EXPERIMENTS ON THE CULTURE OF ISOLATED PLANT CELLS

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(With 1 Plate)

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I.

To my knowledge[7,8], no systematically organized attempts to culture isolated vegetative cells from higher plants in simple nutrient solutions have been made. Yet the results of such culture experiments should give some interesting insight to the properties and potentialities which the cell as an elementary organism[9] [Elementarorganismus] possesses. Moreover, it would provide information about the inter-relationships and complementary influences to which cells within a multicellular whole organism are exposed.

As early as 1898, I had performed a number of such culture experiments with artificially isolated plant cells. Originally, it was my intention to pursue these experiments on a large scale, testing the behavior of cells from different kinds of tissues in different kinds of solutions. Other work prevented this plan from being carried out. Since my sensory

[7]Our footnotes will appear in brackets to distinguish them from Haberlandt's own footnotes.

[8]It seems quite clear that Haberlandt was in fact the first. Harrison (1928, p. 7) described Haberlandt's priority in the following way: "About the time that Haberlandt's first paper appeared Loeb published his experiments with pieces of epithelium from the Guinea pig, imbedded in small blocks of clotted blood or agar which were placed for incubation in the body of another animal . . . The technique was entirely different from Haberlandt's and had nothing in common with that of modern tissue culture, although the underlying purpose of the experiment was essentially the same. Loeb in an earlier paper . . . mentions having made experiments in which the agar blocks containing pieces of living tissue were incubated outside the organism. The results were not stated."

[9]Haberlandt in his *Physiological Plant Anatomy* (1914) emphasized that cells were a unit not only in the morphological sense; e.g., "If the term 'organ' be employed in general to denote the instrument wherewith a definite physiological function is performed, then the cell must be regarded as an elementary physiological instrument or 'elementary organ'! Every cell, namely, performs a definite physiological service for the whole term of its life or at any rate at some period of its existence while the sum total of the physiological functions of the various cells constitutes the vital activity of the entire plant . . . (p. 15). The majority of cells represent not only elementary organs, but also elementary organisms, in other words, a cell, as a rule, does not merely work in the service of a higher living entity, namely the entire plant, but also behaves as a living entity, though indeed as one of a lower order or magnitude (p. 16)."

physiological[10] [sinnesphysiologisches] investigations will probably preoccupy me for some time yet, I should like to briefly communicate, if I may be permitted, the results of my earlier experiments in the following pages.

Of course, only plant parts with cells loosely organized in tissues, so that they were easy to isolate by mechanical means, could be used for these experiments. Since I wanted to try culturing green assimilation[11] cells, it was a question at the outset of finding the appropriately constructed leaf. In this connection, leaves subtending the bracts of *Lamium purpureum*, which possess typical palisade and mesophyll tissue, proved to be most suitable. Small leaf fragments on a slide were teased apart in a few drops of solution with two needles until examination under the low-power objective showed the presence of numerous isolated palisade and mesophyll parenchyma cells. The cultures were initially maintained in hanging-drop slides and later in small glass dishes with covers. The dishes contained approximately 10 cm^3 of solution. Finely drawn-out glass pipettes were used to transfer the cells into the solution. Pipettes of the same sort were also used from time to time to "fish" out [herauszufischen] individual cells from the dishes. These were then transferred to a slide for microscopic examination. The culture dishes stood on a table facing a northwest window of the Botanical Insti-

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[10]Haberlandt argued that terms such as sense-organ, sensitivity, sensation, perception, etc. could readily be used in connection with the physical aspects of stimulation and response. All cells and organs capable of receiving stimuli could, in his view, be termed "sense organs" whether they show any special anatomical features or not. The term "sense physiology" is now outmoded but was never really commonly used in the botanical literature. The English translation of Haberlandt's anatomy book is the only place where consistent usage of the word appears. "In zoological nomenclature, organs concerned with the perception of external stimuli have always been known as sense organs, even among the lower animals, and in other cases in which it is doubtful if the organs in question are responsible for sensation in the psychological sense. It is therefore not only permissible, but necessary in the interests of consistency, to apply the term sense-organ to the analogous structures in plants, especially as the latter often exhibit a close resemblance in plan to some of the perceptive organs of animals (Haberlandt, 1914, p. 572)."

[11]The term photosynthetic [photosynthetisch; Photosynthese] was not then in common usage. Around the turn of the century the noun *photosynthesis* and the adjective *photosynthetic* gained general acceptance (Green, 1900, p. 164) and are of course, now firmly established. Stiles (1925, p. 4) sums up the dilemma of terminology in the following. "The depth of ignorance of this aspect of the subject is clearly indicated by the variety of terms which have been used to describe the process: carbon assimilation, carbon dioxide assimilation, photosynthetic assimilation, chlorophyllous assimilation, photosyntax, photolysis of carbon dioxide. . ."

See also editor's footnote *2,* Pfeffer (1890) p. 302. Ewart discusses the term *photosynthetic assimilation* here for the first time.

tute. Here they were well-lighted but protected from direct exposure to the sun's rays. A few experiments were also carried out in darkness. The temperature, depending on the season of year (April, May, June, September, October, November) varied from 18 to 24°C.

Various precautions were taken, of course, to keep the cultures as nearly bacteria and fungus-free as possible, though in this regard, complete sterilization turned out to be scarcely feasible and really unnecessary. The slides, coverslips, steel needles, pipettes and glass dishes were always passed through the flame of a Bunsen burner several times before use. The solutions were boiled in advance. The shredded leaf pieces were carefully rinsed with sterilized water. This was enough to keep at least some of the cultures sufficiently pure. As for the contaminated cultures, it is worth remarking that, in general, the cultured plant cells were impaired only slightly in their progress by the presence of numerous bacteria in the culture solutions.

The following nutrient solutions were used: tap water, Knop's solution[1], one to five percent sucrose solutions, and Knop's solution with the addition of sucrose, dextrose, glycerine, asparagine and peptone in various combinations and concentrations.

II.

Before I consolidate and discuss the results of culture experiments attempted with the bracts of *Lamium purpureum,* I would like to quote, as an example of the course of a single culture experiment, from the entries of my day book.

Small dish culture. Knop's solution. Started on 21st of April. Average length of palisade cells 50μ, width 27μ. Chloroplasts starch-free.

April 22. Cells unchanged. Engelmann's bacteria method[12] indicates that the isolated cells are assimilating vigorously.

April 23. Cells unchanged. When stained with iodine solution, small

[1]For which Sachs (Vorlesungen über Pflanzenphysiologie, II. Aufl., S. 266) lists the following ingredients: to 1000 cm^3 of water, add 1 gram potassium nitrate, .5 gram calcium sulfate, .5 gram magnesium sulfate, .5 gm. calcium phosphate and a trace of ferrous sulfate.

[12]This elegant technique which was introduced by Engelmann is based upon the fact that certain aerobic bacteria are motile in the presence of oxygen and inactive in its absence. The cells to be tested for photosynthetic activity are placed on a slide in a solution containing the bacteria; the preparation is covered with a cover glass and sealed with a mixture of vaseline and paraffin. In darkness, the available oxygen is depleted by respiration and the bacteria are immobile. As oxygen is generated in the preparation by photosynthesis, the bacteria begin to move and are chemotactically attracted by a bubble of air or oxygen. As the sealed preparation is illuminated, the bacteria aggregate near the photosynthesizing cells (Engelmann, 1882). The method is, therefore, essentially a test for oxygen.

starch grains can be seen in the chloroplasts of several cells.

April 24. Almost all the cells are still alive. Many have grown considerably. Palisade cells grow relatively more in width than in length and show a tendency to round up. Often they take on a pear-shaped form. Chloroplasts have become smaller. They are symmetrically distributed or, in one protoplast, form a compact mass.

April 28. Nearly all cells are still alive. Their growth has progressed even further. Individual palisade cells are now 70μ long and 50μ wide. The chloroplasts have become still smaller and show a tinge of yellow. The bacteria method indicates assimilation still continuing, though less vigorously.

April 30. Majority of the cells still alive. Cell walls have become somewhat thicker. Normal wall thickness .7μ compared to a wall thickness of 1 to 1.4 μ. While the walls of normal cells slowly turn light blue when treated with zinc chloroiodide, this stain is quickly and intensively picked-up by cultured cells. The diameter of the chloroplasts, which have turned yellowish, has diminished by half since the beginning of the experiment: initially 6-8 μ, now 3.2-3. 8μ. Chloroplasts, however, still contain tiny starch grains.

May 2. All cells dead. The very thin plasma membrane has withdrawn from the cell wall, which is furrowed. Chloroplasts still smaller, quite pale, partially disorganized.

I should like to summarize the most important experimental results in the following points:

1. Under culture conditions in diffuse daylight, the photosynthetic cells continued to live many days. In Knop's solution, a simple nutrient solution of inorganic materials, the cells sometimes remained alive for three weeks (for example, in one dish culture, from the 2nd to the 24th of May). In solutions supplemented with 1% sucrose, they lived even longer. After a month, individual cells in these solutions were still alive. The cells quickly died in darkness. In Knop's solution death occurred after only 4-6 days; in Knop's solution plus 1% sucrose, a few day's later. They remain alive no longer in 5% sucrose solution.

2. Initially at least, the chloroplasts photosynthesize quite vigorously. This was established by means of Engelmann's bacteria method and through the demonstration of starch grains in chloroplasts which were free of starch at the beginning of the experiment.

The difference in behavior of chloroplasts in Knop's solution and in 1 to 5% sucrose solution is remarkable. In Knop's solution, they became gradually smaller, soon taking on a yellowish tinge. They then became still paler and were eventually transformed into small, soft and contorted leucoplasts. Their bahavior in sugar solutions was dependent upon

the concentration of the solution. It is true that, in 1%sucrose solution, the chloroplasts also became smaller, but they maintained their green color. In higher concentrations (3-5%) they did not decrease in size and still looked intensively green-colored when the cells died, often just as deep a green as at the beginning of the experiment. It did not matter whether the cells were in the light or in darkness.

How can these differences in behavior of the chloroplasts be explained? One soon assumes that it is a question of nutrition. Since the chloroplasts of isolated cells are entirely dependent upon their own powers of assimilation, they cannot remain intact but must eventually deteriorate. They release their assimilation products so completely to the rest of the cell organelles (which as a result often show vigorous growth), that too little remains for the preservation of their own integrity. It must be assumed that, with the resumption of cell growth, the normal relationship between chloroplasts and non-green organelles is destroyed. A relationship now begins which is similar to that of host and parasite. It would be similar to the simplest form of parasitism, in which the parasite injures the host by depriving it of formative building materials and finally causes its death. That this explanation is correct is suggested by culture experiments with assimilation cells of *Eichhornia crassipes* mentioned below, in which, in darkness, chloroplasts free of starch at the beginning of the experiment quickly perished, while those containing starch remained green until the starch was depleted.

A supply of sugar sufficed to keep the chloroplasts of assimilation cells of *Lamium purpureum* intact. Organic nitrogenous substances were not required. In a solution containing 3% sucrose and 1% asparagine, the chloroplasts remained bright green until the death of the cells, but showed neither growth nor increase. Their tendency to flatten and lie side-by-side was striking, especially in the lobes of the spongy parenchyma cells where uniform, large and trough-shaped chloroplasts were present. Careful observation also showed fine boundary lines present between the individual chloroplasts.

In a culture maintained in darkness in 5% sucrose solution, I observed on the fifth day very distinctive changes of form in the chloroplasts (Fig. 6). Brightly green colored chloroplasts with deep indentations and sinuses were seen. The chloroplasts appeared to be horse-shoe shaped or of an irregularly lobed form. Sometimes the lobes enveloped the rest of the chloroplast. These peculiarities of form are strongly reminiscent of the unusual forms of chloroplasts which I have observed in various species of *Selaginella* (*S. Martensii,* [*martensii*][13] leaf base; *S. Kraussiana*),[1] [*kraussiana*].

[13]The nomenclature has been checked by Dr. William J. Dress of the Bailey

3. A more or less pronounced growth of the isolated assimilation cells was usually associated with their continuing capacity to photosynthesize. Palisade as well as spongy parenchyma cells showed this. The average length of typical palisade cells at the beginning of the experiment was 50 μ, their width, 27 μ. After completion of growth, the maximum values recorded were 108 μ for length and 62 μ for width. The volume increase of palisade cells, computed on the basis of assuming that they have a cylindrical form, was 8 times greater than that of the original. The growth of spongy parenchyma cells was less substantial but still considerable. They increased their diameter from 38-40 μ to 50-68 μ. The palisade cells in general showed more vigorous growth in width than in length. Their tendency to round up and assume a spherical form was especially noticeable. That the volume increase was really due to growth, and not simply the result of an enormous stretching of the cell wall, was shown by plasmolysis experiments and, further, by the growth phenomena of the cell wall discussed in detail below.

The growth of the assimilation cells was not enhanced by the addition of sucrose or asparagine to the medium. The most pronounced growth occurred rather in Knop's solution.

From the previous remarks, it is evident that isolated assimilation cells, which stop growing in the normal course of development are able, in nutrient solution culture, to resume growth in a very vigorous way. What induces them to do so? Little would be gained by regarding this renewed growth as a reaction to injury during isolation (shock stimulus) [traumatische Reizung]. A term of this sort covers a number of separate processes, any of which could act as a stimulus. Among these are the physical strain on the cells during isolation; the rupture of plasmodesmata; the exposure of cell walls, which earlier bordered other cells (involving a presumed increase in transpiration rate coupled to the diminution of water uptake); the interruption of translocation between neighboring cells; the removal of physical and other influences on the part of neighboring tissues and organs; the uptake of decomposition products from cell components destroyed during isolation; or the effect of wounded areas bordering cells which remained uninjured. All these and still other changes in previous equilibrium conditions make up the wound-stimulus in its entirety. Therefore, if a particular reaction, which begins after a traumatic effect, is regarded as a result of this stimulus, what results is only a paraphrase of the facts and not scientific insight.

Hortorium, Cornell University, Ithaca, New York for accuracy and consistency. Where necessary, corrected generic and specific epithets have been inserted in brackets. This help is gratefully acknowledged.

[1]G. Haberlandt, Die Chlorophyllkörper der Selaginellen. Flora, 1888.

In the present case, a careful posing of questions is necessary. In the following pages, a few possibilities which could serve as explanations for the growth of isolated assimilation tissue, will be briefly discussed.

One might next consider the possibility that the cells begin to grow again because they cannot give up the formative building materials which they produce. That this could not be the only or the decisive basis for the renewal of growth is shown by any foliage leaf. The assimilation cells of a leaf, in the course of a sunny summer day, produce far more products of assimilation than they can release in the same time. They do not apply the surplus to their own growth, however. Instead the cells store it up temporarily until, in the course of the night, translocation follows. In the same way, isolated assimilation cells could store up their products without applying them towards growth.

Another basis for the resumption of growth might be found in the removal of physical growth hindrances which restrict cells contained in tissues. But in very loosely constructed palisade and mesophyll parenchyma, these cells, if they had any tendency to grow further, could grow vigorously into the air-filled intercellular spaces.

Most probably, the stimulus responsible for the recommencement of growth is one which the new medium exerts on the cells. In leaves, the cells are partly surrounded by air; in cultures, by solution. The increased water uptake could lead to renewed growth. However, I regard such a direct influence on the part of the surrounding medium as quite unlikely. Years ago in the Botanical Garden at Buitzenzorg, I poisoned the normal hydathodes of foliage leaves of *Conocephalus ovatus Tréc.*[1] [*Poikilospermum suaveolens*] and thus brought about a daily filling of the intercellular spaces with water. The palisade and spongy parenchyma cells flooded by the liquid—disregarding certain spots—showed no changes. It was not the increased water supply as such, but the requirement of the whole leaf for the secretion of surplus quantities of water which lead to the local production of numerous substitute hydathodes. The conductive parenchyma and palisade cells which showed strong growth under these circumstances got no more water conveyed to them than other conductive parenchyma and palisade cells which showed no renewed growth.

The possibility must also be rejected that the uptake of decomposition products from cells disrupted and killed during isolation is largely responsible for inducing the renewed growth of intact isolated cells. Although as a result of isolation, individual dead cells or cell fragments frequently remained attached to intact cells, a sufficient number were

[1]Cf. G. Haberlandt, Über experimentelle Hervorrufung eines neuen Organes bei *Conocephalus ovatus* Tréc. Festschrift für Schwendener, 1899.

also completely isolated. The latter showed just as substantial growth as the former.

In the ambient solution, these unidentified products of decomposition, associated with an extremely small volume of introduced cells, could only have been present in such small quantities in proportion to the dish contents that a stimulating effect on their part was probably excluded.

It is most probable that the renewal of growth of assimilation cells after their isolation is not actually the result of a new stimulus. Rather cells resume an interrupted growth, because some growth inhibiting factor released by the plant as a whole, which induces the assimilation cells of the leaf to cease their growth at a particular stage, disappears after the isolation of cells.

We certainly know that, in an organism, different processes and activities are stimulated or repressed in a self-regulatory way[1] as it meets the needs of the whole. In the interest of the best possible efficiency for a foliage leaf, it is obvious that the photosynthetic cells should not exceed a certain size. The size of the cells comprising a particular kind of tissue, in view of its physiological function, is just as important an attribute as its form and its other morphological characteristics. When this size is reached in the course of ontogenetic development, further growth of the cells will be checked. This happens not because the cells lose their potential capacity for further growth, but because a stimulus is released from the whole organism or from particular parts of it. It could for the moment be a purely dynamic or a material influence, through the action of which growth comes to a standstill. The isolated cell is capable then of resuming interrupted growth.

Even in the whole organism, if it lost control of individual cells or an entire cell complex, as a consequence of some disturbance in the control of the course of regulation, a renewal of growth in these cells could produce a "pathological hypertrophy." This "hypertrophy" could have some similarity to the kind which arises through the direct stimulus of various parasites, for example.

4. The cell walls of isolated assimilation cells show not only surface growth, but also growth in thickness. In the above-mentioned culture (page 71) [pp. 70-71 in this paper], wall thickness increased from .7 μ to 1-1.4 μ in 9 days. Disregarding those in frequent cases where growth in wall thickness was uniform, localized, but very conspicuous wall swellings often occurred. The palisade cells sometimes thickened their end-walls (Fig. 3). These thickenings were most pronounced in the middle and became less conspicuous along the margins. Even more con-

[1]Cf. Pfeffer, Pflanzenphysiologie II. Aufl., 2. Band, S. 160 ff.

spicuous were cushion-shaped swellings of the cell wall in the sinuses between short lobes of the spongy parenchyma cells (Figs. 4, 5). Wall thickness here measured up to 4.6 μ, while the walls of the lobes themselves were usually only .8-.9 μ thick. These thickenings consistently showed the usual cellulose reaction to zinc chloroiodide, just like the rest of the wall parts.

5. The turgor pressure of cells which had grown in culture was greater than that in normal cells found in a tissue. This increase in osmotic pressure occurred in culture in Knop's solution as well as in 1 or 3 percent sucrose solutions. Therefore, it could not be simply an adaptation to a more concentrated solution. The less so since the increase in osmotic pressure was very great in comparison to the concentration of solution. In normal palisade cells at the beginning of the experiment, plasmolysis occurred in a 3% potassium nitrate solution. In cells grown for two weeks in two different cultures (nutrient salt solution and 1% sucrose solution), plasmolysis occurred only after the addition of 5% potassium nitrate solution. This would be equivalent to an increase in turgor pressure from 10.5 to 17.5 atmospheres. However, the rapid deplasmolysis in cultured cells was conspicuous. The tonoplast (wall of the vacuole) was readily permeable to potassium nitrate, which suggests an abnormal modification of the vacuolar membrane. If these cells possessed a specific permeability to potassium nitrate at the beginning of the plasmolysis experiments (which seems quite likely to me) the above turgor pressure value would be too high, of course.

During plasmolysis in 5% nitrate solution, or in dilute glycerine, frequently I observed only the withdrawal and contraction of the tonoplast; the outer protoplasmic membrane [plasmalemma] and the granular cytoplasm, together with the nucleus and chloroplasts, remained adhered to the cell wall. Hugo DeVries[1] recorded the same appearance in protoplasts which were in the process of slowly dying. There is no valid reason to believe that, in my cultures, the cells and cytoplasmic constituents referred to were already dead at the beginning of plasmolysis. Characteristic changes, which will be described, precede the death of the protoplasm; in the above-mentioned case, these had not occurred. It might be supposed, however, that the cytoplasm (with the exception of the more resistant vacuolar membrane) was already so weakened that it was rapidly killed by the sudden introduction of 5% nitrate solution. DeVries also points out that cells whose protoplasts are injured by placing a bit of tissue in solutions of neutral substances for an extended

[1]H. deVries, Plasmolytischen Studien über die Wand der Vacuolen. Jahrb. f. Wissensch. Bot., 16. Bd., S. 466, 467.

time, or through the addition of extremely small quantities of poisonous substances or by gradual heating to the upper temperature limits of life are capable of responding to the sudden introduction of nitrate solution only by withdrawal and contraction of the more resistant tonoplast. In my cultures, however, injury to the cytoplasm was preceded by vigorous growth of the protoplast.

As part of these experiments, a few measurements of the dimensional changes of palisade cells before and after plasmolysis were made. The following short table records the values found in ocular micrometer units.

		Before Plasmolysis	*After Plasmolysis*	*Shortening in Percent*
1. Palisade cells, taken fresh from the leaf	(length	20.8	19.	8.6
	(width	8.	7.5	6.2
2. Palisade cells after 10 days culture in nutrient salt solution	(length	37.5	35.8	4.5
	(width	19.	18.	5.2
3. Palisade cells after 10 days culture in 1% sucrose	(length	38.	37.	2.6
	(width	18.8	18.	4.2

It is evident from this that the shortening of the longitudinal diameter of cells, in response to an increase in turgor pressure, is significantly greater if the cells are taken fresh from the leaf (shortening 8.6%) than if they are taken from a 10-day culture (shortening 4.5% and 2.6%).

The difference in shortening of the tranverse diameters is much less striking. Whether the slighter stretching of the cell wall in palisade cells which have grown substantially in culture (despite the higher turgor pressure) is related to the increase in wall thickness or whether the capacity to stretch has diminished as a result of a qualitative change in the physical properties of the wall remains to be demonstrated.

6. I have only a few observations regarding changes in the condition of the nucleus of cultured cells to discuss. In normal palisade cells, the nucleus, pressed against the cell wall, possesses a half-lens shaped form with a diameter of about 6 μ. In a five-day culture (Knop's solution + 1% sucrose) the nuclear diameter measured from 8 to 10 μ. The nuclei have thus grown with the cells. In contrast to this, in a 16-day culture (Knop's solution) in which cells which had previously grown considerably were on the verge of dying, the nuclei were considerably smaller. Their diameter now measured only 3 to 4.5 μ.

7. In all cultures an irregular sharp convoluting of the protoplasts, now quite watery, preceded the death of the cells. The protoplasmic surface

withdrew from different parts of the cell wall—in spongy parenchyma cells, next to the cushion-shaped wall thickenings in the cell sinuses. Then, forming narrow folds, it invaginated rather deeply into the cell lumen. This convoluting cannot be merely a plasmolytic phenomenon, since it involves a corresponding surface increase of the protoplast. It must rather be regarded as dependent upon an active surface growth of the protoplast. This growth results in the formation of convolutions because the cell wall has previously lost the capacity for surface growth.

Later, during death, the entire protoplasmic surface withdraws slightly from the cell wall. It now shows in surface view irregularly twisted often zig-zag shaped, broken, sharp lines which certainly correspond in part to the rather narrow, sharp convolutions, but may in part also represent fine tear lines.

III.

I will now relate the results of culture experiments carried out on isolated cells of other plants. Actually it is simply a matter of reporting some isolated experiments which, however, still produced some results worth mentioning.

On the 29th of September, two dish-culture experiments were started with isolated photosynthetic cells from the foliage leaves of *Eichhorina crassipes*. In both cultures Knop's solution was used. One culture was placed in front of a window in diffuse daylight; the other was placed in the dark. Both cultures contained completely starch-free photosynthetic cells as well as some whose chloroplasts exhibited numerous starch inclusions.

After 5 days, almost all the cells were still alive in the lighted culture. A few had grown fairly vigorously. The chloroplasts had become conspicuously smaller and paler. A greater number of the starch-containing cells had died; the starch content in a few faded chloroplasts had apparently decreased little or not at all.

In the darkened culture after 5 days, the starch-free cells had grown very slightly or not at all. Their scattered chloroplasts had become conspicuously smaller and were light yellow in color. In the starch-containing cells, where the chloroplasts remained as bright a green as normal, the starch contents had diminished even less than those that had been in the light. After 10 days, the starch-free and starch-containing cells in general showed no further changes. In the starch-free cells, the small yellowed chloroplasts were often pressed around the nucleus. In the starch-containing cells, they were still the same size and a beautiful green color.

This experiment therefore shows that chloroplasts of isolated cells

soon deteriorate in darkness, if they were starch-free at the beginning of the experiment whereas they remain intact if they are able to utilize for themselves at least part of their stored-up starch during the negligible growth of the cells.

Culture experiments with cells lacking chlorophyll were also carried out. Numerous "glandular hairs" occur on the foliage leaves of *Pulmonaria mollissima* Kern. The expanded, club-shaped, often obliquely attached terminal cells of these hairs are completely full of uniformly granular protoplasm. The centrally situated round nucleus possesses a large nucleolus. These glandular hairs were cut off with a razor in such a way that one or two intact stalk cells were usually carried along with the glandular cells into culture.

Roughly the same changes took place in ordinary tap water as in Knop's solution, although the cells remained alive a little longer in tap water. After one day in culture, numerous vacuoles, most flattened lengthwise, appeared in the protoplasm of glandular cells, so that the protoplasm took on a course, fibrous appearance (Fig. 12). The protoplasmic strands were all arranged parallel to the long axis of the cell. In the vicinity of the nucleus, the protoplasm was finely alveolar. A few strongly refractive droplets were irregularly distributed in the protoplasm After three days, the protoplasm of the glandular cells had become highly vacuolated. The fluid cytoplasm was distributed uniformly over the walls and connected by numerous thick or thin cytoplasmic strands to a central cytoplasmic mass containing the nucleus, which had become somewhat smaller (Fig. 13).

After 7 days, cells contained in nutrient salt solution were completely dead; in tap water, a few were still alive. Their protoplasts were now very greatly reduced in mass and poor in substances (Fig. 14). The cytoplasm now formed only a thin wall covering which was somewhat thicker at the base of the cell. The nucleus, grown conspicuously smaller, was situated here. A few cytoplasmic strands connected the thin cytoplasmic mass, which surrounded the nucleus to adjacent parts of the cell wall. No growth of the cells was ever observed.

In a third culture, set up at the same time, the glandular cells were placed in a solution which contained 3% sucrose and 1% asparagine. After one day, the cells had scarcely changed. Only a few individual cells showed a hint of the fibrous differentiation of cytoplasm observed so clearly in the fore-mentioned cultures after the first day. After three days, the cytoplasm showed the same distribution as cells in tap water, yet was still very abundant and quite granular. On the seventh day, many cells were dead. Again, cells which were alive showed cytoplasm dis-

tributed as in cells cultured in water, but were more vigorous and richer in substances. No growth occurred in this culture either.

The deterioration of the initially well developed protoplasts of the glandular cells would be expected under culture in tap water and nutrient salt solution, where the cells were under starvation conditions. To some extent, the rapidity with which loss of substance followed is surprising. Since it is supposed that the plasmalemma uses up negligible quantities of building materials, it must be that very vigorous metabolic processes occur. The end products of these processes are excreted. Above all, respiration must have been very intense. It must also be assumed that respiration in the protoplasm of glandular cells is especially intense on the basis of other reasons. Solutions with organic materials (sucrose and asparagine) delayed the deterioration of the protoplast, but were not able, at the concentrations employed, to stop it completely.

A peculiar response was shown by the stinging hairs of *Urtica dioica,* which were cut off, placed in a 1% sucrose solution, and cultured at a temperature of 33°C in a thermostat. The experiment started on September 22. After two days, all the stinging hair cells were still alive. After ten days, the majority were dead. Those with signs of life remaining had the following appearance. The formerly protoplasm-rich base of the hair cells now possessed only a relatively fluid cytoplasm forming a thin peripheral wall layer and a wide, long, fibrous central protoplasmic strand. The nucleus had shifted out the bulb and was now situated at the base of the cone-shaped part of the hair. A great deal of protoplasm was now present here. Numerous ellipsoidally stretched vacuoles of various sizes now occurred in place of uniform vacuoles. The upper part of the hair was completely filled with protoplasm containing no vacuoles. The protoplasm exhibited slow cytoplasmic streaming and showed the long-fibrous structure so characteristic of streaming cytoplasmic masses.[1] On the seventh of October, i.e., after fifteen days, all cells were dead and badly fungus infected.

Two points are worth mentioning with respect to this experiment. First, the streaming of the protoplasm, a greater portion of which migrated from the base to the upper part of the hair; and secondly, the fact that, after ten days, the total amount of protoplasm had not diminished, but instead, so far as could be estimated, had increased. It is not impossible that regeneration and growth of the protoplasm was accomplished at the cost of introduced nutrient materials. However, it seems more probable to me that the considerable quantities of protein lost

[1]Cf. G. Haberlandt, Über fibrilläre Plasmastructuren. Berichte der deutschen bot. Gesellschaft, 1901, Decemberheft.

from the cell sap of the stinging hairs,[2] represent a food reserve, and that this reserve delays the deterioration of the protoplast and eventually even supports further growth.

An experiment with cultured filament hairs of *Tradescantia virginica* (*sic*) [*T. virginiana*] gave interesting results. Immature hairs were removed from the plant, and 4-8 celled fragments were placed in a hanging drop of solution which contained 2% dextrose and .4% asparagine. Usually all but one or two of the cells of each fragment died, so that individual cells were actually cultured. The long life-span of the isolated cells was especially surprising. After 26 days, numerous cells were still alive.

Their life span above and beyond the usual may have been prolonged by the artificial medium. The cells showed a very vigorous growth, approximately doubling in length and width, and also showed a vigorously developed protoplast (Fig. 7). The distribution of protoplasm was normal.

The response of transverse walls bordering dead neighboring cells was very striking. These walls were (in spite of the turgor pressure) curved into the lumen of the dead neighboring cells and showed, in most cases, a rather conspicuous growth in thickness (Figs. 8-10). The walls were eventually three to five times as thick as they were initially. Frequently the thickening extended into adjacent parts of the cell wall (outside the area of contact) and here gradually disappeared. The location of the nucleus was also worthy of notice. If the cell in question bordered dead cells on both sides so that both transverse walls were thickened into protruding papillas, the nucleus lay approximately in the middle of the cell. If, however, two neighboring cells were alive (Fig. 7), so that the septum between them remained even and unthickened, then the two nuclei moved closely up against the thickened end walls. This is a further informative example of the relationship discussed by me years ago between function and position of the nucleus in growing plant cells.

After injury of a multicellular hair, the end-wall, which now becomes the outer wall, thickens and cutinizes; this is an easily understood and common biological protective device. With hairs connected to a whole plant, however, it is questionable whether the thickening and cutinization of the end wall is a gradual reaction of the hair itself or whether this hair receives instructions, as it were, from the whole organ. Results of the experiments with the cultured hair fragments of *Tradescantia* suggest that the former is the case. The cells in question thicken their end wall on their own initiative and demonstrate in such a manner the instinct of self-preservation of the elementary organism.

[2]Cf. G. Haberlandt, Zur Anatomie und Physiologie der Pflanzlichen Brennhaare. Diese Sitzungsberichte, 93. Bd., I. Abth., 1886.

Ordinary epidermal cells, as far as my very fragmentary observations go, remain alive only a short time in an isolated condition. In small pieces of epidermis peeled from the foliage leaf of *Ornithogalum*, I noted that the shorter cells inserted between long epidermal cells remainded alive a few days longer than the others.

Leitgeb[1] has already referred to the powerful viability of stomatal cells. For example, he allowed a 1 cm. piece of the scape of *Galtonia candicans* to stand for a month in a humid, lighted, room. After this time, it had become discolored, decayed and fungus infected. All tissues were dead, except for the guard cells, which were a vivid green, strongly turgid and extraordinarily strongly curved, so that individual guard cells touched their own ends and formed a ring. No complete closure of the guard cells was seen after the increase of turgor, a fact which Leitgeb attributes to the enormous extension of the cell, which exceeds the limits of elasticity of its wall. Perhaps surface growth of the walls, especially the dorsal walls, had already occurred. I have also observed the phenomena described by Leitgeb in my cultures and, although with different plants (*Ornithogalum umbellatum, Erythronium dens canis,* [*E. dens-canis*], *Fuchsia globosa*) [*Fuchsia magellanica* cv. *'Globosa'*] I can add nothing substantially new to his statements. In any case, the stomatal cells, because of their great viability, suggest themselves as very suitable for this kind of culture experiment.

IV.

In closing, I should like to point out the fact that, in my cultures, despite the conspicuous growth of the cells which frequently occurred, cell division was never observed. It will be the problem of future culture experiments to discover the conditions under which isolated cells undergo division. Certain hints in this connection are given by the well known experiments of Loeb, Nathansohn, and Hans Winkler concerning the experimentally induced extensive development, actually cleavage, of unfertilized egg cells.

Loeb[1] first observed a parthenogenetic development of sea urchin eggs which were exposed for one or two hours to a solution of $MgCl_2$ and then returned to sea water. He concluded on the basis of this experiment that a specific effect of Mg was involved. Later experiments, however, showed that appropriate concentrations of NaCl and KCl, as well

[1]Beiträge zur Physiologie der Spaltöffnungsapparate. Mittheilungen aus dem bot. Institute zu Graz, 2. Heft, 1888, S. 123.

[1]J. Loeb, On the nature of the process of fertilization, etc. Amer. Journ. of Physiology, Bd. III. 1899. Ferner: Experim. on artificial parthenogenesis in Auclids (Chaetopterus) and the nature of fertilization. Ebenda, Bd. IV, 1901.

as sucrose, have the same effect. Only a specific increase in the osmotic pressure of the fluid surrounding the egg is needed to induce the parthenogenetic development. The possibility that experiments of this kind with isolated plant cells could lead to a division of the same sort will now be examined.

It was shown by Nathansohn[2] that, with the genus *Marsilia* (*sic*) [*Marsilea*], the capacity of the eggs for parthenogenetic development can be enhanced by raising the temperature to 35°C. After I had read Nathansohn's publication, I performed a few additional culture experiments with isolated cells at a temperature of 33°C. (in a thermostat). The photosynthetic cells behaved no differently from those at ordinary room temperature. The described changes occurred more quickly and accordingly the cells died sooner. The results of a culture experiment with stinging hairs of *Urtica dioica* were as already described above.

Finally, demonstration has been furnished by Hans Winkler[1] that uncleaved eggs of *Arbacia* and *Sphaerechinus* can be induced to undergo a few cleavage divisions by material extracted from the sperm of the same species. This result finds its analogue, to a certain degree, in the effect (also reported by Winkler) of the pollen tube on the development of the ovule in orchids, the swelling of ovaries, etc.

Probably substances are involved here, "growth enzymes" ["Wuchsenzyme"] which, released from the pollen tube, act as a chemical stimulus to the growth and division of the cells concerned. Of course, these substances are probably not identical to those which generally induce the egg cell to divide. Moreover, the stimulus causing the egg cell to divide could be different from that which causes a vegetative cell to divide.[2] Still, it would be worthwhile to culture together in hanging drops vegetative cells and pollen tubes; perhaps the latter would induce the former to divide. Most promising would be such experiments as those with orchids and other plants, by which the effect of the pollen tube mentioned above has already been established.

Not only pollen tubes could be utilized to induce division in vegetative cells. One could also add to the nutrient solutions used an extract from vegetative apices, or else culture the cells from such apices. One might also consider utilization of embryo sac fluids.

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[2]Über Parthenogenesis bei *Marsilia* und ihre Abhangigkeit von der Temperatur. Berichte der Deutschen bot. Gesellschaft, 1901, S. 99 ff.

[1]Über die Furchung unbefructeter Eier unter der Einwirkung von Extractivestoffen aus dem Sperma. Nachricht der K. Gesellsch. der Wissensch. zu Göttingen, Math.-Phys. Cl., 1900, Heft 2. Ferner: Über Merogonie und Befruchtung. Jahrb. für Wissensch. Botanik, 36., Bd., 1901.

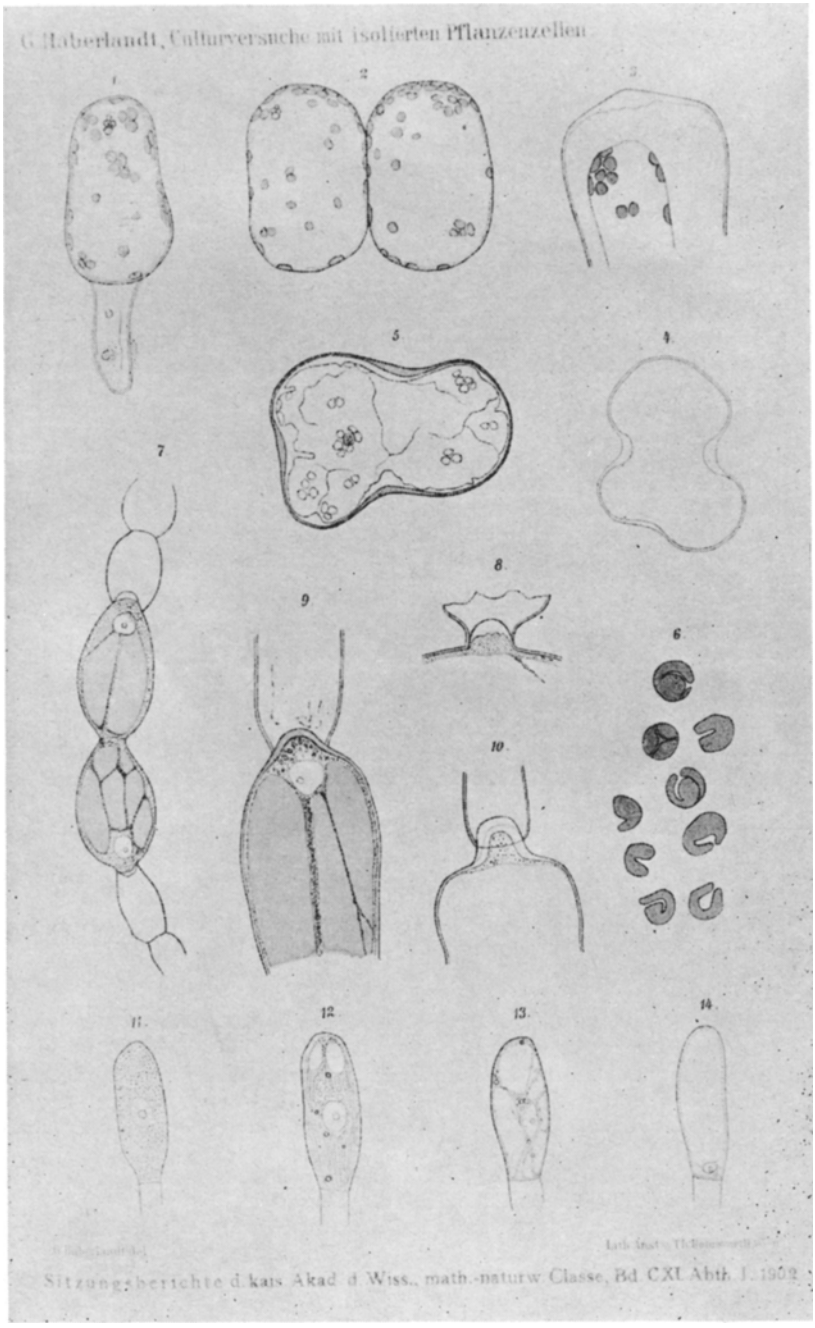
[2]Cf: Winkler, Jahrb. für Wissensch. Botanik, 36. Bd., S. 773.

Without permitting myself to pose further questions, I believe, in conclusion, that I am not making too bold a prediction if I point to the possibility that, in this way, one could successfully cultivate artificial embryos from vegetative cells.

In any case, the technique of cultivating isolated plant cells in nutrient solutions permits the investigation of important problems from a new experimental approach.

EXPLANATION OF THE FIGURES

FIGS. 1-14. FIG. 1. Palisade cell of *Lamium purpureum* after 8 days of culture in nutrient salt solution; it has grown actively. Below is a dead palisade cell which was injured in the isolation. FIG. 2. Two actively growing palisade cells of *L. purpureum* after 8 days of culture in nutrient salt solution. FIG. 3. Part of a palisade cell of *L. purpureum* after 11 days of culture in 1% cane sugar; plasmolyzed with dilute glycerin. The upper cell wall has become thickened. FIG. 4. A spongy parenchyma cell of *L. purpureum* after 12 days of culture in nutrient salt solution; cushion-like thickenings in the cell indentations; the cell contents are not damaged. FIG. 5. Dead spongy parenchyma cell of *L. purpureum* after 16 days of culture in nutrient salt solution; the elevated plasma membrane shows folding. FIG. 6. Chloroplasts from palisade cells of *L. purpureum* after 5 days of culture in 5% cane sugar; cultured in darkness. FIG. 7. Segment of a stamen hair of *Tradescantia virginica* [*T. virginiana*] after 12 days of culture in nutrient solution (2% glucose, 0.4% asparagine). Only two cells have remained alive; these have grown and have thickened on their cross-walls adjacent to the dead cells. FIG. 8. End of a living hair cell after 8 days of culture; the cross-wall has turned-up papillae and is quite thickened. FIGS. 9 and 10. The same after 12 days of culture. The wall thickening extends even up to the cross-wall of the neighboring parts of the outer wall. FIG. 11. End cell of a glandular hair of *Pulmonaria mollissima* Kern. at the beginning of culture. FIG. 12. The same after 1 day of culture in tap water. Vacuolization of the protoplast, which partly assumes a coarse fibrous structure. FIG. 13. The same after 3 days of culture in tap water. FIG. 14. The same after 7 days of culture in tap water. The protoplast has grown thin; moreover, the cell nucleus has become much smaller.


G. Haberlandt, Culturversuche mit isolierten Pflanzenzellen.
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