STUDIES IN THE DYNAMICS OF HISTOGENESIS.

I. Tension of Differential Growth as a Stimulus to Myogenesis.

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(Received for publication, January 17, 1920.)

The prevalent opinion among embryologists in regard to the origin of muscular tissue is that of self-differentiation. This is due largely to the work of Wilson (1904) on *Dentalium*, and Conklin (1896–97, 1905) on *Cynthia* (*Styela*) *partita* and *Crepidula* in regard to the organ-forming elements of the cytoplasm, and to the experimental work of Harrison and Lewis (1904, 1905). Harrison ablated the spinal cord of the tadpole prior to the growth of the peripheral nerves into the limb buds. This operation eliminated any peculiar formation stimulus emanating from the nervous system. Still the differentiation of the contractile substance took place in the normal manner, as did the grouping of fibers into the individual muscles. Lewis (1910) draws the following conclusion based on Harrison's experiments, in regard to the genesis of cross-striated muscle:

> "Thus it is seen that all the constructive processes involved in the production of the specific structure and arrangement of the muscle-fibres take place independently of stimuli from the nervous system and of the functional activity of the muscles themselves. Cross-striated muscle tissue and the individual muscles are thus self-differentiating."

The fact that there is considerable muscular differentiation before nerves establish a connection with their corresponding muscles has been shown by Bardeen (1900, 1906–07), Harrison, and Carey (1918) in the pig embryo. There is also considerable smooth muscle differentiation in the descending colon of the pig before either the myenteric or Auerbach's plexus is detected.

Lewis (1910) endeavored to solve the problem at how early a period in the development of the ovum this power of self-differentiation of muscle tissue begins. He found by transplanting tissue from the lips of the blastopore in the early gastrula stage of the frog that this tissue later on showed muscular differentiation. The conclusion is drawn "that muscle tissue is already predetermined in the early gastrula."

The idea conveyed by the last statement is that muscular tissue is formed, *sui generis*, by some inherent predetermination and not by the agency of its surroundings nor due to its position in the whole. Lewis' view-point is in accord with Conklin's (1905) as seen in the following statement of the latter observer: "The potencies or prospective values of any blastomere are not primarily a function of its position, but rather of its material substances."

There are three theories regarding cellular differentiation; first, the "mosaic theory" of Roux (1881), later modified by Wilson (1904), Conklin (1905), Zeleny (1904), and Boveri; second, the "organization theory" of Whitman and more recently elaborated by Child (1915) in his studies on metabolic gradients and individuality; third, "the homogeneity theory" of Driesch (1894, 1899). Driesch considers the peculiar organizing quality of protoplasm as due to the expression of a mysterious force wholly different from any in the inorganic world.

His, Roux (1881, 1892, 1893), Wilson (1892, 1893, 1897, 1904), and Conklin (1905) lay emphasis upon the cell as the key to all ultimate biological problems. Whitman, on the other hand, points out the inadequacy of the cell theory to development. "That organization precedes cell formation and regulates it, rather than the reverse, is a conclusion that forces itself upon us from many sides," is a summary of his studies. Morgan (1895, 1898) had deduced the idea from his studies on regeneration that the multicellular individual is a whole in the same sense that the unicellular form is a whole. Child (1899, 1915) also lays emphasis on the fact that it is the "organism—the individual, which is the unit and not the cell." Differentiation of a single cell, consequently, according to Child and Whitman, is a function of its position in the whole. This view is also upheld by Driesch (1894). Wilson and Conklin, on the other hand, conclude that potencies are functions of the material substance of the cell.

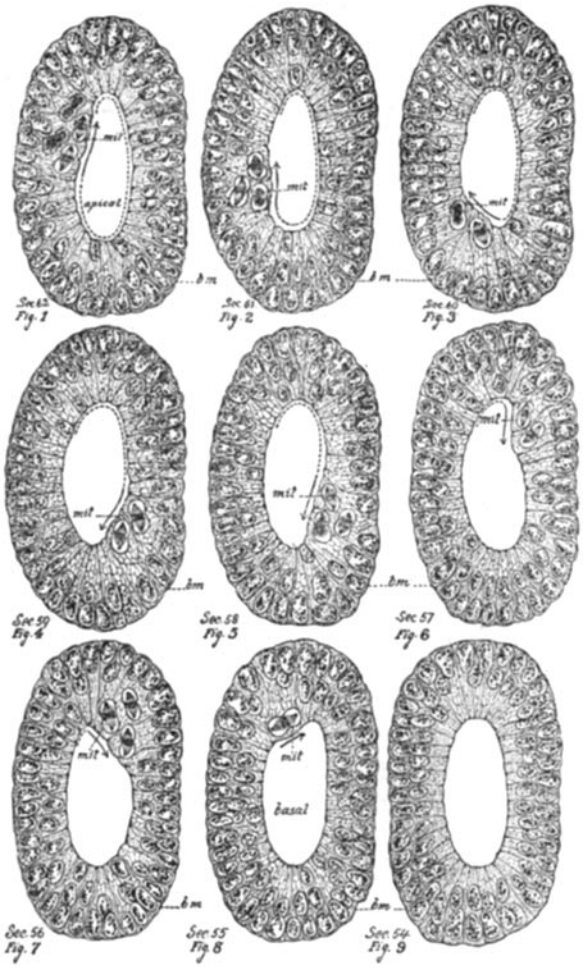
The influence of the organism as a whole in subjugating its dependent parts is convincingly shown by Loeb (1916) in his regeneration experiments on *Bryophyllum calycinum* and in his experiments on *Amblystoma* larva (1897). This influence is exerted through the blood stream by means of "hormones." The sound mechanistic attitude of Loeb toward development may be seen in the following statement: "As soon as we can show that a life phenomenon obeys a simple physical law there is no longer any need for assuming the action of non-physical agencies" (1916).

The object of this paper is not, however, to discuss historically the views of these various authors, but to emphasize certain facts in the development of muscle tissue hitherto overlooked. It is a well known fact that the embryo presents differential rates of growth. It is desired, therefore, to emphasize the fact that in embryological development there are zones of unequal or differential growth, and that the effects of these zones of growth are factors in histogenesis. The active and less active zones are defined with reference to the rate of cell division per mm. of cross-section. This principle was deduced from a series of studies on osteogenesis and myogenesis begun in 1914. Previous reports of a part of this work have been presented to the Association of American Anatomists (Carey, 1917, 1918, 1919).

It will be illuminating to search for the cellular forces outside of the immediate differentiating zone under observation. This search necessitates lower magnifications in order to enlarge our field of view. Heretofore, cytological differentiation has been studied *per se*, with magnifications of 1,000 to 2,000 diameters which considerably reduce our range of view. The higher magnifications are profitable in revealing cytological detail but the interpretation of the process is lost unless, in conjunction with the higher, intermediate magnifications are used. The employment of all magnifications of the microscope in connection with naked eye studies will reveal the interaction of related developing parts.

Early Development of the Descending Colon of the Pig.

The attention of the writer was directed to the fact, after plotting hundreds of intestinal epithelial mitotic figures, that these figures were usually confined to some definite region of the circumference of a single



FIGS. 1 to 9. Sections of the epithelial tube representing one complete turn in a dexiotropic rotation in a spiral manner of the mitotic division. The primary type is the left-handed helix. Spiral path is directed upward toward the ileocecal valve. Section 62 represents the head or apical end of the mitotic path. Sections 55 and 54 represent the tail or basal end of the mitotic spiral path. *mit*, mitosis; *bm*, basement membrane of the epithelial tube. Drawings are made with the aid of a Spencer Camera lucida. Sections 54 to 62 are from Pig Embryo 19, 24 mm. in length (Creighton Embryological Collection).

section (Figs. 1 to 9). This region was found to change at different levels of the serial sections. By graphic reconstruction this plot was found to form the path of a definite spiral. The predominant type was the left-handed helix. In one of the embryos of the twenty that were plotted this spiral was arranged as a right-handed helix (Figs. 1 to 9). The spiral itself presented a head or apical region in which mitotic figures were found to be numerous, and a tail or basal end in which there were fewer and fewer figures. The apical end of the spiral path is always directed towards the ileocecal valve and the basal end towards the rectum. Growth is, therefore, from below upwards in a spiral course. One spiral growth is quickly followed by a second which rifles a path slightly lateral to its predecessor. This in turn is followed by a third in a path still more lateral, and so on around the circumference. This intermittent rhythm of explosive spiral growth may be compared to that of the successive fire balls emitted by a roman candle in fireworks. The paths formed by this explosive spiral growth may be compared to those within the barrel of a Winchester rifle.

The most rapidly growing part of the intestine, therefore, is the epithelial tube. In embryos 10 to 25 mm. in length the descending colon grows relatively more rapidly in diameter than in length (Tables I and II). The increase in diameter is due primarily to the rapid growth of the entodermal epithelial tube and only partially to its surrounding mesenchymal cloak. The latter is relatively passive in growth with respect to the former (Fig. 10). It is during this early increase in diameter that the inner smooth muscle coat is in process of formation. The mesenchymal cells are drawn out gradually in a definite series of concentric rings. These rings appear not unlike those of the planet Saturn and the annular nebula in Lyra.

A definite centripetal force is active in the rapid spiral growth of the intestinal epithelial tube. The surrounding mesenchymal cells are thrown into an obvious series of concentric rings, according to their various densities. Those possessing the greatest density will join the outer ring in the tangential path of the force, whereas the inner ring will be composed of bodies forming a gradient of decreasing densities. The cells forming the outer ring will be most elongated.

TABLE I.

Measurements of Differential Growth of Descending Colon.

Length of embryo.	Thickness of mesenchymal wall.*	Diameter of epithelial tube.	Diameter of descending colon.	Epithelial tubular indices.†
mm.	*mm.*	*mm.*	*mm.*	*per cent*
10	0.085	0.048	0.218	6
12	0.099	0.069	0.267	8
13	0.115	0.075	0.305	8.5
14	**0.128**	0.081	0.337	10
16	0.126	0.089	0.341	14
19	0.124	0.095	0.343	18
20	0.123	0.099	0.345	20
22	0.122	0.119	0.363	25
23	0.121	0.138	0.383	30
24	0.120	0.152	0.392	38
25	0.115	0.164	0.394	40
27	0.109	0.188	0.406	44
30	0.104	0.208	0.416	46
32	0.099	0.220	0.418	47
35	0.098	0.246	0.442	48
37	0.096	0.260	0.452	50
39	0.093	0.279	0.465	52
40	0.092	0.289	0.473	54
42	0.090	0.312	0.482	57
45	0.083	0.321	0.486	61

* The mesenchymal wall begins to diminish in thickness after it reaches a width of 0.128 mm. in the 14 mm. stage of the pig embryo. This diminution is due to the tension caused by the more rapid epithelial tubular growth in diameter. Measurements made with B. and L. filar micrometer, calibrated.

† The ratio of the square of the mean diameter of the epithelial tube to that of the surrounding mesenchyme is referred to as the epithelial tubular index. It has been calculated from the following formula.

$$\left\{ \frac{\dfrac{(x+y)^2}{(\ 2\ )} \times 100}{\dfrac{(X+Y)^2}{(\ 2\ )} - \dfrac{(x+y)^2}{(\ 2\ )}} \right\} = Z$$

x and y are the long and short diameters of the epithelial tube respectively. X and Y are the long and short diameters of the surrounding mesenchymal tube. Z is the ratio of the epithelial tube to the mesenchymal tube.

As this concentric initial smooth muscle layer becomes differentiated it tends to restrict the diametrical growth of the epithelial tube. The epithelial mitotic figures under this restriction shift their planes of division from a right angle to a parallel position with the smooth muscle cells. This shifting results in an elongation of the intestine.

TABLE II.

Ratio of Diameter to Length of Entire Colon.

Length of embryo.	Diameter of descending colon.	Length of entire colon.	Ratio of diameter to length of entire colon.
mm.	*mm.*	*mm.*	*mm.*
10	0.218	1.95	1:9.9
12	0.267	2.00	1:7.5
13	0.305	2.02	1:6.6
14	0.337	2.05	**1:6.0**
16	0.341	2.10	1:6.1
19	0.343	2.20	1:6.4
20	0.345	2.30	1:6.7
22	0.363	2.75	1:7.5
23	0.383	3.00	1:7.8
24	0.392	3.50	1:8.9
25	0.394	3.95	1:10.0
27	0.406	6.00	1:14.0
30	0.416	8.00	1:19.0
32	0.418	11.00	1:26.0
35	0.442	16.95	1:36.0
37	0.452	18.00	1:39.0
39	0.465	21.00	1:45.0
40	0.473	23.00	1:48.0
42	0.482	27.00	1:56.0
45	0.486	29.00	1:59.0
Adult.	50.000	7,000.00	1:140.0

In embryos 25 to 40 mm. (Tables I and II) in length, the elongation of the descending colon is more rapid in growth than that of the diameter. It is during this period that the outer longitudinal muscular coat is in the process of formation. The rapid growth of the epithelial tube in length tends to elongate the peripheral undifferentiated mesenchymal cells which were not directly involved in the formation of the inner smooth muscular coat (Fig. 11).

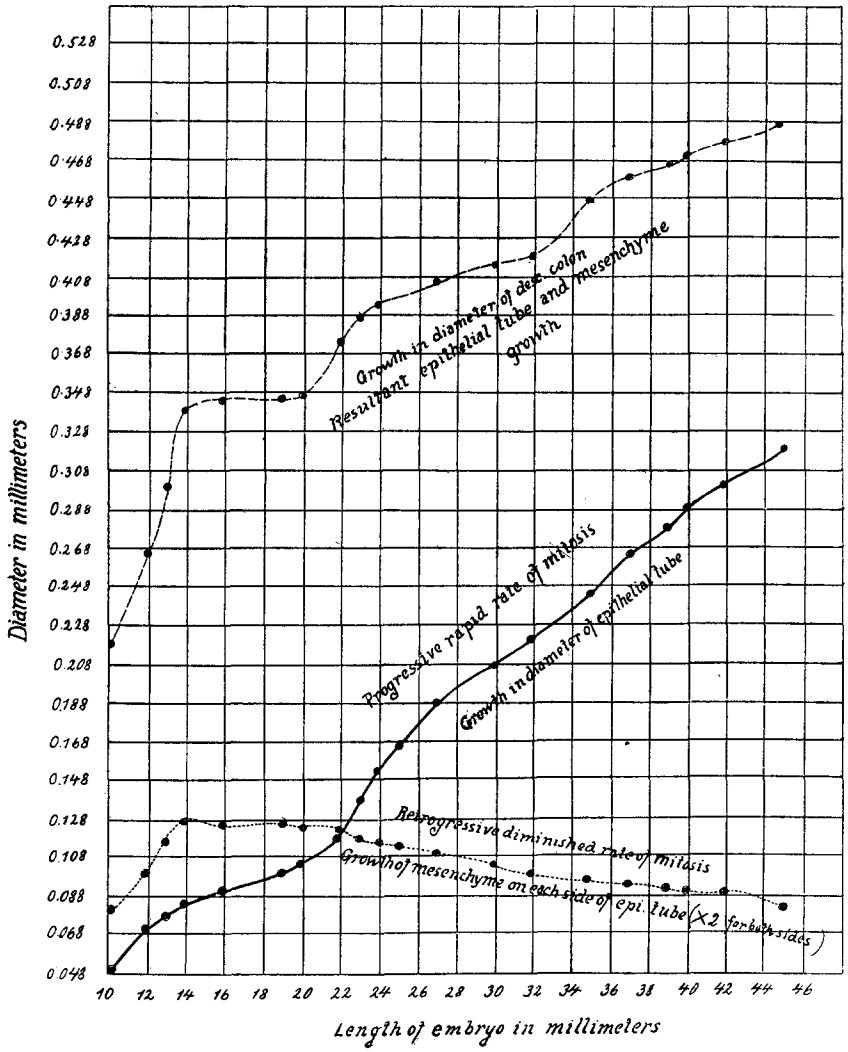


FIG. 10. Curves of differential growth of descending colon. The active growth of epithelial tube is contrasted with the passivity of the mesenchymal wall. An absolute decrease in thickness of the mesenchymal wall is seen after the stage of the 14 mm. pig embryo.

The differentiation of the outer longitudinal muscle coat therefore coincides, in time, with the rapid growth in length of the intestinal epithelium. The inner smooth muscle coat, on the other hand, is formed during the period of the rapid growth of the intestinal epithelial tube in diameter. Once the formation of the inner circular muscular rings is fairly established a resistance to growth in width is

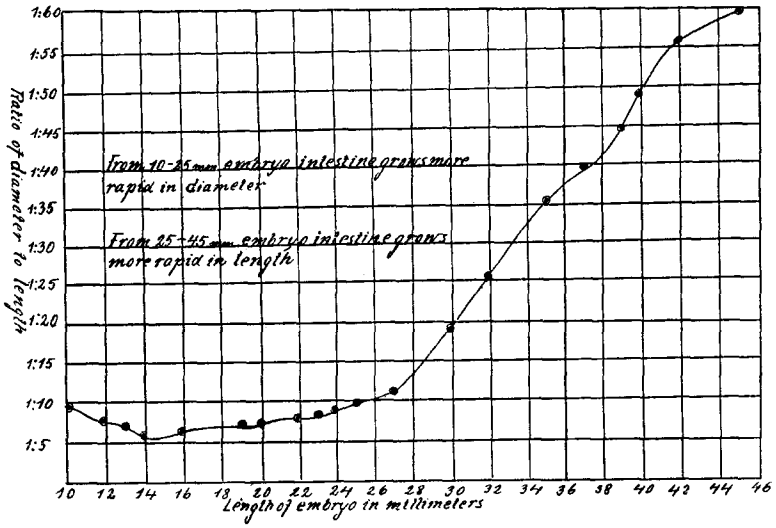


FIG. 11. Curve representing the ratio of the diameter to the length of the descending colon in embryos 10 to 45 mm. in length. Particular attention is directed to the fact that in embryos 10 to 25 mm. in length the intestine grows relatively more rapidly in diameter than in length. In embryos from 25 to 45 mm. in length the intestine grows more rapidly in length than in diameter. The inner circular smooth muscle is formed during the period of rapid growth of the intestine in diameter. During the period of the rapid growth of the intestine in length the histogenesis of the outer longitudinal smooth muscle coat is taking place.

encountered by the cells surrounding the rapidly dilating lumen. These cells then grow primarily along the path of least resistance in a longitudinal manner. At this stage the longitudinal muscle cell, spherical in shape (Fig. 12), is elongated to a spindle-shaped structure (Fig. 13).

An interesting correlation in the development of the esophagus in man may be cited. This correlation was detected in the work of

Jackson and in that of Keibel and Elze. The former investigator studied the developmental topography of the esophagus, the two latter the histogenesis of the esophagus. Jackson states that the descent of the stomach is accompanied by a great elongation of the esophagus. In a 9.4 mm. specimen, the esophagus measures 1.8 mm. At this proportion it should measure 4.3 mm. in a 22.8 mm. embryo but its actual length is found to be 8 mm. A year previous to this, Keibel and Elze reported that the esophagus in 12.5 mm. embryos shows a circular but no longitudinal muscle layer. In 17 mm. embryos, they find a circular layer with the longitudinal layer faintly

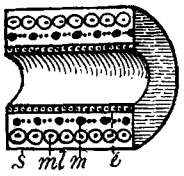


FIG. 12.

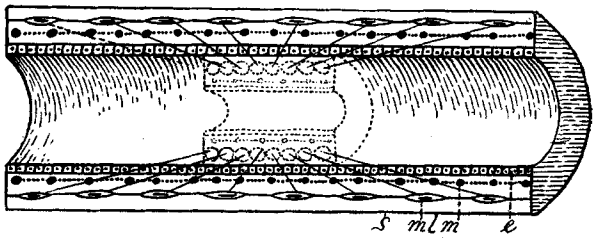


FIG. 13.

FIG. 12. Longitudinal section of intestine; *s*, peritoneal epithelium; *ml*, mesenchyme; *m*, circular muscle; *e*, epithelium.

FIG. 13. Longitudinal section of intestine schematizing the elongation of the intestine represented in Fig. 12. Due to the resistance of the inner smooth muscle layer *m*, the intestinal epithelium grows in the longitudinal path of least resistance. This results in the elongation of the outer mesenchymal cells *ml* (Fig. 12) into the elliptical or spindle cells *ml* (Fig. 13).

indicated. The histogenesis of the outer longitudinal layer of the esophagus as studied by Keibel and Elze coincides in time with the rapid elongation of the esophagus, due to the descent of the stomach, as recorded by Jackson.

Interpretation of Results.

The result of the action of a force on an elastic body is the production of a strain. If mechanical forces are at work on organic matter, they tend to produce similar results to these acting upon inert matter. Too frequently the term self-differentiation is applied to alteration of

form and internal structure of developing cells without searching the immediate environment of the specializing cells or syncytium to ascertain whether or not these changes are attributable to forces outside of the differentiating zone. This applies particularly to the differentiation of bone and muscle tissue. If a cell changes in form successively through the spherical, ellipsoid, and spindle stages it undergoes a strain. A strain is usually due to an external force which elicits internal reacting stresses in the body acted upon. Cytological differentiation is frequently a manifestation of these internal reacting stresses to forces extrinsic to the differentiating zone.

A strain is produced in certain regions of the embryo by the expansion of a rapidly dividing group of cells against a less active or relatively passive group of cells. After their differentiation the relatively passive group of cells in their turn react upon the former. This action and reaction are objectively evident by a retardation or alteration of the rate of growth, or by a change produced in the external form or internal structure of the cells involved.

In this study, the initial zone of rapid growth is found in the epithelial tube. The rapid spiral expansion of the entodermal epithelial tube reacts against the surrounding splanchnic mesenchyme with the result that the less actively growing cells of the peripheral region of the intestinal wall are elongated. Later the elongated, differentiated mesenchymal cells cause a retardation of the growth in diameter of the epithelium. Immediately following this retardation of diametrical growth the period of rapid growth in length of the intestine takes place. In this development, therefore, the influence of unequal growth zones is definitely shown as furnishing a tensional stimulus for the differentiation of muscle.

This action is diagrammatically illustrated in Figs. 14 and 15. In Fig. 15 the growth in diameter of the intestine is schematized; the rapid increase in width is shown as due primarily to the increase in the lumen. This growth is due to rapid mitotic activity of the epithelium (*e*, Fig. 14, to *e'*, Fig. 15). In the lumen of Fig. 15, the former is represented in a spiral manner, *sg*. In this growth the strain upon the surrounding mesenchymal cells *m* is illustrated. These cells are strained by the external applied forces of the progressively diverging radii. The internal reacting stresses are manifested by the changes

in shape through spherical m, ellipsoidal m', and spindle m'' cellular phases in Fig. 15. In addition to the homogeneous strain to which the cells m, m', and m'' are subjected there is a definite pressure exerted by the epithelial cells lining the expanding lumen.

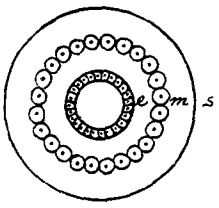


FIG. 14.

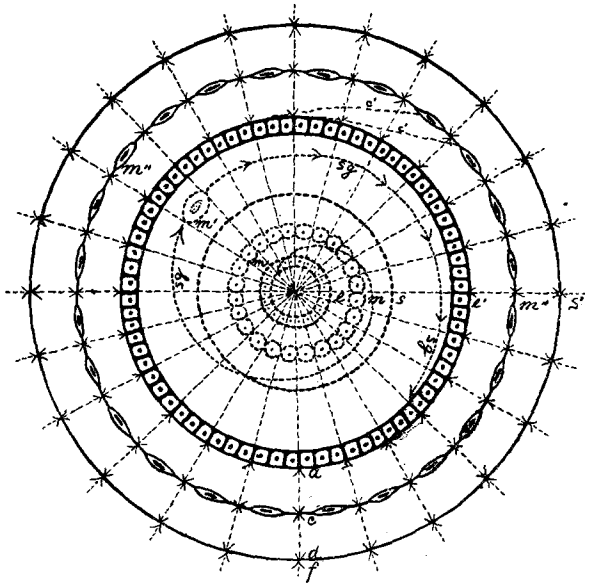


FIG. 15.

FIG. 14. Transverse section of intestine; e, epithelium; m, mesenchyme (spherical nucleus); s, peritoneal epithelium.

FIG. 15. Transverse section of intestine grown to three times the width represented in Fig. 14. Fig. 15 is represented in broken lines within the lumen. The spiral growth of the epithelium is represented by the broken lines sg. The tension, upon the mesenchyme, by the most rapidly growing epithelium, is shown in the elongated muscle cells $m.''$ These cells are homogeneously strained in the centrifugal path c' due to the progressively diverging radii. Cells marked m, m', and m'' represent the progressive steps in the strain ellipsoid in the differentiation of a muscle from a mesenchyme cell. The expansile force of the epithelium is shown by the double arrow a–d; the reacting resistance of the serous membrane by the line d–a. Equilibrium is established in the middle of the mesenchyme and is graphically represented by the double arrows a–b and d–c. This is another factor in the tensional elongation of the middle cells. The smooth muscle ring exerts a centrifugal reaction to the applied centripetal force of the dexiotropic spiral rotation of the epithelial tube. The mitotic figures of the descending colon primarily follow the path of a left-handed helix.

This force of expansion is represented by the arrow *a–d*. A resistance *f*, due to the peritoneal epithelium, is met. This causes a reaction *d–a*, With progressively increasing growth a zone of equilibrium of expansile and reacting forces is established in the middle, represented by the double arrows *b–a*, and *d–c*. This action and reaction of forces is another factor tending to compress the cells in the middle of the mesenchymal wall of the intestine. The action of the centripetal component of the spiral growth of epithelium in forming the rings of dense spindle-shaped muscle cells *m″* is represented by the broken curved arrows. The spiral growth of the epithelial tube in a dexiotropic rotation exerts a centripetal force upon the surrounding mesenchyme. The mesenchyme consequently exerts a simultaneous equal and opposite centrifugal force upon the epithelial tube. This growth is primarily in the form of a left-handed helix from the rectal to the ileocecal valvular regions of the large intestine. In Figs. 14 and 15 the right-handed helix is depicted.

Although by direct observation of serial sections no motion is seen, there is, however, objective evidence of homogeneous and ellipsoidal strains upon the surrounding mesenchyme. The mesenchyme is drawn out into concentric rings, the outermost of which are most viscid, by the spiral growth of the epithelial tube, roughly comparable to the increase of viscosity and concentric annular formation of egg albumin when subjected to an egg-beater.

CONCLUSION.

The genesis and maintenance of muscle tissue represents a resultant or equilibration of converging factors which are active and formative during development. *One of these factors is the tensional stresses to which the mesenchyme is subjected by a force extrinsic to the differentiating zone.* In subsequent involution or degeneration of muscular tissue during the prenatal or postnatal periods, this equilibrium is upset by altering or destroying the tensional reacting stress.

Tension is developed when a muscle contracts. Contractility is a fundamental property of protoplasm and, when manifested, tension is developed. In both, the development and specific function of muscle tissue, tensional stresses are inseparably involved. The

Ameba possesses the property of contractility in all possible directions. The function of contraction in one definite direction characterizes muscle tissue from that of undifferentiated protoplasm. What initiates the progressive series of physicochemical changes in the mesenchyme resulting in an alteration of its attribute from non-specificity to its specificity of direction of contractility? This question is answered as follows.

The mesenchyme before differentiating into muscle tissue must be subjected to a certain minimal homogeneous and ellipsoidal strain. This strain is objectively evident by an alteration of the form of the spherical nuclei, into the ellipsoidal and spindle conditions and by an elongation of the granular cytoplasm into parallel granular and continuous fibrillæ. The fibrillæ are arranged along lines of internal and reacting tensional stresses. The ends of the mesenchymal cells, in tension, must be attached to supports of which one, at least, is mobile. The tensional stresses are reactions to simultaneous forces extrinsic to the zone of myogenesis. The external forces are produced by a progressive divergence or separation of the mobile supports to which the mesenchymal cells are attached. Therefore, muscle tissue is not self-differentiating but is dependent upon an external dynamic stimulus. As regards smooth muscle this stimulus is the *tension of differential growth.*

SUMMARY.

1. The region of most active mitosis per mm. of cross-section in the intestine is the entodermal epithelial tube. The mitotic figures primarily follow the path of a right-handed helix. In one of the twenty embryos the mitotic figures describe the path of a right-handed helix.

2. The region of least active or relatively passive growth per mm. of cross-section is the mesenchyme, derived from the splanchnic mesoderm surrounding the epithelial tube.

3. The rapid expansion, due to epithelial growth in a rotating spiral manner, of the intestinal lumen is greater than the activity manifest in the surrounding mesenchyme. This causes a pressure in the latter resulting in a flattening and elongation of the mesenchymal cells. The successive changes in shape of these cells through the spherical, ellipsoidal, and spindle cellular phases are seen. The

mesenchymal wall decreases in thickness, due to tension caused by epithelial tubular dilation.

4. The rotating spiral growth of the epithelial cells causes the formation of a series of mesenchymal cellular and fibrillar concentric rings due to the centripetal force of the former.

5. The circular, smooth muscle cells are differentiated in the outer, more condensed margins of the ring. At these points the developing tensional stresses are greater than within the ring.

6. The inner circular smooth muscle coat is the first one differentiated and is incident to the rapid growth of the epithelial tube in diameter. The former soon tends to restrict the growth of the epithelial tube in diameter. The tube, pursuing the lines of least resistance, grows in length. During the period of rapid growth in length the outer longitudinal muscle coat is in the process of formation.

7. The tensional stresses to which the elongated strained mesenchymal cells are subjected appear to be a dynamic stimulus to smooth muscle differentiation.

8. From this study of a closely graded and progressive series of sections of intestinal development, the conclusion is drawn that muscle tissue is not self-differentiating, in the strict sense of the term, but that the *tension of differential growth* acts as the stimulus to smooth muscle differentiation.

The writer wishes to express his indebtedness to Professor H. von W. Schulte for his interest and his valuable suggestions; to Madame Helen Ziska for the illustrations; and to his wife for her help in reading the proof.

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