Heritance of Acquired Characters

A unifying concept is developed in relation to the genesis of cancer.

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The title of this article, which does in fact indicate what is before us for consideration, perhaps merits a word of clarification. The title, like the subject, was selected deliberately because it serves to point to new and exciting developments in the biological sciences. The theme does not carry the implication of refutation or support of any theory of inheritance, certainly not of Lamarckian concepts. It is proposed merely that, with all honor and respect for classical theories but without prejudice, we assemble and evaluate what has been learned recently about the genesis of malignant growths. If this should correspond with Mendelian and Darwinian teachings, few will be surprised; if it should not, it may be well to reassess our views.

The history of modern genetics is briefer than is often realized. Although Mendel had established a number of principles which provided a foundation for the science, his work was not recognized until the turn of the century. With De Vries's rediscovery of Mendel's classical studies and his own publication of mutation theory in 1901, modern genetics began its remarkable development. Indeed, almost everything we shall consider has emerged during the present century, and most of it during the last decade. Of interest to the present theme is the fact that the first decisive demonstration of a viral disease in man also appeared in 1901, with the report of Reed, Carroll, and Agramonte on experimental yellow fever. The relevance of these two publications to each other will become clearer as we proceed.

The major issue before us, an understanding of the genesis of cancer, might be said to have begun with the first demonstration that factors such as x-rays, certain organic chemicals, or viruses could induce the disease. These findings also were reported in the early years of this century. But in relation to our current concept, the beginning is considerably more recent and emerged only in 1944. It was then that Avery, MacLeod, and McCarty (*1*) announced that deoxyribonucleic acid (DNA) induced transformation of pneumococcal types. This was the first decisive evidence that nucleic acids possess biological activity. The discovery initiated a revolution in the biological sciences and led eventually to the demonstration that nucleic acids provide the chemical counterparts of the classical gene of heredity. It needs to be emphasized that broad acceptance of the central and controlling role of nucleic acids in the genetic apparatus has been accorded only during the last few years.

Not only did the now classical demonstration of the chemical nature of the transforming principle initiate studies (still far from completion) on the nature and functions of nucleic acids but also it provided the first example of a guided and directed mutation with precisely predictable features. The heritance of an acquired character was no longer a controversial and unsupported theory; it had become a reality. Bacterial cells produced a new type of capsular polysaccharide when new genes were introduced in the form of nucleic acids derived from different bacterial cells. The new character so acquired continued to appear in their daughter cells; the change was enduring and heritable. In addition, bacterial cells transformed in type by such nucleic acids became capable of producing the same nucleic acid, which could in turn transform other bacterial cells in an identical manner. Both the new character and its new genetic basis were handed on from cell to daughter cell in enduring continuity.

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A New Era in Biological Thought

This marked the beginning of a new era in biological thought, one which has not yet reached its full potentiality. In addition, there soon developed an awareness among physical scientists of the implications of this new concept. As a result, nucleic acid chemistry, genetics, virology, and immunology are presently being largely rewritten; they have already undergone upheavals that could not have been foreseen even a few years ago. The abyss between the smallest unit accessible to biologists—a virus particle—and the largest unit available to biochemists—a protein or nucleic acid macromolecule—has been bridged. The absolute distance was not great, but it took a very long time to span.

As is common at times of rapid advancement in knowledge, new information has been acquired too fast for new language to keep abreast of it. This has created some semantic problems which, in response to the tyranny of terms, have led to the invention of some astonishing verbal hybrids, several of which suggest paradoxes. Not only are we now confronted with *crystalline biology* and *molecular biology* but also, and more startlingly, with *infective molecules, infective heredity* and even *molecular disease*. Of the concepts that require such remarkable terminology more will be said a little later.

Before proceeding, it may be of interest to see some examples of objects that are described by such verbal hybrids. The gem shown in Fig. 1 is not a jewel. Clearly, however, it is a crystal, and of such value as not to be bought. To describe it fully and completely would require data usually provided by investigators in some eight different disciplines—five in the physical sciences and three in the biological sciences (*2*). This is a remarkable example of crystalline biology for it represents a crystal of the first human virus to be obtained in this form. The gem is composed of poliomyelitis virus and was prepared only 3 years ago by Steere and Schaffer, at the Virus Laboratory of the University of California.

Figure 2 shows, in the middle frame, an "infective molecule." The threadlike structure in the process of being unsheathed from a single rodlike particle of tobacco mosaic virus is thought to be a single molecule of ribonucleic acid. It is the one chromosome of this virus particle and can, even when fully separated from the particle, initiate infection

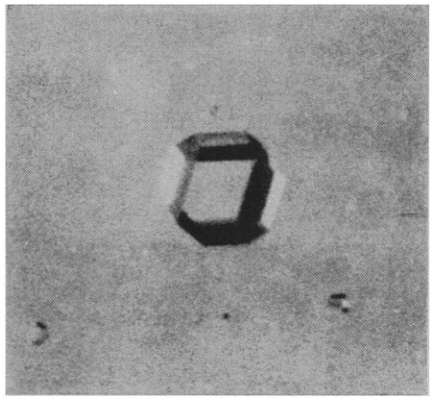

Fig. 1. A single large crystal of Mahoney poliomyelitis virus (× 80). [R. L. Steere and F. L. Schaffer, *Biochim. et Biophys. Acta* **28**, 241 (1958)]

of a host cell (*3*) which will result in the production of several thousand new virus particles identical to that shown in Fig. 2 in the frame at left. A comparable infective nucleic acid can be separated from poliovirus. Some 20 other viruses from plants, animals, or man have yielded infective nucleic acid molecules, and it is of special interest that two tumor-inducing viruses—polyoma of mice (*4*) and Shope papilloma of rabbits (*5*)—have recently been found to yield infective deoxyribonucleic acid.

As these few examples illustrate, the separateness of biology and chemistry seems partly to have vanished with recognition of the duality of nucleic acids, just as the distinction between classical physics and chemistry largely disappeared with the development of the quantum theory some 40 years ago.

Four Terms

It is often said that at present there are four magic terms in the biophysical sciences. All are closely related to each other and to the problems of cancer. These four terms are *nucleic acids, genetics, immunology*, and *viruses*. What do they signify, and how are they interrelated?

Nucleic acids are molecular substances of large dimensions—that is, with molecular weights of 2 to 6 or more million—composed of nucleotide sequences in greatly elongated chains which appear to be responsible for the continuity of life. Genetics is, of course, the science of heredity and variation. Immunology is the science of host response to a recognized difference in fine chemical structure. Viruses are the smallest, most simply constituted, and most remarkable infectious agents. They can lead to a state now designated "infective heredity," which is another way to say that they can lead to the heritance of acquired characters.

These four terms are inseparably related in the following way: Three of them—*genetics, immunology*, and *viruses*—identify biological phenomena which depend on the chemically coded information-bearing capacity of the fourth, *nucleic acids*. Genes are now thought to represent the biological counterparts of chemical fine structure —that is, short nucleotide sequences— in the nucleic acid molecule. Immunological responses are believed to depend on the exquisiteness of the recognition of the distinction between "self and non-self," in Burnet's terms—that is, on differences in the chemical fine structure of substances whose synthesis is under the control of genes. Viruses are considered to serve as vehicles for the transmission of information-bearing —that is, infective—nucleic acid to host cells, which become altered by the new genetic material.

This serves to exemplify the quite unexpected syntheses that have emerged recently among disciplines that, seemingly with but little in common, have for so long been considered separate and distinct. It underlines the importance of achieving still further unification of scientific knowledge. As a result of all that has been learned in the last few years, modern biology, finally and fully associated with the physical sciences and mathematics, appears to have vistas as extensive and as exciting as those of modern physics, whether of the astro- or the nuclear persuasion. In relation to the natural history of man, the implications of this new knowledge are as rich for fuller understanding of malignant tumors, behavioral disorders, and degenerative processess as for metabolic aberrations, endocrine dysfunctions, and infectious processes.

Transmissible Alterations

A beautifully documented example of the heritance of acquired characters is provided by the association of bacterial cells and certain bacterial viruses. Some of these infective agents may induce an alteration in their bacterial

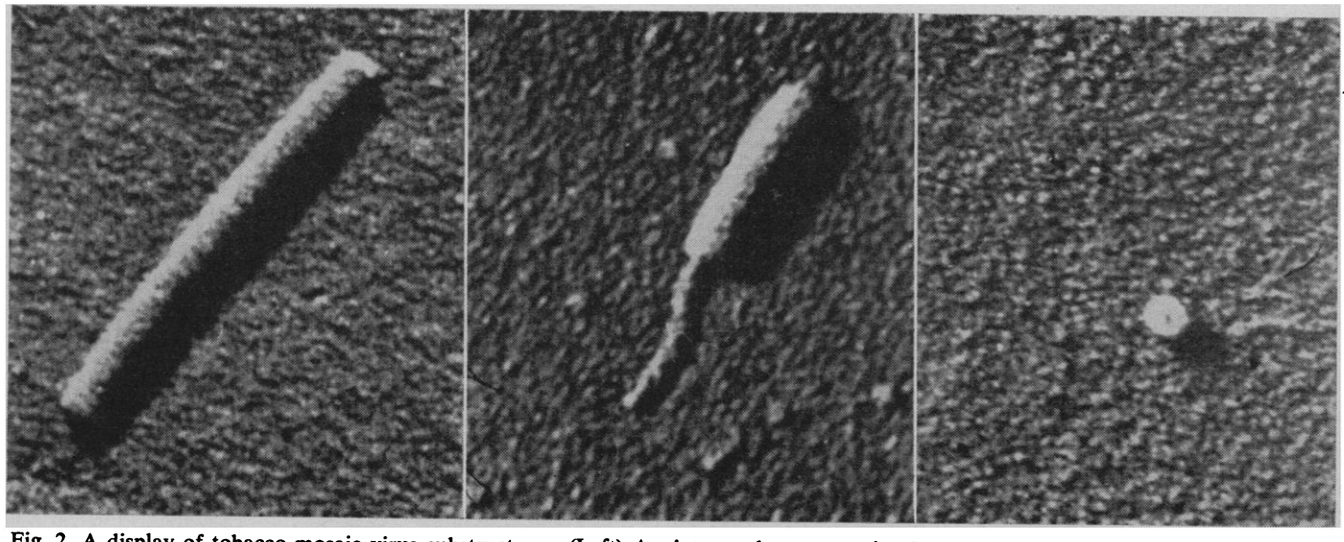

Fig. 2. A display of tobacco mosaic virus substructures. (Left) An intact tobacco mosaic virus particle; (middle) a partially degraded particle from which the ribonucleic acid (RNA) remains as a coaxial filament; (right) a short portion of RNA-free protein, seen end on, with a central hole visible (about × 195,000). [T. M. Rivers and F. L. Horsfall, Jr., Eds., *Viral and Rickettsial Infections of Man* (Lippincott, Philadelphia, ed. 3, 1959), p. 27]

host cells which is characterized by the dormant potentiality to produce more bacterial viruses, identical to that which led to the new character (*6*). Of most importance, the character so acquired is handed on to daughter cells in enduring continuity and has all the features of a genetically determined heritable change. It is now thought that part of the genome of the bacterial virus is incorporated into the genetic apparatus of the host cell and goes along with it in close register during cell division.

Even more startling is the biosynthetic alteration that is induced in nontoxigenic diphtheria bacilli by certain bacterial viruses. Under the influence of appropriate viral genetic material, such bacteria acquire the attribute of synthesizing a new and highly damaging protein—diphtheria toxin (*7*). This new character is clearly heritable and is handed on from cell to cell without the intervention of any additional viral infection.

The states of lysogeny (*6*) and diphtheria toxin production are both examples of infective heredity. Each is induced by a bacterial virus which contributes the imprint of new gene structure on a chromosome of the infected host cell. Daughter cells are marked by the new character, which has become transmissible and heritable. In this manner, certain changes in the genetic apparatus which formally correspond to mutations can be produced at will and, like those first demonstrated with the transformation of pneumococcal types by deoxyribonucleic acid, are predictable as to their nature and the products that result.

It should be emphasized that the examples cited represent enduring and transmissible alterations in the properties of cells that are induced in the first instance by external factors. Whether the change in character is determined by the effects of transforming DNA or by genetic material of a bacterial virus, the initial stimulus comes to the cell from its surroundings. Because the environment provided the material necessary to induce the change, the alteration may properly be designated an acquired character, and the evidence that it is in fact heritable appears conclusive. In essence, the only real difference between induced alterations of this kind and so-called spontaneous mutations, which by definition are heritable, is that the stimulus is known in the case of induced alterations and is not known when spontaneous mutations occur.

As might be expected, the association of viral genetic material with the genetic apparatus of the host cell commonly involves disappearance of the infective agent. For as long as the genetic material of the virus remains associated with a chromosome of the host cell, the virus itself usually cannot be demonstrated directly in that cell. This state characterizes bacterial cells that are designated as lysogenic and diphtheria bacilli that produce toxin. Moreover, it appears also to characterize most cells that have been made cancerous by polyoma virus (*8*). This phenomenon also may provide an explanation of the finding that infective Shope papilloma virus cannot be recovered from transplantable cancers that developed initially from papillomas induced by the agent (*9*).

In certain instances, by markedly altering the cell's environment, it is possible to cause sufficient dissociation between the viral genetic material and the genetic apparatus of some host cells to permit viral multiplication to occur. This results in the production of new infective particles.

Although this procedure provides a means of demonstrating that viral genetic material is being carried by lysogenic bacterial cells, it has not yet been possible to accomplish a similarly decisive demonstration with mammalian cells that have been made cancerous by viruses. The well-known difficulties that have encumbered attempts to recover viruses from the cancerous tissues of man may well be attributed to the possibility that infective viral particles are not present in full-blown cancer cells even though viral nucleic acid may be present.

That nucleic acids, whether of the ribonucleic acid type, as in plant and certain animal viruses, or of the deoxyribonucleic acid type, as in bacterial viruses and animal cells, guide and orient biological processes and function as the memory core of the genetic apparatus is now generally accepted. The discovery that a nucleic acid molecule is a self-replicating entity which can reproduce itself in a living cell, that the molecule carries along its nucleotide chain as much coded information as can be packed into 100 textbooks, and that it controls its own replication with such precision that a mistake—that is, a spontaneous mutation—may occur no more frequently than once in a million replications represents extraordinary advances.

Viral Studies and Cancer

How are these new findings related to cancer and to the problems that this relentless disease presents to mankind? Can the new knowledge of the biophysical sciences, advanced so rapidly through brilliant studies on viruses of several kinds, be rewarding in attempts to understand the genesis of malignant tumors?

On the evidence of history, knowledge of cause has been the most important factor in the development of specific treatment and effective prevention. There can be little doubt that one of the most pressing questions concerning cancer is this: What are its necessary and sufficient causes? As must be obvious, the current era, which has been characterized by remarkable advancement in the control of various infectious diseases, could not have emerged without knowledge of the causes of these diseases. Antimicrobial agents, useful in the specific treatment of a number of infectious processes, are directed, as their name implies, against the microorganisms that induce these diseases. Similarly, bacterial or viral vaccines, effective in the prevention of several infectious processes, produce immune responses in the host which are directed against the infective agents that induce these diseases.

A vast amount of work on the causes of cancer can be summarized as follows: The new properties which characterize cancer cells can be caused to emerge by a variety of factors. Among the best known and most decisively associated are radiant energy at certain wavelengths and in certain quantities, as in the instance of x-rays and ultraviolet light; organic chemical compounds of special types, such as some of those present in coal tar; and a surprisingly large number of viruses of animals of numerous species. Factors which are correlated with the occurrence of cancer but probably stand in a different relation to its inception than those enumerated above include, of course, genetic make-up, endocrine activity, and the age of the host.

Despite the multiplicity of factors that are known to be capable of inducing cancerous changes in cells, it seems probable that the necessary and sufficient alterations in the cell itself are similar, if not identical, in every instance. Important to the development of this general concept is the fact that the inducing factor has relevance to

cancerous changes in cells only at their inception. Once the properties that characterize cancer cells have appeared, such cells continue to produce more cells with similar properties during growth and division, just as do transformed pneumococci, lysogenic bacteria, or toxigenic diphtheria bacilli. This process can continue indefinitely under laboratory conditions, and in cell cultures the cancerous alteration, once it has appeared, continues to characterize the cell's offspring in the absence of the inducing factor which initiated the change. Thus, the new characters are enduring and transmissible in biological continuity, and it seems likely that they result from changes produced in the genetic machinery of the cell. Because the new characters may be led to appear through the influence of environmental factors, they may quite properly be considered to have been acquired. Because the new characters are transmissible in series in the absence of the initiating factor, they may be thought of as heritable. Thus, cancer can be assumed to represent still another example of the heritance of acquired characters at the level of the individual cell. The only important way in which it differs from the bacterial examples cited is that cancer involves the cells of animals, including man.

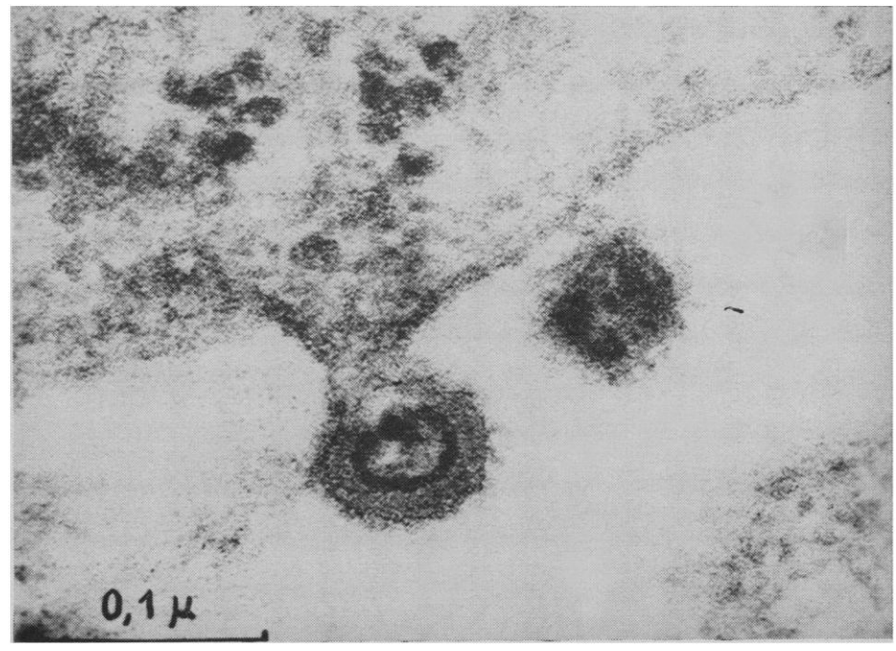

Fig. 3. A particle of Friend leukemia virus emerging from an infected mouse cell (about × 380,000). [E. de Harven and C. Friend, *J. Biophys. Biochem. Cytol.* **7**, 747 (1960)]

In Support of a Unifying Concept

In support of this unifying concept of the genesis of cancer there is a large and impressive mass of evidence. Cancer can be induced in animals by infective molecules—that is, deoxyribonucleic acid, separated from polyoma virus. This virus can lead to the development of some 20 different kinds of cancer in certain rodent species. The agent can also cause animal cells in culture to become cancerous. Not only do such altered cells breed true on continued cultivation but also they may cease to yield infective virus particles. This is closely similar to what occurs when viral genetic material becomes associated with the genetic apparatus of bacteria, as in the state of lysogeny or diphtheria toxigenicity.

Some 30 other viruses are now known to be capable of inducing a variety of cancers in numerous species of animals, including chickens, mice, rabbits, and dogs. These virus-induced cancers do not differ in any recognizable feature from those of similar type that occur in man. Like human cancers, they grow in an uncontrolled manner, produce metastases, and eventually lead to the death of the host.

In Fig. 3 is shown a virus particle emerging from an infected cell. This virus can cause leukemia in adult mice (*10*). Vaccines prepared with the virus are effective in producing immunity against this form of mouse leukemia. Some five other viruses that can induce leukemia in mice are already known. Among 17 lines of mouse leukemia that are being studied intensively at the Sloan-Kettering Institute, infective viruses capable of inducing leukemia have been recovered from six lines, and virus-like particles, closely similar to that illustrated, have been found in the others.

When normal cells are converted to cancer cells by viruses, new properties appear which commonly are expressed as alterations in cell form, in growth rate, in metabolism, and even in chromosome pattern or number. The cancerous change in cells has the hallmarks of a change in genetic make-up—that is, in gene complement—and so may be considered to have formal correspondence to a mutation. Permanent heritable changes—mutations—induced by viruses are well known in bacteria and in certain flowering plants. That such changes can also be produced in animal cells seems probable from recent work with cancer-inducing viruses.

Cancer can be induced both in animals and in man by radiant energy at certain wavelengths and intensities. Such radiant energy markedly increases the frequency of occurrence of a number of mutations and is known to produce permanent and transmissible alterations in the genetic apparatus. The effects of x-rays on chromosomes and even on the fine structure of nucleic acids are beginning to be understood. Similarly, several organic chemical compounds of special character are mutagenic and may also induce cancers in animals or man. As with the alterations produced by x-rays or viruses, the new characters acquired by cells exposed sufficiently to such compounds persist and are transmitted from cell to cell in continuing series. In all of the instances cited, the evidence indicates that the effects of the various cancer-inducing factors are similar and attributable to alterations produced in the nucleic acids that control the genetic machinery.

Thus, it seems reasonable to think that cancers in general can be considered to represent the heritance of acquired characters at the cell level. Such a unifying concept serves to embrace all the types of cancer cells that have been adequately studied and provides a basis for questions to which acceptable answers may be secured through further research on the necessary and sufficient causes of malignant growth.

Implications

In this article, one additional question should be answered. If there are a number of environmental and intrinsic factors that contribute to the occurrence of cancer and if ultimately all lead to the same alteration in the most vital machinery of the cell, why has so much emphasis been placed on work with cancer-inducing viruses?

The emphasis has been deliberate for these reasons. First, it is beginning to appear that most tumors of animals will be found to be virus-induced. Second, there seems to be no valid reason to think that human tumors are in any way unique or that they differ from tumors of other animals in any significant manner. Third, if viruses are associated with the inception of cancer in man, it seems possible that several beneficial advances might be developed. Through extension of principles that have evolved from studies of virus-induced cancers in animals it might be feasible to develop reliable procedures for early and specific laboratory diagnosis, so that effective treatment of early lesions could be instituted well before these lesions would ordinarily be detected on physical examination. Finally, it might be possible to develop specific preventive measures and, through appropriate immunization procedures, to prevent virus-induced cancerous changes at the cell level before they occur. The feasibility of such encouraging developments has already been demonstrated in principle through model experiments with cancer-inducing viruses in animals. Whether such possibilities have any real applicability to man depends on answers to two last questions: Are viruses causally related to human cancers? If they are, what is the frequency of the relationship?

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Medical Scientists in a Château

The traditional social structure creates problems for medical research and researchers in Belgium.

Renée C. Fox

On Sunday afternoon, 15 November 1959, a medical scientific colloquium was held in the château at Laeken, which belongs to the royal family of Belgium. This was a meeting officially devoted to accomplishments and problems in the field of cardiac surgery. The conference was held in honor of three foreign medical scientists, A. G. Brom of Leyden University, André Cournand of Columbia, and Robert Gross of Harvard, who, through their trail-blazing experimental work, have made outstanding contributions to this field. The three men had traveled to Belgium in order to personally receive the "doctor *honoris causa*" degree that each was to be awarded in the course of the following week. Along with King Baudoin of Belgium, who was honored with the same diploma, Cournand subsequently received his honorary degree from the Free University of Brussels on the same day that Gross and Brom were awarded their degrees by the Catholic University of Louvain.

According to accounts in Belgian newspapers, among the persons invited to the medical scientific gathering in the royal château were the following: numerous members of the royal family (King Baudoin, ex-King Leopold, Princess Lilliane, Prince Alexandre, Prince Albert, Princess Paola); the ambassadors of France and the United States; various ministers, present and past, of Cultural Affairs, Public Instruction, Social Security, Public Health, and so on; the rectors and deans of each of the four major Belgian universities (Ghent and Liége, as well as Brussels and Louvain); professors of the medical faculties of each of the universities, and numerous other professors; medical specialists from various university-connected centers; certain young Belgian physicians who were members of cardiac teams; mature physicians in private practice, specializing in cardiology; Belgian physicians who had received some training in the United States; the director and various members of the Princess Lilliane Cardiology Foundation; representatives of the Belgian Academy of Medicine and the Royal Flemish Academy of Medicine; the president of the Fund of Medical Scientific Research; the president of the Red Cross; a commissioner from EURATOM; Belgian patients with heart maladies who had undergone cardiac surgery outside of Belgium, chiefly in the United States (there were aproximately 40 of these); Belgian patients who had undergone cardiac surgery in Belgium (there were 400 such individuals at this time—how many of these came to the colloquium was not specified in the newspapers); some candidates for cardiac surgery; and the families of all these patients.

Before the colloquium, a tea was served in the Palm Rotunda of the château. In the midst of the reception a sudden failure in electricity extinguished all the lights of the château. Members of the palace staff had to be summoned to bring candles, and for a while the reception proceeded in the at once eerie and romantic "ambiance" of candlelight.

After the tea (electricity restored), a speech was delivered in French by a professor of medicine of the University of Brussels, who was also Belgium's delegate to the International

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