ELECTROMETRICS OF ATYPICAL GROWTH*

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Much has been written and much said about the cancer problem. Large sums of money, large for biological research, but small for investigations in the physical sciences, have been expended in the search for the "cause" of cancer and for its cure. While a considerable number of more or less isolated facts have been discovered, the answer is not yet. Instead of a single factor which gives rise to the atypical growth of malignancy, there seem to be many agents which are possible instigators—constitutional characters (possibly inherited), chemical agents, trauma and mutations, perhaps brought about through endocrine imbalance. The very multiplicity of initiators implies that the answer must be found in some more fundamental property of living systems. It is clear enough that malignancy is a distortion of the characteristic design of the organism. For some reason as yet undiscovered, cells which still retain their capacity for continued multiplication escape from an organismic control. Instead of obeying some set of inherent regulating forces, such dividing units participate in a new growth which, while having definable characteristics, nevertheless departs from the teleological character of normal development. Such considerations make it abundantly clear that the cancer problem is but a special case of the more general central problem of biology, the origin of design or pattern in living systems. The forces inherent in the organism which control morphogenesis, and hence the functions of the whole and its constituent parts, are still unknown. For Aristotle, the regulation was accomplished through the activity, among others, of a "formal cause." Hans Driesch, in modern times, postulated the presence in organisms of a force not amenable to examination by the current techniques of science, which guided and controlled morphogenesis and the continued functioning of the adult. This Entelechy, as he called it, was to be thought of as a supernatural agency outside the present horizons of science. Thus, Driesch aligned himself with the Vitalists who believed in the soul as the directing power. Biologists crucified Driesch for his stand, refusing to admit that there were factors in the living system which were not amenable to rigid description and measurement. Nevertheless, the results of the operation of some sort of powerful, precise forces defining normal and experimentally modified development have rarely been more compellingly described than by this great experimental biologist.

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More recently, Child has invoked physiological or axial gradients as an explanation of the nature of the forces which control the unfolding of structure. That such forces exist is unquestionable. His studies have amply demonstrated the oxygen gradient and its corresponding sensitivity to inimical environmental agents. Just how such a decline in oxygen consumption along the axis of an organism could control rates of cell division and the subsequent differentiation of cells *in situ,* to say nothing of the migration of cells, is not clear.

This is the same sort of problem which is found in genetics. No one questions the existence of genes, or that they can be correlated with morphological characters of the adult, but the forces by which genes impose the genesis of form are no less obscure.

It is clear from the above, and from innumerable other studies, that some sort of control of morphogenesis is present in all living beings. No less obvious is the probability that in atypical growth certain cells, retaining the fundamental capacity for division, escape from normal controls and set up what is in many ways a new organism parasitic on the host. Such release from regulation is clearly not just a local phenomenon, for something happens to the whole organism which is revealed in a number of constitutional changes. Moreover, at some period in the development of the new growth, the basic property of protoplasmic movement exhibits itself and cells wander to new environments. This has been likened to population movements by the late Raymond Dodge, who suggested that cells, like humans, set off on journeys to new pastures seeking more favorable environments.

Such considerations as the above make it clear that not only must consideration be given to the local chemical changes which may be found to accompany atypical growth, but also to the forces which in turn regulate the constant chemical flux. The organism as a whole depends on energy transformations for its continued existence and so does the atypical growth. Energy, however, is a scalar property and is itself indifferent to the direction in which it flows, except that the second law of thermodynamics requires that the flow be such as to increase the entropy of the system. The chemistry of the energy transfers is an absolute requirement for any understanding of the cancer problem, but it is equally important that consideration be given to the fact that in the growth and development of the organism there are forces, in addition to the scalar characteristics of chemical changes, which give direction to the energy flow. It cannot be denied that morphogenesis is directed. This is true of the whole organism and also of its constituent parts. Moreover, the very "directedness" of development implies a necessary "relatedness" of the units of which the system is composed, a relatedness which imparts to the organism that property which makes the whole greater than the sum of its parts.

Considerations, such as those briefly sketched above led, in 1938, to an examination of the electrical properties of malignancies in mice. The experiment was designed to determine whether or not the polarity vectors were altered in atypical growth. For a period of years, a considerable number of mice from the colonies of Dr. L. C. Strong were studied weekly. In all, there were four groups of mice:

A. normal controls; B. a strain of mice known to have a high incidence of mammary cancer, occurring spontaneously; C. a strain of mice prone to atypical growth following the administration of carcinogens; and D. a number of mice from a strain which readily accepted implanted malignant tissue.

From four to eight measurements were made each day and, subsequently, both the magnitude of the potential difference and the polarity were subjected to a statistical analysis. Quite early it became clear that any set of measurements, on any particular day, showed little or no correlation with what had been done to the animal. However, comparison of the groups made it clear that, statistically, the control group showed a different electrical pattern from that found in the animals with spontaneous breast cancer, malignancy produced by carcinogens, or the atypical growth of transplants. There were also statistically significant differences between the spontaneous carcinogenic and the transplanted growth.[9]

Under the conditions of the experiment, individual day-to-day variations in both magnitude and potential distribution were such as to obscure any really significant findings. The results provided an interesting clue but no practical concrete consequences. It was felt, however, that there was some item here of probable significance which needed much more intensive study. Revival of the experiment in the last few years has revealed that the day-to-day care of the mice has to be maintained within rather narrow limits in order to reduce the variability factor. Food, water, temperature, and draughts require careful control. If this is done, the individual variability can be reduced significantly.

A less direct frontal attack on the malignancy problem was undertaken, and aimed at a study of the possible relationships between the vector properties of the electrical phenomena and morphogenesis in a number of forms. During the summer of 1938, through the generosity of F. S. Hammett and the Lankenau Laboratory at Truro, Cape Cod, a study was made of the electrical phenomena associated with the life cycle of Obelia.[13] Here was noted the same kind of phenomena that could be recorded in amphibia[6, 17] and in the chick embryo,[5] namely, that during development there is a steady increase in potential difference along the long axis of the organism until the time when morphogenesis as such, is virtually complete. In Obelia, and it was subsequently confirmed in Amblystoma, during the phases of cell division, the changing potential was an uncomplicated, relatively steady rise in potential difference. However, with the onset of differentiation, the steady climb in potential was replaced by fairly irregular but slow oscillations in potential difference. Subsequently, in a study of the growth of corn roots, in which moving pictures of corn root growth were made with a simultaneous electrometric recording, the same phenomena were observed of slow but steady increase during cell division, followed by oscillations during differentiation.[19, 21, 23] It was clear from these experiments that the electrical phenomena associated with growth and differentiation were of such a nature as to fulfill the requirement for a set of forces capable of giving direction to morphogenesis. All the studies referred to above are in accord with the original requirements set down by Burr in 1932,[1] and Northrop and Burr in 1935,[2] that an adequate survey of the electrical phenomena of living systems would provide a set of measurements which would, in part, define an electrodynamic field. This field was conceived of as a set of forces in a continuum capable of giving control and direction to all of the unitary components which come within its sphere of activity.

Further evidence of a significant correlate of the importance of relatively steady state standing potentials in morphogenesis was obtained by two quite different kinds of experiments. In the first of these, the electrical patterns of the developing frog's egg were examined. It was found that in the unfertilized egg and in the fertile egg, following through formation of the morula and the gastrula, there could be observed an electrical axis unrelated to any obvious externally observable differences until the appearance of the longitudinal axis of the embryo. The two axes were coincidental. This clearly demonstrates the existence of a measurable constant through development which was related to morphogenesis. It can be argued, therefore, that these potential differences and the pattern in which they are found, provide the direction necessary for a continuous control of the developmental process.[16]

With the coöperation of E. W. Sinnott, similar studies were carried out on the embryos of cucurbits. Four types of gourds were studied in the embryo stage, each of them having its own electrical pattern which, during development, could be correlated with the changing relative growth rates in round, flat, straight, and hook necked gourds. It is well known that the cellular components of these gourds are essentially alike; but in the course of development they are related to each other in different ways. As a consequence of changes in relatedness, the four different gross forms appeared.[22]

One final bit of evidence, that there is a significant correlation between electrical patterns and fundamentally biological properties, appeared as a result of a study of relatively steady state standing potentials in the corn seed,[21, 28] where it was found that the magnitude of the potential difference measured along the long axis of the kernel was a good prediction of relative growth rates in the field and in the genetic constitution of the embryo. High potential seeds grew faster and taller and yielded more than low potential seeds. Thus, there is a possible relationship between available electrical energy and heterosis. Among the strains studied was a single gene mutant. Kernels from this stock had a significantly smaller potential difference than either of the parents and in the field produced dwarf plants. Since it is well known that genes are significant factors in morphogenesis, another bit of evidence is at hand that potential differences and morphogenesis are related.

If the evidence, briefly reviewed above, is a true picture, a further investigation of the electrometric correlates of atypical growth seems to be in order. It can be argued, therefore, that in atypical growth, with disturbances in the control of normal morphogenesis, an alteration in the electrical pattern, as foreshadowed in the early studies on mice, should be investigated.

Through the very fortunate and enthusiastic collaboration of Dr. Louis Langman, and supported in part by a grant from the United States Public Health Service, a study of the electrometric correlates of malignancy in the generative tract of women was undertaken.[20, 24, 25] In all, more than 900 patients were studied from the Gynecological Service of the Bellevue Hospital. The patients exhibited many varieties of pathological conditions in the generative tract, including malignancy. Eight hundred and sixty of these patients were subjected to statistical analysis. By the clinical evidence, 737 of these were free from malignancy. One hundred and twenty-three, on the other hand, were judged on the basis of complete clinical examination, including biopsies, to be suffering from malignant growth. The voltage gradients, as measured between the posterior fornix and the ventral abdominal wall, showed that the former was positive to the latter in 611 cases, and negative in 126. Of the 123 in the group with malignancy, five patients exhibited positive potential difference in the posterior fornix and 118 a negative one. This gives a chi-square value of 318.5, with 3.841 being the significant number. When this is broken down into age groups, from 21 to 40 and from 40 to 61, the chi-square fact in the younger group was 131.2, and in the older group 80.6. The presence of 56 fornix negative readings in the 509 patients in the younger group can be explained by the fact that all of these patients were still ovulating. It has already been shown in a number of studies that during ovulation the electrical pattern in intact living organisms, from rabbit to man, changes markedly.[3, 4, 7, 8, 10, 13, 15, 18] Furthermore, the existence of five fornix positive readings in the group with malignancy still lacks explanation.

Finally, in the older age group, 53 out of the 198 patients without malignancy gave fornix negative readings. As the study progressed, the incidence of negativity seemed to increase in the period after the menopause. Nevertheless, it is abundantly clear that the great majority of the patients exhibiting fornix negative readings, which could not be accounted for on other clinical grounds, had malignant growths. Conversely, in only five out of 611 instances was the fornix found to be positive in the presence of demonstrated malignancy.

Attention should be called to the fact that this study was carried out in a large hospital service and it is more than likely that although this group was large, it is not a fair sample of the population. The evidence seems clear enough to warrant large-scale investigation of the findings.

All the evidence so far collected make it necessary to reëxamine, under more controlled laboratory conditions, the electrical correlates of malignancy in mice. Again, through the generous coöperation of Dr. H. S. N. Greene, some three hundred mice were intensively studied following implantation of the breast region in the C_3H strain of mice. The implantations made by Dr. Greene in the right axilla were from tumor tissue labelled 4,5,7, and 8 B-PX, and MT8. In addition, foetal and visceral implants were made from normal animals. The technique of measurement which finally evolved was as follows:

The unanesthetized mice were tied down on their backs on a small animal board and the silver-silver chloride electrodes were placed: (i) the reference electrode in the mouth of the animal, and the exploring electrode, in a drop of physiological salt solution, in the right breast region, and then in the left breast region. Since the left side of the animal was not involved in the implantation, it served to some extent as a control for the right side. The potential differences between the two electrodes were determined by a vacuum tube microvoltmeter with an input impedance of 10 megohms. Since the meter drew less than 10^{-10} part of an ampere, there was an insignificant current drain from the system under measure. As a result, the readings were astonishingly reproducible. Daily determinations were made upon these animals, beginning the day after implantation and continuing for approximately two weeks, or until the implant had achieved something like maximal growth. Normal controls were run to provide a baseline. Since some of these tumors grew rapidly, and others much more slowly, four groups of animals can be reported:

A. The control group
B. The rapidly growing group
C. The slowly growing group
D. The foetally implanted group

TABLE 1

POTENTIAL DIFFERENCES IN MILLIVOLTS BETWEEN RIGHT AND LEFT AXILLA ON SUCCESSIVE DAYS AFTER IMPLANTATION IN RIGHT AXILLA. SIGN INDICATES POLARITY OF RIGHT AXILLA TO LEFT AXILLA.

	Potential Differences in Days														
Growth	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
None	—1.0	—0.8	—1.0	—0.2	—0.2	—1.0	—1.8	—0.3	—1.0	—1.5	—1.0	—2.0	—1.7	—1.0	—2.0
Foetal	—0.2	—0.1	—1.0	—0.4	—0.5	—2.0	+2.0	—1.0	+1.0	+0.5	+1.0	+1.0	+0.8	+0.2	+1.0
Slow	+0.2	+0.2	—2.2	0	—1.3	+4.4	—1.1	—1.2	—1.8	—2.4	—1.0	—0.6	+0.3		
Rapid	—1.0	—1.0	—3.0	—1.5	—1.6	—3.0	—2.0	—3.0	—3.0	—4.4	—3.0	—2.7	—3.0		

In all, 118 animals were studied. The results are graphically represented in Figure 1. It will be noted that if the potential difference measured on the operated side is contrasted with that on the unoperated side, a significant difference appears early, within 24 to 48 hours after implantation in the rapidly growing tumor groups. This difference increased steadily and quite smoothly to reach a maximum of approximately five millivolts on or about the eleventh day, following which it decreased. In the foetal implant group the difference began to appear a little later and reached a peak on approximately the sixth day, following which the potential difference dropped to *zero* and reversed its polarity until the end of the experiment. In the implantations of the slowly growing tumors, potential difference began to emerge on the third or fourth day but reached its maximum of approximately three millivolts on the tenth or eleventh day. From there, until the end of the experiment, the difference in potential fell steadily to *zero*. The control and those without growth showed a variable difference between the two sides of less than a millivolt for the entire experiment. For purposes of clarity, the plotted difference is slightly exaggerated. In all of these measurements the axilla containing the implanted foreign material was negative to the opposite axillary region. It is clear from these findings that the presence of atypical growth in a host organism produces measurable and reproducible electrometric correlates. The rapidly growing tumors developed higher potentials more quickly than the slow growing implants. The foetal tissue started off rather promptly but early reached an electrometric peak and thereafter declined to *zero,* subsequently to appear as a polar reversal which in turn, returned to *zero*. The slow growing implants started late but exhibited an electrometric curve paralleling the essential slope of the rapidly growing tumors, but reached their lower maximum at approximately the same time as the rapidly growing tumors. There is a certain similarity in these plots of potential differences to those found in wound healing.[11, 14] Curiously enough, the time factors are very similar. It is not immediately apparent whether this sequence has any significance. The decline of the potential difference in all three experimental groups is interesting, for it seems to be quite closely correlated with the beginning of necrosis in the atypical growth and in the disappearance of foetal tissue.

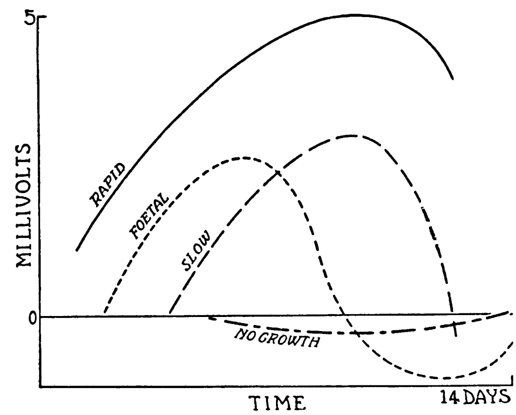

FIG. 1. Smoothed curves drawn from data in Table 1.

Since these experiments were in the nature of fairly acute observations, the presence or absence of any constitutional factor associated with atypical growth was not observed. The success of this last group of experiments was undoubtedly due to the fact that one electrode was placed in the mouth. Thus there was in the mouse experiments an analagous situation to that found in the human patients. In a certain sense it can be argued that in both instances one electrode was in the body cavity and the other on the body surface. As a result, the potential differences were of considerably greater magnitude than when only surface measurements were made. This greater difference made the electrometric correlates considerably more obvious and raised them outside the probable error of determination. Furthermore, it is interesting to note that the atypical growth in both the human cases and in the mice was electrometrically negative to uninvolved tissue.

It would appear from all the evidence seen and reported here that electrometric techniques might be of some value in the early diagnosis of malignancy. However, it is still highly questionable whether the patients used were an adequate sample of the total population. Those drawn from the hospital services were obviously a specialized group. Certainly the procedure is promising, but obviously studies on a much larger scale could well be instituted with a wider selection from the population. Whether or not the procedure would stand up in a general clinical practice can only be determined by further study.

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