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Estudios sobre la dinámica de la histogénesis.

- IV. Tensión del crecimiento diferencial como un estímulo para la miogénesis del miembro.
- V. Compresión entre los centros de crecimiento acelerado del esqueleto segmentario como estímulo para la formación de las articulaciones.
- VI. Las resistencias al crecimiento esquelético como estímulo para la condrogénesis y osteogénesis.

De las pruebas presentadas en el trabajo adjunto se desprende que los diversos estados del desarrollo esquelético son los resultados de resistencias mecánicas al crecimiento. El miembro posterior presenta dos zonas principales que crecen longitudinalmente con diferente velocidad. El área esquelética central se acelera en su crecimiento, mientras que se retarda el crecimiento del mesenquima periférico. El alargamiento de los fascículos musculares en vías de desarrollo en el miembro, sigue la dirección del crecimiento longitudinal acelerado del segmento esquelético domin ante próximo. Este alargamiento se produce por la tensión inducida en las masas premusculares por el segmento esquelético de crecimiento acelerado, rodeado por aquéllas. El cartílago y el hueso no son autodiferenciados, ni son tampoco productos autocristalizados, sino que son las respuestas celulares inmediatas a la intensidad variable de las presiones y tensiones producidas por resistencias (presión) que contrarestan el crecimiento del esqueleto blastémico. Este último, en apariencia continuo, es en realidad segmentario y está compuesto de centros de crecimiento acelerado de acción opuesta. El contorno de la superficie opuesta que forma una articulación depende de la intensidad de la fuerza de crecimiento por milímetro cuadrado de sección transversa de los segmentos que forman una articulación, y también de las resistencias al crecimiento de los segmentos. Las resistencias, producidas por la interacción del crecimiento diferencial del miembro posterior, son activas y formativas en el origen y elaboración del esqueleto, articulaciones y musculatura.

STUDIES IN THE DYNAMICS OF HISTOGENESIS

IV. TENSION OF DIFFERENTIAL GROWTH AS A STIMULUS TO MYOGENESIS IN THE LIMB

V. COMPRESSION BETWEEN THE ACCELERATED GROWTH CENTERS OF THE SEGMENTAL SKELETON AS A STIMULUS TO JOINT FORMATION

VI. RESISTANCES TO SKELETAL GROWTH AS STIMULI TO CHONDROGENESIS AND OSTEOGENESIS

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NINE FIGURES

1. INTRODUCTION

The writer has presented facts ('19-'20 a; '20 b) which prove that the elongation of the developing muscular fasciculi of the digestive tract is in the direction of a dominant growth mechanical-energy extrinsic to the zone of myogenesis. This elongation is dependent on the fact that the primitive gut presents two zones of differential rates of growth. The inner epithelial tube is accelerated in growth, as compared with the outer splanchnic mesenchymal tube, which is retarded in growth. The growth of the inner tube is after the mode of a left-handed spiral, and is at first relatively more rapid in diameter than in length. The inevitable mechanical resultant of this differential growth is a tension of the outer tube in the same direction as the growth of the inner one. The inner close spiral muscle coat of the colon, in embryos of 14-25 mm. in length, begins to form during this initial transversely accelerated growth of the epithelial tube.

This initial muscle coat reacts upon the epithelial tube, thus restricting growth in diameter. This resistance causes the inner

tube to grow more rapidly in a longitudinal direction than in a transverse. The force of the growth of the inner tube during the period of its rapid longitudinal extension, in embryos 30–50 mm. in length, exerts a longitudinal, spiral tension upon the outer tube which had been retarded in growth. Concomitantly with the epithelial tube's period of rapid growth in length, the outer elongated spiral muscle coat is being derived from the splanchnic mesoderm.

According to the evidence, muscle formation in the gut is not due to a self-differentiation, nor to a spontaneous self-elongation of the myoblast, but is a dependent modification of the mesenchyme, due to the tension elicited by an extrinsic growth force. In view of other evidence yet to be presented, the writer is confident that this is the fact as regards all musculature. As an example, we may cite the spiral direction of the cardiac fasciculi corresponding to the changes in the vortical tension caused by the helicoidal blood stream flowing through the embryonic heart. In regard to the lingual musculature, an extrinsic force is found in the accelerated growth of the entodermal epithelium of the tongue.

The facts of direct observation prove that the formation of muscle tissue is a function of its position. Muscle tissue is formed *in situ* and is dependent upon an optimum tension, elicited by a dominant zone of accelerated growth, forcing by traction a connected zone retarded in growth. The direction of the resultant muscular fasciculi serves as a criterion of the direction of the dominant accelerated force which exerted the tension of differential growth.

In view of the evidence supporting the conclusion, that muscles arise through traction exerted upon the mesenchyme by a force extrinsic to the zone of myogenesis, it is as logical to claim that they self-elongate as it is to assert that a rubber band can stretch itself or a balloon can self-dilate. In the latter cases extrinsic forces are implied. As regards muscle origin, these forces are elicited by extrinsic zones of accelerated growth, inevitably drawing by traction retarded zones of growth, this being due to their relative positions to each other.

The reason for incorporating the three headings, involving the development of the muscles, joints, and modifications of the segmental skeleton, in this paper is the fact that the dynamic view-point, as regards limb development, necessitates the consideration of concomitant changes, in order to present the sequence of phenomena and evaluate cause and effect. The cause of the fundamental idea, first expressed in this paper, having been overlooked by previous observers is the fact that each tissue has been studied intensively as an entity isolated from the organism as a whole. The entire field of embryology must be reworked from the dynamic view-point of interaction, before a clean-cut idea of the physicochemical endowments of the primordial germ cell may be distinguished from that which is the mechanical resultant of the interaction of differential growth forces.

The purpose of this paper is to present facts of direct observation which prove that the formation of the skeletal muscles of the hind limb of the pig is dependent upon a tension of the somatic mesenchyme elicited by a force extrinsic to the region of myogenesis. The hind limb, like the gut, possesses two zones growing longitudinally but at different rates. There is an inner blastemal skeleton of accelerated growth and an outer somatic mesenchyme of retarded growth syncytially continuous with the skeleton. By the tensional interaction of this differential growth of the limbs, the skeletal muscles arise.

2. DIRECT OBSERVATIONS ON THE EARLY DEVELOPMENT OF THE HIND LIMB OF THE PIG (*SUS SCROFA*)

In embryos 6 to 10 mm. in length (fig. 1) the hind limb is represented by a convex bud covered with ectoderm and filled with a mass of uniform cells manifesting no signs of differentiation. As development advances, in embryos 10 to 14 mm. in length, a rapid condensation of the blastemal skeleton, proximodistally is occurring *pari passu* with limb extension. The nuclei of the syncytial skeletal core undergo rapid mitotic division. The first increase in size of the blastema is relatively more rapid in width than in length (fig. 2 and table 2).

This apparently continuous blastemal skeleton is in reality segmental in nature (fig. 2). The segments appear progressively from the proximal to the distal end, much in the same manner as that found in the successive caudal formation of metameres in the chick embryo. In contradistinction to the idea that segmentation of the continuous skeleton does not appear until joint formation occurs, the evidence at hand proves that centers of accelerated growth, segmentally arranged in the apparently continuous blastemal skeleton are prior in time to the formation of joints. This observation confirms that made by Bardeen ('10) on human embryos as noted in the following statement: "These cartilaginous anlagen are embedded in a dense blastema which shows lighter areas in the vicinity of the future joints." The same fact is recorded by Schulin ('79).

With the relatively greater increase in width than in length of the incipient femoral blastemal center, the peripheral cells of this rapidly proliferating zone show signs of retarded growth. They become elongated in the direction of the radial force of growth, forming an encircling constricting periblastemal membrane. With the formation of this limiting membrane, more rapid growth in length subsequently ensues in embryos 14 to 25 mm. in length (figs. 3, 4, 5 and table 2). This is due to the shifting of the planes of mitosis from a parallel to a transverse position, as regards the long axis of the limb.

The significant feature of the relatively and absolutely greater femoral growth in length during this period is the concomitant elongation of the nuclei of the premuscle masses in the direction of the skeletal growth. There is a subsequent stretching of the cytoplasmic myofibrils in the same direction. This inception of muscle differentiation of the thigh is more than a mere coincidence. It is an effect, a mechanical cellular response to the traction or tension to which the syncytially continuous and peripheral mesenchyme is subjected during the rapid growth of the femur in length. It would be as impossible for the femur to extend rapidly in length without elongating the continuous surrounding mesenchyme as it would be to expand a rubber balloon without stretching the limiting membrane.

During this period of rapid interstitial growth in length of the femoral blastemal center of the segmental skeleton, resistances are met with, first, at the proximal end in the centers of opposed growth for the ilium, ischium, and pubis; next, at the distal end in the centers of opposed growth for the tibia. The less condensed region, between the centers opposed in growth, becomes more and more condensed until a compression of the blastemal cells occurs along the line of juncture of the centers opposed in growth. Certain observers consider the segmentation of the skeleton as taking place at this stage and view the compression line as representing the beginning of the hereditary joints. The appearance of the joints, however, is the mechanical result of the opposed growth of antecedent, contiguous accelerated growth segments, and not the spontaneous cause of the segmentation.

With the inception of the outlines of, first, the hip and, next, the knee-joints, the contour of each skeletal segment becomes more defined. During this period the blastemal cells toward the center of the femoral segment become chemically changed by the intercellular formation of a cartilage matrix. This gives each cell the clean-cut appearance of a cell membrane. At the same time the primordial femur is bent, with its convexity toward the quadriceps extensor muscle and the concavity toward the hamstrings. The resistance to interstitial growth in length of the femur, due to the opposed centers at the acetabulum and at the knee, together with muscular contractility now manifested in the inception of the normal rotation of the hind limb, causes this characteristic bend of the femoral rod.

The attention of the observer is directed especially to the significant fact, that all cellular differentiation and degeneration occur first on the convex aspect at the center of the bent beam (fig. 5, no. 4). The first change from the blastema is the transformation into cartilage. Soon a dense perichondrial strain fibrosis is formed, followed by the degeneration of the cartilage cells and a calcification of the cartilage matrix. These retrogressive changes are due to the constriction of the blood supply, readily proved by injecting the embryos with india ink while

the heart is beating. All capillaries are then found peripherad to the perichondrium.

The last change in the above sequence of events on the convex aspect of the bent femur is the initial step in the formation of bone. The perichondrial fibrosis which effectively strangled the cartilage cells becomes modified into a periosteal membrane. Where the cartilage cells are degenerating a proliferation of the osteoblasts from the deep aspect of the periosteum is seen. These cells replace the dead cartilage cells. The osteoblasts form a matrix which is mechanically situated to serve effectively as a cellular reaction to the great strain to which the bent femoral beam is subjected. This appositional growth of bone serves to strengthen the femoral beam at its weakest part. The direct mechanical result of this cellular reaction is the progressive formation of a more stable base for the application of muscular forces.

The various steps in the increase of skeletal density, first, from the blastemal to the cartilage period, and, secondly, from the cartilage to the osseous period, in skeletal condensation is next to be considered simultaneously with those changes extrinsic to the zone of skeletal formation. During the early stages of development, the weight of the entire limb is supported by the femur acting like a cantilever beam. The weight of the limb rapidly increases (table 1 and chart A). In an 18-mm. pig embryo the femur constitutes one-fifth of the volume of the limb and supports a weight of 0.013 mg.; in a 38-mm. embryo the femur constitutes one-sixth the volume of the limb and supports a weight of 0.125 mg., whereas, at the 50-mm. stage of the developing embryo, the femur constitutes one-seventh of the volume of the limb and supports a weight of 0.25 gram. Later, at the 20-cm. stage, the femur constitutes only one-sixteenth of the volume of the limb, yet it supports the greatly increased weight of 30 grams. In addition to sustaining the above weight, the femur is opposed in growth by the accelerated growth centers located proximally and distally. Finally, as development continues, the resistance presented to longitudinal femoral growth by the contracting and elastic reacting muscula-

ture is an opposing factor to be considered as extrinsic pressures limiting the relative volume of the femur to the thigh.

With the rapid increase of limb weight and with increase of opposition to growth at the ends of the femur, together with the resistances manifested by muscular reaction, the density of the femur progressively increases (tables 2 and 3 and chart B).

TABLE 1

EMBRYO			HIND LIMB	
Length	Weight	Volume	Weight	Volume
<i>cm.</i>	<i>grams</i>	<i>cc.</i>	<i>grams</i>	<i>cc.</i>
20.0	334.0	330.0	30.0	28.0
18.5	282.0	270.0	27.0	25.0
17.5	252.0	240.0	24.0	21.0
16.5	227.0	225.0	15.0	12.0
16.0	225.0	220.0	13.0	10.0
14.0	140.0	140.0	10.0	9.0
13.0	140.0	130.0	8.0	7.0
11.5	105.0	100.0	7.0	6.0
9.3	46.0	38.0	2.0	2.25
9.0	39.0	36.0	2.0	2.1
6.2	17.0	15.0	0.9	0.36
5.2	9.0	8.0	0.25	0.25
2.5	2.0	3.0	0.025	0.064
2.2	1.5	1.75	0.020	0.048
2.0	1.0	1.25	0.018	0.032
1.8	0.75	0.90	0.013	0.018
1.6	0.60	0.70	0.012	0.014
1.5	0.425	0.52	0.010	0.011
1.4	0.350	0.48	0.008	0.010
1.2	0.125	0.210	0.002	0.003

This increase in density is going on simultaneously with the relative decrease in femoral volume as the growth of the limb advances. In an 18-mm. embryo, the volume of the femur constitutes one-fifth that of the entire limb, whereas its density is 1.025. In a 38-mm. embryo, femoral volume is one-sixth that of the limb and its density is 1.055; whereas, in the 50-mm. embryo, the volume of the femur is one-seventh and the density 1.075. The density of the femur in a 20-cm. embryo is 1.6 and the volume is one-sixteenth that of the limb. (Chart B.)

TABLE 2

EMBRYO LENGTH	LENGTH FEMUR	WIDTH FEMUR	WEIGHT FEMUR	VOLUME FEMUR	DENSITY FEMUR
<i>cm.</i>	<i>mm.</i>	<i>mm.</i>	<i>grams</i>	<i>cc.</i>	
20.0	30.0	3.00	2.9	1.8	1.60
18.5	25.0	2.55	2.1	1.6	1.31
17.5	25.0	2.5	1.9	1.4	1.36
16.5	24.0	2.3	1.2	1.0	1.20
16.0	22.0	2.1	0.8	0.69	1.10
14.0	21.0	2.0	0.7	0.64	1.15
13.0	20.0	1.9	0.69	0.60	1.10
11.5	18.0	1.75	0.60	0.54	1.11
9.3	12.0	1.25	0.34	0.28	1.20
9.0	12.0	1.15	0.29	0.27	1.07
6.2	8.0	1.00	0.063	0.06	1.05
5.2	6.0	0.85	0.037	0.035	1.05
2.5	2.95	0.715	0.010	0.010	1.00
2.2	2.55	0.705	0.009	0.009	1.00
2.0	2.40	0.695	0.008	0.008	1.00
1.8	2.15	0.650	0.006	0.006	1.00
1.6	1.50	0.625			
1.5	1.00	0.608			
1.4	0.95	0.459			
1.2	0.88	0.351			

TABLE 3

EMBRYO LENGTH	FEMUR VOLUME	HIND LIMB VOLUME	RATIO OF FEMORAL VOLUME TO LIMB VOLUME	PER CENT OF FEMORAL VOLUME TO LIMB VOLUME
<i>cm.</i>	<i>cc.</i>	<i>cc.</i>		
20.0	1.8	28.0	1:16.0	6.3
18.5	1.6	25.0	1:16.0	6.3
17.5	1.4	21.0	1:15.0	6.6
16.5	1.0	12.0	1:10.0	10.0
16.0	0.89	10.0	1:11.0	9.9
14.0	0.64	9.0	1:14.0	7.0
13.0	0.60	7.0	1:11.0	9.9
11.5	0.54	6.0	1:11.0	9.9
9.3	0.28	2.25	1: 8	12.5
9.0	0.27	2.1	1: 7.5	13.0
6.2	0.06	0.36	1: 6.0	16.6
5.2	0.035	0.25	1: 7.0	12.8
2.5	0.010	0.064	1: 6.4	15.6
2.2	0.009	0.048	1: 5.3	18.8
2.0	0.008	0.032	1: 4.0	25.0
1.8	0.006	0.030	1: 5.0	33.3

With the progressive increase of femoral density the definitive muscles of the thigh become split from the dorsal and ventral premuscle masses. Those myoblasts favorably located along lines of optimum tension for continued muscle differentiation advance in development; whereas, those not so favorably situated revert to embryonal connective tissue. This split into the separate muscles begins with the first compression line forming the hip-joint. Those mesenchymal cells previously drawn out opposite the location of the future joint by the indefinitely outlined blastemal femur retrogress with the progressive clean-cut development of the joints. This is due to the fact that the lines of optimum tension become more definite with the formation of the joints and with the continued development of the definite contour for the femur.

The premuscle mass for the quadriceps extensor (fig. 3) is seen to present a cleavage line opposite the dorsal aspect of the developing hip-joint composed of retrogressive myoblasts. With the continued morphogenesis of the femur and hip-joint, the dorsal premuscle mass is seen to split into its derivatives. The two muscle derivatives clearly seen in figures 4, 5, and 6 are the vastus intermedius and the rectus femoris. Note that the cleavage separating these muscles in the dorsal premuscle mass begins opposite the hip-joint (fig. 4) and progressively advances toward the patella (figs. 5, 6, 7).

The progressive elaboration of the hip-joint advances *pari passu* with the increasing density and the interstitial growth of the femur, together with the developing definitive thigh muscles. The head of the femur advances farther and farther into the acetabulum formed by the ilium, ischium, and pubis. By reference to figures 3, 4, and 5, it is seen that the growing segment forming the femur possesses a greater longitudinal growing force per square millimeter of cross-section than that of the elements forming the acetabulum. By actual measurement at the 20-mm. stage of the embryo, the length of the longitudinally growing femur is 2.40 mm., whereas the acetabulum possesses a depth of 0.195 mm. This is significant, for the force of longitudinal interstitial growth of the femur per square millimeter of cross-

section is twelve times greater than that of the primordial acetabulum. This fact, together with the muscular restrictions to longitudinal femoral growth, and with the fact that the femur is becoming a more stable bar by the appositional growth of bone at its center of ossification, must be considered in order to understand the continued elaboration of the hip-joint by the femur acting like an electric trip-hammer.

With these dynamic points in view, the omnipresent puzzle to the student of anatomy, as to the processes by which one segment of a movable joint possesses the socket and the other the ball, is solved. Joints, according to this evidence, are not hereditary; they are the mechanical resultants of the opposed centers of accelerated growth, segmentally distributed in the apparently continuous, blastemal skeleton.

3. INTERPRETATION

It is evident that the ultimate differentiation of muscles, joints, and skeletal components from the uniform mass of mesodermal cells in the hind limb of 10-mm. embryos is dependent upon one of two factors or a combination of both. These factors are, first, an intrinsic self-differentiation of the cells and an extrinsic mechanical interaction due to differential growth. At the present time, the majority of the students of development consider the genesis of the structures enumerated above as a spontaneously hereditary and self-differentiating process, intrinsic to each cell involved. If such is the case, the solution of the problem of development of the limb goes by default. But the writer takes a decided stand to the contrary.

Thoma ('07) considered the first formed bone as the resultant of mechanical factors, but the evidence presented was deductions primarily based on the stress and strain of the mature femur supporting the body weight in the erect position. Thoma was right in his mechanical idea, but his evidence was not convincing. The femur is not formed in anticipation of the stress and strain to which it will be subjected in the future, but is an immediate mechanical resultant of the force of its own interstitial growth and the immediate resistances encountered to this growth.

These resistances may be enumerated as follows: first, the weight of the hind limb; second, the reacting muscular force of elasticity of traction of the surrounding mesenchyme retarded in growth; third, the active muscular contractility manifested during the rotation of the hind limb, and, fourth, the restriction to longitudinal, interstitial growth of the femur at the proximal acetabular accelerated growth center and at the distal tibial accelerated growth center.

These resistances to femoral growth are active and formative during development. They are just as efficient in causing femoral differentiation as the intrinsic accelerated growth of the femoral center itself. Intrinsic growth of the femur and extrinsic resistance are factors *pari passu* in the genesis, growth, and perfected maturity of the femur. Growth and resistance are inseparable; one is just as important as the other. The formative influence of this resistance has been hitherto entirely overlooked.

In embryos 10 to 14 mm. in length, the skeletal condensation of the central core of mesodermal nuclei is purely a mechanical function of position (fig. 2). The syncytial nuclei, located at the center of the limb, have less volume to expand in and a greater resistance to overcome than those located more peripherad. The less volume and the greater resistance are the two factors determining the compactness of the skeletal nuclei.

Once the limb begins to grow rapidly in length, the apparently continuous, compact skeletal core presents two centers of accelerated growth. These centers, dense with nuclei, are separated by transverse zones lighter in texture. The lighter zones are the indefinite lines of demarcation between the centers of accelerated growth of the primordial and segmental blastemal skeleton.

The ultimate external form and internal structure of the various components of the mature skeleton are dependent upon the following factors: First, the varying continuation of the differential intensity of the force of each accelerated growth center of the segmental blastemal skeleton, and, second, the varying resistances encountered by this force. Starting with these two

factors, the muscles, joints, and various degrees in the condensation of the skeleton are mechanical resultants of the interaction of the forces of differential growth.

The intensity of the force of the accelerated growth segment of the femur, together with the resisting reactions of the elongated mesenchyme and the restrictions offered to femoral longitudinal extension by the acetabular and tibial centers at the ends, interact to cause the following definite results: First, the definitive muscles tend to split opposite the joints from the pre-muscle masses along lines of specific optimum tension. Second, the traction to which the primitive muscles are subjected causes an increase in the volume of this tissue. This is manifested by cytoplasmic differentiation of myofibrils and the mitotic reaction of the myoblasts. Third, the definitive muscles now present a definite point of application in reacting to the traction to which they are subjected by the rapid longitudinal growth of the femur. This tends to outline definitely a more stable base or framework on which the muscles act. Fourth, in addition to the force of elasticity of traction presented by the embryonal muscles, the femoral growth center meets resistance at the proximal and distal ends by the acetabular and tibial growth centers, respectively. This tends more and more to outline, then elaborate, the opposed surfaces entering into the formation of the hip- and knee-joints. Fifth, all the above factors converge to cause the femur to assume a characteristic bend. At the center of this bend the mechanical stimulus is first applied, causing the cells to react by forming, first, cartilage, then bone, in the initial differentiation of a progressively more rigid skeleton. Sixth, the differentiation, first, of cartilage at the center of the femoral beam; next, of degeneration of the cartilage, and, finally, periosteal (former perichondrium) bone formation at this center shifts the growing points of the femoral rod to the extremities.

The differentiation of cartilage and its subsequent degeneration at the center of the femoral beam is a critical stage in joint formation. Once a skeletal segment begins to show alteration of its cellular components at the center, they cease to proliferate in the active elongation of the segment. The growth of the

femur in length is then due to the proliferation of the cells constituting its extremities. This terminal growth of a skeletal segment opposes the active growth of the cells of a contiguous skeletal segment.

The first objective evidence in joint formation is significant. There is seen (fig. 4) a linear flattening of the blastemal cells along the line representing the zone of juncture between opposed zones of accelerated growth of neighboring segments. The contour of this linear condensation of cells, which outlines the subsequent position of the incipient or primordial joint cleft, is dependent upon the intensity of the mutual forces of growth opposed in action. That particular skeletal segment presenting the greater intensity of growth force per square millimeter of cross-section will bore into and possess the convex component, constituting a diarthroidal joint, whereas the component presenting the lesser force of growth per square millimeter of cross-section will possess the concave component of a ball-and-socket joint.

The formation of a joint, therefore, is a resultant along the lines of juncture between the zones of accelerated growth of neighboring segments opposed in action. The segment presenting the greater force of growth possesses the convex element, whereas, the segment presenting the lesser force possesses the concave element entering into a movable joint. If the opposed forces are equally distributed between the surfaces of the two segments, a joint with more or less opposed plane surfaces, as the intervertebral joints, is the result.

According to this evidence, muscles, bones, and joints are not hereditary nor do they spontaneously crystallize out of embryonic tissue by some unknown, non-biological method of self-differentiation, but they are the mechanical resultants of an apparently continuous blastemal skeleton, possessing segmental centers of accelerated, longitudinal growth opposed in action. This segmental skeleton is in an environment with which it is syncyti-ally continuous. The effect of longitudinal skeletal growth is traction of the surrounding mesenchyme along the lines of optimum tension evidenced by muscle origin. The perfected

limb represents a continuance of this interaction between skeletal segments and developing musculature until equilibrium is established. This is reached at maturity when the forces of growth are counterbalanced by the resistances to growth. This active interaction of a dominant growth force and its concomitant resistances, playing a dynamic rôle in histogenesis and morphogenesis, has never been heretofore considered in ontogenetic development of the limb.

4. GENERAL CONCLUSIONS

From the facts presented in this paper, the generalization is clearly apparent that the volume of a skeletal segment decreases relatively to the increase of the intensity of the external pressure or resistance. The less the extrinsic resistance to growth early in development, the greater the relative volume of the growing skeletal segment is found to be.

With the decrease in the relative volume of the skeletal segment as development advances, another fact is self-evident, namely, there is an increase in density. The greater the resistances overcome by the growing skeletal segment, the greater the density becomes; conversely, the less the resistance encountered in growth, the less the density, the more gelatinous the consistency. These facts may be summarized in the following laws:

The law of density of a growing tissue: *The density of a growing tissue is directly proportional to the resistances (pressure) encountered during growth.*

The law of relative volume of growing tissue: *The relative volume of a given quantity of growing tissue is inversely as the resistances (pressure) which it bears.*

From the evidence leading to these laws it is conclusive that the various stages of the developing skeleton are resultants of the mechanical resistances to growth which, interpreted, means that cartilage and bone are not self-differentiated, nor are they spontaneously self-crystallized products, but they are the immediate cellular responses to the varying intensity of the stresses and strains produced by resistances (pressure) counteracting the growth of the blastemal skeleton.

5. CONCLUSIONS

1. In an embryo 10 mm. in length the hind limb bud is filled with a uniform mass of unmodified mesenchymal cells.

2. The central condensation of the nuclei forming the blastemal skeleton is due to two factors: first, the less volume centrally in which these cells have to expand; second, the resistance of the surrounding mesenchyma.

3. The blastemal skeleton at first grows relatively more rapid in width than in length in embryos 10 to 14 mm. long.

4. With the formation of the periblastemal membrane the subsequent growth of the femur is relatively and absolutely greater in length than in width in embryos 14 to 25 mm. long. This is due to the shifting of the planes of mitosis on account of the compression of the periblastemal membrane. A central skeletal core of accelerated longitudinal growth is now clearly demarked from the peripheral mesenchyme retarded in longitudinal growth.

5. The interaction of these differential growing zones results in a tension of the peripheral mesenchyme. The nuclei of the latter are drawn out in traction, thus resulting in the first step of myogenesis. The direction of the elongated cytoplasmic myofibrils is arranged, therefore, in the line of longitudinal skeletal growth.

6. The formation of skeletal muscle is a dependent differentiation, relying on the accelerated longitudinal growth of the skeleton for its genesis, growth, and continued differentiation. The tension of differential growth is the efficient interacting stimulus to myogenesis in the limb.

7. The following law of direction of the skeletal muscular fasciculi may be formulated from the evidence presented:

The elongation of the developing muscular fasciculi of the limb is in the direction of the accelerated longitudinal growth of the related dominant skeletal segment. As regards developing muscles in general, the elongation of muscular fasciculi is in the direction of a dominant force extrinsic to the zone of myogenesis.

6. CONCLUSIONS

1. The apparently continuous blastemal skeleton is in reality segmental and composed of centers of accelerated growth opposed in action.

2. By the continued opposition to growth between the contiguous centers of the segmental blastemal skeleton, mechanical compression occurs, revealing the location of the future joint cavities.

3. The contour of the opposed surfaces constituting a joint is dependent on the intensity of the force of growth per square millimeter of cross-section of growing segments opposed in action, together with the force of muscular pull. That segment will possess the ball of a ball-and-socket joint, which possesses the greater force of interstitial growth longitudinally per square millimeter of cross-section.

4. Joints, therefore, are not the cause of skeletal segmentation; they themselves are the mechanical resultants of compressive and shearing stresses of prior centers of accelerated growth opposing each other in action in the segmental blastemal skeleton.

5. *Law of joint formation: The contour of the opposed surfaces forming a joint is dependent upon the intensity of the force of interstitial growth per square millimeter of cross-section of the skeletal segments forming the joint and upon the force of muscular pull.*

7. CONCLUSIONS

1. Skeletal condensation, varying through the different degrees of density, beginning with the blastemal period, progressing through the cartilaginous, and terminating in the osseous period, is a direct resultant of the varying intensity of the resistances (pressure) encountered during the period of growth.

2. The resistances to femoral growth are as follows: 1) Weight of the hind limb; 2) reactive force of elasticity of traction of the forming muscles; 3) active muscular pull; 4) opposition to interstitial femoral growth at the ends by the acetabulum proximally and the tibia distally. The evidence presented in this paper warrants the formulation of the following laws:

3. The law of density of a growing tissue: *The density of a growing tissue is directly proportional to the resistances (pressure) encountered during growth.*

4. The law of relative volume of a growing tissue: *The relative volume of a growing tissue is inversely as the resistances (pressure) which it bears.*

5. Cartilage and bone do not self-differentiate, but they are the cellular reactions to the varying mechanical resistances (pressure) encountered by groups of cells in a field of differential growth.

6. The evidence supports the conclusion that resistance to growth is active and formative during development, and that the processes of histogenesis and morphogenesis of the skeleton and muscles are as much dependent upon the mechanical factors extrinsic to the region of the specific developing structure as upon the intrinsic faculty of the modifying tissue to grow. The modifying growing cells receive and respond to the mechanical stimulus. The stimulus, however, is a function of position.

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PLATE 1

EXPLANATION OF FIGURES

- 1 Middorsoventral, longitudinal section of hind limb bud of a pig embryo 10 mm. in length. $\times 40$.
- 2 Middorsoventral, longitudinal section of hind limb bud of a pig embryo 12 mm. in length. $\times 40$.
- 3 Middorsoventral, longitudinal section of hind limb bud of a pig embryo 14 mm. in length. $\times 40$.
- 4 Middorsoventral, longitudinal section of hind limb bud of a pig embryo 16 mm. in length. $\times 40$.
- 5 Middorsoventral, longitudinal section of the thigh of a pig embryo 20 mm. in length. $\times 40$.
- 6 Middorsoventral, longitudinal section of the thigh of a pig embryo 25 mm. in length. $\times 40$.
- 7 Middorsoventral, longitudinal section of the thigh of a pig embryo 29 mm. in length. $\times 40$.

ABBREVIATIONS

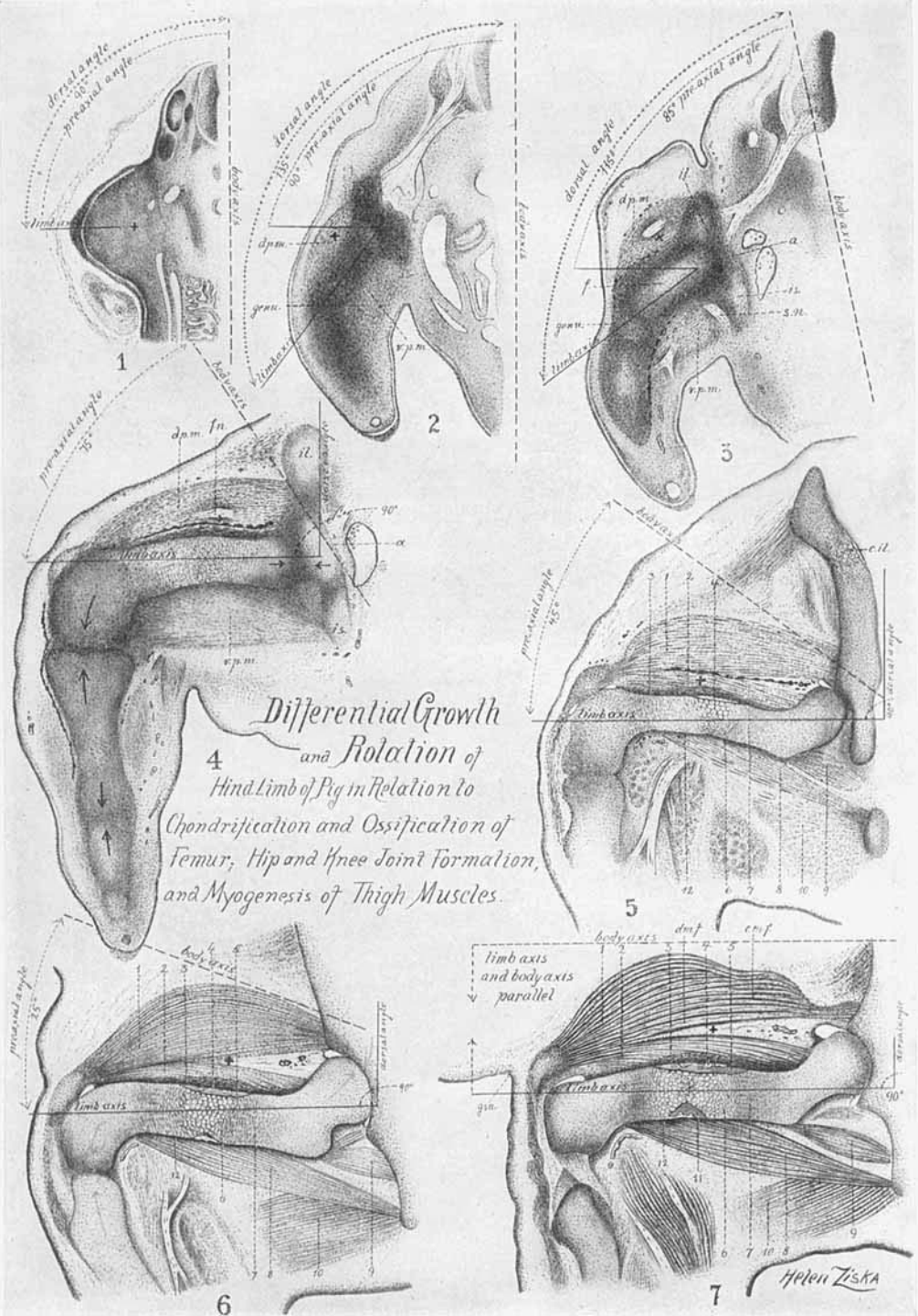
- | | |
|---|---|
| <i>d.p.m.</i> , dorsal pre-muscle mass | 9, pyriformis muscle |
| <i>v.p.m.</i> , ventral pre-muscle mass | 10, semimembranous muscle |
| <i>a</i> , acetabulum | 11, compressive osseous trabecula |
| <i>il.</i> , ilium | 12, compressive perichondrial strain fibrosis (periosteum) |
| <i>is.</i> , ischium | <i>dorsal angle</i> , angle formed by a line through the dorsal aspect of the longitudinal axis of the limb with a dorsoventral line through center of hip-joint |
| <i>s.n.</i> , sciatic nerve | <i>pre-axial angle</i> , angle formed by pre-axial aspect of limb with lateral body wall. By reference to the various changes in these angles the rotation of the hind limb is exemplified on a plane surface. Since the aspect of the limb cannot change in these figures, the body axis is represented as changing. |
| <i>f.</i> , femur | |
| <i>n.</i> , femoral nerve | |
| 1, rectus femoris muscle | |
| 2, vastus intermedius muscle | |
| 3, tensile perichondrial strain fibrosis (periosteum) | |
| 4, Osteogenetic tissue | |
| 5, tensile osseous trabecula | |
| 6, degenerating cartilage cells arranged along tensile and compressive stress lines | |
| 7, proliferating cartilage cells in advance of degenerating zone. | |
| 8, abductor magnus muscle | |

The attention of the observer is specifically directed to the following facts:

1. The volume of the central condensed blastemal skeleton (fig. 2) occupies relatively more space of the thigh than the femur in figures 4, 5, 6, and 7. It is immediately evident that as development advances the relative volume of the femur to thigh decreases. At the same time, the density increases as exemplified by the progressive deposition of bone (figs. 6 and 7, nos. 5 and 11). The bone on the convex side is drawn out in tension, that on the concave side is compressed. The first deposition of bone, therefore, follows mechanical laws.

2. The width of the femoral segment is relatively greater in figure 2 than that in figures 4, 5, 6, and 7. The femur grows relatively more rapidly in length than in width in embryos 14 to 25 mm. in length. Note that during the accelerated

(Continued on p. 112)

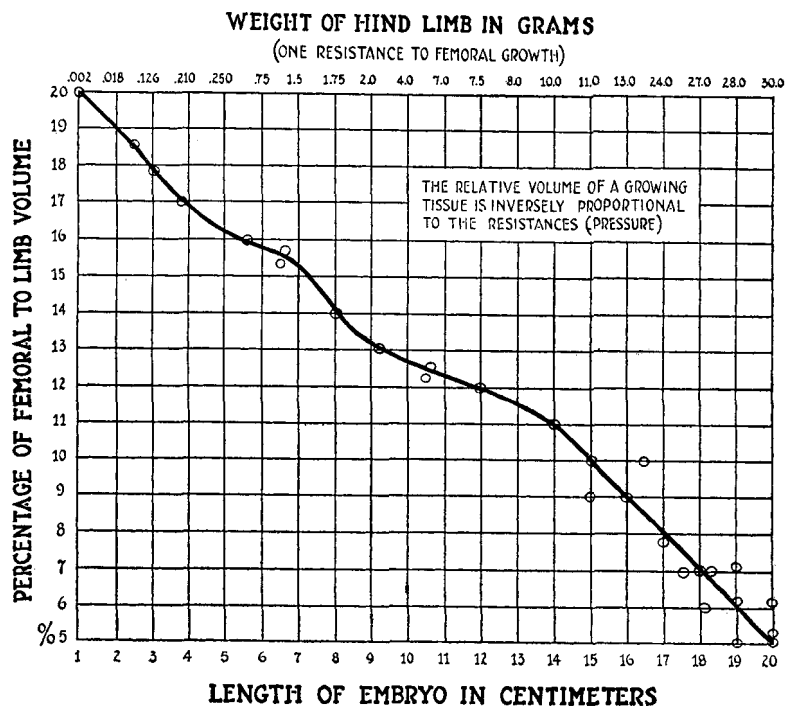


longitudinal growth of the femur the progressive appearance of more and more definite traction lines is seen in the surrounding mesenchyme retarded in longitudinal growth. Along these lines the fasciculi of the developing muscles form, due to the tension of differential longitudinal growth. The determination of accelerated and retarded growth is based on the number of mitotic figures and the compactness of the nuclei per square millimeter of cross-section in a field of differential growth.

3. The force of longitudinal growth of the femur is from ten to fifteen times greater than that of the acetabulum. Compare along the line labeled 'limb axis.' Note, at the same time, the inevitable mechanical effects of muscular pull. These factors, together with the strengthening influence of femoral ossification, determine the location of the convex ball of the hip, ball-and-socket joint, on the femur and not on the region of the acetabulum.

4. The tensile perichondrial strain fibrosis is clearly detected as a limiting membrane, first appearing on the convex aspect of the bent femur (figs. 3, 4, 5, 6, 7, no. 3). Figure 4 represents the limb of a living embryo injected with india ink. Note that the injected capillaries are outside of the perichondrium. This membrane strangles the cartilage cells at the center of the bent femur and later becomes modified into the periosteum from which the osteoblasts proliferate. The osteoblasts, by appositional growth, replace the degenerating cartilage scaffold with a more rigid bony base. These cellular reactions are elicited by the progressive intensity of the strain to which the femur is subjected by the resistances to advancing femoral extension. Longitudinal femoral accelerated growth and extrinsic resistances to this growth are interactions that must be intensively studied in order to appreciate the competition and the resultant products of differential growth.

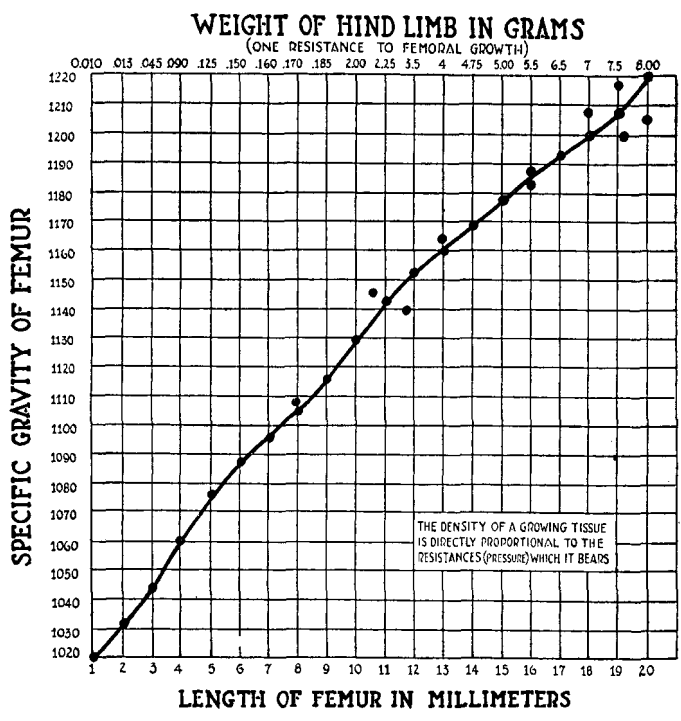
Chart A Curve of relative decrease of femoral volume with increased resistance due to hind-limb weight.



A

Chart B Curve of increase of femoral density with increased resistance due to hind-limb weight. The resistances to femoral growth are as follows: 1) Weight of the hind limb; 2) reactive force of elasticity of traction of the forming muscles; 3) active muscular pull; 4) opposition to interstitial femoral growth at the ends by the acetabulum proximally and the tibia distally.

The specific gravity of these femora was determined by immersion in various concentrations of benzene (sp. gr. 0.879) and chloroform (sp. gr. 1.499). The specific gravity of that solution in which a certain femur would neither rise nor sink was determined by means of the pyknometer; this method gave the specific gravity of the femur. The details of this method will soon be published, with my colleague in biochemistry, Dr. Joseph C. Bock.



B