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What is This?
Megaoesophagus in the mouse: histochemical and ultrastructural studies

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Summary

Histochemical and ultrastructural studies of the muscle coat of the oesophagus from ICRC/HiCri mice (with megaoesophagus) and DBA/2fNCri mice (normal oesophagus) were carried out. The striking observation from histochemical studies was the presence of smooth muscle in the abdominal segment of the oesophagus from ICRC mouse in contrast to the control strain where smooth muscle was present only in the lowermost portion adjoining the stomach. Ultrastructural studies of the oesophageal wall from 5- and 10-day-old ICRC mice revealed an apparently normal muscle coat. In 3-month-old ICRC mice the upper abdominal segment of the oesophagus showed several abnormalities of smooth muscle fibres and paucity of plexus tissue accompanied by interstitial collagen deposition. The abnormalities were more severe in 1-year-old animals and were seen throughout the abdominal segment. From this study it is suggested that the primary cause of megaoesophagus in ICRC mice is neurogenic and not myogenic.

Keywords: Smooth muscle; Oesophagus; Megaoesophagus, Mouse inbred strain

Megaoesophagus is a neuromuscular disorder characterized by an absence of peristalsis, flaccid dilatation, resulting in retention of ingesta in the dilated segment. This condition which results from several causes has been reported in man (Cassella et al., 1964), cat (Soifer & Freeman, 1970), dog (Clifford & Pirsch, 1971), rat (Deerberg & Pittermann, 1972) and mouse (Randelia & Lalitha, 1988). In man, the muscle coat in the upper third of the oesophagus is composed of striated muscle, the lower third of smooth muscle and the middle third has both types, with a transition from striated to smooth (Weisbrodt, 1976). The smooth muscle is innervated by the autonomic nervous system which includes the submucous (Meissner's) and myenteric (Auerbach's) plexuses. In the dog, rat and mouse, the muscle coat of the entire oesophagus consists mainly of striated muscle which is innervated by motor axons of both vagus nerves (Gruber, 1978).

In both dog and rat with megaoesophagus, histological studies have indicated a normal or decreased number of ganglion cells in the myenteric plexus. Histochemical or ultrastructural studies have not been carried out, thus the pathogenesis of this condition in animals has not yet been elucidated. A similar oesophageal anomaly has been observed in one of our inbred strains of mice known as ICRC/HiCri strain. Genetic aspects, histological and histochemical findings of this condition have already been reported (Randelia et al., 1988; Randelia & Lalitha, 1988). The anomaly is recessive, and histologic examination indicated decreased myenteric ganglia and abnormal muscle in the abdominal oesophagus. In the same segment, acetylcholinesterase (AChE) activity was absent at the motor endplates as well as in the myenteric plexus. However, it was not possible to decipher whether the primary lesion was in nerve or muscle.
In the present study, an attempt has been made to define the morphological changes in the oesophagus during early and late stages of development of the anomaly with the aid of histochemical and ultrastructural methods, and so clarify the pathogenesis of the disease.

Materials and methods
The inbred strains of mice used were ICRC/HiCri with megaoesophagus and DBA/2fNCri as normal control. They were maintained under identical conditions: temperature (22–24 °C), relative humidity (55–65%), constant light/dark cycle, fed the same powdered diet No. 2 (Ranadive, 1957) and water ad libitum. The diet contained wheat 70%, bengal gram 20%, fish meal 5%, yeast powder 4%, sesame oil 0·75% and shark liver oil 0·25%.

Histochemistry
The animals were killed by an overdose of ether and oesophagus from 18 mice aged 5 days and 2 months were taken for histochemical studies. A few samples of colon from adult DBA and ICRC strains were also included as controls for normal smooth muscle. The oesophagus was cut just above the diaphragm into two parts before removal from the animals.

For frozen sections, the two portions of the oesophagus and the colon were cut open longitudinally, cleaned, then rolled into a 'Swiss roll', snap-frozen and sectioned with a cryostat (Webster, 1974). This enabled examination of the whole length of the oesophagus and colon in a single section. Histochemical reactions were carried out on 12 μ thick unfixed frozen sections for 1 h at room temperature for the following four enzymes. The first three were used as markers for smooth muscle and the fourth for striated muscle:

Butyryl Cholinesterase (BuChE): The technique described by Karnovsky and Roots (1964) was utilized. Butyryl thiocholine iodide was used as substrate.
5'-Nucleotidase: The method employed was that described by Scott (1965). Substrate used was adenosine-5-phosphate.

Adenosine Triphosphatase (ATPase) at pH 4·3: the substrate was di-sodium adenosine-triphosphate (Padykula & Herman, 1955). Succinic dehydrogenase (SDH): The technique given by Bancroft (1975) was followed. The substrate was sodium succinate along with nitroblue tetrazolium.

Ultrastructure
For electronmicroscopy, samples of the oesophagus were obtained from 8 DBA and 8 ICRC mice. The age groups selected were 5 days, 10 days, 3 months and 1 year. After opening the animals, 3% gluteraldehyde in cacodylate buffer was introduced into the lumen of the oesophagus. Two to four segments, depending on the size of the oesophagus, were marked out with a pointed needle. In 5-day-old animals the oesophagus was divided into upper and lower parts. In 10-day-old animals, it was divided into three parts comprising upper, middle and lower. In adult animals, it was divided into four parts (Fig. 1). Besides the cervical and thoracic parts, the abdominal portion was divided into upper and lower segments. The oesophagus was cut open...
Enzymes | Oesophagus | Colon
---|---|---
ATPase | DBA | ICRC | Colon
5'-nucleotidase | | |
BuChE | | |
SDH | | |

Fig. 2. Diagrammatic representation of the enzyme activity in the muscle walls of the oesophagus and colon from DBA and ICRC mice. The upper bar indicates the cervical and thoracic segments and the lower one indicates the abdominal segments. The three enzyme markers for smooth muscle (ATPase, 5'-nucleotidase and BuChE) are positive over the very short lowermost portion of abdominal oesophagus in the control DBA strain while they are expressed throughout the abdominal segment and also in the lower thoracic segment in ICRC strain. The colon which contains only smooth muscle is also positive for these three enzymes and shows weak reactivity for the skeletal muscle enzyme SDH. ■, strong reaction; ■, weak reaction; □, no reaction.

Fig. 3. Abdominal portion of a DBA oesophagus showing ATPase (pH 4·3) activity only in the lowermost region (arrow) (×19).

along its length, spread on a filter paper and fixed in 3% glutaraldehyde for 10 min. Then the mucosa was removed, the marked segments were separated, sliced into tiny pieces which were processed further. After an hour's fixation, the pieces of tissue were postfixed in 1% osmium tetroxide for 1 h, dehydrated in graded alcohols and embedded in araldite.

Fig. 4. ATPase (pH 4·3) activity found throughout the abdominal segment of an ICRC oesophagus (×19).

One micron thick survey sections stained with toluidine blue were examined and the relevant areas were selected for thin sectioning. Ultrathin sections stained with uranyl acetate and lead citrate were examined in a Zeiss 109 electron microscope.

Fig. 5. Cervical and thoracic portions of an ICRC oesophagus showing ATPase (pH 4·3) activity in the lower part (arrow) (× 19).
Results

Histochemistry

The oesophageal muscle was classified according to its histochemical features. The histochemical patterns of the four enzymes were alike in 5-day-old and adult mice, therefore the following findings apply to both age groups. Figure 2 shows the distribution of activity of the four enzymes in the muscle coat of the two segments of oesophagus from adult mice. The results for the colon are also included.

In the normal DBA mouse, positive reaction for ATPase 4·3 (Fig. 3), 5'-nucleotidase and BuChE was observed only in the lowermost region of the abdominal segment, whereas strong SDH activity was demonstrable in cervical, thoracic segments, and in two-thirds of the abdominal segment. The lowermost region close to the stomach showed only weak enzyme activity. In ICRC mice, intense activity was observed for ATPase 4·3 (Fig. 4), 5'-nucleotidase and BuChE throughout the abdominal segment. Positive reaction for these enzymes was also observed in the lowermost portion of the thoracic segment (Fig. 5) whereas the same regions showed only weak SDH activity. Strong reaction of this enzyme was confined to the upper portion of the oesophagus. The muscle coat of the colon from ICRC and DBA mice was positive for BuChE, 5'-nucleotidase and ATPase 4·3 but SDH activity was weak.

Ultrastructure

DBA mouse

In 5-day-old mouse, the muscle coat in the upper portion of the oesophagus showed striated muscle cells with distinct Z, A,
I, H and M bands. Mitochondria, glycogen and T-tubules were also present. Each cell was surrounded by a distinct basal lamina. Myenteric plexuses were observed in the connective tissue between the two layers of the muscle: well developed motor endplates were not observed. In the lower half of oesophagus there was an admixture of smooth and striated muscle cells which appeared immature. The cytoplasm of smooth muscle cells was filled with thin myofilaments. Mitochondria, rough endoplasmic reticulum, pinocytotic vesicles, dense plaques and scattered dense bodies were also noticed. Individual cells were covered by a thin basal lamina. Intermediate type of cell-to-cell contacts were encountered between a few cells.

In the adult mouse, the muscle coat of cervical, thoracic and upper abdominal portions of the oesophagus consisted of striated muscle cells, some of which showed motor endplates. Between the two layers of muscle, groups of unmyelinated axons of the myenteric plexus were observed throughout. In between the muscle cells, the spaces were filled with collagen fibres, fibrocytes...
and blood vessels. In the lower segment of the abdominal portion, smooth muscle cells were found along with striated muscle cells; only sheets of smooth muscle were seen in the lowermost part adjoining the stomach (Fig. 6). Small nerve endings containing dense-cored granules and some empty vesicles were seen close to some of these muscle cells.

ICRC mouse The muscle coat in the uppermost part of 5-day-old ICRC oesophagus was seen to consist predominantly of well developed striated muscle cells as found in the control specimen. However, a few apparently normal smooth muscle cells were also observed, closely intermingling with striated muscle cells. Plexus tissue consisting of groups of unmyelinated axons was present between the two layers of the muscle coat and collagen fibres surrounded the plexus. The lower half of the oesophagus showed only normal looking smooth muscle cells with intermediate type junctions. In 10-day-old animals, normal striated and smooth muscle cells were seen to constitute the muscle coat in the upper two-thirds of the oesophagus, whereas the lower part contained only smooth muscle cells. Plexus tissue was scanty and embedded in collagen. Nerve fibres were observed near to the smooth muscle cells.

In 3-month-old animals, the cervical portion of the oesophagus showed only well developed striated muscle cells. The thoracic portion contained both striated and smooth muscle cells which appeared normal. Plexus tissue and motor endplates were found as in the control DBA mice. The abdominal portion consisted exclusively of smooth muscle cells, but they appeared different in the upper and the lower segments. In the upper segment, smooth muscle showed several morphological deviations from normal. Some cells showed either long dense plaques along the membrane or dense bodies of anomalous shape.
and size (Fig. 7). Pinocytotic vesicles were normal in appearance. The majority of smooth muscle cells were widely separated from each other and had tortuous cytoplasmic processes on the surface (Fig. 8). The intercellular contacts were not as prominent as in the control DBA mice. There was marked fibrosis with abundant collagen separating the cells. Glycogen granules were found in a few cells.

The plexus tissue was scarce and indistinct. Nerve endings were not detected, but large nerve bundles containing myelinated and unmyelinated axons were observed in between the muscle fascicles.

The lower segment of the abdominal portion adjoining the stomach again showed smooth muscle cells arranged in sheets (Fig. 9) as found in controls. The plexus tissue was found to be normal. The intramuscular nerves were observed in the connective tissue septa. Many nerve endings containing dense core vesicles were seen adjacent to smooth muscle cells, and some had clear vesicles (Fig. 10).

In a 1-year-old animal, the oesophageal wall from the cervical and thoracic portions contained normal striated and smooth muscle cells. The alterations of the smooth muscle observed in 3-month-old animals were more pronounced and more frequent in the upper abdominal segment than in the lower segment. Besides abundant collagen fibres, electron dense masses of various sizes were encountered in between the muscle cells (Fig. 11). Though rare, intramuscular nerves could be seen, but myenteric plexus or nerve endings were not encountered.

The lower abdominal segment showed vacuolar degeneration and electron dense masses in between the muscle cells. In addition, there
were some degenerating cells bearing small irregular nuclei with condensed chromatin.

**Discussion**

The most striking and interesting observation in the ICRC mouse was the presence of smooth muscle over a greater extent of the oesophagus than that in the control DBA strain. The enzyme activity patterns were helpful for the identification of fibre types (striated and smooth muscle) in the entire oesophagus. In the normal DBA mouse, striated muscle was the exclusive component of muscle coat throughout the oesophagus except in the lowermost segment which is composed of smooth muscle. In the ICRC mouse, smooth muscle extended from the lower part of the thoracic segment to the gastro-oesophageal junction.

The difference in AChE activity between normal and ICRC oesophagus which we have already reported (Randelia et al., 1988), could be explained by our present findings on the difference in muscle fibre types in normal and ICRC oesophagus. The motor endplates found throughout the oesophagus stained for AChE in the control DBA strain, whilst they were absent in the lower third of the oesophagus in the ICRC mouse. This deviation is due to the fact that in ICRC mouse the muscle coat of the lower third of the oesophagus was constituted exclusively of smooth muscle.
The greater component of smooth muscle in ICRC oesophagus seems to be a genetically determined trait. It is possible that due to a disturbance in development, smooth muscle develops in the lower oesophagus instead of striated muscle. With this change, there is a need for a concurrent switchover of the innervation from somatic motor to autonomic component of the vagus nerve. This is perhaps defective with the result that smooth muscle (particularly the upper abdominal segment) was most affected because of faulty innervation. On the other hand, smooth muscle adjacent to upper striated muscle in the thoracic segment did not show any abnormality and, in addition, a paucity of plexus tissue and nerve endings were observed at an early age. It is therefore more likely that the muscle changes are secondary to nerve lesions in the pathogenesis of the disease.

The occurrence in man of 'achalasia' a neuromuscular disorder of the oesophagus has prompted a number of investigators to develop an animal model (Deloyers et al., 1957; Harris et al., 1960; Gruber, 1978) but techniques like vagotomy or destruction of ganglion cells in dogs, cats, and monkeys, were not successful. Even spontaneously occurring megaoesophagus in dogs and rats are not suitable models as evidenced from manometric, pharmacological and histopathological findings (Diamant et al., 1973; Harkness & Ferguson, 1979). However, the presence of smooth muscle in the thoracic and abdominal segment of oesophagus in the ICRC mouse makes it closer to human oesophagus. Lower oesophageal sphincter dysfunction is a prominent feature of achalasia in man and this could be investigated in the ICRC mouse.

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References
Harkness JE & Ferguson FG (1979) Idiopathic megaoesophagus in a rat. Laboratory Animal Science 29, 495–498
Randelia HP & Lalithia VS (1988) Megaoesophagus in ICRC Mouse. Laboratory Animals 22, 23–26