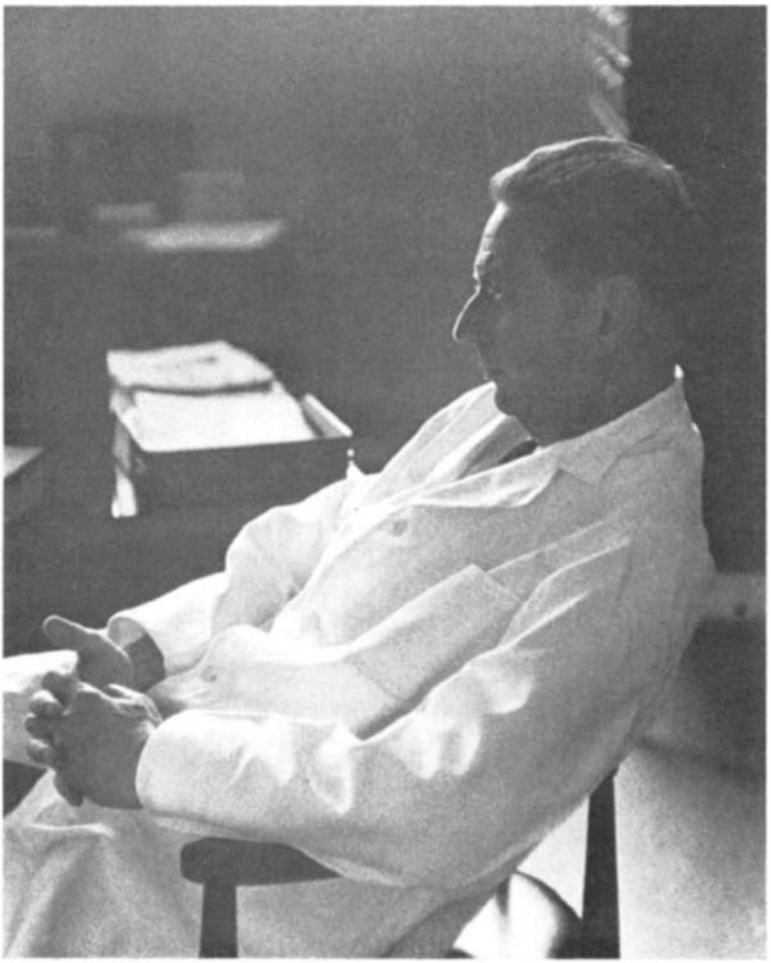

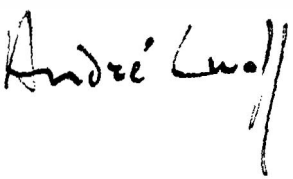

FROM PROTOZOA TO BACTERIA AND VIRUSES 1560
Fifty Years With Microbes

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To Marguerite

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Introduction

My father, born in Russia, in Sinferopol, came to France in 1880 at the age of 21. As a student, he had been involved in political activities and was sentenced to a few months imprisonment in the Peter and Paul prison in Saint-Petersburg, later Petrograd, then Leningrad. My father then left Russia, came to Paris, where he studied medicine and became a psychiatrist. My mother, too, was Russian. She had studied sculpture in Saint-Petersburg with Antokolski and, as a young girl, came to Paris as so many artists did.

My father soon became head physician in a psychiatric hospital and I

was born in the small village of Ainay-le-Château (Allier) in the center of France, on May the 8th, 1902. Then we went to Neuilly-sur-Marne, close to Paris, to another hospital. The psychiatrist had a house just at the outskirts of the hospital. At home, the servants, the cook, the chambermaid, and the gardener were inmates of the hospital. The cook sometimes answered voices which were speaking to her and which I could not hear. The chambermaid sometimes behaved strangely, and also the gardener. This was perfectly normal, of course, and things went all right. Maybe I should mention that one person was not an inmate of the hospital, namely, "Fraulein." She taught me German and at the time I became bilingual. However, Fraulein used to speak to herself . . . Have I been influenced in one way or another by this unusual environment? I am the last to be able to answer this question.

The house was surrounded by a garden with beautiful trees and flowers. There were also fruit trees and a vegetable garden. In the vicinity was a tennis court, and not far away a river where we used to swim and row. Moreover, I had been presented with a small rifle and used to kill rats and sparrows with bullets and even, once—a great event—a partridge in the field behind the garden. From time to time my father interrupted the games and I was summoned to his office to read Plato or Kant but, on the whole, life was marvelous.

Guests came to visit us. Relatives from Russia, of course, and from Italy and Austria, because the family had been scattered by emigration. There were also Russian socialists just out of jail or from Siberia, freed in one way or another. Also members of illustrious aristocratic Russian families living in Paris who had taken the wrong path and needed the care of a psychiatrist.

My father had decided that the study of mental illnesses should be part of my education, therefore at a tender age, I was taken to the wards. I do not know if this exposure has been useful but I am under the impression that the contacts, perhaps somewhat premature, with the manifestations of mental disorder considerably reinforced my inclination for scientific disciplines.

I was twelve when the first World War started. The German troops had reached a point twenty miles from our place. Later on during the battles, I could hear the roaring of the cannons from the battle line. From time to time at night German planes were trying to bomb Paris. The antiaircraft guns were close and the splitters were whistling and drumming on the roof. I listened to the strange music with curiosity, perfectly unaware of the danger. Later on, the psychiatric hospital was turned into a military hospital but I was not mature enough to realize the depth of the war tragedy.

I went to school, read an incredible number of books, and suddenly I decided to study biology in order to do research. How this idea germinated in my head, I do not know. Perhaps because of Elie Metchnikoff who was a friend of my father. When a patient had died of a disease which interested

Metchnikoff, he went to the hospital for the autopsy, and then came home for lunch. Cotton-plugged tubes showed outside the pocket of his coat and the cotton was soiled with blood. My poor mother was horrified.

It was Elie Metchnikoff who showed me a microbe for the first time. It was in 1915 when I was thirteen. My father had taken me to the Pasteur Institute. Metchnikoff asked, "have you ever seen a microbe?" I had not. So, Metchnikoff took a glass slide, put it under the microscope and said "it is the typhoid bacillus. Look." I was very excited. What happened, I remember very well. I looked into the microscope and saw nothing. I was very impressed. Such was my first contact with bacteria.

Anyhow, at seventeen, I decided to study biology and to do research. My father explained to me that research is not always successful and as I would have to earn my living, I had better study medicine. At the time, before entering the medical school the student had to spend a year in the *Faculté des Sciences* studying biology, chemistry, and physics. This was not enough. As I was very enthusiastic I had spent two months in the Marine Biological Laboratory at Roscoff in Brittany during the summers of 1919 and 1920, and during my first years of medicine I took the botany and zoology examinations at the Sorbonne and again in 1921 went to Roscoff where a determining event took place.

Impressed by my youthful ardour, the assistant recommended me to Edouard Chatton. Thus, I had the privilege of becoming the pupil and the collaborator, later on the disciple and friend, of the most brilliant representative of the brilliant French school of protozoology and, in my judgment, the greatest protozoologist of all time. Could I note for the readers of the *Annual Review of Microbiology* that it is Chatton who, in 1928, separated the Eukaryotes from the Prokaryotes, and coined the two names. From 1921 on, we worked together two or three months each year in the marine laboratories at Roscoff, Banyuls, Villefranche-sur-Mer, Wimereux or Sète. Our collaboration was interrupted by the war and ended only with the death of Edouard Chatton in 1947.

In 1921, Chatton was professor in Strasburg. He recommended me to Felix Mesnil who was head of the Department of Protozoology at the Pasteur Institute. He had been the secretary of Louis Pasteur and the collaborator of Alphonse Laveran. In October 1921, I received a fellowship and worked part time whilst studying medicine. I earned 350 francs a month, that is, 70 U.S. dollars—and felt rich. I had, of course, a tendency to sacrifice my medical studies to my passion for ciliates. But still I learned to examine patients; I swallowed anatomy, histology, embryology, bacteriology, parasitology, physiology, and biochemistry, etc., and succeeded in passing the examinations for pathology, obstetrics, therapeutics, and the rest.

The Eyes of Copepods

So, I passed the examinations. But in order to become an M.D. one had to defend a thesis. Whilst in the marine station in Roscoff I had worked on

parasitic ciliates which evolved in copepods engulfed by coelenterates. Often they were red and soon it was clear that the eye of the copepod was the origin of the pigment. So I started working on copepod eyes and in 1927 I presented my thesis at the Medical School. The title was "Le cycle du pigment carotinoide chez *Idya furcata* Baird, Copépode Harpacticide." For the first time, a carotinoid pigment was described in an eye. The eyes of copepods are normally dark red. I raised copepods on a carotinoid-poor diet. The red layers became less important and a beautiful blue structure appeared which is normally masked. I showed, among other things, that the blue pigment is a combination of a carotinoid with a protid and proposed the hypothesis that it plays a role in the vision. The president of the "Jury" was the well-known histologist, André Prenant, but a gynecologist was a member of the examination board. He shrugged his shoulders in disgust. Who knows, perhaps he was not really interested in copepods. Anyhow, as a result of the work, my name is now on the list of world copepodologists!

However, there was a byproduct: whilst working at the Kaiser Wilhelm Institut in Heidelberg in 1932, I called to the attention of Richard Kuhn and Edgar Lederer the fact that carotinoids were present in crustaceans. They bought a few lobsters and discovered astacin.

Morphology and Morphogenesis of Ciliates

However beautiful a copepod with blue eyes might be, I was in love with ciliates. During the summer of 1921 in Roscoff I had been asked by Edouard Chatton to explore the gills of acephals. The first mollusc examined, *Dosinia exoleta,* showed a strange organism devoid of cilia which reproduces by ciliated buds. This was the type of a new genus, *Sphenophrya,* and of a new family, the *Sphenophryidae,* to which we later added two other genera, *Pelecyophrya* and *Gargarius.* The systematic investigation of the ciliates of the gills brought the discovery of many new organisms and the organization of new families into a new suborder, the *Thigmotricha.* I should make clear that the work on ciliates, with a few exceptions, was performed with Edouard Chatton and, from 1925 on, with Marguerite Lwoff.

The study of the morphology and morphogenesis of ciliates was greatly facilitated by the introduction of a new technique of silver staining. The Klein method, silver staining of dried specimens, was not applicable to marine ciliates owing to the formation of precipitates of silver chloride. But we noticed the absence of a precipitate and nice staining of encysted ciliates. So, the idea germinated that cysts could be replaced by gelatin. After proper fixation the ciliates were embedded in salted gelatin, then impregnated with silver nitrate which was reduced by ultraviolet light. The shape of the specimens was preserved and the kinetosomes were beautifully stained as also were the trichocysts and many fibers. Moreover, the whole organism was stained. So the ciliary system of many ciliates was described accurately for the first time, new structures were discovered as well as the intimate process of stomatogenesis. In *Tetrahymena piriformis,* before divi-

sion, the kinetosomes of the stomatogenic kinety n° 1 start dividing toward their left. It is in or around the newly formed field of kinetosomes that the membranelles are organized and that the morphogenesis of the mouth takes place (with E. Chatton, M. Lwoff, and J. Monod).

One of the groups studied was *Apostomata*. Numerous new species were discovered and studied. We described the life cycle in the two hosts and the ciliary metamorphosis which take place during the cycle.

Some general features of the morphology of ciliates were disclosed: 1. the genetic continuity of kinetosomes and the concept of the "infraciliature"; 2. the origin of trichocysts; 3. the kinetodesma; and 4. the law of desmodexy. We shall examine them separately.

1. When one looks at a silver-stained ciliate one sees that new kinetosomes always appear in the immediate vicinity of a persisting one and that the two kinetosomes are united by a "desmose." Therefore, it was concluded that kinetosomes are endowed with genetic continuity and reproduce by division. We know today that only a double-stranded nucleic acid made of two complementary parts can "divide" and reproduce its kind. No other molecule is reproduced by division. So, ***sensu stricto,*** a kinetosome cannot divide. How, then, does a kinetosome reproduce? The simplest approach is to assume that a kinetosome is located in a specific morphogenetic field or territory. The molecules of this territory can increase in number. A new kinetosome can be organized only within the vicinity of a pre-existing one. The reproduction of a kinetosome is the result of the division of a specific morphogenetic territory.

Let us now consider a ciliate as a whole. Ciliates reproduce by binary fission or division. Before division, two new ciliates are organized within the framework of the parent. Division separates two new ciliates, one anterior, the other posterior, for which we proposed the name "proter" and "opisthe," respectively. Whatever the case might be, if the reproduction of a ciliate is called division, then the reproduction of a kinetosome is also a division.

Some adult ciliates are deprived of cilia but are reproduced by ciliated buds. This is the case for most suctorians. The study of the suctorian *Podophrya* revealed that kinetosomes devoid of cilia are present in the adult and that they are the origin of the kinetosome of the bud. The ensemble of kinetosomes was called an "infraciliature."

2. Let us consider the origin of trichocysts. In the Apostomatous ciliates, trichocysts are formed only during a short and unique definite period of the life cycle. One then sees all the kinetosomes "dividing" to their left and, from the daughter kinetosomes, one sees trichocysts growing. Needless to say, the electron microscopists studying apostomous ciliates were, up to now, unable to see this phenomenon which is very clearly visible with an ordinary microscope when one examines silver stained ciliates *at the right phase of the cycle.*

It is currently said that cilia are produced by kinetosomes. The kineto-

some as such cannot synthesize the proteins of the cilium, but they have the property to organize and to orient the ciliary proteins, and also the proteins of the trichocysts and of the fibers. A kinetosome and its territory represent a remarkable morphogenetic machine.

3. The study of ciliates revealed the existence of a fiber which we called the "kinetodesma" and which always runs parallel to the rows of kinetosomes on their right. This is the law of desmodexy. The kinety is thus asymmetrical—one can recognize an anterior and a posterior end, a right and a left. With Chatton, we also saw that the kinetodesma was formed by fibers connected to the kinetosomes. This was rediscovered much later by the electron microscopists.

In some ciliates at a given phase of the life cycle, the kinetodesma of a given kinety disappears. As a result, the corresponding kinetosomes are scattered in disorderly fashion. This exemplifies the role of the cortex in the maintenance of the kinetodesma and the role of the kinetodesma in the organization of the kinetosomal pattern.

Thigmotricha and Apostomata were our main concern. But, when working in a marine laboratory, one comes across strange organisms which nobody yet has seen. We thus described a curious peritrichous ciliate, *Ellobiophrya,* which is appended to the gills of the acephalus *Donax vittatus* like an earring and reproduces by budding a strange and beautiful "embryo." We also described the *Conidophrys pilisuctor* a ciliate which, as embryo, empales itself on the secretory hairs of the amphipod *Corophium,* loses its cilia, encysts, and reproduces by budding.

Finally, I discovered an amazing ciliate whose nuclear apparatus is a protocaryon: *Stephanopogon mesnili* is homokaryotic. It was not possible to state any longer that ciliates are characterized by the differentiation of the nuclear apparatus into a macro- and a micronucleus. Maybe I should add that *Stephanopogon* is a true ciliate which possesses mouth characteristics both of Gymnostomes and of Hymenostomes, and is a most devilish animal. We may note here that Opalinids, which are homokaryotic, were generally and are sometimes still considered as ciliates despite the fact that Chatton and Brachon, a long time ago, showed that their division is longitudinal; it cuts between two sets of longitudinal kineties, whereas in ciliates the kineties are cut transversally by division.

I started playing with ciliates in 1921. The milestones of the work were monographs on *Stephanopogon, Conidophrys, Ellobiophrya, Apostomata,* and the two monographs on *Thigmotricha.* In 1948, I was invited to Harvard to deliver the Dunham Lectures. The theme was the morphogenesis of ciliates and a book was published, *Problems of Morphogenesis in Ciliates,* which I will try to summarize.

The study of the development of ciliates shows that the activity of the kinetosomes, their organization into specific patterns, their division, and the expression of their potentialities, depend on their position and on the phase of the life cycle. As already said, before division of *Tetrahymena piriformis*

occurs, the kinetosomes located around the middle of the "stomatogenic kinety" n° 1 start dividing actively toward their left; a dense field of kinetosomes is formed which is the base for the organization of the membranelles and the mouth. It is clear that kinetosomes are induced to reproduce by the properties of the underlying cortex. Hence, the conclusion that "if kinetosomes are necessary for morphogenesis, they seem not to command but to obey some mysterious force . . ." In certain ciliates, trichocysts, for example, are formed only at one phase of the cycle. The analysis of the movements of the cortical structures in various ciliates led to the hypothesis that the cortex differs according to its location and that cortical structures command morphogenesis. Morphogenesis is in part the result of the response of an apparently homogeneous population of kinetosomes to their environment, the cortex. An orderly and organized asymmetry like that of an egg or of a ciliate may be only the reflection of cortical properties. A constantly flowing or potentially flowing endoplasm cannot be asymmetrical. The organelles may be asymmetrical. But when the ciliate is considered as an organism the conclusion is reached that organized asymmetry, or simply organization, can belong only to a more or less rigid, a more or less permanent system, that is to say, the cortex. Finally, one finds that, in well-defined evolutionary series of ciliates the structure of the daughter ciliates just before division corresponds to that of the primitive type. Ontogeny repeats phylogeny.

It came as a surprise when Beisson and Sonneborn, in a paper which appeared in the *Proceedings of The National Academy of Science* in 1965, made me responsible for the statement that: ". . . kinetosomes . . . are directive or instrumental in morphogenesis" (this sentence is the only reference to my book). Impressed by this statement, Nanney wrote later: "Persistent reports of DNA associated with the ciliate cortex . . . lend plausibility to the suggestion (see Lwoff 1950) that gene-like elements imbedded in the cortex represent peripheral "nucleic" reservoirs responsible in some way for cortical characteristics." This, supposedly a view of mine, is stated by Nanney to be opposed to Sonneborn's ideas concerning the role of cortical structures in morphogenesis!

Of course, kinetosomes have a morphogenetic role in the sense that they are responsible for the production of cilia, trichocysts, and fibers. However, I have never thought nor said that kinetosomes were controlling the cortex or were directives in the morphogenesis. As a matter of fact, my conclusions as can be judged by the above quotations point to the opposite concept, namely, that the presence, location, organization, and activity of cortical organelles—including kinetosomes—are not determined by the kinetosomes but by the cortical structures and their "internal" environment as determined by the phase of development. Beisson and Sonneborn have reached the same conclusion. I am, however, unable to understand why my colleagues made me say *exactly the contrary* of what I clearly said. Owing to the authority of Sonneborn, his distorted interpretation of my concepts is now spreading. I regret to have been obliged to put things straight myself.

Nutrition of Free-Living Protozoa

The work on morphology and morphogenesis of ciliates was performed each year at several marine biological laboratories during the months of July, August, and September and sometimes during the Easter holidays. At the Pasteur Institute I first took the course of microbiology. Later on, I became responsible for the classes in protozoology and parasitology and had the responsibility of maintaining pure cultures of trypanosomides, trichomonas, and amoebas. So, I gained some experience in handling protozoa and decided to work on the nutrition of ciliates. This was mad. So far as I can remember, I had read a review of an article dealing with the alleged pure culture of a ciliate in a medium in which ammonium sulfate was the nitrogen source and dextrose the sole organic substance. The author of the review said that these results shattered the doctrinal corpus concerning the power of synthesis of protozoa. The literature was searched for data concerning this power of synthesis. Nothing was found except concepts such as "heterotrophy" defined as the need for organic substances, and "autotrophy," the absence of need for organic substances which was considered as bound to photosynthesis. I could not discover anything about the nutritional requirements of protozoa. This is why I decided to investigate the nutrition of ciliates. In order to do so, "pure," that is bacteria-free, cultures were needed. From a wild culture of *Tetrahymena piriformis,* I obtained in 1923 a bacteria-free pure culture. A ciliate was put in a drop of sterile medium and transferred from drop to drop with a micropipette under a dissecting microscope. The ciliate grew in peptone solutions but refused to multiply in media with an ammonium salt and with dextrose as sole organic source. So it needed other "organic substances" which I tried bravely to identify. Knowing today the large number of amino acids and of vitamins necessary for this organism, it is retrospectively not surprising that my work failed, but not entirely as will be seen later. However, the first pure culture proved to be a useful tool for the solution of biological and morphological problems.

Flagellates seemed more promising. A number of groups are interesting because they comprise chlorophyll-bearing organisms as well as their chlorophyl-less counterparts. The nutrition of the "green" and "white" species was systematically investigated with the collaboration of Marguerite Lwoff first and, later on, Hisatake Dusi and Luigi Provasoli.

Some species existed in culture collections and I isolated a few strains by washing single individuals. The green flagellates utilize nitrates as nitrogen source. Most white organisms thrive in the presence of an ammonium salt but not with a nitrate. However, it was found that one species, *Polytoma ocellatum,* utilizes nitrates. This was the first example of a protozoan able to reduce nitrates.

It was known that *Polytoma uvella* utilizes acetic acid as a carbon source. The systematic study of white Chlamydomonadinas, Phytomonadi-

nas, Cryptomonadinas, and Euglenidas revealed that some of them can thrive on acetic acid only, whereas others are satisfied with a number of lower fatty acids and some also lactic and pyruvic acids. The obvious idea was to study the green counterpart of the white flagellates in the absence of photosynthesis. *Chlamydomonas* grows beautifully in the dark if provided with acetic acid, as does *Haematococcus* also. It turned out that in the presence of acetic acid only, *Haematococcus* manufactures large amounts of carotenoids in the dark as well as in the light. Finally, we investigated a few Euglenidas. For *Astasia* (with H. Dusi) the fatty acids were the right carbon sources and this gave the key to the culture in the dark of green Euglenidas. They had been grown in the dark, but the cultures were always poor except when acetic acid was added. Acetic acid was supposed to act by lowering the pH, but we showed that, in fact, acetic acid acted as a carbon source. The green *Euglena* in the absence of photosynthesis behave as do the other flagellates (with H. Dusi).

All the investigated flagellates manufacture starch or paramylon. None of them, however—as already said—utilize glucose or any other sugar as an extrinsic carbon source. However, *Polytomella caeca* contains a phosophorylase which can transform starch into glucose-1-phosphate in the presence of phosphate (with Hélène Ionesco). It could be that the synthesis and utilization of starch in these organisms takes place without glucose as intermediate except in the phosphorylated form.

Hematin as a Growth Factor

In 1932, I received a Rockefeller fellowship to work with Otto Meyerhof in Heidelberg at the "Kaiser Wilhelm Institut für Medizinische Forschung." I stayed there fifteen months, until the end of the year 1933. It was a very interesting year, not only from the scientific point of view. The government of Germany changed in the spring of 1933 and a tragic era began. It was clear that war would come sooner or later. The laboratory was very active and everyone behaved apparently as if science only was important. However, the numerous foreign workers attracted by the fame of Meyerhof did not fail to observe and to comment on the situation without any illusion for the future. Later on, Meyerhof, as a Jew, had to leave Germany with his family. His relatives who stayed disappeared in extermination camps together with millions of other human beings.

In the Pasteur Institute, Marguerite Lwoff had shown that hematin could replace blood for the growth of *Crithidia fasciculata*—at this time called *Strigomonas fasciculata*. I decided to investigate the role of hematin as a growth factor. The field of growth factors was rather confused. Microbiologists were convinced that a "trace" of a growth factor would induce an unlimited development of microorganisms. Growth factors were supposed to act on multiplication as catalysts. At the time, a few essential amino acids had been shown to be necessary for the development of bacteria, but no "vitamin" had been identified. There was only one exception, namely hema-

tin. So, hematin as a growth factor for *Crithidia fasciculata* was investigated. It was first shown that blood and hematin acted quantitatively; the number of flagellates which developed was, within certain limits, proportional to the amount of hematin. Each flagellate needed 520,000 molecules of hematin. What was its function?

The respiration of flagellates grown in a medium in which the hematin concentration is the limiting factor is lower than the respiration of "normal" flagellates. When hematin is added the respiration increases, and the increase is proportional to the amount of hematin added. It was then easy to calculate the amount of hematin needed by one flagellate in order for its respiration to be normal: it was 720,000 molecules, in good agreement with the number 520,000 found by measuring the growth as a function of the growth factor. Thus, it was clear that hematin was not acting as a "catalyst" either on multiplication or on respiration. It entered into the constitution of the catalytic respiratory system and its action was quantitative.

Why is hematin necessary? Blood was known to be necessary for the growth of the bacteria *Hemophilus influenzae*. As it was found that hematin works, but that hematoporphyrin is inactive, the action of hematin had been ascribed to its iron atom. A number of "active" iron preparations had been proposed as a substitute for hematin and were supposed to work. However, hematoporphyrin does not differ from protohematin only in the absence of iron. Therefore, the specificity of the hematin molecule was investigated.

Protoporphyrin proved to be active. Thus, iron—the catalytically active atom of hematin—was not the growth factor. Moreover, a number of hematins were investigated, all of which were inactive. The activity of hematin was bound to the structure: tetramethyl 1,3,5,8-divinyl 2,4-dipropionic 6,7-porphyrin. This was the first study of the specificity of a growth factor. The need for hematin was the consequence of the inability to synthesize it.

The properties of hematin as a growth factor for the flagellate *Crithidia fasciculata* turned out to be the general feature of growth factors. Growth factors act quantitatively and not catalytically on growth. They enter into the constitution of catalytic systems. Their activity is bound to a specific structure.

Growth factors were for the first time defined as specific substances which the organism is unable to synthesize and which are necessary for its growth and multiplication. Trypanosomids are parasitic flagellates and the need for hematin is found only in parasitic organisms. It was concluded that the need for hematin was the result of the loss of the power to perform its synthesis. This concept was to be extended to all growth factors.

There is today a general consensus about growth factors. Let me tell what happened to me at the 2nd International Congress of Microbiology held in London in 1936. I had to open a session on growth factors. The title of the paper was "study of lost functions." I discussed especially the results concerning hematin. When I had finished, an eminent microbiologist and biochemist, the head of a brilliant school, stood and said, "I do not like sub-

stances which produce miracles." A beautiful execution—which I survived. The judge-executioner also survived. The miracle soon became the daily bread provided by text books but I still feel the rope around my neck, and how I was thrown into the emptiness.

Moraxella

The results concerning *Crithidia* were extended to *Hemophilus influenzae*. Moreover, with Ignacio Pirosky we showed that hematin was a growth factor for *Hemophilus ducreyi*. So it was decided to investigate systematically all the members of the *Hemophilus* group. Among them, in *Bergey's Manual,* the Morax bacillus was included. At the time, the group was defined essentially by the "need for body fluids." Officially, the Morax bacillus was unable to grow in the absence of serum. So I secured a culture of the bacillus and realized that it was widely different from *Hemophilus influenzae,* that it could belong neither to the genus nor to the family, and that it could not fit in any known bacterial genus. So I proposed the new name *Moraxella* given in the honor of Victor Morax who "invented" the organism known today as *Moraxella lacunata*. It grows in broth only if serum is added, and the question of the nature of the substance involved was posed. It turned out that the serum acts by neutralizing the toxic action of fatty acids present in broth. *Moraxella lacunata* grows in broth provided it is diluted with distilled water.

The systematic study of the various species of *Moraxella* was started and Alice Audureau discovered a new species (*Moraxella lwoffii*). We tried to grow it in synthetic media with an ammonium salt and glucose. It multiplied only when peptone was added. So I tried to identify the responsible growth factors. Thiamin was active, but was the organism really unable to synthesize thiamin from pyrimidine and thiazole? An astonishing phenomenon was observed. *Moraxella* grew in the presence of pyrimidine as well as thiazole. It was then realized that thiamine, pyrimidine, and thiazole were dissolved in ethanol. The addition of ethanol permitted growth. It was found that *Moraxella lwoffii* is unable to utilize any sugar as carbon and energy source but utilizes ethanol. It does not need any growth factor.

The wild strain of *Moraxella lwoffii* is unable to utilize malic acid as carbon source. I found a mutant able to do so and which possesses an enzyme converting malic acid directly into pyruvic acid without oxaloacetic acid as an intermediary step. The enzyme requires K^+ which could be replaced by rubidium or caesium but not by sodium.

Growth Factors for Free-Living Protozoa

Since 1932 the situation concerning vitamins had undergone considerable changes. The first vitamin, vitamin C, had been identified. This was followed by the identification of vitamin B_2 and B_1. During investigations on the carbon sources for flagellates it had been noticed that some flagellates would not grow in a medium containing acetic acid as sole organic

substance. A "trace" of something was necessary. The growth factors for some of the flagellates were identified with the thiazole or pyrimidine moiety of thiamine.

Thiazole (methyl-2, β-hydroxyethyl-5, thiazol) is the only growth factor for *Polytoma obtusum, P. ocellatum,* and for *Chilomonas paramoecium.* Both thiazole and pyrimidine (methyl-2, amino-4, aminomethyl-6 pyrimidine) are necessary for *Polytomella caeca* (with Hisataka Dusi). The investigation was extended to an amoeba, *Acanthamoeba castellanii,* which was available in pure culture. In addition to numerous growth factors, it needed pyrimidine. All the investigated organisms are thus able to manufacture thiamine from pyrimidine and thiazole. This is not possible for the ciliate *Tetrahymena piriformis* which needs the complete molecule of thiamine (with M. Lwoff). Thiazole, pyrimidine, and thiamine were the first growth factors identified for free-living protozoa. The specificity of thiazole and pyrimidine was investigated.

The substitution of the -hydroxyethyl in the molecule of thiazole by one hydrogen or by methyl leads to an inactivation of the molecule, but the replacement of -hydroxyethyl by an acetoxyethyl is compatible with utilization as a growth factor by the flagellates (with H. Dusi).

The replacement of the aminomethyl in position 5 in the pyrimidine by thioformylaminomethyl or by a hydroxymethyl is compatible with the utilization. The substitution by a methyl inactivates the molecule as a growth factor. The substitution of the NH_2 in position 4 by hydroxyl or by $-OCH_3$ leads to inactivation. The transfer of the methyl from position 2 to position 6 also leads to inactivation.

The suppression of the hydroxyethyl of thiazole, the transfer of the methyl group of pyrimidine from position 2 to position 6, and the presence of a supplementary bond -C-N=C- between pyrimidine and thiazole inactivates the molecule of thiamine as growth factor for the ciliate *Tetrahymena.*

Growth Factor V

In 1936, the Rockefeller Foundation gave me a second fellowship to work in Cambridge with David Keilin, then Director of the Molteno Institute.

Hemophilus influenzae can be grown in broth only if blood is added. It was known that blood provides two factors: one, the factor X, is hematin, the other one being the growth factor "V." "V" is often interpreted as a Roman figure for five, whereas it is a V as in victory. In fact, it stands for "vitamin-like" because it is known to be destroyed by heat; sensitivity to heat being long considered as a characteristic property of vitamins. At any rate, the growth factor V is not destroyed by "heat" in an acid medium. Nothing was known about its nature.

With Marguerite Lwoff, we decided to try to identify it. Very valuable help was received from David Ezra Green, who was then working in the Department of Biochemistry, and from Tadeusz Mann, David Keilin's col-

laborator. Thirty pounds of yeast were extracted and fractionated and the fractions tested for their "V" activity. The active substance was finally identified with coenzymes I or II, later on, phosphopyridino-nucleotides. Bacteria grown with factor V as limiting factor had a very low respiration rate and were unable to reduce methylene blue. The addition of coenzyme I or II restored the movement of hydrogen within 60 seconds. The need for growth factor V was found to be due to the lack of power to synthesize phosphopyridino-nucleotides. A new growth factor had been identified and its physiological role determined.

Nicotinamide

After Paul Fildes had discovered that nicotinamide was the only growth factor for *Proteus vulgaris,* we investigated, together with Andriès Querido, the specificity and established the effects of various substitutions on its activity. Finally, a quantitative test for the estimation of nicotinamide was devised. We estimated nicotinamide in various organs and the first value for blood was found to be 0.75 mg per 100 ml. Vitamin PP had not yet been detected in milk and the hypothesis was put forward that the newborn synthesizes nicotinamide. With Madeleine Morel, we showed that nicotinamide was present in colostrum of human milk and that the nicotinamide content decreases for two to nine days and then increases up to a value of 15 to 34 mg/100 ml. The administration of nicotinamide was followed by a rapid but limited (0.5 mg/100 ml) increase. We also estimated vitamin PP of various tissues in various pathological conditions.

A System of Nutrition

In the "good old days," autotrophy was defined as the ability to grow in the absence of any organic substance, and was considered to be correlated either with photosynthesis or with chemosynthesis. Heterotrophy was defined as the need for organic substances.

It was clear that organic substances could represent either energy and carbon sources or growth factors. Moreover, it was shown by Hisatake Dusi that some photosynthetic *Euglenas* need one or many growth factors not yet identified (later shown to be vitamin B_{12}). Thus, it was proposed in 1932 to consider separately the energy and carbon sources and the growth factors.

The problem was considered anew in 1946 in Cold Spring Harbor with C. B. van Niel, F. J. Ryan, and E. L. Tatum. We took into account the latest developments in microbial physiology. A nomenclature of nutritional types was proposed which was based upon energy source on the one hand, and the ability to synthesize essential metabolites on the other. Phototrophy, of course, corresponded to energy provided by photochemical reactions, of which there are two types: photolithotrophy and photo-organotrophy, depending on whether the exogenous hydrogen donor was inorganic or or-

ganic. Chemotrophy corresponded to the energy provided by dark chemical reactions with two types, chemolithotrophy and chemo-organotrophy, depending on whether growth depended on inorganic or organic substances. Autotrophy corresponded to the synthesis of essential metabolites—the term, prototrophy, is now commonly used; heterotrophy, to the need for growth factors.

For the definition of categories one considers separately the energy source, the hydrogen donor, and the power to synthesize essential metabolites. This principle is now widely accepted and the terms proposed are commonly used.

Lysogeny

In 1949, I started working on lysogeny. At a time when genetic material had not been identified, a few bacteriologists had understood the strangeness of lysogenic bacteria, but knowledge concerning viruses and their reproduction was too cloudy. Moreover, a number of papers were obscured by useless polemics. Some textbooks of microbiology contained a paragraph about lysogeny but their reading did not bring much light; no adequate review or discussion was available.

My work had led to the following conclusions: (*a*) In lysogenic bacteria the phage is perpetuated in a noninfectious form which was called the prophage. (*b*) Bacteriophage is not secreted but is liberated by the lysis of a lysogenic bacterium. (*c*) The production of bacteriophage is the result of an "induction." Irradiation with ultraviolet light induces the quasi totality of the lysogenic *Bacillus megaterium* to produce bacteriophage (with Louis Siminovitch and Niels Kjelgaard). (*d*) Hydrogen peroxide is an inducer but only in organic media. Organic peroxides are inducers; the development of bacteria in an organic medium containing copper ends with the oxidation of thio compounds, the formation of hydrogen peroxide, and of organic peroxides which account in part for the "spontaneous" production of bacteriophage. (*e*) In *Salmonella typhimurium,* the fate of a bacterium infected with a temperate bacteriophage is decided within seven minutes (with Evelyne Ritz). (*f*) Lysogeny was defined as the perpetuation of the power to produce bacteriophage in the absence of infection.

Finally, the hypothesis was proposed in 1953 that the potential power of a cell to become malignant may be perpetuated in the form of a genelike structure—the genetic material of the oncogenic virus—and that carcinogenic agents induce the expression of the potentiality of this genetic material, which would culminate in the formation of virions. The history of lysogeny, together with the development and state of the new concepts, were discussed in the review "Lysogeny" which appeared in 1953.

In 1966, a collection of essays was dedicated to Max Delbrück on the occasion of his sixtieth birthday. The stories had been written by his friends, colleagues, and disciples. Max Delbrück had paradoxically played a

role in the development of lysogeny. I say paradoxically because the founder of the "phage church" did not believe in the existence of lysogeny. Falling from the lips of Max Delbrück, the death sentence, "I do not believe" had been often heard by many of us. It was an excellent catalyst.

I contributed a paper entitled "The Prophage and I" which is the story of my own contribution—and I am not going to repeat myself. However, I would like to say that the lysogeny period had been something quite apart in my scientific life. In 1949, the "occupation" and its sequels were just over. After a long tragic period of isolation the outside world had flowed in with all its marvelous news, an awakening in flourish after a long sleep. Around us, groups were forming again, Louis Rapkine and Jacques Monod joined the Pasteur Institute. Young men, freed from the war, were starting their career, foreign scientists were visiting.

The 1946 Cold Spring Harbor Symposium had marked the rediscovery of freedom and the beginning of the new era. It was in this rather exceptional atmosphere that the experiments on lysogenic bacteria were started, that the work developed and that unexpected results cropped up.

The Poliovirus

In July 1953, I attended a Cold Spring Harbor Symposium on Viruses. It was sponsored by the National Foundation for Infantile Paralysis which gave considerable support to fundamental work on viruses, including bacteriophage, wisely considered as a good model.

After the meeting, I visited Harry Weaver, head of the Research Department of the Foundation. He invited me to lunch at the Banker's Club. The National Foundation for Infantile Paralysis was on Broadway, close to Wall Street. The Banker's Club was located on the top floor of a skyscraper. The weather was perfectly clear and the view of New York Bay extended for miles and miles. When I returned to earth, I suddenly became conscious of what had happened. The Foundation had invited me to spend a few months in the States, to visit a few laboratories in order to learn tissue culture and make contact with animal viruses. Thereafter, it would provide my laboratory with the necessary equipment and support my research for a few years. I would work on the poliovirus. I was abashed.

I have always been extremely sensitive to the charm, beauty, and personality of soul-inhabited cities, even if the soul is of stainless steel. Each one exerts its own specific influence and induces a given mood. The standing city was obviously not conducive to dreams but to action. I gave some thoughts to the wealth, power, and efficiency of American foundations. Of course, there was still time for reflection, but in a way it was too late; squashed on the bottom of the black Wall Street Canyon I was muddled by vertigo. Antivertigo would be more correct; and more fashionable too.

It was of course foolish to abandon the still quiet—although not for long —field of lysogeny in order to intrude into the jungle of animal virology.

To enter a new field at the age of fifty-two is unwise anyhow; but unwise decisions debouch on the unexpected which is the salt of research. Beforehand, the unexpected is necessarily entirely hypothetical. The aposteriori nature of these remarks will not escape the perspicacity of the reader.

I forgot, or tried to forget, that I was worried about my future as an animal virologist and in March 1954 we, that is, my wife and myself, embarked courageously for a long trip in the United States. It started in Bethesda where we spent two weeks in the National Institutes of Health with Wilton Earle. Later on we visited, in succession, Joseph Melnick in New Haven, John Enders in Boston, Raymond Parker in Toronto, Jonas Salk in Pittsburg, Gerome Syverton in Minneapolis. I suppose there are a number of recipes for the rejuvenation of aging scientists. One of them is to become a student.

After a fascinating and beautiful drive we finally reached Pasadena. At the California Institute of Technology, we worked with Renato Dulbecco and Marguerite Vogt from July to December. How was the poliovirus released from the cell? Throughout the vegetative phase or at its end? In order to solve the problem it was necessary to study single cells, and a number of technical difficulties had to be overcome. Finally, the question was answered. Infectious particles are liberated all at the same time by the burst of the infected cell. On the way back I stopped in New York and delivered a Harvey Lecture, "Control and interrelations of metabolic and viral diseases of bacteria." The year had been busy.

Back in Paris, something unexpected happened. A colleague from the Pasteur Institute tried to persuade the Foundation that he, and not I, should receive a grant: he was defeated. He also made efforts that I should be forbidden by the Director to work on poliovirus but without success. I did what I had decided to do. Bad or good, I have always done.

It took some time to start the experiments. The Service de Physiologie Microbienne was really crowded. Jacques Monod had not yet completed the organization of his new laboratory and the density of scientists per square foot—I wish the United States had adopted the metric system—was high. As a matter of fact, the research on the poliovirus started only in the fall of 1955.

I had no idea, not the slightest idea, in what direction things would go. I knew only that I was expected to meet with success. A grant is more or less an investment. Of course, foundations are aware of the hazard of research and the National Foundation for Infantile Paralysis was very kind. It was only after two years when research was developing that Theodor Boyd, who had succeeded Harry Weaver, told me that the Foundation had been for a time worried because it felt responsible for throwing me into the adventure. The work had developed slowly. I had started to play with the virus, to study its multiplication under various conditions, and it was necessary to know the optimal temperature.

One-step growth cycles were performed at various temperatures. The curves of viral multiplication as a function of temperature showed that different strains exhibited different patterns. Beforehand, and even now, the sensitivity of development to temperature was expressed by a^+ or a^-. We proposed to express the sensitivity by the temperature at which the viral development is decreased by 90 percent. This was the *rt* (*r* for reproduction, *t* for temperature). To determine *rt* is a rather long procedure. First, one needs a series of water baths at different temperatures which have to be rigidly controlled. For certain critical values, a difference of 1° may modify the yield by a factor of 2. One needs to have a growth curve at each temperature, that is to say a number of estimations. It is probably the reason why the ++, +, ± and − are still popular.

The multiplication of the virulent strains was less sensitive to "high" temperatures—between 37 and 41°C—than the multiplication of the nonvirulent strain including the vaccine strains.

The vaccine strains of Hilary Koprowski and of Albert Sabin have a *rt* of 37.8. By growing the type I vaccine at 41°C a strain of *rt* 40°8 was obtained. An injection of 600,000 virions of the vaccine strain in the spinal cord of the monkey—the most sensitive route—does not produce lesions. With Albert Sabin, we injected the "hot" virions into the brain, the less sensitive route; three particles killed the monkey. A similar type of experiment was performed with the MEF strain of type II. The hot strain was much more virulent for the mouse than the normal one. The LD_{50} was correlated with the *rt*.

This type of experiment was extended to the virus of encephalomyocarditis. One particle of the wild strain kills the animal; the virulence is maximum. By growing the virus at lower temperature, strains of lower *rt* and of a higher LD_{50} were obtained (M. Lwoff, Y. Perol-Vauchez, and P. Tournier). This, a relation was established between virulence and the sensitivity to high temperatures. What does this mean?

Fever and the Fight of the Organism Against a Primary Viral Infection

It had been known that by growing the poliovirus at low temperatures (23°C) strains devoid of virulence were obtained. That a strain, unable to grow, or growing poorly, at the temperature of the animal, would be devoid of virulence was not in the least surprising. The existence of a correlation between the ability to multiply at temperatures above the normal temperature of the animal and virulence posed a problem.

It had been known that an elevation of temperature can decrease the severity of a viral disease. Experiments showed that the value of the LD_{50} is increased when fever is induced within an animal. Suddenly, everything became clear. Fever is one of the mechanisms by which the organism fights against the primary viral infection. A virus is virulent when it can multiply

despite fever. Fever is a byproduct of the inflammatory reaction. Moreover, in an inflammatory zone the pH can drop to values below 6.00 and the poliovirus is unable to multiply below 6.8. It thus appeared that the inflammatory reaction played an important role during a primary infection. Anyhow, prior to the production of antibodies only nonspecific reactions could be responsible for the fight of the organism against the virus.

During the past ten years many examples have been given of the relation between virulence and the resistance of viral development to temperature. The importance of fever in viral infection was recognized very slowly. Text books of virology are now often written by molecular virologists who are not interested in infectious diseases, and the books are strangely lacking in discussions concerning the fight of the organism against the viral infection. Is it so strange after all?

Viruses: Definition, Terminology, Classification

A virologist is necessarily bound to ask questions. One of them is, what is a virus? The question was asked and has been answered. He is also faced with problems of terminology; new terms were proposed. Finally, when one enters the field of virology rather late one can experience difficulties in recognizing the place of each virus, hence the need for a classification.

Definition.—Words should have a meaning. The "Concept of Virus" was discussed in a Marjory Stephenson Lecture delivered in 1957 before the Society for General Microbiology in London. It was proposed to define viruses as infectious particles possessing only one type of nucleic acid and which reproduced from their sole genetic material. A few other characteristics were sifted out: inability to grow and to divide, absence of metabolism, absence of the information for the enzymes of energy metabolism. We added later on, the absence of transfer RNA and of ribosomes and also of the corresponding information. Thus, by the virtue of a few discriminating traits, viruses were separated from nonviruses: the category, virus, was at last defined. An infectious particle could no longer belong to the group of viruses by the sole virtue of its size. A number of "small" bacteria were thus excluded from viruses and reinstated where they belong.

The concept of virus as it was proposed is now of universal acceptance. For historical purposes I should note that the concept had already been proposed in 1953 in the review "Lysogeny." At the time, however, nobody paid any attention to the proposals which were a few years in advance on the viral calendar.

Terminology.—Virologists interested in the structure of the infectious particle came across the inadequacy of terminology. With Thomas Anderson and François Jacob, we proposed three terms: *virion, capsid,* and *capsomere.* Later on, in Cold Spring Harbor, a group of virologists added *nucleocapsid.* All these terms are now part of the virological vocabulary.

Classification.—A synoptic table of viruses is certainly useful. Now, either you like order or not. If you like it, your love can be either active or platonic. If it is active then you are thrown into systematics. To classify is an amusing game—one tries to select characters and to define categories. However, there are drawbacks. First, categories do not exist in nature. They are creations of our mind; a category is the result of an arbitrary grouping. This does not matter as long as you are aware of the arbitrariness. If you were alone there would be no problem. But if you are not . . . The hell, according to Sartre, the hell, it is the others.

Moreover, when one builds categories, one has to provide them with names, hence nomenclature. A nomenclature has to be international. No wonder that there are conflicting views on nomenclature as well as on classification, hence discussions and even polemics. Before the war, I had been a member of the Judicial Committee of Bacterial Nomenclature. One day, I complained to one of my colleagues, an eminent biochemist, about the total lack of interest in the sessions. He said that if one would leave nomenclature to people interested in nomenclature the result would be a catastrophy.

This being said, with Robert Horne, Paul Tournier, and Peter Wildy, we discussed the problem of classification and finally succeeded in producing a system with the use of four characters. Once the work was brought to an end, Peter Wildy decided that he could not sign the paper because of hierarchy, the hierarchy of viral characteristics, of course. I have always regretted this decision. So the proposed system had to be issued without him. It became the L.H.T. system. It suffered the fate of all classifications, adopted by some, villipended by others. This system, however has a few advantages: 1. it exists, 2. it is the only one to exist, and 3. it allows us to classify viruses. If I were not a co-father I would be inclined to say that it is not such a bad system after all.

We had made use of four discriminating characteristics and I am still convinced that discriminating characteristics should be the basis for a classification. It is clear, for example, that the category, virus, can be defined only by the use of discriminating characteristics. What could be the use of nondiscriminating ones?

However, a number of virologists have not yet understood that the principles which apply to the category virus necessarily apply to categories of lower hierachial rank. They have escaped the difficulty by forgetting to provide their own definition of viruses. So the selective use of discriminating characteristics and the LHT system are not universally accepted and battles are raging. A taxonomical war, because it deals with categories which do not exist in nature and with opinions, is the equivalent of a religious war. There is, however, a difference. The heretics, that is the others, not being burned, the war cannot come to an end.

The Cancer Institute

In October 1966, the director of the Centre National de la Recherche Scientifique asked me if I would within two years consider taking the direc-

torship of the Institut de Recherches Scientifiques sur le Cancer in Villejuif (one mile south of Paris). This could only be a full time job. Acceptance would therefore mean abandoning the Pasteur Institute where I had worked for forty-five years and where I felt quite at home, and also the Sorbonne (Faculty of Sciences) where I had taught microbiology since 1959.

The decision was postponed. In 1967, I spent seven months with Renato Dulbecco at the Salk Institute and we worked together on the biology of the Simian virus 40. Back in Paris, I considered the situation.

The "Délégation Générale à la Recherche Scientifique et Technique" had, in 1961, offered the Pasteur Institute the funds necessary to build an Institute of Molecular Biology. However, the director and the board of trustees ruled that molecular biology held no interest whatsoever for the Pasteur Institute.

In 1965, the obstruction ceased. A new director took up the matter again and after a number of vicissitudes, the question was settled. But the edification would start in the spring of 1969 and the building be ready in the fall of 1971—only a year before my retirement. No opportunity was offered to me anywhere else. The attic still harboured various residues and I had no chance to develop what was since the adventure of lysogeny, my principal and enduring interest, namely, the cancer problem.

Good groups were at work at Villejuif. Why not spend a few years helping them to develop research on cancer? So I decided to move and on February the first, 1968, started my last scientific—or maybe parascientific—endeavour.

Remembrances

During the first part of my career at the Pasteur Institute, I had the fatherly and efficient support of Felix Mesnil who, just before his death in 1938, had obtained for me the creation of the Service de Physiologie Microbienne. So I organized an attic into a laboratory. Later on, I experienced some difficulties. For example, a director told me once that my work was devoid of any interest for the Pasteur Institute and I should throw out a few workers in order to save money! I paid no attention whatsoever to this preposterous command for I had decided long ago and once forever that the scientists transcended the director and the board of trustees, and that everything good for science was good for the Pasteur Institute.

In the Institute, salaries were low, promotions almost nonexistent, and the budget of the laboratories poor, but freedom, holy freedom, was provided with unlimited generosity. In passing, freedom, if provided without discernment, can be very costly. Yet, freedom is not enough. If the work could be pursued and developed it was, thanks to the help of the Centre National de la Recherche Scientifique, of the Institut National de la Santé et de la Recherche Médicale, of the Délégation Générale à la Recherche Scientifique et Technique, of the National Foundation for Infantile Paraly-

sis, and of the National Institutes of Health. Thus, the Service de Physiologie Microbienne was amply provided with technicians, equipment, and a budget for daily life in such a way that money has never been the limiting factor for the work. May I be allowed here to express my deep gratitude to all those who have given a testimony of their confidence by generously supporting our work.

A scientist should be aware of the existence of competition and not be obnubilated by "the others," but I am now more and more conscious of the intensity of competition and of the pace of scientific development. Of course, in the past few decennaries, competition and pace have increased markedly. So, the alteration I observe in my mind might be a sign of the time as well as an evidence of maturation, or a symptom of aging, who knows? Whatever the case might be, science has always been competitive and it has certainly never been wise to enter, necessarily unprepared, widely different new fields of research. I have never given any thought to this aspect of scientific endeavour, and it is why, with perfect unconsciousness I have worked in succession on the morphology and morphogenesis of protists, on growth factors, and on various aspects of cell physiology, on lysogeny, on the virulence of viruses, and on the role and mechanism of nonspecific factors in the fight of the organism against viral infection.

In fact, during many years, research was performed simultaneously in different disciplines: the work on ciliates had started in 1921 and ended with the monograph on Apostomes in 1935, and with "Problems of Morphogenesis in Ciliates" in 1950. The work on nutrition of protozoa started in 1923 and was discussed in "Recherches biochimiques sur la Nutrition des Protozoaires."

The milestones of the work on growth factors were the papers on hematin (1933–34), on growth factor V (1936), and the book "La vitamine PP et les avitaminoses nicotiniques" (1942), l'Evolution physiologique, the editing of *Biochemistry and Physiology of Protozoa* (1951). The work on lysogeny extended from 1949 to 1953 and ended—almost—with the review "Lysogeny" (1953). The "Concept of Viruses" was published in 1957, the "System of Viruses" in 1962. In the meantime, "Biological Order" had seen the light (1962) and also the new concepts concerning the virulence of viruses and the fight of the organism against a primary viral infection (1959), and finally the mechanism of the action of fever on viral development (1969).

Retrospect

Biology in its widest sense has, since 1921, undergone extraordinary development: the structure and functions of vitamins, of growth factors and coenzymes, the steps of anabolism and catabolism, the activation and movements of oxygen and hydrogen, the cytochromes, the storing and utilization of energy, the antibiotics, the antimetabolites, the nature and structure of genetic material, one gene–one enzyme, the messenger, the operator, the

repressor, the transcription and translation, the code, colinearity, the structure of proteins, allostery, the nature of mutations, the sexuality of bacteria, and, more widely speaking, molecular biology and also molecular virology.

It happens that I have been associated with, or known many, if not most, of the scientists responsible for these revolutions in our knowledge and in our thinking. In one way or another, by their achievements or their personality, they have influenced what I may describe as my evolution. A few men, however, played an especially important role in my scientific life. Edouard Chatton, my master, with whom I collaborated intensively for sixteen years; Otto Meyerhof who accepted me in the Kaiser Wilhelm Institut in Heidelberg; David Keilin who provided to me a kind hospitality in the Molteno Institute in Cambridge; Louis Rapkine, the friend too soon disappeared and, finally, the members of the Service de Physiologie Microbienne. I had been fortunate enough to attract a few exceptionally gifted scientists to the Pasteur Institute and to be able to provide everyone with everything needed for research. My collaborators have certainly influenced me at least as much as I might have influenced them. Their names and achievements are well known. Thanks to them, the attic has been for many years the theater of remarkable successes. The work was pleasurable despite its intensity, and the atmosphere festive. As research pertains to ludic[1] activity I should perhaps have described the attic as a playground. Anyhow, it has been for me a constant ravishment to see important problems solved, great discoveries blooming, and new concepts piling up day after day. I sometimes said to my friends that I never felt jealous . . . and that this was meritorious.

[1] From the latin ludus, meaning game.

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