# Development of the Anal Canal Muscles

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Levi AC, Borghi F, Garavoglia M. Development of the anal canal muscles. Dis Colon Rectum 1991;34: 262-266

The anal canal muscles development is studied in 18 human embryos. The external anal sphincter results to origin common with the urogenital sphincter from the cloacal sphincter. The muscle, after its appearance, is subdivided into two portions from a thin mesenchimal layer. Moreover, our embryologic study clearly confirms that the puborectalis muscle is a portion of the levator ani, its primordium being common with the ileo and the pubococcygeus muscles. The anal smooth musculature appears later than the striated one. [Key words: Anal canal; Levator ani; External sphincter; Development]

The anal canal muscles development is a matter of controversy. The external anal sphincter can be observed in human embryos at 30 mm crownrump length (CRL).<sup>1, 2</sup> Its origin could be considered either from the cloacal sphincter<sup>3, 4</sup> or common with the levator ani muscle.<sup>5, 6</sup>

There are no embryologic studies particularly concerning the puborectalis. Human anatomical researches and phylogenetic studies give different results: they demonstrate that the puborectalis is a portion of the levator ani<sup>7–9</sup> or of the external anal sphincter.<sup>10, 11</sup>

Our work purposes an attempt to demonstrate the real origin and pertinence of the anal striated musculature. The knowledge of the age of appearance and of the morphogenetic processes of the muscles is essential for an understanding of their significance and behavior both in physiologic and pathologic conditions.

# METHODS

Our study was carried out on 18 human embryos and fetuses ranging from 15 to 134 mm CRL (6 weeks to 3.5 months as stated in the classic embry-

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ologic work of Hamilton and Mossman<sup>6</sup>). The CRL in millimeters of each single embryo and fetus is reported in Table 1.

The whole embryos and the separated caudal part of the fetal trunks (under L 5) were fixed in a liquid containing picric acid (Bouin). After embedding in paraffin, the specimens were sectioned in the different planes (transverse, sagittal, frontal): particularly the embryos, because of their typical caudal curvature, were oriented during cutting to obtain always transverse sections. All the microtomic sections (7  $\mu$ m thick) were stained with hematoxylin-eosin and Mallory's trichrome method for microscopic observation. Furthermore, the histologic specimens were serially microphotographed to reconstruct the muscular structures which are the object of this study.

#### RESULTS

The development of each single muscle of the anal canal progresses as follows:

1. Near the ninth week circular musculature appears below the epithelial layer, tightly continuing the musculature of the primitive rectum, gradually increasing by the apposition of new layers to be completely formed at the third month as the internal sphincter muscle. The longitudinal muscle fibers appear in the primitive rectum at the ninth week, but they run in the anal canal later in comparison with the circular layer. At the second month the longitudinal muscular bundles intermingle with the pubococcygeus muscle, and at the third month descend to the intersphincteric space.

2. The levator ani primordium at the sixth week is recognizable in the form of some promyoblasts and myoblasts scattered into mesenchymal tissue around the rectum (Fig. 1). In earlier embryos we were not able to demonstrate any identifiable muscular structure. Afterwards the levator ani extends anteriorly towards the pubis, posteriorly to the coccyx, and laterally, in both sides, toward the

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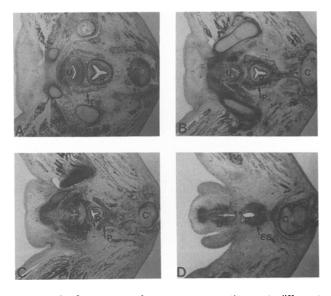
Table 1   Crown-Rump Length of Embryos and Fetuses (CRL)		
Human embryo		
1	15	
2	18	
3	23	
4	25	
5	25	
6	30	
7	32	
8	33	
9	35	
10	35	
Human fetus		
11	42	
12	45	
13	50	
14	54	
15	56	
16	80	
17	80	
18	134	



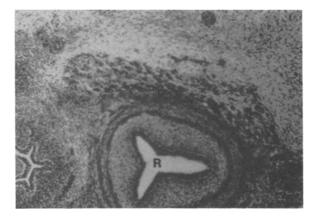
**Figure 1.** Transverse section of the pelvis in a 18-mm CR human embryo (Azan Mallory, original magnification  $100 \times$ ). The arrow indicates the promyoblasts of the levator ani primordium (L). R: rectum.

internal obturator muscle (Fig. 2A); in front of the coccyx the anococcygeal raphe is developing.

Two portions of the levator ani take connections with the rectal wall: the pubococcygeus and the puborectalis muscles. The pubococcygeus muscle, medialis part of the levator ani, at the end of second month adheres to the longitudinal muscular fibers (Figs. 2B and 3); some myoblasts move medially

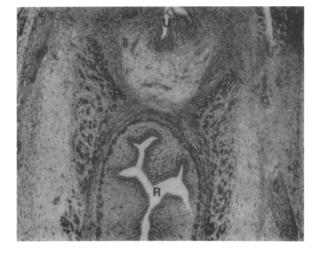


**Figure 2.** Sequence of transverse sections at different levels (from above downwards) of the caudal trunk in a 35-mm CR human embryo (Azan Mallory, original magnification  $25\times$ ). U: urogenital sinus; R: rectum; C: coccyx. A. In the upper section the lateral part of the levator ani (L) is not in contact with the pelvic viscera. B. The pubcoccygeus muscle (Pc), medialis part of the levator ani, adheres to the rectal wall (see also Fig. 3). C. The puborectalis sling (Pr) around the rectum is clearly demonstrated. D. In the lower section the external sphincter (ES) surrounds the anal canal, which is separated from the urogenital sinus by the perineal body (PB). The different stage of development of the external sphincter in respect of the levator ani is clear.



**Figure 3.** Transverse section of the pelvis in a 35-mm CR human embryo (Azan Mallory, original magnification  $50\times$ ) showing the tight connections between the pubococcygeus (Pc) and the longitudinal muscle of the rectum (R).

interposing between the urogenital sinus and the primitive rectum (Fig. 4). The limits between pelvis and perineum are defined at the third month when the levator ani muscle insertions have been established (Fig. 5A). In the same period (week 7–9) transverse sections demonstrate that the pu-



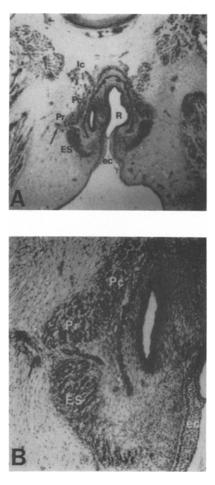
**Figure 4.** Transverse section of the pelvis in another case of 35-mm CR human embryo (Azan Mallory, original magnification  $50\times$ ). Some myoblasts of the pubococcygeus (Pc) are interposed between the urogenital sinus (U) and the primitive rectum (R).

borectalis muscle is a typical sling around the rectum (Fig. 2C).

3. In week 7, the primitive rectum and the urogenital sinus, separated by the primitive perineal body, are surrounded by promyoblasts forming a ring: this is the cloacal sphincter. On coronal sections the muscle is clearly at a different level in respect of the levator ani (Fig. 6).

During the second month, between the primitive rectum and the urogenital sinus, the myoblastic wall collapses and furthermore subdivides itself forming anteriorly the urogenital sphincter, in connection with the urogenital sinus, and posteriorly the external anal sphincter, in connection with the anal canal (Fig. 2D); this muscle is in a less differentiated stage in respect of the levator ani of the same embryo.

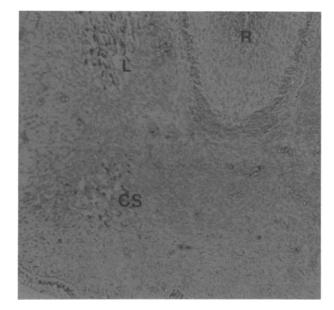
At the third month a mesenchymal thin layer separates the external anal sphincter into two parts: the superficial one is in connection with the cutis, the deep one underlies the puborectalis muscle. Inferior hemorrhoidal vessels and the anal nerve are running in the connectival space between the external anal sphincter and the puborectalis muscles that correspond to the ectodermal and endodermal epithelium respectively (Fig. 5, a and b). The anal nerve leaves some branches to the external anal sphincter. Furthermore, only growth affects the external anal sphincter and the other muscles, their connections and shape being firmly established.



**Figure 5.** A. Frontal section of the anorectal region in a 45-mm CR human fetus (Azan Mallory, original magnification  $25\times$ ) showing the division between pelvis and perineum. Ic: ileococcygeus; Pc: pubococcygeus; Pr: puborectalis; ES: external sphincter; R: rectum; ec: ectodermal epithelium. B. The same section at a higher magnification ( $50\times$ ). The arrow shows the inferior hemorroidal vessels and the anal nerve between the external sphincter (ES) and the puborectalis muscle (Pr). Pc: pubococcygeus. The correspondence of the site of the external sphincter to the ectodermal epithelium (ec) is clear.

# DISCUSSION

Some conclusions about the anal canal muscles development can be derived from the present embryologic observations. Wendell-Smith<sup>10</sup> observes muscular bundles passing from the urethra to reach the pubis in anthropoid apes. The author affirms: "these fibers are the homologues of the puborectalis muscle which is thus part of the external sphincter system and sphincter cloacae group." Conversely, recent anatomic<sup>7</sup> and electrophysiologic studies<sup>8</sup> demonstrate that the puborectalis muscle and the ileo and pubococcygeus muscles have identical innervation. Moreover, phylogenet-



**Figure 6.** Frontal section of the anorectal region in a 25mm CR human embryo (hematoxylin-eosin, original magnification  $50\times$ ) showing the separation between the cloacal sphincter (CS) and the levator ani primordium (L). R: rectum.

ically the puborectalis is present only in primates with erect posture and has been regarded as a special adaptation of the pubococcygeal part of the levator ani.<sup>9</sup>

Our research gives evidence of the common origin of the puborectalis, ileococcygeus, and pubococcygeus muscles. The puborectalis must be considered as a part of the levator ani: it is never connected with the external anal sphincter in different stages of the embryos development. Careful examination of embryos in different developmental stages demonstrates that both the external anal sphincter and the urogenital sphincter originate from the cloacal sphincter.

The two muscles are clearly delineated at the end of the second month<sup>12</sup> but are yet connected in the mesenchymal space interposed between the anal canal and the urogenital sinus. Furthermore, it is demonstrated that the time of appearance of the external anal sphincter and levator ani is different: particularly, up to the second month, the sphincter is essentially formed by promyoblasts that are less differentiated than the myoblasts of the levator ani. These results agree with those obtained by Popowsky<sup>3</sup> and also with that phylogeny and comparative anatomy report.<sup>9, 13, 14</sup>

The external anal sphincter, according to Thompson,<sup>13</sup> Milligan and Morgan,<sup>15</sup> and Stelz-

ner,<sup>16</sup> consists of three components: the subcutaneous one, the superficial, and the deep one. Conversely, Courtney<sup>17</sup> and others,<sup>18–20</sup> by histologic observations or anatomic dissections, hold that the external anal sphincter is only formed by two portions. Our embryologic study confirms this last opinion, demonstrating that the external sphincter is subdivided into two parts, the superficial and the deep one, without any connection with the puborectalis, which is a dependence of the levator ani. Our interpretation confirms the study of Popowsky<sup>3</sup> and is different from that of Courtney<sup>17</sup>: the author suggests that the superficial sphincter originates from the primitive cloacal sphincter, whereas the deep portion is a part of the levator ani muscle.

The anal canal smooth musculature appears later than the striated musculature: according to the present observations the internal anal sphincter is delineated at the third month. This data differs widely from Nobles,<sup>1</sup> who affirms the origin of the internal anal sphincter at the sixth week. The longitudinal fibers descend in the intersphincteric space in the same stage of development.

The different ages of development of the anal canal musculature confirm its subdivision into two concentric cylinders proposed by Parks<sup>21</sup>: an internal cylinder made of smooth muscles and an external one, striated, that precedes the descent towards the perineum of the visceral cylinder. This consideration is also important to define the functional significance of the anal canal muscles.

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