IOPscience iopscience.iop.org

This content has been downloaded from IOPscience. Please scroll down to see the full text.

Download details:

IP Address: 18.117.183.150
This content was downloaded on 06/05/2024 at 20:12

Please note that terms and conditions apply.

You may also like:

Climate Change for Astronomers

Aptasensors for Point-of-Care Diagnostics of Cancer

INVERSE PROBLEMS NEWSLETTER

A Grain of Truth: The Media, the Public, and Biotechnology
Susanna Hornig Priest

Principles of Plasma Spectroscopy
A.L. Osterheld

Part II

Foresight

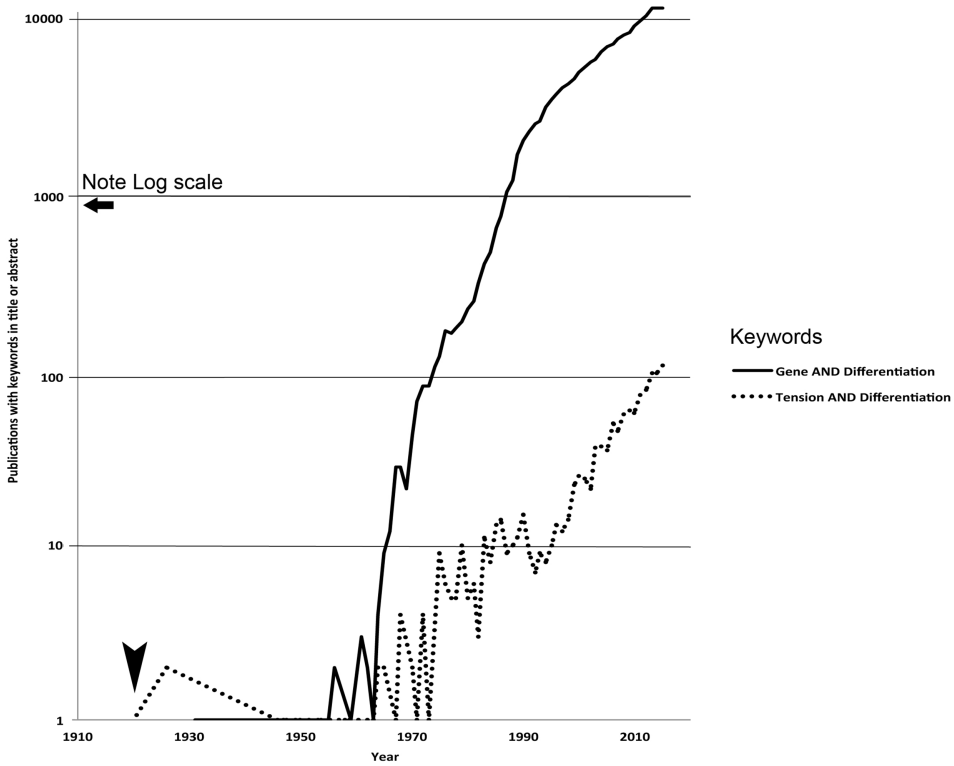
IOP Publishing

Ahead of the Curve
Hidden breakthroughs in the biosciences
Michael Levin and Dany Spencer Adams

Chapter 2

Physics in cell and developmental biology

Case study 3: Development Employs Forces



Carey (1920) Studies in the dynamics of histogenesis: tension of differential growth as a stimulus to myogenesis.

doi:10.1088/978-0-7503-1326-1ch2

© IOP Publishing Ltd 2016

Perspective on *Studies in the Dynamics of Histogenesis. I. Tension of Differential Growth as a Stimulus to Myogenesis*

Raymond E Keller
Professor of Biology
University of Virginia

Even today, many biologists do not want to think about morphogenesis and the importance of forces. A more popular idea is 'self assembly' of embryos, a concept that is rather strange: what other choice do embryos have? The idea sprang from the 'self-assembly' of the tobacco moaic virus, spread to self assembly of molecular aggregates of all kinds, and got a big boost from Steinberg's differential adhesion hypothesis, the DAH: genes encode proteins, including cell adhesion molecules, these impart different cell adhesive strengths, and borrowing from mass action chemistry, motility stirs the pot, bringing cells of differing adhesive strengths in contact, and they sort out based on the thermodynamics of adhesive strengths. The DAH kept genetics and molecular biology at center stage—in the minds of many, everything subsequent to the encoding of adhesion was thought to be irrelevant—motility, polarity, cell sociobiology etc were not needed, only generic motility was needed. Overt generation of forces by patterned cell behavior was ignored. Sometime in the 1980s, a then prominent geneticist, told me 'Ray, physics has absolutely nothing to do with morphogenesis'. It was a sign of the times. Cell adhesion is important but it is a regulated property, but one of many among the integrated force-generating behaviors of cells that have rightly taken center stage. Today, the Carey paper looks pretty modern.

STUDIES IN THE DYNAMICS OF HISTOGENESIS

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

by Eben J Carey.

(*From the Department of Anatomy, College of Medicine, Creighton University, Omaha.*)

(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.

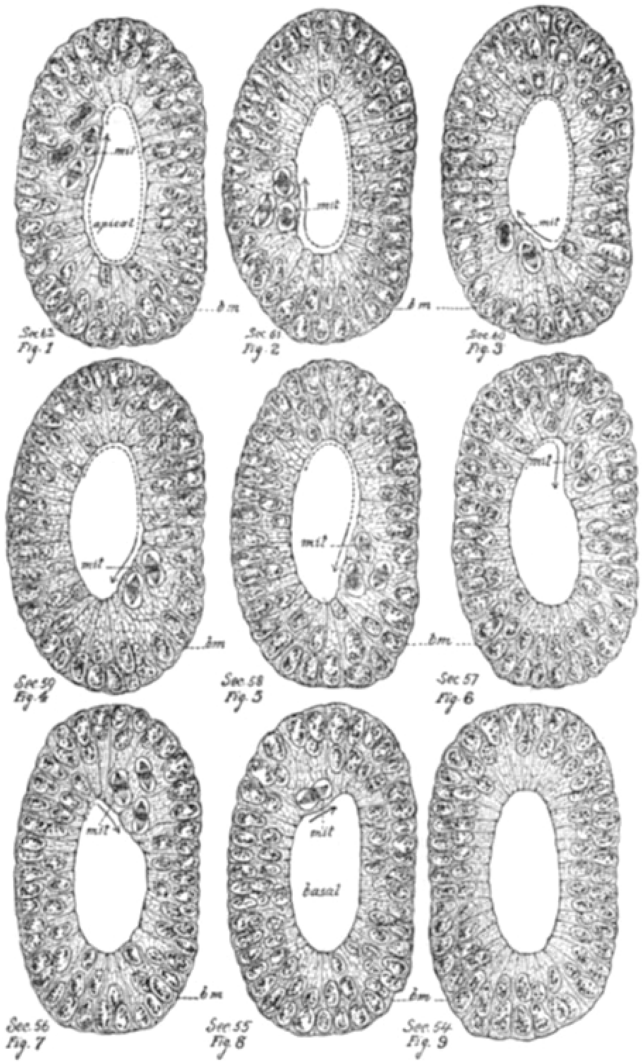
The influence of the organism as a whole in subjugating its dependent parts is convincingly shown by Loeb (1916) in his regeneration experiments on *Bryophyllum calycinum* and in his experiments on *Amblystoma* larva (1897). This influence is exerted through the blood stream by means of "hormones." The 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 section (Figs. 1 to 9). This region was



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).

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.

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.

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).

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

TABLE I. Measurements of Differential Growth of Descending Colon.

Length of embryo. *mm.*	Thickness of mesenchymal wall.* *mm.*	Diameter of epithelial tube. *mm.*	Diameter of descending colon. *mm.*	Epithelial tubular indices.† *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.

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 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).

TABLE II. Ratio of Diameter to Length of Entire Colon.

Length of embryo. *mm.*	Diameter of descending colon. *mm.*	Length of entire colon. *mm.*	Ratio of diameter to length of entire colon. *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

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 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

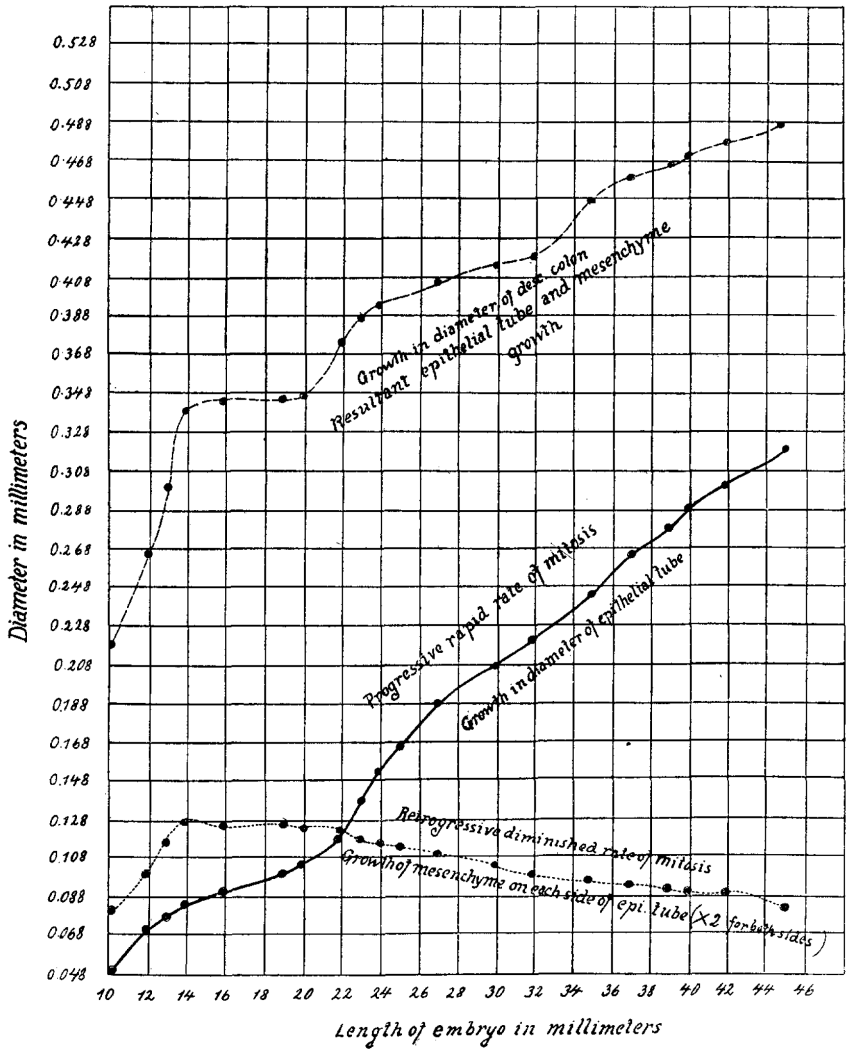


Fig. 10. Curves of differential growth of descending colon. The active growth of epithelial tube is contrasted with the passivity of the mesenchymal wall. An absolute decrease in thickness of the mesenchymal wall is seen after the stage of the 14 mm. pig embryo.

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.

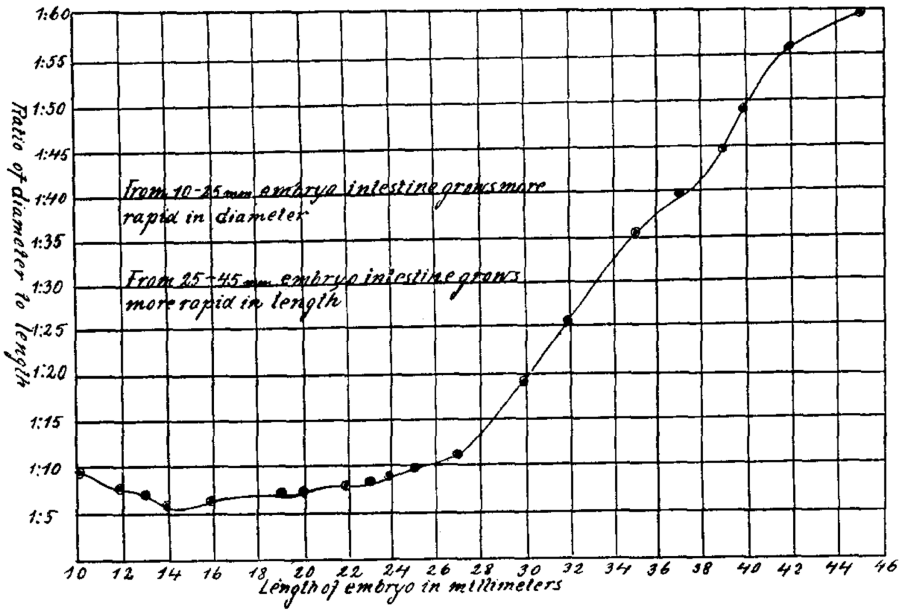


Fig. 11. Curve representing the ratio of the diameter to the length of the descending colon in embryos 10 to 45 mm. in length. Particular attention is directed to the fact that in embryos 10 to 25 mm. in length the intestine grows relatively more rapidly in diameter than in length. In embryos from 25 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.

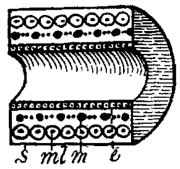


Fig. 12. Longitudinal section of intestine; *s*, peritoneal epithelium; *ml*, mesenchyme; *m*, circular muscle; *e*, epithelium.

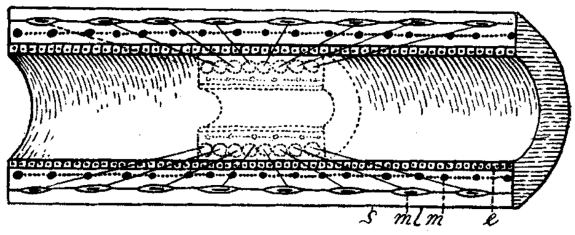


Fig. 13. Longitudinal section of intestine schematizing the elongation of the intestine represented in Fig. 12. Due to the resistance of the inner smooth muscle layer *m*, the intestinal epithelium grows in the longitudinal path of least resistance. This results in the elongation of the outer mesenchymal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

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.

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

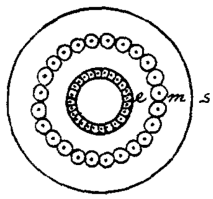


Fig. 14. Transverse section of intestine; *e*, epithelium; *m*, mesenchyme (spherical nucleus); *s*, peritoneal epithelium.

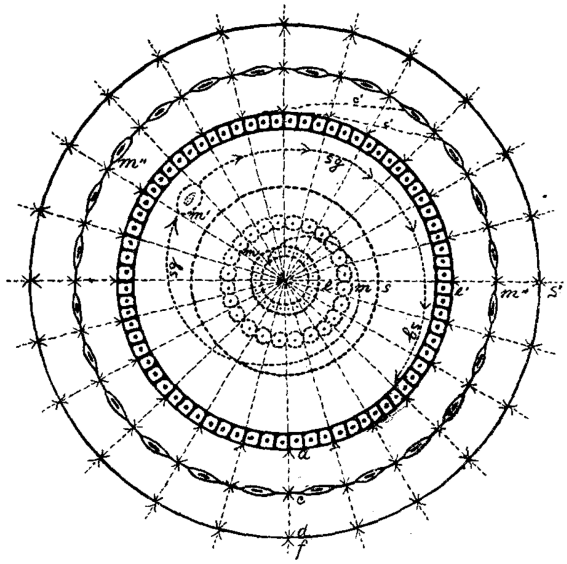


Fig. 15. Transverse section of intestine grown to three times the width represented in Fig. 14. Fig. 15 is represented in broken lines within the lumen. The spiral growth of the epithelium is represented by the broken lines *sg*. The tension, upon the mesenchyme, by the most rapidly growing epithelium, 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.

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.

Bibliography

Bardeen C R 1900 *Johns Hopkins Hosp. Rep.* **ix** 231 *Am. J. Anat.*, 1906–07, vi, 259

Boveri T *Sitzungsb. phys.-med. Ges. zu Wurzburg*, 1904, 16; Ergebnisse über die Konstitution der chromatischen Substanz des Zelikerns, Jena, 1904, 115

Carey E. J. *Anat. Rec.*, 1917, xi, 1; 1918, xiv, 30; 1919, xvi, 45, 114

Child C. M. Biological lectures from the Marine Biological Laboratory of Woods Hole, 1899, 231; Individuality in organisms, Chicago, 1915, 5

Conklin E G Biological lectures from the Marine Biological Laboratory of Woods Hole, 1896–97, 17; *J. Exp. Zool.*, 1905, ii, 145

Driesch H Analytische Theorie der organische Entwicklung, Leipsic, 1894, 97; *Biol. Centr.*, 1899, xix, 225; *Arch. Entwcklngsmechn. Organ.*, 1899, viii, 123

Harrison R G 1905 *Am. J. Anat.* **iii** 197

His W Unser Körperform und das physiologische Problem ihrer Enstehung, Leipsic, 1874, 165

Jackson C M 1909 *Anat. Rec.* **iii** 361

Keibel F and Elze C Normentafeln zur Entwicklun-geschichte der Wirbeltiere, Jena, 1908, viii, 1

Lewis W H *Am. J. Anat.*, 1904, iii, 505; *J. Exp. Zool.*, 1905, ii, 431; in Kiebel, F., and Mall, F. P., Manual of human embryology, Philadelphia and London, 1910, i, 456

Loeb J *Arch. Entwcklngsmechn. Organ.*, 1897, iv, 502; The organism as a whole, New York, 1916, 11, 152

Morgan T H Biological lectures from the Marine Biological Laboratory of Woods Hole, 1898, 196; *J. Morphol.*, 1895, x, 419

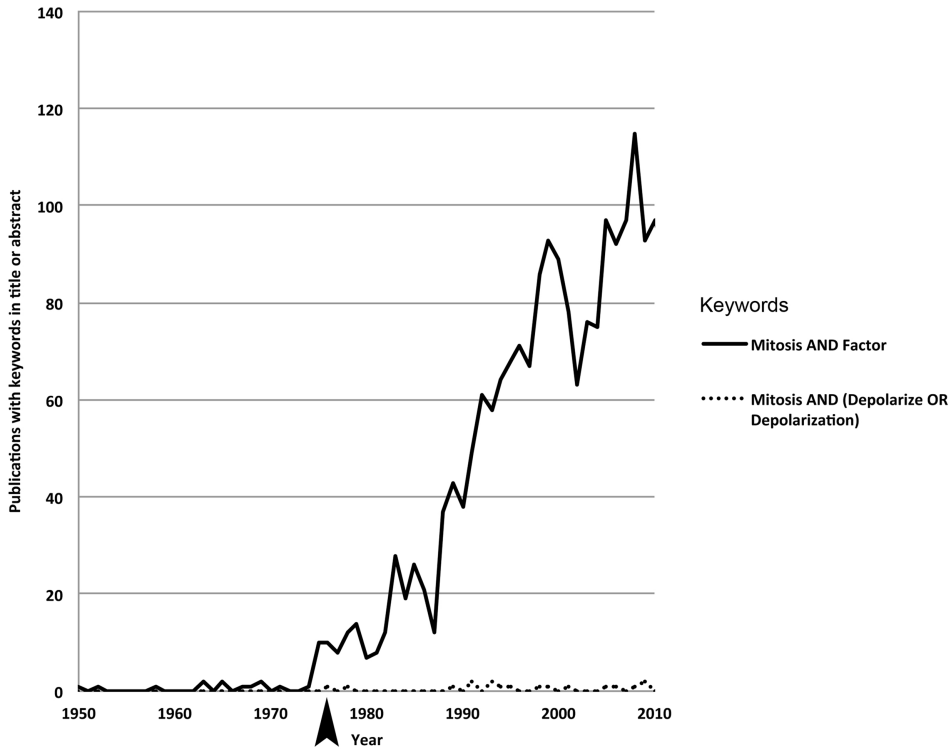
Roux W Der Kampf der Theile im Organismus, Leipsic, 1881, 152; in Merkel, and Bennet, Ergebnisse der Anatomie und Entwickelungsgeschichte, 1892, ii, 415; *Zool. Anz.*, 1893, 115

Whitman C O 1893 *J. Morphol.* **viii** 639

Wilson E B *J. Morphol.*, 1892, vi, 361; 1893, viii, 579; The cell in development and inheritance, New York, 1897, 23; *J. Exp. Zool.*, 1904, i, 1, 197

Zeleny C 1904 *J. Exp. Zool.* **i** 293

Case study 4: Depolarization Induces Neuron Division



Cone and Cone (1976) Induction of mitosis in mature neurons in central nervous system by sustained depolarization.

Perspective on *Induction of mitosis in mature neurons in central nervous system by sustained depolarization*

Michael Levin, PhD
Allen Discovery Center,
Professor, Tufts University

'Electrical Potential: a new control knob for cell functions'

Salamanders can regenerate large portions of their brain after injury. A major limitation in the regenerative capacity of the brain in other species is the fact that post-mitotic neurons generally do not divide. Cone's work in the late 1960s and early 1970s showed that mitosis can be triggered by sustained depolarization of resting potential [1–8]. His group also extended it to the control of mitosis in cancer cells [9], formulating a theory of electrical control of cellular proliferation [10–12]. This work, though not widely recognized in subsequent decades, has major implications for a number of areas of biology and medicine. Its ramifications have begun to bear fruit in recent years [13], extending beyond the control of single cell behaviors toward a new aspect of developmental bioelectricity as a regulator of large-scale biological shape.

The resting potential (V_{mem})—the voltage difference between the inside and the outside of the cell surface—is established by ion channel and pump proteins in the cell membrane which facilitate the inward or outward movement of specific charged molecules. This electrical aspect of cell behavior is a crucial part of the living cell, being responsible for many aspects of its physiology and considered a hallmark of life [14]. Cone's work was perhaps the first to recognize the importance of this parameter for guiding key cell behaviors—proliferation and differentiation, beyond basic homeostasis. The study of endogenous, electrical control of cell functions other than excitable nerve and muscle is called developmental bioelectricity, and forms an exciting part of developmental, regenerative, and cancer biology today.

Over the last century, a number of waves of research studied the bioelectric aspects of life. Lund [15], Burr [16], Marsh and Beams [17], and many others in the 1960s–1980s studied the electric fields and ion currents that contribute to developmental pattern formation and regenerative repair [18–20]. The development of electrode strategies and breakthrough vibrating probe technology [21], which allowed accurate measurements of ion flux across membranes in vivo, led to an accumulating mass of data implicating endogenous electric fields in events like wound healing, guidance of migratory cell behavior, and regenerative response. Cone's work uniquely examined a different cellular parameter: the resting potential. While V_{mem} is related to ion flux and electric fields, it is a distinct property that must be studied separately. In the following paper, Cone and Cone used pharmacological depolarization and electrophysiology, and were able to show the instructive role that V_{mem} plays: depolarization is associated with a plastic, proliferative state (e.g. cancer cells), while hyperpolarization is associated

with a post-mitotic, mature condition—a relationship that has been recently validated in a number of cell lines, including human stem cells [22, 23].

Cone's group's work served as an inspiration for the recent advances in developmental bioelectricity focused on resting potential. New methods using fluorescent voltage reporter dyes allow the electrical potentials of many cells to be imaged at once, without having to impale cells with electrodes. In this way, scientists detect the voltage potentials throughout the body, to observe the electrical conversations that cells are having with each other. Modern techniques of molecular biology (altering the expression of ion channels and pumps using genetic methods) have allowed us to manipulate those signals. By specifically altering the resting potentials of cells, we can re-write the natural electric patterns; recent work has shown that this has dramatic effects on gene expression and pattern formation. Bioelectric gradients, of the kind Cone studied in single cells, form patterns across tissues in vivo that are part of the way cells coordinate their activity towards constructing embryos and repairing/regenerating organs in adults [13, 24–29]. Thus, Cone's model of mitosis appears to be correct, but more than that, these voltage gradients control the types of organs that appear during embryogenesis and the geometric relationships between those structures. As conjectured by Burr, bioelectric gradients in vivo really do seem to be subtle prepatterns that guide growth and form. For example, recent work has taken advantage of the ability to control resting potentials in biological circuits in vivo to kickstart regeneration of spinal cord and muscle [30], to understand how mutations in ion channels induce craniofacial defects in embryogenesis [31, 32], and to re-specify the entire body plan [33], of model species like frog and flatworm.

Cone's conjectures about cancer were validated as well; recent work has shown that not only can tumors in vivo be recognized early by their aberrant electrical signature, but they can actually be prevented or reprogrammed into normal tissue by enforcing correct resting potentials [34–39]. Ion channel-targeting compounds are thus an important aspect of the search for new cancer drugs [40, 41], as well as an emerging class of 'electroceuticals' for applications in birth defects and regenerative medicine. C D Cone and his colleagues (including C M Cone, M Tongier, E F Stillwell) were way ahead of the community in recognizing the importance of resting potential as a key control parameter for cells, not just a housekeeping physiological side-effect. He would probably have been really pleased to see the exciting applications emerging in the modern study of developmental bioelectricity.

References

[1] Cone C D and Tongier M 1971 Control of somatic cell mitosis by simulated changes in the transmembrane potential level *Oncology* **25** 168–82

[2] Stillwell E F, Cone C M and Cone C D 1973 Stimulation of DNA synthesis in CNS neurones by sustained depolarisation *Nat. New Biol.* **246** 110–1

[3] Cone C D 1974 The role of the surface electrical transmembrane potential in normal and malignant mitogenesis *Ann. NY Acad. Sci.* **238** 420–35

[4] Cone C D and Cone C M 1978 Evidence of normal mitosis with complete cytokinesis in central nervous system neurons during sustained depolarization with ouabain *Exp. Neurol.* **60** 41–55
[5] Cone C D and Cone C M 1978 Blockage of depolarization-induced mitogenesis in CNS neurons by 5-fluoro-2′-deoxyuridine *Brain. Res.* **151** 545–59
[6] Cone C D, Tongier M and Cone C M 1977 DNA content of daughter nuclei from ouabain-induced nuclear divisions in central nervous system neurons *Exp. Neurol.* **57** 396–408
[7] Cone C D and Cone C M 1976 Induction of mitosis in mature neurons in central nervous system by sustained depolarization *Science* **192** 155–8
[8] Cone C D 1980 Ionically mediated induction of mitogenesis in CNS neurons *Ann. NY Acad. Sci.* **339** 115–31
[9] Cone C D 1969 Autosynchrony and self-induced mitosis in sarcoma cell networks *Acta. Cytol.* **13** 576–82
[10] Cone C D 1971 Unified theory on the basic mechanism of normal mitotic control and oncogenesis *J. Theor. Biol.* **30** 151–81
[11] Cone C D Jr. 1970 Variation of the transmembrane potential level as a basic mechanism of mitosis control *Oncology* **24** 438–70
[12] Cone C D and Tongier M 1973 Contact inhibition of division: involvement of the electrical transmembrane potential *J. Cell Physiol.* **82** 373–86
[13] Levin M 2014 Molecular bioelectricity: how endogenous voltage potentials control cell behavior and instruct pattern regulation in vivo *Mol. Biol. Cell.* **25** 3835–50
[14] Deloof A 1993 Schrodinger 50 Years Ago—What Is Life—the Ability to Communicate, a Plausible Reply *International Journal of Biochemistry* **25** 1715–21
[15] Lund E 1947 *Bioelectric fields and growth* (Austin: Univ. of Texas Press)
[16] Burr H S and Northrop F S C 1935 The electro-dynamic theory of life *Quarterly Review of Biology* **10** 322–33
[17] Marsh G and Beams H W 1952 Electrical control of morphogenesis in regenerating dugesia tigrina. I. Relation of axial polarity to field strength *J. Cell Comp. Physiol.* **39** 191–213
[18] Pullar C E 2011 *The physiology of bioelectricity in development, tissue regeneration, and cancer*Biological effects of electromagnetics series (Boca Raton: CRC Press)
[19] Borgens R, Robinson K, Vanable J and McGinnis M 1989 *Electric Fields in Vertebrate Repair* (New York: Alan R. Liss)
[20] Nuccitelli R, Robinson K and Jaffe L 1986 On electrical currents in development *Bioessays* **5** 292–4
[21] Jaffe L F and Nuccitelli R 1974 An ultrasensitive vibrating probe for measuring steady extracellular currents *J. Cell Biol.* **63** 614–28
[22] Sundelacruz S, Levin M and Kaplan D L 2009 Role of membrane potential in the regulation of cell proliferation and differentiation *Stem cell reviews and reports* **5** 231–46
[23] Sundelacruz S, Levin M and Kaplan D L 2013 Depolarization alters phenotype, maintains plasticity of predifferentiated mesenchymal stem cells *Tissue engineering. Part A* **19** 1889–908
[24] Bates E 2015 Ion Channels in Development and Cancer *Annu. Rev. Cell Dev. Biol.* **31** 231–47
[25] Tseng A and Levin M 2013 Cracking the bioelectric code: Probing endogenous ionic controls of pattern formation *Communicative & Integrative Biology* **6** 1–8
[26] Levin M 2013 Reprogramming cells and tissue patterning via bioelectrical pathways: molecular mechanisms and biomedical opportunities *Wiley Interdisciplinary Reviews: Systems Biology and Medicine* **5** 657–76

[27] Adams D S and Levin M 2013 Endogenous voltage gradients as mediators of cell-cell communication: strategies for investigating bioelectrical signals during pattern formation *Cell Tissue Res* **352** 95–122

[28] Mustard J and Levin M 2014 Bioelectrical Mechanisms for Programming Growth and Form: Taming Physiological Networks for Soft Body Robotics *Soft Robotics* **1** 169–91

[29] Levin M 2014 Endogenous bioelectrical networks store non-genetic patterning information during development and regeneration *The Journal of Physiology* **592** 2295–305

[30] Adams D S, Masi A and Levin M 2007 H^+ pump-dependent changes in membrane voltage are an early mechanism necessary and sufficient to induce Xenopus tail regeneration *Development* **134** 1323–35

[31] Adams D S *et al* 2016 Bioelectric signalling via potassium channels: a mechanism for craniofacial dysmorphogenesis in KCNJ2-associated Andersen-Tawil Syndrome *J. Physiol.*

[32] Vandenberg L N, Morrie R D and Adams D S 2011 V-ATPase-dependent ectodermal voltage and pH regionalization are required for craniofacial morphogenesis *Dev. Dyn.* **240** 1889–904

[33] Beane W S, Morokuma J, Adams D S and Levin M 2011 A Chemical genetics approach reveals H,K-ATPase-mediated membrane voltage is required for planarian head regeneration *Chemistry & Biology* **18** 77–89

[34] Chernet B T and Levin M 2013 Transmembrane voltage potential is an essential cellular parameter for the detection and control of tumor development in a Xenopus model *Disease models & mechanisms* **6** 595–607

[35] Chernet B and Levin M 2013 Endogenous Voltage Potentials and the Microenvironment: Bioelectric Signals that Reveal, Induce and Normalize Cancer *J. Clin. Exp. Oncol.* Suppl 1

[36] Lobikin M, Chernet B, Lobo D and Levin M 2012 Resting potential, oncogene-induced tumorigenesis, and metastasis: the bioelectric basis of cancer in vivo *Physical biology* **9** 065002

[37] Chernet B T, Adams D S, Lobikin M and Levin M 2016 Use of genetically encoded, light-gated ion translocators to control tumorigenesis *Oncotarget*

[38] Chernet B T, Fields C and Levin M 2015 Long-range gap junctional signaling controls oncogene-mediated tumorigenesis in Xenopus laevis embryos *Front Physiol* **5** 519

[39] Chernet B T and Levin M 2014 Transmembrane voltage potential of somatic cells controls oncogene-mediated tumorigenesis at long-range *Oncotarget* **5** 3287–306

[40] Arcangeli A 2011 Ion channels and transporters in cancer. 3. Ion channels in the tumor cell-microenvironment cross talk *American journal of physiology. Cell physiology* **301** C762–771

[41] Arcangeli A and Becchetti A 2010 New Trends in Cancer Therapy: Targeting Ion Channels and Transporters *Pharmaceuticals* **3** 1202

Induction of Mitosis in Mature Neurons in Central Nervous System by Sustained Depolarization

Abstract

DNA synthesis and mitosis have been induced in vitro in fully differentiated neurons from the central nervous system by depolarization with a variety of agents that produce a sustained rise in the intracellular sodium ion concentration and a decrease in the potassium ion concentration. Depolarization was followed in less than 1 hour by an increase in RNA synthesis and in 3 hours by initiation of DNA synthesis. Apparently normal nuclear mitosis ensued, but cytokinesis was not completed in most cells; this resulted in the formation of binucleate neurons. The daughter nuclei each contained the same amount of DNA as the diploid preinduction parental neurons; this implies that true mitogenic replication was induced.

The hypothesis has been advanced [1, 2] that intracellular cation levels associated with generation of electrical transmembrane potentials in somatic cells may be functionally involved in control of mitogenesis and, hence, of cell division. Results from studies with a variety of cell systems have supported this premise [3, 4]. As a corollary of the hypothesis, it was proposed [2] that mitogenesis might be activated in highly polarized, nondividing cells such as neurons in the central nervous system (CNS) and muscle by treatments that would produce and maintain a substantial increase in the level of intracellular Na^+ and a decrease in K^+. This proposition was recently investigated for the case of mature neurons from chick spinal cord depolarized with ouabain [5]; DNA synthesis was induced in a significant percentage of the neuron population by a range of ouabain concentrations. The question remained, however, as to whether the observed synthesis was truly mitogenic, rather than the result of an anomalous thymidine exchange or repair activity. We now report the induction of full DNA synthesis and subsequent mitosis in much larger percentages of chick spinal cord neurons by use of more favorable ouabain treatments, and an equally effective induction of mitotic activity in such neurons by other agents that depolarize by quite different mechanisms. The daughter nuclei from the induced mitoses each contain an amount of DNA identical to that of the mitotically quiescent G_1 (or G_0) neurons; this implies that true mitogenic replication has been induced by the imposed ionic changes.

The neurons were obtained by trypsin dissociation of spinal cords from chick embryos 7 to 10 days old and cultured as described [5]. Mature 16- to 20-day cover slip cultures of well dispersed, fully differentiated neurons were used in the experiments. The cultures contained numerous small neurons and similar-sized neuroglial cells in addition to the large, distinctive motoneurons, but primarily the latter were utilized in the present investigation to preclude any possible confusion with the glial cells; fibroblasts were also quite numerous. The motoneurons were readily identified by their large size, angular cytons with long axon and dendrites, extensively granular cytoplasm, distinctive vesicular nucleus with prominent nucleolus, and differential staining properties [6]. The glial population in the cultures increased continuously by mitotic division up to 14 days after plating but declined thereafter, although many

glial mitoses were still observable; the ratio of glia (including the numerous microglia) to neurons at the time of culture use in experiments was approximately 10 to 1. In general, the glial cells tended to aggregate in clumps while the motoneurons were well dispersed. The cultured neurons had resting potentials of −35 to −60 mv, and were entirely devoid of mitotic activity after plating.

The ability of ouabain, veratridine, and the ionophore gramicidin to induce neuronal DNA synthesis and mitosis was determined over a range of concentrations for each agent. For DNA synthesis determinations, cultures were incubated for 6 hours with medium containing the test agent and [^{3}H]thymidine (0.1 μc/ml). The cover slips were rinsed, fixed, coated with Kodak NTB-3 nuclear track emulsion, and exposed for 14 days as described [5]. The time of onset of DNA synthesis following exposure to ouabain was determined by pulse labeling for 1 hour prior to serial fixations at 1 to 6 hours. Similar pulse labeling with [^{3}H]uridine (2.5 μc/ml) was used to study changes in RNA synthesis rate following ouabain treatment; the same autoradiographic procedures were used as with DNA, but with a 7-day exposure period. For mitotic activity determinations, cultures were incubated for 24 hours with medium containing the test agent, then fixed, stained, and examined for binucleate neurons [7]. Control cultures were assayed as were the test cultures, but were incubated in normal culture medium without addition of the depolarizing or other agents. Several supplementary time-lapse film studies were made of treated neurons to elucidate features of the mitotic process and the behavior of daughter cells. Relative amounts of DNA in the nuclei of control phase (G_1) neurons and in the daughter nuclei resulting from induced mitoses were determined by Feulgen microspectrophotometry of individual nuclei [8]. The nuclear DNA content of G_1 fibroblasts in the cultures was similarly determined by using an excess-thymidine blocking procedure for accumulating G_1 cells, to establish the basic diploid nuclear DNA level. The neuron cultures were Feulgen-stained by the procedure of DeCosse and Aiello [9], and the relative absorbency of each nucleus was determined at 550-nm wavelength by using a Zeiss fluorescence microscope integrated with a model 240 Gilford monochrometer and digital photometer.

The percentages of neurons induced by the various agents to initiate DNA synthesis and to complete nuclear division are given in Table 1. All agents tested were effective in inducing both DNA synthesis and nuclear division. Since significant electroosmotic swelling [1] was found to accompany neuron depolarization in our initial studies with ouabain [5], an attempt was made to reduce the swelling (and to increase the Na^+ influx) by increasing the total medium osmolality 5 percent by addition of NaCl. This addition not only reduced the swelling but also lowered the ouabain concentration required for mitogenesis activation. Depolarization by the alkaloid veratridine produced less rapid swelling and detachment of neurons, and yielded the highest percentage of activated cells [10]. Like ouabain, the ionophore gramicidin produced substantial swelling and detachment upon depolarization, and was as effective as ouabain in its activation of neurons. Since both glial cells and fibroblasts showed spontaneous mitotic activity in control and test cultures, no quantitative assay was made of their mitotic activity in treated cultures. However, in some gramicidin-treated cultures a pronounced increase in the number of fibroblasts labeled with [^{3}H]thymidine and in the grain density of such labeling occurred in

Table 1. Percentages of CNS neurons induced to initiate DNA synthesis and nuclear division by various depolarizing agents. From 300 to 700 neurons were assayed at each test condition; four to six separate cultures were examined per condition. Grain density of labeled control cells (DNA synthesis) was one to two grains per nucleus, only slightly above background; that of labeled induced cells was severalfold higher (five to six grains per nucleus). The binucleate cells found in control cultures (mitotic activity) apparently resulted from neuroblasts in mitosis at the time of cord removal; the binucleate level in control cultures remained constant or decreased after 2 days in vitro. Data presented are for near-optimum concentrations of activating agent. A few cultures treated with 10^{-6}M ouabain plus NaCl had labeling frequencies as high as 58 percent.

		DNA synthesis (% of cells labeled)		Mitotic activity (% of binucleate cells)	
Agent	Agent (*M*)	Test	Control	Test	Control
Ouabain	10^{-5}	34	4	20	3
Ouabain	10^{-4}	34	2		
Ouabain + NaCl	10^{-6}	31	3	23	3
Veratridine	5×10^{-5}	63	3	30	8
Gramicidin	10^{-8}	32	6	29	6

portions of tightly confluent fibroblast monolayer areas in the neuron cultures. Numerous mitotic figures of fibroblasts and glia were seen in both control and test cultures, but very few binucleate cells of either type were found, which indicates completion of full cytokinesis in these cells. Phase-contrast photomicrographs of two binucleate neurons resulting from ouabain treatment are shown in Fig. 1.

Although the present experiments were concerned with assay of binucleate induction and the treatment time used was intended to permit full completion of nuclear division, a number of clearly discernible neuronal mitotic figures was found in the fixed test cultures. Metaphase, anaphase, and even prophase figures were clearly distinguished in neurons whose processes had not detached during treatment and which retained characteristic morphology and cytoplasmic staining properties. Also, a few neuronal mitotic figures on stained slides were independently identified by the periodic coordinate location procedure [7].

As shown in Fig. 2, DNA synthesis began in an appreciable fraction of the ouabain-treated cells within 3 hours. DNA synthesis was preceded by a significant rise in the RNA synthesis rate, which essentially reached its maximum level within 1 hour; this indicates that mitogenesis activation was induced quite rapidly with the ouabain concentrations used. A frequency distribution of the [^{3}H]uridine grain count per nucleus for control neurons and those treated with ouabain plus NaCl indicated that 78 percent of the treated cells had rates of RNA synthesis significantly greater than the mean level for the control neurons. In the time-lapse films of binucleate neuron formation, clear observation of individual chromosomal dispersion and movements was generally precluded because of the pronounced refractiveness of the rounded cyton, but the time of chromosome separation and formation of

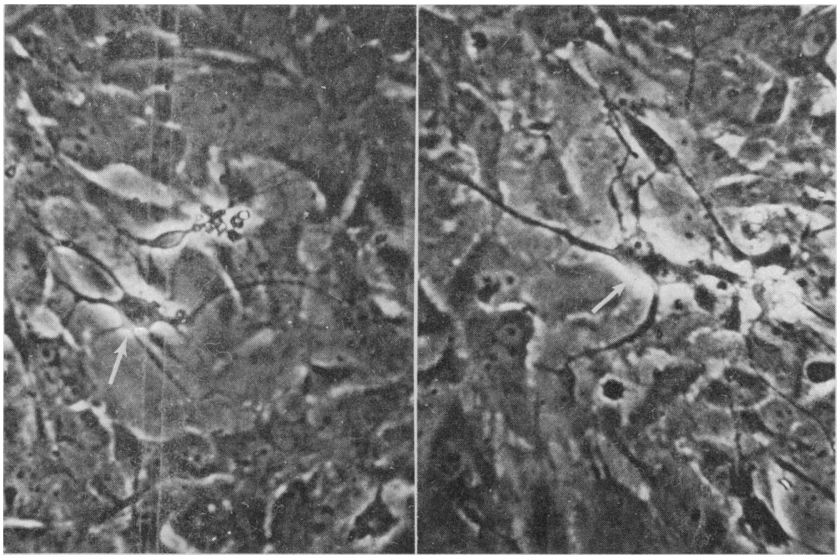


Fig. 1. Phase-contrast photomicrographs of two live binucleate neurons resulting from the ouabain treatment of 15-day test cultures. Ouabain was removed from the cultures after a 24 hour treatment. Several other binucleate neurons appear in these photographs, but are not so clearly discerned as those indicated by the arrows.

two nuclear masses could be discerned in several films. The elapsed time from beginning of treatment through completion of cytokinesis in one film of a gramicidin-treated neuron undergoing full cytokinesis was 12 hours. Although maximum swelling and "rounding up" [1] of the cell occurred within 2 to 3 hours after gramicidin addition, the actual onset of mitotic prophase did not begin until approximately 10 hours, and mitosis was completed by 12 hours. In cases where full rounding of the neuron took place with absorption of its processes, complete cytokinesis usually occurred; the resulting daughter cells were quite transient in shape and initially not morphologically identifiable as neurons.

Measurements of the DNA content of each of the daughter nuclei in 62 binucleate neurons resulting from ouabain-induced divisions revealed that each of the 124 daughters contained identical amounts of DNA. The DNA content of each daughter nucleus from the induced divisions was found to be identical also to that of the mononucleate neurons in control cultures. Since each of the 101 control neurons assayed contained the same amount of nuclear DNA as the G_1 (diploid) fibroblasts in the cultures, the control and test neurons were diploid cells prior to treatment.

The premise is generally accepted that mitotic activity ceases in fully differentiated neurons of the CNS and dorsal root ganglia, both in vivo and in vitro [11], although observations of divisions in such neurons in vitro have been reported [12]. In the present experiments, neurons that were apparently fully differentiated [13] were obtained by using cords in which neuronal mitotic activity had essentially ceased [5] and by maintaining the cultures for several days after attainment of full morphological and electrophysiological development before use in experiments. Neuronal mitotic activity was completely absent in pre-experimental cultures, as

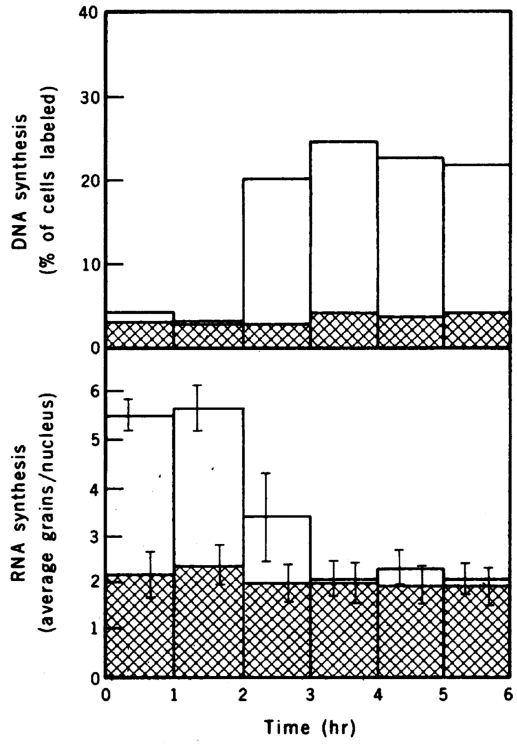


Fig. 2. Time sequence of changes in DNA and RNA synthesis induced in CNS neurons by treatment with 5×10^{-5} *M* ouabain plus NaCl, added to the culture medium at zero hour. Shaded bars refer to control cultures, and open bars to treated cultures. The labeling pulse was given 1 hour before culture fixation; approximately 350 neurons were assayed per time interval. Cells were considered to be synthesizing DNA only if their nuclear grain count was three times background (two to three grains per nucleus). Autoradiographic grain counts (RNA synthesis) are the mean per nucleus, averaged over 350 randomly chosen neurons in each time interval. The range of the mean at the 95 percent confidence level is indicated for each interval (the range for test neurons is indicated at left of interval bar, that for controls at right).

demonstrated by (i) constancy of the number of binucleate neurons after culture establishment (that is, beyond 2 days in culture; see Table 1 legend), and (ii) direct observations of some 200 individual neurons on a daily basis during 3 weeks of culture. Consequently, the mitoses reported herein were induced in neurons which had achieved the state of complete mitotic quiescence characteristic of the fully differentiated cells. An obvious and important extension of the present experiments, however, is determination of the mitotic response of neurons from adult animals and humans of various ages to these treatments.

The observation of neuronal mitotic figures in test cultures, along with the finding that daughter nuclei of binucleate neurons induced from diploid mononuclear parental neurons also contain the exact diploid level of DNA, strongly implies that the induction treatment activated true mitogenic replication of the neuronal nucleus. It is highly unlikely that anomalous nuclear fragmentation would result consistently in the production of daughter nuclei

having equal amounts of DNA, corresponding exactly to the normal diploid level. Likewise, it is unlikely that the daughter nuclei resulted from nuclear amitosis, in view of the presence of neuronal mitotic figures in the test cultures and the dearth in observations of true amitosis in higher somatic cells. It is clear that the daughter nuclei did not result from the mitosis or fission of tetraploid parental nuclei already existing in the preexperimental cultures, since both the daughter and parental cell groups possessed the diploid level of DNA. Rather, the results indicate that the treatments induced an exact doubling of parental DNA followed by an exact halving to produce identical daughter nuclei, as in normal mitosis. The fact that most of the treated neurons retained some degree of process attachment and did not complete cytokinesis, while those few which fully rounded up underwent complete division [7], suggests the possible involvement of the neuronal microtubule system in preventing completion of cytokinesis, thus fostering binucleate rather than individual daughter neuron formation.

In regard to the validity of the basic hypothesis on ionic modulation of mitogenesis activity which the present experiments were primarily designed to test, it appears particularly significant that apparently normal nuclear mitosis has been induced in such highly differentiated and mitotically refractory cells as CNS neurons by three distinct agents whose only basic commonality of action is to effect an increase in cellular Na^+ and decrease in cellular K^+ [5, 14]. The present results strongly imply that the induced mitoses follow the usual sequence of mitogenic events and reinforce the previously advanced view that activation of mitogenic RNA synthesis by significant shifts in the intracellular Na^+ level or Na^+ to K^+ ratio (or both) may be a key event in the initiation of mitogenesis [1, 2, 4]. Timelapse film and direct visual observations have demonstrated that viable daughter cells result from such induced division when cytokinesis is completed. Whether the daughter cells of such neuron divisions still possess full neuronal phenotypic properties and functionality remains to be determined, but this question obviously constitutes a subject of considerable basic, and ultimately perhaps clinical, importance. In this regard, elucidation of those conditions that favor completion of cytokinesis, rather than binucleate neuron formation, is essential.

Clarence D. Cone, Jr.
Charlotte M. Cone
Cell and Molecular Biology Laboratory, Veterans Administration Center, Hampton, Virginia 23667

References and Notes

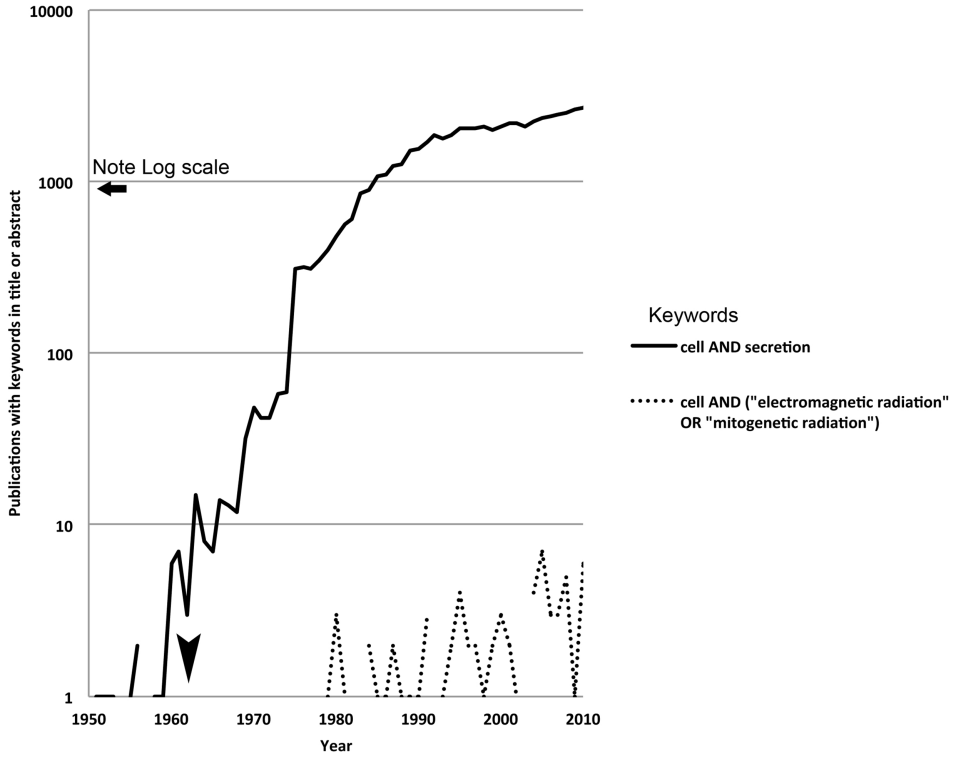
1. C. D. Cone, *Trans. N.Y. Acad. Sci. Ser. 2* **31**, 404 (1969).
2. ———, *J. Theor. Biol.* **30**, 151 (1971).
3. ——— and M. Tongier, *Oncology* **25**, 168 (1971); C. W. Orr, M. Yoshikawa-Fukada, J. D. Ebert, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 243 (1972).
4. C. D. Cone and M. Tongier, *J. Cell Physiol.* **82**, 373 (1973).

5. E. S. Stillwell, C. M. Cone, C. D. Cone, *Nature (London) New Biol.* **246**, 110 (1973).
6. In addition to their distinctive nuclear and morphological characteristics the neurons were clearly differentiated by staining with methylene blue [O. Costero and C. M. Pomerat, *Am. J. Anat.* **89**, 405 (1951)], neuron polychrome [5], or methyl-green pyronin [L.- W. Chu, *J. Comp. Neurol.* **100**, 381 (1954)]. The latter stain was particularly useful with autoradiographic emulsions and for differentiating the larger ependy mal neuroglia.
7. In most of the dividing neurons some processes remained attached during mitosis. However, cytokinesis was not fully completed, and clearly discernible binucleate cells were thus produced. By determining the cover slip (location) coordinates of many individual neurons prior to treatment and then periodically relocating and observing these same cells during treatment and up to the time of fixation, it was found that substantial numbers of the neurons were lost during rinsing and fixation. Those few neurons that were observed to complete cytokinesis had rounded up fully during treatment, with detachment and absorption of their processes; the small daughter cells were also lost during fixation. In the present study, only clearly identifiable binucleate neurons (remaining on the fixed culture slides) were included in the assay of mitotic activity; the percentages of mitotic neurons given herein (Table 1) are thus minimums. The true percentages are possibly double those indicated, as estimated from the periodic observations. A similar situation existed in the case of the DNA synthesis determinations; detachment and loss of activated cells also occurred in substantial numbers, but with somewhat less frequency than in the mitotic cells, due perhaps to the shorter treatment period. The possibility that the binucleate cells observed arose from induced or spontaneous fusion of adjacent neurons, rather than from nuclear division, was precluded by use of cultures in which the individual test neurons were well dispersed.
8. C. D. Cone and M. Tongier, in preparation.
9. J. J. DeCosse and N. Aiello, *J. Histochem. Cytochem.* 14, 601 (1966).
10. Although the possibility exists that the activated cells might comprise an immature neuronal sub-population specifically susceptible to induction, it appears more likely that all of the neurons are potentially capable of being activated; the non-activated fractions probably consist of neurons that had not adequately depolarized within the treatment periods used. Also, the high apparent percentages of nonactivated cells indicated by Table 1 may be misleading, due to the substantial loss of activated cells (but retention of the nonactivated population) during fixation [7].
11. M. J. Hogue, *Anat. Rec.* **108**, 457 (1950); *Am. J. Anat.* **93**, 397 (1953); C. E. Lumsden, in *The Pathology of Tumours of the Nervous System*, D. S. Russell, L. T. Ruben stein, C. E. Lumsden, Eds. (Arnold, London, 1959), pp. 272–309; J. Nakai and M. Okamoto, in *Morphology of Neuroglia,* J. Nakai, Ed. (Igaku Shoin, Tokyo, 1963), p. 65; H. A. Hansson and P. Sourander, *Z. Zellforsch. Mikrosk. Anat.* **62**, 26 (1964); M. R. Murray, in *Cells and Tissues in Culture,* E. N. Willmer, Ed. (Academic Press, New York, 1965), vol. 2, p. 373; P. Weiss, in

Mitogenesis, H. S. Ducoff and C. F. Ebret, Eds. (Univ. of Chicago Press, Chicago, 1956), p. 54.
12. M. R. Murray and A. P. Stout, *Am. J. Anat.* **80**, 225 (1947); R. S. Geiger, *Exp. Cell Res.* **14**, 541 (1958); M. R. Murray and H. H. Benitez, in *CIBA Symposium on Growth of the Nervous System*, G. E. W. Wolstenholme and M. O'Connor, Eds. (Churchill, London, 1968), p. 148.
13. Neurons obtained by culturing procedures identical to those used in the present experiments have demonstrated the capability of attaining such advanced developmental activities in vitro as formation of functional neuromuscular junctions [G. D. Fischbach, D. Fambrough, P. G. Nelson, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **32**, 1636(1973)].
14. R. C. Thomas, *J. Physiol.* (*London*) **220**, 55 (1972); M. Ohta, T. Narahashi, R. F. Keeler,7. *Pharmacol. Exp. Ther.* **184**, 143 (1973); B. C. Pressman, *Proc. Natl. Acad. Sci. U.S.A.* **53**, 1076 (1965).
15. We thank Burroughs-Welcome and E. R. Squibb and Sons for gifts of gramicidin. Dr. B. Chance originally suggested to us the use of ionophores as possible depolarizing agents for cells. Sponsored jointly by the Veterans Administration and the Eastern Virginia Medical Authority, Norfolk.

4 September 1975; revised 31 October 1975

Case Study 5: Cells Talk to Each Other via Electromagnetism



Gurvich *et al* (1961) Mitogenetic radiation of the neuromuscular system as a method of analyzing its molecular substrate.

Perspective on *Mitogenetic Radiation of the Neuromuscular System as a Method of Analysing its Molecular Substrate*

Lev V. Bellousov
Faculty of Biology
Moscow State University

Alexander Gavrilovich Gurwitsch (Gurvich in English transcription) is known for two main contributions: (1) a discovery (in 1923) of an ultraweak photon emission in UV range coming from a number of living objects and claimed to stimulate mitotic divisions at a certain distance, and (2) formulation and further extensive elaboration of a field concept, defined firstly as a 'force field' (1912), then 'embryonic field' (1922) and at last as a 'biological' or 'cellular' field (1944; posthumous edition 1991). The both had a dramatic and controversial fate. We start from the first one (for detailed account see Volodyaev and Beloussov 2015).

The 1923 crucial experiment was the result of G.'s prolonged interest to the causes of cell divisions. He came to the conclusion that at least one of these causes should be of a physical nature, able to 'resonate with cell membrane'. For detecting this factor, he brought into close vicinity two perpendicularly oriented onion roots, using one of them (oriented by his tip towards a side wall of another) as a sender of a physical signal while the second one as a receiver. As a result, he observed a statistically significant increase of the number of dividing cells in the receiver's area just opposed to the sender's tip. This effect was blocked by inserting between the both roots a glass plate (non-permeable to emission in the UV-range) but non-affected by inserting a UV-permeable quartz plate. In addition, the signal was shown to pass via the optical root of a UV spectrograph. Similar 'mitogenic' effects were reproduced in bacteria, yeasts (which became in G.'s labs the main every-day detector) and several kinds of animal cells.

This discovery quickly attracted a broad interest, going well beyond the borders of the former Soviet Union. Several groups in Germany, Netherlands, France, Italy, USA and other countries checked this effect and contrary to wide-spread opinion, most of them confirmed it. A properly balanced review of this period is given in Rahn's (1936) monograph. G. was several times nominated for a Nobel Prize. However, the only prize which he received was from the Soviet government. In the years 1930–48, G. was the head of large labs of a medical orientation in Leningrad (former and future St Petersburg) and Moscow, and used his discovery as a highly sensitive non-invasive tool for exploring a molecular basis of cells' and tissues' reactions to non-specific stresses (discovering what he called the 'degradation radiation'), different functional states of neuro-muscular system, and, most exciting, the cancer problem: it turned out that the blood of cancer patients stopped emitting UV photons at the very beginning of the malignant process (well before the appearance of any clinical symptoms), while the blood of healthy persons (or those suffering other diseases) permanently emitted photons. This discovery was successfully used for diagnostic and prognostic purposes not only in the Soviet Union in the 1930s and 1940s, but also in Germany before WWII.

This does not mean that the pathway of G. discovery was always triumphant. Its weak point was the capricious behavior of routinely used biodetectors unexpectedly changing their sensitivity. This paved the way for a huge paper by Hollander and Claus (1937) who claimed that G. results are at all irreproducible. At the same time the authors overtly claimed that they deviated from theexperimental protocols recommended by G. as mostly reliable ones. This paper played a fatal role, much reinforced by the events of WWII and subsequent Lysenko's campaign in the Soviet Union. As a result, to the end of the 1940s, the work on this direction was practically cancelled outside Russia, to be continued only in Moscow in a very small scale.

A new revival of interest in G.'s discovery was associated with technical progress in photo-registration techniques. Some of the most remarkable contributions were those by Quickenden and Tilbury (see Tilbury and Quickenden 1992) who fully confirmed the existence of UV radiation just of the same wave-length and emitted from the same sources (bacteria and budding yeasts) as described much earlier in G. labs. On the other hand, the authors did not confirm the mitogenic effect itself (stimulation of cell divisions). However, this could be expected, because similarly to Hollander and Claus, the authors deviated from G.'s protocols.

All in all, today's situation in this area of science looks paradoxical and controversial: if indeed the mitogenic effects of biogenic UV emission do not exist (which is still a dominating view) how could the previous investigations completely based upon the usage of biodetectors correctly predict the data confirmed later by physical measuring technique? In addition to Quickenden and Tilbury results several other examples of such correlations can be mentioned (see Volodyaev and Beloussov 2015). This urgently invites to revisit G. discovery using in a more adequate context the powerful modern technique.

The concept of a field arose in G.'s mind at the beginning of the second decade of the XXth century as an opposition to 'genocentric' ideas just emerging at that time and regarding a developing organism as a passive executor of a set of 'instructions' encoded in genes. Instead, the field concept tended to treat an embryo as a system of physical forces coordinated both in space and time and obeyed essentially wholistic laws, which G. stubbornly tried to express in mathematical terms. In the same years or slightly later, several other authors also used the term 'field' giving rise to the claims that G was not first in this topic. However, G.'s concept differs from all the others in making an accent on the temporal component of development ('our position different from the common one is that from an adequate analysis of a momentary state [of embryo] its passage to the next one should immediately follow'—G., published in 1991), rather than being restricted by a mere static registration of holistic effects. In other words, G.'s concept related to all the others as differential equations to algebraic ones. One may also say that G.'s concept, contrary to all the others, suggest certain generative rules aimed to reproduce the observed sequences of embryonic stages. In a number of his constructions G. intuitively previewed some principles of a self-organization theory. Although in spite of several reformulations, G. did not succeed in constructing a concise theory of embryonic morphogenesis (which was virtually impossible in his time due to poor knowledge of cellular and molecular basis of development), the fate of this direction

of his work was probably happier than of that discussed beforehand. While being overshadowed in the midst of the XXth century by monopolistic domination of a synthetic theory of evolution, the field approach was rehabilitated in the framework of the contemporary Evo-Devo (Gilbert *et al* 1997) and G.'s contribution was properly acknowledged (see Commentary to Beloussov 1997).

References

Beloussov L V 1997 and additional commentary by J.M. Opitz and S.F. Gilbert Life of Alexander G. Gurwitsch and his relevant contribution to the theory of morphogenetic fields *Int.J.Dev Biol* **41** 771–9

Beloussov L V 2008 "Our Standpoint Different from Common…" (Scientific Heritage of Alexander Gurwitsch) *Russ J. Dev Biol* **38** 307–15

Gurwitsch A G 1991 *(in Russian) Principles of Analytical Biology and a Theory of Cellular Fields* (Moskva: Nauka)

Hollander A and Claus W D 1937 *An experimental study of the problem of mitogenetic radiation* (Washington, DC: Bull. Natl Res. Council)

Tilbury R N and Quickenden T I 1992 Luminescence from the yeast *Candida utilis* and comparisons across three genera *J.Biolumin. Chemilumin.* **7** 245–53

Rahn O 1936 *Invisible Radiations of Organisms* (Berlin: Gebruder Borntraeger)

Volodyaev I V and Beloussov L V 2015 Revisiting the mitogenetic effect of ultraweak photon emission *Front. Physiol.* **6** 241

MITOGENETIC RADIATION OF THE NEUROMUSCULAR SYSTEM AS A METHOD OF ANALYSING ITS MOLECULAR SUBSTRATE REPORT III. THE REGULATING EFFECT OF THE SPINAL CORD CENTERS ON THE MOLECULAR SUBSTRATE OF THE MUSCLES AT DIFFERENT AGE PERIODS OF THE ANIMALS AND THE SIGNIFICANCE OF THIS REGULATION FOR THE MUSCLE METABOLSIM

A. A. Gurvich, V. F. Eremeev, and M. A. Lipkind

From the Office of Mitogenesis (Head : A. A. Gurvich) of the Institute of Normal and Pathological Physiology (Director: Active Member of the Akad. Med. Nauk SSSR V. V. Parin) of the Akad. Med. Nauk SSSR, Moscow
(Presented by Active Member of the Akad. Med. Nauk SSSR V. V. Parin)
Translated from Byulleten 'Eksperimental' noi Biologii i Meditsiny, Vol. 51,No. 4, pp. 57-61, April,1961
Original article submitted April 25, 1960

In previous publications [2,3,6], data were presented on the antibathic processes (processes of a reverse nature) arising in spinal cord centers (presumably areas rich in cells—the motor cells of the anterior horns, the inter-neurons) and in muscle fibers (neuromuscular junctions).

The action of the muscles imparts a prepolymer character to the substrate, while the effect of the centers, on the other hand, results in a dispersed arrangement. The resultant state of the neural and muscular substrates is extremely labile, and may be characterized as a state of unequilibrated molecular regularity.* The unequilibrated nature of the substrate in the muscle fibers has been demonstrated particularly clearly during the resting state of the muscles in experiments in situ.

Along with this, a previous report [3] introduced the concept of the constant regulation of the three-dimensional parameters in the molecular substrate of the muscles as a specific physiological phenomenon.

Further study of this regulation, serving as the goal of the present work, was carried out in the following directions.

1. We studied the evolution of the molecular substrate character in the muscles throughout the development of the animal, i.e. in the period when the full functional relationships are formed.
2. We studied the action of the centers on the muscle substrate as a factor limiting and, in this way, regulating the character of the metabolism. In this case we were thinking of mediators, specifically acetylcholine. Reducing the influence of the centers, we investigated the possibility of acetycholine arising under this condition in a resting muscle.

* The unequilibrated state of the molecular substrate in living systems is one of the basic concepts in the problem of mitogenesis [4], This state, of course, does not exclude the presence of myosine protein chains, etc.

METHOD

The first part of the work was performed on rabbits, the second, on frogs. We investigated the mitogenetic radiation of the gastrocnemius muscle in the resting state within the living animals. The adult rabbits were secured to a horizontally placed stand. The muscle was exposed, and the radiating area, 10–12 mm^2, was delineated in the upper portion. The detector was placed at a distance 10 mm from the muscle. The same conditions were observed in the work with the young rabbits, except that because they were so active it was necessary, in addition to the regular fastenings, to hold them in place manually.

The adult rabbits were also left in the horizontal positon for the spectral analysis of the radiation, directed into the objective aperture of the spectrograph* by reflection from a concave mirror. The young rabbits were placed in a the vertical position, directly in front of the spectrograph aperture.

The frogs were also centered in the vertical position, fastened to a cork disk.

Degradation radiation [3, 4, 6] was caused by irrigation of the muscle with chilled physiological saline(6-8° for the rabbits, and 1-2° for the frogs).

Separation of the narrow spectral bands was accomplished with the aid of a monochromatic aperture. However, the majority of the data was obtained by coarser separation of the spectrum into portions 100 A in width.

The biodetection method was used for the observation of the radiation. This method has been described in detail in a number of publications [4, 5, 6].

RESULTS

Just as in the case of the spectrum described for the corresponding muscle in the frog [3], the radiation spectrum of the gastrocnemius muscle in the adult rabbit is characterized by a single radiation maximum in the range of 2200-2300 A (Table 1). Only with finer separation was a small difference seen in the spectra: the greatest radiation intensity for the frog muscle lay in the range 2000-2260 A, while for the rabbit it was in the range 2210-2270 A.

The same type (as in the frog) and spectrum of degradation radiation was seen to arise in the rabbit muscle in association with its cooling.

Spectral analysis of the radiation encountered in the resting gastrocnemius muscle of the rabbits in the different age periods yielded results which are presented in the figure.

The clear evolution of the spectra toward reduction in the number of active bands makes these data completely conclusive, despite the small number of animals. The radiation spectrum of the muscle in the 16-day old rabbit is already comparable to the spectrum observed in the adult animals. It must be noted that at this age, i.e., after maturation of the animal, degradation radiation of the muscle also acquires the same spectral composition.

These data make it possible to postulate that the molecular substrate of the muscle fibers gradually acquires the character of an unequilibratedly regular system.

* Fuess Quartz Spectrograph, possessing adequate dispersion.

TABLE 1. Radiation Spectra of the Rabbits'. Gastrocnemius Muscle

Wave length (in A)	Resting radiation	Degradation radiation
	15-18 second exposure effects (in %)	6-8 second exposure
1 900–2 000	−3	3
2 000–2100	4	−7
2 100–2 200	−1	5
2 200–2 300	52	45
2 300–2 400	4	0
2 400–2500	8	−3
2 500–2 600	6	5
2 600–2 700	−2	–
2 700–2 800	−8	13
2 800–2 900	10	−3
2 900–3 000	4	−3
3000–3 100	−1	5
3 100–3 200	−3	–

Note: Averages of 5 trials are presented in column 2; averages of 2 trials are presented in column 3.

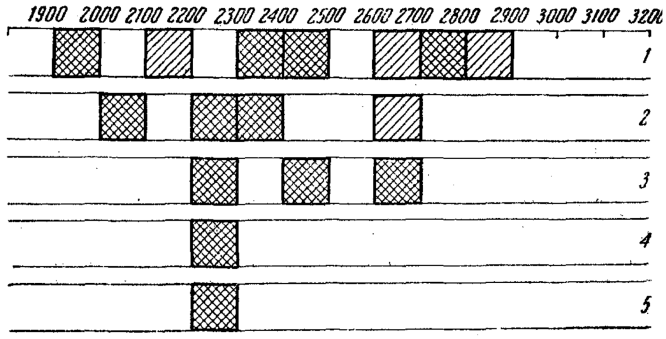
TABLE 2. Intensity of the "Resting" and Degradation on Radiation of the Gastrocnemius Muscle in Rabbits at Various Age Periods

	Resting radiation		Degradation radiation	
Age (in days)	exposure, sec**	effect (in %)	exposure, sec	effect (in %)
1–2	2–3	**40**	4–5	5
			8	**36**
4–5	2–3	−3	2–3	−2
	4–5	**28**	**4**–5	**27**
9	2–3	10	2–3	−10
	4–5	**36**	**4**–5	**20**
16	4–5	2	2–3	**48**
	8	**24**		
Adults	4–5	−3	2–3	**39**
	8	**45**		

Note: Averages of the figures from 2 trials are presented. **The threshold exposures are indicated in boldface type.

The data were supported by the results obtained from comparing the intensity of "resting" and degradation muscle radiation in the young and adult rabbits (Table 2).

Intensity and threshold duration were inversely related: I.t = const. [5, 6] (I – intensity, t—threshold exposure). In these experiments the constant radiation area (10 mm^2) was strictly observed, as was the constant distance of the detector from the muscle, 10 mm.



Spectra of radiation encountered in the resting gastrocnemius muscle of the rabbits at various age periods:
1) 1-2 days; 2) 4-5 days; 3) 9 days; 4) 16 days; 5) adults.

Spectra of radiation encountered in the resting gastrocnemius muscle of the rabbits at various age periods: 1) 1-2 days; 2) 4-5 days; 3) 9 days; 4) 16 days; 5) adults.

Thus, we can speak of significant intensity of the resting radiation from the rabbit muscle at early ages of the animals, and of approximately the same elevation in the intensity of the degradation radiation, beginning with the rabbit's second weak of age.

The entire body of results permits drawing the following conclusions.

In the first days following the birth of the animal the radiation of the muscle in the resting state is directly related to metabolic processes, and must be very varied in that period. Later the radiation arises as a result of natural disturbances in the unequilibrated molecular substrate, i.e., appears as degradation radiation of a physiological type. Energy for the support of this unequilibrated state is supplied by the muscle metabolism. The three-dimensional regularities in the substrate's molecular order reproducible in general outline, are, as we already stated earlier, the product of an uninterrupted interaction of processes whose sources are the centers and the muscles.

Judging form the evolution of the spectra, the general steric regularities of the substrate gradually transform into more well defined structural parameters. Nevertheless the state of the substrate completely retains its unequilibrated character, i.e. requires a considerable expenditure of energy for its support.*

This latter conclusion leads to the concept, already advanced earlier, that the resting state of the neuromuscular system must be regarded as an active setting, and emphasizes that a high energy potential is specific for the muscle resting state.**

Judging from the low intensity of the "resting" radiation of the muscle, it is consumed very expeditiously and economically. The unequilibrated structural state of the substrate doubtlessly aids this.

* The concept that a more limited spectrum reflects greater uniformity of the substrate's structural organization has been completely substantiated.

** Especially manifest, apparently, for heavy muscles.

We considered it of interest to compare these data with the results of detailed investigations by I. A. Arshavskii and V. D. Rozanova [1, 8], demonstrating a low lability and excitability, and also low resistence of the neuromuscular apparatus in the early age periods of the animals, and elevation of these properties with development of the animal. The gradual formation of the unequilibrated structural arrangement corresponds to the rise in lability and excitability. The constant reproducibility of the structural arrangement formed may serve as one of the factors elevating the resistence of the muscle tissue.

But along with this the question may be raised as to whether such dynamic constancy of the structural arrangement does not serve as the kind of regulating factor which limits the accomplishment of various chemical activities.

In this case, reduction in the interaction between the spinal cord centers and the muscles could lead to "enrichment" of the spectrum for the resting radiation of the muscle and to inclusion of bands in it which are characteristic of metabolites not found under normal conditions. We settled on acetylcholine, whose spectrum is not observed during analysis of the resting radiation from the frog gastrocnemius muscle, and which arises during its excitation [7]. The experiments presented below were also carried out on frogs.

Reduction of the action of the centers was accomplished by cooling them. This, along with direct stimulating action, yields an increasing inhibitory effect on the enzymatic activity of the tissue, i.e. indirectly also on the functional activity of the centers.

TABLE 3. Radiation Spectrum of the Frog Gastrocnemius during Cooling of the Spinal Cord Centers (the acetylcholine line is shown in boldface type)

Wavelength (in A)	Exposure (in seconds)	Effect (in %)	Wavelength (in A)	Exposure (in seconds)	Effect (in %)
1 900–1 930	8–10	43	1 940–45	20–30	0
1 930–1 960	8–10	0	1 945–50	20–30	40
1 960–2 000	8–10	12	1 950–55	20–30	−10
2 000–2 100	8–10	3			
2 100–2 200	8–10	−3	2 330–35	20–30	6
2 200–2 300	8–10	−6	2 335–40	20–30	39
2 300–2 400	8–10	2	2 340–45	20–30	0
2 400–2 500	8–10	−8			
2 500–2 600	8–10	3			
2 600–2 700	8–10	12	2 370–75	20–30	3
2 700–2 800	8–10	6	2 375–80	20–30	45
2 800–2 900	8–10	−1	2 380–85	20–30	6
2 900–3 000	8–10	33			
3 000–3 100	8–10	20	2 610–20	20–30	0
3 100–3 200	8–10	16	2 620–30	20–30	60
			2 630–40	20–30	10

Note: The averages of two trials are presented in column 3, and the averages of 3 trials in column 6.

A flat piece of ice from frozen phsiological saline was affixed to the corresponding portion of the vertebrate, which had first been freed of the muscles covering it. After 5-8 min we began the spectral analysis of the radiation from the gastronemius muscle (Table 3).

We see, in this manner, that not only the general character of the spectrum changed, but weak acetylcholine lines also arose. The parallel occurrence of these two phenomena are of great interest from out point of view.

These data thus confirm the concept that regulation of the structuro-energy state in the molecular substrate of the muscle in association with the overall state of all systems involves the principle of limiting the various chemical processes, i.e., it determines, to a definite degree, the character of the metabolsim.

In line with this one must refer to the additional orientating data, showing that with adequate excitation of the muscles there occurs an "enrichment" of the total radiation spectrum in addition to the simultaneous genesis of acetylcholine,

SUMMARY

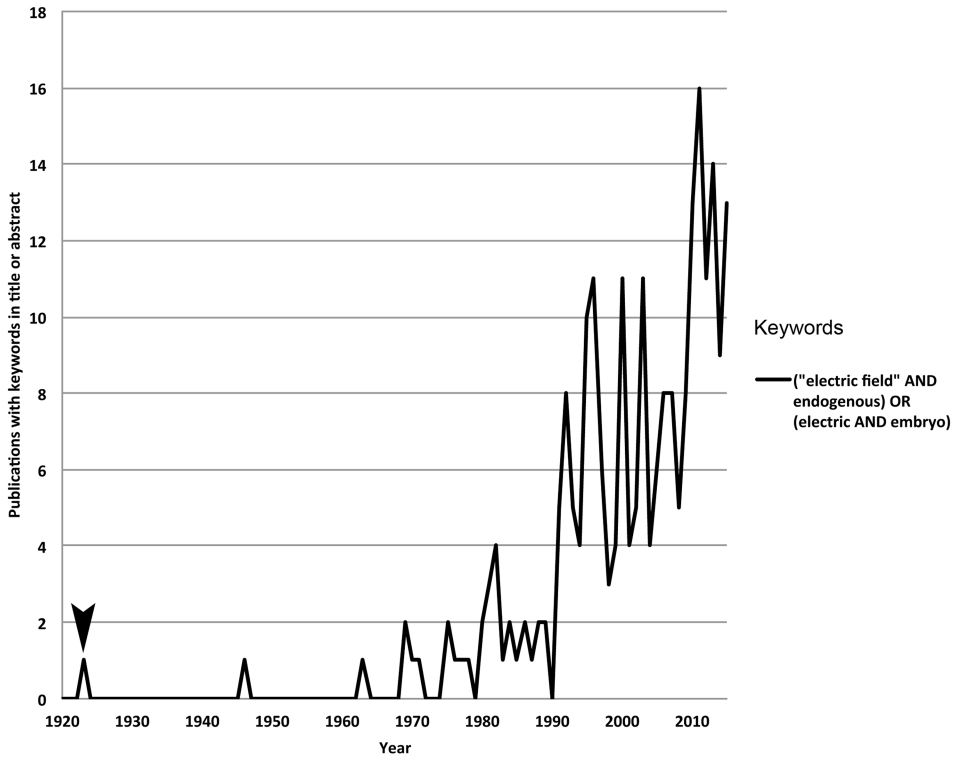
The authors present data which broadens the concept of the regulatory effect exerted by the spinal cord centers on the molecular substrate of muscles. Evolution of mitogenetic spectra of the resting rabbit gastrocnemius (connected with age) and the prevalence of mitogenetic radiation of degradational type in adult animals point to the gradual formation of the nonbalanced structural state of the substrate.

It was shown that the nature of metabolism is also connected with the substrate regulation. This follows from the altering of the mitogenetic spectrum lines of the resting frog gastrocnemius caused by reduced influence of the centers. In such conditions, the spectrum contains acetylcholine lines as well.

LITERATURE CITED

1. Arshavskii, I. A., Izv. Akad. Nauk SSSR, Seriya Biol., No. 1, p. 71 (1958).
2. Gurvich, A. A., Byull. Ekspt. Biol, i Med., No. 5, p. 67 (1960).
3. Gurvich, A. A., Byull. Ekspt. Biol, i Med., No. 10, p. 82 (1960).
4. Gruvich, A. G. and Gurvich, L. D., Mitogenetic Radiation [in Russian] (Moscow, 1945).
5. Gurvich, A. G., and Gurvich, L. D., Introduction to the Study of Mitogenesis [in Russian] (Moscow, 1948).
6. Gurwitsch, A., Die mitogenetische Strahlung, (Berlin, 1932).
7. Lipkind, M. A., Byull. Ekspt. Biol, i Med., No. 6, p. 65 (1958).
8. Rozanova, V. D., Fiziol. Zhurn. SSSR 25, p. 202(1938).
9. Rozanova, V. D., Fiziol. Zhurn. SSSR 30, No. 3, p. 346 (1941).

Case Study 6: Ion Currents Regulate Cell Polarity



Lund (1923) Electrical control of organic polarity in the egg of Fucus. This paper is just one example of the range of ideas under study prior to the rise of molecular biology.

Perspective on *Electrical control of organic polarity in the egg of fucus*

Richard Nuccitelli, PhD
RPN Research and BioElectroMed Corp.

A true pioneer in the discovery of bioelectricity

Life as we know it relies extensively on electric fields and ionic currents. Not only is our main energy currency, ATP, generated by an electromotive force of 200 mV across our mitochondria membranes, but every sense that we have from vision to hearing utilizes electrical signals.

Consider our vision for example. Our retinal rods are highly polarized with Na^+ channels localized to the outer segment and K^+ channels localized to the inner segment. This polarization of ion channels generates a steady 'dark' current through these cells that is controlled by the concentration of cGMP that gates the Na^+ channels. When light hits the rhodopsin in the outer segment, it triggers a signal transduction cascade that results in the hydrolysis of cGMP and subsequent reduction in the Na^+ current. This leads to a hyperpolarization of the membrane potential of that rod and influences secretion to the next cell in the visual system to influence image formation in the brain. We are truly electric beings.

With this in mind, it is not surprising that our development utilizes electrical signaling as well. The first investigator to propose that natural electric fields might influence growth was Albert Mathews in 1903 [1]. He measured potential gradients along the surface of regenerating hydroids, indicating current entering the regenerating polyp end. He suggested that this current polarized the protoplasm or cells. However, the most significant and influential early investigations of developmental fields were surely those of Elmer Julius Lund [2, 3]. These studies began in 1921 and culminated in his book, *Bioelectric Fields and Growth* in 1947. His work stimulated a considerable number of contemporary investigators such as Spek [4] and Burr [5, 6] as well as later investigators such as Lionel Jaffe, Ken Robinson, Benjamin Peng, and myself. His paper included here describes a very simple method for applying an electric field to cells in sea water while measuring their polarized growth. He determined that rhizoid outgrowths of *Fucus inflatus* eggs would form towards the positive electrode if the eggs were exposed to an electric field of about 25 mV/egg throughout a period of about 10 h prior to rhizoid formation. These observations were both confirmed and improved upon by Ben Peng [7] and we now know from our more recent work [8, 9] that the direction of this imposed current is the same as the endogenous one that enters the egg in the rhizoid end and leaves the thallus. These eggs begin life completely spherically symmetrical with no preformed axis and can generate a rhizoid on any part of their surface. The selection of the optimal.

Growth axis can be guided by external cues such as light, pH or nearby neighboring eggs. Light is the most common guiding influence as they would normally grow away from the light to generate a holdfast on the intertidal rocks. Investigators stimulated by Lund's initial observations of fucoid egg sensitivity to imposed electric fields have determined that light triggers an influx if Ca^{2+} into the dark region of the egg surface [10] and an efflux of K^+ on the opposite end, and have

measured the endogenous extracellular current flow resulting from these transmembrane ion movements [8]. We now know that these transcellular currents are found to be associated with cell function on most cells that have been investigated with the appropriate tools [11]. Thus, Lund's contributions 93 years ago have been seminal and inspired a great deal of investigation into the bioelectric properties of cells and tissues.

References

[1] Mathews AP Electrical polarity in the hydroids *American J Physiology* 1903**8** 294–9

[2] Lund EJ 1923 Electrical control of organic polarity in the egg of Fucus *Bot. Gaz.* **76** 288–301

[3] Lund EJ 1947 *Bioelectric Fields and Growth* (Austin, Texas: Univ. Texas)

[4] Spek J 1930 Zustandsänderungen der Plasmakolloide bei Befruchtung und Entwicklung des Nereis-Eies *Protoplasma* **9** 370–427

[5] Burr HS 1941 Field Properties of the Developing Frog's Egg *Proc. Natl Acad. Sci. U S A* **27** (6) 276–81

[6] Burr HS and Bullock TH 1941 Steady State Potential Differences in the Early Development of Amblystoma *The Yale journal of biology and medicine* **14**(1) 51–7

[7] Peng HB and Jaffe LF 1976 Polarization of fucoid eggs by steady electrical fields *Dev. Biol.* **53** 277–84

[8] Nuccitelli R 1978 Ooplasmic segregation and secretion in the pelvetia-fastigiata egg is accompanied by a membrane generated electrical current *Dev. Biol.* 62

[9] Jaffe LF 1966 Electrical currents through the developing Fucus egg *PROC NATL ACAD SCI (USA)* **56** 1102–09

[10] Robinson KR and Jaffe LF 1975 Polarizing fucoid eggs drive a calcium current through themselves *Science (Washington D C)* **187** 70–2

[11] Nuccitelli R 1983 Transcellular ion currents: Signals and effectors of cell polarity *Modern Cell Biol.* **2** 451–81

ELECTRICAL CONTROL OF ORGANIC POLARITY IN THE EGG OF FUCUS

E. J. Lund

(with plate xxvii, and two figures)

Introduction

Organic polarity in animal and plant cells, organs, and whole organisms is one of the fundamental common properties of living material, and therefore it makes little difference whether the phenomena are considered in plant or in animal cells, the problem is probably, if not certainly, the same. This fact has long been recognized, but the problem of polarity is only a special (although in all probability primary) part of the general and more comprehensive problem of the origin and causes of symmetry in living material. An excellent treatment of certain aspects of the principle of symmetry in its relations to chemical and biological science is given by Jaeger (3).

The facts of physiology relating to growth, differentiation, and dedifferentiation have shown beyond question that the visible structure of the cell or organism is only a result of preceding physico-chemical processes, in the same sense that the formation of a precipitate is the result of a chemical or physical process, which in turn is always associated with an energy change. From this fundamental fact it follows that the energy changes, or forces which determine the polar or symmetrical arrangement of the material of living cells, organs, and whole organisms must to a greater or less degree be directed forces. Such energy changes or forces must have an axial or polar orientation, corresponding in degree to the axial or polar arrangement of the material laid down as structure. The kind of information desired, therefore, is facts relating to the type, magnitude, and distribution of the associated energy changes, and not primarily a knowledge of the quality or quantity of the materials laid down as structure. The primary problem, therefore, would appear to be the nature of the forces which bring about the characteristic orientation of the materials in the process of formation of the colloidal structure of cells.

It is well known that light is capable of determining the polarity of the germinating spores of many lower plants. This has been shown in the recent experiments by Miss Hurd (I) on the egg of *Fucus*. Effects of mechanical pressure and centrifugation of animal eggs in relation to orientation of cell cleavage have been described by many workers (see Kellicott 4), but so far as the writer is aware, the following is the first successful attempt at controlling the polarity of an egg and the future body axis by means of an electric current. The object in the present paper is (I) to show that by the application of a difference of electrical potential to the egg of *Fucus*, it is possible to determine the axis of symmetry of the egg and future plant body; and (2) to give a method by means of which it has been possible to calculate the magnitude of the potential difference necessary for this orientation.

Fucus inflatus is monoecious, and consequently the eggs are fertilized at the time of shedding. The first cleavage, which is equal, begins approximately sixteen hours after the shedding of the eggs, and is completed in a few hours at room temperature.

The eggs are perfectly spherical, and no axis of symmetry is evident before cleavage begins. As is well known, one of the cells of the first cleavage becomes the rhizoid or holdfast, while the other gives rise to the thallus, thus determining definitely the future polar axis of the organism.

Methods

The procedure in obtaining the eggs of *Fucus* was the same as that described by Miss Hurd. Plants with mature tips were collected from midtide level near the Friday Harbor laboratory, and wrapped in moist cloths for periods varying from ten to eighteen hours, and then exposed to air for about half an hour. The tips were then removed and floated in flat dishes containing fresh sea water. Within a few minutes or half an hour the tips began to shed the eggs, which then settled down upon the bottom of the dish and could be collected and removed with a pipette. The possibility of carrying out the experiments described depends entirely upon the fact that after fertilization the *Fucus* egg fixes itself permanently to the solid substratum (cover glass, for example) upon which it comes to rest. Permanent fixation takes place in about six to eight hours, or in some cases within a shorter time. All of the experiments with the electric current were carried out in total darkness, so that none of the results are attributable to light.[I]

The apparatus used is shown in text fig. I, which is about one-half the actual size. *A* is a general view of the arrangement, while *B* is a longitudinal section through the arm d of the trough shown in *A*. The V-shaped arrangement of the two arms *c* and *d* of the trough in *A* provided for a continuous flow of a stream of sea water, whose rate of flow was regulated by the stopcocks e and *f*. The stopcocks were connected by siphon to the same supply of fresh sea water kept in glass jars. The level of the water in the two arms of the bath was regulated by the adjustable outflow *g*. The apparatus was made from a glass plate having three holes bored through it. The two holes at the ends of the V-shaped trough were covered with porous clay diaphragms (*x*) sealed to the glass by means of de Kotinsky cement, as shown in *B*. Into the other hole (*h*) was fitted the outflow tube *i*, in which the zinc rod electrode was held by a special support not shown in the figure. The electric current was led into the arm (*d*) of the trough from the dish of sea water (*j*), in which the other zinc electrode was supported. This is shown best in the section *B*. The sea water in the dish *j* could be changed continually if necessary, thus removing all possibility of any products of electrolysis from entering the stream of water over the eggs. The products of electrolysis at the electrode *z* were washed away by the constant stream of sea water from the two arms of the trough, thus insuring for the eggs absolute freedom from contact with any foreign chemicals. As a result of this arrangement, the electrical resistance of the arm (*d*) of the trough was relatively low, so that a relatively large adjustable resistance (*r*) could be inserted, thereby making possible a complete control of the density of the electric current through the arm of the trough (*d*). The electric current from the battery *b* (about 18 volts) was passed through an accurately

[I] The writer is greatly indebted to his assistant, Miss Gladys Hamm, for the work connected with the preparation of the material for experiment.

calibrated milleammeter reading to 0.05 of a milleampere. The two arms of the trough provide for identical conditions except for the electric current passed through *d*. The trough was made from accurately fitted glass strips held together by de Kotinsky cement, and covered by glass plates (*l*, *m*), so as to provide a definite constant cross-sectional area of the stream of sea water in which the eggs were placed. Since the total electric current flowing through the arm *d* of the bath is known from the milleammeter reading, and the cross-sectional area of the column of sea water over the eggs is constant and known, then the current density (the number of microamperes per sq. mm. cross-section of the sea water) is known. Finally, if we can determine the resistance per unit length of the column of sea water under the

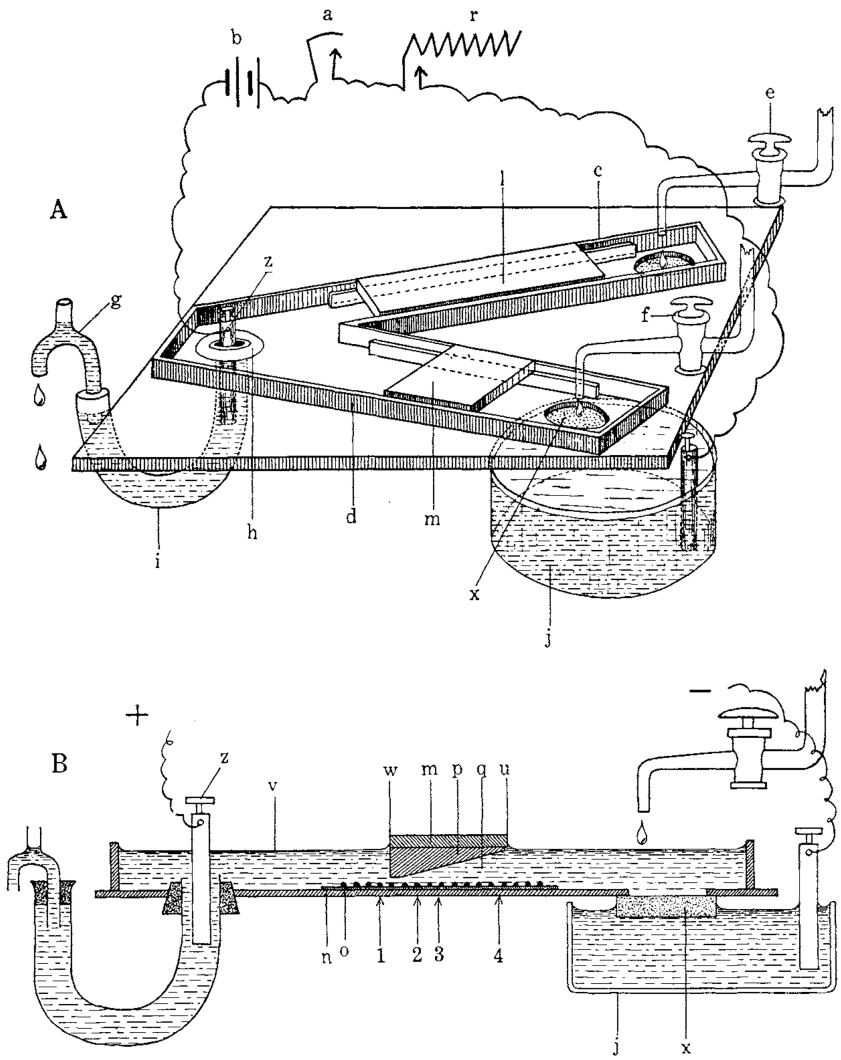


Fig. I. —*A*, general view of apparatus; *B*, longitudinal section of arm; explanation of details in text.

glass cover *m*, then of course the fall of electrical potential over any cross-section of water column can be calculated. Text fig. I*B*, which is a longitudinal section of the arm *d*, shows the essentials of the arrangement to better advantage. The cover glass (*n*) bearing the eggs (*o*) is fixed in place on the bottom of the trough by a thin film of vaseline. To the under side of the glass cover (*m*) is fixed a glass wedge (*p*), whose width is such that it fits snugly between the sides of the arm d of the trough. This provides for a sharply defined wedge of sea water (*q*) whose dimensions can be determined, and therefore provides for a gradient of known density of the electric current, and therefore a gradient of known fall of electrical potential over the eggs spread upon the cover glass. The electrical resistance of this wedge of sea water of known dimensions was determined in the ordinary way, using platinum electrodes which were inserted in the positions *u* and *w* covering completely the two ends of the water wedge. To check the accuracy of this method of determining the resistance of the wedge, one of the electrodes was placed in the position *u*, while the other was placed at some definite position *v*. The resistance of the sea water column was then determined with the electrodes at these positions. The electrode at *u* was then removed to the position at *w* and the resistance again determined. The difference between these values was the resistance of the water wedge *q*. Identical values (195 ohms) for the wedge of sea water were obtained by the two procedures.

Experimentation

Several preliminary experiments were undertaken to discover the appropriate current density at which effects of the electric current upon the eggs appeared. The use of the wedge facilitated the work; for, as was found in the writer's previous work on the electrical control of polarity in regenerating internodes of Obelia (6), a definite threshold of current density for orientation of the first cell cleavage exists in the *Fucus* egg.

In the first experiments the wedge was not used, but instead the current density was varied by placing the cover glasses holding the eggs upon glass squares of different thicknesses, which were then placed on the bottom of the trough under the glass covers (*m*, *l*). The results of a successful experiment, in which the appropriate current density for orientation was accidentally found in this way, is given in Plate XXVII, *a* and *b*, in which *a* is a typical field on the cover glass, the eggs upon which had been subjected to the electric current, while *b* is a field from the cover glass kept as a control in the other arm of the trough without the electric current. Other controls were also kept in finger bowls. These showed identical conditions of growth with that of the control *b*.

The photomicrographs *a* and *b* were taken seventy-one hours after seeding the eggs on the cover glasses, and seventy-two hours after shedding of the eggs by the plant tips. The electric current was turned on immediately after the eggs were placed on the cover glass, and at the same time the water current was turned on through the arm *c* of the trough, thus serving to wash the electrode *z*. The sea water in the dish *j* was changed several times during the experiment, thus insuring freedom of the eggs in *d* from chemicals, even though no water current was flowing through *d.* Since it takes about six to nine hours for the eggs to become firmly fixed, the current of sea

water was not turned on in *d* until nine hours after seeding. The velocity of flow of sea water over the eggs in the arm *c* was slower than that over the eggs in arm *d,* because of the greater cross-sectional area of the water column in *c;* hence the control eggs in arm *c* were not in danger of being dislodged by the water current, even though this was started immediately after seeding the eggs upon the cover glass. This procedure permitted the eggs on the cover glass in *d* to become fixed firmly in relatively quiet water, while the electric current was being passed through the eggs from the beginning.

When the eggs in *d* had become fixed, the water current was turned on without danger of losing any of them. As time went on after seeding, the eggs became more and more securely fixed, until at the end of about twenty-four hours the swiftest current did not seem to be able to dislodge them.

In one of the preliminary experiments eggs were placed upon two glass squares of different thicknesses. These two glass squares were then placed on the bottom of the same trough *d* and the electric current passed through. The water was turned on in the arm *c* at the beginning, as in the experiment just described. Later the water current was turned on in *d.* The result was that the eggs upon the thicker glass square (higher current density) were oriented, while those on the thinner glass square were not.

A cover glass with control eggs previously kept in sea water in a finger bowl until they had become fixed was then put into the arm *c,* and the water current allowed to flow much more swiftly through the arm *c* than over the eggs in the arm *d.* No trace of any orienting effect of the water current upon the eggs in *c* could be seen. These and other similar preliminary experiments convinced the writer that the orienting effects observed in the electric current were not due to the flow of water, nor were they due to any reaction by the egg cells to chemicals from the electrodes. In figs, *a* and *b* no difference in the amount of growth of the eggs can be seen. The eggs in the control *b* appear slightly larger than those in *a,* but this is simply due to an accidental difference in magnification. Under the microscope no difference between the two lots of eggs, except that of orientation, could be seen. These experiments, together with others giving similar results, show that it is possible to orient the first cleavage of the egg, and therefore the polarity and future body axis by an electric current.

The following experiment is reported in full because the procedure enables us to determine the quantitative relations between the current density, or electrical potential, and the effects upon the eggs. The eggs were placed on cover glasses within one hour after being shed. Some of these cover glasses were kept as controls in finger bowls containing fresh sea water. One of the covers was placed in the arm *d* of the trough under the glass wedge exactly as shown in text fig. I*B.* The water current was turned on through the arm *c* to wash the electrode *z*, and the electric current turned on in *d* immediately after seeding the cover glass. Twelve hours after the beginning of the experiment the water was turned on in *d.* Fourteen hours after the beginning of the experiment a cover glass containing control eggs was removed from the finger bowl and placed in the arm *c* of the trough. Forty hours after the beginning of the experiment the cover glass in *d* was removed for examination, and photomicrographs of typical microscopic fields were taken at the positions numbered I, 2, 3, and 4 in text

fig. I*B.* These figures are correspondingly numbered I, 2, 3, and 4 in plate XXVII. It will be seen that the eggs shown in figs. I and 4, corresponding to positions I and 4 in text fig. I*B,* do not show any orienting effects of the electric current.

Growth in these positions was not different from growth in the control kept in the arm *c,* or those in finger bowls. In all such lots of eggs there are always some eggs which do not divide, similar to what occurs for example in echinoderm eggs, so that the few eggs showing no growth have no special significance.

Fig. 2 from position 2 shows practically 100 per cent orientation. Examination of the eggs in position 2 showed that the planes of the first cleavage were very nearly at right angles to the direction of the electric current. This fact was verified by several members of the laboratory at the time.

Fig. 3 from position 3 in text fig. 1*B* shows that there is a very distinct threshold of current density (or *PD*) at which orientation occurs. The writer believes that this may prove to be an important fact for the significance of the phenomena of orientation as a whole. Similar well defined thresholds of electrical potential have already been described for *Obelia* (6). On the left side of fig. 3 most of the cells are oriented, while on the right side of the same field no orientation effects are evident. The attempt was made to show the region of the threshold in the middle of the photograph, but unfortunately manipulation of the camera resulted in a slight displacement, so that the orientation threshold lies at the left of the middle of the figure.

Under the glass wedge between the positions I and 2 a definite inhibition (delay) in cleavage occurred. Development occurred in most of these delayed eggs after removal of the cover glass to a finger bowl with sea water. As seen from the experiments (figs, *a* and *b*), orientation by the electric current does not necessarily involve inhibition (delay) of cleavage and growth. This is again evident in figs. I,3, and 4, and consequently places the fact of orientation in a definite category of what might be called normal response comparable with the response to light as found by Miss Hurd (i).

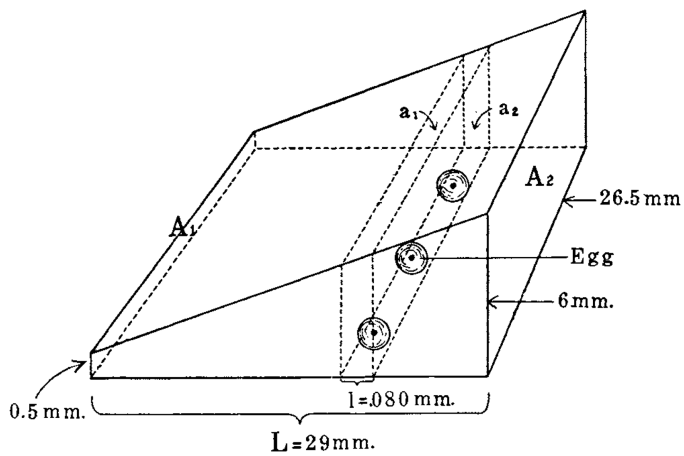


Fig. 2. —Diagram of water wedge q in fig. I*B*; numbers give actual dimensions in millemeters.

Figs, *a,* 2, and 3 show that orientation of cells to the electric current is not perfect wherever the eggs lie in close groups. This may be interpreted simply as due to the fact that the electrical resistance of the group is high, and therefore the fall of electrical potential across an egg in such a group is less (that is, below the threshold value, other things being equal) than it would be if that same egg were isolated. An instance of group orientation as described by Miss Hurd is shown in fig. 4. This orientation by the cell to adjacent cells undoubtedly modifies the response to the electric current, similar in a way to the interference effect of a contact stimulus upon *Paramecium* when reacting to an electric current.

Calculation of threshold value of electrical potential for orientation

Let fig. 2 represent the shape of the water wedge q in text fig. I*B*. Since the total electric current (C) flowing through the wedge from face A_{I} to face A_2 was known, and equal to 37.4 milleamperes in the experiment from which figs. I, 2, 3, and 4 were taken, and the total resistance (R) of the wedge was 195 ohms, then by Ohms' law, E, the total fall of electrical potential between the faces A_{I} and A_2, was 7.29 volts. The P.D. causing the current to flow through any given egg is the P.D. between two planes parallel to A_{I} and A_2, and separated by a distance equal to the diameter of an egg. Since the diameter of the *Fucus* egg is nearly 0.08 mm., the problem becomes one of calculating the magnitude of the P.D. between any such two planes 0.08 mm. apart, at any position along the length of the wedge, as for example a_{I} and a_2 in fig. 2. Since the specific resistance K of the sea water (in this case the resistance of a millimeter cube of sea water) is given by the equation $K = \mathrm{R}\frac{\mathrm{I}}{L}\frac{(A_{\mathrm{I}} + A_2)}{2}$ (I), in which all the terms except K can be determined by direct measurement, and where $R = 195$ ohms (total resistance of water wedge); $L = 29$ mm. (total length of water wedge); and A_{I} and $A_2 = 13.5$ and 159 sq. mm. respectively (areas of ends of water wedge); then $K = 580$ ohms.

The form of the equation for calculating the resistance of the wedge when K is known is therefore $R = \frac{KL_2}{(A_{\mathrm{I}} + A_2)}$(2). Since in equation (2) R is the total resistance of the water wedge, and since any cross-section of this wedge is similar to the whole, for any such section of the wedge as shown in fig. 2 between the parallel planes a_{I} and a_2, we have $r = \frac{580 \times 2 \times l}{a_{\mathrm{I}} + a_2}$(3); where r = resistance of wedge section between any two planes with areas equal to a_{I} and a_2, and $l = 0.08$ mm., the diameter of the egg.

Position of photograph	$\frac{580 \times 2 \times \mathrm{I}}{a_{\mathrm{I}} + a_2}$	r (ohms)	P.D. across egg (volts)	Current density (microamperes per sq. mm.)
I	$\frac{580 \times 2 \times 0.08}{351.0}$	0.26	0.009	210
2	$\frac{580 \times 2 \times 0.08}{76.6}$	I.2I	0.036	970
3	$\frac{580 \times 2 \times 0.08}{109.4}$	0.84	0.25	680
4	$\frac{580 \times 2 \times 0.08}{111.3}$	0.4I	0.012	330

The values for the areas of the planes a_1 and a_2 for each one of the positions I, 2, 3, and 4 were determined by direct measurement from a plaster cast of the space below the glass wedge p in text fig. I*B*. Since the distance between a_1 and a_2 is very small, the areas of a_1 and a_2 at any one position may be considered equal.

Applying equation (3), we can now determine *r* for these sections, and, since the total current is known, the P.D. between their faces, that is, the actual fall of electrical potential through an egg at these positions. Table I gives the values for the positions from which the photographs were taken. It will be seen from the table that the threshold P.D. for orientation is 0.025 volts. This value is several times greater than the electrical potentials which are effective in producing the results described by the writer for *Obelia* (**6**).

The difference between these thresholds of P.D. may be due in part to the fact that the *Fucus* egg possesses a tough membrane, which may have a high resistance to the electric current. It will be clear, of course, that the density of the electric current in the egg may be entirely different from that in the sea water, depending upon the ratio of the specific resistance of the egg protoplasm to that of the sea water. The last column in table I gives the densities of the electric current in the sea water at the positions of the photographs. The limits of accuracy of the method were tested by making calculations based on different sets of measurements of the water wedge. These showed, for example, that the different values for the threshold P.D. did not differ by more than 0.002 volt. Consequently it is believed that the values given lie within an error of 15 per cent of the actual values.

Discussion

Our present knowledge concerning the phenomena of orientation of the axis of symmetry of cells to an electric current is too limited to warrant any final conclusions as to its actual significance for morphogenesis, but attention may be called to certain facts related to the problem. Many free living cells orient to the electric current by movement of the cell as a whole, but in these experiments we are confronted with a process involving orientation of cell division and growth (synthesis of protoplasm). A close similarity appears to exist between the results of Ingvar (2) on the oriented growth of nerve cells in tissue cultures placed in an electric field, and the growth processes described here. The main difference is that in the Fucus egg we have cell division superposed, so to speak, upon the growth process (synthesis of protoplasm) as such. The orientation of Amoeba to an electric current is also of interest, since in this case morphological polarity is a fluctuating property, and in all probability may be considered identical with fluctuating functional polarity. A series of striking cases of what appear to be transitional conditions between functional polarity and morphological polarity and their reversal, in a cell (Bursaria), has been described by the writer elsewhere (**7**). In these cases it is clear that a highly specialized and apparently fixed visible morphological polarity in a single cell may disappear by a process of dedifferentiation (autolysis?), part of the material of the cell reappearing with a different polarity from what it had before. The facts cited show a very close interrelation between definitely directed energy

transformations and the processes of orientation of the materials laid down as morphological structure (= colloidal crystallization?) in the process of cell morphogenesis, as stated in the first part of this paper. The experiments and suggestive speculations of Lillie (**5**) are to the point in this connection. If the physico chemical process in the cell, which determines morphological polarity, can be oriented by the application of a difference of electrical potential of external origin, does not this imply that the mechanism determining the morphological polarity possesses electrical properties?

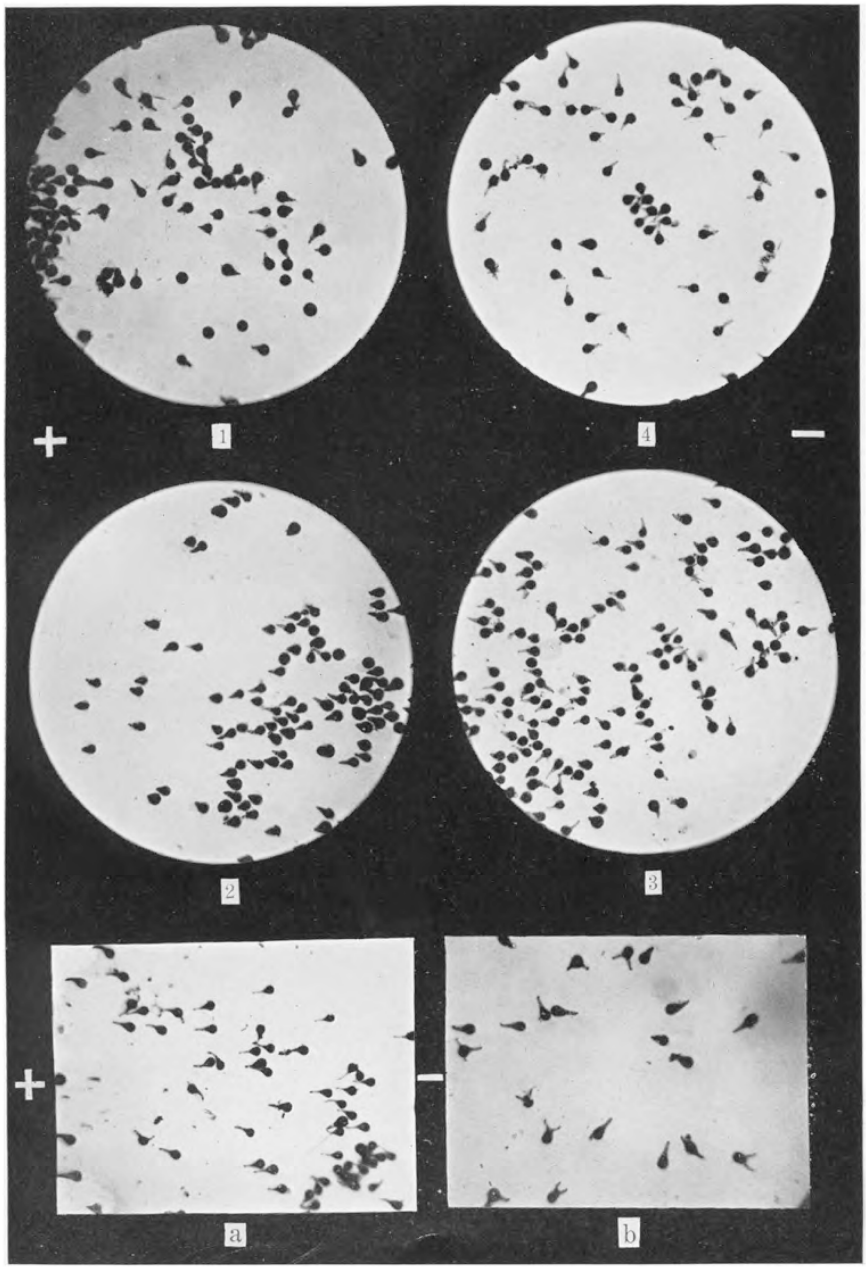
The orientation by light presents no necessary difficulty, of course, for it is a well known fact that bioelectric currents are associated with photochemical changes, for example, plants, retina of the eye. The occurrence of constant electrical potentials in tissues and organs have long been known, for example, in epithelia, roots and shoots of seedlings, Muller-Hettlingen (1**0**), hydroids, Mathews (**9**), Lund (**8**). These electrical potentials correspond to the polarity and symmetry properties of the tissues.

From these facts the following interesting question arises. May not such electrical differences of potential present in cells and tissues act upon adjacent cells and cell groups in such a way as to bring about orientation effects such as appear in phenomena of cell correlation, where cells in contact with one another behave in morphogenesis as an ordered whole? The writer believes that such an assumption may prove a valuable guide to the investigation of the nature of the phenomena of cell correlation. Experimental facts bearing upon this question will be presented elsewhere.

Summary

1. Polarity of the egg of *Fucus* can definitely be determined by applying a difference of electrical potential to the egg. Apparently this is the first egg, among either animals or plants, the polarity of which has been controlled by means of an electric current. The response of the *Fucus* egg to the electric current in total darkness is similar to its response to light.
2. A method is given by which it is possible to measure the P.D. across a single egg which is placed in an electric current flowing in a stream of sea water of constant cross-section.
3. The magnitude of the electrical potential applied to opposite sides of an egg, which is just able to cause orientation (threshold P.D.) of the axis of symmetry of the protoplasm of the *Fucus* egg, is 0.025 ± 0.002 volts.
4. This threshold P.D. is definite and does not appear to involve any marked inhibitory effect (delay) upon growth. A P.D. of 0.036 volts applied to the egg does show an inhibitory effect (delay) upon cleavage.
5. The establishment of an electrochemical polarity of some sort may possibly be a fundamentally associated condition for the development of morphological polarity, because the physiological mechanism in the *Fucus* egg which determines morphological polarity can be controlled and directed by the application to the egg of a difference of electrical potential of external origin.

University of Minnesota
Minneapolis, Minn.



LUND on FUCUS

LITERATURE CITED

1. HURD, ANNIE M., Effect of unilateral monochromatic light and group orientation on the polarity of germinating *Fucus spores*. BOT. GAZ. **70**:25–50. 1920.
2. INGVAR, SVEN, Reactions of cells to the electric current in tissue cultures. Proc. Soc. Exp. Biol, and Med. **17**:198–199. 1920.
3. JAEGER, F. M., Lectures on the principle of symmetry. Amsterdam. 1920.
4. KELLICOTT, W. E., Textbook of general embryology. 1913.
5. LILLIE, R. S., The formation of structures resembling organic growths by means of electrolytic local action in metals, and the general physiological significance and control of this type of action. Biol. Bull. **33**:135–186. 1917.
6. LUND, E. J., I. Effects of the electric current on regenerating internodes of *Obelia*. Jour. Exp. Zool. **34**:471–494. 1921.
7. ———, Reversibility of morphogenetic processes in *Bursaria*. Jour. Exp. Zool. **24**:1–33. 1917.
8. ———, II. The normal electrical polarity of *Obelia*. A proof of its existence. Jour. Exp. Zool. **36**:477–494. 1922.
9. MATHEWS, A. P., Electrical polarity in the hydroids. Amer. Jour. Physiol. **8**:294–299. 1903.
10. MULLER-HETTLINGEN, J., Über galvanische Erscheinungen an keimenden Samen. Pflüg. Arch. **31**:193–214. 1883.