"VEGETABLE DYNAMICKS" AND PLANT TISSUE CULTURES[1]

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SOME time ago, at a dinner at the Yale Medical School, I was asked to occupy the chair specially built for the Honorable William Howard Taft when he was professor of jurisprudence at Yale—and was made to feel very, very small thereby. Now you have done me the honor to link my name, however tenuously, with that of the father of our discipline, Stephen Hales. It is hard to know whether to be inflated by the connection or deflated by the comparison. Whatever should be my reaction, I deeply appreciate the honor. In accepting that honor, I shall try not to impose upon your patience after a good meal by talking to you too long.

Stephen Hales's interests, as a minister of the gospel and a humanitarian, led him to touch upon anything which could contribute to the welfare of his fellow men and redound to the glory of Almighty God. The processes by which God's creatures function in their everyday lives could, according to his lights, only show the perfection of the works of the Great Planner. Thus, Hales set to work to discover what details he could in that plan, in a spirit of humility. And, since plants were also God's creatures and Hales was a good gardener as well as a good shepherd, he turned much of his interest to a study of what he chose to call "Vegetable Staticks."

In Hales's day, the classification of plant parts into tissues, proposed by the Greeks, and especially by Theophrastus, had been for the most part forgotten, and his considerations were wherever possible of the plant as a whole. But the philosophical analyses of Theophrastus and the anatomical work of Grew and Malpighi, Hooke and Loewenhoeck had already laid the foundations for another point of view. As our knowledge of the living functions increased, so did the complexity of the plan revealed increase, until it became an almost hopeless task to unravel the details while trying to encompass the whole picture, even as regards a single creature. Thus it happened that, about a century after Hales's epoch-making work, there came to be formulated the so-called cell theory which said, in essence, "It is not necessary to encompass the plan of a plant or animal in its entirety and all at once, for each creature is made up of a sum of many smaller, simpler, potentially autonomous organisms, the cells. Learn to understand the plan of a single cell. From that the greater Plan can be built up." That theory, while sharing the field with the older concept of the organism as a whole, has nevertheless dominated much of our thoughts as cytologists, as anatomists, as geneticists and as physiologists for the past century.

Hales developed a series of techniques for studying the organism as a whole which are still useful. But, as we have passed from the larger aspects to smaller and still smaller ones, the technical difficulties have increased. To deal with single cells, we must have methods adapted to that end. Haberlandt saw that clearly forty years ago and set in train the series of explorations of which I am but one of the momentary advance guard.

We can not yet grow single somatic cells of higher plants, as Haberlandt would have had us do. Some day I am certain some one will be able to do so. But we can grow comparatively simple organs and tissue complexes. A few of you, I know, have followed the steps by which that has been accomplished. Others I am sure have not, and I hope I will be forgiven if I review briefly for their benefit the development of the field. The aim was, as we have said, the study of single somatic cells of higher plants. The method aimed at was the cultivation of those cells, their study as separate entities, and their subsequent aggregation into more and more complex groupings until full-blown organisms could again be attained. Haberlandt tried to grow single green somatic cells, unsuccessfully, as did many of his students. Kotte and Robbins in 1922 compromised by using, not cells, but organs—root tips—with some success. But the results were not sufficiently promising to encourage further work and the attempt was not pushed further. A decade later the problem came to my attention and I decided, on theoretical grounds, to study three types of materials—embryos, seed primordia and root tips. Of these, the last proved most satisfactory. With them a beginning has been made toward a solution of the problems involved.

Fundamental, of course, was the need of an environment that should replace all the essential features of that in which the cell or tissue or organ finds itself in the organism while discarding the non-essentials—as regards physical and chemical composition of that environment. Temperature, light, oxygen tension, acidity, physical state, motion, conditions of contact, as well as ionic composition, content in energy sources, hormonal and vitamin content and similar aspects all

[1] Abridged from the Stephen Hales Prize Address, given before the American Society of Plant Physiologists, Dallas, Texas, December 29, 1941.

had to be studied. And, while many of these requirements were likely to prove similar for all cells, others might not prove similar. We have solved all these problems in a preliminary way. But those solutions are in many cases obviously not yet the most perfect ones possible, and the work still goes on. We are on fairly firm ground as regards the physical conditions, the inorganic nutrition and the carbohydrate supply. Requirements as regards organic nitrogen supply, vitamins and hormones are less clearly established, although conditions which are "satisfactory" are known.

We are, however, now in a position to grow a variety of plant materials, root tips of many species, embryos, undifferentiated tissue masses from *Salix, Nicotiana, Helianthus, Daucus, Ulmus,* and a few other sorts, *in vitro,* for long periods and to control the environments in which they grow with a considerable degree of precision. The technique for which Haberlandt was searching is thus now available and it is, I trust, because of the part I have played in developing that technique, rather than for any particular application, that you have honored me this evening.

It would be superfluous for me to review our findings in detail here. It is my task, rather, to point out the directions in which we have already moved and perhaps to outline briefly some of the new fields in which we may hope to find opportunities for further work.

As I have already noted, problems in nutrition were among the first to present themselves and the way in which they have been solved points to other possibilities in the same direction. How significant is the calcium-magnesium balance in the economy of an isolated tissue? Less so, I think, than we have long been led to believe. What is the value of iron for cells which possess no chlorophyll? Our results show an unusually clean-cut picture of its importance. Iron is not tolerated at an ionic concentration above about 2×10^{-5} ($= 2/100{,}000$) M; yet its omission from the nutrient results in an abrupt and complete, but reversible, cessation of growth. That its function is an indispensable one is evident. What that function is we do not yet know, but the tissue culture technique offers us a hope of finding out. How far is the effectiveness of sucrose in the nutrient to be attributed to the energy it supplies, and how far to its function in maintaining an isotonic state? Our studies emphasize its energy-giving function and show that the isosmotic function is relatively unimportant. How does iodine affect the morphogenetic pattern? How does oxygen tension affect this same pattern? And how does sucrose, a classic plasmolytic agent, penetrate to the interior of the cell? When I published experiments showing that excised tomato roots preferred sucrose to dextrose, I let myself in for some criticism from those who followed the text-book story. But I also received a very heartening and interesting letter from Dr. Annie May Hurd-Karrer, saying that in 1926 she had been puzzled by evidence of a similar preference by *Ustilago* infecting corn. Perhaps the question needs to be re-examined. The text-books have sometimes been wrong. How important is auxin in the economy of an excised member? What is the function of thiamin in a growing tissue? What is the relation between pyridoxine and protein synthesis in the plant? These are but a few characteristic nutritional problems that have been or can be attacked by means of a tissue culture technique.

But the value of the method is by no means limited to nutritional problems. We were from the start interested in using it in dealing with a variety of problems in plant pathology. Some of these have led us into unexpected paths. The method by which viruses travel through the host has long been a puzzle to phytopathologists. This is paralleled and perhaps intimately bound up with the methods by which foods move in the plant. And it is also conceivably bound up with the methods by which water may sometimes be moved through the plant when certain external forces are temporarily in abeyance. The picture of water movement as a function of vital activity of plant tissues has long been a puzzling one. Five years ago, I set out to study this picture as exemplified in growing excised roots, where the complicating factors of photosynthesis, evaporation, trunk expansion and contraction under temperature influences, local hydrolysis, etc., could be eliminated. We have not solved the problem as far as demonstrating a detailed mechanism is concerned. But we have been able to establish certain significant facts in an unexpected and striking way, facts which tend to support and enlarge upon Stephen Hales's old concept of water secretion as a vital function of root tissues, bound up with respiratory processes. Those results at the time seemed again to run counter to accepted text-book theories. But they have since found support in work of Rosene, Grossenbacher, Mason and Phyllis, and others. And the picture which Mason and Phyllis draw certainly corresponds rather closely to that of the contractile vacuole in protozoa, which is another little-understood phenomenon.

Recently we have come back to one of the first problems for the solution of which the value of a tissue culture technique was foreseen, that of the etiology of neoplasia. This we have attacked in plants, using tissues affected at a previous time by crown-gall organisms. The method permits us to maintain growing tumor tissues under continuous and detailed observation, to establish by appropriate tests their freedom from contamination, to subject them to controlled environments, and then, at suitable intervals, to return them to appropriate hosts, where they give rise to new tumors. We have already followed by similar means the processes of growth and differentiation in a neoplasm of genetic origin—that arising in the hybrid cross between *Nicotiana glutinosa* and *N. langsdorffii*. The step from there to neoplasia of biologic (parasitic) origin has not proved a difficult one. May we not hope later to proceed a step further, to neoplasia of recognizable and controllable physiological origin?

These are but a few examples to indicate the proven as well as potential scope of the field which the technique of tissue cultures opens to us. Stephen Hales was not thinking in terms of this sort. But these problems are such that their solution, by whatever means, can give us greater insight into the workings of biological entities, cells, tissues, organs, and thereby of the organisms which were Hales's interest. They may well help us to understand some new bits of the universal plan which he sought to elucidate.

OBITUARY

JAMES J. WALSH

DR. JAMES J. WALSH, of New York, died on March 1. Son of Martin J. and Bridget Golden Walsh, he was born at Archbald, Pennsylvania, on April 12, 1865, so that he was in his seventy-seventh year. He obtained the degree of bachelor of arts from Fordham College in 1884, and that of master of arts in 1885, and then entered the Society of Jesus, intending to pursue a career in the Church, but a few years later was released of his vows, when he felt that he was not fitted for the priesthood. The training with the Jesuits made a lasting impression upon him and gave him skill in dialectics. In 1889, Walsh received the degree of doctor of philosophy from Fordham College.

He began the study of medicine at the University of Pennsylvania and required only two years to complete his course, graduating M.D. there in 1895, with his younger brother, Joseph, whose studies of Galen have delighted the readers of the *Annals of Medical History*. During the next three years he studied in Paris, Vienna and Berlin, where he and his brother had a place in Virchow's laboratory. Whilst in Europe, Dr. Walsh began his career as a medical writer, acting as correspondent for several American journals, and when he returned in 1898, he became assistant editor of the *Medical News*. Later he wrote much for the *New York Medical Journal* and the *Journal of the American Medical Association;* he was the medical and scientific editor of the *Independent* and the medical editor of the New York *Herald*.

In 1900 he was appointed an instructor in medicine and an adjunct professor in 1904 at the New York Polyclinic Medical School, where he taught until 1907, when he was made acting dean and professor of neurology at the Medical School of Fordham University. Here he remained until he resigned in 1913. At Fordham he gave regular lectures on the history of medicine, which were amongst the early ones to be established in the United States. He also lectured on physiological psychology, of which subject he was professor at Cathedral College, New York (1907–1938).

Dr. Walsh was the author of many books, and he established the Fordham University Press. Some of his more important works were "Catholic Churchmen in Science," Philadelphia, 1906; "Makers of Modern Medicine," New York, 1907, which was dedicated to his friend, William Osler; "The Thirteenth, the Greatest of Centuries," New York, 1907; "The Popes and Science," New York, 1908; "History of Medicine in New York," 5 volumes, 1919. Dr. Walsh was always a loyal son of the Roman Catholic Church. His paper, "The Popes and the History of Anatomy," appeared in the *Medical Library and Historical Journal,* Vol. 2, 1904, and that on "The Supposed Warfare between Medical Science and Theology," in the *Messenger,* New York, July, 1906. Dr. Walsh was an authority upon the history of the Roman Catholic Church. He was made a Knight Commander of the Papal Order of St. Gregory and also a Knight of Malta and received many honorary degrees. He belonged to numerous societies, was a life member of the New York Historical Society, a fellow of the New York Academy of Medicine and a member of the American Medical Association.

In 1915 Dr. Walsh married Miss Julia Huelat, who, with a son and daughter, survives him.

ARCHIBALD MALLOCH
NEW YORK ACADEMY OF MEDICINE

RECENT DEATHS

DR. RAYMOND LEE DITMARS, curator of reptiles and of mammals of the New York Zoological Park, died on May 12 in his sixty-sixth year.

BRONISLAW K. MALINOWSKI, Bishop Museum visiting professor at Yale University, who had been appointed professor of cultural anthropology at the university, effective on July 1, died on May 16. He was fifty-eight years old.