TRANSCRIPTIONS OF NARRATIONS FOR EMBRYOLOGY OF THE BRANCHIAL REGION

Branchial Arch Development, slide 2
This is a very familiar picture - a median sagittal section of a four week embryo. I have actually done one thing correctly, I have eliminated the oropharyngeal membrane, which does disappear sometime during the fourth week of development. The cloacal membrane, as you know, doesn't disappear until the seventh week, and therefore it is still intact here, but unlabeled. But, I've labeled a couple of things not mentioned before. First of all, the most cranial part of the foregut, that is, the part that is cranial to the chest region, is called the pharynx. The part of the foregut in the chest region is called the esophagus; you probably knew that. And then, leading to the pharynx from the outside, is an ectodermal inpocketing, which is called the stomodeum. That originally led to the oropharyngeal membrane, but now that the oropharyngeal membrane is ruptured, the stomodeum is a pathway between the amniotic cavity and the lumen of the foregut. The stomodeum is going to become your oral cavity.

Branchial Arch Development, slide 3
This is an actual picture of a four-week embryo. It's about 5mm crown-rump length. The stomodeum is labeled - that is the future oral cavity that leads to the pharynx through the ruptured oropharyngeal membrane. And I've also indicated these ridges separated by grooves that lie caudal to the stomodeum and cranial to the heart, which are called branchial arches. Now, if this is a four- week old embryo, clearly these things have developed during the fourth week, and I've never mentioned them before. Previously, I treated the fourth week simply as if it were a period of embryonic folding. But obviously, other things are going on during the fourth week, and the development of branchial arches is one of those things. Let me point out, that in our own bodies the region between the mouth and the heart is largely composed of the neck. So when I talk about branchial arch development, I will be talking about development of the neck.

Branchial Arch Development, slide 4
Let's go back to a median sagittal section of a four-week embryo. I hope you've realized that I have pretended that the folding was completed before the brachial arch development even began. But in fact, these things are all going on simultaneously. But allowing me this pretense, let's take a kind of section that we haven't taken before. This will be equivalent to a coronal section in which some dorsal portion of the embryo is removed from some ventral portion.

Branchial Arch Development, slide 5
And we will ask Dr. Larson to look down on to the cut surface of the ventral portion of the embryo. We can predict what she will see as you look at this from left to right, she is going to see the ectoderm which covers the head region, then she will see a cut through the brain, then the notochord beyond that, and then some mesoderm which fills the head region, and then she will come across the cut surface of the pharynx, and then she will look down through the opening of the ruptured oropharyngeal membrane, and then further to your right as you look at the slide, she will be seeing the floor of the pharynx.

Branchial Arch Development, slide 6
Here are all those structures I had mentioned that Dr. Larson will see. I've done one thing, obviously; had she looked down on it as I had drawn, the brain would be up towards her forehead and the heart would be down towards her chin. But I've rotated everything 90 degrees so the brain is to your left (not up by your forehead) and the heart is to your right (not down by your chin), so I would have a wider area in which to draw these things. We can also see things to the left and to the right of the median sagittal plane that we hadn't talked about. First, we realize that even in the region where the neural tube will form the brain, there are clumps of neural crest cells on either side of the developing neural tube. And also, there are somites on either side of the neural tube (I haven't labeled them here). We do see in the median sagittal plane the region of the ruptured oropharyngeal membrane that I have encircled with little yellow dots for reasons that elude me. But out to the side of the pharynx, we see something that you may have forgotten about. We see pericardioperitoneal canal portions of the coelom on the left and on the right, and we also see the aortic arches which lie cranial to the coelom. These have emanated from the

heart tubes, swept around the sides of the foregut, joined one another dorsal to the gut tube to form the aorta, which then runs caudally in the embryo ventral to the notochord. And now I can give a more precise definition of the neck. It is the region cranial to the coelom and caudal to the mouth, which is represented here by the ruptured oropharyngeal membrane. You look at this picture, this embryo has a very short neck; there is not much cranial to the coelom and caudal to the mouth. And what there is of the neck contains the first aortic arch running through it.

<u>Branchial Arch Development, slide 7</u>
Let's take a cross-section through this neck region, a cross-section through the aortic arches.

<u>Branchial Arch Development, slide 8</u>
Here's that cross-section through the neck. Many things are unlabeled. Neural tube is not labeled, neural crest is not labeled. I haven't labeled somites or notochord, but you should be able to identify them. Ventral to the notochord on either side of the midline are two arteries, these are coming from the dorsal regions of the aortic arches and are traveling into the head of the embryo. I didn't give them names. On either side of these two arteries are the anterior cardinal veins, which I mentioned briefly when we talked about the chest. But I have labeled on the right the first aortic arch and there's one clearly on the left as well, and I have labeled the pharynx in the midline. All the light pink stuff which fills up the embryo is just intraembryonic mesoderm derived from the primitive streak.

<u>Branchial Arch Development, slide 9</u>
Now we really need to make this neck very much longer. And we're going to do it, or the embryo is going to do it, by growth predominantly in the region between the first aortic arch and the cranial end of the coelom.

<u>Branchial Arch Development, slide 10</u>
Now we might expect that this growth will come about by replication of cells in the lateral plate mesoderm to fill in the enlarging area, and I'm sure some of this must occur. But actually, something very surprising accounts for most of the new material. Neural crest cells associated with the developing brain migrate into this newly created neck region of the embryo, and in fact they make the major contribution to its cellular composition.

<u>Branchial Arch Development, slide 11</u>
The neural crest cells that do this are really odd, because they turn into a kind of tissue that is really indistinguishable morphologically from other mesodermal tissue. But of course, those cells are not derived from mesoderm as we know it, they are derived from neural crest, which is ultimately derived from ectoderm, and so the special kind of tissue created by this migrating neural crest is called ectomesenchyme. I want to emphasize that the ectomesenchyme cells are morphologically indistinguishable from lateral plate mesoderm cells. However, the ectomesenchyme cells express different gens than the lateral plate cells and will have a different fate by virute of that.

<u>Branchial Arch Development, slide 12</u>
Now I want to go off on a little side track to tell you all the things that neural crest becomes. Neural crest is a very important tissue. Many of the structures of the head and neck that we would expect to be derived from mesoderm, are in fact derived from ectomesenchyme. I have listed these in item 1 of the table. Some anomalies of the head and neck are thought to be due to problems with neural crest formation and migration in this region. Some things you know come from the neural crest are the spinal ganglia, that is the dorsal root ganglia. I will add here that some of the cells in cranial nerve sensory ganglia come from neural crest as well. All of the autonomic ganglia, that is sympathetic and parasympathetic ganglia, come from neural crest. The adrenal medulla, which is like a sympathetic ganglion, but with no postganglionic axons, comes from neural crest. Dr. Larson may have told you that the spiral septum, which separates truncus arteriosus into a pulmonary outflow tract and an aortic outflow tract, is derived from neural crest of the head region which has migrated down to the heart region, and probably some of the cusps of the aortic and pulmonary valves are derived from such migrating neural crest. And in fact there are associations of head and neck anomalies with heart anomalies probably all accounted for by problems with neural crest migration from the head region. Melanocytes of your skin that produce pigment come from neural

crest. Schwann cells, which provide the myelin of the peripheral nerves, come from neural crest, and item 8 lists some odds and ends which you really don't have to know about.

<u>Branchial Arch Development, slide 13</u>
Now I could have left these ectomesenchyme cells green to remind us that they come from ectoderm, but since in fact they are going to become mesodermal structures, I decided to change their color to brown, which is as close as I could come to mixture of green and pink.

<u>Branchial Arch Development, slide 14</u>
And the next thing we'll see is that arising on either side of the pharynx in series are these endodermal outpocketings called pharyngeal pouches - four on the left and four on the right. And opposite these pharyngeal pouches are ectodermal inpocketings of the outer surface of the body, and these are called branchial clefts - again four on the left and four on the right. And that means that the ectomesenchyme and lateral plate mesodderm of the neck is starting to be broken down into chunks between one branchial cleft / pharyngeal pouch pair and another branchial cleft / pharyngeal pouch pair. Each of these chunks is called a branchial arch. We'll number them in later slide. This slide also shows that as the branchial arches develop, the lateral plate mesoderm of each one aggregates into the center of the arch to form a mesodermal core surrounded by the ectomesenchyme of that arch.

<u>Branchial Arch Development, slide 15</u>
This shows some considerable progress since the last slide. First, the pharyngeal pouches have grown further, and they have contacted the branchial clefts. The zone of contact, an ectodermal/endodermal plate, if you will, is called a branchial septum. The branchial arches are clearly demarcated from one another. The ectomesenchyme that lies cranial to the first branchial cleft and pharyngeal pouch is called the first branchial arch, and it houses the first aortic arch. And then in sequence caudal to it, are the other branchial arches, the last one being immediately cranial to the coelom. Now you will have noticed that there are five branchial arches. But they are not numbered 1, 2, 3, 4, 5. They are numbered 1, 2, 3, 4, 6, because of this little structure to which an arrow is pointing. This is an abortive fifth pharyngeal pouch. There is no corresponding fifth branchial cleft. As a result, people believe that there is an undeveloped fifth branchial arch, which should have been given the number 5 had it been fully developed, but since it isn't there, the fully developed arch which lies cranial to the coelom is called branchial arch number 6. This slide shows several other things. It shows that for each branchial arch there is an aortic arch. In truth, the branchial arches are created in sequence _ number 1 being created first and number 6 being created last _ because the pharyngeal pouches and branchial clefts are created in sequence. The aortic arches, as Dr. Larson I'm sure mentioned, are created sequentially. There is one for each branchial arch. The aortic arches are also numbered 1, 2, 3, 4, 6. You see, however, I have drawn in a tiny little fifth aortic arch associated with the abortive fifth pharyngeal pouch. Even that tiny little fifth aortic arch is rarely present, and if so, it quickly degenerates.

<u>Branchial Arch Development, slide 16</u>
And finally, this slide shows one of the things that the ectomesenchyme of an arch forms. In each arch it provides the cells for a bar of cartilage. The cartilage runs dorsoventrally within the arch; that means we caught it in section here. It starts behind the plane of the computer screen, and passes in front of the plane of the computer screen. There are five branchial arch cartilages on each side; they form the skeleton of a branchial arch.

<u>Branchial Arch Development, slide 17</u>
I would like you to know what happens to the branchial arch cartilages. As this slide indicates to you, the first arch cartilage becomes some bones - the malleus and incus of the middle ear; and then its perichondrium, that is its connective tissue sheath, becomes a ligament called the sphenomandibular ligament, which you will see in dissection. The second branchial arch cartilage becomes some bones - the stapes, the styloid process of the temporal bone, the lesser horn of the hyoid bone, and the superior portion of the body of the hyoid bone; and its perichondrium becomes the stylohyoid ligament that connects the styloid process of the skull with the lesser horn

of the hyoid bone. The third branchial arch cartilage becomes the greater horn of the hyoid bone and most of the body of the hyoid bone. Very recent studies have shown that the thyroid, cricoid, and arytenoid cartilages come from ectomesenchyme. Presumably they come from the branchial arch cartilages of the 4th and 6th arches, but that has yet to be shown definitively.

Branchial Arch Development, slide 18
Let's take a cross-section through the second branchial arch, specifically through the back end of the second arch so that we will be going through its mesodermal core and the the second aortic arch.

Branchial Arch Development, slide 19
Here is that cross-section. I've indicated the right and left second branchial arches as bulges out to the side.

Branchial Arch Development, slide 20
This will be a new kind of cross-section because, rather than going through a branchial arch, it goes through a branchial cleft, branchial septum, and pharyngeal pouch.

Branchial Arch Development, slide 21
Here is that cross-section as labeled; it's pretty self-explanatory. The previous slide indicated it was taken through the second pharyngeal pouch and branchial cleft, but at this stage it would look the same no matter which pharyngeal pouch and branchial cleft it was taken through.

Branchial Arch Development, slide 22
You've heard the phrase "ontogeny recapitulates phylogeny", which means that in many ways the embryological stages through which an organism goes is similar to the evolutionary stages through which it went over many millions of years. I want to point out to you that the development of the neck in mammals, humans being a mammal,   really does seem to go through stages which are very much like those of the neck during evolution. For example, we have now reached the stage in development which is not terribly different from what a fish's neck looks like. Now I have indicated on this slide something that does not happen in amniotes, but which, if we were going to become fish, would happen. The branchial septa, each one of them, would dissolve, and a communication would be established between the lumen of the pharynx and the external environment of our creature. If we were going to become a fish, we would suck fluid in through the mouth, that is the ruptured oropharyngeal membrane, and then we would push that fluid out through a pharyngeal pouch and a ruptured branchial septum and through the branchial cleft back into the external environment. We would also have our aortic arch develop a capillary system that lay beneath the endoderm and ectoderm of this system, and as the fluid was swept past this capillary system, oxygen would be transferred from the fluid to the blood stream. What I have described are the gill slits and gills of a fish, and we see that although this doesn't actually happen in human development we get pretty close to it. We start to develop these gill_like structures, but the branchial septa will not rupture, and instead the branchial arches and the associated structures will follow a different path that will lead to a human neck.
skull, in particular the occipital bone. Occipital somites also have dermomyotomes, and these give rise to some of the striated skeletal muscle of the head region. Somitomeres are not fully developed somites. They do not give rise to sclerotomes, and therefore they do not contribute to any bone of the skull. On the other hand, they do give rise to striated skeletal muscle. And, in fact, that's all they do, and all of the striated skeletal muscle of the head is derived either from somitomere tissue or from the dermomyotome tissue of the 4 occipital somites.

Branchial Arch Development, slide 23
Up to now I've talked about the segementation of the neck into branchial arches filled with lateral plate mesoderm and extomesenchyme. I've not mentioned at all what happens to the paraxial mesoderm of the head and neck which was derived from the primitive streak about a week earlier in development. We know that in the trunk region of the body, paraxial mesoderm gives rise to somites, and this happens in the cervical region as well. But things are a little different in the head. This drawing is an idealized coronal section through the neural tube of the head, which will become the brain, I've indicated by a line labeled "myelospinal boundary" the demarcation between the portion of the neural tube that will become the spinal cord (that is down near the bottom of the slide)

and all the neural tube cranial to this boundary, which is going to become the brain. I've labeled bits and pieces of the brain here, but you don't need to know them for this course. Among the things I would like you to know is that the paraxial mesoderm near the back end of the brain does in fact organize itself into regular somites, as we saw elsewhere in the body. There are 4 such somites around this hindbrain region, and each one is called an occipital somite. Their sclerotome portions give rise to the bones of the hind part of the skull, whereas their dermomyotomal portions (which are not really separable into hypaxial and epaxial parts) give rise to all the muscles of the tongue, which as you may know are innervated by the cranial neve XII. Cranial to these 4 occipital somites, the paraxial mesoderm never segments. It forms on each side one continuous column of loosely packed cells that is simply called Cranial Paraxial Mesoderm (or CPM). The most cranial part of this CPM, along with cells from prechordal mesoderm, migrate into the developing orbit to become the extraocular muscles, which are innervated by cranial neres III, IV and VI. The rest of the CPM divides itself into 5 portions, each of which migrates into a branchial arch.

Branchial Arch Development, slide 24

Within each branchial arch, the CPM joins with the lateral plate mesoderm of the mesodermal core to form the dorsal portion of what is an enlarged mesodermal core of each branchial arch. I have already mentioned that although the ectomesenchyme cells of a branchial arch express different genes than the the lateral plate mesoderm cells of the arch, they do not differ morphologically from them. I can now add that the CPM cells which migrate into the arch do not differ morphologically from either of the other two cell types. You can tell them apart only by virtue of the fact that they have their own unique pattern of gene expression. The fate of these five branchial arch mesodermal cores is complicated. The mesodermal core of the first rach gives rise to striated muscles innervated by the trigeminal nerve, with the CPM portion giving rise to the major movers of the lower jaw, i.e., masseter, temporalis and pterygoid muscles, whereas the lateral plate component of the first arch’s mesodermal core gives rise to the anterior digastric and mylohyoid. The source of the tensor veli platini and tensor tympani has not been established. All the striated muscles innervated by the facial nerve derive from the lateral plate component of the second arch’s mesodermal core. The fate of its CPM component is unclear. Similarly the striated muscles innervated by the glossopharyngeal nerve, vagus nerve, and accessory nerve arise from the lateral plate component of the mesodermal cores of arches 3, 4 and 6. Again, what happens to the CPM components of the mesodermal cores of these arches is unknown. To add to the complexirty, it turns out that both components of the first arch mesodermal core, and the lateral plate component of the second arch mesodermal core, give rise to a very substantial portion of the muscle of the heart.

Branchial Arch Development, slide 25

I’ve said all I plan to say about the fates of the ectomesenchyme and both components of the mesodermal cores of the branchial arches. Since they don’t differ morphologically, I have decided to show all three types of cells in light pink, which means you will not be able to tell them apart in future slides. Now we can return to what happens specifically to each of the pharyngeal pouches, branchial clefts, and branchial arches. The first pharyngeal pouch separates ever so slightly from the first branchial cleft, and ectomesenchyme moves in between them to form the connective tissue of the eardrum, which therefore is lined externally by an epithelium derived from ectoderm and lined internally by an epithelium derived from endoderm, which is the endoderm of the first pharyngeal pouch. The first branchial cleft persists as the external auditory meatus; the ectoderm of the first branchial cleft becomes the epidermis of the skin of the external auditory meatus. The first pharyngeal pouch persists as the Eustachian tube and the cavity of the middle ear, the endoderm of that first pharyngeal pouch therefore becomes the lining epithelium of the Eustachian tube and middle ear cavity. I see that on this slide I've also taken out the first and second aortic arches, you know that they degenerate. I've left in the 3rd, 4th, and 6th. I'm not going to be talking about them anymore; Dr. Larson discussed with you what they become.

Branchial Arch Development, slide 26

The second branchial cleft and second pharyngeal pouch separate from one another more substantially.

Branchial Arch Development, slide 27
Then, lymphoid tissue accumulates adjacent to the floor of the 2nd pharyngeal pouch. That lymphoid tissue is going to become the palatine tonsil. And the endoderm of the 2nd pharyngeal pouch is simply going to become the epithelium that covers the palatine tonsil. We'll see what happens to the second branchial cleft later.

Branchial Arch Development, slide 28
The third pharyngeal pouch and branchial cleft also substantially separate from one another, and I've shown a change in the shape of the third pharyngeal pouch, which will be explained in a moment.

Branchial Arch Development, slide 29
That change in shape of the third pharyngeal pouch is best understood if we now take a cross-section through it.

Branchial Arch Development, slide 30
After the third pharyngeal pouch pulls away from the third branchial cleft, it develops a dorsal and a ventral expansion. Each pouch sort of looks like a hollow dumbbell connected to the pharynx through the middle.

Branchial Arch Development, slide 31
The endoderm of the third pharyngeal pouch then proliferates making this dumbbell a solid structure, so now we have a solid dumbbell with a dorsal clump of cells and a ventral clump of cells connected to one another by an endodermal cord, and this solid dumbbell-like structure separates from the pharynx, and the pharynx reconstitutes its circular wall.

Branchial Arch Development, slide 32
This is what our coronal section would look like after this has occurred. You see that instead of a third pharyngeal pouch you now see a solid structure separated from the pharynx itself. We can pretend this is either the dorsal clump or the ventral clump of cells of the now solid third pharyngeal pouch.

Branchial Arch Development, slide 33
The fourth pharyngeal pouch is going to undergo a change very similar to what happened to the third pharyngeal pouch. First, the fourth pouch will separate from the fourth branchial cleft, as indicated here.

Branchial Arch Development, slide 34
Then just like the 3rd pouch, the cells of the 4th pharyngeal pouch will proliferate and obliterate the lumen of the pouch. A dorsal clump of cells will form, a ventral clump of cells will form, and they will be connected to one another by a thin endodermal cord. And the result on cross- section would look like an endodermal dumbbell, which will have separated itself from the pharynx, which in turn will reconstitute its lateral wall. I have also indicated on this picture that the abortive 5th pharyngeal pouch proliferates a little bit, and forms its own tiny clump of endodermal cells adjacent to the ventral clump derived from the 4th pharyngeal pouch.

Branchial Arch Development, slide 35
And now I'm showing something that is happening to the 2nd branchial arch itself. The ectomesenchyme of the second branchial arch grows laterally and caudally, pushing ectoderm in front of it, and forms a sheet of tissue that extends lateral to the 3rd and 4th branchial arches. I call this the opercular process because it reminds me of the opercululum of a bony fish, but in fact this not a generally accepted term. A smaller version of this - a sort of mirror image of this - forms from the caudal end of the 6th branchial arch and it grows cranially, and it is called the epipericardial ridge.

Branchial Arch Development, slide 36
Let's take a cross-section through the region of the third pharyngeal pouch after the opercular process has begun its development. This cross-section will look a lot like one we've seen before, but we'll have to include the opercular process in it.

Branchial Arch Development, slide 37
And indeed it looks just like slide 31, but the opercular process is seen extending along the lateral side of the embryo.

Branchial Arch Development, slide 38
The epipericardial ridge doesn't grow very much, but the opercular process does, and eventually approaches the epipericardial ridge. In so doing, it now covers the second, third and most of the sixth branchial arches. The fluid filled space between the opercular process and the lateral surface of these arches is called the cervical sinus. A sinus is just a fluid filled space connected to the external environment by a channel. And this space, the cervical sinus, is connected to the external environment by the channel between the opercular process and the epipericardial ridge.

Branchial Arch Development, slide 39
Let's take a cross-section through the embryo at this later stage in development. You might think it would look identical to the one just shown, but go on to the next slide and you'll see a slight change that I have made.

Branchial Arch Development, slide 40
And that change is that the dorsal and ventral surfaces of the opercular process have merged with the rest of the ectomesenchyme of our embryo, so that cervical sinus really has become a fluid-filled space whose only means of communication with the external environment is through the gap between the opercular process and the epipericardial ridge.

Branchial Arch Development, slide 41
And I'll bet most of you guessed that eventually the free edge of the opercular process contacts and merges with the free edge of the epipericardial ridge, making a smooth lateral surface to the neck region caudal to the external auditory meatus, except that between the deep aspect of the opercular process and the lateral surfaces of the third, fourth, and part of the sixth brachial arches is a fluid-filled cyst of some sort.

Branchial Arch Development, slide 42
That cyst does not normally have a very long life, though we will see how it might in cases of anomalies of development. But normally, the ectoderm on the deep aspect of the opercular process contacts the ectoderm on the lateral aspects of the 3rd, 4th and 6th branchial arches, and these two will fuse. The ectomesenchyme will bridge across the zone of fusion, and this cystic area will be obliterated and filled in with solid ectomesenchymal tissue.

Branchial Arch Development, slide 43
This shows you what the embryo looks like after the opercular processes is incorporated into the rest of the neck tissues, giving a nice smooth contour to the lateral aspect of the neck. The bulge here is an artifact of the way I drew this; most of our necks do not have big bulges on the side.

Branchial Arch Development, slide 44
Although there is a written document associated with this lecture that describes some of the anomalies associated with the developmental processes I've just discussed, I apparently made up slides which illustrate these things, so lets proceed to considering anomalies associated with problems in formation of the opercular process, or closure of the cervical sinus, or development and disappearance of the branchial septa.

Branchial Arch Development, slide 45
One such anomaly is persistence of that fluid-filled space between the deep aspect of the opercular process and the lateral surfaces of the third, fourth, and part of the sixth branchial arches. When this fluid-filled persists, it is called a branchial cyst, and indeed may present as a swelling beneath the skin of the lateral surface of the neck.

<u>Branchial Arch Development, slide 46</u>
Or, not only may this fluid-filled space persist, but the opercular process may not completely merge with the epipericardial ridge; and there may be an opening connecting the branchial cyst with the external environment. Well, if you have a fluid-filled space that is communicating with the environment, that's not called a cyst anymore, it's called a sinus. This particular communication is to the outer surface of the embryo, so it's called an external branchial sinus, and it may in fact leak fluid through the opening onto the lateral aspect of neck.

<u>Branchial Arch Development, slide 47</u>
Another variant of this kind of anomaly is persistence of this fluid-filled space, but there is successful merger of the opercular process with the epipericardial ridge, so the space is not open to the external environment on the outside of your neck, but instead there is rupture of the second branchial septum, allowing communication between the lumen of your pharynx and this fluid-filled space deep to the skin on the side of your neck. In this case, we have a sinus whose communication is to an internal space, so it's called an internal branchial sinus. Usually, it's the second branchial septum that ruptures. In this case the opening of the internal branchial sinus will be in the bed of the palatine tonsil. The sinus itself is prone to infection because of bacteria that can travel down from your throat into the sinus. Less commonly it is a third branchial septum that ruptures through. You still have an internal branchial sinus; it's just that its opening is into the throat somewhere below the hyoid bone.

<u>Branchial Arch Development, slide 48</u>
This is sort of a worst case scenario. Not only did the opercular process not merge with the lateral aspects of branchial arches, and therefore a fluid-filled space persisted, but the opercular process did not join the epipericardial ridge, and so a communication to the external environment was created through the gap, and the second branchial septum also ruptured, so a communication to the lumen of the pharynx was created. And in such a person, you could actually thread a fine wire through the hole in the bed of the palatine tonsil all the way down through a narrow channel and have that wire emerge on the lateral surface of the neck, usually in the front of the sternocleidomastoid. At any rate, when you have an abnormal connection between two spaces that are not supposed to be connected, that's called a fistula. And this is called a branchial fistula, connecting the lumen of the pharynx with the external environment.

<u>Branchial Arch Development, slide 49</u>
There are still more developmental changes that will occur in the neck region. I know if I were you, I could not wait to find out what they would be. But some of you might feel this is an appropriate time to take a break. If I were giving a regular lecture, this would be the end of one hour and the next part of this would be the second hour of the lecture.

<u>Branchial Arch Development, slide 50</u>
This is a reminder that on each side there are these two dumbbell-shaped objects of endodermal cells, one derived from the 3rd pharyngeal pouch, one derived from the 4th pharyngeal pouch. And since we see a little clump derived from the abortive 5th pouch, this coronal section must be through the ventral clumps not the dorsal clumps.

<u>Branchial Arch Development, slide 51</u>
If we take a cross-section either through the third pharyngeal pouch region or through he fourth pharyngeal pouch region, the cross-sections will look identical, as shown in the next slide.

<u>Branchial Arch Development, slide 52</u>
We've seen this before. There's nothing more to say about it.

Branchial Arch Development, slide 53

Here is a new view. It is sort of a median sagittal section, but I have shown things that can't be seen in median sagittal section. Number one, I have indicated where the branchial arches are, and those structures are to the left and to the right of the median sagittal plane, so we wouldn't actually see them. At the level of the pharynx, between the first and second arches, there is an oval made of little yellow circles; that's meant to represent the auditory, or Eustachian, tube. At the level of the pharynx, between the second and third branchial arches, there is a circle made of little yellow circles, and that's meant to be the bed of the tonsil, derived from the second pharyngeal pouch. And then I've shown these dumbbell-shaped clumps of cells. One between the third and fourth branchial arches, and that dumbbell is derived from the third pharyngeal pouch; and the other one, more caudally, between the fourth and sixth branchial arches, is derived from the fourth pharyngeal pouch. And now I've started to tell you what it is that the dorsal and ventral clumps of these dumbbell-shaped endodermal objects will become. The dorsal clump derived from the third pharyngeal pouch is called parathyroid III, and it will become on each side a parathyroid gland. The dorsal clump derived from the fourth pharyngeal pouch is called parathyroid IV, and again on each side, it will become a parathyroid gland. The ventral clump of cells derived from the third pharyngeal pouch will become the nonlymphoid part of the thymus. There is some uncertainty about what the ventral clump of cells derived from the fourth pharyngeal pouch, and the little clump derived from the abortive fifth pouch, will become. I've put in the most popular theory right now, and that is they become the C-cells of the thyroid gland; these are cells thar secrete calcitonin. In the adult, the parathyroid glands do not lie lateral to the pharynx, and the thymus lies in the thorax, and the C-cells of the thyroid gland lie inside, really, the thyroid gland. So we are not yet finished with what's going to happen to these pharyngeal pouch derivatives. We are just, right now, saying what they will become, and later we'll say how they get to where they're going to end up in the adult.

Branchial Arch Development, slide 54

Many other things are going on simultaneously. One thing that's going on is that the ectomesenchyme ventral to the pharynx, but between the right and left first branchial arches, starts to proliferate and to bulge into the pharyngeal lumen, carrying a layer of endoderm with it. This structure is called the median tongue bud.

Now I want to add a trivial postscript to what I just said. I referred to the epithelial covering of the median tongue bud as endoderm, and I colored it yellow in this picture, but I really should have probably colored it blue, and that’s because the oropharyngeal membrane, before it disappeared, attached at a site that will became the junction of the anterior two-thirds of the tongue with its posterior one-third. And it turns out that the proliferating ectomesenchyme between the first branchial arches is destined to become the anterior two-thirds of the tongue, so its epithelial covering comes from cells that lay in front of the oropharyngeal membrane and really are ectoderm. I have to admit that I was too lazy to color this stuff in blue in this slide, and I’m too lazy to do it in all the slides that come after it, becasuse I really don’t care if anybody knows this.

Branchial Arch Development, slide 55

And we'll get a different view of it if we take a cross-section of the embryo through the level of the first branchial arches.

Branchial Arch Development, slide 56

We've seen a section like this before, but now we can see that there is a proliferation of ectomesenchyme in the midline that pushes up into the pharynx. We also see that, lateral to this median tongue bud, are additional proliferations of ectomesenchyme, pushing endoderm in front of them, and encroaching upon the lumen of the pharynx. These are called the lateral tongue buds, and I want to emphasize that the two lateral tongue buds, and the median tongue bud, arise in the interval between the two first branchial arches.

Branchial Arch Development, slide 57

Back to our sort of median sagittal section, and let's take a look at what is happening ventral to the pharynx in the region between the second branchial arches. Rather than there being a proliferation of ectomesenchyme which will grow up and encroach on the lumen of the pharynx, there's a midline outpocketing of the endoderm that forms the ventral floor of the pharyngeal wall. Soon after arising, this diverticulum turns caudally and will start to extend ventral to the pharynx down the length of the neck, it is called the thyroid diverticulum; it's name tells you what it will become. This shows it at the earliest stages of its development. We'll see later what happens to it.

<u>Branchial Arch Development, slide 58</u>
I don't know if it's necessary, but it can't hurt to take a cross-section through the embryo at the level of the second branchial arches so we can see what this thyroid diverticulum would look like in such a section.

<u>Branchial Arch Development, slide 59</u>
Surprise, surprise! It looks like a ventrally directed outpocketing, in the midline, of the endoderm that forms the floor of the pharynx between the second branchial arches.

<u>Branchial Arch Development, slide 60</u>
And again the faux median sagittal section showing not only the median tongue bud and the thyroid diverticulum, but also that the ectomesenchyme ventral to the pharynx between the third branchial arches starts to proliferate and bulge into the pharynx encroaching on its lumen, and the same thing happens with the ectomesenchyme ventral to the pharynx between the fourth branchial arches. Now this swelling between the third branchial arches is called the copula, and the one between the fourth branchial arches is called the epiglottis, and together (because they sort of merge together they are not entirely distinct), together they are called the hypobranchial eminence.

<u>Branchial Arch Development, slide 61</u>
We could take a cross-section of the embryo at the level of the third branchial arches and copula, or we could take one at the level of the fourth branchial arches and epiglottis - they would look the same - and it will be shown in the next slide.

<u>Branchial Arch Development, slide 62</u>
And of course, all this really shows that we haven't seen before is a midline proliferation of ectomesenchyme encroaching upon the lumen of the pharynx. And if it were the third arch that we were looking at, it would be the copula; and if it were the fourth arches we were looking at, it would be the epiglottis.

<u>Branchial Arch Development, slide 63</u>
And now let's focus on what is happening ventral to the pharynx between the 6th branchial arches. And just as we saw a midline endodermal outpocketing between the 2nd arches, which we called the thyroid diverticulum, we see a ventrally directed, midline endodermal outpocketing between the 6th arches, which is called the laryngotracheal diverticulum. Its name tells you that it's going to become the larynx and the trachea. And of course, the trachea (I think we mentioned this earlier), when it gets into the chest, is going to bifurcate into the lung buds which grow and become the lungs.

<u>Branchial Arch Development, slide 64</u>
This is a cross-section at the level of the 6th branchial arches. I haven't fooled you, it's actually the same one that I showed at the level of the 2nd branchial arches. I've just labeled this midline diverticulum made of endoderm, from the floor of the pharynx, as now being called the laryngotracheal diverticulum.

<u>Branchial Arch Development, slide 65</u>
I've tried to place all this stuff that's going on ventral to the floor of the pharynx, or things that are going on with the floor of the pharynx, into the perspective of this coronal section that we've worked with so often. So up at the level of the first branchial arches, I show a little yellow circle, which is meant to be the endoderm covering the median tongue bud, and then I show these two projections coming in from the side of the pharynx, which are the

lateral tongue buds. There is a black hole between the second branchial arches, which is meant to be the hole that leads to the thyroid diverticulum. Then there is a yellow rectangle attached to a yellow oval. The rectangle, at the level of the third branchial arch, is meant to be the endoderm covering the copula. The yellow oval, which is partially joined to the copula, is meant to be the endoderm covering the epiglottis. These structures are swelling into the floor of the pharynx. And then, finally, the black oval at the level of the sixth branchial arches is the opening into the laryngotracheal diverticulum.

<u>Branchial Arch Development, slide 66</u>
Let's take a cross-section through the lateral and median tongue buds at the level of the first branchial arch (we did it before).

<u>Branchial Arch Development, slide 67</u>
And I just show you this again because we are going to modify it as these lateral tongue buds grow.

<u>Branchial Arch Development, slide 68</u>
The lateral tongue buds are going to push into the floor of the pharynx more than does the median tongue bud. The two lateral tongue buds will in fact meet one another, and bury the median tongue bud beneath them.

<u>Branchial Arch Development, slide 69</u>
And eventually, the median tongue bud loses its integrity as a separate structure, which makes me wonder why I even bother to mention it, and also a septum indicating the site where the two lateral tongue buds met one another is formed, and this is called the median septum of the tongue. It persists throughout life and you'll see it in dissection.

<u>Branchial Arch Development, slide 70</u>
And this picture shows that not only do the lateral tongue buds grow towards one another to meet, but they also extend forward in the pharynx and actually stick out your mouth. The final product of the lateral tongue buds is the anterior two-thirds of the tongue, that's the part that has a median septum. In a moment we'll talk about where the posterior one- third of the tongue comes from; it does not have a median septum.

<u>Branchial Arch Development, slide 71</u>
The posterior 1/3 of the tongue comes from that thing we called the copula, which is an ectomesenchymal proliferation covered by endoderm, in the midline, that grows forward and meets the back edge of the fused (or nearly fused) lateral tongue buds. And together, then, the lateral tongue buds and the copula form the entire tongue, with the copula making up its posterior 1/3. And there is that little pit in the midline, between the posterior 1/3 of the tongue and the anterior 2/3 of the tongue, which leads down into the thyroid diverticulum at this stage in development. That pit is called the foramen cecum of the tongue. This drawing also shows that the epiglottis becomes a flap -like structure, which protects, if you will, the entrance to the laryngotracheal diverticulum.

<u>Branchial Arch Development, slide 72</u>
If you compare this slide to the previous one, you'll see that all that I've done is allow the thyroid diverticulum to grow a little longer so its tip extends a bit further caudally. And I've shown when the laryngotracheal diverticulum reaches the region dorsal to the heart, it bifurcates into the two lung buds.

<u>Branchial Arch Development, slide 73</u>
As the slide says, the thyroid diverticulum continues its growth caudally.

Branchial Arch Development, slide 74
And the thyroid diverticulum continues its caudal growth, to stop only when its tip reaches a position in front of the developing trachea.

Branchial Arch Development, slide 75
Now that may be when the thyroid diverticulum stops its caudal growth, but that's not the end of its development. And in order to see what happens next, we have to take a very peculiar section, indicated by this bent blue line, which is sort of a coronal section through the thyroid diverticulum and its tip.

Branchial Arch Development, slide 76
Well, all it really looks like is a hollow endodermal tube. I've left out all the cranial bit that connects to the foramen cecum of the tongue.

Branchial Arch Development, slide 77
And what happens to the thyroid diverticulum when its tip reaches a position in front of the future trachea, is that tip divides into two. This is very much like what happens to the laryngotracheal diverticulum when its tip reaches a position dorsal to the heart.

Branchial Arch Development, slide 78
The two branches of the thyroid diverticulum, right and left, grow bigger.

Branchial Arch Development, slide 79
And then the endoderm that forms these two branches proliferates, and makes a solid structure out of the bilobular tip of our thyroid diverticulum. There is a left lobe of the thyroid gland so created, a right lobe of the thyroid gland, and the connecting endoderm is called the isthmus of the thyroid gland. And the isthmus is connected by a hollow endodermal tube all the way up to the foramen cecum of the tongue. And hence, that hollow tube is called the thyroglossal duct, which means the duct between the thyroid gland and the tongue.

Branchial Arch Development, slide 80
So now in this median sagittal section, I've shown the solidification of the thyroid diverticulum at its caudal end to become the thyroid gland, and I show the full length of the thyroglossal duct. We're not finished with the thyroid gland, but before I bring it to completion, I want to go now and take a look now at what is going to happen to the clumps of endodermal cells that were derived from the third and fourth, and abortive fifth pharyngeal pouches.

Branchial Arch Development, slide 81
One of the main things that's going to happen to these cells is that they are going to migrate caudally. And all I mean to show by this slide is that the material derived from the 3rd pharyngeal pouch will migrate further caudally than the material derived from the 4th pharyngeal pouch or the abortive 5th pouch.

Branchial Arch Development, slide 82
And associated with this caudal migration is a slight ventral component to the migration as well.

Branchial Arch Development, slide 83
The endodermal cells derived from the fourth pharyngeal pouch, that is those cells that are going to be become parathyroid IV and the ventral clump, which along with the abortive fifth pouch, will become the C cells of the thyroid - those cells don't migrate very far caudally; they migrate to take up a position on the dorsal aspect of the thyroid gland. And here parathyroid IV takes up permanent residence, and the cells derived from the ventral clump of the fourth pouch and the abortive fifth pouch disperse throughout the other thyroid glandular tissue to become as I said the C cells of the thyroid gland. Endodermal cells of the third pharyngeal pouch migrate further caudally, and when they reach the lower pole of the thyroid lobes, the dorsal clump of cells, destined to become parathyroid III, separate from the ventral clump of cells, destined to become the nonlymphoid part of the thymus.

Branchial Arch Development, slide 84
Parathyroid III is going to take up a position on the posterior surface of the lower pole of the thyroid gland, but the ventral clump of cells that will become the nonlymphoid part of the thymus will continue a caudal migration into the superior mediastinum of the chest. The reason parathyroids III end up more caudally than parathyroids IV is because they are dragged along with the thymic part of the third pharyngeal pouch, and they don't separate from it until they are already caudal to parathyroids IV.

Branchial Arch Development, slide 85
This is as far as I'm going to take development of the third and fourth pharyngeal pouches. Parathyroids IV, so named because they came from the dorsal clump of cells of the fourth pharyngeal pouch, become the definitive superior parathyroid glands. Parathyroids III, so called because they come from the dorsal clumps of cells of the third pharyngeal pouches, become the definitive inferior parathyroid glands. And you know that there has been this reversal of position because parathyroids III have been dragged further caudally in migration by their attachment to the portion of the third pouch that will become the non-lymphoid part of the thymus. You can well imagine that some of these migrations can go awry, and they do from time to time; these are described in the accompanying clinical sidelights document. One of the most interesting ones is when the inferior parathyroid glands end up in the superior mediastinum because parathyroids III did not separate from the non-lymphoid part of the thymus until it had dragged parathyroids III all the way into the superior mediastinum.

Branchial Arch Development, slide 86
And now let me complete development of the thyroid gland. In most people the thyroglossal duct completely degenerates.

Branchial Arch Development, slide 87
You are left with a thyroid gland that is more or less H-shaped: a left lobe, a right lobe, and an isthmus that connects them, and then the only other remnant of the thyroid diverticulum is a little pit in the midline of the tongue between it's anterior two-thirds and posterior one-third, which is called the foramen cecum, and it marks the original site of origin of the thyroid diverticulum.

Branchial Arch Development, slide 88
This shows the definitive thyroid gland positioned ventral to the trachea, and it shows that little pit in the midline of the tongue that is called the foramen cecum.

Branchial Arch Development, slide 89
What can go wrong with thyroid development: this is described also in the clinical correlates document, but since I have some slides to illustrate it, I'll discuss it here as well.

Branchial Arch Development, slide 90
What's illustrated here is not really anything that's gone wrong. This is a normal variant. The distal end of the thyroglossal duct may undergo proliferation and solidification to become normal thyroid glandular tissue, and in which case it constitutes what's called a pyramidal lobe of the thyroid gland. I am certain that in dissection some of your cadavers will demonstrate pyramidal lobes of the thyroid gland. It extends cranially from the isthmus a centimeter or two.

Branchial Arch Development, slide 91
Again what's illustrated here is a normal variant. When you have a pyramidal lobe of the thyroid gland, sometimes the thyroglossal duct cranial, or superior, to it turns into a connective tissue band linking that pyramidal lobe to the hyoid bone. This is called the thyroglossal ligament.

Branchial Arch Development, slide 92
And now we're getting into something which is actually an anomaly of development. Some middle portion of the thyroglossal duct may persist as a hollow fluid-filled sac. Such a structure is called a thyroglossal cyst, or a

thyroglossal duct cyst. This is the most common anomaly of head and neck development. It is the second most common cause of a neck mass in a young child. When a thyroglossal duct cyst does exist, it is in the anterior midline deep to the skin and inferior to the hyoid bone. One clue that a mass in this location is a thyroglossal duct cyst is to ask the child to stick his or her tongue out, and a thyroglossal duct cyst will move superiorly in that case. A rare anomaly of the thyroid gland is a lingual thyroid , which you should read about in the clinical sidelights document.

Branchial Arch Development, slide 93
No narration.