Stage 16  Closure of Posterior Neuropore;  
Hind Limb Bud and Tail Bud  
10 Days, 30–34 Somites, 3.1–3.9 mm (fresh)  

External Shape  
Some typical features of stage 16 are represented in Fig. 134. The hind limb bud becomes 
visible as a distinct bulge at the level of the 23rd–28th somite. The tail rudiment appears 
as a short stump (Fig. 127). The surfaces of the third and fourth branchial bars are distinctly 
concave, in contrast to the second. In this way the formation of the cervical sinus is initiated. 
The lens plate is usually slightly indented (Figs. 129–131). This corresponds to the early 
horizon XIV of Streeter. Lens development seems to be slightly advanced in mouse com-
pared to human embryos. The olfactory placode is clearly indented, and is also slightly 
advanced in development compared to the human embryos. 
The otocyst is invariably closed. It is no longer spherical, but more or less pear-shaped in 
its dorsal segment (Fig. 127). 
The posterior neuropore begins to close.  
Length. In the unfixed state, the overall length varies between 3.3 and 3.9 mm.  

Circulatory System  
The cardinal veins may be clearly recognized in intact embryos (Fig. 129). The heart forms 
a prominent bulge in the vicinity of the branchial bars. The structure of the heart is essen-
tially the same as in stage 17 for which a detailed reconstruction is represented in Fig. 148.  

Intestinal Tract  
The most conspicuous change in this age group is the formation of the lung anlage (Fig. 
132): the laryngo-tracheal groove branches from the esophageal gut and the two primary 
bronchi sprout out in a T-like manner (Figs. 132, 133). The development of the intermediate 
portion of the intestinal tract was shown in Fig. 123. The hindgut still has the same appear-
ance as in Fig. 126.  

Urogenital System  
The nephrogenic cord is now widely separated from its origin in the somite stalks. In the 
pronephric region, anterior to the 12th somite, only small clusters of cells are visible, and 
there is no structural organization. At the level of the 12th somite, spherical groups of 
nephrogenic cells may be recognized. They are arranged as vesicles at the level of the 13th 
to the 15th somite (Fig. 135). Generally, two such vesicles will form in each segment. Matu-
ration proceeds slowly: at the 32 somite stage, the nephrotome of the 20th somite is not 
much more developed than in the 26-somite stage (Fig. 135). In one 26-somite embryo, 
there is a peritoneal funnel. There are no mesonephric glomerula at this stage.
The Wolffian duct is recognizable in the younger specimen examined, and it extends from the 13th to the 26th somite. In the older specimen, it has already reached the cloaca. At the point of contact, numerous pycnotic nuclei may be seen.

Primordial germ cells [103] can be seen as large cells containing much alkaline phosphatase. Some are already situated within the genital ridge, on both sides of the dorsal mesenteric attachment.

Central Nervous System

In most cases the neural tube is now completely closed. Compared to the human embryo of similar somite number, closure is delayed in mice. As an example, specimen KT 939/4 has 32 somites, and its posterior neuropore has just closed.

The separation and differentiation of the otic vesicle behaves nearly exactly the same in mice as in humans. The vesicle is now closed and completely separated from the epidermis. The endolymphatic appendage is marked off as a dorsal recess from the main cavity of the vesicle, giving it a more elongated appearance.

The olfactory placode is considerably thickened and slightly indented.

The ganglia of the cranial nerves appear as distinct blastemal condensations. In transparent specimens, the large trigeminal ganglion can easily be recognized, situated just anterior to the pontine flexure of the brain tube (Fig. 127). Posteriorly, the spinal ganglia are differentiated from the neural crest.

In older specimens, the lens plate is slightly indented (Fig. 130).

Placenta

The loosely structured chorionic plate is traversed by the allantoic vessels. The ectoplacental plate is transformed into the labyrinth, and its margins are turned inward (Fig. 136). Consequently, the transition zone of the two yolk sac layers is also turned inward. The yolk sac cavity will later communicate here with the interplacental cavities, which develop secondarily as small clefts within the labyrinthine cell mass after disappearance of the original ectoplacental cavity [33].

The zone of "giant cells" (Fig. 136A) borders the decidua, which has many multinucleate cells in this region. Toward the mesometrium, the deciduous cells penetrate the myometrium and start to form the "metrial gland" [25].

<table>
<thead>
<tr>
<th>Material</th>
<th>Age</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>KT 938–40</td>
<td>10 days</td>
<td>4 with 25, 26, 27 and 28 somites (listed previously). 1 with 32 somites. Posterior neuropore just closed. 1 with 32 somites. Posterior neuropore still open. 1 with 34 somites. Posterior neuropore closed.</td>
</tr>
</tbody>
</table>
Figs. 127–133:

Fig. 127. Embryo of 32 somites, from the right. Formalin fixed.
The trigeminal ganglion (V) is visible through the skin as well as the slightly pointed otic vesicle (O).
*Dashed line* indicates level of the section Fig. 130. 13.5:1

Fig. 128. Embryo of 30 somites, on millimeter scale. Bouin fixed.
Nominal age 11 days, developmental age 10 days.
KT 945. 13.5:1

Fig. 129. Embryo of 33 somites, from the left.
For explanations, see Fig. 134. 13.5:1

Fig. 130. Horizontal section through eye primordium, low magnification. Plane of section indicated in Fig. 127.
*D = diencephalon* (3rd ventricle), *Tel = cerebral hemisphere.*
KT 939/4, 32 somites. 100:1

Fig. 131. Detail of Fig. 130.
*L = lens invagination, R = retinal sheet.* 270:1

Fig. 132. Cross section, level of lung primordium. The apparent asymmetry of the lung bud is mainly due to the oblique plane of section.
*V.ca. = vena cardinalis anterior, Ao = aorta dorsalis (paired).*
KT 939/4, 10 days, 32 somites. 100:1

Fig. 133. Detail of Fig. 132.
*L = laryngotracheal groove.* 270:1
Fig. 134. Externally visible features of embryo Fig. 129, 33 somites

Fig. 135. Development of the mesonephros, 10 days. (A) Drawing shows level of cross section; (B) cross section, embryo of 26 somites; (C) shows same cross section, embryo of 32 somites; Pe = peritoneal funnel; Ne = nephrogenic cord (dotted area); W = Wolffian duct. KT 939/2
Fig. 136. Development of the placenta.
(A) Disappearance of the ectoplacental cleft (broken line between laminae);
(B) margins of laminae turned inwards, (C) formation of labyrinth (dashed area indicates maternal tissue).
A KT 1001/4, B KT 935/9, C KT 938/4