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REFLECTIONS AND SPECULATIONS

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When I was invited to write an introductory chapter for this Annual Review, I accepted with alacrity, especially since it was meant to be a "personal, philosophical, or historical essay." Present-day editorial policies do not allow a scientist to be

personal any more: an author should appear to be emotionally completely detached from his subject, which the born scientist of course never is. Just as the serenity of Vermeer and Delius, or the gigantic minds of Rembrandt or Beethoven are expressed in their creations, thus the personalities of the early botanists and those of the turn of the century became living persons in my mind, as they revealed themselves in their publications. It was a source of great satisfaction, when meeting them in person, to find that their physical appearance often resembled the mental image I had formed of them. It was probably no coincidence that the person of H. Fitting did not resemble his scientific image, for he seemed more detached in his writings than most other botanical authors. It is a source of regret that I never had a chance to meet N. Cholodny, A. Paal, nor P. Boysen Jensen to compare my mental image of them with reality. Yet a present-day meeting with Charles Darwin or Julius Sachs could hardly be satisfying because of the exalted image I have developed of them, based entirely on their writings and work.

What Makes a Botanist?

In my youth I had an extraordinary opportunity to become a botanist. I was born and grew up in a botanical garden. My father (F. A. F. C. Went) was professor of botany and director of the garden and botanical laboratory at the State University of Utrecht, a provincial town in the center of the Netherlands. His official residence, a very roomy 300-year-old house, was located in the botanical garden, just across from the newly rebuilt botanical laboratory, which under his guidance had become the model of a modern botanical installation, attracting visitors from all over the world. Just because of the obvious environmental pressure, my father was very careful not to push me into a botanical profession, and my early direct contacts with science and botany were: (*a*) my high school professors in biology, chemistry, and physics, who were all extraordinary teachers; and (*b*) a fellow high school student, C. G. G. J. van Steenis, with whom I regularly made bicycle trips to collect plants for our herbaria, and with whom I have been bound by a lifelong—and occasionally explosive—friendship. But it was not until I went as a student to the University of Utrecht that my fixation on botany became permanent.

What goes into the making of a professional biologist? Often it is environment, in which usually intellectually inquisitive parents and a plethora of plants or animals in garden or field produce the winning combination. If the home environment has not brought the stimulation, it usually is an inspiring high school or college teacher which produces a biologist. Any collecting activities resulting in herbaria, terraria, aquaria, or collections of insects or fossils are important too. And in the past as well as in the present, inquisitive minds became intrigued with the problems plants presented, such as their medicinal qualities (many of the 16th-18th century botanists were physicians), their diseases, their agricultural problems or horticultural possibilities. While all these factors contributed to my becoming a botanist, perhaps the most powerful was the fact that as a boy I spent many hours in the botanical laboratory. In the brightly lighted (or dark) rooms students could work the whole day with plants and discover their secrets, studying them under the microscope, or in the mysterious physiological darkrooms, or on the clinostat, or in a constant

temperature waterbath, or in complicated machines. In the greenhouses of the botanical garden were sensitive plants, Venus fly traps and other insectivorous plants, cacti and desert plants, morphologically interesting forms, exotic flowers, and any number of other growing wonders, from algae to fungi to palms, which would whet the research appetite of any budding scientist. It is a pity that nowadays campus development has broken the close ties between students and teachers on the one hand and botanical gardens and biologically interesting plant collections on the other hand. In the old days botany professors profited by living in a botanical garden, and much of the work of J. Sachs, H. de Vries, K. von Goebel, E. Bünning, or Charles Darwin was based on the extensive plant collections they had growing next door. They verified their discoveries on a wide variety of plants, stressing the universal occurrence of a phenomenon rather than its presently overemphasized statistical significance. Many physiological problems can be solved with "oats, peas, beans, and barley," as the nursery rhyme says, even with their seedlings grown in physiological darkrooms, or their fruits or seeds bought in a supermarket, but for a balanced view of plant processes we need an overall view of the plant kingdom, which can be so inspiringly demonstrated in a botanical garden. Therefore, I consider myself lucky in having been connected most of my life with botanical gardens: I was born in the one of Utrecht, my first job was as a botanist at the famous botanical garden in Bogor, Java; then I was president of the California Arboretum Foundation, the sponsor of the Los Angeles State and County Arboretum, and for 5 years I was director of the Missouri Botanical Garden. In addition, extensive travels have given me a good overview of the plant kingdom. This gave me the advantage of coming close to the living plant, to acquaint myself not only with its appearance and occurrence, but also with its workings. And it has prevented me from becoming a narrow specialist, spending my life on the response of a single plant or organ.

Botanical Problems of Yore

When I look back on the problems that faced botanists 50 years ago, I realize the big ones are still with us. Although we know a lot more about the why and how of plant growth and development, about form and morphogenesis, about metabolism and nutrient uptake, or about response to the environment, the elemental problems remain basically the same, as enigmatic now as then. Only the emphasis has changed.

At the turn of the century there was an intense interest in tropic responses of plants, at least in European laboratories. It was thought that the response of a stem, a root, or a leaf of a plant to light or gravity could tell us much about inner processes controlling these responses. There was a curious duality in basic thinking on this subject. On the one hand it was thought, especially by the school of Pfeffer in Leipzig, that complex tropistic responses reflected an equally complex inner mechanism of stimulation and activation, to some extent the way Darwin compared the control of stem and root behavior by the stem and root tip with the action of the brain in lower animals. Thus Pfeffer was greatly puzzled by the basic experiment of P. Boysen Jensen on transmission of the phototropic stimulus across a cut surface,

which was carried out in his laboratory in 1907. Later, in 1914, when A. Paal worked in his laboratory, Pfeffer let him repeat the transmission experiment, since the results were contrary to the extensive research of Fitting. But until his death in 1917, Pfeffer never drew the conclusion that a simple substance, not a complex stimulus, was involved in phototropism.

A different line of thought about tropism emanated from my father's laboratory, starting with the basic research of one of his students, A. H. Blaauw, on phototropism. The exceedingly clear mind of Blaauw could not accept the imprecise concept of "stimulation," and in some basic experiments he showed that a definite phototropic curvature was a response to a definite amount of light energy. Soon Blaauw, being a superb photographer, related the phototropic curvature to a photochemical process in the plant. Later, in a sensational series of experiments, Blaauw showed that the inhibitory effect of light on straight growth could—at least qualitatively—account for the phototropic curvature (since unilateral light produces a light gradient in the phototropically responding organ). He then pronounced his famous dictum: the problem of phototropism has become empty: only the problem of the effect of light on growth remains. During the next 10 years this resulted in a whole series of investigations for or against Blaauw's theory.

Curiously enough, this controversy was entirely bypassed in America. The practical New World botanists had very little interest in the tropistic behavior of plants to the extent that the word tropism was not even mentioned in the American textbook of plant physiology most used in the thirties, that of Miller.

Another burning question during my student days was that of limiting factors, a subject first broached by Liebig in the 1840s in the form of the "law of the minimum," and revived in 1905 by F. F. Blackman in a remarkable publication. In the U.S. this problem took the form of "master reactions" which were analyzed by means of their temperature sensitivity (e.g. by Crozier), or later by their differing chemical dependencies.

AUXIN PROBLEMS

The great advantage of working in my father's laboratory was not only (for those days) the superb instrumentation available (clinostats, Koningsberger auxanometer, thermostats, light sources, etc), but also the intellectual inquisitiveness of the entire staff and the broad range of scientific problems under investigation (temperature effects, respiration, photosynthesis, tropisms, growth responses, biochemistry, morphogenesis). There was a complete openness of discussion of each student's work, which resulted in a good deal of mutual stimulation and education. Perhaps most important in my father's laboratory were the availability of experimental techniques and material, the controlled temperature and controlled humidity chambers, and the oat coleoptile. Then as now, there were some students dissatisfied with too great an emphasis on the mechanistic approach towards life. I well remember endless discussions with some fellow students on why the growth-promoting principle of the oat coleoptile was not a spirit and weightless, ghostly and immaterial. It induced

me to measure the diffusion constant of this "spirit," which indicated that auxin had a molecular weight ranging between 300 and 400, which made it impossible to classify it as a "spirit."

To get started in research in my father's laboratory, for the predoctoral degree (MS) a student had to repeat some work previously done for a PhD degree. So I started by remeasuring the growth response of oat coleoptiles to light with the auxanometer of Koningsberger. With a simple device to prevent the coleoptiles from circumnutating, I was able to obtain some excellent growth responses. They showed that the tip and the base of a coleoptile were differentially sensitive to light, the tip producing a much larger response. Discussing this result with my fellow students, it became clear that in some way this agreed with Boysen Jensen's and Paal's conclusions about the special function of the coleoptile tip in tropisms and growth. Since at that time I discharged my military obligations by attending a gas-warfare school in Utrecht during the day, I had my evenings and nights available for more productive activities, so I worked all night in the laboratory. After having established that decapitating coleoptiles did not basically abolish their ability to grow, I laid the basis for using such decapitated coleoptiles to test growth factors. When it was then established that a unilateral application of coleoptile diffusate caused a unilateral growth increase, the foundation for a quantitative growth hormone analysis was laid. The first experiment applying the diffusate of coleoptile tips into gelatin onto decapitated coleoptiles succeeded at 3 AM on April 17, 1926, and the next morning (when I had no military duties because it was Prince Consort Henry's birthday) I could repeat the experiment to my father's satisfaction. Then a whole series of experiments ensued, in which (*a*) the quantitative response of decapitated oat coleoptiles to the growth hormone could be established; (*b*) the thermostability of the growth substance was proved; (*c*) the light stability of auxin was evident; and (*d*) its significance in normal coleoptile growth was shown. This led to the dictum: no auxin—no growth.

After completing my military duties, I chose auxin (the word assigned by Kögl to the plant growth hormone) as my doctoral thesis subject. The newly developed technique of quantitative auxin analysis made it possible to study a number of other auxin and growth problems in a completely impartial and direct manner. I would like to stress that a number of facts thus turned up in my thesis work were complete surprises for me, such as the polar auxin transport and the lateral deflection of auxin transport under the influence of unilateral light. They definitely were not based on preconceived ideas or theoretical considerations, and in my later work as well, experimental facts rather than theoretical considerations have guided me. They also were supported by a number of studies by others, especially in my father's laboratory. Later in the 1940s and 1950s, not only the polar and lateral auxin transport, but also the light stability of auxin was challenged. Remarkably enough, this challenge was not based on facts, but founded on an artificial theoretical construction. Misused statistics were employed to show that my conclusions of 1927 were wrong, and it took considerable effort on the part of many investigators to reaffirm my original conclusions. This makes one wonder how often, in fields with which one

is not familiar, wishful and clever theoretical constructions have warped facts or influenced their interpretation. And it is not even necessary to warp facts to reach completely erroneous conclusions, as the phlogiston and ether theories attest.

I would like to discuss some of the problems opened up by the development of a quantitative auxin test in more detail.

Polar Auxin Transport

The discovery of the polar transport of auxin was not only an unexpected phenomenon, but it promised a better insight into a puzzling morphogenetic problem, that of polarity in general. Why does an initially undifferentiated zygote produce a structure with head-tail or shoot-root differentiation? Grafting and cutting experiments had shown that the polarity exhibited by a stem or tuber or root in regenerating new roots or buds was a fundamental property of each tissue and each cell. All this came into the realm of polar auxin transport when I could show that the polarity in root initiation on stem cuttings should be attributed to polar auxin transport to the basal cut surface. Thus morphological polarity could be explained by the polar transport of a particular substance: the hormone auxin. At long intervals I have returned to this polarity problem.

First I found that dyes with chromophoric anions penetrated into a polar tissue (*Phaseolus* seedling stems) preferentially from the apex (like the acid auxin), whereas basic dyes penetrated over a greater distance from the base. This seemed to suggest that the polar auxin transport was based on an electrical polarity in the polar tissue.

I was able to attack another aspect of the polarity problem when radioisotopes became available. If auxin was moved polarly in coleoptiles by way of an electrical gradient, would other ions also show a tendency towards polar transport in coleoptiles? In my experiments, carried out in Berkeley with radioactive tracers, I applied agar blocks with radiophosphorus, radiosodium and radiobromine to the basal or apical cut surface of Avena coleoptile segments. I was unable to measure any differential transport in the two directions; actually these ions did not move at all, although simultaneous measurements showed polar auxin movement in the same coleoptile segments. Among the many possible interpretations of these results, I prefer the one that polar transport of auxin is only possible in a lipid medium in which an electrical gradient could be maintained.

Later I made another attempt at the polar transport problem in measuring auxin transport in upright and inverted *Tagetes* cuttings. When placed upside down in sand, they will root at the original apex, and the sprouts growing from a basal node will produce perfectly normal plants. Auxin transport in the inverted stem section of the cutting originally was strictly polar, but after several weeks a second, base-to-apex, auxin transport became superimposed on the original apex-to-base polar transport. The original intent of this experiment, planned jointly with E. J. Kraus to compare the physiological changes with anatomical studies, was never followed up because of Kraus' retirement. My own interpretation of these results is that in the course of several weeks, new vascular strands are produced in the inverted *Tagetes* stem, with an opposite polarity.

Seed Dormancy

A fully dormant seed retains its storage food for many years whether this dormancy is induced by complete dryness or otherwise, as, for example, *Amaranthus* seeds, which retain their viability for years after submergence in water, or *Lotus* seeds, buried for thousands of years in bogs. In the latter case it is obvious that the maintenance of the living condition—of the polarity of the cell structure, and of the accumulation of foods and salts inside the cells—does not require any metabolic activity. Yet as soon as a seed dies, it releases all its accumulated storage food. This is very obvious in germination tests. Viable seeds laid out on wet filter paper may remain dormant for many months, during which time they are not infected by fungi or bacteria. But the dead seeds among them within a few days are overgrown by mycelia, fed by the food oozing out of the dead cells. If the maintenance of the living membranes of 3000-year old buried *Lotus* seeds required any metabolic processes, then not more than one sugar molecule would have been available each minute per hundred million protein molecules, truly negligible for any maintenance work. Such dormant *Lotus* seeds obviously stay alive while fully saturated with water and maintain their semipermeability. Their cells must preserve all accumulated salts and nutrients without any appreciable energy expenditure. This can be accomplished in most seeds in the absolutely dry condition, as established in the first 20 years of a 1000-year experiment on seed longevity. These same seeds, when not maintained under vacuum, lose their viability in 5–10 years.

Second Law of Thermodynamics

Much biological work is directed at applying physical and chemical principles to biological systems, and—not surprisingly—in most cases these biological systems conform to physical laws or rules of chemical reactivity. This only proves that conventional physical and chemical processes prevail in biological systems, but it does not explain the basic problems of life. The Second Law of Thermodynamics, which requires that the equilibrium state is the state of maximum entropy (or randomness), cannot encompass the living condition, which is a continuous refutation of this second law, for living matter evades the decay to equilibrium. Life is not an equilibrium condition; it is not a condition of maximum entropy. Actually life is a question of increased enthalpy, in which energy is gathered at a greater rate than it is dissipated, building up more and more living cells, which only after death follow the general rule of increasing entropy. In the absence of life, in the absence of water, there are no energetic cycles, and the Second Law of Thermodynamics rules absolutely, as on the surface of the moon.

The dividing line between a living and a dead cell has not been found; as far as we know, the structure and configuration of an instantly killed cell remains unchanged, and the chemical constitution initially also is identical. These premises are the essential foundation for almost all biological and biochemical research carried out in the past.

A number of explanations have been given for this discrepancy between living and dead. It has been claimed that an essential part of the fine structure, not caught by

the finest of our observational instruments, is destroyed. Thus the separation of the cellular components would be violated; enzymes and substrates would come into contact indiscriminately, and no proper sequence of reactions would be possible any longer. Another viewpoint is that certain higher levels of integration have been destroyed. Thus the death of a person usually involves only the nervous and functional integration of the body as a whole, whereas the individual cells remain alive for a considerable time; hair continues to grow. These two viewpoints are diametrically opposed: one seeks the problem of life on the molecular level; the other looks for it on the integrated level.

To me the most fundamental aspect of life is polarity or directedness, which we find on the molecular level in the synthesis of compounds, in the accumulation of ions, in the maintenance of a low level of entropy. We also find it in development, which is directed continuously towards increased size and differentiation or ontogenesis; it is unidirectional. And in evolution we find the same principle again: the tendency towards greater and greater complexity, which is often called orthogenesis.

The fact that any living organism is a refutation of the Second Law of Thermodynamics, which requires in all physical systems a continual increase in randomness, the entropy, is often glossed over by pointing out that the organism plus its environment as a totality follows the second law. This is essentially a reaffirmation of the first law. But this does not explain how parts of this system continuously violate this law up to the moment of death, when immediately the Second Law takes over again. That much more clarification and clear thinking is needed in the application of the Second Law of Thermodynamics to life phenomena is evident from the conclusions reached in two recent books on thermodynamics. Spanner, in his 1964 *Introduction to Thermodynamics,* questions the applicability of the Second Law to life. He claims that certainly memory, evolution, and life itself fall outside its bounds. Morowitz, in his *Entropy for Biologists* (1970), holds the opposite view: "At all levels life is very much subject to the Second Law of Thermodynamics. . . . Dissipative processes inherent in the random distribution of thermal energy act to constantly degrade biological structures. . . ." But this holds only when death supersedes life. Earlier Morowitz states: "The very ordered state of a biological system would, if left to itself, decay to the most disordered possible state." This, of course, is what happens after death. To counteract this leveling, "work must constantly be performed to order the system. The continuous performance of this work requires a hot source and a cold sink, which are ordinarily provided on the earth's surface by the heat of the sun and the cold of outer space." This is quite true, but Morowitz fails to add that just the existence of life makes the process go; on the moon the same sources of heat and cold exist, but without the ordering effects of life, no work can be performed and the moon surface remains completely inert.

Phototropism

I discovered another aspect of organismal polarity while trying to analyze phototropism in terms of auxin. Since coleoptile growth was completely limited by auxin supply and auxin was light stable, it should be possible to measure the phototropic curvature—a process involving growth—in terms of the amounts of auxin diffusing

from the tip. When I measured the amounts of auxin produced by the coleoptile tip, I found that the total amount was only slightly decreased by illumination (16%), but that this auxin was redistributed by lateral illumination; compared with an oat seedling in darkness, during the first hour 54% diffused down the illuminated side and 114% diffused down the dark side, whereas in the second hour after illumination practically all auxin moved down the dark side. This was the experimental evidence for the Cholodny-Went theory of tropisms, for which Dolk provided the proof for geotropism. This evidence was completely clear cut, yet for almost two decennia it was attacked until work with radioactive indoleacetic acid fully confirmed our results. The fascination of radioisotope work was finally able to overcome prejudices against equally exacting pre-isotope work.

Auxin and Cofactors for Growth

One aspect of my thesis with which I felt most satisfied never has been commented on since. This was the analysis of the growth of the whole oat coleoptile. As in other plant organs, the region of maximal growth occurs at some distance from the stem tip. Since I could show that auxin was produced in the coleoptile tip only, there had to be some explanation of why the tip did not grow at the greatest rate. The explanation came by considering two sets of facts: (*a*) the region of maximum growth in the coleoptile shifts with age: when young its base has the fastest growth rate, but as it grows the zone of most rapid growth moves up and stays at an even distance from the tip; and (*b*) in the quantitative *Avena* test for auxin there is a sharp break in the auxin concentration-growth rate relationship; below a critical curvature angle there is a direct proportionality between applied auxin and curvature, and above this angle (17–20°) there is no effect of an increased auxin concentration at all. These two facts could be explained by a single assumption: auxin had to interact with another growth factor to produce growth, and this growth substance X was supplied from the base of the coleoptile. This meant that near the coleoptile tip auxin was in excess and did not limit growth, but nearer the base, where factor X was in excess, any variation in auxin concentration would show up in a variation in growth rate.

This two-factor model for growth has fascinated me all these years and has been the source of much frustration as well. After it was found that auxin was involved in many other growth and morphogenetic processes such as root initiation, it became very clear that one single substance could perform so many different functions *only* if it interacted with a whole array of different cofactors, which I referred to as calines. Indirect evidence for their existence was very clear, yet attempts to extract and to isolate them failed.

Translocation of Growth Factors Across Graft Unions

I then tried to approach the problem of the existence of other growth factors in a different way, namely by grafting. When stems are cut off, their growth stops almost immediately in spite of the fact that auxin production in the stem tip and leaves continues for some time. This cessation of growth is not due to lack of water or nutrients or any other materials which can be supplied to the cut stems. However,

when new roots are produced on these cut stems growth resumes, and this also happens when the stems are grafted on a root stock, after vascular connections between stock and scion have become established. This seemed to indicate that a factor X was produced by roots, and was translocated to the growing point through the phloem.

Etiolated peas were used as experimental material since (*a*) they could be raised under the completely controlled conditions of a physiological darkroom (this was in the days before air-conditioned greenhouses); (*b*) many pea varieties were available, differing in leaf form and size and in morphological characters; and (*c*) their seeds have so much storage food that pea plants can grow for several weeks in darkness without running out of food. The outcome of these experiments, published with Hayward, was interesting for several reasons. In the first place, there were no differences between stem growth of the scions when put on stocks (their cotyledons attached) with different growth rates. This indicates that factor X, required to produce stem growth in conjunction with auxin, was not specifically stored in cotyledons, but was produced in sufficient quantities by the root systems regardless of variety or growth habit (dwarf, tall, and slender). In the second place, big differences were seen in leaf growth on the scions according to the genetic constitution of the stocks: pea varieties with large leaves (e.g. Daisy or Marvel) produced much larger leaves on the scions than small-leafed varieties like Alaska or Perfection. Leaf size of the scions apparently was determined by different amounts of stored phyllocaline in the cotyledons of the stocks. The third conclusion was perhaps the most interesting. Morphological characters such as multijugateness ("Acacia leaf") or "stipuleless" were not transmitted by grafting: they depended exclusively on the genetic constitution of the scion. Therefore the following statement seems pertinent: quantitative characters under genetic control are expressed through hormones, transmittable from organ to organ. Qualitative genetic characters are expressed through intracellular processes and are not hormonally controlled.

In the late 1930s, while I carried out these pea grafting experiments, I also tried my hand at grafting tomato varieties. My technique was poor, however, and I made only a few successful grafts between potato-leaf, wiry, and other tomato varieties, with a slight indication of transfer of leaf characters across the graft union. With a better technique I might have become just as famous as Lysenko, about whose grafting experiments I learned several years later.

CONTROL OF THE PLANT ENVIRONMENT

After Thimann and I had written *Phytohormones,* and when my quest for the elusive cofactors of auxins bogged down, a remarkable event occurred. Dr. H. O. Eversole, a retired physician, offered to build two air-conditioned greenhouses for me after he had succeeded in air-conditioning his own orchid house with the help of an engineer, A. J. Hess.

These first air-conditioned research greenhouses were financed by Miss Lucy Clark and built at Cal Tech in 1939. The preoccupation of the geneticists and plant

scientists at Cal Tech with biochemistry kept them from realizing the extraordinary opportunities these Clark Greenhouses afforded for studying the environment and its role in plant development. Besides, they viewed heredity exclusively from the genic standpoint, disregarding a possible environmental involvement. This of course enabled me to use these greenhouses myself without any outside interference, and with a number of collaborators who came to employ these new facilities, many new facts were established. I could operate and modify these greenhouses without any objections, getting a very liberal education in air-conditioning and climatic control. And at the same time, having the greenhouses always full of plants, I learned a lot about the proper growing conditions for plants and the effects of temperature on them.

With the new greenhouses I had to develop new experimental plants. After comparing many plants I finally chose the tomato, which responded usually within 24 hours to changes in the environment. Their response could be measured easily in terms of stem length, provided they were kept pruned to a single stem. And their fruit set was very sensitive to the proper temperature regime: at 17°C night temperature fruit set was excellent, especially when combined with 26°C day temperature. This diurnal thermoperiodicity turned out to be one of the most important climatic responses of most other plants as well.

Amateurs in Science

When he offered to build the air-conditioned greenhouses for me, Dr. Eversole had expected such a specific temperature response, based on his experience with *Phalaenopsis* growing in his own air-conditioned greenhouse. Not being a practicing scientist himself and therefore not bound by the rigid rules of scientific experimentation, he had manipulated his thermostat until he found the ideal conditions for growing *Phalaenopsis,* and that was 26°C during the day and 20°C during the night. Only a person with the remarkable observational powers of Dr. H. O. Eversole could have thought of such decontrolling of a thermostat, and it violated all rules of a rigid scientific approach demanded by reviewers of papers to be published in scientific journals. This excludes most amateurs from contributing to science, which is a great loss, for we have to admit that all experimental science started through amateurs. It was not the official scholars, professors at universities, who started the era of the experiment. It was Francis Bacon, whose thinking stimulated men like R. Boyle and R. Hooke to carry out the first systematic experimentation, who originated a causal approach to nature and who started what now has become the Scientific Revolution. Transpiration, plant nutrition, and photosynthesis were discovered by country gentlemen and practicing physicians, and it was a country gentleman, Charles Darwin, who brought on the greatest change in biological thinking.

Thermoperiodicity

In the succeeding years I found more and more cases in which optimal growth and development of plants occurred at a higher daytime temperature and a cooler night, leading to the concept of diurnal thermoperiodicity. In the course of the next 30 years it became accepted with the same universality as photoperiodism, and any

air-conditioned greenhouses or controlled environment growth rooms used for the routine growing of plants are now maintained at higher light and lower dark temperatures. The optimal daily temperature differential differs from plant to plant, but it is lowest for tropical rainforest plants (3°C for coffee), intermediate for most crop plants (6°C for tomato and corn), higher for dry region plants, and extreme for desert plants.

In addition to crop plants, about a dozen garden flowers were tested in the Clark greenhouses, and two dozen California wild flowers. The latter had not been selected for genetic uniformity, and varied considerably in their response, but all showed a preference for daily thermoperiodicity, and most were long-day plants.

In addition to thermoperiodicity, the experiments in the Clark greenhouses showed that greenhouse-grown plants would resemble field-grown plants in appearance, sturdiness, and productivity when the climatic regime in the greenhouse approximated that in the field. This became apparent when several tomato varieties grown in the field plots in coastal and inland locations in California were compared with the same varieties grown in the Clark greenhouses. I could estimate within close limits the night temperature in each field location by observing the appearance and fruit production of the different varieties, especially Beefsteak and Earliana, with whose behavior I was familiar from the greenhouse experiments.

When I had similar experiences with garden plants and wild California plants that also showed a correlation between greenhouse and field behavior, it obviously was time to launch a campaign for extending the air-conditioned greenhouse approach to plant growing in general. I convinced Dr. R. A. Millikan, chairman of the executive council of Cal Tech (equivalent to the position of president), of the importance of building a set of air-conditioned greenhouses and artifically lighted growing rooms in which the whole range of naturally occurring climates could be maintained. He in turn persuaded his friend, Mr. H. Earhart from Ann Arbor, Michigan, to provide the funds for them, and in June 1948 the building of the Earhart Plant Research Laboratory was started, to be completed a year later. Since nothing like this Pasadena phytotron (under which name the Earhart Plant Research Laboratory became known) had ever been built, it would have been very difficult to obtain tax funds for it. We can compare this situation with the first astronomical observatories and cyclotrons which also were privately financed.

Productivity

Of the many problems that have been investigated with the facilities of the phytotron, I would like to mention only a few. One of these is productivity, based on actual dry matter production over a period of 1–2 weeks' growth. The tomato plant was again the main experimental object, one reason being that it does not store food but uses all recently produced photosynthates for growth. Plants placed in darkness stop growing within 36 hours, when all available sugar has been consumed. After that, growth can resume upon sugar application in darkness. Sugar analyses had shown that in a tomato plant only sucrose is photosynthesized and metabolized; monoses remain constant and apparently are stored in the vacuole of the cells where they produce turgor but are unavailable for growth. During the first 7 hours in

daylight, the sucrose content of a tomato leaf increases form 1% to 7% in a sigmoid way: first slowly, then for a few hours at a maximal rate, and in the final hour slowing to practically a standstill. The maximal photosynthetic sugar production within 7 hours of exposure to light was confirmed in a field experiment with tomato plots being covered with black cloth 5, 6, 7, and 8 hours after uncovering them in the morning. After 7 weeks, those receiving 7 hours of daylight were the heaviest and had produced 10 times the fruit weight of the uncovered controls.

Work based on dry matter production over a week's period showed that total photosynthesis was entirely limited by the amount of growth; for example, reducing growth by cutting off roots or stem tips reduced dry matter production to the same extent. Therefore, growth of the tomato plant is not controlled or limited by the amount of photosynthesis, but photosynthesis is limited by the amount of growth and the degree to which the plant can utilize its photosynthates. Under ideal growing conditions young tomato plants can transform 9.4% of the light energy falling on them into chemical energy, and this is only a fraction of the efficiency of the photosynthetic process.

This same limitation of photosynthesis by growth was found in experiments illuminating tomatoes with different colors of light. The efficiency of light utilization was the same for blue, red, and a combination of red and blue light. It was less for white light because of the poor absorption of the green part of the spectrum. But total growth was many times greater in a combination of red and blue light, and therefore, even though the efficiency at low light intensities was the same, maximal yield was low in either blue or red light.

In white light the saturating light intensity (when supplied at an 8-hour photoperiod per 24 hours over a 7–12 day growing period) for tomato plants was about 1200 fc. This means that even though for short periods the saturating light intensity for photosynthesis might be much higher, any light supplied for a week over an 8-hour photoperiod above 1200 fc is wasted. With the same experimental setup (young plants covering the pot surface fully with their leaves), the saturating light intensity was the same for sugar beets, strawberries, and other plants as it was for tomatoes. I believe that my experiments on efficiency of light absorption came closer to field conditions than short-term determinations obtained with gas-analytical methods. In this connection I showed that the geometry of leaf position greatly influenced the saturating light intensity of a leaf canopy, and that in most crop plants the leaf inclination was such that daylight was used at an optimal rate.

Circadian Rhythm

On the basis of the information presented earlier, I concluded that plant production could be improved by supplying the photosynthetic light in shorter bursts, allowing the sugar content to decrease in the photosynthetic cells between illuminations, and presumably having the plants make better use of the supplied light. This worked only when a 2 or 4-hour dark period was interposed between two 4-hour light periods, but growth and dry matter production was greatly reduced when the second 4-hour light period came 8 hours after the first. In the latter case the explanation obviously had to be Bünning's, namely that light during the skotophil phase was

inhibitory. This brought me right into the problem of circadian rhythms, which were already under investigation in the Earhart laboratory. It had been found that tomato plants could grow well in a constant environment *only* if there were dark interruptions of the light on a 24-hour cycle; or if there was a temperature fluctuation on a 24-hour basis. Kristoffersen actually found that the optimal length of either the light interruption or the low temperature treatment was 6 hours per 24 hours. This strongly suggested that it was not a dark reaction or a low temperature process that was essential for normal growth of a tomato plant, but that an external *rhythm* could satisfy its circadian requirement.

This external rhythm could be treated quite quantitatively in experiments. When tomato plants were grown at 23°C, a light-dark succession of 12–12 hours was optimal; when the light-dark periods had a 22 or 27-hour cycle length, growth was less, and it was still less on a 20-hour cycle. When the optimal cycle length was determined at 15°, it was 27 hours, and at 30°C it was 20 hours. This indicates a Q_{10} of the circadian rhythm length of about 1.2–1.3. When other plants were investigated this way *(Baeria, Saintpaulia),* they showed the same response to a circadian rhythm in the environment, with a similar temperature dependence.

This is the first case in which it has been shown that an external circadian rhythm is essential for the normal functioning of an organism. This proves that in these plants an internal circadian rhythm exists that has to be driven by an external rhythm. Without this induced internal rhythm the plant cannot function properly. This is essentially what Bünning called the photophil and skotophil phases of a plant, and for which he showed that they could be "set" by an external clock. And it may well be this process that Brown stresses in his solar-day, lunar-day, and annual clocks controlling biological processes, which he shows being synchronized by an external clock.

The Nature of the Circadian Clock in Higher Plants

Throughout this work I have, of course, been speculating on the basis for the need of a plant for such an external circadian rhythm. When we observe tomato plants or African violets that did not receive the proper rhythm from an external clock being grown under constant conditions, we see several abnormalities. The first is a gradual general deterioration of the plant, a process that takes weeks or months, and if continued long enough leads to death. An African violet plant dies in 4–5 months when kept in a constant 10°C temperature, yet it grows well at 26°C, whereas an English daisy dies within 2 months when kept at 26°C but grows very well at a constant 13°C. The second deviation of the plant kept at the wrong constant temperature is a decrease in leaf size and the production of malformed and chlorotic leaves. And the third is a gradual decrease in stem growth rate, increase of the plastochron length, and an abnormal growing tip. These can all be reduced to one common denominator: a disturbed apical meristem. How can we imagine a mechanism for this disturbance?

For a long time investigators have looked for the process that regulated cell divisions in the apical meristem of plants. It was thought that it might be something

like Spemann's organizer or at least some hormone controlling the sequence of cell divisions that leads to leaf differentiation and flower initiation. But no real evidence was found for the existence of such substances or hormones. The closest we have come to them is florigen. But active extracts have been found effective only on plants that need a single short-day cycle for induction, and the effect of these extracts never exceeds the response to a single inductive cycle.

Cytologists had known for a long time that to obtain good preparations of mitosis, growing points had to be fixed at a particular time of day, usually around midnight. This was measured quantitatively by Bünning, who showed that most mitoses in the stem apices of *Tradescantia, Perilla,* and spinach actually occurred just before or after midnight. As has been shown for algal and other cultures, such synchronization of mitosis can be induced by a rhythm in the environment. Therefore I assume that the cells in the meristem are synchronized by an environmental signal such as light-dark or high-low temperature rhythms. Thus the rhythm in the environment performs the same controlling role in a meristem as a hormone plays in stem elongation.

Taking this argument one step farther, I conclude that the photoperiodic stimulus in the apical meristem of a long or a short-day plant also is a rhythmic one. Since it comes from the leaves and can be transmitted by grafting, I further conclude that "florigen" or "anthocaline" is a factor periodically produced in the leaves of photoperiodically induced plants. This might be related to the daily periodicity of auxin production which Yin discovered in papaya leaves. And it suggests that extracts applied to induce flowering be supplied on pulses of 24 hours.

Which Factors Ultimately Limit Growth?

In our age of biochemistry we think of all biological processes as being controlled by chemical reactions, hormones, enzymes, or DNA. This cannot be the case in circadian rhythms, which have a Q_{10} of slightly above 1, indicating that a physical process such as diffusion is in control. This was found to be the case also for the "master" process in growth, under optimal growing conditions when no chemical processes limited growth. Ever since the Clark and Earhart greenhouses were built, I have been trying to increase the growth rate of tomato plants by changing their temperature and light regimes, water and chemical supply, genetic constitution, root environment, and CO_2 supply, and finally I reached a steady state rate of 42 mm/24 hr. This can be exceeded for a few days only when the plants are kept at a suboptimal night temperature, followed by nights at a supraoptimal temperature. Why can't a tomato plant grow at a greater rate? If a specific chemical were limiting, we should be able to supply this, either by application or by breeding, but no growth factors have been found to increase tomato stem growth beyond 42 mm/24 hr. If it were a chemical process, again a breeding program or a temperature treatment should be able to overcome this limitation, especially because the optimal temperatures for the growth of mature tomato plants (25° C during the day, 17° C during night) lie far below the optimum temperature of most physiological processes (at or above 40° C). Also, growth limits photosynthesis rather than photosynthesis limiting growth.

Taking these considerations and many other facts into account, I have come to the conclusion that it is the sugar supply to the growing tissues that becomes insufficient for faster growth. It would be interesting to breed a tomato variety with more and wider phloem tubes. But this might have to be done in a completely insect-free greenhouse where the plants could be fully protected from insect and mechanical injury, because any injury to such wide-vesseled phloem plants might be lethal if the sugar flow from a cut of the phloem could not be stopped.

Yet even if the sugar supply of the growing cells could be increased, there is another—and absolute—limitation on growth. This is an internal diffusion process inside the growing cells, where nucleic acids and messenger RNA have to interact with the cell constituents that do the growing. Since a diffusion process varies inversely with the second power of the linear dimension, one would expect that the maximum growth rate of 1 μ bacterium would be 100 times as fast as that of a meristematic cell with a linear dimension of 10 μ. Since a mitotic division in the growing point of a higher plant occurs about once a day, a 1 μ bacterium should be able to divide every quarter hour (which actually has been found), whereas the growth rate of a tetraploid with bigger meristematic cells should be only half that of a diploid (although the overall size of the tetraploid could be more). This certainly would explain also why mature plant cells seldom divide.

My general conclusion is, therefore, that while the immediate control of plant growth is based on hormonal supply and metabolic processes, the ultimate control is by diffusion processes (including a circadian one), which are not as yet experimentally managable.

Variability and Air-Conditioned Rooms and Greenhouses

It is obvious that under well-controlled conditions the variability of plants should be less. But it is not generally recognized to what remarkable extent the variability is reduced in air-conditioned greenhouses. Significant treatment differences of only 10% in weight or size can be established with groups of 4–10 genetically uniform plants. Even more important is the degree to which reproducibility is improved. Since there are far fewer unintentional variables of temperature, light, nutrition, soil, pests, diseases and weeds, the response of plants under controlled greenhouse conditions is very much alike from experiment to experiment. An unexpected benefit from growing plants in air-conditioned rooms and greenhouses is that under optimal growing conditions variability of plant material is least. This has to be explained by the fact that even though growth rates are highest, it is not a single factor which controls growth, and therefore fluctuations in any one factor will have little effect on overall response. In general, the work under properly controlled conditions makes it possible to eliminate almost all variability deriving from the environment. This leaves only (*a*) the genetic variability, which can be reduced to very low levels either by using clonal material or by breeding; and (*b*) the basic variability within any physical system. Yet this innate statistical variability is unexpectedly low for a system as complicated as a living one, a fact on which both Bohr and Schrödinger have commented. Since it is possible to work so close to this low innate statistical variability of biological material, it is illogical and inefficient to continue using

ordinary uncontrolled greenhouses for any research work. Commercial greenhouse growers have discovered this, and most rose or orchid or carnation growers have installed quite effective evaporative cooling systems in their greenhouses to control daytime temperatures, in addition to their heating systems for night temperature control. But too many university greenhouses are still uncontrolled, partly because of a lack of comprehension by administrators who consider money spent beyond salaries of research personnel as wasted, even though each research worker would be many times more efficient if he could carry out his experiments under controlled conditions. The National Science Foundation has recognized this fact by supporting the construction and operation of a number of phytotrons, but obviously it cannot underwrite the construction of every research greenhouse.

ECOLOGY

One of the major fields of research in the Earhart and Clark Laboratories obviously had to be ecology, since at last most of the environmental factors in the growth of a plant could be controlled, and their effect could be assessed. Thus the autecology of quite a number of plants, especially of cultivated ones, was determined to the extent that their behavior in the field could be predicted. Fortunately, in most plants only one or two environmental variables have an overriding effect on their climatic response, variables such as night temperature in tomatoes and day temperature in peas. This has not been realized by some ecologists who demand that all temperature fluctuations and photoperiods occurring in nature be religiously recreated in a phytotron to study the response of their plants. This defeats the purpose of a phytotron, for the significance of none of the factors or fluctuations can then be interpreted; such experiments should be conducted in nature, using the fluctuations of the natural environment. The facilities of the Pasadena phytotron and of most others were not designed to operate that way. Each greenhouse and each controlled environment room is being used by many investigators in many different experiments to study the effect of particular day and night conditions in many plants. The differences in response between tomato, potato, pea, sugar beet, strawberry, coffee, orchids, ecotypes of *Poa*, and *Mimulus* were thus dramatically illustrated and showed us many generalities, which would have been lost in the minutiae of exact climatic duplications.

In general it was found that the optimal growing conditions of a plant agree closely with the prevailing climate of the native habitat of the plant. Thus the range of plants being grown in field or garden gives an excellent idea of a local climate, provided their climatic responses are known. The further a plant is removed from its optimal climate, the more it has to be babied by the grower to keep it growing, and the more it has to be kept free from weeds. The work also showed the irrationality of maintaining separate orchid and fern and cactus greenhouses. What is needed are greenhouses in which specific climates are maintained, and plants with those climatic requirements should be grown together in them regardless of taxonomic relatedness. This was the basis for the air-conditioning arrangement in the climatron-greenhouse which I built in 1959-60 at the Missouri Botanical Garden in St.

Louis, where in different areas different climatic conditions could be maintained, resulting in optimal growth for plants from different regions of the world in the various sectors of the climatron.

The *Annual Review of Plant Physiology* is not the best place to discuss work on the ecology of plants. But since ecological studies have been an important part of my research work, I want to at least mention them. In Java, where for 5 years (from 1928–1932) I was employed at the Bogor Botanical Gardens, I started ecological work for two reasons: first, purely physiological studies can be carried out anywhere in the world, and the laboratories in temperate climates were better equipped for such work; and secondly, I wanted to lay a basis for more detailed physiological studies. I worked on a typically tropical subject, epiphytes, and found in a tropical rain forest that the different species of most trees harbored quite different communities of epiphytes. Only the epiphyte communities growing in humus accumulations in crotches of trees or in nest ferns were nonspecific. Especially orchids were very specific in their host tree, and I could identify trees by the orchid communities growing on them.

Desert Plants

While living in Pasadena (1933–1958) I soon became fascinated with the desert, and our family spent many weekends in the Mohave and Colorado deserts. A number of biological desert problems attracted my attention, none of which could be answered on the basis of available information. So I started to observe and measure desert plants, and after the Clark greenhouses were built, I could study the behavior of them under controlled conditions.

The first problem was their curious seasonal response. There are two periods when occasional rains occur in the Southern California deserts: midsummer, and late autumn and winter. And rain of more than an inch is followed by extensive germination. Most seedlings are annuals, and they occur in any one locality as two completely different communities: the summer and the winter annuals, with hardly a single species in common. Being reared on proper Darwinian principles, I looked for general germination of all species and survival of either summer or winter annuals. But in summer only the seedlings of summer annuals were found, and after an autumn or winter rain only winter annuals occurred. This could be confirmed in the air-conditioned greenhouses. The upper half centimeter of the desert soil with its normal seed complement was collected and this was spread thinly over containers with sand. When watered properly and placed in greenhouses at different temperatures, only summer annuals germinated at 26°C, only winter annuals at 8°C, and a combination of both at intermediate temperatures. Therefore, the species composition of the vegetation is not determined by selection and survival of those seedlings adapted to the prevailing temperatures, but by preferential germination. I also found that simple wetting of the desert seeds was insufficient for germination; a soaking rain was required. This was based on the leaching of inhibitors from the seeds by the rain. All this laboratory information was taken back into the field, and now it has become possible to predict desert blooming many months ahead, when the

amount of rain and the temperatures following them are known. Conversely, it is possible to deduce the amount of rain and the date of its occurrence from the vegetation; it is even possible to tell summer rains many years after their occurrence, because of shrub germination and growth. Thus field observations led to laboratory analysis that could be taken back to the field to explain and understand what is happening in nature.

Mycorrhiza

Another subject that I first observed in the field and subsequently brought into the laboratory for further study was mycorrhiza. As a member of an expedition of the research vessel "Alpha Helix," I spent 1½ months in 1967 in the center of the Amazon basin. Impoundment of my laboratory equipment by Brazilian customs made it impossible to carry out my intended research program, so I spent my time in the Amazonian rain forest. There I found a tremendous activity of fungi in the upper soil layer where dead leaves, branches, and all other debris from the rain forest produced a litter layer completely pervaded by tree roots, fungal hyphae, and rhizomorphs. With this mass of hyphae digesting so much organic material, one might expect a very extensive development of mushrooms and other fungal fruiting bodies on the rain forest floor, but mushrooms are remarkably rare in the tropics. What I actually observed was an intimate network of hyphae and rhizomorphs between litter and tree roots, and most of these roots pervading the litter were mycorrhizal. Thus it became clear that mycorrhiza is not just a tree root-fungus association, but that it is part of a tripartite system. The fungi digest the litter and pass much of the extracted nutrients back to the tree roots, closing a nutrient cycle without which a rich rain forest never could exist on the very poor and leached soils of most of the Amazonian basin.

I hope that realization of this basic fact will become generally accepted by developers of the Amazonian rain forest. Utilization of temperate-zone agriculture (based almost exclusively on annual crops) has led to irreparable damage to untold Amazonian acres. If the original rich rain forest is replaced with an equally rich forest of economically useful plants such as Brazil nuts, oil palms, or cacao trees, the Amazon basin could become a real food basket of the world. But to this end a typical tropical agriculture must be developed based on leached soils, perennial crops, and mycorrhiza.

A somewhat similar situation was found in the desert, where most decomposition of plant litter is accomplished by fungi, and where mycorrhiza also occurs on the roots of a number of desert shrubs. And a considerable part of the consolidation of the desert sands, and even the fixing of dunes, is due to hyphae weaving sand grains together. But this is only possible, of course, when sufficient organic matter is present for fungal growth.

The realization of the overriding importance of litter in the mycorrhiza picture led me to a number of experiments in which pine seedling growth was quantitatively linked with the amount of decomposing litter in the pots. Humus, the end product of litter decomposition, had much less influence on the pine growth.

AIR POLLUTION

Much of my research work has been centered around effects of the environment on plant growth, and this brought climate to my attention. Then in the late 1940s another environmental factor, air pollution, started to require more of my attention. And ultimately this led to a realization that plants not only were passively responding to climatic factors, but actively changed them, in addition to their known role in energy transformation.

I have had occasional experience with toxic gases in the air. The first was SO_2 from a zinc smelter in Holland, then SO_2 from an undersea crater in Indonesia, and later SO_2 from a sulfuric acid factory in California. In all cases white bleached areas appeared on leaves between the main veins. Then I became acquainted with HF damage, caused by smoke stack emissions from a steel plant, an aluminum metal reduction plant, and superphosphate factories. This was typified by leaf-edge burn and brown discolored areas on corn and grape leaves. Then in the autumn of 1948 an entirely new type of plant damage started to appear on tomato seedlings and spinach plants in our Pasadena greenhouses. We first tentatively attributed it to fungicides. But this did not make much sense since (*a*) it occurred irregularly; (*b*) it never had occurred before; and (*c*) commercial spinach growers around Los Angeles suddenly started to complain about damage to their crops too, on the same days the spinach in our greenhouses was injured. This was not due to an emission of toxic materials from a point source, as in the case of SO_2 damage, but it was an areal occurrence including the whole Los Angeles metropolitan area. This was a new phenomenon. It occurred each time there had been an excessive number of complaints by the public of eye irritation, which was associated with dense blue hazes in the morning, the so-called Los Angeles smog.

Smog

Up to that time it had been assumed by the air pollution control authorities that the Los Angeles smog was just another form of SO_2 pollution. I rejected this assumption because (*a*) the plant injury symptoms did not agree with the intercostal bleaching of leaf areas caused by SO_2; (*b*) my nose had never alerted me to excessive SO_2 concentrations; and (*c*) SO_2 damage on plants normally had occurred only near concentrated SO_2 emission points, which hardly existed in the Los Angeles area. No SO_2 damage to vegetation had ever been observed in or near any city. Besides, my colleague, A. J. Haagen-Smit, a biochemist with a most remarkably sensitive nose, had identified Los Angeles smog with oxidants (ozonides and peroxides) produced when unsaturated hydrocarbons (olefins) react with ozone. He not only produced a product which looked and smelled like smog by reacting olefin vapors with ozone, but he showed how in the Los Angeles atmosphere these oxidants were produced by a photochemical process. This occurred whenever a high enough concentration of gasoline vapors or of exhaust gases from internal combustion engines was exposed to full sunlight in the presence of a catalyst such as nitrogen oxides. Such high concentrations of gasoline vapors could develop under low atmospheric temperature inversions, which were common in the Los Angeles area.

As a joint venture of Cal Tech, the University of California, and the Los Angeles County Air Pollution Control District, I organized a research team that used the facilities of the Earhart Research Laboratory (just inaugurated at that time) to identify the phytotoxic component of smog. In this team I was fortunate enough to combine the services of both A. J. Haagen-Smit, the later chairman of the important California Air Resources Board, and J. Middleton, the later U.S. Pollution Commissioner. In the specially designed gas chambers of the newly opened phytotron they tested all organic gases that were or could be present in the Los Angeles smog. Many different organic acids, hydrocarbons, aldehydes, ketones, and chlorinated compounds were tested singly or in combinations, but none produced smog injury symptoms on the five different test plants (spinach, endive, beets, alfalfa, and annual blue grass). It was not until we tried Haagen-Smit's olefin-ozone mixture that the typical smog injury symptoms developed on our test plants. This plant work signaled the change in attitude of air pollution control officials towards the identity of the toxic materials that had to be combatted in smog. Although more than half the SO_2 emissions had been removed from the Los Angeles atmosphere during the years that this gas was thought to be responsible for smog, the latter had not diminished. Combatting the oxidants, however, has prevented further deterioration of the smog situation in Los Angeles after 1954, which is more than can be said for most other metropolitan areas here and abroad.

The blue smog haze that accompanied eye irritation and the acrid smog smell apparently were due to the photochemical production of submicroscopic particles such as had been experimentally prepared by Tyndall a century ago. At that time he passed a beam of actinic light through air charged with organic vapors of amyl nitrite or allyl iodide. The developing "blue cloud" as he named it was due to the production of submicroscopic particles on which water vapor could condense and which could be measured conveniently with an Aitken condensation nucleus counter. This instrument is now available in a very convenient form, the Gardner small particle counter, which gives an excellent measure of the degree of air pollution.

We had thus a number of independent methods of measuring "smog": (*a*) an acrid smell and eye irritation due to oxidants; (*b*) a blue smog haze due to condensation nuclei; and (*c*) leaf damage to various plant species. By these criteria I estimated the amount of smog in different parts of the world, and I found typical photochemical smog in most of the metropolitan areas of the world: in all big North American cities, in South America (Sao Paulo, Rio de Janeiro and Bogota), in Australia (Melbourne, Sydney), and in Europe (London, Paris, Cologne, Copenhagen). An approximate analysis indicated that as soon as gasoline consumption in a city exceeded 12 tons per square mile per day, smog damage was visible.

Natural Smog in Nature

In my preoccupation with photochemical smog, I started to see smog hazes not only in cities, but also in the surrounding countryside. At first these were attributed to Los Angeles smog spilling over mountain passes. But when flying cross country I saw these blue hazes over the intermountain area and all over the east, becoming

denser near big cities like Chicago and New York, but being essentially constant in between. Therefore, I had to conclude that there were sources other than gasoline vapors and exhaust gases that gave rise to the blue hazes in the countryside. Many geographical names were based on such hazes. Thus there are the "Blue Ridge" and the "Smoky Mountains" in Virginia and North Carolina, or the "Blue Mountains" in Australia. This blue haze is not a smoke (consisting of visible particles), since a smoke has the color of the actual material of which it consists, such as brown iron oxide, white calcium carbonate, black soot, or grey or yellow clay dust. And there are hardly any blue minerals. But submicroscopic particles of any kind will seem blue. On this basis it could be concluded that the blue "summer" or "heat" haze consisted of submicroscopic particles that did not arise by diminution of larger particles, but which only could arise from originally molecularly dispersed chemicals aggregating to particles with a molecular weight of millions. Therefore, I started to look for gases in the air which might react like the gasoline vapors that produce smog. And I found them in the aromatic substances given off by plants. Then it became clear that blue hazes were seen all over the world where vegetation occurred. In the Amazon basin I measured low numbers at ground level, but higher up in the atmosphere enormous numbers of Aitken condensation nuclei (ACN) occurred: over 1,000,000 ACN/cc. Over forested areas in the Midwest 30,000 ACN/cc occurred during summer, while in deserts the numbers were 2,000–10,000 and over oceans there were less than 1000/cc. There was also a strong positive correlation between ACN and the density of the vegetation in the desert. After a rainy winter, when lots of annuals had developed on the desert floor in March, the number of nuclei was at least double that of other years.

With a gas chromatograph the terpenes free in the country air can be measured. They fluctuate from about 2×10^{-9} g/liter of air in winter to 10×10^{-9} in summer and occasionally 20×10^{-9} in autumn. The pattern of their release by plants is much like that of transpiration: a maximum rate around noon, and a complete release upon death of the cell. In addition, ionone and irone are released in autumn upon decomposition of carotenoids in the fallen foliage. They provide the typical late-autumn smell of forests, and after condensation and coagulation of their photochemical reaction products they, together with terpenes released from the dying cells, are responsible for the dense autumn hazes.

Both in the laboratory and in nature I was able to reproduce the photochemical production of condensation nuclei, combining terpene vapors with ozone, or mixing terpenes and a catalyst (such as nitrogen oxides or iodine vapors) in strong light. There is no doubt about terpenes being able to produce ACN and blue hazes. In darkness, such as in caves or during night, no ACN are produced at all. But there are also other sources of ACN. In and around cities they come from combustion processes: exhaust from car engines, burning of coal, oil or gas, cigarettes, etc. And perhaps most of the pink haze one sees over the ocean consists of salt particles produced by bursting air bubbles. And a small amount of haze may be of volcanic origin. But otherwise all atmospheric hazes are derived from the vegetation. How does this fit quantitatively?

Quantity of Natural Smog on Earth

Photosynthesis produces yearly 2×10^{11} ton of organic matter. Upon decomposition most of this escapes as CO_2 into the air. But 0.2% of photosynthates are carotenoids and phytols, which probably after decomposition become terpenoids. The amount of terpenes produced by the vegetation is hard to estimate, because ultimately they all volatilize. It varies much from plant to plant, but probably is at least 0.5% of all dry matter formed. This would amount to 1×10^9 tons of terpenes, and together with the decomposition products of carotenoids, as much as 1.4×10^9 tons of volatile plant products are produced over the whole world per year. They are photochemically transformed into oxidants or free radicals which condense to particulate matter, first of a size of $10^{-2}\mu$, but gradually grow by coagulation to particles of $0.1-1\mu$ diameter. The latter particles can be filtered out by sucking air through "absolute" filters. The filter paper turns grey or greyish brown, and microscopically the filtered particles are brown droplets or black soot-like clusters. These have been named combustion nuclei and were supposed to be industrial smoke coming from coal and oil fires. Their concentration is actually twice as high in cities as in the countryside, but I collected them equally in southern Patagonia, the middle of the Amazon basin, Death Valley, the Sierra Nevada, eastern Nevada, and Point Barrow (the northernmost tip of Alaska). Therefore, I have to conclude that they are of natural origin and that they are the end products of photochemical terpene decomposition. This is supported by their quantity. In big cities the average particulate loading of the air is $150\gamma/m^3$, in smaller cities it is $100\gamma/m^3$, in the forested areas of southeastern USA it is $50\gamma/m^3$, and in the west and northwest it is $20\gamma/m^3$. The few data I have from the tropics indicate $100\gamma/m^3$ or more. Averaging this as 50 γ/m^3 for the land area of the world (90×10^6 square kilometers) to a height of 2 km, there are 9×10^6 tons of particulate matter of plant origin in the air at any one time. If we assume a dwelling time of 10 days for each particle, there would be 0.32×10^9 ton of particulates in the air over the world in one year, about one-fourth of all volatile matter produced by the vegetation.

Some of the major questions about this particulate matter concern what it is, what it does, and where it goes. In answer to the first question, chemical analysis of the aerial soot has shown it to be very high in carbon (80–90%) and hydrogen, with little oxygen or nitrogen. This agrees with its origin from terpenes (80% carbon). As to the function of the hazes, they contribute perhaps as much as a quarter of the total long-wave radiation of the atmosphere. This is many times as much as the CO_2 radiation, and therefore, together with water vapor, they are largely responsible for the greenhouse effect of the atmosphere; the effects of an increase in CO_2 caused by the combustion of fossil fuels can be disregarded.

Fate of the Natural Smog

As to the question, where this black air soot goes, this is partly answered by the black or grey color of drapes in windows, collars and cuffs of shirts, and the dark covering of older leaves of trees and shrubs, especially if they contain sticky oils. But this

takes care of only a minute amount of all particulate matter. Most of it collects in the inversion layer of the atmosphere and in the surface of cumulus clouds, which especially in stagnant air masses become very dark. I have measured the condensation nuclei in cumulus cloud surfaces and found them to be concentrated 2–30 times. And we know from the dirt in rain and snow that much air soot is precipitated this way. The brown or black material that comes down by precipitation attaches itself to clay particles or accumulates under anaerobic conditions in bogs. The material on clay particles (microscopically visible on clay minerals in ponds and rivers) washes down rivers and accumulates in delta areas, which are the main source areas for oil accumulations. In bog areas the air soot is safe under anaerobic conditions and accumulates to produce anthracite or hard coal. If viewed this way, coal, which is largely an amorphous material, is an aerial product, and it is found in bogs not because it was formed there but because it is preserved there under anaerobic conditions. I was able to show that the high carbon content of fossil wood can be accounted for by impregnation of the fossil wood remains with high-carbon materials rather than decomposition of the low-carbon (cellulose) materials of wood. Thus I completely dissociate brown or soft-coal production (humifications of lignin) from bituminous coal formation.

I have no idea why either liquid drops or clusters of carbon particles result from the condensation of haze, but it is interesting that the major oil and coal forming periods in the world history coincide in time. And there are all intermediates between anthracites (low in bituminous material) through bituminous coals, asphalts, tars, and oil deposits.

CONCLUSION

Since I believe that my success as an investigator is partly due to qualities and training differing from those of my colleagues, I would like to analyze these. There is no doubt about the advantages I had in my early environment and training. Through teenage opportunity programs or high school summer training programs many young people have similar opportunities today as I had in my teens by associating as apprentices with mature scientists. And many children get a great deal of stimulation from science fairs or from a chance to work after school hours in high school laboratories. This may lay the motivation for their future careers and life. Children or students should be aware of the fact that their chosen profession will be with them throughout their lives, and that this choice better satisfy them. Let no one ever talk a child out of his own choice of a career, especially not if the objections are based on economic arguments. A dedicated scientist or technologist, no matter in what field, will find economically satisfactory employment.

Beyond opportunity, I was fortunate in my training. In Holland the high school training was very thorough, so that all language (French, German, and English) and humanities training, plus most of my chemistry, physics, and mathematics needs were satisfied when I left high school at 17 years of age. This meant that during my entire university training I could concentrate on biology. Its subject matter was certainly much more restricted than it is today, with only a little biochemistry,

cytology, or genetics, but that was compensated for by physiological, taxonomical, and morphological training, even more than most physiologists, taxonomists, and morphologists receive today. This is inevitable with the increasing specialization today, but I greatly profited from the thorough knowledge my father had of botany in general. He knew the entire plant physiological literature, having read every important paper ever published (and remembering its content). He personally subscribed to most botanical journals. He knew and was friendly with most botanists all over the world, and thus I came to know many of them.

As to my own attributes, the most important is probably an insatiable curiosity about the world around me, not just about auxins or temperature effects on plants, but about nature in general. This extends beyond physiology to evolution, morphology, and ecology, and beyond that to climate and other elements of the physical world. When I can figure out why a particular plant grows in a particular place, or how some desert plant is able to take up enough water to survive, or why different plants in different habitats have the same shapes, or why there are no unbearably hot places in nature (outside of volcanoes, of course), then my intellectual curiosity is satisfied and I can go on to other problems. Yet all my most interesting and elusive problems are rooted in nature, and that is where I obtain my inspiration and motivation. The solution of these problems requires in most cases a laboratory, but ultimately my satisfaction comes when the answer I obtain in the laboratory is applicable in nature. In this way I am still basically a naturalist, or perhaps a biologist. A second and I think important quality I have is the inclination to generalize conclusions, but not to the extent of excluding facts which oppose these generalizations. Rather I tend to remember exceptions more than general rules. Thus I do not accept hypotheses or theories or even laws which disregard too many exceptions. Thus I reject the Second Law of Thermodynamics, or the concept of florigen as a specific substance, or the official theory of coal formation. This is not opposition to the establishment, but acceptance of exceptions.

As to work habits, I try many simple experiments. If these give positive results, I will try to follow them up, but if the results are equivocal, probably requiring intricate statistics, I drop them like a hot potato. It is of course very difficult to decide which problems will yield to an experimental approach, but I have been lucky in that respect.

I am very little impressed by complicated and clever theoretical or mathematical constructions; in fact, I don't understand many of them. Nor can I follow or accept statistical analyses: if the facts don't speak clearly for themselves, no statistical treatment will make them palatable. I still stand on my earlier criticism of the over-use of statistics in biology: statistics tend to smear variability evenly over an experiment; the good biologist should try to eliminate variability as much as possible.

Finally, I feel that research work should be fun, or rather that it should give me satisfaction. If a field becomes too controversial or too theoretical, I prefer to leave it, as I did the growth factor field in the early 1940s. After Thimann and I had written *Phytohormones,* I felt that I degenerated to a policeman, overseeing the auxin field, checking doubtful statements or questionable results. If they turned out

to be correct, I had not achieved anything new, and if they were wrong, I had not discovered anything either.

This is a plea to change from time to time one's field of enquiry, to enter a new field where few preconceived ideas need to be fought, where little literature needs to be consulted, and where any discovery tends to be new. Besides, it is likely that discoveries in new fields can still be made with a minimum of sophisticated equipment. Such relatively new fields that I would like to enter are those of insect galls, sociology and physiology of ants, competition and other interrelationships between plants, evolution on an experimental basis, mimicry, symbiosis, and many other presently neglected fields which may not find their solution in DNA or RNA. Excessive preoccupation with this subject presently so popular has impoverished biology as a whole.