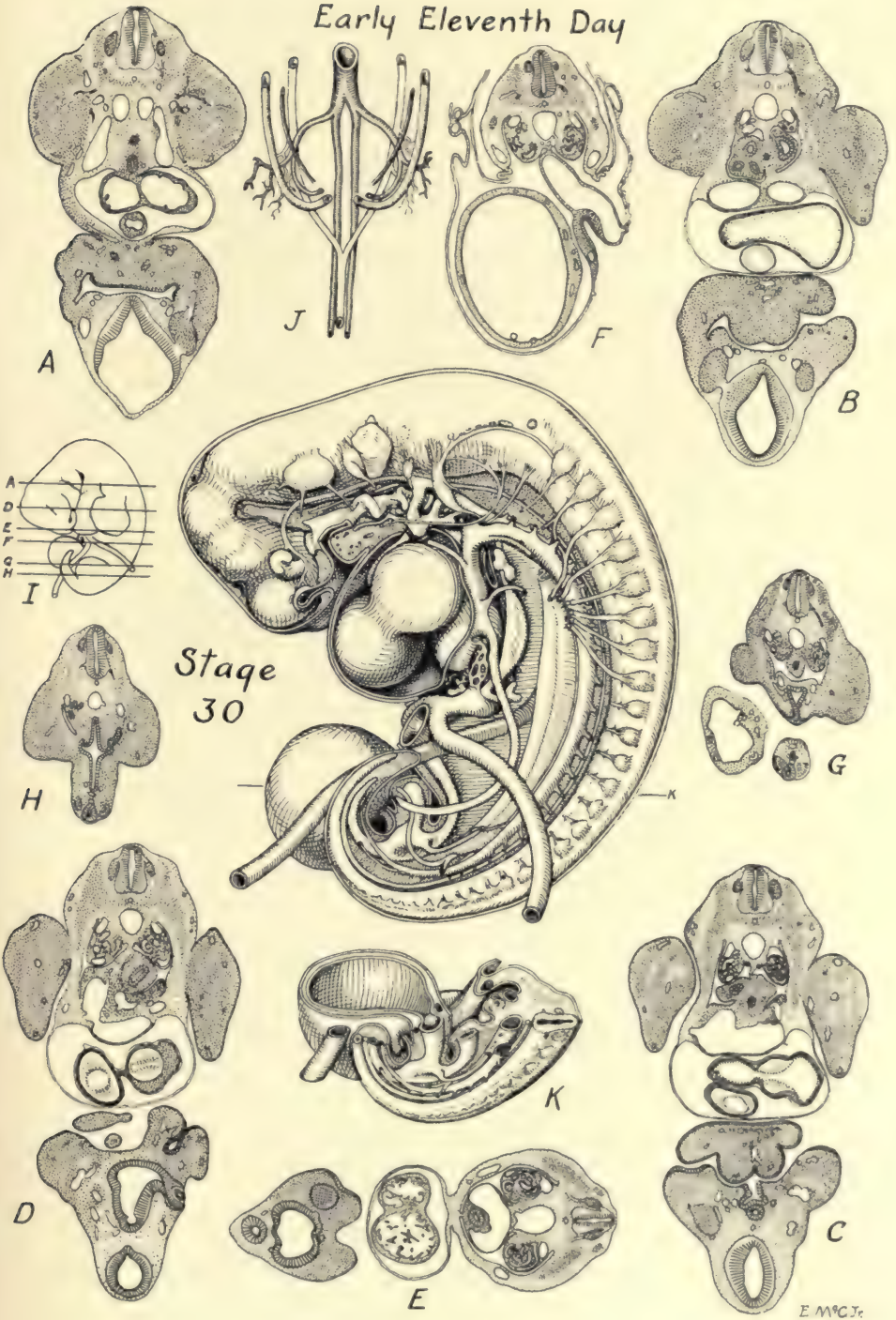
If it is true that the primary (concave) lumbar flexure was due to the rapid development of the mesonephros, which made the ventral portion of the trunk elongate more rapidly than the dorsal portion, then it is probably true that the secondary (convex) lumbar flexure is due to a spurt of growth in the lumbar spinal cord which makes the dorsal portion of the trunk elongate more rapidly than the ventral portion. This suggestion has not been verified by precise quantitative studies, but it is in harmony with the fact that the posterior limb bud first becomes conspicuous at this time, and there is a corresponding and easily visible development of the region of the spinal cord concerned with the prospective lumbosacral plexus. In stage 29 there is a very early neural crest formation in the lumbosacral region and no sign of motor

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Fig. 42 Reconstructions and sections of stage 30. The central reconstruction is from 17132. The sections and J and K are from 16158. I represents only the levels of the sections, not the size of the specimen.



Early Eleventh Day



Stage 30

ganglion cells or of differentiation of the three layers within the spinal cord. In stage 30 all of the sensory ganglia for the limb segments become organized out of the neural crest and develop connections both with the cord and with the limb bud; and motor ganglion cells within the cord send out axones to the limb bud. After noticing the striking similarity between the cranial, cervical, and thoracic parts of the body in stages 29 and 30, to which attention has already been called, one has only to compare section G of figure 39 with section H in figure 42 to see at once that the principal developments in stage 30 are in the posterior end of the body and especially in the spinal cord.

*Bronchiolar buds.* In chapter X the development of the lungs was followed from the earliest, dorsolateral, paired anlagen to the stage where they become ventral buds connected to the pharynx by means of a single, ventral tube—the trachea. In stage 30 the process of bronchiolar budding begins. This seems best described as monopodial. The original bud on each side elongates to form a stem bronchus, and as it grows caudad secondary bronchial buds arise as diverticula from its side. In stage 30 one such secondary bud has arisen on the left and two on the right. This stage corresponds to that of a 10-mm. pig or a 7-mm. human embryo as far as the lungs are concerned; but as the lungs in the opossum are very precocious this comparison does not apply to the embryo as a whole. For the less specialized organs the stage 30 opossum is better compared with a 5-mm. pig or a 10½-day (5 mm.) rabbit.

This budding goes on principally in the endodermal portion of the lung. The mesodermal blastema seems to receive quite passively the general shape of the endodermal tree as the latter expands within it; and the lobes are not well indicated on the external surface of the lungs until a later stage.

*The pulmonary arteries.* The origin of these arteries in the opossum has not heretofore been described. Bremer's remarks about them in his 1909 paper do not refer to the early stages. Of more interest is his description ('12) of the

aortic arches in the rabbit embryos, in which he points out that the pulmonary arteries are extensions of the ventral aortae—not outgrowths of the sixth aortic arch. In fact, they grow past the level of the sixth aortic arch before that arch is formed. This is quite definitely the case in the opossum; Federow (see Bremer, '12 b) has found it in the guinea pig; Buell ('22) found it in the chick; and Congdon ('22) found it in man. What is, therefore, usually interpreted as the ventral half of the sixth arch is really part of the ventral aorta; and the arch, which is entirely dorsal to the point of origin of the pulmonary artery, is also entirely lost in later development, no part of it ever being incorporated

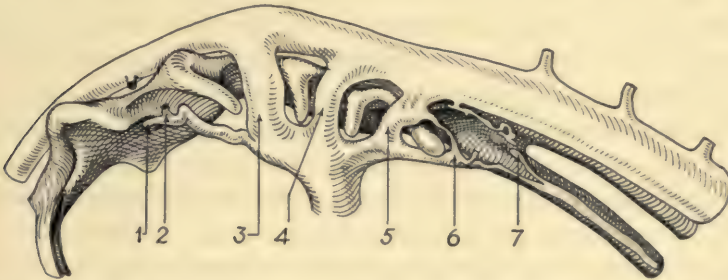


Fig. 43 Lateral reconstruction of aortic arches and pulmonary artery in stage 30, 17132.

into the pulmonary artery. Thus far, the course of events in the origin of the opossum's pulmonary artery coincides with what Bremer found in the rabbit; but in stage 30 I have found in three specimens a condition not heretofore described, and possibly of considerable significance.

Figure 43 shows a reconstruction of the aortic arches and pulmonary artery on the left side in specimen 17132. The most interesting feature of this specimen is the presence of a plexiform and not quite complete seventh aortic arch. Whether this is a normal structure I am not prepared to say with finality. The embryo is normal in every other respect. On the right side in the same specimen the rudiment is less well developed but it is represented by a ventral sprout at the



same level as the one on the left. In another specimen of the same litter (17156) there are well-developed dorsal and ventral sprouts on both sides. And finally, in one specimen (17135) from another litter there are small ventral sprouts on both sides at the proper level.

The fact that these rudiments have been found in three embryos of the same stage of development, and taken from two litters, suggests that they may be normal structures of short duration. This interpretation is strengthened by the evidence already presented that the lungs are first formed as a pair of gill pouches. For if so, the assumption that at some time in the phylogeny of the pulmonates there must have been a seventh aortic arch, is a necessary part of the theory.

I therefore follow Bremer in saying that the pulmonary arteries are certainly not derivatives of the sixth aortic arch, and that portion of what has been called the sixth below the origin of the pulmonary artery is really part of the ventral aorta. But I go one step further and suggest that beyond that point the pulmonary artery may represent still another aortic arch, the seventh or true pulmonary arch, the dorsal component of which in the embryology of most pulmonates has been lost or reduced to a vestige.

*The liver cords.* The originally simple and broad ventral diverticulum from the duodenum is now becoming organized into several anlagen which will have very different fates. The portion which runs most nearly craniad is the hepatic duct (fig. 42, reconstruction), and from it many solid cords of cells are growing out into the mesenchyme of the ventral mesentery. These cords ramify in the direction of the sinus venosus and between the most proximal portions of the omphalomesenteric veins, which latter respond to the presence of the cords in a remarkable way. Numerous twigs grow out from the veins and branch among the cords. In the case of the left vein this process rapidly breaks up the original channel into a complex of anastomosing sinusoids running between the cords.

A conspicuous bud running more ventrally from the original liver diverticulum is the anlage of the gall bladder (fig. 42, reconstruction, just above the omphalomesenteric vein). And finally, at the junction of the liver diverticulum and the duodenum a small ventrocaudal bud (not shown) represents the ventral pancreatic diverticulum.

*The septum transversum and the pleuropericardial membrane.* In the opossum the omphalomesenteric veins play no part in the formation of the septum transversum. In figure 26 it was shown how the two heart tubes and the veins which are their posterior extensions ran in the splanchnopleure before there was a foregut; how the splanchnopleure folded ventrally to form the floor of the foregut, and the heart tubes came together to form a single median heart with only a dorsal mesocardium—no ventral one. The dorsal mesocardium forks posteriorly, descends around the edges of the anterior intestinal portal (fig. 33, stage 27, d.m.l.), and continues into the extra-embryonic splanchnopleure as the attached face of the omphalomesenteric vein. In the region of the heart proper the line of attachment of the tube is dorsal; at the level of the anterior intestinal portal it spirals around the omphalomesenteric vein on its medial side; and in the extra-embryonic region it is ventral. The peritoneal cavity is thus continuous with the pericardial cavity by a passage which in stages 27, 28 and 29 is lateral to the omphalomesenteric veins at the level of the anterior intestinal portal.

The closure of this passage begins, however, back in stage 25 (fig. 25, H) when the sinus venosus expands until it touches the opposite somatopleuric wall at the level of the anterior end of the umbilical veins. In stages 26 and 27 the umbilical gains access to the sinus through the resorption of the walls at this point; and the anterior cardinal also joins in the anastomosis.

The point at which the sinus meets the somatopleure is well shown in figure 36, section D. Dorsocaudal to the sinus and on either side of the mesentery are the pleural canals—the dorsal remnants of the original wide communication between

the pericardium and the peritoneal cavity. Section C shows that these pleural canals pass the ducts of Cuvier on their medial sides. This is also particularly well shown in section B of figure 39. The central reconstruction in figure 36 shows that the pericardium also still communicates with the peritoneal cavity ventrolaterally by the pericardioperitoneal canal which passes over the omphalomesenteric vein. On each side of the body the original, single, wide communication between the anterior and posterior portions of the splanchnocoel has thus been divided in two by the developing sinus, leaving a narrow dorsal passage (the pleural canal) and a narrow ventral passage (the pericardioperitoneal canal).

The latter of these two is the first to close, and its closure is accomplished by the continued lateral and ventral expansion of the sinus venosus. Figure 39, reconstruction and sections C and D, shows the fusion of the sinus with the lateral body wall continued almost to the point of closure ventrally. In stage 30 (fig. 42) this closure is complete, and the septum transversum is thus formed by the expansion of the sinus venosus—not by the omphalomesenteric veins.

The closure of the pleural canals, however, is like the same process in other mammals. The central reconstruction shows the one on the left passing medially behind the duct of Cuvier and emerging between the duct and the lung buds. On the right (not figured) this great vein has pressed medially until its wall has fused with the mesentery and thus obliterated the pleural canal on that side. By the same process the thin slit which remains on the left side will be closed in stage 32. The membrane formed by the inward migration of the ducts of Cuvier is the pleuropericardial membrane.

The septum transversum in the opossum is thus formed by the lateral and ventral expansion of the sinus venosus. The pleuropericardial membrane, on the other hand is formed by the medial migration of the ducts of Cuvier, as in other mammals.



*Advances in the nervous system.* All cranial nerves (fig. 42, reconstruction) are present in stage 30 except the motor nerves of the eye. The olfactory is represented by only a few fibers from the olfactory pit to the forebrain. The optic is represented in a sense by the optic stalk, but the nerve fibers proper are only beginning to appear. The retina shows the first steps of differentiation of the cellular and fibrous layers. The gasserian ganglion has a strong connection with the hind-brain, and the three branches of the trigeminal (ophthalmic, maxillary, and mandibular) are well formed. The facial nerve has not yet developed a chorda tympani. The dendrites of the eighth nerve from the otocyst are now separable for the first time into two groups, a dorsolateral or superior group, and a ventrolateral or inferior group. The geniculate and acoustic ganglia are fused dorsally, but their ventral thirds are separated, the acoustic being medial to the geniculate. The anlage of the spiral ganglion is recognizable as a less compact bud on the ventrolateral tip of the acoustic complex. The glossopharyngeal nerve has a well-developed petrosal ganglion, but the superior ganglion is indistinct and diffuse. The vagus similarly shows a good nodosal ganglion, but the jugular accessory root ganglia are poorly formed. The spinal accessory is well developed proximally but not separated from the vagus distally. The hypoglossal can be followed all the way to the tongue anlagen. It may arise either lateral or medial to the precardinal vein. If it arises lateral, then during stage 30 it migrates through the precardinal, or the latter grows around it, so that the nerve becomes medial. It is always medial in stage 31 and later. Froriep's ganglion is present, but I have not seen any connection between it and the hypoglossal.

The first cervical nerve has no ganglion at any time. The fourth, fifth, sixth, seventh, eighth cervicals and the first thoracic form a loose and irregular fusion which is the primordium of the brachial plexus. There is no indication of a lumbosacral plexus as the nerves at that level are just beginning to grow out, and the ganglia are just becoming organized from the neural crest.

In the caudal region the neural crest is still in a primitive condition; and in very early specimens there is even a posterior neuropore in the tail.

Within the central nervous system the ependymal, mantle, and marginal layers are well differentiated in the hind-brain and cervical cord (fig. 42, sections A to E).

Larsell ('35) has found two commissures arising in the cerebellum in stage 30. The cerebellar commissure, which is the more anterior of the two, arises in connection with the trigeminal; and the lateral commissure, the more posterior, is associated with the seventh nerve. According to his interpretation these two primitive commissures form the basis of two functionally and phylogenetically different parts of the adult cerebellum—the corpus cerebelli, associated primitively with the fifth nerve, and the flocculo-nodular lobe associated with the seventh.

*Miscellaneous details.* In the heart the interventricular septum (E), the interatrial septum primum (A), and the endocardial cushions in the atrioventricular canal (fig. 42, C and D) are just beginning to form. Capillaries from the left part of the atrium invade the mesoderm of the lungs. From the proximal part of this plexus a single large pulmonary vein is organized during stage 30. It runs between the ducts of Cuvier and empties into the atrium just to the left of the septum primum. Some transitory capillary twigs from the anterior cardinal also ramify in the region of the trachea.

The first and second aortic arches are reduced to capillary plexuses. The third is at the height of its development. The fourth and fifth are well formed (fig. 43). It is interesting that the fifth is well developed and arises before the sixth—a primitive condition not heretofore described in a mammal.

The lens cup pinches off to form a vesicle (fig. 42, D). The optic vesicle begins to invaginate to form a cup (D). The naso-oral groove deepens very markedly (D).

The thyroid diverticulum pinches off to form a vesicle situated just in front of the fork of the ventral aortae (reconstruction). Rathke's pouch deepens and constricts, but is not yet pinched off (reconstruction).

The chondrostyle is forming around the notochord, and pre-muscle masses are prominent in the forelimb (fig. 42, A, B, C and D).

Figure 42, J, is a reconstruction of the wolffian duct and the blood vessels of the lumbosacral region. The wolffian duct is the very slender, black tube. The aorta and its branches are shaded with curved lines. The umbilical vein is stippled. The postcardinal vein is unshaded. It can be seen that the aorta, which became single in the lumbar region in stage 29, is in stage 30 still double at the level of origin of the common iliac arteries. The paired aortae are continued into the tail as the caudal arteries which run on either side of the tail gut. The common iliac arteries divide at the level of the limb bud into the external iliac and the hypogastric. The latter passes ventrally and cranially around the cloaca and out to the allantois. The single, median, caudal vein runs on the ventral side of the tail gut (section G), divides near the cloaca (section H) into the two postcardinals, which latter receive many small veins from the limb bud before they reach the mesonephros.

The capillary connections between the omphalomesenteric veins dorsal to the gut anterior to the liver bud, and ventral to the gut posterior to the liver bud, described in stage 29 (p. 123) have now developed into large anastomoses, so that the veins form two complete loops around the duodenum. However, the left arm of the anterior loop has already become plexiform, as described in connection with the liver cords.

A slight thickening of the peritoneum in a longitudinal line along the medial wall of the mesonephros is the anlage of the genital ridge.

The tail gut is at the height of its development (central reconstruction).

#### *The second half of the eleventh day*

Stage 31. External distinctive features. Changes in the chorion. Changes in the venous system. The earliest lymphatic anlagen. Changes in the arterial system. Changes



in the pancreas. Pleuroperitoneal membranes. The ureteric bud. The primitive choanae. The motor nerves of the eye. Miscellaneous details.

*External distinctive features.* Stage 31 is distinguished from stage 30 by the loss of the naso-oral groove, the development of the frontal process, the auricular tubercles, the fusion of the mandibular and hyoid arches beneath the external auditory meatus, the appearance of prominent digital ridges on the forefoot, the enlargement of the allantois until its diameter is about one-third the greatest length of the body. It is distinguished from stage 32 by the smaller allantois, the fact that the digital anlagen on the forefoot are not yet buds; and the hind limb is still a bud, not a club, that is, its length is not appreciably greater than its width and it has no terminal enlargement.

*Changes in the chorion.* Up until the middle of the tenth day all of the vesicles are spherical and roll or float about freely in the cavity of the uterus. During stages 29 and 30 the animal pole of the vesicle comes to rest against the uterine mucosa. This relation is consistent in all of the vesicles, as if the area vasculosa were for some reason sticky and inclined to adhere to the mucosa. Selenka (1887, S. 128) was of the opinion that the shell membrane (which he erroneously called the 'granulosa membrane') over the area vasculosa becomes soft and adhesive at this time, and acts as the anchoring agent. At any rate, the vesicles all come to rest in this position with the area vasculosa against the mucosa and the non-vascular chorion on the free side. Osborn (1883 a and b, 1887) described the vesicles at this time as aligned in two longitudinal furrows of the uterine mucosa, but no such arrangement has been seen by any other investigator. The vesicles are usually scattered at random throughout the uterus.

After coming to rest the vesicle continues to enlarge, but the enlargement is accommodated in different ways at the attached and unattached faces of the vesicle. The attached face wrinkles into great folds which sink into corresponding

crypts of the uterine mucosa (fig. 17, also Hartman, '23, fig. 31). These folds and crypts become exceedingly complicated as development continues, but no fusion between foetal and maternal tissues ever occurs. The use of the extensive folding is only to increase the surface of contact. At any time during development the vesicles may be separated from the mucosa without a tear and without loss of either foetal or maternal blood.

The non-vascular portions of the vesicle enlarge by expanding medially into the lumen of the uterus and laterally until they meet each other. The surfaces of lateral contact

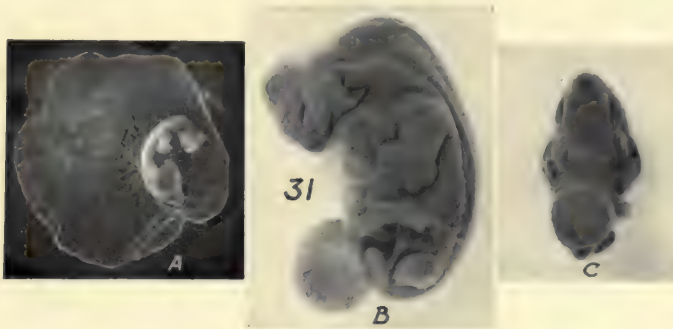


Fig. 44 Photographs of external views of stage 31. A, lateral view of 16164 photographed alive in Ringer's solution. B, lateral view of 16162 with all extra-embryonic membranes except the allantois removed. C, ventral view of same specimen.

of adjoining vesicles then fuse together so that each vesicle assumes the shape roughly indicated in figure 45, G. The vesicles thus develop non-vascular, membranous attachments to each other, but never any sort of attachment to the maternal tissues. All of the changes just recounted were accurately described by Selenka.

*Changes in the venous system.* The omphalomesenteric veins have enlarged enormously. The left side of the anterior loop, which was plexiform in stage 30, is altogether gone in stage 31, so that there is only a single large vein at the level of the stomach, which passes dorsad around it and up to the

right side of the liver (fig. 45, central reconstruction). On the other hand, it is the right side of the posterior loop which has been reduced to a vestige, as seen in the cross-cut portion of the central reconstruction; and this will soon be likewise lost. Though the process is not quite complete, it is obvious that within the body the two omphalomesenteric veins are being converted into a single large vessel entering the body on the left side of the duodenum and spiralling around behind it to pass into the liver on the right. Within the liver the right omphalomesenteric is not completely destroyed or replaced by sinusoids as in placental mammals, but maintains its open channel right through to the sinus venosus up until the time of birth. It thus comes to play a part in the formation of the postcava which will be described in the next chapter.

The enlargement of the liver and the constriction of the connection between the mid-gut and the yolk sac have caused important changes in the umbilical veins. These will be discussed in more detail in the next chapter, but it should be mentioned here that the liver has grown around the anterior ends of the umbilicals (fig. 45, reconstruction) and the posterior ends are being drawn toward the midventral line (fig. 45, D).

In the mesonephros many branches from the postcardinal ramify among the tubules. In stage 31 these branches develop longitudinal anastomoses along the ventral medial border of the mesonephros. The new longitudinal channels are the subcardinals (fig. 45, D).

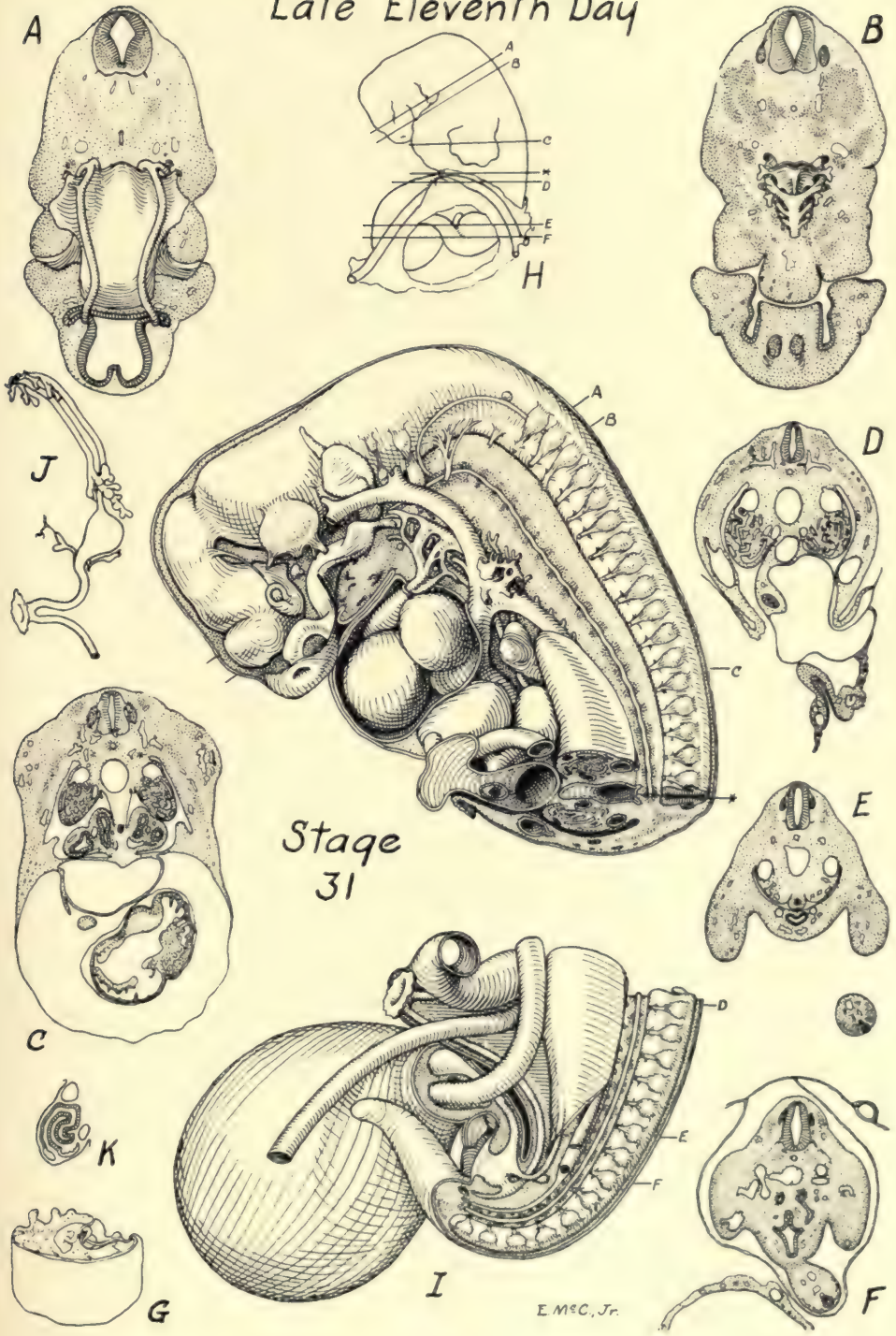
The primitive radial vein, which drained the ventral border of the anterior limb into the umbilical, seems to be finally lost in stage 31. In previous stages its mouth had migrated anteriorly along the umbilical until in stage 30 it emptied into the angle between this vein and the duct of Cuvier. It is possible that this same process of migration has now carried it up the duct of Cuvier to the cardinal anastomosis or promontory, in which case it is preserved as the cephalic vein of

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Fig. 45 Reconstructions and sections of stage 31. The central reconstruction and I and J are from 17146. A and B are from 17147. The sections are from 16163. G shows the real size of the vesicle.



Late Eleventh Day



E. M. C., Jr.

adult anatomy, which may be seen in that position in the reconstruction in figure 45. But I have not a sufficiently closely graded series between stages 30 and 31 to establish this point, and as far as the evidence goes it seems more likely that the cephalic is a new vein.

The primitive ulnar vein has migrated along the post-cardinal to the promontory. And the inferior jugular has migrated up the duct of Cuvier until it has become a tributary to the precardinal (fig. 45, reconstruction).

*The earliest lymphaticanlagen.* The origin, development, and adult anatomy of the lymphatic system in the opossum has been worked out in great detail by Dr. A. A. Zimmermann of the University of Illinois Medical College. This work was done partly at The Wistar Institute and partly at his own University. He very generously allowed me to read his manuscript before it went to press, and to incorporate an abstract of his embryological observations and conclusions in this book. The account of the development of the lymphatics in this and succeeding chapters is based entirely upon his work, and I am very much indebted to him for the privilege of citing it here.

The first steps in the development of the jugular lymph sacs and of the thoracic duct occur independently of each other during stage 31. In stages 27 and 28 there was formed at the angle between the pre- and post-cardinals a plexus of veins which I called the paracardinal plexus (p. 110). In stage 31 this plexus is divided into dorsomedial and dorsolateral portions. The former is the more cranial of the two. It is lateral to the intersegmental veins 1 to 5, and medial to the cervical nerves 4 and 5. Anteriorly it communicates with the first intersegmental vein and posteriorly with the jugular promontory near the mouth of the external jugular (jugulocephalic) vein. The dorsolateral portion is medial to the blastema of the scapula and lateral to the cervical nerves 3 to 7. It communicates with the jugular promontory dorsolateral to the common stem of the third and fourth intersegmental veins (fig. 45). This connection coincides with the mouth of the primitive ulnar vein. It also communicates by a small venule with the dorsomedial portion described above.



Both of these venous plexuses show signs of degeneration in stage 31 (16163), but particularly the dorsomedial or more cranial of the two. The channels are becoming sinusoidal or sacculated, and the endothelium is breaking down. Perivenous spaces form in the mesenchyme immediately around these veins and develop an independent mesenchymal endothelial lining before the endothelium of the veins breaks down (fig. 48). These perivenous spaces are lymphatic anlagen. They do not form in the mesenchyme surrounding the functional or permanent vessels in the same region, but are consistently found around all venules which are to be replaced by lymphatic channels. When the endothelium of the vein thus surrounded breaks down, the blood is liberated into the primitive lymphatics, which are thus at first hemophoric.

At the same time that these changes are occurring in the region dorsolateral to the confluence of the cardinal veins, another set of changes is in process in the mesenchyme ventral to the aorta in the lower cervical and upper thoracic region. Here the mesenchyme is becoming distinctly vacuolated as if the intercellular fluid is increasing. As the mesenchymal clefts enlarge, the cells lining the spaces assume endothelial characteristics. At first the lymphatic anlagen thus formed are isolated, but later they run together to form a plexus which is continuous with other mesenchymal spaces of perivascular origin like those which coalesce to form the jugular sacs.

The thoracic duct thus is derived from two sorts of anlagen—some which form altogether independently of veins, and others which form around degenerating venules. In all cases, however, the anlagen develop their own independent endothelium from mesenchyme.

*Changes in the arterial system.* Among the aortic arches the principal flow of blood seems to be shifting progressively caudad, so that now the fourth arch has become the largest of the group (fig. 45). The fifth is longer and slenderer than in stage 30, but it still has a larger caliber than it ever attains in most mammals. The endocardial ridges of the conus are causing a partition to be formed between the base of the fifth arch and the ventral aorta, pushing the fifth forward with the fourth. The sixth has enlarged until it is about the same size as the ventral aorta, which latter correspondingly looks



like the ventral half of the arch, and the impression is thus produced that the pulmonary artery is a branch from the middle of the sixth arch. Actually the sixth is entirely dorsal to the origin of the pulmonary, as pointed out in connection with stage 30.

The first seven intersegmental arteries become plexiform and develop longitudinal anastomoses with each other dorsal to the aorta. The first six lose their connections with the aorta, and the anastomoses plus the remnants of the intersegmentals form the vertebral arteries in stage 31. The seventh intersegmental artery, from which the vertebral now appears to arise, is at the level of the limb bud and becomes enlarged to form the subclavian artery.

*Changes in the pancreas.* In stage 31 the dorsal pancreatic diverticulum loses its connection with the duodenum, so that the anlage of the duct of Santorini is lost, and correspondingly no accessory duct appears in the adult. The ventral pancreatic diverticulum has migrated away from the angle where it originally formed, and now appears as a branch of the hepatic diverticulum or ductus choledochus. It is not yet in contact with the dorsal pancreas, which is thus completely detached from the digestive tract at this time. With the exception of the absence of the duct of Santorini this stage is like that of a 10-mm. human embryo. In figure 45, J, the ventral pancreatic bud is not well shown, as it is almost completely hidden by the duodenum.

*Pleuroperitoneal membranes.* The anterior end of the pleuroperitoneal cavity is roughly pyramidal in shape. In cross section it appears triangular with the mesonephros at the apex of the triangle, the lung at the medioventral corner, and a new fold of mesodermal tissue at the lateroventral corner (fig. 45, C). If traced anteriorly this fold, the pleuroperitoneal membrane, can be followed to the anterior apex of the pyramid where it merges with the mesonephric fold. In the middle sections the inner edge of the fold is directed toward the interval between the lungs and the mesonephros; and at its extreme caudal end it is continuous with the

coronary appendage of the liver (also called the dorsal pillar of the diaphragm). The part that these folds play in separating the pleural cavity from the peritoneal cavity will be described in connection with stage 34.

*The ureteric bud.* About 48 hours before birth the first rudiment of the ureter is just forming as a very small bud from the wolffian duct near the place where it empties into the cloaca (fig. 45, I and F). Even at this early stage the mesenchyme immediately around the bud shows a definite condensation, the metanephric blastema. This mesenchyme is part of the same nephrogenic mass in which the mesonephric tubules are formed. At first (16174) it is situated right in the posterior end of the mesonephros. Later (16173) it migrates medially and dorsally out of it.

*The primitive choanae.* The disappearance of the naso-oral groove noted in connection with the external distinctive features of stage 31, is caused by the overgrowth and fusion of its edges, converting the groove into a tube connecting the roof of the mouth with the surface of the snout (fig. 45, reconstruction and section B). This involves the fusion of the median and lateral nasal processes and the maxillary process. This direct conversion of the oral end of the naso-oral groove into the primitive choana is characteristic of the Sauropsida, but is not supposed to occur in mammals.

*The motor nerves of the eye.* With the appearance in stage 31 of the third, fourth, and sixth nerves the list of cranial nerves is complete. The oculomotor develops rapidly, and can be followed all the way to the eye, but the trochlearis and the abducens are extremely rudimentary at this time.

*Miscellaneous details.* The stomach is noticeably enlarged. Rathke's pouch has pinched off from the oral cavity. Sympathetic trunks have formed in the thoracic region. The cerebral hemispheres are enlarged. Periotic mesenchyme has condensed slightly as the first stage in the formation of the otic capsule. The allantois is as big as the head and hangs free in a pocket of the yolk sac. It is probably already functioning as an urinary reservoir, for the wolffian duct became

patent into the cloaca in stage 30, and the mesonephros gives every indication of being functional in stage 31. Somites are still forming in the tail. Auricular appendages are forming on the heart.

XII. THE TWELFTH DAY. Stages 32 and 33

*The first half of the twelfth day*

Stage 32. External distinctive features. The veins of the liver and mesonephros. The thoracic duct and the jugular lymph sacs. The adrenal cortex. The foramen ovale. The subintestinal vein. Changes in the branchial pouches. Changes in the digestive tract. Miscellaneous details.



Fig. 46 Photographs of external views of stage 32. A shows a ventral view of 16173 with all extra-embryonic membranes removed. B shows a lateral view of the same specimen. C shows 16174 with the allantois still attached and partly dissected.

*External distinctive features.* Stage 32 is distinguished from stage 31 by having the diameter of the allantois approximately two-thirds the length of the body (instead of one-third); by the hindlimb being a club instead of a bud; by having definite digital buds on the forelimb instead of ridges; by having the ventral thoracic wall opaque instead of transparent; and by having the interval between the first and sixth auricular tubercles very much enlarged. It is distinguished from stage 33 by the absence of the upper eyelid folds, the smaller size of the allantois, and by the hindlimb being a club, not a paddle.



*The veins of the liver and mesonephros.* In connection with stages 30 and 31 I have described the conversion of the original paired omphalomesenteric veins within the body into a single vein starting on the left of the gut (original left omphalomesenteric) then swinging dorsally around behind the gut (dorsal anastomosis between the omphalomesenterics) and finally entering the liver on the right side (original right omphalomesenteric). It was also mentioned that within the liver the right omphalomesenteric, though it develops numerous branches in all directions, retains its principal channel intact. Both right and left omphalomesenterics can be recognized at their anterior extremities just before they join to empty into the sinus, but posterior to this point only the right can be followed continuously through the liver.

It was also mentioned that during stage 31 the folding in of the lateral body wall, which is converting the original wide communication between the mid-gut and the yolk sac into the narrow yolk stalk, causes the anterior third of the umbilical veins to swing ventrally and approach each other near the middle line. Here they become involved in the growing liver and during stage 32 are broken up into sinusoids just as the hepatic portion of the left omphalomesenteric was before them. Further changes in these vessels will be described in connection with stages 33 and 34.

The liver is enlarging very rapidly in stage 32 and on the right side, where there is no stomach in the way, the so-called right coronary appendage of the liver expands dorsad against the mesonephros and the dorsal mesentery. Small vessels from the liver and from the subcardinal vein invade the mesentery, unite, and enlarge to form a conspicuous anastomosis which leads directly from the subcardinal to the right omphalomesenteric vein within the liver (fig. 47, C).

At the same time another important change is taking place in the posterior portion of the subcardinals. From the time of their origin the subcardinals have always communicated with the postcardinals by means of numerous sinusoids running through the mesonephros (the renal portal system). Now in stage 32 two of these anastomoses at the level shown

in section D become very large, and the principal flow of blood from the posterior part of the body is diverted ventrad at this point into the subcardinals. These changes constitute the first steps in the formation of the postcava, which will be discussed further in connection with stage 33.

*The thoracic duct and the jugular lymph sacs.* It was mentioned in the previous section that the earliest anlagen of the thoracic duct are mesenchymal spaces not associated with veins, but that these later coalesce with a second set which are formed around collapsing venules. Zimmermann has pointed out that the first perivascular lymphatic anlagen of the thoracic duct arise in stage 32 (16174 and 17127). They are located in the mediastinum between the levels of the second and seventh thoracic vertebrae. "Caudal to these levels," he says, "there are no signs of thoracic duct anlagen in Normal Stage 32. Cephalad, only the ill-defined mesenchymal clefts of the preaortic mesoderm are found."

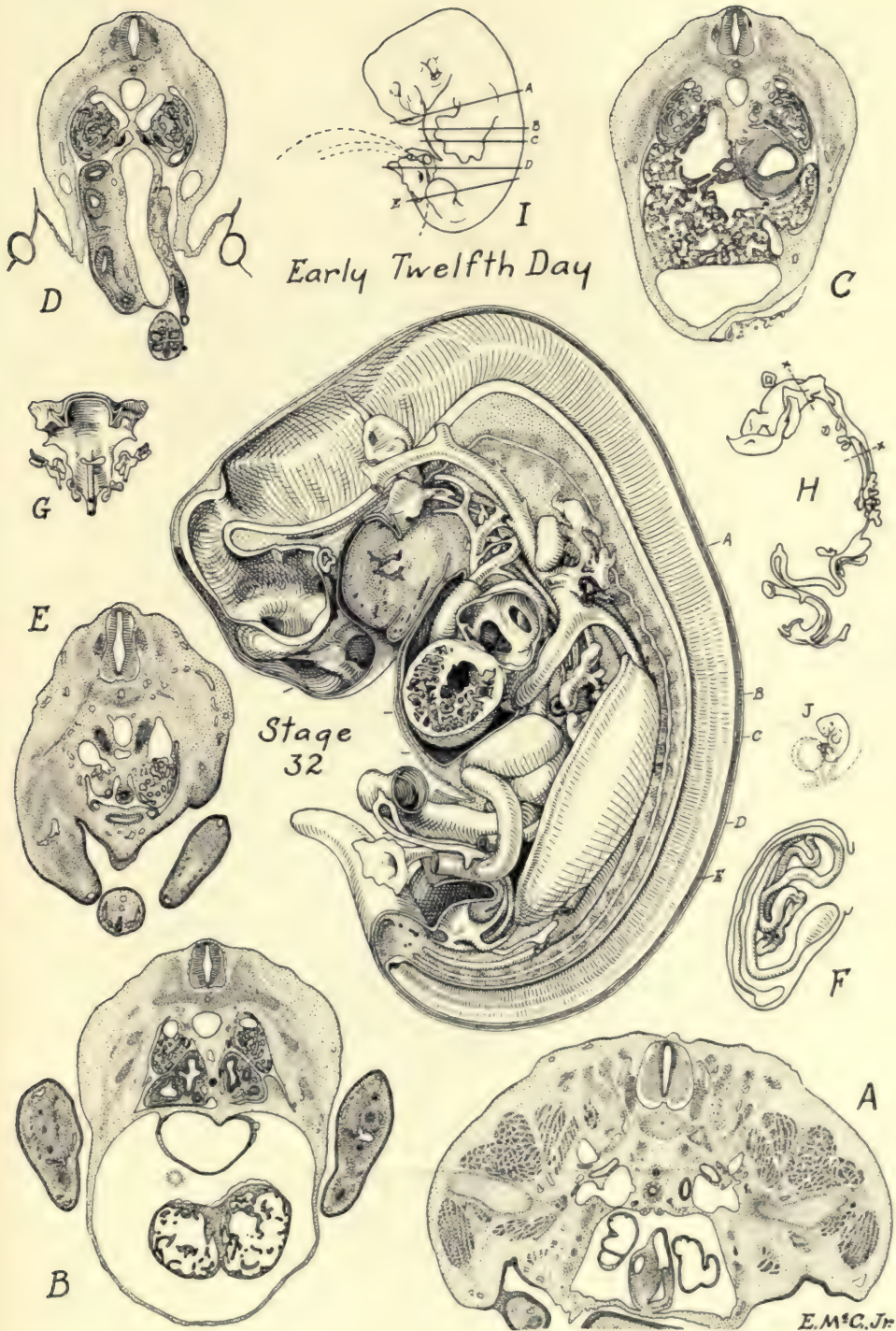
The degenerating venules around which these first perivascular thoracic duct anlagen form, are tributaries of the thoracic portion of the postcardinal veins. Almost immediately after they begin to form, these peri-intimal spaces run together and establish a lymphatic plexus. The fusion of the separate anlagen occurs first at the levels of the fifth and sixth thoracic segments, and later spreads from this point in both directions longitudinally. As the process extends cephalad the mesenchymal clefts formed in stage 31 are met and incorporated in the lymphatic plexus. Between the most anterior of these and the anlagen of the jugular lymph sac there is still a long gap in which no lymphatic anlagen are found.

The jugular lymph sacs are at a similar stage of development. The remnants of the degenerating paracardinal venous plexus have pinched off and are completely detached from the venous system. The perivascular lymphatic anlagen are coalescing to form a continuous lymphatic plexus not in com-

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Fig. 47 Reconstructions and sections from stage 32. The central reconstruction is from 16173 except for the membranous labyrinth which is added from 17127. H and G are from a wax model made by Dr. C. H. Heuser and now in The Wistar Institute. A, E and F are from 17127. The other sections are from 16174. J shows the real size of the embryo and allantois.







munication with the venous system at all. The endothelium of the lymphatics is of mesenchymal origin and is not derived from, or until a later stage even connected with, the venous endothelium.

Heuser's note ('19) about the anatomy of a specimen of this stage referred to the jugular lymph sac as having "become transformed into a much-divided structure with smaller and larger spaces." Instead of the sac having become divided into the several elements referred to, the latter are really in process of uniting to form the sac. His further note that "In an injected embryo of the same litter the lymph sac received no ink, although the injection of the blood vessels is complete" is in harmony with Zimmermann's observations that the jugular lymph sac has not yet acquired communication with the venous system.

Though the lymphatic system has only begun to form at this time, the method of origin thus far described will apply equally well to the later stages. In far the majority of cases the original lymphatic anlagen are spaces which form around collapsing venules. Such a peri-intimal space acquires an endothelium of its own (fig. 48) before that of the vein has disintegrated. When the venous endothelium breaks down, blood is liberated into the lymphatic which is thus for a while hemophoric. The separate lymphatic anlagen coalesce to form a plexus which secondarily acquires communication with the venous system.

The fact that the lymphatics usually, though not invariably, form around collapsing venules, accounts for the frequently recorded observation that they nearly always follow the path of a pre-existing venous plexus. In all cases they are of mesenchymal origin, and they never derive their endothelium from veins.

*The adrenal cortex.* In late specimens of stage 31 and in all specimens of stage 32 cords of mesodermal cells proliferate from the peritoneum near the mesentery at the level of the posterior end of the lungs and caudad as far as the region of the stomach. These cords of cells, the anlage of the cortical

part of the adrenal gland, form fairly compact masses just dorsal to the subcardinal veins (fig. 47, C).

*The foramen ovale.* In stage 32 (fig. 47, reconstruction) the septum primum has descended some two-thirds of the way toward the atrioventricular canal, and has become second-

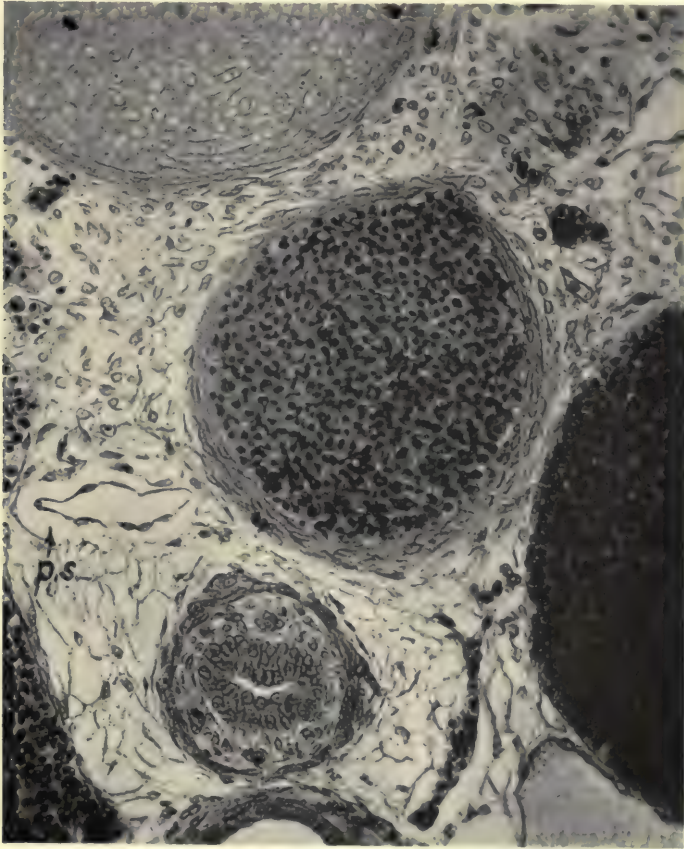


Fig. 48 Photograph by Dr. A. A. Zimmermann showing a peri-intimal space with its own endothelium forming while that of the venule is still intact.

arily perforated by one or more foramina. When there are more than one, which is always the case in later stages, usually one of the more ventral ones is decidedly larger than the others (fig. 51). This multiple condition is a primitive feature not found in placental mammals.

*The subintestinal vein.* Senior ('25) was the first to observe that the subintestinal vessel in the opossum is a vein, and he pointed out that in this respect the opossum is like some of the lower vertebrates, and unlike the placental mammals, in which the vessel is always an artery. Kimball ('28) made a comprehensive study of the vessel, and concluded that it is a vein in all chordates in which there is no allantoic placenta, and that it acquires new connections and becomes converted into an artery when an allantoic placenta is formed. This seems well established by her extensive investigation.

In early stages of the development of the allantois in the opossum (e.g., stage 30, 16160, slide 4) two very small veins on its dorsal wall drain caudally into the subintestinal vein and thence forward into the omphalomesenteric. I shall refer to these as the primitive allantoic veins to distinguish them from the much larger and more important umbilical veins which become the definitive veins of the allantois. As the allantois is a diverticulum from the hind-gut, it is natural that it should carry with it some of the latter's circulatory apparatus, but these vessels never become large enough to be of much functional significance, and they disappear altogether by stage 31.

In stage 32 (16174, 16173) the subintestinal vein thus has no connection with the allantois. Its posterior end is very plexiform and communicates by a few small branches with the postcardinals, but its anterior portion is still well developed (fig. 47, D) and drains forward into the omphalomesenteric. This is the stage which both Senior and Kimball studied.

*Changes in the branchial pouches.* The third pair of pouches become detached from the pharynx in early stage 32 (16174, fig. 47, G). At this time no migration has occurred, so all the elements are readily identifiable.

In late stage 32 (17127) all of the gill pouches are detached and considerable shifting has occurred so that determination of the origin of the different elements is impossible without a large number of closely graded specimens. I have only three



specimens of stage 32, which is not enough, as the shifting seems to take place quite rapidly.

Accordingly I can only say that if the shifting is in general similar to that which occurs in other animals (and I have no reason to think it is not) then the situation at the end of stage 32 is as follows: The thyroid has migrated caudally until it is just ventral to the ultimobranchials (fifth pair). The third pair, including both dorsal and ventral components, has migrated most caudad of all so that it lies posterior to the aortic arches and just anterior to the pericardium. The fourth pair lies between the thyroid and the third pair. The only peculiar detail here is that the dorsal (parathyroid) elements of the third and fourth complexes migrate caudally with the ventral (thymus) components instead of remaining near the thyroid.

*Changes in the digestive tract.* The greater curvature of the stomach (original dorsal border) is bulging very prominently to the left. The yolk stalk has pinched off leaving a temporary hollow diverticulum at the level of the union of right and left omphalomesenteric veins (fig. 47, reconstruction). A new outgrowth from the intestine, the caecal diverticulum, can be seen where the umbilical vein curves to run out into the allantois. This diverticulum was noticed, but not identified, by Heuser ('19).

*Miscellaneous details.* The otocyst has acquired its first semicircular canal. A nasolachrymal chord has grown down from the anterior corner of the eye toward the nasal epithelium, but has not yet reached it. The turbinals are developing.

The mesenchymatous anlagen of all cartilaginous bones except some of the caudal vertebrae are present. The vertebral anlagen become less and less differentiated in caudal progression, until in the tail itself there are still somites in early stages of differentiation. There are about sixteen of these in the tail. Chondrification is beginning in all cartilage bones except the periotics and some of the caudal vertebrae. No membrane bones except the clavicle are present.

The six rhombic grooves are quite conspicuous in the floor of the hind-brain. The lower eyelid fold is recognizable. Epitrichial cells are making their first appearance.

During stage 32 mesoderm invades the interval between the pericardium and the ectoderm so that the ventral and lateral thoracic walls become opaque. Within this mesenchyme the anlagen of sternal bars, ribs, and coracoid become differentiated.

Heuser studied two of The Wistar Institute specimens of this stage (16173 and 16174) and the following notes are taken from his abstract ('19): "The ganglion nodosum is fused with the cervical sinus, and there arises from the ganglion a cord of cells which, with a strand from the thymus, makes another connection with the sinus. The hypoglossus is embedded in the nodosum, but does not touch the thymus. The superior laryngeal nerve is distinct: it extends from the mesial border of the cord above referred to and runs between the thymus and the parathyroid." All of these points are illustrated in figure 47, G, which is taken from his wax model.

In the heart there is a large septum spurium and no septum secundum. The foramen ovale has already been described. The interventricular septum is forming but is not large (fig. 47, B). The pulmonary trunk has twisted about the aorta until it leaves the conus on the left instead of the posterior side.

#### *The second half of the twelfth day*

Stage 33. External distinctive features. The veins of the liver and the postcava. The septum primum. The jugular lymph sacs. The thoracic supracardinal (azygos) veins. The paravertebral lymphatics and the thoracic duct. The migration of the gill pouch derivatives. The allantois at its height. Miscellaneous details.

*External distinctive features.* Stage 33 is distinguished from stage 32 by the following details: upper and lower eyelid folds are quite distinct; there are real digits, not just digital buds, on the forefoot; the beginning of the pontine flexure

has shortened the distance between the cephalic and cervical flexures and lifted the head somewhat away from the chest; the mouth is sometimes open and the tongue protruding; the allantois has attained its maximal size with a diameter equal to or greater than the body length (fig. 49, C). It is distinguished from stage 34 by the fact that the epitrichium has not yet covered the eyes, ears, or mouth; and there are no claws on the forefeet or digital ridges on the hind feet.

*The veins of the liver and the postcava.* In stage 32 the right subcardinal attained communication with the right omphalomesenteric vein in the liver by way of the dorsal mesentery and the right coronary appendage of the liver.

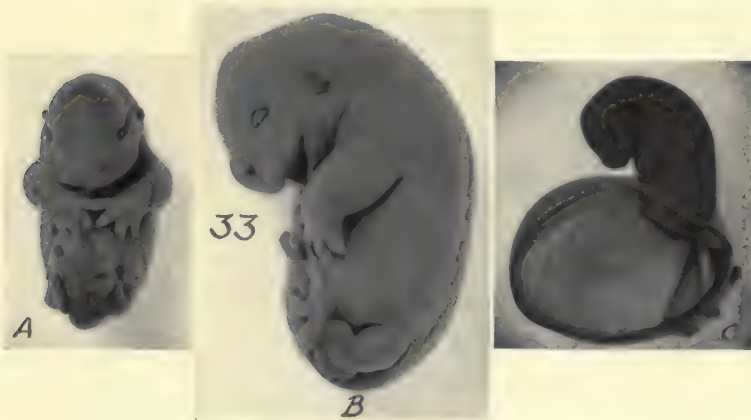


Fig. 49 Photographs of external views of stage 33. A, ventral view of 16176. B, lateral view of 16176. C, lateral view of 16177 showing maximal size of allantois.

This affords a very direct path for the blood from the posterior part of the body to the right atrium, and in stage 33 this channel enlarges until it becomes the most conspicuous vein in the body (fig. 50, postcava). The posterior portion of the omphalomesenteric appears now as a tributary to it. This infrahepatic portion of the omphalomesenteric receives the subintestinal vein, and is the anlage of the hepatic portal; but as it opens directly into the postcava, it is not yet, strictly speaking, a portal, taking portal to mean a venous channel interrupted by capillaries.



The two umbilical veins have fused into a single vessel from the umbilicus craniad to the liver. Within the liver a new vessel of hepatic origin, the ductus venosus, drains this common umbilical into the postcava (right omphalomesenteric portion).

The left subcardinal anterior to the anastomosis is a very small vessel which will be preserved only on account of its

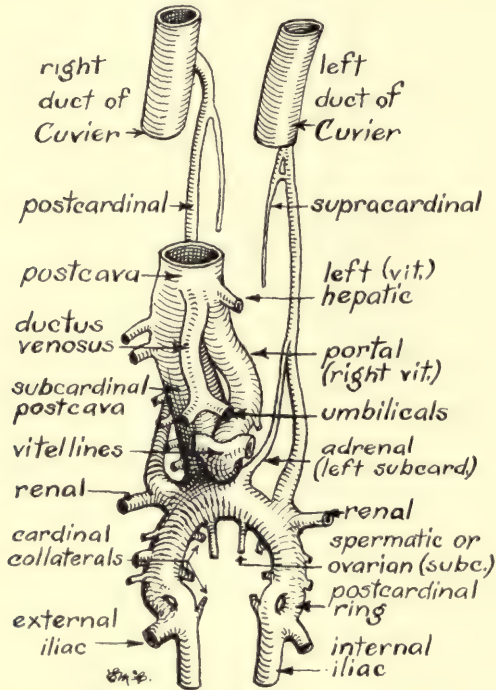


Fig. 50 Reconstruction of principal veins in stage 33 (slightly modified from McClure, '06).

association with the developing left adrenal gland. The right adrenal gland is drained by a branch directly from the postcava which is at this level the right subcardinal.

The fact that the right omphalomesenteric vein does not become broken up into sinusoids as the left does, results in its being transformed into most of the hepatic portion of the

postcava. The branches from it which extend through the coronary appendage and dorsal mesentery to communicate with similar branches from the right subcardinal, constitute a new vessel, the vein of the caval mesentery, but this contributes a relatively small part of the definitive postcava connecting the omphalomesenteric and subcardinal portions. From the liver back to the level of the metanephroi the postcava is the original right subcardinal. At the level of the kidneys it is the subcardinal anastomosis. Posterior to this point up until birth the only important channels are the subcardinal-postcardinal anastomoses and the postcardinals; for the subcardinals posterior to the anastomosis are not well developed, and there are no supracardinals.

The posterior portion of the postcardinals originally passed the common iliac artery on its dorsal side (fig. 42, J). In stage 33 it has sent sprouts around the artery which fuse to form a circumarterial ring (figs. 50 and 52), which was first described by McClure ('06). At this time another set of vessels is developing ventral to the aorta. These cardinal collateral veins, as McClure named them, develop posterior to the large subcardinal-postcardinal anastomosis from the mesonephric venous sinusoids which connect the postcardinals and subcardinals. They later become organized into two channels which replace first the subcardinals and later even the postcardinals in this region. In the formation of the postrenal division of the postcava they play the part which in placental mammals is played by the supracardinals; but unlike the latter the cardinal collaterals are entirely ventral to the aorta. In accordance with Butler's generalization ('27) the high development of the mesonephros in the opossum prevents the postcardinal from taking any part in the formation of the postcava; but why the vessels which replace it should be unlike those of all eutherians is not explained.

Posteriorly the cardinal collaterals are connected with the venous ring around the common iliac arteries already described. And about 1 week after birth when the postcardinals in this region degenerate, the circumarterial rings discharge

all of their blood into the cardinal collaterals. McClure has shown that either the right or the left or both cardinal collaterals can persist, and either the dorsal or the ventral or both halves of the circumarterial ring can persist. By various combinations of these possible routes several types of postcava are commonly formed in the opossum. In the adult the postcava posterior to the spermatic veins may be single or double with about equal frequency. It may also pass ventrad, dorsad, or both, around the common iliac artery. All of these types must be considered 'normal' (McClure, '03). The opossum is simply plastic in this respect, and has not settled upon a single 'normal' method of forming the postcava.

Aside from the plasticity, the two principal peculiarities about the opossum's postcava are: 1) that the hepatic portion is formed almost entirely from a persistent right omphalomesenteric vein, instead of from hepatic sinusoids; and 2) that the postrenal portion is formed from the unique cardinal collateral veins instead of from the postcardinals or supracardinals.

*The septum primum.* The interatrial septum has grown ventrad until it has reached the endocardial cushions. In stage 33 it is always cribriform, and the older the specimen the finer the mesh (fig. 51). Since in the previous stage there is sometimes only a single foramen (fig. 47, central reconstruction), and in later stages there are always many, and the later the stage the smaller the individual foramina, it seems necessary to assume that the muscular slips grow across and subdivide the original aperture. This process carried to its limit is presumably the mechanism of closure.

No septum secundum forms. At birth there are still a few small perforations through the septum primum, but they become gradually grown over and a few days after birth the two atria are separated by an imperforate partition.

In having no septum secundum, and in having many small interatrial foramina instead of a single large one, the opossum is similar to the chick and unlike the placental mammals. It has been known at least as far back as 1868 (see reference to



Owen in Cunningham, 1882, p. 150) that the adult marsupial heart has no fossa ovalis. I believe, however, that the explanation of this fact, namely, that it is due to the total lack of a septum secundum in the embryo and the presence of an avian type of cribriform septum primum, has not hitherto been recorded.

Evidently the equalization of pressure on the two sides of the septum due to the increased circulation of blood through the lungs after respiration begins, has nothing to do with the

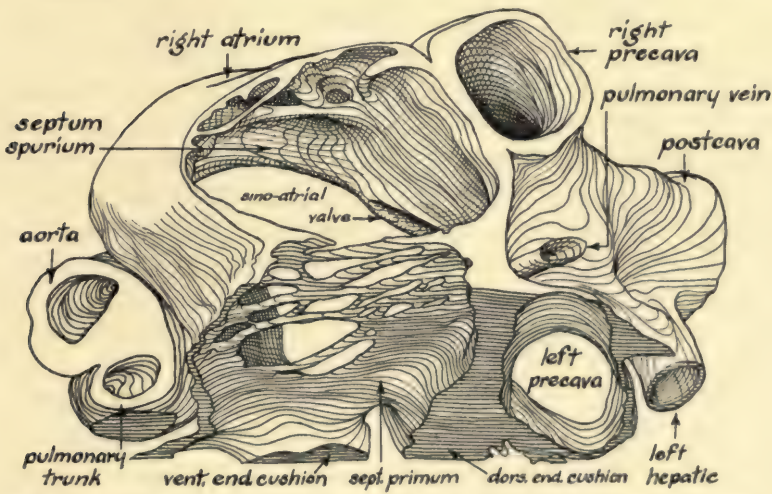


Fig. 51 Reconstruction of dorsal half of heart from 17125 showing the cribriform septum primum and the absence of any septum secundum.

closure of the interatrial foramina in the opossum, as the process of closure is almost complete before respiration begins. Also in prer respiratory stages there is no tendency for the septum to bulge into the left atrium. Figure 51 shows the foramina wide open, but the septum flat. If the pressure is unequal, then the septum is rigid enough to withstand it. Under these circumstances there is no way in which equalization of pressure could close these foramina. They must be closed by growth and fusion of the muscular bands.

*The jugular lymph sacs.* During stage 33 the many, small, separate, lymphatic spaces formed in the paracardinal region fuse together to form a continuous, though somewhat complicated, jugular lymph sac; and this sac acquires a connection with the venous system by means of a bicuspid valve located on the ventral side of the junction of the internal jugular (anterior cardinal) and external jugular (jugulocephalic) veins.

The anatomy of the jugular sac will be treated in detail in Doctor Zimmermann's monograph, so only a few words are necessary here. It is composed of two principal divisions: 1) a cephalic division (fig. 52), which is penetrated by the levator scapulae ventralis muscle (also called the m. atlanto-scapularis anticus) and branches from the third and fourth cervical nerves; and 2) an axillary portion lying mediad of the scapula, which is derived from the dorsolateral lymphatics previously described as paralleling the dorsal ulnar vein. Two small extensions or diverticuli from the cephalic portion should be mentioned on account of their prospective significance. One of these, the medial jugular extension, runs mediad from the slender, lower segment of the cephalic portion. It represents part of the jugular arch which will later connect the jugular sac with the thoracic duct. The other, the intermediate caudal extension, runs laterad from the same slender neck of the cephalic portion of the jugular sac, and will later become the cervical approach of the lymphatic channel which parallels the external mammary artery.

*The thoracic supracardinal (azygos) veins.* The thoracic intersegmental veins from the postcardinal at first (i.e., up until stage 32) lie consistently lateral to the sympathetic trunks. During stage 32 supplementary, minor channels pass around the nerves on the medial side and there is thus a double drainage into the postcardinals. In stage 33 longitudinal anastomoses form between the medial radices of the intersegmentals, the lateral radices drop out, and the postcardinal trunk ahead of the mesonephros degenerates. The longitudinal vessels thus left on the medial side of the sympathetic trunks and draining into the common cardinal, are the supracardinal or azygos veins.



Late Twelfth Day

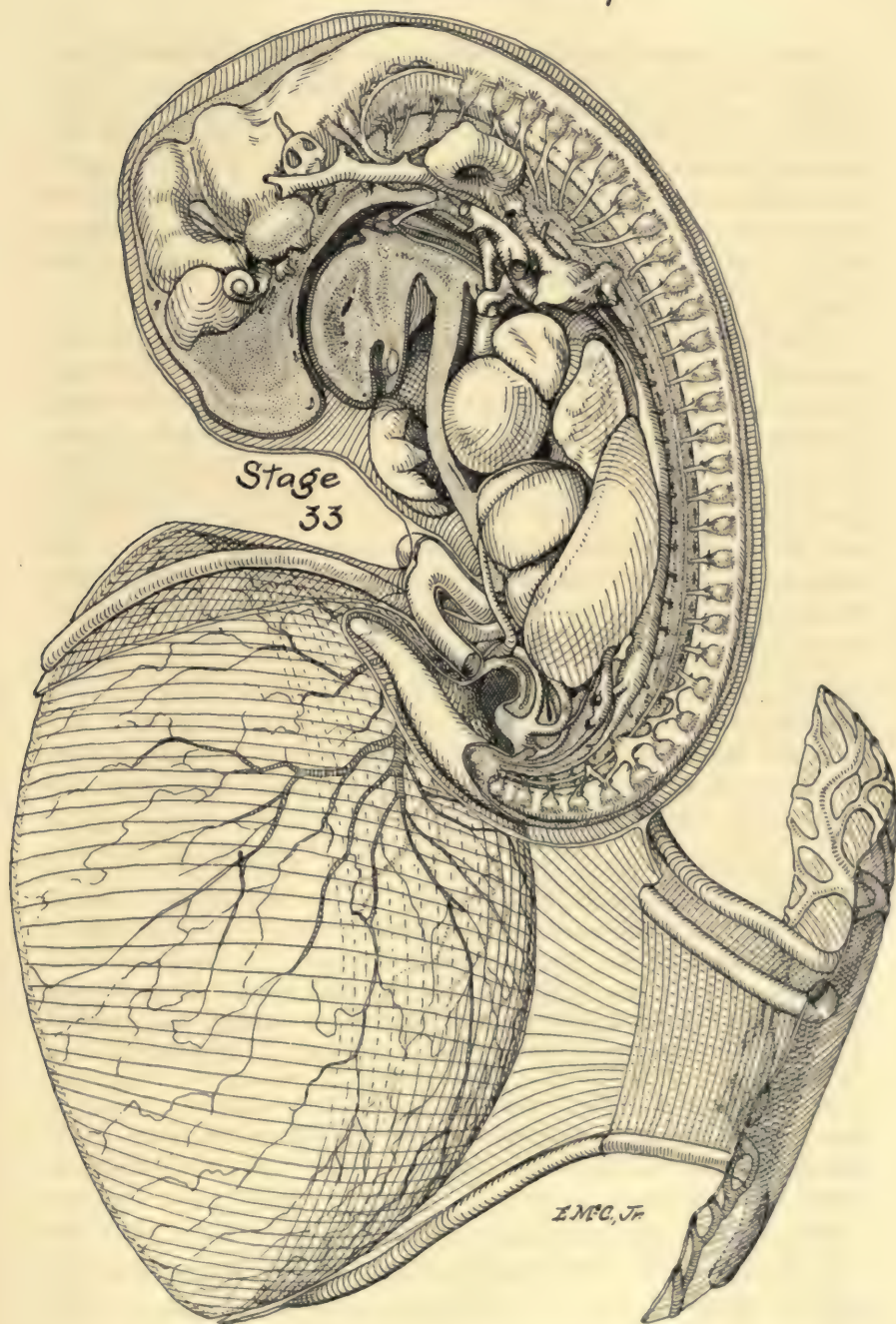


Fig. 52 Reconstruction of stage 33 (17126). Compare with frontispiece.



From the beginning the right and left supracardinals differ in a few minor respects. The right vessel gains access to the common cardinal through a short segment of the postcardinal, which segment is thus preserved and incorporated into the right azygos vein. The left supracardinal, on the other hand, drains directly into the common cardinal, and the entire thoracic portion of the postcardinal degenerates. Both supracardinals are sometimes preserved in the adult opossum, but the left is always the better developed of the two.

*The paravertebral lymphatics and the thoracic duct.* In connection with the dropping out of the lateral radices of the original intersegmental tributaries to the postcardinal, Zimmermann has pointed out that the degenerating vessels go through a history quite like that of the paracardinal plexus. That is to say, they become detached from the stem veins, break up into sacculated, discontinuous, remnants surrounded by the usual peri-intimal spaces. These spaces in the usual way develop an endothelial lining, sprout longitudinally, and coalesce to form a lymphatic channel running longitudinally on the lateral side of the sympathetic trunk. This new channel he calls the paravertebral lymphatic.

At the time of birth (stage 35, 17173) the left paravertebral lymphatic has grown craniad around the dorsal side of the left azygos vein and attained communication with the left thoracic duct. The right at this time is still independent. Both vessels receive the intercostal lymphatics which are apparently derived as sprouts from them.

When in stage 33 the supracardinals replace the postcardinals in the thoracic region, ventromedial sprouts from them into the mediastinum serve, as in the case of the similar ones from the postcardinals, as the skeleton around which more anlagen of the thoracic duct form. The thoracic duct thus grows craniad. Its most cranial anlagen in stage 33 are located at the level of the junction of the common cardinal with the sinus venosus, and instead of being grouped together in the midsagittal plane, they tend to separate into two bilaterally symmetrical channels. This foreshadows the fact that in the adult opossum the thoracic duct branches symmetrically at its anterior end to empty into both jugular lymph sacs.

*Migration of the gill pouch derivatives.* In early stage 32 (16174) the third gill pouches become detached from the pharynx (fig. 47, G). In late stage 32 and in stage 33 the

fourth and fifth gill pouches are also detached and considerable migration has occurred. As there is no histological differentiation between the derivatives of the different pouches at this time, identification becomes complicated. This much can be established with certainty: 1) the thyroid has migrated caudad until it is just ventral to the ultimobranchials (fifth pair); and 2) the third and fourth pairs, including both dorsal and ventral components, have migrated further caudad. The fact that the dorsal (parathyroid) elements of the third and fourth descend with the ventral (thymus) elements into the mediastinum is clear. The only point about which there is any uncertainty is which are the third derivatives and which are the fourth. As both sets have a similar history the question is not important. If, as in other mammals, the third gill pouches migrate most caudad of all, then the four epithelial bodies caudal to the aortic arches and just cranial to the pericardium are probably the two ventral and the two dorsal elements of the third pair. Correspondingly, the four units situated around the common carotid arteries just anterior to the fourth aortic arches are probably the dorsal and ventral elements of the fourth pair. At any rate, in later stages all eight derivatives of the third and fourth branchial pouches come together in the mediastinum to form the thymus and the parathyroids.

It has been known for some time that in the adult opossum no parathyroids can be found in or near the thyroids. This is due to the fact just mentioned, that all four parathyroids become incorporated into the thymus in the mediastinum.

During stage 33 the ultimobranchials come into contact with the dorsal walls of the wings of the thyroid (17151) and fuse with the latter just as the isthmus of the thyroid disappears. These are the so-called 'lateral thyroids' of older authors. Whether they contribute any real thyroid tissue or not, I am unable to say, as they seem to become completely lost in the gland before differentiation of colloid vesicles occurs.

*The allantois at its height.* The principal facts in the development of the allantois have been described in connection

with the discussion of the extra-embryonic membranes in stage 29. But as it reaches its peak of development in stage 33, a few more words about it are necessary at this point.

Figure 49, C, shows how large the allantois becomes at its maximum. In the specimen here photographed the chorion and amnion have been dissected away. The frontispiece and also figure 52 show the relations of the allantois to these other extra-embryonic membranes. Looking at the frontispiece it is easy to visualize the complete vesicle, and to see that the embryo proper and the allantois are in a pocket hanging into and completely surrounded by the endodermal yolk sac, which lines the inside of the entire vesicle.

In this position deep inside the yolk sac the allantoic vessels cannot serve in a respiratory capacity. This function is left entirely to the vitelline vessels, which are permanently superficial. Accordingly, the allantoic vessels do not develop beyond the extent necessary to serve the immediate needs of the thin-walled allantois itself. As the mesonephroi have been functional for at least 24 hours (probably as early as stage 30), and as no mechanism is provided for the transfer of the urine to the maternal blood, the urine has to pile up in the allantois, which correspondingly enlarges to accommodate it.

Finally, in stage 34 the cloacal membrane breaks through and releases urine into the amniotic cavity. From this time on, the allantois looks collapsed as if it had expelled a considerable portion of its contents back through the urachus and cloaca and out into the amniotic cavity. And in harmony with this interpretation the amnion in stage 34 appears rather bloated. These changes occur between 12 and 24 hours before birth.

*Miscellaneous details.* The lateral palatine processes are forming in the mouth on either side of the tongue. The caecum and small intestine still protrude through the umbilicus.

All cranial nerves reach the blastemata of their end organs. The third, fourth, fifth and sixth lumbar, and the first and second sacral nerves develop intercommunications which represent the beginning of the lumbosacral plexus.



There is a well-developed septum spurium in the heart. The conus is divided into pulmonary and aortic halves; and the twisting of the conus has advanced until the pulmonary half is now anterior.

The posterior semicircular canal of the inner ear has been formed. A diverticulum from the nasal epithelium into the septum represents Jacobson's organ. Mihalkovics (1898) commenting on Rose's (1893) description of this organ in the opossum, points out that it is like that of rodents except that it opens into Stenson's duct as in monotremes. Also the fact that its duct arises not from the anterior end of the organ but from the floor behind is a sauropsidan character.

The metanephroi have migrated up to the lumbar region but still consist only of the pelvis and condensed mesenchyme surrounding it.

Hanson ('20) observed the coracoid cartilage in an early stage 33 opossum embryo (H.E.C. 924) extending all the way from the scapula to the sternum without sutures. Later in the same stage (16177) the middle portion of the coracoid disappears and the sternal and scapular ends remain in cartilaginous form. This is the condition when born. In later stages the sternal portion fails to ossify and is resorbed. The scapular portion becomes ossified and attached to the scapula as a typical mammalian coracoid process.

The membrane bones of the skull are layed down in stage 33 but not ossified. All cartilage bones show chondrification except the periotics. Ossification has begun in the clavicle and in the mandible and maxilla. Precartilaginous mesenchyme is recognizable around the trachea.

The first definite anlage of the spleen can be recognized in the thickening of the peritoneal epithelium of the left side of the dorsal mesogastrium. Cells are proliferating from this point into the mesenchyme below.

The dorsal and ventral pancreatic anlagen unite into a single pancreas. The single duct is that derived from the ventral portion.

## XIII. THE THIRTEENTH DAY. Stages 34 and 35

*The first half of the thirteenth day*

Stage 34. External distinctive features. The abdominal, epigastric and umbilical veins. The diaphragm. The perineum. The oral shield. The palate. The mammary anlagen. Connection between thoracic duct and jugular sacs. Miscellaneous details.



Fig. 53 Photographs of 16179 (stage 34). A, ventral view showing oral shield very clearly. Lateral view showing epitrichial growth over eyes, ears, and sides of mouth.

*External distinctive features.* Stage 34 is distinguished from stage 33 by the epitrichium, which covers the eyes, ears, and the sides of the mouth; by the oral shield (fig. 53, A); by the claws on the forefoot and the digital ridges on the hindlimb paddle; by the absence of any external intestinal loop; and by a noticeable elongation of the trunk. It is distinguished from stage 35 by the well-developed oral shield; and by the fact that the digital anlagen on the hindlimb are ridges, not buds.

*The abdominal, epigastric and umbilical veins.* With the collapse of the allantois in stage 34 retrogressive changes become noticeable in the umbilical veins, so that this seems to be a good point at which to review their history and comment on their significance.

In stage 24 when angiogenesis begins, one of the first vessels to appear in the body is a vein running along the lateral body wall. This vein, which has been described as the umbilical, is at first the vein of the anterior limb ridge. In later stages it grows caudad through the lateral body wall until in stages 28 and 29 it reaches the level of the developing posterior limb ridge, which it then invades. At this time the postcardinal has also reached this level and the two form numerous anastomoses within the posterior limb ridge. Simultaneously another complication is introduced by the developing allantois, which becomes involved in this umbilical-postcardinal plexus. As the allantois enlarges and projects beyond the body wall in stages 29 and 30, it carries its portion of the plexus with it, and is thus connected with both the umbilical and postcardinal veins.

In stage 30 the rounding up of the body wall carries the umbilical vein further ventrad, and causes its direct connection with posterior limb ridge to become reduced and eventually lost. Its connection with the anterior limb ridge (the radial vein) is preserved for a longer time, but also eventually (stage 31) disappears. The connection with the allantois, however (and thus indirectly with the hindlimb bud through the postcardinal-allantoic plexus), is strengthened, and from this time on the umbilical vein is devoted almost exclusively to the transfer of blood from the allantois to the liver, and is homologous with the umbilical or placental vein of higher mammals.

The connection between the allantois and the postcardinal is retained at first as a plexus of small veins in the middle of the ventral abdominal wall, running caudad and joining the postcardinal at and around the mouth of the external iliac vein. Later (stages 33 and 34) this plexus is reduced to two



veins in the body wall which empty into the external iliacs, and two in the bladder which empty into the internal iliacs or hypogastrics. McClure ('06) saw the two abdominal veins in the body wall of an 8-mm. opossum (my stage 33). These are strictly embryonic, disappearing in stage 35. The two which with their tributaries drain the wall of the bladder into the hypogastric become the vesical veins of adult anatomy.

The developmental history of this abdominal-umbilical series of vessels has an interesting phylogenetic parallel. In some elasmobranchs there is on each side of the body a lateral abdominal (or epigastric) vein which takes blood from both the pelvic and pectoral fins to the duct of Cuvier. This corresponds to the umbilical vein in the opossum embryo of stage 28. In amphibians and reptiles the hindlimb drains into both the abdominal and postcardinal veins, which is the situation in the opossum of stages 28, 29 and 30. In Amphibia and some reptiles the lateral abdominal veins may migrate to the midventral line and fuse into a single anterior abdominal vein which runs from the external iliac up to the liver and through the latter to the sinus venosus (Kingsley, '26, p. 326). In the opossum the umbilical veins thus unite in stage 34. In the birds the same thing occurs, but in addition, the bladder, instead of remaining entirely within the body in embryonic stages, expands through the umbilicus to form an allantois which, being in the path of this abdominal vein, utilizes it and gives it a new significance. This as already described, is precisely the history of the umbilical vein of the opossum during the last  $2\frac{1}{2}$  days of development. The paired, lateral, abdominal veins thus become the single, ventral, umbilical.

In the placental mammals this umbilical vein is the vein of the placenta, which is formed from allantoic mesoderm. We can, therefore, trace the placental vein of the highest vertebrates back to the lateral abdominal vein of the sharks. The location and course of this lateral abdominal vein, its connection with both limb buds in the opossum embryo, and with both fins in the shark, suggest that if the paired limbs and paired fins of vertebrates were evolved from a primitive paired fin-fold, as is usually taught, then this vein was originally the

vein of the finfold. At any rate, it is the original vein of the limbs from whatever source they came.

*The diaphragm.* The origin of the septum transversum by the lateral and ventral expansion of the sinus venosus, the origin of the pleuropericardial membrane by the medial migration of the ducts of Cuvier; and of the pleuroperitoneal membrane as a ridge which forms at the junction of the pleuropericardial membrane and the lateral body wall, have already been described.

The pleuroperitoneal membrane was described as continuous anteriorly with the anterior tip of the mesonephros, and posteriorly with the coronary appendage of the liver (i.e., dorsal pillar of the diaphragm). Between these two ends the edge of the membrane was in stages 31 to 33 directed toward the interval between the lungs and the mesonephros. Between stages 33 and 34 a considerable elongation of the trunk occurs, which results in carrying the mesonephros caudad away from the lungs. This movement draws the pleuroperitoneal membrane caudad around the posterior tip of the lung. It then fuses with the dorsal mesentery and separates the pleural cavity completely from the peritoneal cavity. Muscle tissue appears in the pleuroperitoneal membranes at this time, and the diaphragm is complete.

*The perineum.* Although figure 54 is taken from a specimen of stage 35, except for the prominence of the phallus it will equally well represent stage 34. Here it may be seen that the tail gut has completely disappeared and the perineal fold has advanced ventrally until it has divided the cloaca into a cranial urinogenital sinus and a caudal rectum. It is important to notice that the true cloaca has thus been eliminated before birth and the anal and urinogenital apertures are separate. There is a widespread impression, which dates at least from the time of Selenka, that the adult female opossum has a cloaca. This is due to a misleading appearance caused by a common sphincter muscle which develops secondarily around the anal and urinogenital apertures as will be described in the chapter on the pouch young.

*The oral shield.* Figure 53, A, shows a front view of a curious, cornified epithelial structure which develops around the oral aperture during the last day of gestation. This apparently useless structure is the most conspicuous and characteristic external feature of stage 34. Selenka (1887, S. 157) first described and figured it, and called it the 'Schnabelschild.' His account is quoted below with the exception of his timing data, which are incorrect.

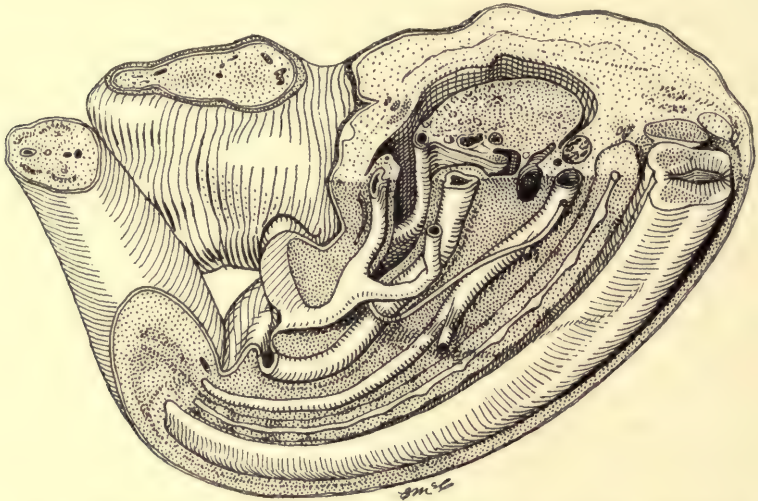


Fig. 54 Urinogenital reconstruction from 17173 (stage 35), showing well-developed mesonephros, only the beginning of a metanephros, and no müllerian duct.

The most remarkable structure of the epidermis is the oral shield, which is illustrated in Plate XXVII . . . . Some hours before birth it shows signs of degeneration, and in the new-born there is only a trace of it left. The complete shield (fig. 5) surrounds the mouth like a flat collar. Its circumference is raised into six prominent points, the lower ones of which are quite sharp. The whole structure is composed of nothing but cornified, epidermal cells.

When I first observed this remarkable lobate structure I was reminded of the horny bill of the monotreme, *Ornithorhynchus*, and I believe I make no mistake in recognizing in this



extuberance the vestige of a horny bill which served the ancestors of the opossum as a grasping organ. It can hardly have any function in the opossum, since at the time of birth it has already degenerated, and can not, therefore, participate in tearing the embryonic membranes.

This structure sometimes appears as early as the end of the twelfth day and then persists through the early part of the thirteenth. Similarly I have obtained one batch of stage 33 embryos 11 days and 9 hours post coitum (i.e., before the middle instead of after the middle of the twelfth day). These last four stages are of rather long duration, and, of course, like all other stages, they are not sharply demarcated but grade into one another. I have placed them in the temporal scale where they seem most typical. Some sources for errors of a few hours in the timing of stages are mentioned in the next section in connection with the discussion of the length of gestation.

*The palate.* Presumably as a result of active opening of the mouth, the tongue drops below the level of the lateral palatine processes which then come together in the midline and fuse below the nasal septum. In their anterior third they also touch the septum and fuse with it. In regard to the opening of the mouth and the relation of the position of the tongue to the lateral palatine processes, it should be mentioned that though the mouth is open to some extent in stage 33 (see frontispiece and fig. 49) the tongue never slips below the palatine processes at that time. The tip of the tongue in the frontispiece is projecting through the incisive foramen, the body of the tongue lies dorsad of the palatine processes, the frenulum of the tongue lies in the narrow slit between the palatine processes. Only the tip of the tongue is seen in this figure. In the posterior part of the angle between the lips the lateral palatine process hides the body of the tongue from view. The oblique line across the mouth is the anterior edge of the palatine process.

In stage 34 though the epitrichium has covered over the sides of the mouth, which gives the impression that the jaws

have been brought together, actually they are farther apart than ever before and the mouth cavity internally is quite large.

*Mammary anlagen.* The cross-cut surface in figure 54 shows an epidermal thickening between the midline and the base of the hindlimb. Medially the thickening projects deeply into the mesenchyme, and on the surface of the body it lifts up slightly above the surrounding epithelium. This is the anlage of a single mammary gland. There is no continuous mammary ridge at first, but these separate anlagen arise in pairs which are arranged into two lines. The most anterior pair appears first, and is situated just cranial of the level of the umbilicus. The more caudal pairs appear in succession forming two lines of separate anlagen which converge toward the phallus. There are usually six such pairs forming a sort of horseshoe, and in addition, a single, unpaired, median nipple at about the level of third nipple from the posterior end in the lateral series. Fairly often there are additional pairs at the anterior ends of the lateral series so that the total may be fifteen or seventeen. In the case of one embryo (17173) I have found the 'median' nipple also paired, making a total of eighteen. In adult females the most anterior pair is sometimes lost, leaving only eleven glands in all. The average number, however, is thirteen. These appear in both male and female embryos, and sex will not be distinguishable in any way until stage 35.

*Connection between thoracic duct and jugular sacs.* In stage 33 a medial sprout from the jugular lymph sac on either side was described as growing toward the region of the thoracic duct. And it was mentioned that at the same time separate lymphatic anlagen of perivascular origin were forming in the mediastinum in two longitudinal rows which diverged anteriorly from the median, single portion of the thoracic duct. By the coalescing of the perivascular lymphatic anlagen and the medial sprouting of the jugular sac extensions the thoracic duct attains connection with both jugular sacs in stage 34. This makes its anterior end complete, but posteriorly there is no cisterna chyli as yet.

The venules around which the arms of the Y-shaped thoracic duct form are tributaries of the common cardinal and precardinal veins principally, with a few in the more posterior levels from the supracardinals.

The thoracic duct proper, exclusive of the cisterna, is thus seen to be derived from three sources: 1) medial outgrowths from the paired jugular lymph sacs; 2) mesenchymal vacuoles in the mediastinum not associated with venous channels; 3) peri-intimal spaces around degenerating mediastinal venules. The venules referred to are tributaries of the postcardinal, supracardinal, common cardinal, and precardinal veins. The postcardinal and supracardinal contribute the plexus around which the anlage of the single median part of the duct forms. The common cardinal and precardinal veins supply the plexus around which the bilateral arches connecting with the outgrowths from the jugular sacs develop.

In the same way, the external mammary lymphatics, which also arise independently from perivenous spaces, attain communication with the intermediate caudal jugular extension. Further caudad the external mammary lymphatics develop connections with the deep axillary portion of the jugular lymph sacs, and sometimes with the subclavian vein near its origin.

*Miscellaneous details.* The chorioid plexus is beginning to form in the fourth ventricle. The cerebellar thickening extends across the midline (Larsell, '35, figs. 20 to 37). The pontine flexure is sufficiently far advanced to cause the head to lift away from the chest.

Semilunar valves have formed in the aorta and in the pulmonary trunk. Mesenteric branches of the omphalomesenteric veins have become the principal channels, as the vitelline plexus is showing retrogressive changes. The allantoic vessels are also becoming reduced in caliber.

The lateral semicircular canal of the inner ear is formed. Jacobson's organ elongates.

The tail contains about 22 somites.



Chondrification has at last begun in the dorsal part of the periotic capsule or petrosal bone. Tracheal cartilages are forming.

*The second half of the thirteenth day*

Stage 35. External distinctive features. The gestation period. Parturition. The migration to the pouch. The negative geotropism. The attachment to the nipple. The respiratory bronchioles. The intranarial epiglottis and the pumping of milk. The 'tubular' muscle fibers. Testis cords and the urinogenital organs. The heart and the cardiac veins. The stomach and the intestines. The sensory organs. The cisterna chyli. The auditory ossicles. Miscellaneous details.

*External distinctive features.* Stage 35 includes specimens shortly before birth (17173) and shortly after birth (17174 and 17175). All of these specimens are distinguished from stage 34 by the almost complete resorption of the oral shield (fig. 55). Of course, the specimens after birth also show some external changes associated with the new mode of life, as the navel in place of the umbilical cord, and the thicker trunk due to the inflation of the lungs and the distension of the stomach with milk.

*The gestation period.* Estimates of the duration of gestation, ignoring for the present the uncertainty about the exact time of fertilization, should be based upon the observation of both copulation and parturition in a considerable number of cases. Such information is not easy to obtain in the opossum. The complicating factors are several. Both copulation and parturition occur most often at night. Copulations which take place during daylight hours seem to be most frequently sterile (see chapter I). But whether fertilization occurs or not, a series of changes simulating pregnancy follows each ovulation. There is no visible enlargement of the abdomen even in late pregnancy, for the newborn opossums are so small that even a large litter takes up no more room than a spoonful of baked beans. Any meal will change the shape of the female

more than pregnancy will. The changes in the mammary glands and genital organs during pseudopregnancy are so similar to those of true pregnancy that so far no one has been able to distinguish the two by any means but one—removing one whole uterus and opening it to see whether it contains embryos or not. Selenka, Hartman and I have all used this method. If the technique has been sterile and hemorrhage has been avoided, the incision may then be sutured and the



Fig. 55 Photograph of stage 35 (17175).

remaining uterus left for any desired number of hours or days before being removed in turn. Or it may be allowed to complete its normal gestation. As the embryos in the two uteri are always at the same stage of development, by removing the two at different times one can obtain accurate information about the intervals between the different stages of development. The operation does not retard the development of the embryos in the untouched uterus or interfere with parturition in any way.

Selenka did not have the good fortune to observe parturition, but by removing the two uteri at different times and at known intervals after copulation he obtained accurate information about the timing of twelve of the thirty-five stages I have defined. According to my stage numbers the stages he thus located are 13, 14, 18, 22, 24, 27 (slightly abnormal specimen, but accurately timed), 28, 29, 32, 33, 34 and 35. His timing is accurate only when computed from copulation, which can be done from the data he gives; but the ages as he recorded them (in terms of hours after the beginning of cleavage) are inaccurate on account of the mistake he made about the time of cleavage. His cleavage stages are all degenerating unfertilized ova, as already pointed out by Hartman. The cases in which he described internal structures accurately are noted in the appropriate parts of this text, but in many stages he omitted the internal anatomy.

Though he did not observe parturition, on one occasion he found a litter of young in the pouch 13 days after an observed copulation. And on another occasion he found embryos still in the uterus 12 days and 18 hours after an observed copulation. He concluded that birth must take place about 12 days and 21 or 22 hours after copulation.

Hartman ('28) using the same method was able to assemble a large number of stages, the intervals between which were accurately known. But in only one case did he observe parturition, and in this case, unfortunately, he had not observed copulation. So his estimate of the length of gestation was based upon a known maximum—the age of the youngest litter he found in the pouch—and corroborative evidence derived by totalling the known intervals between a large number of separate stages. In this way he arrived at a figure similar to that suggested by Selenka—i.e., in the neighborhood of 13 days.

I have found a litter in the pouch 12 days and 19 hours after an observed copulation. In conjunction with Selenka's case of unborn embryos at 12 days and 18 hours, this would seem to locate the time of parturition pretty accurately at about 12 days and 18½ hours.



It is probably correct to think of gestation in the opossum as so consistent and precise in duration as to be defined in terms of hours, not days; for when the whole period is so short and development is so rapid, a difference of a few hours makes a considerable difference in the stage of development, and it is not likely that there can be any great irregularity about the stage of development at birth. On the other hand, all of the above estimates suffer from one defect. Copulation in the opossum is not a momentary affair. It sometimes lasts for 5 hours. When, then, is one to say copulation occurred? My own notes always refer to the time when intromission was first observed. If the time of fertilization could be determined accurately, that would be the proper starting point, but as long as our information about that is only approximate, our knowledge of the length of gestation must be only approximate.

With the above reservation, then, all the evidence seems to point to a rather sharply defined gestation period of about  $12\frac{3}{4}$  days.

*Parturition.* The mother sits on her haunches and licks the vulva, the abdomen, and the inside of the pouch. Contractions of the uterus probably force the young into the vaginal cul-de-sac. The chorions, which are all fused into a single mass and intimately associated with the mucosa of the uterus by complex folds, are left in the uterus. The amnion and fragments of the allantois go with the young into the vaginal cul-de-sac. Contractions of the maternal abdominal wall then cause the lateral vaginal canals, which are bent upon themselves, to become completely occluded, and the increased intra-abdominal pressure can be relieved at only one point—the pelvic canal, which leads to the outside. The posterior wall of the vaginal cul-de-sac is thin and lies in contact with the anterior wall of the urogenital sinus, the two together forming a delicate membrane across the pelvic canal (fig. 5). This membrane ruptures under the increased pressure from the inside, and an artificial, but direct, passage—the pseudo-vaginal canal—is formed. The young pass through this into the urinogenital sinus, and thence to the outside.

The amnion and the allantois are exceedingly thin and delicate. If the surrounding fluid is removed, the allantois will usually break under the weight of its own contents. The amnion is so close-fitting and thin that a single stroke of the foreleg will rip it open. These two membranes are correspondingly torn to shreds very early in the course of parturition, and are usually left in fragments in the vaginal cul-de-sac and urinogenital sinus, though some fragments may sometimes be still adhering to the foetus when he emerges. No such fragments are ever found in the lateral vaginal canals, for the foetus does not pass out that way.

On emerging from the vulva the newborn is thus either entirely free of its membranes, or attended only by fragments of the amnion and allantois. The statement sometimes made that no navel is visible at this time is due either to special use of the word 'navel' or to failure to look in the right place. There is no depression, but the interrupted epidermis and the torn end of the urachus are always distinctly visible a short distance above the tip of the phallus (fig. 56). In this position the navel tends to be concealed by the hind legs and the tail (fig. 55), and careful inspection under the dissecting microscope is necessary to identify it.

*The migration to the pouch.* The method by which the newborn marsupial gets from the urinogenital sinus to the pouch has long been a subject of dispute, and though exact information with reference to the opossum has been available for some time (Hartman, '20), a large number of naturalists remain somehow unconvinced.

The original theory, and one which is still widespread today, is that the mother picks up the newborn young with her lips, places it in the pouch, and holds it there until it is attached to the nipple.

Middleton Michel published in 1847 what purported to be a first-hand account of parturition in the Virginiana opossum. I do not mean to reflect upon the good faith of Doctor Michel's report, but in the light of subsequent observations by several others, I think it possible that he was misled by appearances

*The End of the  
Thirteenth Day*

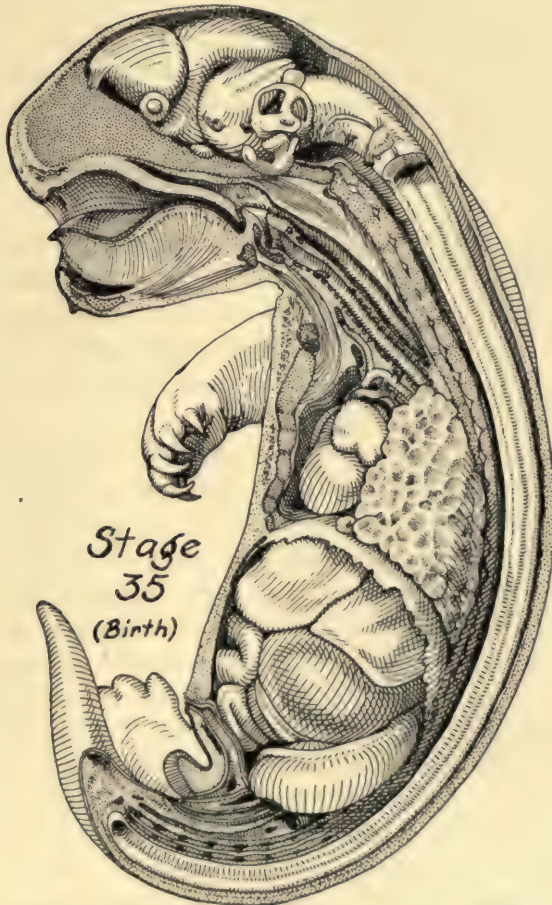


Fig. 56 Drawing from dissection of specimen 17184 (stage 35). To obtain this view of the membranous labyrinth the periotic capsule was removed and cleared in aniline oil.



into thinking that he had observed parturition when he really had not.

On January 28, 1847, Michel observed the copulation of two opossums, and 14 days and 17 hours later he observed what he described as follows:

The pregnant female was found standing on her hind legs; her body was much bent, and propped up against the corner of the cage; her muzzle in immediate contact with the cloacal opening, which was red, tumified and distended; a young appeared at the opening, and was conveyed by the mother's mouth to the pouch, or perhaps was rather licked in, as her tongue seemed busily employed within, around and about the pouch.

The details which make this account questionable are several. In the first place, the period of 14 days and 17 hours is about 2 full days too long for normal gestation. As mentioned in a previous section, it is unlikely that the period varies more than a few hours. A variation of 2 days is certainly abnormal, if possible at all.

In the second place, the external genital region is not 'tumified and distended' at the time of parturition; though to one not thoroughly familiar with its appearance, it might easily appear to be so at any time, for it always has a 'swollen' appearance. That he was not very familiar with its appearance is definitely shown by his reference to the 'cloacal opening.' A closer observation would have shown him that there is no cloaca at all in the opossum. The urinogenital orifice and the anus open separately to the outside (fig. 5). Finally, the wording of his last sentence clearly expresses uncertainty about what he saw. The female was licking the perineal region, the lower abdomen, and the inside of the pouch. The young that he thought he saw at the opening may very reasonably have been the tip of the mother's tongue, for I have often seen it when it could easily be thus misinterpreted. It seems rather likely that the young he found in the pouch had been born about 2 days previously, and all he observed was the mother grooming herself.

Twenty years later another observer (E. S. Hill, 1867) thought that he had seen a similar thing in the kangaroo. While concealed behind a bush watching some wild kangaroos he thought he saw a female pick up 'a stone' or something in her lips. He then saw her put her head into her pouch; and later when he shot her, he found a young foetus inside. The female very commonly licks out the inside of her pouch. He did not recognize the foetus in her mouth; in fact, did not even think of a foetus until he found one in the pouch. Then, of course, it was very easy to interpret what he had seen as he did; especially, as one must admit that a very young marsupial looks altogether incapable of getting into the pouch by its own efforts.

These two observations form the basis for the theory that the mother transfers the young from the vulva to the pouch by means of her lips. One of the very serious objections to the theory is the fact that in some marsupials (e.g., the bandicoots, Peramelidae, and the marsupial mole, *Notoryctes*) the opening of the pouch is directed backward, and it is unlikely that the mother can insert her snout into the pouch at all.

The next important evidence was published in an Australian newspaper in 1913, but was not made generally available until Hartman republished it in his 1920 paper. Mr. A. Goerling had been attracted by the curious behavior of an adult female kangaroo (*Macropus rufus*) which he had in captivity. She had refused food, and he had observed blood marks in the cage. She was sitting with her "tail passed forward through the legs," and "was busy licking and cleaning herself." Goerling says,

Presently she lifted her head, when I was astonished to see a young kangaroo clinging to the long fur about four inches below the opening of the pouch. It moved about slowly, very slowly, through the fur upwards, using the arms in its progress, and continually moving the head from side to side, thus assisting the upward movement. Nearly 30 minutes were required by the little wanderer to reach the top of the pouch, the last end in a semicircle. During the whole

of this time the mother paid no attention to her offspring, offering no assistance, and leaving it entirely to its own exertions.

This was the first time anyone had seen a very young marsupial crawl up to the pouch by its own efforts, but as the author had not actually seen the young emerge from the urinogenital sinus, he could not prove that it was actually newborn. The next question to be settled was: What is the earliest age at which the marsupial young is capable of crawling by its own efforts?

Hartman ('17) made some experiments which answered this question, but which he did not publish until 1920. He removed embryos near term from the uterus, freed them from their envelopes, and allowed them to crawl about over the mother, "which they did for at least fifteen minutes." This definitely established the fact that they are capable of this complicated feat even before birth.

The final link in the evidence was supplied on February 6, 1920, when Doctor Hartman and Mrs. Hartman both witnessed the whole migration from the first appearance of the foetus at the vulva until its attachment to the nipple in the pouch. "Unerringly the embryo traveled by its own efforts; without any assistance on the mother's part, other than to free it of liquid on its first emergence into the world" (Hartman, '20, p. 5).

I have given this rather lengthy summary of the evidence because it does not seem to have found its way into the textbooks of zoology, and the impression that the mother places the young in the pouch is still almost universal. This must be due partly to the extremely immature appearance of the newborn and the consequent difficulty of believing it possible that such a foetus could accomplish so difficult a feat. It may also be due in part to unsuccessful attempts by others to demonstrate this ability in young removed from the pouch for experimental purposes.

I have performed such experiments many times, and for a while without success. My first success came on an occasion



when I had called in two witnesses. Thirteen days and 2 hours after an observed copulation a young opossum had been found in the pouch (its actual birth not having been observed). I removed the foetus from the nipple and placed it on the mother's abdomen. The hair was dry and the foetus stuck to it so that although he struggled vigorously he was unable to make progress in any direction. Wetting my finger I moistened all the mother's hair in that region with saliva, and also moistened the foetus' skin. The latter's movements quickly became effective. In less than 3 minutes it had clambered up the inclined plane of its mother's abdomen and disappeared into the pouch. Three minutes later I open the pouch and found it safely attached to another nipple—not the one it had first appropriated.

The significance of this experiment seems to be that though the mother does not pick up the young, her licking the hair of the abdomen seems to be an essential service.

*The negative geotropism.* Hartman ('20, pp. 255 and 256) pointed out that, "If the skin be tilted, the embryos can be made to travel upward and even away from the pouch, for they are negatively geotropic." Langworthy ('28, p. 214) noted that the vestibular mechanism does not begin to function until about 41 days after birth. So it is evident that if the young does orient itself away from gravity, it does so without the assistance of any vestibular mechanism.

Both of these observations have been confirmed and an explanation suggested as follows (Larsell, McCrady and Zimmermann, '35, pp. 106 and 107):

The effect of gravity on the position of the body of the pouch young, before vestibular reflexes appear, is obvious when the young are placed upon a rough towel or a hairy arm. The forefeet are well enough developed at birth to make possible grasping of hairy or similarly rough surfaces, but the hind feet do not have this capacity. When the surface upon which the young is placed is tilted at the proper angle, the body, held in position only by the grasping action of the forefeet, swings in obedience to the pull of gravity, the outspread hindlegs serving only to brace it and prevent it from rolling. In this manner the head is always directed upward.

The young thus orients itself away from gravity without the assistance of a righting reflex or of any consciousness of 'up or down.' This is a true tropism, or simple, automatic orientation, conditioned solely by the structure of the body at the time—the functional forelegs and the non-functional hind legs.

The mother sits in such a position that the path away from gravity leads to the pouch. And as a result the young wanders blindly into the place of safety and food. Probably many of them miss it, for the young found in the pouch are nearly always fewer than the embryos found in the uteri shortly before birth.

The crawling movements have been studied in some detail. The youngest foetuses which I have tested for reflexes were 11 days and 9 hours after copulation (34 hours before their litter mates from the other uterus were found in the pouch). These stage 33 foetuses were placed in mammalian Ringer's solution. Their vascular system was intact—no embryonic or extra-embryonic vessels either ligated or cut. Their hearts could be seen beating. No foetal respiratory movements were observed. Hairs and fine gut sutures (medium dermis suture—size 000) were used as probes. No reflexes could be evoked by tactile stimuli on any part of the body. Unfortunately, only superficial stimulation was tried, so I am unable to say whether the muscles can respond to deep, direct stimulation, but this is not likely, as not even fixation in Bouin's fluid will cause any muscular contractions at this time. The most serious objection to this experiment is that the Ringer's solution was not artificially heated. The temperature of the room and of the solution at the time was probably about 85°F.

Late stage 34 foetuses, on the other hand, if removed from the uterus and freed from their membranes, execute perfect crawling movements like those of the newborn to be described next. So the development of this complicated behavior is very rapid—the non-motile to the crawling stages in 24 hours or less.

The crawling foetuses of stages 34 and 35 are so ceaselessly active that I have not so far devised a successful method of testing them for reflexes to tactile stimulation. But the crawling movements themselves are interesting. These begin with the lateral flexion of the head and neck followed closely by the forward movement of the ipsilateral foreleg. The head then starts a flexion toward the opposite side while the claws of the forefoot close in a clasping movement and the foreleg is moved backward in an effective, propulsion stroke. The caudal end of the body is then flexed as a whole toward the side on which the effective stroke of the forelimb is being executed. This constitutes a complete cycle. A similar cycle is initiated on the contralateral side when the head moves in that direction. The whole cycle seems to represent a wave of contraction which starts at the anterior end of the body on one side and travels posteriorly, the similar wave of the opposite side starting before the first wave has progressed beyond the anterior limb. This is a typical Coghill ('29) S-reaction except for the precocious individuation in the forelimb.

*The attachment to the nipple.* When the young enters the pouch he finds himself in a maze of tangled, curly hairs among which he must locate one of thirteen, tiny mammary nipples—about the size of the head of a common straight pin. The sensory equipment which he has to assist him in this search, is very scanty indeed, as will be described below. And perhaps the most amazing thing that he does is to succeed in this search; but once in the pouch, he is almost certain to do so. I have watched them do it many times, and I do not yet know what clues they use—certainly not sight, possibly smell, possibly cutaneous sensations of warmth, moisture, and touch. The head is moved in wide excursions to right and left, and when the snout finally touches the soft, moist, and warm nipple, the wandering stops and the young promptly sucks the nipple into its mouth.

The sealing of the lateral portions of the lips by means of epitrichial cells in stage 34 leaves the mouth large inside,



but with only a small aperture to the outside. When the soft nipple is sucked through this narrow opening it swells up on the inside and 'buttons' the young to the mother. For several days this is a weak attachment, but the more the nipple is sucked upon, the more it enlarges at its tip, and the more secure the attachment becomes. After about the fourth day in the pouch the young cannot be removed without considerable effort and danger of tearing the corners of the mouth. This is not due to any organic union between maternal and foetal tissues, as has been popularly supposed, but simply to the fact that the oral aperture is not large enough to permit the

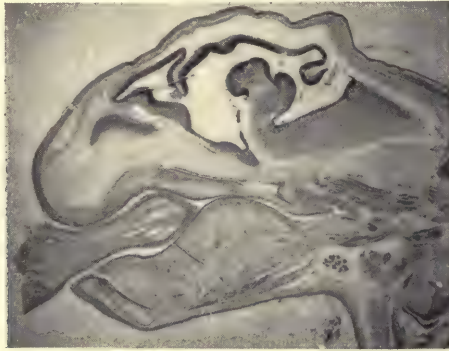


Fig. 57 Photograph of section from an 8-day post partum opossum with the mammary nipple still in its mouth. No fusion of maternal and embryonic tissue occurs.

passage of the bulbous end of the nipple. Figure 57 shows a sagittal section through the head of a young mammary foetus with the nipple in the mouth.

There is also a traditional belief that the young swallows the nipple all the way down to the stomach. This is based upon the observation that after about 6 or 7 weeks of nursing, the nipple stretches to some  $1\frac{1}{2}$  inches in length. But only the tip of this is ever in the young's mouth. The epiglottis is so constructed (v.i.) that the nipple cannot pass it. The long nipple allows the advanced young to nurse from outside the pouch when they have become too large for the pouch to contain them all at once.

*The respiratory bronchioles.* Selenka (1887), who was the first to describe the lung of the newborn opossum, said:

The construction of the lung is precocious in the sense that alveoli capable of respiratory function are developed in an extraordinarily short time; but it is retarded in the sense that the multiplication of the alveoli is deferred to a later time. Probably also hereditary phenomena are at work in this strange developmental history of the opossum lung, for the lung of the new-born has exactly the appearance of a reptilian lung; and since the simple structure of the latter is manifestly adequate for the marsupial young, it can be retained during the early part of life. Not so with the Placentalia, among which the embryo is retained much longer in the uterus. Indeed, even with them the first anlage of the lung is much like that of the reptile and the opossum, but these simple alveoli do not function any longer as respiratory organs, having now become the building loci for the definitive infundibula and alveoli.

Bremer ('04) confirmed Selenka's description in general, but suggested that it is incorrect to call these first functional air chambers 'alveoli.' Histologically, they are really peculiar, modified bronchioles, which function as respiratory chambers for awhile, and then revert to typical bronchioles after the true infundibula and alveoli have grown out from them. The difference between these first 'alveoli' and the true alveoli seems to have been recognized by Selenka in his last line where he distinguishes between 'die einfachen Alveolen' and 'die definitiven Alveolen,' but Bremer's terminology is probably better. A rather more fundamental point was made by Bremer in correcting Selenka's impression that the true alveoli are formed by the ingrowth of partitions which subdivide the primitive respiratory chambers. Bremer pointed out that the infundibula and true alveoli arise as diverticula from the respiratory bronchioles.

One interesting point about these first respiratory chambers is that they are lined with an easily demonstrable respiratory epithelium. The illustration of this epithelium shown in figure 58 is taken from Bremer. The true alveoli do not appear until quite late. Bremer found them in a 14-cm. opossum.

On the average such an animal would be between 70 and 80 days of age. The latter figure represents the usual time of weaning. I have found that the early pouch young cannot control their own body temperature. They are 'incubated' within the marsupium. The control of body temperature seems to begin between the sixtieth and seventieth days. One cannot help but wonder whether the reptilian lung and the poikilothermal condition are correlated. As long as he does not have to maintain a high rate of metabolism to provide his

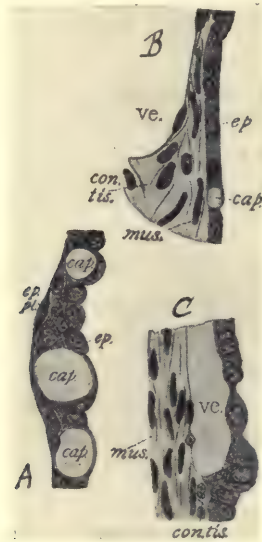


Fig. 58 Functional respiratory epithelium (from Bremer, '04). A and B from a 12.5-mm. opossum. C from a lizard.

own source of heat, the relatively inefficient oxidation accomplished through a visible epithelium may be as adequate for a mammal as it is well known to be for reptiles and amphibians. In the adult opossum, which does control his own body temperature, an epithelial lining is no more demonstrable than it is in any other adult mammal. A careful investigation of the temporal relation between the change in metabolism and the change in alveolar lining should be made (see remarks in the Alveolar Epithelium Conference reported by Macklin, '36).



A few words about the gross anatomy of the lungs are in order. The left lung in the possum is composed of only one lobe, instead of two (fig. 56). The right lung, on the other hand, has in addition to the usual three, an accessory or infracardiac lobe the bronchus of which arises near the hilum of the lung on a level with the first hyparterial bronchus, and runs medially and caudally beneath the heart. Jazuta ('32) observed that the right and left pleural cavities in the opossum communicate with each other in the dorsomedial line. This interpleural opening has been found in *Didelphys*, *Perameles*, and various birds. This fact and the fact already referred to, that the functional lung of the opossum at birth is composed only of bronchi and bronchioles without alveoli, Jazuta regards as strong evidence that the Marsupials represent a connecting link between the Eutheria and the Sauropsida.

I have not been able to find in any of my specimens, either before or after birth, a left eparterial bronchus at the level of or anterior to the left hyparterial. Bremer ('04) described such an eparterial bronchus in five newborn opossums, but mentioned that it is always "smaller and slightly lower placed, and the chambers supplied by it *do not form the apex of the lung.*" As in all my specimens the only such bronchus I can find is well below the left hyparterial, I am forced to suppose that what he saw was either an abnormality, or, more likely, the first left dorsal bud, which occurs also in man and in mammals generally, and is certainly of no phylogenetic significance. There are many mammals, both high and low in the phylogenetic series, in which a true left eparterial bronchus has been described—*Bradypus*, *Phoca*, *Equus*, *Auchenia*, *Elephas*, *Phocaena*, *Delphinus*, and *Cebus* (to take a list from Goodrich, '30, p. 610)—and at best their phylogenetic significance is very uncertain.

*The intranarial epiglottis and the pumping of milk.* As the term implies, the epiglottis in the opossum projects into the posterior nares or choanae (fig. 56). It is rather tubular in form, and in this position fluid can pass around it on both

sides, without necessarily interrupting breathing, and without danger of choking. Incidentally, this condition persists in the adult.

Such an arrangement is found also in all the Cetaceae and in scattered members of all the other mammalian orders. In the case of the marsupial mammary foetus it lends credibility to the theory first proposed by Seiler (1828) that the young does not suck, but has its milk pumped into it by a muscle in the maternal marsupium which is homologous to the cremaster of the male and which he called the iliomarsupialis or compressor mammae. This so well developed in some forms (e.g., *Thalacynus*—see Cunningham, 1882, pl. 4) as to provide an obvious mechanism for the purpose.

On the other hand, in the opossum there seems to be no mechanism for the pumping of milk, and the newborn foetus can be seen and heard to suck. Indeed, it is only by suction that it draws the nipple through the narrow, external aperture of its mouth. It seems certain that not only the opossum but all newborn marsupials can suck. The pumping of milk by the mother is not universal, and is only a supplemental mechanism if it occurs.

*The 'tubular' muscle fibers.* The functional muscles in the newborn opossum are histologically unique. Selenka has illustrated them beautifully in longitudinal and cross-sectional views (1887, pl. XXIX, figs. 6 and 7). The fibers are striated, but the nuclei, instead of being disposed peripherally, are aligned in a single axial row. All the striated fibrillae are confined to the periphery. The cytoplasm between the nuclei is clear and undifferentiated.

I know of only one other instance in which the nucleus of a striated muscle cell is located in a non-fibrillar central core. This is in the heart muscle of the gasteropod mollusc, *Sycotypus canaliculus* (Dahlgren and Kepner, '08, fig. 91), but in this case, as in heart muscle generally, there is only one nucleus in each cell; whereas, in the opossum's tubular fibers there are numerous nuclei in tandem along the non-fibrillar core.

This curious arrangement, however, is only transitional in the opossum, for by the time the pouch young is 2 weeks old all of the nuclei have migrated to the periphery, and the whole fiber is quite like that of any other mammalian striated muscle.

*Testis cords and the urinogenital organs.* Though sex cannot be distinguished externally until some 12 to 14 days after birth, the first histological differentiation can be recognized in stage 35. The female gonad at this time still appears indifferent, but the male gonad develops clearly defined testis cords containing the primordial germ cells.

At this time there is still no sign of a müllerian duct (figs. 54 and 59). The ureters as they enter the bladder are medial to the wolffian ducts. Tubule anlagen are just beginning to form in the metanephroi. The mesonephroi are highly developed and functional. These primitive kidneys remain functional for about a week after birth in the opossum.

*The heart and the cardiac veins.* A short resumé of previous stages will help to clarify the following description. During the concrescence of the heart tubes the primary head vein and the umbilical vein on each side fuse together and then develop a communication with the vitelline vein on each side to form a sinus venosus (stage 25). At this time the paired heart tubes have fused only in the bulbar region. In stage 26 the ventricles fuse, and in stage 27 the atria fuse and the two venous sinuses open into the single atrium separately on either side of the foregut. At stage 30 the two sinuses have come together so that there is only one valve into the single atrium, and the septum primum now begins to form just to the left of this valve, so that when the single atrium becomes divided into two again, this valve empties into the right one of the two new atria.

The twisting of the heart has progressed so far at this time that the left precava is pulled up tight against the atrium of the left side, passes beneath the pulmonary vein, and at the back of the heart lies in the crease between the atrium and the ventricle. Here in stage 35 it receives one or more small tributary veins from the wall of the left ventricle before it



empties into the sinus venosus. This condition persists in the adult, for the two precavae do not develop any cross anastomosis as in *Eutheria* but remain permanently independent of each other. The left precava thus serves to drain



Fig. 59 Photograph by Dr. A. A. Zimmermann of a section through the cloaca in specimen 16175 showing entry of ureters into cloacal horns medial to wolffian ducts. No müllerian ducts are present.

the blood from the left wall of the heart, but at the same time retains its original function of draining the left side of the head and neck. It is thus both coronary sinus and precava.

The veins just described as emptying into the precava are the left cardiac veins, but in the opossum the largest cardiac vein, the vena cordis magna, has been shown by McClure ('03) to run in the ventral interventricular furrow until it reaches the auriculoventricular groove, then to pass dorsad between the root of the pulmonary artery and the left auricle and empty into the right atrium as in birds. This is also characteristic of *Thalacynus* as Cunningham (1882) has shown. Such a condition is never found in placental mammals.

*The stomach and the intestines.* Heuser ('21) studied the digestive system in stages 34 and 35. He found that the stomach becomes enormously larger immediately after birth due to distension with milk (fig. 60), and that its gross anatomy is like that already described for the adult opossum by Bensley ('02). "Glands however," he says, "are nowhere present, nor can any of the elements be regarded as cells associated with the formation of acid as found in the functioning stomach of higher mammals." The stomach, therefore, is a reservoir at this time, and does not otherwise participate in the digestive process.

The same author observed that:

Villi are present in all parts of the small intestine, although they are older and more advanced in the duodenum . . . . In many places between the villi there are patches of epithelial cells which are smaller and more darkly stained than those covering the villi . . . . The large intestine had changed to a much smaller degree relatively in the transformation from the late embryo to the pouch young. Although the external caliber of the colon has increased considerably in the interval of growth, there is but little advance in the epithelial or mesoblastic differentiation . . . . The relatively slight dilation of the colon and the histological findings indicate that the residue of the foodstuffs reaching the colon in the opossum pouch young is relatively small; practically all of the milk must be disposed of in the upper portions of the alimentary canal.

*The sensory organs.* The eye of the opossum at birth is in a very embryonic condition. The lens vesicle is in process of developing a fiber nucleus; that is to say, the cells of the medial

wall have elongated, but have not yet obliterated the cavity of the lens. These primitive lens fibers are still nucleate. The pars optica of the retina shows only the cellular and fibrous layers, no ganglionic or rod and cone layers having

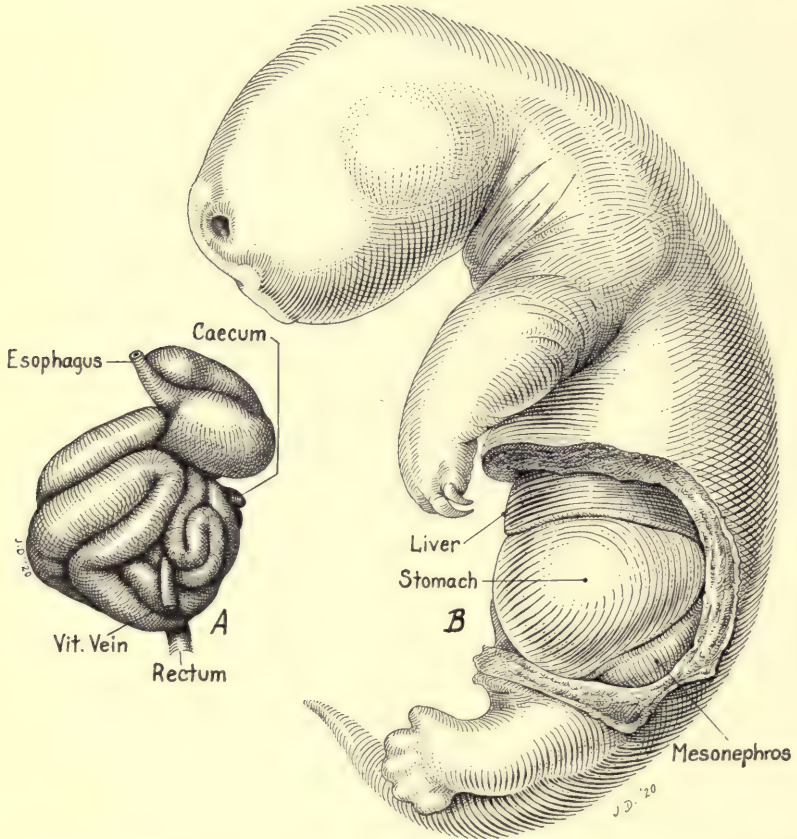


Fig. 60 Dissection of abdominal viscera of embryo and early pouch young (from Heuser, '21). A, stage 34. B, stage 35.

yet differentiated. Pigmentation of the outer layer has just begun. The cornea has not cleared. Mesenchyme which will later give rise to the vitreous body is beginning to filter into the optic cup, through which a single vascular loop runs. This stage is similar to that of a 12.5-mm. human foetus except



that in the opossum the eyelids have become fused together and covered over by the epitricium. Externally there is no indication of the eye, except that in the living specimens the pigment of the retina can be faintly seen through the epitricium.

I have removed the lens from a newborn opossum, and found that 15 days later (17183) there was no regeneration, and in the absence of the lens the cornea had not cleared and the retina had atrophied to a considerable extent. The control eye in the same individual had advanced to a stage similar to that of a 3-month human foetus.

Like the eye, the ear at birth is completely non-functional (Larsell, McCrady and Zimmermann, '35). The cochlear duct (fig. 56) has grown out to about one-half turn. It contains no organ of Corti, but the epithelium of the tympanic wall is slightly thicker than that of the vestibular wall. There are no hair cells, tunnel of Corti, tectorial membrane, scala tympani, or scala vestibuli. The external auditory meatus is completely plugged with epitrichial cells, and the latter also overlie the developing pinna.

In the vestibular portion of the inner ear the ampullae of the semicircular canals are recognizably indicated, and the utricular and saccular anlagen, though not yet separated from each other, are identifiable. The only indication of the cristae and maculae is the slightly thicker epithelium at the appropriate points. There are still no signs of cupulae, otolithic membranes, or perilymphatic space. No nerve endings are present in either the acoustic or vestibular part of the inner ear.

The olfactory organs, on the other hand, may possibly be in a functional condition. The conchae are not well developed (about like those in a 3-month human foetus), but the olfactory epithelium is ciliated, and nerve fibers from this epithelium enter the olfactory lobe of the forebrain in large numbers. Selenka, also judging from histological appearances, considered this organ functional.

There are no papillae or taste buds in the tongue.

Selenka (1887, p. 160) claimed that no organs of touch are present, but perhaps this question has not been approached with adequate techniques. It is hard to imagine that the complicated crawling movements with their necessary component—the clasping of the mother's hair by means of the claws and toes of the forefeet—can be accomplished without some sort of exteroceptive apparatus in the skin.

*The cisterna chyli.* In the opossum the cisterna chyli is not developmentally a dilatation of the thoracic duct. It is derived principally from a mesenteric lymph sac which develops in stage 35 and which is homologous with the mesenteric lymph sac of rabbit, pig and cat embryos. This secondarily establishes communication with a peri-aortic lymphatic plexus which leads into the caudal end of the thoracic duct. The peri-aortic lymphatic plexus later enlarges and forms the cranial portion of the cisterna, which is permanently rather plexiform.

In addition to the rather poorly developed connections of the mesenteric sac with the peri-aortic plexus in stage 35 there is a well-developed connection with the peri-oesophageal lymphatics. This curious arrangement is later abandoned, only the peri-aortic connections being preserved. Doctor Zimmermann has suggested that the temporary connection with the peri-oesophageal lymphatics is associated with the precocious development and functioning of the digestive tract.

Among other minor changes in the lymphatic system at about the time of birth, the jugular lymph sacs attain their maximal size, and the axillary portions establish communications with the deep lymphatics of the forelimb. The thoracic duct enlarges markedly in caliber, and caudally it attains communication with the mesenteric lymph sac as described above.

No lymph nodes have been developed in any part of the body at birth.

*The auditory ossicles.* In examining the earliest stages in the development of the ossicles in the opossum I have been unable to find any clear connection between the stapes and

the hyoid arch at any time. As such a connection has been described in a great many mammals this matter needs further study, and I have referred the problem as well as the detailed study of the development of all the ossicles to Mr. J. A. McClain who is now working in this laboratory. Accordingly, I shall content myself in this paper with a brief comment on only two stages.

Figure 61 shows the condition of the ossicles at birth. This is a familiar arrangement and needs very little comment. Meckel's cartilage extends from its articulation with the incus all the way forward through the core of the mandible. The

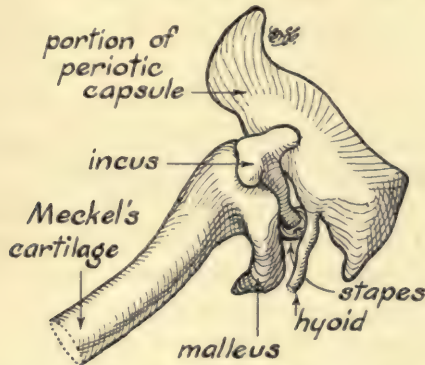


Fig. 61 Reconstruction of cartilaginous auditory ossicles at time of birth (17175). Lateral view.

stapes is penetrated by the stapedial artery, a branch of the internal carotid. The hyoid cartilage is continuous with the periotic capsule and makes no contact with the stapes, which is medial and slightly anterior to it.

Figure 62 shows a much later stage near the time of the beginning of auditory function. This specimen was 49 days of age (i.e., post partum), and was tested for auditory reflexes before he was killed. No reflexes were observed, though in the light of later studies (McCrary, Wever and Bray, not yet published) it seems likely that the first electrical responses of the cochlea could be detected at this time.



The principal point to be noticed here is the extraordinary anterior process of the malleus. This process extends in a semicircle halfway around the tympanic annulus, and it persists in the adult. It is derived, not from Meckel's cartilage, which is also shown in the figure, but from a membrane bone, presumably the prearticular, which partly encloses the posterior end of Meckel's cartilage just as the dentary encloses the anterior end. The portion of Meckel's cartilage lying in the groove of the prearticular degenerates without ossification. All of the manubrium and possibly also the head and neck of the malleus, however, are formed from the extreme tip of Meckel's cartilage. The foramen shown in the anterior process admits the passage of the chorda tympani nerve.

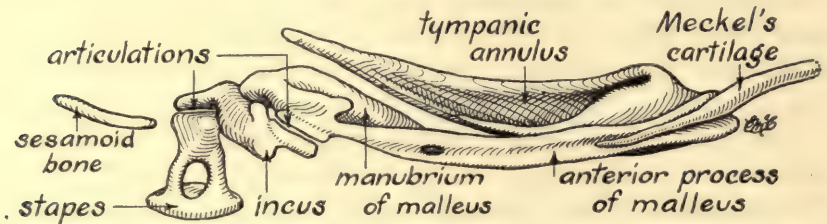


Fig. 62 Reconstruction of ossicles and tympanic annulus 49 days after birth showing the extraordinary anterior process of the malleus. Dorsal view.

My tentative view of the homologies of these structures is: The original mandibular arch becomes subdivided into an upper palatoquadrate and a lower Meckel's cartilage. The posterior end of the palatoquadrate ossifies to form the quadrate of reptiles, which becomes the incus of mammals. The posterior end of Meckel's cartilage ossifies into the articulare of the reptiles, which becomes the manubrium, and possibly the head and neck, of the malleus of mammals. The anterior process of the malleus, which is exceptionally large in the opossum, is formed from a membrane bone, the prearticular of reptiles. Another membrane bone, the angulare of reptiles, becomes the tympanic annulus of mammals.

This accounts for all but the stapes, the only one of the ossicles about which there has been general agreement in the

past. Mr. McClain's more detailed study may yet disclose the connection between the hyoid arch and the anlage of the stapes which I have been unable to find in the opossum. But even if it does not, this connection has been found in so many other mammals that the weight of the evidence will still favor the homology of the stapes with part of the hyoid arch, and with the medial end of the columella of lower vertebrates.

*Miscellaneous details.* The nasal septum fuses completely with the palate. Stenson's canals remain open. Labiodental ledges are forming in upper and lower jaws. The nasolachrymal cord is still solid and still does not reach the nasal epithelium. The vomer is represented by two cartilages as in reptiles, rather than by one as in mammals. In the adult opossum, however, there is a single, median vomer, as in other mammals. This is strong evidence of the homology of the reptilian and mammalian vomers, usually considered a moot point.

The interatrial septum still shows a few very small perforations. The ductus Bottali is constricting, but still patent. The interventricular septum is incomplete in the bulbar region. The twisting of the bulbus is complete, so that the pulmonary trunk, which was originally caudal to the aorta, has passed through three-quarters of a turn and now lies to the right of the aorta.

The tail has about 24 somites, which makes the total number of segments formed 52. This is the final number.

The medulla of the adrenal gland is in process of formation from chromaffin cells.

The splenic epithelium separates from the underlying mesenchyme, but there are no splenic sinuses and practically no blood vessels. This situation is similar to that of a 15-mm. pig.

There is no corpus callosum between the cerebral hemispheres, and none ever develops in the opossum. Johnston ('13), however, by means of degeneration experiments has shown that in the adult opossum certain fibers in the dorsal commissure serve the same purpose and probably represent the phylogenetic anlage of the corpus callosum.

The erythrocytes at birth and for some days afterward are oval and nucleate like those of lower vertebrates.

#### XIV. APPENDIX

Postnatal development. Some experimental techniques. The evolution of the mammals.

*Postnatal development.* As its title indicates, the proper province of this book does not extend beyond the time when the newborn young reaches the pouch and becomes attached to a mammary nipple. But though embryology proper may be said to end at that time, so much of development remains to be accomplished that a few words about the pouch young seem necessary before closing.

The earliest pouch young (i.e., the newborn opossum described in the preceding chapter) averages about 11 mm. in crown-rump length, though many individuals may be as small as 10 mm. C.R.L. It has a fairly well-developed circulatory system; lungs which are functional, though unfinished; a digestive system which is also functional, but unfinished; a muscular system which is partly functional, but unfinished; a skeletal system which is almost entirely in the cartilaginous and membranous stages; a central nervous system which has virtually no cerebellum (Larsell, '35), but is well developed in the hind-brain and cervical cord; a functional mesonephros and a non-functional metanephros; only the earliest stage of histological sex differentiation in the gonads; non-functional eye and ear, and only non-functional endocrine gland Anlagen.

At this stage of development the young opossum becomes so securely attached to its mother's mammary nipple that it cannot detach itself for some 50 days. And during this interval it brings the development of all its organs to a condition roughly approximating that of most newborn placental mammals. A few details of the changes during this 50-day period will be mentioned.

About 1 week after birth the metanephros begins to function and the mesonephros shows retrogressive changes. At about the same time the müllerian duct Anlage appears. It arises as an invagination from the coelomic epithelium into the ventral border of the extreme anterior end of the mesonephros. A very early stage of the diverticulum is found in a 17-mm. male specimen lent to me by Dr. C. F. W. McClure of Princeton University. The development of this duct and the part it plays in the formation of the female genital tract have been studied by Dr. J. S. Baxter ('35). The following details are taken from his paper.



The müllerian duct tunnels into the mesonephros and grows caudad as a solid cord which follows the course of the wolffian duct but does not apparently receive any cells therefrom. When it reaches the urinogenital sinus it fuses with its wall at the point which represents the old cloacal horn. The epithelium of this horn meanwhile has proliferated until it has filled the lumen and converted the horn into a solid cord separating both the müllerian duct and the wolffian duct from the cavity of the urinogenital sinus. Of course, the wolffian duct cannot function at all after it loses communication with the cavity of the sinus, and the retrogression of the mesonephros thereafter is rapid. The solid sinus cord later becomes the caudal third of the lateral vaginal canal which (as Hartman, '23, has shown) remains uncanalized until just before the first prooestrus. The müllerian duct forms the cranial two-thirds of the vagina. This is the situation which Buchanan and Fraser ('18) found in *Trichosurus*, and which Hill and Fraser ('25), on the basis of an histological study of the adult, predicted would be found in *Didelphys*.

To revert now to the 1-week-old opossum, in addition to having a functional metanephros and the beginning of a müllerian duct, it also has the beginning of a tectorial membrane in the inner ear, and the cochlear duct has grown out to one and one-half turns (fig. 63). The lagena or apical tip is still actively growing. The vestibular part is just completing the growth which will soon separate it from the sacculus everywhere except at the very slender *canalis reuniens*. The only part of the duct which has already ceased to grow is the second half of the first turn. This region which ceases to grow first gets a 'head start' in differentiation, and the first appearance of the tectorial membrane is at this point. The differentiation of other structures in the inner ear will follow this same course, that is, each new structure appears first in the apical part of the first coil and later spreads in both directions from this point.

About  $1\frac{1}{2}$  weeks after birth sex becomes distinguishable externally on account of the appearance of the pouch rudiments in the female and the scrotal rudiments in the male. Both pouch and scrotum are represented at first by paired bilateral folds. The position of the scrotal folds in the male corresponds to the position of the posterior end of the pouch folds in the female. Just as in the female the posterior ends of the marsupial folds approach each other and fuse to form the posterior part of the pouch at a point just cranial to the clitoris; so in the male the scrotal folds approach each other and fuse to form a scrotum just cranial to the penis (fig. 64). In later stages both the pouch and the scrotum migrate further cranially so that they are finally situated a considerable distance anterior to the clitoris and penis respectively (fig. 5).

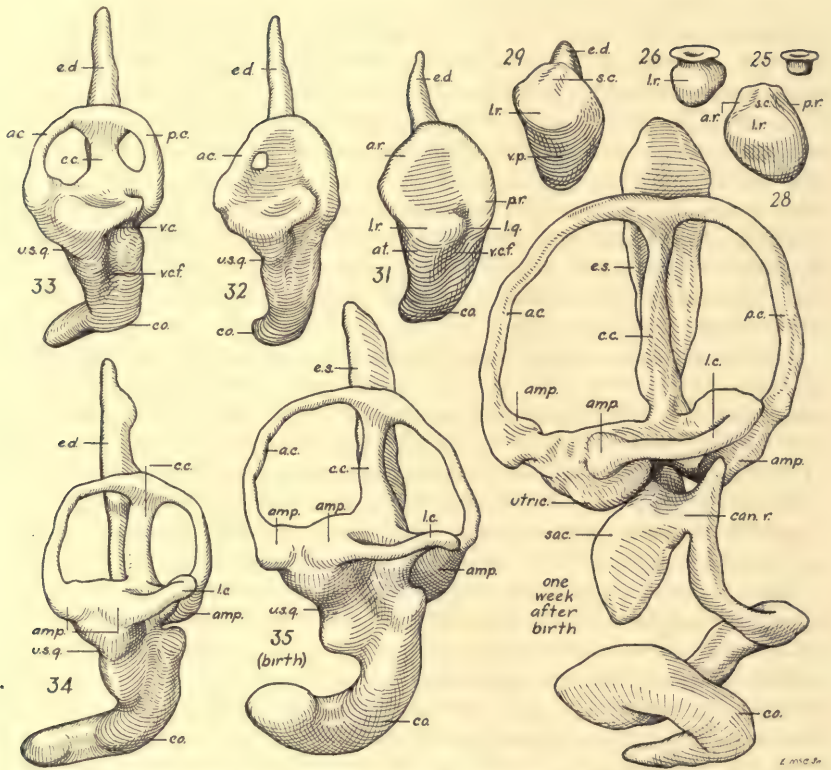


Fig. 63 Lateral view of reconstructions of inner ear from stage 25 until 1 week after birth (from Larsell, McCrady, and Zimmermann, '35).

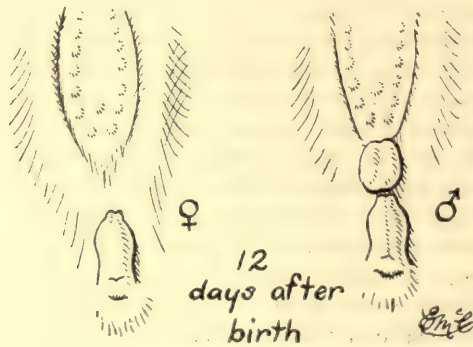


Fig. 64 Rudiments of pouch and scrotum 12 days after birth.

This perfect parallelism between the development of the scrotal anlagen and the lips of the pouch makes it impossible for the present author to avoid the conclusion that the lips of the pouch represent the labia majora, the homologues of the scrotum in higher mammals. The situation of the labia majora in this case some distance anterior to the clitoris is certainly no more surprising than the situation of the scrotum at the same distance anterior to the penis. The fact that this homology has apparently never been suggested before, though a number of investigators have studied the development of the pouch and the descent of the testes, suggests that there must be some obvious and fundamental objection to it; but I confess I have been unable to think of any such objection.

Nerve endings appear in the cochlear duct and in the vestibular parts of the inner ear about 4 weeks after birth, but neither the acoustic nor the vestibular apparatus is functional at this time. Vestibular reflexes appear about 6 weeks and acoustic reflexes about 7 weeks after birth. The range of hearing is at first restricted and sensitivity is poor. As sensitivity improves, the range of hearing extends to higher and lower notes. The first notes heard are around 1500 c.p.s. which is about the center of the adult opossum's range.

The 50-day-old opossum is about the size of a mouse, and has a short and sparse coat of fur. Sometime between then and the sixtieth day it opens its eyes and mouth, and for the first time since birth is free to crawl about on its mother's body. It cannot yet control its body temperature, however, and does not leave its mother. Also it is not yet weaned. It must continue to nurse for some 30 more days, and it returns frequently to the pouch for this purpose. If separated from its mother at this time, its body temperature soon goes down approximately to room temperature, and it goes into a sort of coma resembling hibernation.

By the time it is 70 days of age the young can control its body temperature, can hear five or six octaves, and can venture on slight excursions away from its mother; but it still returns to the pouch for food. By 80 days it has become the size of a large rat. The mother's pouch has enlarged enormously to accommodate the growing young, but it cannot hold a large litter of 80-day-old specimens. Accordingly special arrangement has to be made for the last part of the nursing period. This arrangement is that the mother's nipples elongate to some  $1\frac{1}{2}$  inches, which makes it possible for the young to lie outside the pouch while they nurse.

The first tooth to erupt is the deciduous third premolar, which appears at about 60 days of age and has a curious molariform shape. It functions as a molar until sometime between the ninetieth and the three-hundredth days when it is shed and replaced by a permanent



premolar. With this one exception the milk dentition is abortive—that is to say, the other deciduous teeth do not erupt, but remain as functionless vestiges until a comparatively late stage. The second tooth to erupt belongs to the permanent set. It is the second premolar and is present at 75 days. Between the seventy-fifth and eighty-fifth days the last four incisors, the canine, and the first premolar, erupt. By the ninety-fifth day the first molars and the first incisors are present, and only the last three molars and the third premolar remain to be cut.

With teeth ready for solid food, the young are ready to be weaned. This usually occurs between the eightieth and the ninetieth days. At this time the animal has developed his adult auditory range—about eight octaves (McCradly, Wever and Bray, '36). Curiously enough, the notes he hears best from this time on (about 7000 c.p.s.) are not at the center of his range, but are decidedly nearer the high end. And this fact is probably responsible for the fact that he can be startled more violently by rustling leaves or hay than he can by talking or even loud shouting. The sounds made by the rustling of leaves are composites of short wave lengths, and are much louder to the opossum's ear than they are to ours.

The sound which the young makes when separated from the mother is also a composite of short wave lengths and is probably very loud to the opossum's ear.

Very soon after the mother has weaned a litter and ceased to lactate, she goes into another oestrus. I have seen a female copulate on the second day after she was separated from her litter. She produced a new litter 13 days later.

*Some experimental techniques.* It is hoped that one of the uses of this description of normal stages of opossum development will be to serve as a background for various experimental studies. In this connection it may be of interest to mention some of the experiments which have been performed on embryos and pouch young in this laboratory to indicate the type of procedure employed and the suitability of the animal for operative work.

The only experiment with embryos so far is the preliminary study of the behavior of specimens of stages 33 and 34 already described on page 182. The advantageous feature of the opossum embryo for such studies is that it has no placenta, and correspondingly, can be removed from the maternal uterus without the ligation or section of any part of its circulatory system.

On specimens of stage 35 two operations have been performed. The lens vesicle of the eye has been removed, and the limbs have been amputated. In neither of these cases was any anaesthetic employed on the pouch young. If the mother was not too excited she also was

not anaesthetized. My assistant laid her on her back on the operating table, held the back of her neck with his left hand, and held her pouch open with his right. This is the simplest procedure, and the most desirable when possible; but if the mother becomes too excited, as is sometimes the case, she must be anaesthetized. We have usually used an ether cone for this purpose. If any of the ether passes through the mammary gland into the milk, it is not enough to have any recognizable effect on the young in the pouch.

In the removal of the lens from the eye the epitrichium between the lids was cut with a small pair of spring scissors the tips of which had been specially ground to a very fine point. Then a small sliver of steel broken from the edge of a thin safety razor blade, and held in a small needle chuck, was used as a knife for slitting the anlage of the cornea. When this was cut, gentle pressure on the side of the optic cup with the tip of a dull probe, caused the lens vesicle to pop out. If the original incision through the epitrichium is along the line of fusion of the lids, there is no bleeding in this operation. The incisions were allowed to close by themselves.

In the amputation of the legs three methods were tried by Dr. Olof Larsell and myself, and only one method found satisfactory. Cauterization and simple snipping off with fine scissors both lead to high mortality. On the other hand, if a loop of fine, white, cotton thread is tied tightly about the limb, the portion distal to the ligature can be cut off immediately or can be left until the following day and then cut off. Survival in this case is usually excellent, and not even a scar can be found on the stump at later stages. Occasionally the ligature slips, or is not made tight enough to begin with, allowing blood to get into the limb. In several such cases the result was hemostasis (due apparently to occlusion of the veins, which are superficial, but not the arteries, which are deep), with great swelling and discoloration of the limb and eventual death of the individual so affected. Both hind limbs can be amputated at one time without danger, but when a hindlimb and forelimb were removed from the same individual it was found advisable to amputate one at a time, with an interval of 24 hours for recovery after the first operation.

Though regeneration was not the subject of these studies, it may be of interest to note that none occurred in any of these cases. At the time of the operation the hindlimb was only an embryonic paddle with small digital buds, and the opossum is more primitive than any mammal the embryo of which has hitherto been tested for regeneration.

Both of these operations were done with the young still attached to the mammary nipple. In fact, this attachment was found to be a convenient mechanism for immobilization of the head. The rest of the body was immobilized for the eye operation by means of a strip of adhesive tape fastening the young to the mother's abdominal wall.

When the operations are performed less than 4 days after birth, it is fairly common for the young to become detached and fall out of the pouch. After about a week no such danger is encountered—even if the specimen dies, he remains attached to the nipple. From this time until about 45 days after birth, if the young is forcibly removed from the nipple, he is usually unable to re-attach, and so dies. After about 45 days they can be removed without danger of tearing the lips, as the mouth is nearly ready to open spontaneously, and when replaced in the pouch the young will resume nursing.

In connection with studies of the development of hearing (done in collaboration with several others) I have operated upon pouch young between 48 and 80 days of age in order to place an electrode upon the

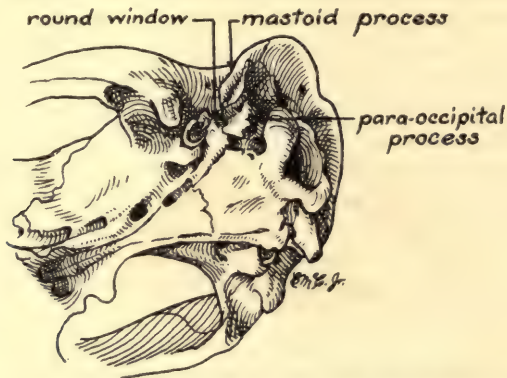


Fig. 65 Ventrolateral view of posterior part of adult skull (from McCrady, Wever and Bray, '37 a).

round window for the purpose of observing the Wever and Bray phenomenon. The operative procedure is rather different from that employed on placental mammals, because in the opossum the squamosal, petrosal, and tympanic bones do not fuse to form a single temporal bone, but remain permanently separate as in reptiles (fig. 65). Accordingly, the middle ear is not encased in bone. In adults prolonged muscular traction has caused the mastoid portion of the squamosal bone to elongate and slightly overhang the round window. For this reason, animals less than 100 days old are preferable for this operation.

Figure 66 shows the operative field in a specimen 78 days old. The following summary of the operative procedure is taken from McCrady, Wever and Bray ('37):



After anaesthetization with Avertin, injected intraperitoneally in doses of 0.0001 cc. per gram of body weight a skin incision some  $\frac{3}{8}$  inch long was made in the direction indicated in this figure. With a long, slender, pointed scalpel the loose connective tissue behind the facial nerve and immediately ventral to the mastoid process of the squamosal bone was then trimmed away. The sterno-mastoid muscle is continuous with the trapezius from their common origin on the mastoid process down to the point where the great auricular nerve comes between them. It was sometimes necessary, particularly in the older specimens, to cut away the muscle tissue for a few millimeters at this common origin to expose the endodermal lining of the middle ear. The exact point at which the endoderm was then cut is caudal to the exit of the facial nerve, ventral and as close as possible to the mastoid process, and dorso-cranial to the origin of the digastric muscle. Little harm can come from cutting somewhat too far caudad,

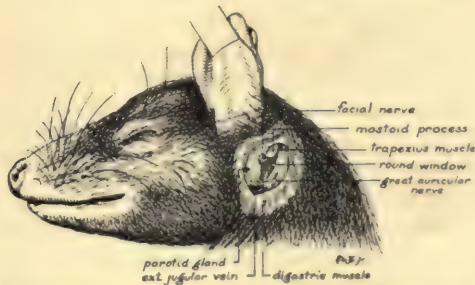


Fig. 66 Middle ear operation in 78-day-old opossum (from McCrady, Wever and Bray, '37 a).

as the paraoecipital process (fig. 65) limits digression in that direction; but it is important not to cut too far cranial as the tympanum and ossicles may be injured. The best limiting landmarks in the cranial direction are the exit of the seventh nerve and a small tubercle on the petrosal bone . . . seen in figure 65 below and slightly to the left of the round window.

My former conjecture that this tubercle "probably represents the styloid process of higher mammals" was quite wrong. The attachment of the hyoid cartilage to the periotic capsule at the end of the embryonic period (fig. 61) is dorsocaudal to the stapes and the fenestra ovalis instead of ventrocranial to the fenestra rotunda, and there is no indication of this tubercle at this time. The latter arises much later as a process of the petrosal bone not associated in any way with the hyoid cartilage. It provides a surface for the attachment of the caudal border of the petrosal-alisphenoid ligament which forms part of the ventral wall of the middle ear cavity.

After a similar surgical approach I have used a very small dental drill to destroy parts of the cochlear duct (McCrary, Wever and Bray, '37). After the lesion had been made in the inner ear a few dermal sutures were made and the deeper structures were left to take care of themselves. The sutures were sometimes covered with thin celloidin. Aseptic precautions were observed throughout the operation, and healing was found to be very rapid. Survival was almost 100%. From 1 month to 3 months after the first operation a second operation was performed for the purpose of making electrical tests of the experimental ears. A comparison of the range of hearing before and after such operations is in progress.

*The evolution of the mammals.* One cannot examine a large number of embryos of any species without pausing from time to time to reflect upon the subject of evolution. The study of a particularly primitive mammal leads one almost irresistably to the subject of mammalian phylogeny.

The theory which has been the basis for all discussions of this subject in modern times was published by Prof. T. H. Huxley in 1880. He divided living and extinct mammals into three great subclasses—the Prototheria, the Metatheria, and the Eutheria. Of these, he conceived that the Prototheria in an unspecialized form evolved first. They then split into two branches. One branch by a relatively slight departure from the original stem became the modern Monotremata. The other gave rise to the Metatheria, which in turn split. One group of Metatherians by relatively slight specialization became the modern Marsupials; the other gave rise to the Eutheria. So in the modern world we have somewhat specialized representatives of all three of the ancestral stocks—the Monotremes are slightly specialized Prototherians; the Marsupials are slightly specialized Metatherians; the Placentalia or Monodelphia are Eutherians in various degrees of specialization.

This has been, and is today, the most widely accepted theory of the evolution of the mammals; but it has met with considerable opposition—principally as a result of the discovery of many supposedly Eutherian characters among the modern marsupials. Such discoveries have led a few to believe that the modern marsupials are a degenerate group descended from Eutherian ancestors (e.g., Dollo, 1898, and Hubrecht, '08).

Wilson and Hill (1897) and Hill (1897), while not accepting that terminology, that is to say, not granting that the marsupials are descended from Eutheria, at any rate, feel that they are descended from a stock which was both diphyodont and placental. In other words, they believe that the Metatheria developed a double dentition and an allantoplacenta before they split up into the Marsupialia and Eutheria.



Bensley ('01 a, '01 b, '03) points out that as far as the diphyodont condition of the Metatheria is concerned, that is exactly what Huxley hypothesized in his original paper (1880, p. 655); and it seems to be well substantiated by the finding of a suppressed milk dentition among modern marsupials. But as to the placental condition, only one marsupial is known to have an allanto-placenta comparable to that of Eutheria, and that marsupial, *Perameles*, is one of the more specialized forms, not a very primitive one. Accordingly, he thinks it more likely that this is a case of evolutionary convergence.

Flynn ('22) argues that the extraordinary similarity of the allanto-placenta in *Perameles* to that in the Monodelphia makes convergence seem an inadequate explanation, and that all of the aplacental marsupials (i.e., most of the marsupials) must be degenerate in this respect, not primitive. This seems unsound for two reasons. There are too many other instances of striking convergence between the marsupials and the placental mammals; and there are too many other anatomical indications that the most primitive marsupials are aplacental. In a similar way one might argue that the extraordinary similarity between the incisors of the Diprotodontia and those of the Rodentia proves that the marsupials are derived from rodent ancestors, and that all of the Polyprotodontia are degenerate, not primitive.

Striking examples of homoplasy or convergence are not to be wondered at among members of a single Phylum and Class. If the eye of a mollusc is found to be identical in almost every detail with the eye of a vertebrate, one may justifiably express consternation; but among animals universally believed to have had common ancestors at a not very remote geological period, this kind of convergence is not only natural, but common. The detailed parallelism between the adaptive radiation of the Australian marsupials and that of the monodelphia, which has been known ever since the days of Cuvier, furnishes numerous instances in point. Witness the extraordinary similarity between the marsupial mole and the eutherian mole, the marsupial rodent and the eutherian rodent, the marsupial cats and the eutherian cats, the flying phalangers and the flying squirrels, etc.

In regard to the evidence that the most primitive marsupials are aplacental, we may ask what features we may regard as indications of primitiveness. If we take the same criteria which have been used in working out the evolutionary tree of the Eutheria, we can establish a very similar tree for the marsupials.

Dollo (1899) showed very convincingly that the feet of all modern marsupials can be derived from an ancestral form very remarkably like that of the modern opossums (*Didelphyidae*), but that even this primitive foot pattern included one specialized feature—the hallux of



the hind foot is opposable, an arboreal adaptation. This is in line with Huxley's theory that one of the distinctive features of the marsupials when they first emerged from the primitive Metatheria was the assumption of an arboreal habit. So this evidence points to the opossum, an aplacental marsupial, as near the bottom of the evolutionary tree, and *Perameles*, with its enlarged fourth digit, reduced hallux, and syndactylous second and third digits, as one of the more specialized forms.

Bensley showed that the teeth of the American Didelphyidae are nearer the original stem than are those of any other living marsupials; and the "The molar tooth patterns of the stem form are almost exactly reproduced in those of the Oligocene Opossums (*Peratherium*)" ('01 b, p. 249). Both in number of teeth and in elaboration of the molars, *Perameles* is more specialized than is the opossum.

Brass (1880) showed that the most primitive form of the vaginae is found in the Didelphyidae and that every gradation can be found between this form and the peculiar median vagina in the specialized Macropodidae.

Wallace (1876) pointed out that during the Tertiary period in Europe there were no other marsupials than the Didelphyidae, and Lydekker (1896) considered the most primitive Australian marsupials, the Dasyuridae, to be descended from the Oligocene Didelphyidae of the Northern Hemisphere.

All these lines of evidence converge to indicate that the aplacental opossums are the most primitive living marsupials, and that the nicely graded series which Hill (1897) pointed out between the allanto-placenta of *Perameles*, the allantois in contact with the serosal membrane, but not forming a placenta (*Phascolaretus* and *Aepyprymnus*), and the small allantois enclosed in the yolk sac (*Didelphys*), probably indicates an evolutionary line in the opposite direction from that inferred by him. That the non-placental allantois is among marsupials the more primitive, is also suggested by its widespread distribution (*Didelphyidae*, *Phalangeridae*, and *Macropodidae*). In the light of the development of the respiratory allantois in the Sauropsida, there can be no doubt about the fact that the opossum's allantois is degenerate; but the evidence indicates that it has degenerated from the sauropsidan condition, not from the eutherian. The early birth and precocious functioning of the lungs in marsupials removes the need for a respiratory allantois. The opossum's lung begins to function at a much earlier stage of general body development than does that of the chick (see chapter IX, p. 90). With its respiratory function usurped by the precocious lung, it is not surprising that the allantois of most marsupials is to some extent degenerate.

Bensley ('01 a) has also pointed out the following facts about the mammary apparatus and the pouch. The observation of Gegenbaur that the mammary glands of monotremes are specialized sudoriparous glands whereas those of marsupials and placentals are of sebaceous origin is sometimes cited as evidence that the marsupials are derived from the Eutheria, not from the Prototheria. Bensley quotes Gegenbaur (1880, p. 35) to the effect that he found some sebaceous milk glands even in the monotremes and he recognized the probability that in the original prototheria both sorts of glands were present. In the modern monotreme one type has been emphasized, so to speak, and in higher mammals the other. Klaatsch (1892) found both sorts present in the artiodactyl ungulates, which confirmed this interpretation.

Klaatsch also recognized the homology of the tubular teat cavities of the artiodactyl ungulates and the circular folds which surround the nipples in marsupial embryos. He was of the opinion that the pouch of the marsupials is formed by the fusion of these teat cavities, and correspondingly he argued that the ungulates could not have passed through a marsupial stage. Bensley mentions that this is beside the point, as Huxley had regarded the pouch as a character of modern marsupials, and had suggested that the Eutheria were derived, not from the marsupials, but from the primitive Metatheria. Furthermore I am dubious about these teat cavities having anything to do with the formation of the pouch. If the theory of pouch formation that I suggested in a previous section of this paper be correct, then the primitive Metatheria must have had paired, longitudinal, labio-scrotal folds, and from these are derived both the pouch of the marsupials and the labia majora of the placental mammals. In this feature, as in most others, the modern Didelphyidae and Dasyuridae would be nearer the original ancestral stock than any other living marsupials.

Lest in this emphasis on a few debatable points we lose sight of the myriad anatomical features in which the marsupials are nearer the Sauropsida than are the placental mammals let me call to mind again: the presence of both ducts of Cuvier; the absence of a septum secundum in the heart; the absence of a corpus callosum between the cerebral hemispheres; the separate squamosal, petrosal, and tympanic bones; the presence of a complete coracoid cartilage in the embryo. And then we may add a few which have not been mentioned before in this paper.

The cells of Paneth are not confined to the glands of Lieberkühn, but are distributed all over the surface epithelium of the intestine, and are primitive in structure. This is like the condition in the lizard, and unlike that of placental mammals (Klein, '06). The

tapetum lucidum of the eye is in the pigment layer instead of in the chorioid, and is composed of crystals of guanin instead of fibrous cells. This is like the condition in the crocodile family, and in contrast to the condition in the ungulates and carnivores (private communication from G. L. Walls). The subintestinal vessel is a vein as in lower vertebrates, not an artery as in all placental mammals (Kimball, '28). The capillaries of the cerebral cortex form simple loops between arterioles and venules instead of being plexiform as in higher mammals. This is like the condition in amphibians and reptiles (Wislocki, '37).

It is difficult to imagine that all or even a large proportion of these features are the result of degeneration from a eutherian condition. It seems much more reasonable to interpret them as evidence that the marsupials have diverged less from the original common stock than have the eutheria. Everything seems to bring us back to Huxley's original theory essentially unscathed. The only modification of it which seems possible is the one advocated by Wilson and Hill, and Flynn—that the metatheria acquired an allantoplacenta before they gave rise to the marsupials and the eutheria. This is possible; but neither compelling, nor, I think, likely.

One point on which almost all the evidence is in agreement is that the Didelphyidae are the most primitive marsupials alive today, and that fact adds another source of interest to the embryology of the Virginia opossum.

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In response to numerous requests from this country and abroad for an opossum bibliography, it has been considered desirable to bring together at this place a rather comprehensive list which may be of wider interest than the volume to which it is attached. The references listed below, therefore, are not all cited in the text. Many deal with subjects other than embryology. A few are concerned with related animals, not with the opossum itself. Fewer yet refer to animals not even closely related, but yielding data which throw light upon some observation or principle referred to in the text.

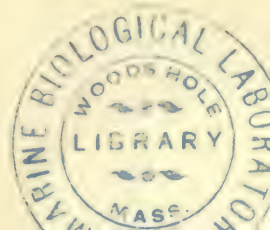
The original list compiled by the author included a much larger number of titles. It has been contracted to the present size by the elimination of those which could not be consulted on account of inaccessibility of the journal, or incompleteness or incorrectness of the reference data; those which deal with distantly related marsupials a knowledge of which is not very pertinent to anything discussed in the text; and finally, a few which are apropos in subject matter, but so superficial or incorrect as to be considered more disconcerting than useful. This last category of eliminations might have been made more extensive, but the author was anxious to err in the direction of over-comprehensiveness rather than of unduly personal selectiveness.



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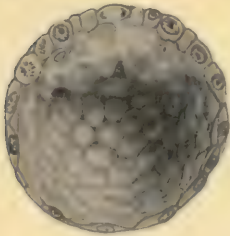
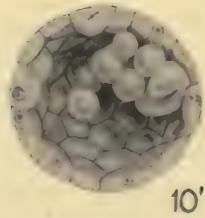
PLATES

## PLATE 1

### EXPLANATION OF FIGURES

- 67 Normal embryonic stages 1 to 13.
- 1 One-celled ovum.
  - 2 Two-celled ovum.
  - 3 Three-celled ovum.
  - 4 Early 4-celled ovum with large blastomeres.
  - 4' Late 4-celled ovum with small blastomeres.
  - 5 Six-celled ovum.
  - 6 Eight-celled ovum.
  - 7 Twelve-celled ovum.
  - 8 Sixteen-celled ovum.
  - 9 Thirty-two-celled unilaminar blastocyst.
  - 10 First endodermal mother-cells.
  - 11 Earliest medullary plate and considerable yolk in gastrocoele.
  - 12 Expanded and attenuated vesicle with endodermal cells still almost entirely under medullary plate.
  - 13 Ellipsoid blastocyst eccentric in albumen and with endodermal cells spreading toward equator.

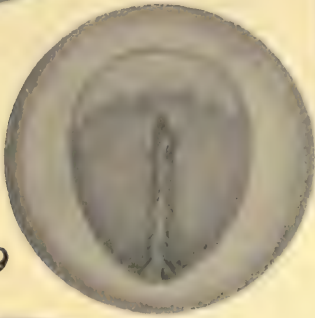
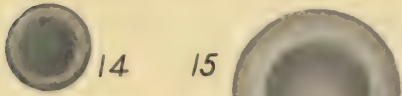




## PLATE 2

### EXPLANATION OF FIGURES

- 63 Normal embryonic stages 14 to 23.
- 14 Spherical bilaminar blastocyst.
  - 15 Clear spot in medullary plate.
  - 16 First mesodermal cells forming cloud in the clear spot.
  - 17 Primitive streak with mesodermal crescents.
  - 18 Hensen's node.
  - 19 Primitive groove. Mesoderm still only beneath medullary plate.
  - 20 Clear spot in front of Hensen's node. Mesoderm extending beyond medullary plate.
  - 21 Notochord equal to or shorter than primitive groove.
  - 22 Notochord longer than primitive groove. First somites forming.
  - 23 First coelomic rudiments. No heart. No optic cups.





### PLATE 3

#### EXPLANATION OF FIGURES

- 69 Normal embryonic stages 24 to 35.
- 24 Optic cups but no contact of medullary folds.
  - 25 Contact of medullary folds, but no proamniotic fold.
  - 26 Proamniotic fold, but no caudal amniotic fold.
  - 27 Caudal amniotic fold, but anterior neuropore and otocysts still open.
  - 28 Anterior neuropore and otocysts closed. Amniopore still open.
  - 29 Amniopore closed. Forelimb becomes a club.
  - 30 Forelimb paddle. Secondary lumbar flexure.
  - 31 Diameter of allantois approximately one-third length of body. Hind limb bud. Frontal process.
  - 32 Diameter of allantois approximately two-thirds length of body. Hind limb club.
  - 33 Diameter of allantois equal length of body. Hind limb paddle. Eyelid folds.
  - 34 Oral shield. Epitrichium covers eyes, ears, and sides of mouth. Claws on forelimb digits.
  - 35 Oral shield practically resorbed. Umbilical cord lost or very much constricted.



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