Succinic dehydrogenase activity of the 15 to 20 day murine (CD-1) mandibular joint was determined by incubating cryostat sections at 37°C for one hr in a medium containing sodium succinate. A series of adjacent sections were incubated in a medium from which the substrate had been deleted. The SDH activity in the mandibular joints of 15 and 16 day embryos was minimal and thereafter a gradual increase in the enzyme activity was evident. The cells involved in intercellular matrix elaboration (chondroblasts and osteoblasts) and those concerned in remodeling of existing structures (osteoclasts/chondroclasts) demonstrated high levels of enzyme activity. The activity observed in chondrocytes varied between slight and moderate, however, the osteocytes were non-reactive. Intense SDH activity was observed in the medial and lateral pterygoid muscles as well as in the trigeminal ganglia.

The development of squamosomandibular articulation, the only freely movable joint of the skull, has been studied in vivo both in prenatal and postnatal rodents (1, 2, 5, 6, 8) and in vitro (10). The processes occurring during the embryogenesis of this joint are unusual, in that its development is closely associated with the appearance of secondary cartilage, instead of following the pattern of classic endochondral ossification. Silbermann and Frommer (19) demonstrated the presence of hydrolytic enzymes such as aminopeptidase, B-glucosaminidase, arylsulfatase and nonspecific esterases in mouse mandibular condyle chondrocytes, as well as in chondrocytes within mineralized zones and adjacent to the ossification fronts. It thus appears that the cartilage cells, unlike the epiphyseal plate chondrocytes, remain viable and demonstrate positive reactions to hydrolytic enzymes. The presence of a mitochondrial enzyme, succinic dehydrogenase (SDH), has also been demonstrated in various zones of condylar cartilage of pre- and postnatal gerbils (7) as well as in postnatal mice (17). However, the activity of this enzyme has not been determined in the prenatal mandibular joint of the mouse. The purpose of the present investigation, therefore was to localize SDH activity in the prenatal murine squamosomandibular articulation.

MATERIALS AND METHODS

Pregnant mice, CD-1, (Charles River) 15–20 days of gestation, were sacrificed by chloroform inhalation and the fetuses, were extirpated and decapitated. The severed heads were frozen and mounted on a chuck to obtain 14μm thick serial sections...
in a frontal plane. The cryostat sections were placed on alcohol-cleaned slides and air dried at room temperature. Demonstration of succinic dehydrogenase activity was accomplished by incubating the sections for one hr (37°C) in a phosphate-buffered sodium succinate medium containing a ditetrazolium salt, Nitro-BT (15). A series of adjacent sections were incubated in a medium from which sodium succinate had been omitted. Following incubation the sections were washed in distilled water and counterstained with 1% Safranin 0. The sections were dehydrated in an ascending series of alcohols, cleared in xylene and mounted with Permount.

**RESULTS**

Succinic dehydrogenase activity in the developing squamosomandibular joint (SMJ) was observed as bluish deposits of diformazan crystals. The specificity of the enzyme-substrate reaction was determined by incubating tissues in a medium from which the substrate (sodium succinate) had been omitted. Tissues incubated in the absence of the substrate lacked reaction deposits, indicating that the diformazan crystals were the product of SDH activity. The degree of enzyme activity in various components of the joint and its adnexa, was graded as negative (0), slight (+), moderate (++) and high (+++) (Table 1).

In the 15-day embryo the site of the future mandible was identified as mesenchymal condensation lateral to the Meckel's cartilage (Fig. 1). The cranial portion of this cellular mass will eventually differentiate into the mandibular ramus. The cells located in the center of the mesenchymal condensation demonstrated a slight (+) SDH activity, whereas the peripherally located cells were negative. The differentiating medial and lateral pterygoid muscles were slightly positive while the nerve cell bodies of the trigeminal ganglia and the developing tongue musculature displayed

| Activity code: | 0 negative | + slight | ++ moderate | ### high |

Table 1. Distribution of succinic dehydrogenase activity in the prenatal murine mandibular joint

<table>
<thead>
<tr>
<th>CELL AND TISSUE TYPE</th>
<th>15</th>
<th>16</th>
<th>17</th>
<th>18</th>
<th>19</th>
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<td>Mesenchyme</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Perichondrium</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Chondroblasts</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Chondrocytes</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Chondroclasts</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Periosteum</td>
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<tr>
<td>Osteoblasts</td>
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<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
</tr>
<tr>
<td>Osteocytes</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Osteoclasts</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Articular disc</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Trigeminal ganglia</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>
a moderate reaction for SDH. Cell differentiation and internal organization was very evident in the developing condyle of 16-day embryos. A majority of the cells in the future condyle were still undifferentiated, however, in the anterior inferior part of the condyle foci of differentiating chondroblasts were observed. These cells had a rounded morphology and demonstrated a moderate (++) reaction for SDH. The peripherally located cells of the future perichondrium and the undifferentiated mesenchymal cells of the posterior part of the condyle were slightly positive (+). Superior to the developing condyle, differentiation of cells was evident in the area of the future squamosal plate and zygoma. The differentiating osteoblasts were slightly active for SDH (+), even though matrix elaboration had not been initiated. The areas of prospective joint cavities were non-reactive, while the developing tongue and pterygoid muscles demonstrated enzyme activity which was more intense than in the 15-day embryos (Table 1). The enzyme activity of the squamosomandibular joint and adnexa in 17-day embryos appeared to have increased generally over that of the previous day (Table 1), however, in the SMJ of 18-day prenatal embryo a substantial increase in SDH activity was observed (Fig. 2). The chondrogenic core initially located in the anterior part of the condyle had advanced posteriorly and was now present throughout the condyle. The hypertrophied chondrocytes and the chondroblasts of the core demonstrated a moderate (++) enzyme reaction. The activity in the cellular layers of the perichondrium remained similar to that of the previous day (+ to ++). At the anterior inferior pole of the chondylar cartilage osteoblasts involved in periosteal bone formation demonstrated moderate enzyme activity. The medial and lateral pterygoids and the nerve cell bodies of the trigeminal ganglia displayed an intense reaction for SDH. The osteoclasts in the ossification centers of the squamosal plate were strongly reactive, the osteoblasts moderately active while the osteocytes demonstrated a complete lack of diformazan reaction deposits (Fig. 3). The future articular disc separating the upper and lower synovial cavities demonstrated moderate enzyme activity (Fig. 4).

By the 19th day of gestation extensive vascular networks were evident in the perichondrium and the periosteum. The inner cellular regions exhibited moderate, while the outer fibrous region displayed slight SDH activity. Osteoblasts involved in the elaboration of the subperiosteal bone collar demonstrated moderate (++) enzyme activity whereas the activity of the osteoclasts was strong (+++) (Fig. 5). The activity of the chondroblasts was moderate and the hypertrophied chondrocytes (unlike their 18-day counterparts) exhibited slight to moderate enzyme reaction. Intense activity was noted in muscle cells, chondroclasts/osteoclasts (Fig. 6) and nerve cell bodies of the trigeminal ganglion.

Control sections, incubated in a medium from which the substrate had been omitted, lacked SDH activity (Fig. 7).

By the 20th gestational day, the activity of the hypertrophied chondrocytes in regions of erosion and those located in the intact cartilage of the superior as well as the posterior aspects of the condyle was similar (+) (Fig. 8). Chondrocytes liberated from their surrounding lacunae demonstrated viable nuclei and an enzyme activity which was similar to that of chondrocytes in the intact lacunae. The osteoclasts in the osteogenic zone of the subperiosteal bone collar as well as those of the squamosal plate and zygoma demonstrated moderate enzyme activity (Fig. 9), however, osteocytes were not active. Osteoclasts involved in bone resorption were
observed routinely in the ossification fronts and demonstrated intense enzyme activity (†) (Table 1). The articular disc interposed between the two well defined synovial cavities had developed into a thick cellular layer displaying slight to moderate SDH activity (Fig. 9).

DISCUSSION

Despite the numerous studies on the postnatal mandibular joint in man, rat, mouse, gerbil and guinea pig (1, 2, 3, 8, 18, 20), little information is available concerning the prenatal histogenesis of this joint. Frommer (6) described the development of mandibular articulation in mouse fetuses between 15–20 days insemination age, Cunat (5) reported on the development of this joint in the rat from 16 days insemination age to 30 days after birth and Gartner et al. (8) investigated the joint in Mongolian gerbil from its inception (18th day of gestation) through the 8th postnatal day. The present study describes the squamosomandibular articulation in prenatal mouse embryos 15 through 20 days of gestation, with special emphasis on the SDH activity in various components of the joint. Succinic dehydrogenase is an important enzyme in the citric acid cycle and its presence indicates an active metabolic state. The enzyme, involved in biological oxidation, is localized in mitochondria and is reported to be more active than the other dehydrogenases, (13).

Studies of succinic dehydrogenase activity in oro-facial tissue, to a great extent, have been limited to palatine shelves and odontogenic tissues (9, 41, 16), where its presence is paralleled by the acquisition of functional competency of cells. A similar situation was observed in the cellular components of the squamosomandibular articulation. The cells actively involved in matrix elaboration (chondroblasts and osteoblasts) and those involved in remodeling of existing structures such as osteo-

Fig. 1. SMJ of a 15-day embryo. Mesenchymal condensation (arrow) lateral to the Meckel's cartilage indicates the site of future mandible. T. G. Trigeminal Ganglion, L. P. Lateral Pterygoid. ×45
Fig. 2. SMJ of a 18-day embryo. The chondrogenic core (C) demonstrates moderate SDH activity and the lateral pterygoid displays intense enzyme reaction. M. C. Meckel's cartilage. ×45
Fig. 3. A higher magnification of the SMJ in Fig. 2. The osteoblasts in the ossification centers of the squamosal plate exhibit moderate enzyme activity while the osteoclasts (arrow) are strongly active (C) chondrogenic core, A.D. Articular disc. ×115
Fig. 4. Synovial cavities and the articular disc of 18-day SMJ. The articular disc (AD) consisting of 3–4 layers of cells is moderately active for SDH. ×448
Fig. 5. SMJ of a 19-day embryo. Chondrocytes (C) display slight to moderate activity, perichondrium (PC) slight activity and the osteoclasts/chondroclasts (arrow) display intense SDH activity. ×448.
Fig. 6. SMJ of a 19-day embryo. Note the intense SDH activity in the medial and lateral pterygoid muscles. ×45
Fig. 7. SMJ of a 19-day embryo incubated in a medium from which the substrate had been omitted. Note the lack of SDH activity in the chondrocytes (C), perichondrium (PC), lateral pterygoid (LP) and the articular disc (arrow). ×115
Fig. 8 SMJ of a 20-day embryo. Note the intense enzyme activity of the lateral pterygoid (LP), osteoclast/chondroclast (arrow) and the slight activity in the perichondrium and periostium. ×45
Fig. 9. SMJ of a 20-day embryo. The chondroblasts (arrow) show a moderate activity, the articular disc (AD) slight to moderate while the hypertrophied chondrocytes (C) demonstrate a slight SDH reaction. ×115
clasts and chondroclasts demonstrated the highest levels of enzyme activity (Table 1). The intensity of enzyme reaction in these cells increased with embryonic age from slight to very strong. The enzyme activity in chondrocytes varied between slight and moderate and remained at these levels throughout various stages of cartilage maturation and erosion. It has been suggested that chondrocyte mitochondria release Ca++ in the region of initial calcification, where this mineral is transferred to matrix granules. This release is supposedly preceded by depressed O₂ tension, necessitating a switch from aerobic to anaerobic glycolysis in those chondrocytes. Such a shift to the monophosphate shunt should result in decreased Krebs cycle activities with a concomitant depression of SDH activity. While the present results indicate a slight reduction in chondrocytic SDH activity, the degree of reduction was not sufficient to support or oppose that suggestion.

The present results, however, are in accord with those reported for succinic dehydrogenase activity in the mandibular joint of the gerbil (9) as well as for certain hydrolytic enzymes active during chondrogenesis and mineralization of murine secondary cartilage (19). It thus appears that, in the secondary cartilage, hypertrophied chondrocytes released from their surrounding lacunae are not dying cells, as observed during normal endochondral ossification but cells which appear to have retained their vitality. The fate of these cells is not clear, though it has been proposed that following their release from the calcified matrix they use to form multinucleated chondroclasts (17). In mammalian long bone the liberated chondrocytes may be transformed into chondroclasts as well as osteoprogenitor cells which differentiate into osteoblasts and osteocytes (4).

REFERENCES