

## BIO-ELECTRIC POTENTIAL GRADIENTS IN THE CHICK.\*

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### *The Problem*

Gradients of many kinds have been described in a variety of living organisms, including invertebrates as well as vertebrates. The extensive studies of Child and his co-workers have shown rather wide differences in physiological activity between apical and basal poles in certain invertebrates and between head and tail in longitudinally organized animals. The rates of O<sub>2</sub> consumption and CO<sub>2</sub> formation, as well as reaction to destructive agents, have been used as indices of these differences. Moreover, there seems to be good experimental evidence that these physiological gradients, whether they be axial, radiate, or surface-interior, in some way exert a controlling influence on differentiation. In numerous papers, Child<sup>8</sup> has suggested that bio-electric factors might be important in determining these physiological gradients.

Direct evidence for this hypothesis was presented by Mathews.<sup>14</sup> Studying electrical polarities in Hydroids by means of a capillary electrometer, Mathews showed that in the stem of *Parypha* with the cut surfaces placed on two zinc-zinc sulphate, clay electrodes moistened with  $\frac{5}{8}$  n. sodium chloride solution, the polyp surface was electro-negative to the stolon surface. The current was probably about 5 millivolts. Moreover, Mathews related regeneration to these bio-electric phenomena and in an illuminating sentence presented a far-reaching generalization. He said, "Every excess of action, every change in physical state of the protoplasm of any organ, or of any area in the embryo or in the egg produces, it is believed, an electrical disturbance." And again: "These currents probably play a larger part in the determination of rates of growth, in the orientation or polarization of the cells, and the differentiation of the organism, in its polarity, in other words, than has been supposed." (p. 297)

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However, older technics of measuring bio-electric phenomena have been far from satisfactory. The available instruments were untrustworthy, for they were unstable, drew current for their operation from the system being measured, or were insufficiently sensitive. It is only recently that the development of a new procedure by Burr, Lane and Nims<sup>4</sup> has made possible rigorous analysis of potential differences in living things. By means of the Burr-Lane microvoltmeter, it is possible to determine with reasonable certainty bio-electric potential differences in the living organism without upsetting significantly a very sensitive electro-chemical balance. This can be accomplished because the instrument draws approximately  $10^{-14}$  amperes—a current drain which, so far as is known, is insufficient to disturb the polarization of membranes. Moreover, since the technic is simple and easily controlled, it is possible not only to determine the presence of a gradient in any individual or group of individuals, but it is also possible to study the changes in the gradient with time. Thus, there is made available a new measuring stick for growth. If it is true that gradients rest on bio-electric phenomena and that they exert a controlling influence on differentiation, it should then follow that the establishment and the measurement of an electrical gradient during the development of the nervous system would give evidence of one of the determining factors in the differentiation of that system. Moreover, this should provide a clue to the mechanisms which underlie the determination of neural patterns. Support for this argument can be found in the numerous papers by Ariens Kappers (Cf. particularly, Ariens Kappers, Huber and Crosby<sup>1</sup>). His original and stimulating ideas give credence to the above hypothesis.

### *The Technic*

The first step in a series of studies directed toward a solution of this problem was to investigate the presence and development of potential gradients in the chick embryo. Eggs were incubated in an oven of the proper temperature for from 20 to 72 hours. The eggs were opened under warm physiological salt solution and the embryonic disc was carefully separated from the yolk. The disc was then transferred to a watch glass containing warm salt solution, and the

whole transferred to a stage of the microscope mounted in a micro-manipulator. By means of the latter, micropipettes filled with physiological salt solution, in continuity with the salt solution surrounding the silver-silver chloride electrodes, could be brought into contact with the surface of the embryo at any desired point. The points of election were at the cephalic end of the nervous system, usually between the two cerebral hemispheres in the midline, and at the root of the tail and again in the midline. Since the instrument draws no current, the determinations are independent of resistance and, therefore, of the distance between the electrodes.

As soon as the electrodes were in place, a direct determination of the voltage difference was made from the galvanometer deflection. Using a stage which could be raised and lowered, it was possible to withdraw the embryo from the two electrodes simultaneously. The embryo was then returned to its original position with the electrodes in contact. Usually five such determinations were made, whereupon the axis of the embryo was reversed and the readings repeated. This procedure was carried out over the entire group of eggs and gave strikingly reproducible measurements throughout the series. It was noted in the course of the movement of the embryo away from the electrodes that there was a gradual falling off of the potential difference and not an abrupt cessation. In the same way, when the embryo was brought up to the electrodes, voltage differences could be detected from  $\frac{1}{2}$  to  $\frac{1}{4}$  of a millimeter distant from the surface of the embryo and these could be seen to increase as the distance between embryo and electrode decreased, reaching a peak at the instant of contact.

### *The Results*

Four groups of fifteen embryos each were studied. The groups contained embryos of 0-10, 10-20, 20-30, and 30-40 somites, respectively. One hundred determinations of electrical potential were made upon each group.

The average values of the electrical potential readings of each of the groups of embryos are given in Table 1. The variability of the data is also indicated by the accompanying standard deviations and ranges of the distributions. Graphical representation of these

TABLE 1

MAGNITUDES OF CEPHALO-CAUDAL ELECTRICAL POTENTIAL (IN MICROVOLTS OF EACH OF EMBRYO GROUPS)

Group	Somites			
	0-10	10-20	20-30	30-40
Mean .....	9.0	105.0	403.0	996.0
S. D. Dist. ....	72.3	154.8	233.7	376.2
Range .....	390	780	1180	1890

data is made in Figure 1. Frequency distributions of electrical potentials for each of the groups are plotted in shaded histograms.

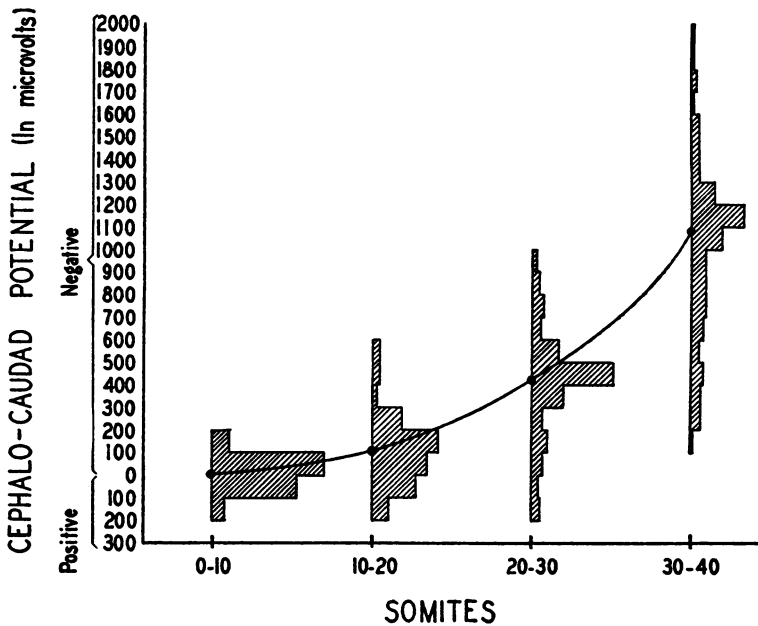


FIG. 1. Histogram showing the development of axial gradient potentials.

The size of the bar at each magnitude of potential is proportional to the number of cases of that potential. The solid line indicates the general trend of potential values by connecting the black dots which indicate the medians of the distribution. The increased size of the cephalo-caudad potential with increased age of the embryos is

apparent. The curve is positively accelerated. The same change in potential with increased age is reflected in the measures of variability. The standard deviations of the distributions and the ranges are seen to increase linearly at first, then more rapidly. This is in support of the results of Crozier and Hoagland<sup>9</sup> on other biological material where the same law, obtained from the means of the distributions, is found when the measures of variability are considered.

The reliability of the differences between the means of the various groups is indicated by the critical ratios  $\left(\frac{\text{Diff.}}{\text{P. E. diff.}}\right)$  presented in Table 2. Each of the groups differs significantly (critical ratio >4.00) from every other.

TABLE 2

RELIABILITY OF OBTAINED DIFFERENCES IN ELECTRICAL POTENTIAL BETWEEN VARIOUS GROUPS OF EMBRYOS

<i>Groups compared</i>	<i>Diff. (microvolts)</i>	<i>P. E. diff.</i>	<i>Critical Ratios Diff. P. E. diff.</i>	<i>Per cent equalling or exceeding median of comparison group</i>
0-10 with 10-20	96.0	11.49	8.35	29.4
10-20 with 20-30	298.0	18.85	15.81	12.0
20-30 with 30-40	593.0	29.87	19.85	10.7
0-10 with 20-30	394.0	16.11	24.42	7.7
10-20 with 30-40	891.0	27.44	32.21	0.1
0-10 with 30-40	987.0	25.86	38.21	0

The same positively accelerated function found for the means and for the standard deviations is obtained from the use of the critical ratios. The size of the critical ratios increases when successive groups of embryos are compared (0-10 compared with 10-20, 8.35; 10-20 compared with 20-30, 15.81; 20-30 compared with 30-40, 19.85). The consistency of the data is further demonstrated by the regularity of the increase. The critical ratios of the differences of groups differing by 20 to 30 somites can be approximated by summing the critical ratios of the constituent groups:—

Critical ratio of Group 0-10 compared with 10-20 = 8.35

Critical ratio of Group 10-20 compared with 20-30 = 15.81

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Computed sum = 24.16

0-10 compared with 20-30; Observed sum = 24.42

Critical ratio of Group 0-10 compared with 10-20 = 8.35

Critical ratio of Group 10-20 compared with 20-30 = 32.21

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Computed sum = 40.56

0-10 compared with 20-30; Observed sum = 38.21

Critical ratio of Group 0-10 compared with 20-30 = 24.42

Critical ratio of Group 20-30 compared with 30-40 = 19.85

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Computed sum = 44.27

0-10 compared with 30-40; Observed sum = 38.21

By plotting the magnitude of electrical potential obtained when the electrodes are at varying distances from the embryo an *approach gradient* can be obtained. Simple gradients are presented for embryos 10 and 29 somites respectively (Figs. 2 and 3). The increase in potential with approach to the embryo follows the formula  $\log E = \log ad^n + k$  obtained with other physical systems. A discrepancy between theoretical values and the obtained values is found at the point of actual contact with the embryo. It is possible that this is due to potentials resulting from actual contact. To check this possibility a bi-metallic compound of copper and solder generating a potential of about 400 microvolts was substituted for the embryo. The approach gradient, plotted from readings at points varying distances from the solder-copper compound, is presented in Figure 4. It will be seen that the form of the curve obtained is identical with that obtained from the embryos, except that with decreased distance, and finally contact, the equation remains unchanged.

### *The Argument*

It is clear from the data presented above that voltage differences between the extremities of the embryo can be measured with considerable accuracy. Similar studies, as yet unpublished, reveal corre-

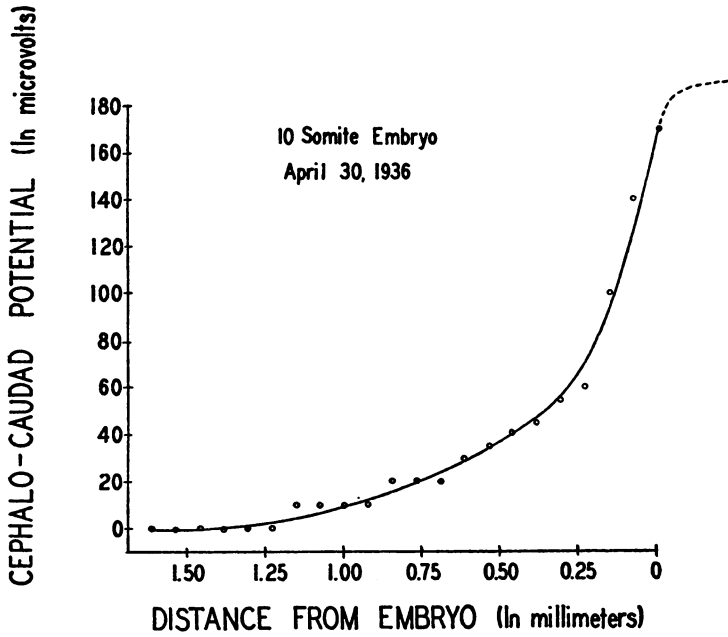


FIG. 2. Approach gradient in a 10-somite embryo. The dotted portion of the curve indicates that the curve becomes asymptotic.

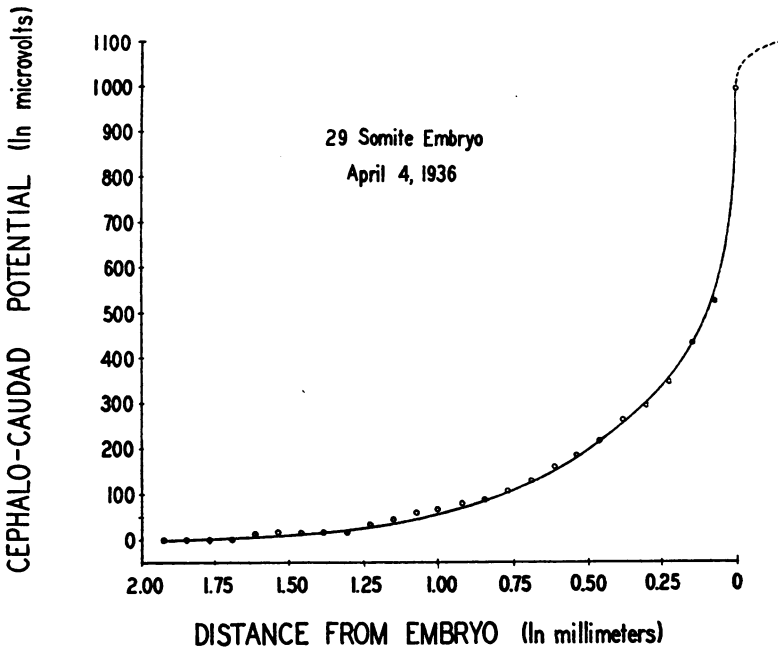


FIG. 3. Approach gradient for a 29-somite embryo.

sponding phenomena in salamanders and in mice. Moreover, not only do embryos show the presence of potential gradients but they also give evidence of a change in voltage difference as the embryo grows.

While we have evidence from other sources (Burr, Hill, and

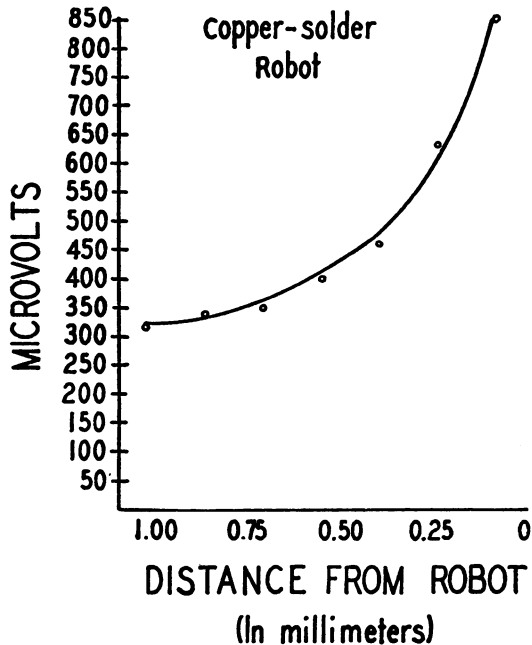


FIG. 4. Approach gradient for the copper-solder robot.

Allen;<sup>3</sup> Burr, Smith, and Strong;<sup>7</sup> Burr and Musselman<sup>5</sup>) that in all probability the activities of the entire organism are accompanied by these potential differences, the chick studies would seem to indicate that the potential gradients also are associated with the development and differentiation of the nervous system. It may be argued that during the latter part of the period studied, the vascular system is being developed and, therefore, might contribute to these voltage differences, for it is well known that movement of solutions is accom-



panied by potential differences in the wall of the restraining vessels. Furthermore, it was noted that the cessation of the heart beat was followed quite promptly by a marked lowering of the voltage differences. While all of this lends support to the idea, consistent potential differences are to be found long before the vascular system is laid down. Although this argues against the vascular system playing any large part in the phenomena, it is not impossible that the acceleration in the growth curve of the gradients may be associated with the development and differentiation of this additional organ system.

Two interesting characteristics of the plotted curve of the axial gradients should be noted. The plot, based on massed data, itself falls fairly exactly on a mathematical curve. This suggests that the phenomena follow physical and mathematical laws. Even more striking is the fact that it is possible to predict the voltage differences of any group of embryos from those obtained from the preceding groups. Moreover, statistical analysis shows that the likelihood of these measurements being due to chance is so remote as to fall into the realm of astronomical figures.

Additional evidence that the electrical phenomena here recorded are truly physical in their nature and not some obscure, ill-defined biological equivalent is to be seen from the records taken from the copper-solder robot. These observations, suggested by Dr. Leslie Nims, show that it is possible to devise a system made of two different metals whose interaction with each other and with the salt solution develops voltage differences which are strikingly analogous to those found in the embryo. The voltage differences between solder and copper and the character of the approach gradient as the electrodes are brought closer and closer to the robot, resemble those obtained from the embryo. Only one difference between the two systems could be noted. The last portion of the approach gradient in the robot follows somewhat more closely the mathematical curve than it does in the chick embryo. The gradient in the chick is somewhat steeper than in the robot up to the point of contact. Possibly surface electrical phenomena enter in, since the semi-permeable limiting membranes of the embryo are certainly different from either the surface of the copper or of the solder.

Probably the most fundamental questions raised by the observations here recorded are those which arise in connection with the

consideration of the origin and meaning of these potential differences. As to the origin of these potential relations little can be said. An examination of the fundamental theory of physical chemistry indicates that there is no physical meaning for the notion of an electrical potential for each atomic unit of solutions (Gibbs,<sup>10</sup> Guggenheim,<sup>11, 12</sup>). Rather, potential differences in solutions can only be measured when two electrodes are immersed in an electrolyte so that there is at least one other junction. This is simply another way of saying that present physical chemical theory forces us to consider potential differences in fluid systems as an irreducible relational factor not to be derived from the physical property of single atomic constituents. On the other hand, it may be argued that these potential differences and the fields, which by definition accompany them, are the mere by-products of ionic movement.

As indicated above, it is well known that fluids moving through restricted channels generate potential differences. Thus movements of ions within the living system might generate currents whose potential differences and fields have been measured in this study. That this is not true is clearly shown by the fact that the copper-solder robot developed an electrical field quite analogous to that found in the embryo, although there is certainly no ionic-movement within either the solder or the copper. On the contrary, the electronic activity within the metal generates an electrical field which moves the chlorine ions of the physiological salt solution in such a characteristic fashion that voltage differences appear at the silver-silver chloride electrodes.

The only alternative is to hold that the electrical properties are truly fundamental. In other words, it is to be concluded that the ionic movements are the result of the electrical relations and it is more than probable that the orientation of molecules, as suggested by the hypothesis of Harrison<sup>13</sup> is brought about by the same mechanism.

### *The Conclusion*

Finally, the data here presented, demonstrate clearly that in the developing chick there is a steady state bio-electric potential gradient with its accompanying bio-electric fields. These fields exist continuously in space throughout the whole organism and they persist

continuously in time, present before, continuing through, and remaining after such local energy transfer phenomena as are recorded in the alternating current readings of neural activity. In other words, these relatively steady state fields are as universally present as are the atomic chemical constituents of the organism. Thus, the conclusion that the electrodynamic fields herein described for the chick embryo provide the missing irreducible relational factor required to account for the organization of the physical chemical constituents of living organisms can hardly be avoided. This means that with this new technic it is possible to measure with certainty and accuracy at least one of the organizing agents of living things. Furthermore, it is quite clear that all of the energy intake of the organism is confined not to metabolic activities alone but rather that a measurable portion of it appears as electrical energy. Therefore, we have another bit of evidence which indicates that these electrical fields are not mere by-products of metabolic activity but in all probability are irreducible organizers of the pattern of living organisms. It is concluded, therefore, that the electrodynamic theory of living systems, first postulated by Burr in 1932 and elaborated by Burr and Northrop in 1935 into a consistent whole, has been found to hold true for, at least, the early development of the chick embryo.

#### *Summary*

1. Steady state bio-electric potentials have been measured between the head and the tail in chick embryos during the first 72 hours of incubation.
2. These potential differences increase steadily during development.
3. They can be determined at a distance of from  $\frac{1}{4}$  to  $\frac{1}{2}$  millimeter outside of an embryo.
4. They seem to be quite similar to those which may be obtained from a suitably designed copper-solder robot.
5. The gradients are an expression of one part of the energy intake of the organism and the fields which they produce are believed to be an irreducible relational factor which has an important function in the determination of the pattern of the organization of living things.

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