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# THE HISTORY OF EARLY VIRUS RESEARCH

## HOW TECHNICAL PROGRESS IN THE INVESTIGATION "FILTERABLE" INFECTIOUS AGENTS UNDERSTANDING THE NATURE OF THE VIRUS HAD DEVELOPED[1]

[1] This work was created during a guest residency of the author at the Max Planck Institute for the History of Science in Berlin from July 1997 to January 1998. The author worked in the department of Professor Dr. Hans-

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## INTRODUCTION

In general, scientific experiments are attributed a compulsory character, to which the gain in logical knowledge of nature is owed. Methods for the appropriate control and execution of procedures and their repetition are understood as a means of eliminating differences of opinion as to what may be considered the "right" extension of scientific knowledge (see Collins 1985b: 137). In evaluating an instrument, its reliability is considered a key criterion for enabling information transformation, the transformation of input information from the outside world into outputs that can be absorbed by our sensory apparatus[2], a view that is cultivated in education and wherever spectacular experiments are used for demonstration purposes. For "normal" science, this understanding, which avoids reflection on assumptions of reality, has proved to be very useful. From this point of view, the development of scientific knowledge presents itself as a process of a progressive elimination of subjective perceptions in favour of measurable quantities and theoretically founded invariants, as a process in which subjective constructions are continuously replaced by objective knowledge. Contrary to this, the more recent sociology of science, when expressing itself on the connection between empirical laboratory practice and theoretical knowledge, works towards an understanding according to which objects of research, as they are "given", cannot be distinguished from the way in which they are recognized.

Scientific meanings are not something that is already contained in the facts and is unalterably predetermined for researchers, as if experiment and observation not only help to develop practical skills for reproducing the phenomena under study, but at the same time reveal otherwise hidden "information" to the senses of researchers, which could be led to an adequate (with the phenomena "coinciding") theory-language expression (see Latour 1987: 27 and 30; Latour/Bastide 1986; Collins/Pinch 1982: 7 ff.Collins 1985a, 1985b; Krohn/Küppers 1989: 28; Woolgar 1988: 28 f.).

The development of scientific knowledge is not due to the adaptation of interpretation patterns to found phenomena. Rather, what is investigated and interpreted is shaped according to patterns by the researchers themselves. Research activity is instructed by given theories and

Jörg Rheinberger, to whom he owes the friendly invitation and many suggestions. He also owes much to Dr. Skúli Sigurdsson and Dr. Ton van Helvoort, who were always willing to discuss and critically review his manuscripts.

[2] „The history of instruments shows that a general approach to improve the reability of an instrument is to narrow its application scope, that is, to make it special for a limited range of subjects ... The proliferation of instruments provides a material base for the specialization of science“ (Chen 1997: 271).

methods of a discipline, so that the results are to a certain extent guided back to the conditions of research. If discrepancies are found between what was observed in the experiment and what should have occurred according to the theory, efforts are made to change the experimental procedures and conditions so that the objects examined behave as expected. This connection can be fixed in abstraction as a cycle; Collins speaks in this respect of an "experimental circle".[3] This also applies to the so-called key experiments. Gilbert and Mulkay describe how different the stories that researchers tell about such an experiment turn out to be when the circle of addressees changes, and it turns out that there are different ways in which key experiments are used to construct stories: They can be presented as if they had necessarily led to a theoretical version, but they can also be described as evidence for the validity of an already preconceived theory (Mulkay/Gilbert 1984: 117 ff.).[4]

[3] He explains this using the example of the history of radio astronomy: In the late 1960s, the US physicist Weber claimed that an instrument he had constructed could be used to measure gravitational radiation, of which it was not known at the time whether it actually existed. There was no unanimous opinion on the part of physicists. Some agreed with Weber, others considered his opinion to be an unproven assertion. To prove it, the device developed would have had to be proven reliable, but this would have depended on whether it actually registered what was to be registered - gravitational radiation. It must therefore deliver "correct" results. But what is a "correct" result had to be made dependent on whether gravitational radiation actually exists, which, however, should first be determined with the device (Collins 1985a: 79 ff.).

[4] From which part of the circle the story is started to be told is influenced by social conditions (the relationship of the narrator to the audience). There is a noticeable difference whether the circle of addressees is made up of closer professional colleagues with whom informal conversations can be held, or whether it is a not so close audience, an audience with whom only text-language communication takes place. In the latter case, the contingent stories of origin of the experiments are faded out, in the other case "those experiments ... defined as key, not because of any particular objective features of the experiment itself or the reception the experiment received, but by the way they are presented when participants construct a particular kind of justificatory historical account" (Gilbert/Mulkay 1984: 117 f.).

Concepts that are debated in the professional public of a scientific discipline can only be justified by reference to applied technical procedures. Since reality is thus always experienced concretely only through information processing processes, guided by the experiences and processing rules established in the subject, the proportion of what is realistic can never be precisely determined (see Graber 1984), so that the perfection or further development of experimental techniques in the hope of a better approximation to "reality" cannot be understood as something that would reduce the "blind spots" more and more. Thus it cannot be determined once and for all which is the one correct theory in which a phenomenon, an object is to be described. Empiricism does not control the discourse of researchers in a way that excludes rivalries about how to understand the nature of an object. And thus the interpretation of findings can never be brought to a final conclusion. For any given set of experimental results and empirical data there is not only one theory that can explain them, and the question arises whether - if observations are theory-driven - theoretical differences in a given field can be understood at all as different interpretations of the *same* observational data (see Hanson 1969: 18).

From the foregoing, a number of questions arise: What drives the development of theoretical knowledge if experimental and other empirical data are not sufficient to determine the theory in which they can be explained? And what exactly is the "part" that perfection and improvement of research techniques and methods play in this? The independence of the structure of theoretical knowledge from empirical knowledge does not mean that progress achieved in the development of knowledge that instructs practical activities is irrelevant to the development of theory. How the transition from the level of empirical knowledge to the level of logical-theoretical knowledge takes place when empirical research practice cannot be understood as an effective test of the validity of assertions separate from the wishes and intentions of the researcher, when the idea that the repeatability of results in experiment would create a fixed relationship between theory and observation must be discarded[5], is a question that still provokes controversial debate. It will be examined below using an example from the history of science. We refer to sections of the earlier history of research on viral infections.

## THE DISCOVERY OF A FILTERABLE INFECTIOUS AGENT AND ARGUES ABOUT WHAT IT IS: A CHEMICAL SUBSTANCE OR A MICRO-ORGANISM

The virus is defined as a biological entity consisting of nucleic acid and protein, as a complex of macromolecules whose genetic material consists of either DNA or RNA and for whose replication suitable host cells must be present. This definition (which is not reproduced here in

[5] According to Bijker (1994: 242), the functioning or non-functioning of a technology is not an inherent characteristic, but the result of social construction.

full) differs markedly from the one that still applied in the early 20th century: the virus was determined as a filterable, submicroscopic pathogen of infectious diseases that could not be cultivated on inanimate culture media. [6] In aetiological disease research, two further characteristics were added, the ability to reproduce in the infected organism and unlimited transferability from one susceptible organism to another. This definition boils down to the verbal manifestation of a specific research practice by explaining the pathogen by its reactions to bacteriological experimental conditions common at the time. We are mainly interested in the transition from the early to the modern virus concept and the role that the development of procedural conditions played in this process.

From the beginning, very different views were taken on the nature of the virus. It was thought of either as a soluble substance, an enzyme, a ferment, high molecular weight proteins that can survive a series of chemical processes without losing their infectivity (i.e. that are organic substances without life of their own), or the virus was thought of as a particularly tiny microbe. Plant pathologists in particular concluded that a soluble substance or an enzyme was a microbe. The history of their subject led them to think primarily of chemical compounds. Animal and human pathologists, who were more closely connected to bacteriology and cytology, favoured the microbial concept.

In 1879, Adolf Mayer discovered the infectious nature of tobacco mosaic disease at an agricultural experimental station in Holland. However, he did not succeed in isolating a pathogen causing this disease. He initially considered that the disease might have been caused by nutritional deficiencies. However, after a comparative chemical analysis of the healthy and diseased tobacco leaves, he found that the diseased plants did not lack nitrogen, potash, lime or other substances. Nor could the soils have caused the disease; they were uniformly fertilised and suitable for growing tobacco. Modifications in the layout of the forcing beds (for example, variation of the heat) could not provide information either. Targeted injuries to the roots of young plants also proved harmless (Mayer 1886: 455 f.). Mayer then made the discovery that "the sap of diseased plants obtained by friction is a safe infective agent for healthy plants. If a diseased leaf is finely rubbed with the addition of a few drops of water and the resulting emulsion is allowed to soak up through finely drawn capillary glass tubes and pierced into the leaf veins of an older leaf, the disease can be transmitted to healthy plants (ibid., 461 f.). Mayer was now looking for "formed content bodies". But the infectious substance proved to be something that could not be examined under a microscope. Robert Koch's methods of cultivation on inanimate nutrient substrate - the cultivation of pure cultures was, according to

[6] In the second half of the last century, at the height of bacteriology, the term "virus" was associated with any type of infectious microscopic agent. Shortly before the turn of the century, however, it was used, following Beijerinck (1899), only with regard to filterable infectious agents.

Koch, the actual "main focus of all studies on infectious diseases" (Koch 1912: 131) - also failed.[7] Mayer ruled out the possibility of a ferment, because then the reproduction of the agent could not be explained. He substantiated this decision with a filtration experiment: when using double filters (consisting of filter paper) he obtained a clear filtrate and assumed that a non-cellular substance had passed through the layers of paper. According to Mayer's report, however, the filtrate was not infectious (loc. cit., 465). "This would already rule out the possibility of an infectious effect by an enzyme-like body; for it is almost contradictory to all known properties of these strange substances to be removed from a liquid by simple filtration. His observation that the infectiousness of the juice was destroyed by heating it to 80° Celsius after several hours, he interpreted as meaning that the pathogen organizes that it must be cellular. Mayer finally thought of a bacterially caused disease, although "a closer knowledge of the form and way of life of the guilty bacterium ... of the guilty bacterium cannot be obtained in this way" and must be reserved for future research (ibid., 466).

Contrary to Mayer, who had only discovered the infectious nature of tobacco disease, but not the pathogen itself, the Russian plant physiologist Ivanovskij (1892) was able to prove that it is the liquid filtered from mosaic-diseased tobacco leaves that causes the infectious effect. Ivanovskij presented the results of his observations in an essay entitled "O dvuch boleznjach tabaka", where he critically evaluated the observations made by Mayer in Holland and based on his own studies in Crimea and Bessarabia, described the mosaic disease of the tobacco plant, ascertained its infectious character and announced the surprising fact that the cell sap with the pathogen passes through bacteria-proof filters without losing virulence (Ivanovsky 1892). Such a phenomenon had not been encountered before when investigating the transmissible agents known up to that time, and it immediately posed serious problems in explaining bacteriology. With the filtration technique used in this field, infectious material was to be sifted out of liquids, so that only sterile filtrates were to be expected, "waste products" which were produced when handling infectious material and therefore seemed to be of no importance.

The Dutchman Beijerinck (1899) noticed a little later, without knowing Ivanovskij's discovery, that the examination of sick tobacco leaves produced a filtrate that ran contrary to this expectation. He too succeeded in spreading the disease with filtrates from diseased plants. In his experiments, Beijerinck drew juice from mosaic-diseased tobacco leaves through porcelain filters, after microscopic examinations of the pressed juice and cultivation had always produced

[7] Behring emphasised in 1894 that Koch's methods allowed "the targeted search and identification of suitable animal species for experimentally produced infections, the separation of the various microorganisms in the disease products by breeding on solid culture media, the excretion of those microorganisms, which are insignificant for the emergence of the specific character of the disease, the artificial generation of the suspected pathogens in pure culture and the exact morphological study of them, finally the arbitrary generation of an infection by the pure cultures of the parasitic pathogen" (1894; quoted after Zeiss / Bieling 1941: 31).

negative results and biological manifestations of the pathogen could not be detected. After an unsuccessful search for anaerobic bacteria[8]that might have passed through the filter (which were known to have extremely small, filterable spores)[9], and the fact that no corpuscular pathogens could be detected under the microscope, Beijerinck denied the agent a cellular nature and characterized it as a living liquid contagium vivum fluidum, a substance that, in order to replicate, exerts its influence in solutions. He considered water solubility to be a characteristic of all filterable contacts. As molecular agents capable of replication, they should only be effective when incorporated into the living protoplasm of the cell.

The assumption that the pathogen was a living infectious substance in the form of a liquid met with widespread resistance at the time, because it was difficult to imagine a dissolved living substance, a substance which, although non-cellular, could reproduce. A number of researchers were more inclined to assume that there was a Contagium inanimatum. Centanni, who had identified an infectious filtrate as the cause of the chicken plague, considered the possibility that the pathogen might be a chemical agent of the autocatalyst type, capable of irritating the host cells and, by a pathogenic deviation of their metabolism, stimulating them to produce a substance identical to it. However, he did not rule out the possibility that his investigations might have led to the discovery of a reproducing living organism (1902: 198).
At about the same time as Beijerinck, the American plant pathologist and physiologist Woods (1899) was also studying this phenomenon. He used enzyme research to explain the phenomenon and came to the conclusion that the mosaic disease of tobacco was not infectious at all, but the result of the overproduction of certain oxidising enzymes in the plant, which could also be detected in increased quantities in the sick tobacco leaves. For him, the aim was therefore to find the cause of the tobacco mosaic disease in the plant itself and not in an exogenous agent. Woods was particularly interested in the role of enzymes in cell physiology. In the late 1990s, he studied the relationship between certain enzymes and plant diseases that were associated with chlorophyll destruction. One of the subjects of his research was the discoloration of chlorophyll, the green dye in plant cells. Woods believed that the discolouration of the leaves in autumn could be shown to be an effect of oxidising enzymes. In certain disorders such as tobacco disease, where chlorophyll degradation is clearly visible - the stains on the leaves could be seen as symptoms of that degradation - the enzymes oxidase[10] and

[8] In the absence of oxygen (under exclusion of air) growing microorganisms, which gain their vital energy through fermentation. When respiration is carried out under anaerobic conditions, inorganic compounds serve as hydrogen acceptors instead of oxygen.

[9] He also found that infectivity could be eliminated by applying heat once, at a level where spores could not yet be destroyed.

[10] Enzymes that activate oxygen and transfer hydrogen or electrons directly to molecular oxygen, forming water or hydrogen peroxide.

peroxidase[11] could be the cause of the disease. Although the enzymes in question could not be filtered, they switched to the culture medium (agar) used for cultivation.

A microbial nature of the virus was also denied by Hunger (1905), but Woods' position was rejected by him on the grounds that the unlimited transmissibility of the tobacco mosaic disease pathogen speaks against the assumption of an oxidizing enzyme. Instead, he proposed to assume a non-living "phytotoxin". This toxin, which is normally a harmless metabolic product of the plant cell, causes physiological disorders (such as mosaic disease) when it is accumulated as a result of a very high plant metabolism. The toxin can then penetrate into normal cells where it induces a multi-product of toxin via a physiological contact effect. The transferability was to find its explanation in the fact that the poison had the property of acting in a physiological-autocatalytic form (1905: 415 f.). That the virus of the mosaic disease is a metabolic product of the tobacco plant itself (the stimulation sequence of a pathogenic deviation of the metabolism, which is accompanied by the new formation of the irritant substance), that it has an endogenous history of origin as a product of the infected host body, was later advocated by Doerr (1923), among others. Accordingly, results of laboratory experiments on the production of diseases such as tobacco mosaic disease were not interpreted as the result of activating latent infections by means of any kind of intervention, but in the sense of stimulating a pathological deviation in the metabolism of the respective organism (see Fust 1944: 202 f.).

Neither Ivanovsky, Beijerinck, nor Woods were able to satisfy those demands on which the probative force of claims of infectious diseases was made dependent at the time, demands which are recorded in the so-called Koch postulates (Koch 1881). In the[12] following period, such difficulties were also encountered in the investigation of other diseases, and not only in plant pathology, but also in animal and human pathology. The work of Loeffler and Frosch on the etiology of foot-and-mouth disease, which they published in 1897 and 1898, played an important role in further virus research. They found that animals treated with bacterially sterile filtrates derived from lymph became ill in the same way as control animals treated with non-filtered lymph. Spoonbills and frogs had initially expected to obtain a toxin similar to the

[11] Enzymes which oxidise substrates with hydrogen peroxide, whereby hydrogen peroxide is reduced to water by the hydrogen split off from the substance to be dehydrated.

[12] Koch had already noticed that a large number of infectious diseases, which were later frequently identified as virus-induced, elude bacteriological understanding. As early as 1881, he warned against the assumption that all causes of infection were of a bacteriological nature. Other microorganisms could also be effective in the animal body. At a congress in 1890, he explained that bacteriological research had failed in the case of those infectious diseases which, because of their pronounced infectiousness, seemed to offer particularly easy targets for research. "This concerns first and foremost... exanthematic infectious diseases... Not a single one of them has been able to find even the slightest clue as to the nature of the pathogens that cause them ... I would like to incline to the opinion that the diseases mentioned are not bacteria at all, but organized pathogens which belong to completely different groups of microorganisms" (Koch at the 10th International Medical Congress in 1890, quoted from Gildemeister 1939: 1; see also Koch 1890: 756).

diphtheria toxin when they discovered that laboratory animals injected with bacteria-free filtrates from calf lymph became ill in the same way as the control animals. Bacteria as the pathogen of foot and mouth disease could not be found. In bacterially sterile lymph, morphological elements of various kinds could probably be found. However, it was not possible to detect any structures that could be regarded as pathogens. The surprising result that the effectiveness of the lymph was not influenced by filtration could be reproduced by experiments on numerous calves and pigs: Again and again, the same clinical picture could be produced in animals infected with foot-and-mouth disease with bladder contents from animals suffering from foot-and-mouth disease, which had been filtered through diatom candles. Spoonbills and frogs saw two possibilities to explain this phenomenon: Either the bacteria-free filtered tissue fluid contained a dissolved, extremely effective toxin, or the undetectable pathogens of foot-and-mouth disease were so small that they were able to pass through the pores of a filter that could retain the tiniest known bacteria. The discoverers of the filterable agent of foot-and-mouth disease chose the latter option. In 1898, in a report of the German Commission for the Study of Foot and Mouth Disease, they wrote the following: "If the further investigations of the Commission were to confirm that the filtrate effects are indeed, as it would appear, caused by such minute organisms, it is reasonable to think that the pathogens causing many other infectious diseases of humans and animals, such as smallpox, cow pox, scarlet fever, measles, typhus, rinderpest, etc., could also be the cause of these diseases, which up to now have been searched for in vain, belong to the group of these very smallest organisms" (1898: 371).

Ivanovsky had not continued his observations of the phenomenon that the juice of the mosaic leaves retained its infectious properties after filtration through porcelain filters for a number of years. He did not tackle them again until 1897/1898 in the context of his habilitation thesis, which was published in 1902 (on the basis of this work he published an essay in a German journal in 1903). In this work he also dealt with the observations and views of Beijerinck (1899), Woods (1899) as well as Löffler and Frosch (1898), which were already available to the public at that time. He was particularly interested in the first two researchers, both of whom - as explained above - were convinced of the non-bacterial character of the cause of tobacco mosaic disease, which was beyond our control. Ivanovskij considered Beijerinck's concept, which suggested the assumption of a non-corpuscular character of the pathogen, to be not compelling. He also considered Woods' view to be unfounded. The artificial transmission of the disease by inoculating healthy plants over a large population and several generations was not compatible with the assumption that the disease was caused by a plant-specific enzyme, because the infectious effect would have to be exhausted at some point. On the basis of his own research, he was convinced that it was an infectious exogenous pathogen which must be of a corpuscular nature but could not be cultivated on artificial media. Ivanovsky alternately called

the pathogen a virus or a microbe, although he was inclined to believe that the agent he was looking for might be a bacterium with spore formation.

Ivanovsky carried out various experiments to substantiate his view that the pathogen had particle character. And so he looked for microorganisms that were small enough to pass through filters. As a result of microscopic studies he noticed inclusions and crystalline deposits in the cells of diseased leaves in the form of colourless leaves (see 1953: 109-110), in which he believed to have found the pathological origin of tobacco mosaic disease. However, their discoverer did not yet suspect that these "Ivanovsky crystals" - as they would later be called - could be the virus themselves. In his opinion, the crystalline inclusions showed a reaction of the cells to the irritation caused by the pathogens. However, the small amoeba-like structures discovered in fixed and stained cells and believed by him to be the causal pathogen of tobacco mosaic disease, which he called "zooglea", could not be isolated. Ivanovsky proposed to understand the agent as a spore-forming microorganism. The spores, and not the microorganism itself, could be filtered. He wanted to explain the infectivity of a filtrate that could not be cultivated on artificial culture media. If the spores could only germinate in living plants or generally only under optimal conditions, this would also explain the failure of attempts to cultivate the microbe in vitro from infectious filtrate. In heat resistance and resistance to dehumidification, Ivanovsky saw further evidence that there could be spores in the filtrates.

The view that the virus was not a living organism (a tiny bacterium, an "ultra-microbe") but an enzyme-like substance was closely linked to the expectation that a chemically pure virus could be obtained. The understanding that viruses were chemical molecules and that they appeared spontaneously in host bodies without exogenous infection gained plausibility after Stanley succeeded in 1935 in presenting the tobacco mosaic virus in crystalline form. The virus revealed itself to him as something that behaved in all its properties like a chemically pure protein, without admixtures of fat, lipoids, carbohydrates and salts. Such a body could hardly be imagined as an individual organism. The virus presented itself as an elongated molecule of very high molecular weight. The substance obtained in crystal form proved to be something between 100-1000 times more infectious than the viral plant raw material from which it was obtained. Even repeated recrystallization did not reduce the infectious power. Stanley identified the virus as a globulin or protein molecule.[13] After this discovery, other types of plant viruses

[13] Bawden, Pirie et al. argued somewhat later (1936) that they had discovered phosphorus in the crystalline substance extracted from mosaic-diseased tobacco plants and that this element was contained in the form of nucleic acid. They wondered whether the crystalline substance they had isolated was the virus itself or not. For them it had not yet been proven, "that the particles we have observed exist as such in infected sap" (ibid., 1052). Stanley conceded that the isolated substance is not just protein. After that his research was widely recognized. A few years later, Schramm reported that the tobacco mosaic virus is still capable of producing a new generation of viruses even after its protein envelope has been chemically modified and pieces broken out of it. Schramm decomposed the particles with weak alkali. Nitrous acid was then added, after which the

proved to be crystallizable. Finally, it was reported that chemical structure research had also shown that a number of animal viruses had a defined material composition. "Viruses such as foot-and-mouth disease and rabbit papilloma are no less uniform than the tobacco mosaic virus. The investigation of the polyhedron disease of insects showed that the polyhedra occurring in the virus diseased caterpillars can probably be regarded as crystallisates of pure viral proteins. These animal viruses are therefore chemical compounds and not organisms," said Schramm (1942a: 258). And in another essay of the same year it is stated that a protein substance of completely uniform molecular weight was isolated from the vesicles of cattle suffering from foot-and-mouth disease. A uniform protein was also obtained from the warts of the cottontail rabbit. They had to be regarded as chemical molecules, even if it had not yet been possible to obtain them in crystallised form (Schramm 1942b: 793).[14]

The disease-causing effect of filtrates was subsequently demonstrated in a number of other types of infection, but the accumulation of empirical knowledge has not led to a uniform understanding of the nature of these pathogens. It remained open whether such tiny infectious agents are really microorganisms or mere chemical substances. Whether this or that position was taken or rejected - in any case, empirical evidence could be presented for both defence and attack purposes.

The microbial nature of the virus was demonstrated by the unlimited transmissibility of infectious diseases caused by filterable pathogens from one susceptible individual to another, whereby only minimal amounts of substance were required each time, which had to undergo a very considerable dilution in the body of the recipient. It could be thought that even the most effective substance would be rendered ineffective immediately by this continued dilution, unless an oppositely directed process intervened compensatory, the ability to increase in quantity from within, to multiply by assimilation of foreign substances while constantly maintaining the original properties, which was, however, exclusively considered an attribute of living substance (see Doerr 1923: 909). "That a protein molecule should grow out of itself and divide is still incomprehensible with the previous view of life and reproduction", as Seiffert explains (1938: 9).

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particles were restored to their original form. But they no longer contained nucleic acid, and they were no longer capable of reproducing, which led to the conclusion that the protein in the virus does not contain the information for its reproduction. In 1955/56, Schramm and Gierer together succeeded in obtaining the protein-free RNA of this virus by adding phenol to a tobacco mosaic virus suspension. With this "pure" RNA it could be shown that it alone contains all the information for virus replication (note from Munk 1995: 37 f.; no sources given). Independently of these investigations, Fraenkel-Conrat at the Virus Laboratory in Berkeley near Stanley came to the same conclusion.

[14] The crystallization of an animal virus was not successful until 1955, namely the polio virus (Schaffer/ Schwerdt 1955).

Because the various types of virus could be inactivated ("killed") by certain physical and chemical influences (so that the altered material was no longer infectious) without affecting the chemical and serological properties and the shape of the microscopic crystals - they remained intact - this also helped to understand the virus as a microbe: The fact that bacteria are considered to have the ability to reproduce and therefore their
Microbiologists and immunologists were familiar with infectiousness by killing without visibly modifying the chemical composition of their body substances and without affecting the antigen functions in any way.

Burnet and Andrewes referred to "the occurrence of immunologically or functionally different types, the transmission of which, within fairly wide limits, always maintains the initial type. Each type of foot-and-mouth disease virus causes the same clinical picture in the guinea pig, and yet the immunological character of the different types remains unchanged, whether the passage occurs in the guinea pig or in another susceptible animal" (1933: 169; see also Munk 1995: 7 ff.). In the case of herpes, Burnet and Andrewes continue, it is possible, "by means of suitable passages, to obtain strains that ... are neurotropic or dermotropic and are ... ...and reproduce with these properties." Avian tumor viruses and bacterial viruses had the same property (these species are discussed below), "a property probably common to all living organisms of any species. Each pure passage strain will have certain inheritable traits that are characteristic of it. ...that are independent of the surrounding environment and distinguish it from other strains." The occurrence of such type-individuality in transmissible pathogens of the species in question suggests "that these are independent microorganisms with self-multiplication" (ibid.).

Gratia (1921: 217 ff.) considered the idea of the virus as a metabolic product to be justifiable only if it could be shown that the process always involves host cells of the same type; how else could one and the same protein, when acting as a stimulus, modify the metabolism in a constantly identical way and with the formation of identical metabolic products? Viruses, however, would retain their original character during serial transmission even when the host species changes - an unmistakable sign of autonomous behaviour. The fact that, for example, herpes viruses only ever become herpes viruses, regardless of whether they reproduce in human skin or in the rabbit brain, was for him proof against the concept of endogenous virus formation. He did not want to succeed in empirically proving a chemical or serological relationship between the virus protein and the normal protein of the host, which would have supported the hypothesis of endogenous virus production. Chester (1936) was initially convinced that he would have been able to prove cross reactions between crystalline mosaic virus protein and normal tobacco plant protein by means of complement fixation and anaphylaxis. However, subsequent tests showed that the preparations of the virus protein were contaminated with

normal protein (see Doerr 1938: 36). Seiffert referred to immunity research: "On the basis of immunity research, we know that every virus investigated so far has its own antigen structure. Vaccine virus, obtained from humans, cattle, rabbits, from tissue cultures, from egg culture, always produces the same specific reactions with immune sera. Biologically, it would be even more difficult to understand that a virus of the same protein structure should be formed uniformly in cells of such different animals. But the same applies to the very small virus of foot-and-mouth disease. It is quite unlikely that its three types, whose structure can be sharply separated serologically, can be produced quite uniformly in cattle, guinea pigs and cultures. Such a development of a virus from the components of the cells is much more difficult to grasp than the equally incomprehensible self-proliferation of vira, which are apparently pure protein molecules" (1938: 9).

Cases have also been known of one and the same plant being infected with two or more types of virus at the same time, for example tobacco with mosaic virus and ring spot virus. In such cases, in line with the idea of endogenous virus development, one would have to assume that the pathogenic protein metabolism is capable of producing several types of high-molecular proteins in the same host, but which nevertheless retain their special properties, since they can be isolated by a number of methods. This made it difficult to adapt the observed facts to the idea that virus species are nothing more than protein molecules (see Smith 1935: 21 ff.).

The view that the filterable pathogen is a microbe could be supported by reference to its ability to change and adapt. With regard to tobacco disease, it could be said that "in addition to the usual light and dark green patches, yellow patches rarely occur. If these are cut out and inoculated on other plants, only the yellow variant appears. Now it could be that the first plant had three different types of the virus from the beginning. But if the green virus, which always remains green on the same type of tobacco, is transferred to another type of tobacco, yellow spots will suddenly appear. So environmental change plays a role" (Heilmann 1940: 657).

However, there were also empirically based arguments to defend the concept of endogenous virus production and the idea that the virus is a Contagium inanimatum, a single protein that acts as an organic autocatalyst. The understanding of the virus as a filterable microbe was already doubted by many virus researchers because, in their opinion, submicroscopic dimensions were not compatible with the minimum of organisation and structure that was required for a living "wholeness" according to widespread opinion. Guided by the prevailing doctrine that living things must be organized in cellular form, it seemed more plausible to interpret the phenomenon as a chemical substance, because such tiny cells, as would be assumed in the case of the microbial nature of filterable viruses, were difficult to imagine. The filterable agent also seemed to be much too small to satisfy the "space requirement of the

protein" (Errera 1903: 73), without which it was impossible to think of life. Even in the 1930s it was still a mystery to many how a particle consisting of a few molecules could be made in such a way that it was able to carry out all the complex functions of a living, autonomous organism. Elementary organisms seemed to have to be at least large enough to meet this requirement. [14] Andriewsky (1915: 90) found that the chicken pest virus passed through filters which retained haemoglobin. The diameter of the haemoglobin molecules was given as 2.32.5 mμ, and Andriewsky concluded that the molecules or micelles of the virus must be even smaller, so that the virus particles could not be structures similar to the animal or plant cells known to date. [15]

The existence of living beings with submicroscopic dimensions could also be questioned with the argument that the pathogenic ultra-microbes, if they should exist, would have to be contrasted with saprophytic organisms that could be easily cultivated in vitro. It could be pointed out that all the efforts made at that time to detect them had not been successful (Molisch 1919). [16]

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14 Later studies by Stanley (1935), Best (1936), Beard and Wyckoff (1937), however, proved that even small virus types such as the mosaic disease virus of tobacco or Shope's rabbit papilloma contain protein. According to another variant, the impossibility of imagining a cell of such tiny dimensions that not even the indispensable protein, the absolutely necessary building material of every cell, would find room to be no longer a problem, seemed to be impossible if one were allowed to understand the elementary particles not as cells but as molecules.
The idea that the cell was the most primitive, indivisible basic form of all life had largely been abandoned. Initially, structures of the cell plasma such as the granula (mitochondria) were defined as independent living beings (symbionts) which were originally foreign to the cell but had become dependent on it (Buchner 1930: 809 ff.). Or the cell structures were probably seen as the cell's own form elements, which however had a certain independence of life functions within the cell group. Morphological cell research and, above all, the investigation of the processes involved in mitotic cell division and in the fertilisation of oocytes had to constantly give new impetus to the idea that the cell is not a unit but already a complex of much smaller units. It was also considered that life on earth did not begin with a cell.

15 In view of this he felt tempted to agree with the Contagium vivum fluidum hypothesis. However, the "living protein molecule" hypothesis was linked to the difficulty of how to attribute to isolated protein molecules the ability to feed, multiply, inherit and adapt. It was also sometimes considered that the virus may correspond to a borderline state between the animate and inanimate, that they are equivalent to mere molecules or molecular assemblies.

16 For Doerr, this is not a compelling conclusion, because it cannot be excluded that only pathogenic ultra-microbes exist. "...these life forms then sink to mere regressions in phylogenetic observation and have not lost the significance of the question of the origin of life and the problem of abiogenesis as the opening up of a world of ultra-microbes" (1923: 910).
In defence of the view that viruses are microbes, the idea was also developed that viruses were possibly incapable of saprophytic growth because they had suffered an unusually high loss of the enzymes required for this during development, so that they had become obligatory parasites - an explanation variant that was still being advocated in the 1950s, according to Pirie (1973: 45, note from: van Helvoort 1994a: 199; see also Hershey 1957: 230 f.), which made it possible to stick to the understanding of the virus as the simplest form of life despite the lack of evidence of saprophytic growth, which included the idea, already criticised in the late 1940s, that virus reproduction is achieved by cell division.

The fact that infectivity could only be transmitted by artificial means was also used as an argument against the assumption of a microbial nature of the virus. In view of the artificial

transmission, it seemed more appropriate to assume that a toxin capable of causing physiological contact action in normal cells, with the result that the same toxin is produced there secondarily, was hungrier. The toxin of mosaic disease has the property of acting in a physiological-autocatalytic way (1905: 296). Baur also considered the artificial transmission of the disease to be incompatible with the microbial nature of the virus (1904).

Virus species such as cowpox exhibited a behaviour against mechanical, osmotic and chemical influences that made the existence of a surface membrane appear questionable, but which most microorganisms exhibit (see Schramm 1942b: 794).

The type of composition was considered to be an experimentally testable criterion for the assignment of viruses to organisms or to chemical agents, whereby uniformity and a defined chemical composition suggested the latter variant, whereas a dimensional variability of the virus elements, which some researchers claimed to have observed, was more likely to give these entities the character of organisms. Reproduction by cell division would have led to heterogeneous virus particles, whereas the extensive homogeneity which Svedberg and Erikson-Quensel claimed to have determined for the tobacco mosaic virus in the ultracentrifuge and electrophoresis (1936; note from: van Helvoort 1996: 288)[15]was regarded as a property of a chemical substance. The fact that viruses could now be represented in the form of macromolecular proteins - that is, of proteins whose large molecules could be identified with the viral elements in the solution state - meant that those researchers who were convinced of an endogenous virus origin had discovered that the infectious agents, according to Schramm (1942b: 791), "are uniform among themselves and defined in their chemical composition, so that they must be regarded as chemical agents after all. The ability to crystallize "comes ... generally only chemical molecules, but not organisms of complex composition" (ibid., 792). The chemical composition of the agent would have to be "variable within certain limits and not defined in such a way that the assignment of a chemical formula appears to be meaningful", if it was a question of individuals of "a weighable quantity of microorganisms of one and the

[15] Against the idea that with the crystallizability the virus proved to be something inanimate, several analogies could be put forward, which spoke for the fact that a crystalline structure can be quite compatible with vital properties and functions. "One of the best known examples of 'biocrystals'", according to Doerr (1944a: 44), "are the muscle fibres... ; the carriers of contractility are the elongated filamentary molecules of myosin, a globulin-like, highly birefringent protein, which in a suitable experimental arrangement provide the same X-ray images as the muscles themselves ... Even in a 3% solution, the myosin ... shows the ability to solidify into a gel if left standing for a long time; shaking destroys this regular arrangement by throwing the filament molecules into disarray, thus liquefying the jelly. In a similar way, the elongated and thin particles of phytopathogenic viral proteins are stored ... are stored parallel to each other, only that higher concentrations are required than for myosin ... Therefore, if there are multiple and remarkable relationships between myosin and crystalline viral proteins, this applies to a greater extent to another biological counterpart, namely the heads of spermatozoa, whose substance has the properties which prove the para-crystalline structure and consists of nucleoproteins, probably in the form of filamentous molecules.

same species". The construction of a crystal lattice, however, presupposes a large degree of agreement and regularity in the structure of the individual particles (ibid., 791).

## THE DISCOVERY OF "BACTERIOPHAGY

A special chapter in the history of virus research was heralded at the end of the 19th century with the discovery of bacteria-dissolving substances. The dissolving element, the "bacteriophage", also known as "lytic agent" or "bacteriophages lysate" (von Preisz 1925: 2), had dimensions that were also attributed to the particle size of a large number of animal and plant pathogenic virus species (see, among others, Elford/Andrewes 1932; Schlesinger 1932). It passed through porcelain filters and required the presence of bacteria to grow, just as a virus could only be cultivated in the presence of living cells. And with the same techniques that allowed the chemical purification of different types of viruses, it was also possible to obtain purified concentrates from phage suspensions, the effectiveness of which was up to six powers of ten greater than that of the starting solution (see Schlesinger 1934; Northrop 1938), and like animal and plant viruses they seemed to be chemically similar, i.e. to consist of nucleoprotein (see Alloway 1933: 255). Some researchers therefore considered the phage to be a virus-like phenomenon (see Seiffert 1938: 194; Bloch 1940) and named it a "bacterial virus". The analogies mentioned suggested investigations to find out to what extent processes of bacteriophagy can be equated with infection in viral diseases and whether the phage also behaves in a virus-like manner in other, more biological respects (see Bloch 1940: 481). [18]

Bacteria-modifying (-"damaging" and -dissolving) substances were first observed in the late 80s of the last century. Nuttal (1888) and Buchner (1889) reported a bacteria-destroying effect of the blood serum on the typhoid bacillus, whereby this effect was attributed to the protein it contained. Kruse and Pansini (1892) reported the disappearance of pneumococci in older bouillon cultures that had come to a standstill in their growth. In 1899 findings were reported that bacteria would dissolve by pyocyanase (Emmerich, Loew 1899). [19] Conradi and Kurpjuweit were able to prove the presence of selectively growth-inhibiting, thermolabile substances in the cultures of bacteria of the typhoid coli group, which were also found in the intestinal contents of humans, substances which they believed were formed by the bacteria in the course of their growth and were closely related to intracellular enzymes. To name such "inhibitors" they proposed the term "autotoxins" (1905a: 1764; see also Conradi/Kurpjuweit 1905b).

In 1915, Twort, a British bacteriologist, reported that he had come across the phenomenon of *transmissible* bacterial dissolution, the continued transmissibility of antibacterial effects from

one quantum of culture substrate to another. The thermolabile agent capable of bacterial dissolution (lysis) was still effective in high dilutions (transfer of small amounts of a lysed to a fresh broth culture) and was filterable through pores of porcelain candles (Berkefeld candles). Twort initially aimed at the following: It was to prove the existence of filterable ultramicroscopic microorganisms20 , i.e. viruses, not only in pathogenic material (for example in calf lymph), but also in soil, dung, etc. The existence of saprophytic ultramicrobes was considered very likely at the time. Since it was assumed that for every pathogenic microorganism, in addition to ordinary bacteria, many non-pathogenic variations of the same type occur in nature, it was obvious to assume that filterable viruses behaved in a similar way (Twort 1915: 1241), despite the fact that it was difficult to support the assumption

18 There was also a practical research interest in the question of whether the phage could be used as a model object for virus research, where essential aspects of virus behaviour could be studied. A large animal colony was required to test a virus suspension that had to be tested against animals. In addition to the associated costs and the problems this posed for the controllability of experimental conditions, a relatively long time was needed for a single test, whereas only a few hours were needed to test a phage suspension. "Working with plant viruses such as the tobacco mosaic virus was very limited in terms of the time required and the amount of laboratory equipment needed... ...was halfway between animal viruses and phages. So it was clear that the bacteriophage was by far the best material from this point of view. It was therefore sensible to try to learn everything possible from this easy-to-handle experimental subject before moving on to more difficult viruses requiring plant or animal substrates for testing," said Ellis, who had specifically studied virus-induced cancer growth (<1966> 1972: 63).

19 Antibiotically acting metabolic product of Pseudomonas seruginosa, a species of the genus Pseudomonas, like inflammatory, mixed pathogens. Emmerich and Loew mentioned the following experiment with red pig bacilli: In broth culture of these bacteria, agglutination and sedimentation occurred over time. If 1 cm3 of the liquid above the sediment, clouded by agglutinated bacterial flakes, was brought into new broth, agglutination and sedimentation also occurred during incubation in regularly shorter time periods. Repeated transfers resulted in a dissolution of the entire sedimented bacillus mass. In the end (as Emmerich and Loew thought, due to accumulation of bacteriolytic enzymes) the transfer of the culture was not successful at all.

20 According to Burnet and Andrewes (1933: 162), all viruses "that are smaller than 0.2 µ" could be described as "ultra-microscopic". This did not mean, however, that they had to be outside of the visualisation range of the light microscope. "Characteristic tiny corpuscles have been observed in several virus diseases and seem to be causally related to the infectious properties of the material. They can be brought into the visibility range of the microscope in various ways" (ibid.), for example by suitable staining methods in smears.

empirically justified. Twort's original assumption was that if non-pathogenic variations occurred in nature, they would probably be easier to cultivate than pathogenic ones. Attempts have been made to grow them from such materials as manure, grass, water, etc. on tested and specially prepared media (agar, serum, etc.). Various amounts of chemicals or extracts (mushrooms, seeds) were added to them. The material to be tested for viruses was mixed with water, heated to 30° Celsius (also at different times) and then filtered through a candle. Afterwards different media were inoculated with the filtrate.

However, these experiments did not result in growth of the filterable virus. In the hope of inducing the virulence of the filter-passing virus, various animal experiments were also carried out. But the results were always negative. It was never possible to grow a filterable microbe ("a true filter-passing virus") from the filtrates by re-inoculating them on the various culture

substrates. However, results were obtained which were not originally intended, results which were obtained during the investigation of the breeding possibilities of filterable microbes, for which Twort had sown glycerized calf lymph on agar. Inoculated agar tubes, after being warmed up to 37° Celsius for one day, showed a growth of colonies of coccus, which at first looked white and opaque (watery looking areas), but after some time most of them appeared glassy. When smears were applied from the colonies that were only slightly glassy, opaque and glassy colonies were formed. If, on the other hand, a trace of a glassy colony was applied to the edge of an opaque colony, the glassy dissolution of the colony started from this point. The whole colony appeared glassy after a short time and microscopically consisted of finest granules (and no longer of coccus) which could be stained according to Giemsa. Twort proved that the effective agent of such transparent colonies is filterable. Experiments with certain bacilli of the typhus coli group led to comparable results.

Twort also noted that these processes are faster and more comprehensive when fresh and young cultures are used instead of old ones, and that hardly anything was happening with dead or young, recently killed cultures. The glassy material, when diluted with water, passed the finest filters with ease. And one filtrate drop, transferred to an agar tube, was sufficient to make the tube unsuitable for micrococci. At first growth occurred, but soon glassy spots appeared, which then expanded. The score depended on the dilution of the glassy material. In some cases it was so active that growth stopped and the phenomenon became directly effective. It turned out that the effective agent could be carried on from generation to generation of bacteria and that it was not capable of growth by itself.
Twort had initially considered that he had detected the effect of an ultramicrobe when trying to draw definitive conclusions from the results. In the end, however, he regarded this as an autolytic principle (ibid., 1242 f.).

A few years later, the phenomenon of transmissible bacterial dissolution was also described by the Canadian bacteriologist d'Herelle. He had observed that the filtrate of dysentery convalescent stool is able to dissolve living dysentery bacilli in culture (1917; published in 1922). D'Herelle carried out the following experiments: Drops of a dysentery patient's bowel movements were added to a sterile broth. The mixture was then placed in an incubator for a whole day. He then filtered it through a Chamberland candle, which retained all bacteria. In the next step, part of the clear filtrate liquid (10 drops) was added to a fresh sterile broth tube previously inoculated with bacterial dysentery pathogens (Shiga bacilli) and also incubated. Initially, normal darkening occurred in the tube by multiplication of the added dysentery germs (after incubation). Afterwards, the sample was filtered again and a part of the filtrate was added to a new tube and so on. Surprisingly, one day the tube of the last experiment remained clear (sterile). In a control tube (without addition of filtrate), which had also been loaded with bacilli,

the germs reproduced normally and the broth clouded. This proved for d'Herelle that something could be filtered out in the faeces which would dissolve the bacilli and which, as could be deduced from the dilution series, would multiply.

D'Herelle determined that this substance could be bred in series. If a suspension of fresh Shiga bacilli (obtained from an ordinary agar culture) was sown into the tube in which growth had ceased, these bacteria were dissolved after several hours; the tube appeared completely clear. In detail: D'Herelle added a drop of the dissolved culture to a fresh broth culture of bacilli After 15 hours this was also dissolved. In the same way he added another drop of the dissolved culture to a new suspension and so on. Instead of weakening, the lytic activity accelerated after each passage. In other words, the more passages that preceded the dissolution, the less time it took for the dissolution to reach a minimum level that no longer changed. This serial continuation of the lytic principle and its multiplication when the bacteria dissolved was now evaluated by d'Herelle in the opposite direction to Twort's view - as proof that this was a being living at the expense of the bacteria, a parasite of the bacteria (d'Herelle 1922), so that its study was the study of "the pathology of the bacteria" (d'Herelle 1921: 665). The size of this "ultra-microbe", which he also calls "a living collodial micelle" in one of his essays (1928: 541), would not exceed that of a protein molecule (1921: 664).

A further experiment was intended to substantiate this thesis, an attempt to make visible the bacteria-dissolving effect on solid culture media: D'Herelle added a small amount of a dissolved culture (about 0.00001 cm3) to a bouillon culture of Ruhr bacilli. Immediately and after incubating for one, two and three hours, one drop of each culture was spread on a bacillus lawn (on sloping agar tubes). The variation of the contact time led to the following results: In the first tube (without incubation) the agar was covered with a normal bacillus lawn with two holes, i.e. places where no bacterial growth could be observed. The tube inoculated after one hour of incubation had six holes, the tube inoculated after two hours had only one hole, and the tube inoculated after three hours had no culture at all. If a tube containing shiga bacilli and a few drops of a dissolved culture was left to itself, secondary turbidity occurred some time later after the clarification, which indicated sterility, caused by shiga bacilli that were or had become obviously resistant to the dissolving effect. D'Herelle interpreted the results of his experiments as confirmation of his view that what dissolves the bacteria multiplies and takes on visible forms. From the "holes" he concluded that colonies formed as they multiplied, and therefore it could only be a corpuscular organism. The lytic agent, which he assumed was not only found in the chairs of dysentery convalescents, but was also widespread in nature, he first called "Bacteriophagum intestinale", later "Protobios bacteriophague", meaning an ultra-microscopic (invisible) microbe which acts against bacilli and passes through the pores of a porcelain filter.

The opponents of d'Herelles' position - after Hoder (1932: 4), Otto and Munter (1928: 410) and von Gutfeld (1925: 413) they formed the majority of researchers[16] - saw a bacterial decay product in the phage. And so a large number of researchers also reported that they had produced "lysine" from bacteria alone: Gildemeister and Herzberg reported in the mid-1920s that they had investigated "spontaneous lysine formation" under the influence of varied culture conditions (culture media, temperature and time were varied), and the investigations had shown that the bacteriophages had developed in a bacteriophage-sterile reaction chamber, whereby spontaneous lysine formation was primarily governed by temperature (1925). It was claimed by Rosenthal (1926: 612) that he had obtained numerous phages from phage-free cultures (dysentery, typhus, coli, etc.) after a few passages, so that spontaneous phage formation must be assumed. Bordet and Ciuca, who attributed the d'Herelles phenomenon to a metabolic disorder of the bacterium ("viciation nutritive"), stated that after repeated injections of normal coli bacteria into the abdominal cavity of guinea pigs pretreated with colic culture, a transferable lysine for the cola strain used was formed which could be easily obtained with the exudate. In other words, they claimed to have obtained lysine directed against coli bacilli experimentally without using stool filtrates from that peritoneal exudate (1921). The experimenters then identified this or that condition, whereby normal bacteria were to be placed under special conditions which would influence their living conditions in a certain direction and which would enable or favour the formation of lysine (addition of immune serum, weak sublimate solution, leucocyte enzymes, certain poisons, etc.), and such factors were also the subject of controversy (see Otto 1923: 255).

Researchers who were convinced of endogenous virus formation relied mainly on concepts of a biochemical nature (fermentation theory, catalyst theory, protein theory, etc.).According to Hoder (1932: 13), an analogy to secretion processes in yeast species could be used and the autolytic enzyme effect in yeast cultures (see Preisz 1925: 90) or the lytic ability of some fungi (self-digestion) could be referred to. Some researchers presented the phage as a bacterial toxin that changes the metabolism and is regenerated by the diseased bacteria (e.g. Doerr 1922). For Kabéshima (1920) this was just a normal, inanimate bacterial ferment, which was released by autolysis. He suspected that the bacterial dissolution was caused by leucocytes. Kuttner (1921a: 1921b) reported that he had obtained a bacteria-dissolving filtrate from leukocytes, from intestinal mucosal cells and from liver cells of guinea pigs, which had a dissolving effect on typhoid and dysentery germs (shigabacillus). According to Proca (1926: 125, 153), lysine was one of the endotoxins or intracellular enzymes. Von Gruber and von Angerer saw in "lysine" digestive enzymes which were already present in normal bacteria, but which were not normally

[16] According to Doerr, however, the majority of phage researchers were undecided (1923: 909).

effective (von Gruber 1923: 204 f.; von Angerer 1923: 205 f.). Von Gruber recalled the self-digestion of the yeast juice by endotryptase and the rapid melting of the yeast under the influence of small amounts of benzene, ether, etc. Here, Ehrenberg's observations on protein enzymes could be referred to (1922: 432). In his experiments, Ehrenberg was able to artificially "cultivate" and continue a certain degree of specificity in the protein enzymes, whereby filtration proved to be beneficial to the formation of fermentations. Based on this, Otto and Munter determined the "biological nature of bacteriophagous lysine... due to its chemical-physical behaviour as a high-molecular solution of bacterial protein ..., whose properties ... ...can be explained by laws governing how they govern colloidal solutions..." (1928: 400). Bacterial dissolution was to be initiated and maintained by the decomposition of living bacteria into inanimate, fermentatively active protein particles (Otto/Munter 1923: 403); Otto and Munter determined the bacteria-dissolving substances as the "smallest bacterial protein particles equipped with fermentative properties" (1928: 410 ff.). Bail (1925), who was inclined to the idea that the bacteriophage was a part of the generative substance of the bacterial body, thought of released fragments of cells (especially chromosomes): The protective forces of the body cause the bacilli to be broken down, whereby these certain properties are lost, and in some cases they are reduced in size to the "size of fragments" so that they can pass through bacteria-tight filters. If such splinters, which are probably still viable, were brought together with normal bacilli, they would remove the substances lost during decomposition and turn these bacilli back into splinters.

A further representative of an enzyme-theoretically supported understanding of phage reproduction is Northrop, who in the 1920s was engaged in kinetic enzyme studies, in a period when the protein nature of the enzymes had only been proven.[17] For the isolation and purification of enzymes, Northrop worked with methods and procedures that had previously proved their worth in the chemical isolation of enzymes (such as crystallisation and salt fractionation). The investigations produced crystalline products, but these did not show any enzymatic activity. It was discovered that these products were precursors of proteins with such activity, and their autocatalytic properties were demonstrated.[18] For Northrop, autocatalytic processes provided a suitable starting point for the interpretation of biological phenomena such as protein synthesis and propagation in the context of biochemistry and physiology. And similar

[17] Sumner (1926) was the first to succeed in isolating the urea-cleaving enzyme urease, presenting it in crystalline form and identifying it as a protein. It was not until the early 1930s that Northrop was able to demonstrate the same for crystalline pepsin and trypsin. Crystallisation of the proteolytic enzymes pepsin, trypsin and other proteases was widely recognised as a significant advance in the study of biochemical processes. This was because, in the 1930s, it was not yet possible to form clear ideas about protein formation in general and enzyme formation in particular. One possible explanation was that the proteolytic enzymes involved in the degradation of proteins also played a role in their synthesis.

[18] In other words, in some enzymes the precursor in the enzyme was transformed under the influence of the active enzyme, which allowed the conclusion that the formation of these enzymes is an autocatalytic process.

to the way active enzymes are formed by autocatalysis, Northrop also imagined the formation of phages (Northrop 1937; see Olby 1974: 149 f.), which for him and his colleagues could not be a living and complex organism (Krueger/ Northrop 1931; Krueger/Scribner 1939; note from: van Helvoort 1994b: 108). According to this concept, the phage develops from a precursor already present in the bacterium in a reaction analogous to the conversion of pepsinogen and trysinogen into the relevant enzymes in vitro (see Krueger 1937: 379).[19] Doerr (1938: 65) believed that dormant bacterial cells produce precursors of phage which are transformed into active phage in the presence of active phage, a "rather doubtful hypothesis". But even if one did not agree with it, one would have to acknowledge the importance of the experimental results "if they were to stand up to careful scrutiny; in any case, they argue against the possibility that the phages could be exogenous parasites of the bacteria. Appropriate investigations were carried out to isolate the phage in pure form and to prove the existence of phage precursors. The results of investigations for which a staphylococcus strain had been used were used to justify the judgment that when staphylococci reproduce, a phage precursor stage develops in them which, when brought into contact with phages, converts itself into phages.[20] Phage formation was determined by a rapid increase in the phage titer in the precursor phage mixture. Northrop turned to the phage because the bacterial virus offered him a prototype for studying protein synthesis, especially since he could rely on researchers such as Twort, Gratia, Bordet and others who believed that phages were enzymes produced by bacteria.[21]

A number of researchers soon included[22]observations of variability phenomena in bacteria in their investigations of the bacterial dissolution phenomenon, following the idea that bacterial properties were caused by phage action, properties that were retained over several generations, so that it also seemed permissible to "speak of inheritance of properties and assume a genotypic

[19] According to Krueger (ibid.), the conversion into phages could either be based on hydrolytic protein cleavage or be regarded as the final phase of a synthesis in which the full phage would act as a catalyst. These statements were mainly based on investigations which succeeded in isolating staphylococci phages in the form of a nucleoprotein, so that their precursor could also be ascribed protein character.

[20] However, the theory of the existence of a phage precursor was only based on work with one and the same staphylococcal strain and an associated phage. There were no attempts to detect precursor stages in other phage species.

[21] „The multiplication of bacteriophage during bacteriophagy, combined with the supposed non- living nature of bacteriophage, constitued an interesting issue for Northrop“ (van Helvoort 1994b: 106).

[22] The first detailed observation of the variability was, as Fleck explains, related to the so-called bact.coli mutabile. The observers (Neisser and Mansini 1906) had examined cultures after 24 hours as well as after a few years, contrary to dogma. Today, this observation is not called "'classical' ... variability, but as bacteriophage effect" (<1934> 1980: 122 f.). Certainly, the possibility of noticing a bacteriophages dissolving and modifying agent and its relationship to certain chemical and physical substances had arisen with changes in the way certain bacterial strains were created and cultivated and with the application of certain chemical and physical forces. The obtaining of pure cultures of lubricating plates (initially, mixed or lubricating cultures were obtained in the case of smears), which were needed, for example, to test the virulence of suspicious germs. If they were stored for a corresponding period of time, variability of the bacteria could develop, which are less visible on lubricating plates.

change in the bacterium by the phage in question" (Hoder 1932: 10). That phages were supposed to cause a change in bacterial characteristics and the emergence of new types had to be asserted contrary to the prevailing doctrine of bacteriologists at the time, who adhered to a rigid scheme of groups of bacteria.[23] That new (lysine-resistant) bacterial strains (secondary cultures) with different morphological characteristics, with different fermentative capacity, fermentative behaviour, etc., could emerge in the course of experiments, that as a result of lysine action bacteria disappeared and returned, such as colibacillus at the onset of dysentery, cholera, etc. disappearing and reappearing with convalescence, "disrupted" the bacteriological practice, which aimed to extract a well-defined micro-organism from pathogenic material (e.g. pus) and to detect the quantity and distribution of the bacteria to interpret the signs of disease[24]. Since the bacteriologists were primarily interested in identifying bacteria as pathogens - for which the stability of the morphological characteristics had to be assumed to be unaffected by experimental access to the cultures - they were hardly interested in variability phenomena. Findings which seemed to indicate such phenomena were therefore often attributed to the effect of technical errors.[25]

Bordet and Ciuca (1920) assumed that bacteriophage reproduction is initiated by leukocitic elements (that leukocytes could cause bacterial dissolution was - as mentioned above - also assumed by Kabéshima 1920). In order to explain the continued effect of this stimulus, they used the concept of heredity: Under the influence of a stimulus emanating from leukocytes (which are abundant in the Ruhrstuhl, for example), variants would form in the colony forms containing the lytic agent. Under the damaging influence of the cells, variants of the dysentery bacilli containing an autolysis-promoting substance would appear. The autolysing variants should be able to inherit this property. When the bacilli die, the autolytic ferment would be released, which could attack normal dysentery bacilli, which would then also develop a tendency to autolysis. Gildemeister (1917) determined that in a number of cases a group of strangely irregularly shaped colonies formed on stool smear plates (including dysentery and colibacillus), the main characteristics of which were the same for the different types of bacteria.

[23] It failed "because of the resistance of nature ... which does not tolerate schematization" (Hoder 1932: 115).

[24] Also, the "self-healing" hoped for by the lytic principle - the therapeutic efficacy of the bacteria-dissolving parasite with regard to bacterial infections - could not easily be reconciled with the idea of a causal therapy of infectious diseases by isolating, identifying and characterising a pathogen. However, the expectation of many physicians that phages could be successfully used for a targeted therapy against some infectious diseases was later not fulfilled.

[25] In the "classic age of Pasteur cooking ... a rigid bacteriological way of thinking developed", said Fleck, "because only a strictly orthodox method was recognized, the results were very close and uniform. For example, only a 24-hour inoculation of the cultures was generally used; very fresh, approximately 2-3 hour old cultures and very old (approximately 6 months old) cultures were not considered worthy of investigation. Therefore, all secondary changes of the cultures, which are the starting point of the theory of variability in the new style, escaped attention" (Fleck, loc. cit., 122).

In these colonies a more or less restricted growth of the bacillus was observed. Individual forms of this group constantly turned over into each other during further breeding, and the "constantly turning over clans" split off normal forms (ibid., 54). Gildemeister inoculated them with a bacteriophagous "lysine" (as he called this phenomenon) from bacterial suspensions to which he had added a bacteriophagous "lysine" (as he called this phenomenon), and he received the same forms of colonies (ibid., 56). He called them "flutter forms", which he later claimed to have discovered even before d'Herelle that the lytic agent formed colony forms (1923: 181). After d'Herelle's work had become known to him, he assumed that bacteriophagy had entered the field of
Variability phenomena belong (ibid.).

The investigations of the bacteria-dissolving phage effect, the proof of which, according to Hoder, "is a considerable complication of bacteriology and ... a definitive breakthrough in their all too rigid systematics", "which, thanks to mutation research... which had begun to falter thanks to mutation research anyway, and which showed alarming gaps" (1932: 100 f.), did not immediately lead to a unanimously accepted new theory with which the crisis situation could have been ended. That both the "d'Herellesian phenomenon" (brightening of the bouillon cultures without visible residue) and the "Twortsian phenomenon" (a glassy material that was formed during the dissolution of cocci colonies spread out on agar) would refer to the "same natural phenomenon" was not accepted by all.a. by Gratia (Gratia/Jaumin 1921: 880); he was able to transfer the one phenomenon into the other (this in contradiction to d'Herelle, who at first had been of the opinion that the phenomenon he had discovered was not identical with the Twisted phenomenon).[26] However, this did not at all level the rift between the views of both discoverers. Rather, a controversy developed between supporters of Tworts and followers of d'Herelles' view, which was to be renewed again and again with empirical advances in phage research. "The polemic that revolved around the Twort-d'Herellesian phenomenon was pointless," Anderson said in retrospect (<1969> 1972: 72), "lasted several decades and only became irrelevant with the advent of molecular genetic phage research. The results obtained were not such that they spoke unambiguously for or against the animate nature of the phage, so that "many assessments of the nature of the bacteriophage are subjective", as Gildemeister and

[26] In the early 1920s, he had proposed the term "bacterioclasis" to describe the Tworts phenomenon, meaning fragments, tiny granules that could be stained reddish with Giemsa, whereas what he had discovered should be called "bacteriophagis" because it was something else. Lysis, for example, was of an extension that left no residual, the phenomenon extending to the whole culture, whereas the phenomenon he discovered was circular, stable plots on the culture (1923). This view was rejected by Gildemeister (1923: 182), among others. The objections raised by d'Herelle to the identity of his discovery with the Twort phenomenon were not considered valid; the phenomenon should therefore be named after Twort and d'Herelle. However, in an essay by Lisch (1925), published a few years later, it is stated that different strains of Bac. pyocyaneus showed two distinct phenomena which corresponded to the Twort and d'Herelle phenomena. A transition between the two phenomena could never be observed. It seems as if one of the phenomena is a solution of the older individuals, while the other is an inhibition of growth or division.

Herzberg had to conclude in the mid-1925s (1925: 403). The fact that the bacteriophage effect could be observed with the eyes to a certain extent - it could be detected as inhibition of turbidity in broth cultures or as brightening of the already turbid broth and as the formation of growth-free spots in bacterial lawns on agar plates - did not contribute to a generally accepted understanding. "Neither the one nor the other way of making the bacteriophage effect visible is completely suitable", said Hoder in the mid 1920s, "for the determination of bacteriophages" (1925: 424). Each side was able to give experimentally supported reasons for its position, so that the decision for or against the living being theory "ultimately depends on the position of the author, how he evaluates his results", as von Gutfeld judged in 1925 (1925: 427). In the same way, the thesis that bacteriophages could spontaneously appear in pure cultures was evaluated "by the various authors according to their attitude to the virus theory of d'Herelles...". (Gildemeister/Herzberg (1925: 406). Doerr (1922: 1538) describes the situation as follows: "...between an ultramicrobe that is only pathogenic for bacteria, microscopically invisible and only capable of reproduction within living bacterial cells, and an inanimate, colloidally dissolved substance that is toxic only to living (growing) bacteria and is reproduced on an enormous scale by the bacteria influenced by it, there is de facto such a sum of relationships that it must be possible to interpret many observations and experimental results in both senses.

The understanding of the virus as a parasite benefited from the fact that the filterable agent reproduced only at the expense of living bacteria. Since the effect claimed to be parasitic could spread to several species of bacteria, it was reasonable to assume that adaptation would probably be necessary. According to Bruynoghe (1921), the virulence of the individual phage strains had to be regarded as different and passages as a possibility for increasing virulence. According to Hoder (1932: 10), only one species or group was attacked at a given time. In this case, the intensity with which the individuals of the group are attacked is not the same for all.

The formation of aseptic spots in the bacterial lawn after a drop of bacterial suspension to which a small amount of virus has been added is applied to agar, could be understood as colony formation of the virus, brought about by phage multiplication. It could be assumed that the phage develops here at the expense of the bacteria which were inoculated at the same time. The formation of the peculiar holes, which appeared when lysine solutions mixed with bacteria were spread on the surface of solidified culture media (like "colonies" of the bacteriophages), supported the argument that they could only form because germs remained in these places, which then found the possibility to multiply by infecting the surrounding bacteria. The possibility that the sterile spots, instead of showing colonies, could have been formed by

bacteria which were weak and therefore unable to develop was refuted by d'Herelle in one of his experiments.[27]

It has been shown that serial breeding of bacterial cultures preserves certain properties of phage obtained at different times against certain or different bacterial strains, just as certain species characteristics are preserved or inherited over generations. Based on their work, D'Herelle and his followers saw an agar passage or passage in vivo as something analogous to a generation of specimens of an animal species. In vitro, only a faster generation sequence was aimed at (by increasing virulence).

It was pointed out that the phage can be destroyed by chloroform and glycerine, i.e. by substances which are particularly capable of attacking living elements (other researchers, in turn, attributed glycerine resistance to all viruses; see Gildemeister 1939b: 103). Phages also proved to be very little resistant to quinine. It could be shown that neutral quinine salts in a 1% concentration of the solution can render the bacteria-dissolving agent ineffective within a few hours (in higher, 3% concentration even within 30 minutes). This was taken as proof that the lytic principle must be a microorganism, since quinine is probably toxic for bacteria and protozoa, but has no harmful effect on diastases and toxins (see Doerr 1922: 1537).

Evidence has been provided that the phage was "accustomed" to certain conditions under which it was not originally able to develop its lytic effect. Prausnitz, for example, had succeeded in making phages insensitive to the neutralizing effect of their antiserum by habituation, i.e. to produce antiserum-resistant "lysine" (1923: 187). An increase in the resistance of the phage to the effect of antiseptics has been reported when cultured in cultures (Prausnitz 1922). Janzen and Wolff (1922) reported that the phages they had obtained at different times became accustomed to antiseptics (achievement of "poison resistance"). Asheshov announced that he had succeeded in accustoming a phage to exert its effect even in an acidic medium, which he had not originally been able to do (1925: 643 f.). And under suitable breeding conditions, the phage could gradually be made insensitive to certain influences or regain a partially lost (bacteria-dissolving) effect. Such properties were only known from animate beings (see von Gutfeld 1925: 426).

[27] This attempt can be described as follows: If increasing amounts of bacteria and a constant dose of bacteriophage suspension are added to several bouillon tubes and the same amount is added to agar from each tube after shaking, the number of spots formed from each tube is the same. If, on the other hand, increasing quantities of phage are added to the same quantities of bacteria, the number of stains is parallel to the quantity of phage used. If each stain was caused by the presence of a particularly weak bacterial cell, then in the first test arrangement the number of sterile sites should correspond to the quantity of bacteria used, and in the second arrangement the same number of sites should occur everywhere. But the experiment produced just the opposite result: d'Herelle concluded from this that the bacterial suspension contained the element which produced the sterile sites and that the active element was a parasite of the bacteria, an ultra-microbe.

When comparing these reasons for accepting d'Herelle's position, it is striking that they are those which, despite all their differences, can be compared with one another: They are compatible with the understanding of the phage as a living being, but with the understanding one had of life at that time. "It is impossible", says von Gutfeld (ibid.), "to
to characterize the term "life". We call something living when it has those qualities which, according to our experience, belong to those beings which we tend to regard as living. If these are large enough, it has no difficulty. But it is also possible for beings below the visibility limit. However, observation alone is not enough for this ... but to examine the characteristics of the being in question. The "experiences" that one had in general about the properties of life forms were met by the proof of the adaptability of the phage to certain influencing factors (assimilation, "habituation"), as well as by the determination of special characteristics independent of the bacterial species at the expense of which the phage reproduces, or the retention of specific properties of phage against different strains, which made one think of hereditability.

But there were also plausible reasons for the assumption that the phenomenon was a bacterial decay product. This was supported in particular by the dependence of the bacteriophage on the metabolism of the bacteria, which, as many researchers thought, was hardly compatible with the existence of a microbe (see Doerr 1922: 1489 f. and 1537 f.; 1923: 909 ff.).[28]
Bordet, who had given the phenomenon the expression "transmissible autolysis", and Ciuca (1920; 1921: 748 and 754; see also Bordet 1924: 969; von Gutfeld 1925: 428) had combined a small amount of lysine with a large amount of bacilli and found that the lysine did not regenerate under these conditions. They interpreted this as proof that the transferable lytic principle is not organized, i.e. it is not a living being, but only a lifeless ferment, since no reproduction had occurred despite the best nutrition. According to Bordet and Ciuca (ibid.), there is nothing more than a bacterial variation - the product of a metabolic disorder of the bacteria. This view was also made plausible by reference to reports that a lytic effect could have been achieved after damage to bacteria (for example colibacillus).

It was also known that certain inactivated enzymes can be activated. This insight was based on the fact that in the cultures prepared with heated phages, lysine formation did occur again later after initially negative results (see Otto/Munter 1928: 400). According to Otto, observations on

[28] This argument could also be used to deny that viruses of any kind are alive. In order to reject it, supporters of the theory of living beings considered, among other things, that filterable viruses could be a case of retrograde evolution, resulting from a process in which an organism has lost some functions - and has become smaller and simpler - which would explain the virus' dependence on living cells. This assumption has become known as the Laidlaw-Green hypothesis. It states that filterable viruses are unable to reproduce autonomously because they have lost certain metabolic functions, so that they depend on certain growth substances available from host cells (Green 1935; Laidlaw 1938).

the formation of such enzymes from bacterial protein provided plausible reasons for the assumption that the bacteria-dissolving phenomenon originated from bacteria alone (1923: 257).

It was also reported that the "lysines" were highly resistant to higher temperatures that killed living beings (according to d'Herelle, however, the bacteria-dissolving substances lost their biological effectiveness when heated to 60° Celsius for one hour) and that an ether treatment that an animate being would not have survived could not have destroyed the bacteria-dissolving principle (see von Gutfeld 1925: 427 f.). The resistance to chemical disinfectants also spoke against d'Herelles' position. Kabéshima concluded from the ineffectiveness of chloroform and sodium fluoride on the bacteriophages that the latter must not be an animate being but a ferment (1920: 471).

If, as d'Herelle assumed, phages were able to reproduce in an extracellular medium, respiration processes would have been observed in them, as was argued. Bronfenbrenner (1926) and other experimenters had also tried to prove this, using a specially constructed microrespirator that was able to register even extremely weak amounts of carbon dioxide. But even after several days of use, no traces of CO were detected in the filtrate. At the time, however, this failure could still be explained by reference to inadequacies in the design of experimental conditions, so that the results of the experiments, according to Seiffert (1938: 7), need not yet be regarded as final. It should be added that Breinl and Glowazky believed to have detected respiration in vaccine virus purified by centrifugation, from which they concluded that the pathogen causing the vaccines must be a living being (1935: 1149). Although these findings did not appear to be certain to other researchers (Seiffert 1938: 7), they did nourish the idea that one day respiration processes could be detected in phages as well.

Werthemann found that "intravenously injected lysine disappears from the circulation of guinea pigs, rabbits and frogs according to the laws determined for collodially dissolved proteins, but not suddenly 'critically', as is usually the case with ultramicrobes" (1922: 255).

## ON RESEARCH INTO THE VIRUS AS A TUMORIGENIC AGENT

The controversy surrounding the filterable virus and the phenomenon of bacterial dissolution also had a certain impact on cancer research, after a number of researchers reported that malignant tumours from chickens, rats or other animals could be transferred to healthy carcasses using cell-free substances from tumour material obtained by filtration and other methods: The fact that vaccination with filtrates of tumour juice in turn produced tumours -

even with tumour tissue dried and pulverised in a vacuum over sulphuric acid, and also when the tumour material was kept in glycerine for many weeks - suggested that viruses were the cause of tumour formation. However, there were very different views on the nature of the "cancer virus". A number of researchers regarded the supposedly cell-free tumour filtrates as an endogenously produced and subsequently autocatalytically and intracellularly multiplying element, while other researchers saw the agent as an exogenous pathogen. A concept that attributes the development of tumours to virus-like agents did not oblige us to regard this process as an *exogenous* infection. Even researchers who rejected the concept of an exogenous pathogen of cell-free transmissible sarcomas or other cancerous growths and instead thought of a substance that develops in the host organism, believed in the viral nature of cancer-causing agents, although the question of how the tumour virus is formed in an organism could not yet be answered (see Doerr 1938; Graffi 1940).[29] They considered the cell to be the origin of the virus, which, however, was transmissible through cell-free filtrates. The majority of the cancer researchers, however, rejected both the one and the other variant of the virus concept, in the conviction that all phenomena of cancer proliferation were due to the spread of cancer cells, that the cancer problem was a problem of regulation of cellular processes in the organism.[30]

In a certain sense, the search for filterable agents was linked to the direction in cancer research in which the formation of malignant tumours was regarded as an infectious disease caused by parasites that needed to be clarified, combined with the idea that therapies could be developed that were directed against a pathogen instead of the tumour cells. That an invigorating agent would cause cancer was accepted by clinicians and doctors in particular. It was considered whether it could cause certain worms (nematodes, see Fibiger 1921), blastomycetes (Roncali

[29] However, there have also been individual researchers who equated the virus with an exogenous pathogen and therefore refused to give it a role in cancer. For example, Murphy, who believed that he had produced filtrable tumours from chicken germ cells, believed that tumour-generating agents were something that would be fundamentally different from the types of virus because tumours developed endogenously and were due to the effectiveness of the body's own chemical substance (1935; note from: Seiffert 1938: 9). He compared the agent causing avian tumours with the transforming principle of pneumococci. He called these two groups of agents "transmissible mutagens".

[30] A special hypothesis on endogenous cancer formation was put forward by O.Warburg (1926). He regarded cancer as the result of irreversible damage to cellular respiration. He examined the metabolism of tumour cells in comparison to normal cells and found significant differences. While normal cells gain the energy necessary for life through respiration alone, malignant cells show another source of strength of their existence, namely the ability to keep themselves alive even when oxygen is completely cut off, namely through the fermentation of sugar into lactic acid. Cancer cells have an increased consumption of sugar, so that the blood that has passed through the tumours is richer in lactic acid than the blood that flows into them. Normal tissues do not ferment because their respiration is so great that the fermentation of sugar into lactic acid in the cell is suppressed. In all growing cells, respiration produces orderly growth. In cancer, however, respiration *and* fermentation cause disorderly malignant growth. Only when there is a lack of oxygen do normal cells also produce lactic acid from sugar. In tumour cells, however, respiration is normally not large enough to suppress sugar fermentation. All poisons and damages that damage artificially normal cells in their respiration change these cells in such a way that they finally derive their life energy primarily from sugar fermentation. Thus the cancer problem would ultimately be a metabolic problem.

1914; Pentimalli 1916)[31], cockroach larvae, mites (Saul; note from: German Nationals 1927: 231; no source mentioned), protozoa (van Calcar; reference from: Deutschlaender 1927: 225; no source mentioned), certain strains of bacteria (Blumenthal 1918; Reichert 1925) or other organisms that cause tumours. And since bacteriology has existed, there have been repeated attempts to detect specific carcinogens according to Koch's postulates. The reports of alleged carcinogens or sarcomere pathogens were sometimes linked with the claim to have discovered the sole "universal pathogen".[32]

The idea of "injecting cell-free cancer juice" in order to trace the formation of tumours was first put forward by Lubarsch in 1902 (reference from: Teutschlaender 1927: 242; no source cited). After Lewin (1925: 456 f.), Borrel (1909) was probably the first to discuss the etiological significance of an invisible virus for the question of tumour formation. However, he had not achieved a positive result by experimental means. Wunderlich and Uckert (1984: 7) cite Ellermann and Bang in 1908 as the earliest evidence of the viral nature of cancer diseases the successful cell-free transmission of a chicken leucosis (see also Ellermann 1918).[33]

Observations that Rous had begun in 1909 proved to be particularly important for the further development of this research direction. He stated that he had discovered in his experiments that chicken sarcoma could be transmitted with filtrates (1911a; full text reproduced in : Lechevalier/Solotorovsky 1965: 198 f.). In his first experiments, ordinary filter paper had still been used, on the assumption that the thin layer of paper, which allowed the passage of red blood cells and lymphocytes, would retain the tumor, so that a harmless filtrate would result, especially since other researchers who had observed tumors in mice and dogs thought that the filtrates produced were sterile. But Rous found out that tumors could grow if he injected some of the watery filtrate into chickens he used for his experiments, and only a few drops were enough. Even when, after centrifuging the tumour suspension, he used the clear liquid above the sediment for inoculation, he came to this conclusion, which prompted him to carry out further experiments: Rous grinded tumour material taken from the breast of chickens with sand,

[31] Blastomycetes" are unicellular fungi that reproduce by sprouting.

[32] Ochsner described the streptococcus discovered by Nuzum in 1919 as "the ultimate cause of cancer". He had been able to regularly isolate the micrococcus from human breast cancer and produce carcinoma by repeated injections of pure cultures in mice and a dog. Ochsner reported similar success with the same micrococcus. In 1921 Glover reported the discovery of a micro-organism which was said to have been cultivated not only from breast tumours but also from tumours of the bladder, uterus, lips, liver, even from lymph nodes of cancer patients and also from mouse tumours. Van Calcar saw the cause of cancer in a protozoon (references from: Teutschlaender 1927: 225, 240 f.; without references). In the opinion of the Germans, tissue alterations were often presented as cancer formation without regard to their histological behaviour, without proof that the allegedly carcinogenic agent was not only capable of causing atypical epithelial proliferation but also clinically and morphologically provable cancer formation (ibid., 225 and 226).

[33] According to von Hansemann, these experiments "proved ... that chicken leukosis is an infectious disease ... (and) a communicable disease" (von Hansemann 1919: 472 f.).

mixed it with Ringer's solution and shook it mechanically for a while (20 minutes). The sand and the tumor pieces were then centrifuged out over the course of 5 minutes (at a rotational speed of 2800 per minute). The excess liquid was then removed with a pipette and centrifuged for a quarter of an hour (at 3000 rpm). Sufficient liquid for the vaccination was then taken from the upper layers and injected into one side of the chicken breast (0.2 cm3 each), while a small piece of tumour tissue was injected into the other side. Rous achieved positive results with the tumor pieces in all (92) chickens, while in some specimens (7) sarcoma development was also achieved with the filtrate. In another experiment (see Rous 1911b) the liquid was passed through Berkefeld filters after centrifugation. 9 chickens were injected 0.2 cm3 of the filtrate into each side of the breast, 22 chickens only into one side, while some tumor tissue was added to the other side. One of the 9 chickens gradually developed sarcoma on each side. And in 5 of the 22 chickens, which had been injected with both filtrate and tumor fragments, sarcoma development was also observed on each side of the breast, with the process occurring particularly rapidly at the site injected with tumor tissue.

Rous regarded the results of his experiments, which had received little attention from the scientific public for a long time (see Studer/Chubin 1980; note from: Fujimura 1996: 32), as proof that after filtering a tumour emulsion and inoculating the filtrate into the breast muscles of a healthy chicken, a tumour of the same type can be produced in the same chicken. Rous was able to point out some characteristics of this agent which helped us to understand that it was a living but extremely small microbe: one of the reasons for this was that saturation with chloroform cancelled out the virulence of the material. Moreover, the agent was already destroyed at a temperature of 55° Celsius in a relatively short time (15 minutes). However, Rous was not yet conclusive about the nature of the cell-free filtrate of a chicken sarcoma, which could be used to create sarcomas in other chickens. There was no proof that the agent was animated; for this, it would have had to be shown that it could be cultured outside the body. Nor did Rous see any valid reasons for the assumption that the natural occurrence of chicken tumors was due to an exogenous infection.

One of the researchers who, although the cultivation problem was still waiting for a solution, took advantage of the fact that they had come across a cancer virus in their experiments was Keysser, who even claimed to have discovered earlier and independently of Rous that the tumour-causing agent was not carcinoma cells but a filterable virus (1913: 1665). Keysser had started from the question whether "experimentally infiltrating tumours...can be achieved in mice, which can be regarded as equivalent to human tumours" (ibid.,1664). In order to achieve infiltrative growth of the tumours, however, he considered it necessary to carry out vaccinations in organs instead of continuing the "previously practised subcutaneous transfer by transplantation of tissue pieces or injection of undiluted, crushed tumour pulp". As a result of

this method, the subcutaneous tumours appeared as foreign bodies in the mouse, as it were, which bear no resemblance to human tumours. He considered the eye to be a particularly suitable organ for his experiments, obeying the idea "that it is possibly mainly protective substances in the blood and juices that prevent the tumours from attacking. Now we have in the eye a self-contained organism of which we know that the fluids of the vitreous body as well as the anterior chamber contain no or only small amounts of protective substances, that the proteins contained in the eye belong to the lower and simple types of proteins which do not have any specificity" (ibid.). In order to use an eye (or another organ) in the indicated sense, a method was required in his opinion with which gross injuries to the test animals superimposed on the intended experimental effect could be avoided, as was the case with the transplantation of tissue pieces or injection with undiluted tumour pulp. He therefore carried out the vaccination with the thinnest possible suspensions of subcutaneous mouse tumours, which were passed through hair-thin cannulas. According to his report, inoculation of just one or two drops of this thin emulsion was enough to cause tumours to form in the organs. Tumours inoculated in this way grew after only 8 to 14 days and reached hazelnut to walnut size in 4 to 6 weeks. All organs were completely penetrated by the tumour. The tumours grew in the same percentage as the subcutaneous tumours after inoculation with undiluted tumour mash, whereas with subcutaneous inoculation with these thin emulsions there was never or only a very small percentage of subcutaneous tumours.

In addition, Keysser started to carry out transmission experiments from mouse spontaneous tumours as well as from human tumours to rats. Vaccinations into the eye led to macroscopically visible tumours. They could also be achieved by vaccination into the spleen of rats. In one case, this was also achieved by vaccination into the testicles. In these new formations, cell complexes were found which had cells of the same type as the original tumour. Keysser also regarded the occurrence of necrotic (dead) masses with small-cell infiltration as characteristic for the development of organ tumours in mice derived from mice. "... we have the same microscopic picture of the development of foreign tumours as that obtained by organ vaccination with mouse tumours in mice" (ibid., 1665). However, the vaccination of rat tumours originating from mice and humans was only successful in one or two passages. And the vaccination of foreign animals was only successful in 5 % of cases. Keysser explained that when heterologous tumours are approaching, one has to reckon with still unknown dispositional moments which can only be excluded for the time being by applying large series of vaccinations.

Since the organ vaccination with such thin and extremely small amounts of tumour juice helped to induce tumour development, Keysser thought it obvious to assume that the cancer cells might not be important for the further vaccination. In order to test this, he started to carry out

experiments with vaccine material that he wanted to make cell-free by centrifugation. He repeatedly inoculated organs with ascites from mice (with a fluid that accumulates in the free abdominal cavity in the case of dropsy), which had formed in the mice as a result of a large liver tumour. It was possible to achieve tumour formation in organs with the clear substance centrifuged from liquefied tumours. In his opinion, these findings suggested that in a material in which macroscopically no cancer cells are present and with which successful vaccinations can be carried out, there must be virus present which is capable of producing new tumours independently of cancer cells. To substantiate this assumption, he extended the vaccination experiments in a certain direction. He produced filtrates of mouse tumours (using porcelain filters) and used them to inoculate the animals into organs. He succeeded in obtaining a macroscopically visible tumour in the eye of a rat and in proving that a tumour had developed from the filtrate which pathologically and anatomically corresponded to the original tumour from which the filtrate had been produced. In his opinion, this finding corresponded to the investigations carried out by Rous on the filterability of a chicken sarcoma, which had shown that sarcomas of the same cell structure could be obtained in chickens with one filtrate and that new passages could be constantly further cultivated with this filtrate.

The fact that a mouse carcinoma can be transmitted by filtered starting material was also reported somewhat later by Henke and Schwarz (1914). They used a very virulent carcinoma strain. Besides several failed attempts, they were able to achieve a positive result once in 8 vaccinated mice in 3 cases. These animals had been vaccinated with a filtrate produced as follows: After trituration of two living mice of removed tumors with quartz sand, a largely homogeneous emulsion was suspended with 6 cm3 saline solution and centrifuged for a longer period of time. The already quite clear liquid above the sediment was then filtered to achieve cell-free conditions. Cells were then also no longer visible under the microscope. Henke and Schwarz were led to suspect that there might have been pathogens in the filtrate which reproduced the tumour in the new animal body. The newly formed tumours had formed at the vaccination site itself. At the same time, Fujinami and Inamoto (1914) described a myxosarcoma, with whose filtrate the same tumour could be formed by inoculation. In the same way, other sarcomas could be inoculated on chickens. Morris (1917) had been able to produce new tumours in about 3000 rats and mice by filtrating tumour tissue, tumours which, however, differed significantly from the original tumour in histological terms. Some of these animals developed glandular carcinomas, some of which showed a mucilaginous degeneration. Morris also assumed that an invisible virus was the cause of tumor development. A similar view was held by Teutschlaender (1920) with regard to chicken sarcomas (later he moved away from this - see Teutschlaender 1925). According to him, positive tumour inoculations could be achieved

with filtered tumour juice as well as with dried tumour powder and tumour cells stored in glycerine for weeks.

The idea that viruses could cause cancer had also emerged from another direction in cancer research, namely from transplantation biology, which had already gained in importance in the early 20th century, a direction which was concerned with answering the question of whether tumours from one animal could in turn produce tumours in another animal or not. The researchers were interested in the susceptibility to cancer and the development of specific types of cancer, and in this context they thought about heritability and transmission, so that it was necessary to uncover genetic factors that might be involved in the etiology of tumours. The question arose, among other things, because there were results of experiments according to which tumours of rats and mice could only be transplanted to animals of the same species. It was therefore necessary to examine whether the tendency to tumours was a problem of genetic control or not. In this case it was obvious to include experimental animals with a largely identical genetic composition in the investigations. To make this possible, in the 1920s inbred lines of mice (later also rats and guinea pigs) were created by sibling mating over several generations. Genetically, inbreeding means the multiplication of homozygous (homozygous) gene pairs and the reduction of heterozygous (mixed) gene pairs. Populations with strongly homozygous individuals also had a tendency to develop the same species and structures of tumours. Two species were created, one with a strong and one with a weak tendency to develop breast tumours. Individuals of the first were then matched with individuals of the second Art crossed. After the crossbreeding experiments, however, it turned out that only offspring of dams from the group suffering from breast tumours developed tumours again. When males of this group were included in the experiment, the offspring remained free of tumours. This result contradicted the thesis of the genetic inheritability of tumours: the sex could not play a role in genetically controlled tumour formation, since males and females had the same genotype. It was thought that the cancer was caused by a virus which was passed on from mothers to their offspring during suckling (see Bittner 1936 and 1942).

In the mid-1920s, it seemed that the difficulties which had only been suspected of the existence of cancer-causing viruses - difficulties which had arisen in the visualisation of tumour-causing agents and in attempts to cultivate them - had finally been overcome. From the United Kingdom came the sensational announcement to the public, hailed in the[34]British press as a turning point in cancer research, that it had been possible to photograph something causing tumours in ultraviolet light. Barnard (1925), who had developed the technical conditions for this40 ,

[34] The sensationalism of the daily press was the main reason for the publicity that Gyes and Barnard's works caused at the time, and he feared that this could spread an unfounded fear of infection and cancer.

believed that he could distinguish it from other similar bodies found in most organic liquids after attempts to make it visible using various staining techniques had failed. It was possible to identify granules on wafer-thin layers of tissue by staining, but a number of researchers felt that these could not be the viruses they were looking for. "The films," says Gye, "showed innumerable pink granules on the border-line of resolution. Such experiences as these have led me to the opinion that such granules are not the virus. The visual discovery of such small organisms is obviously a special problem in optics" (Gye 1925: 114).

The messages were also of sensational importance because they brought to light successful cultivation attempts. Gye (1925) reported that it was now possible to cultivate the agent of the chicken sarcomas first described by Rous in vitro (with the addition of rabbit serum), that the filterable pathogen of the cancer disease could continue to reproduce from culture to culture in specific nutrient media. It was based on the Rouss discovery that filtrates and extracts from pulverized chicken tumor, which should no longer contain living cells, injected into healthy chickens, produce sarcoma-like tumors. Gye was able to make the agent from the chicken tumors multiply enormously by adding pieces of the tumor to bouillon, to which he had added potassium chloride, rabbit serum, and often sugar. A fragment of a 12 to 16-day-old chicken embryo was added to such broth. The whole was kept anaerobically at 35° and 36° Celsius. A drop of the first culture was added to this mixture. If a small amount of such a subculture was repeatedly brought to new nutrient medium, the tumour could be produced again and again by inoculating a healthy chicken with the liquid obtained, despite the final dilution of the starting material down to one quadrillionth. In another experiment, Gye added pieces of various mouse and rat tumors to the culture fluid described above, produced subcultures that were kept anaerobically, and inoculated chickens with them. The results were negative. He then mixed the culture with kieselguhr and filtrate from chicken sarcoma treated with chloroform. With this mixture he was able to produce tumours in chickens that showed the same structure as Rouss' tumours. From this he concluded that he had propagated the same virus from mouse and rat carcinomas and sarcomas, which was the pathogen causing the chicken tumours.

Gye had discovered that the agent under investigation lost its effectiveness after a number of culture passages, i.e. the tumor vaccine yield became less and less. The fact that it was possible to produce typical Roussarcomas was therefore initially just as likely to be attributed to the transfer of a chemical substance as to a filterable living agent.

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40 Barnard had already been working on the development of microscopic techniques for making filterable infectious agents visible from 1916 (see Barnard 1939: 3f.).

The results of dosed filtrate inoculations, which showed that the effectiveness of the filtrates increases or decreases with their quantity, seemed to speak more in favour of the former variant.

Vaccination with 1 cm3 of pure filtrate produced a palpable tumour after only 2 weeks, whereas with vaccination of 0.5 cm3 the tumour was approximately the same size only after 3 weeks and with vaccination of 0.25 cm3 after 4 weeks. With an even smaller quantity the tumour did not develop. On the other hand, the fact that the virulence of the "primary cultures" obtained in the experiments to first grow the tumour in broth containing potassium chloride was lost after 48 hours, and after one week if rabbit serum was added or under anaerobic conditions, i.e. more slowly than in the absence of serum or in the presence of oxygen, spoke in favour of an invigorated agent. In explaining the decreasing effectiveness of the material, Gye now came up with the idea that this phenomenon was not due to the death of the virus, but to the disappearance of a chemical substance originally contained in the primary cultures and originating from the tumour cells, on the presence of which the infectivity of healthy cells with the virus during vaccination depends (Gye 1925: 116). Certain chemical substances contained in tumour tissue are necessary to maintain the virulence of the filterable pathogen. Neither sterilised filtrate alone nor virus alone is capable of producing tumours. "Neither of these factors operating alone will cause the formation of a sarcoma" (ibid., 113). The chicken tumours would probably be transmitted by an animated virus capable of multiplying, but the resistance of the tissues would first have to be broken by a chemical factor that could be extracted from the tumours.

In order to regenerate the agent, therefore, it had to be a matter - in accordance with this thesis - of adding the substance in question to culture again. Fresh tumour filtrate, in which the pathogens had been killed by the addition of chloroform, was mixed with cultures that had become ineffective, and this mixture of cultured pathogens and effective but killed extract substance resulted in full vaccination yield again. The subcultures containing the virus in question, which was reproduced in them, were in themselves ineffective, i.e. they produced chickens injected, not a tumour. They only became effective when, in addition to diatomaceous earth, the filtrate pre-treated with chloroform was added to them after the chloroform had been expelled. In contrast, the experiments described above did not succeed in treating tumours in rats and mice. Gye suspected that the active chemical substance was apparently present in them in too small quantities. However, with mixtures of cultured rat and mouse tumor virus and the active chemical factor of chicken tumors, he was able to cause tumors of the chicken sarcoma type in chickens. "This indicates", as Lehmann (1926: 226) concluded, "that the same virus is present in all other malignant tumours, but the active chemical substance must be specific for each animal species and for each type of tumour". With the introduction of such a species- and tissue-specific factor, it was possible to take into account that only the animal species and tissue from which the tumour extract originated could be made infectious with the agent (otherwise one would have to assume at least one group of viruses for each species and a specific virus for each tissue; see Gye 1925: 110).

At a cancer conference in Düsseldorf in September 1927, Blumenthal et al. reported that in several cases they had succeeded "in producing tumours in other healthy rats with injections of spleen mush from tumour rats in which no metastases could be detected, which apparently deviated in their histology from the injected tumours. It was assumed that in these experiments a transferred cancer cell could not be the cause of the newly formed tumour, rather we believed that a cancer agent was transferred with the spleen mash in these cases" (Blumenthal et al. 1927: 229; see also Blumenthal 1925: 1306). There were also reports that a number of bacterial strains could be isolated from malignant human tumours and from the malignant growth in dogs (see Blumenthal 1925), some of which have the ability to produce malignant tumours in rats. Reichert (1925: 449) saw in the fact that these are, from a bacteriological point of view, very different germs for the formation of tumours, the expression for the fact "that an ultravisible virus originating from the tumour adheres to the bacteria, which must be regarded as the actual tumour pathogen.[35] In the early 1930s, Shope (1932, 1933) reported that rabbit papilloma (a villi tumour) could also be successfully transmitted by cell-free filtered tumour juice. In 1936 Bittner was able to trace the mamma carcinoma of the mouse back to a filterable agent.[36]

The assertion that experimental transfers of carcinoma filtrates with the effect of new cancer growth in previously healthy organisms had succeeded, i.e. the idea that malignant tumours in animals could be transferred cell-free, which suggested a virus-induced transformation of normal into malignant cells, met with fierce resistance in the 1920s from those researchers who shared the traditional conviction that cancer cells living alone were capable of transferring the tumour to other animals (see Darányi 1937: 1267). The transmissibility of transplantable animal tumours should be linked to the presence of intact cells in the vaccination fluid. This understanding corresponded to the cellular theory or cellular pathology represented by Virchow, according to which the cell is the fundamental physiological and morphological unit of the organism and disease is the disruption of its normal physiological processes (Virchow 1885).[37] In 1930, Ludford characterized the contrast between the infection theory and the cell

[35] Borrel (1909) had already previously proposed the thesis that higher ecto- and entoparasites could be considered as carriers of an as yet unknown, invisible virus and that hair follicle mites in particular played this role in the development of breast cancer.

[36] In the early 1950s, such a connection was also discovered with regard to mouse leukemia (Gross 1951).

[37] However, Virchow himself was not at all averse to the assumption of an infectious etiology of malignant tumours: "The increasing number of parasitic microorganisms in diseased parts of the body over the last few years has led many people to hope with increasing confidence that it will also be possible to detect a cancer bacillus. Up to now, the results of even the most eager researchers have not yet been presented in a convincing demonstration. However, the possibility of such an occurrence is not easy to dismiss; indeed, one can admit that the discovery of a specific bacillus would represent an important advance in the diagnosis and prognosis of carcinoma. The attempt to trace all phenomena of cancer proliferation up to dissemination and metastasis back to the spread of cancer cells is by no means supported by anatomical and experimental findings so reliably that no room would be left for another mode of explanation. Conversely, the need for a cancer bacillus is not

theory approach to cancer research as follows: "The unbiased critic will probably agree that the filtrable tumours of the fowl afford the strongest objection that can be raised to the acceptance of the mutation theory of cancer, while the ardent advocate of the theory will adduce evidence to justify making the necessary assumptions that are required to explain the filtrable tumours by its aid.[38] The incompatibility of both theories is also emphasized by Gye and Purdy (1931): "The one, which is inseparable from the cell theory, assumes that the cause of cancer is something which is operative only at the time when the primary cells of a cancer take on their malignant qualities, the disease afterwards progressing independently; the other assumes that cancer is due to the continuous action of some persisting cause, such for example as a living virus. It will be seen that the two theories are mutually incompatible" (1931: 501, quoted after van Helvoort 1994b: 138).

The Norwegian researchers Margit and Magnus Haaland (1927) were among those who resisted the infectious theory of malignant tumours. They reviewed Gye's attempts to use centrifugation-derived, cell-free meat broth containing pieces of tumor to inoculate mice with tumors. For comparison purposes, cells were also inoculated. Suitable liquid culture media (meat broth with addition of animal protein) were loaded with sterilely removed tumor material (pieces of mouse tumors with part of the affected organ) and incubated partly aerobically and partly anaerobically (by pumping out the air and introducing hydrogen into some test tubes). The inoculation was carried out separately, both with the pipetted clear supernatant - which should be infectious after gye - and with the sediment containing the remains of the inoculated tumor. With a total of 168 inoculations with the clear fluid, Haaland and Haaland could in no case cause tumor growth in mice. The inoculation of the tumor piece after 24 hours of anaerobic incubation was positive in 7% of the cases, after an equally long aerobic incubation in 11%. The inoculation of the fresh tumor had a positive result in 95% of cases. These tumours also grew faster than those obtained from the non-incubated material, which the two researchers attributed to the fact that incubation had damaged the cells; they would partially dissolve, which could be determined microscopically. However, it is certainly to be expected that these are still surviving cells - even in the anaerobically incubated tubes - which transmit the tumour. Where these are missing, as in the clear supernatant culture fluid, a successful tumour vaccination is not possible. The fact that they only succeeded in tumour transmission when cells were

so great that without it we would be deprived of any possibility of understanding. Animal or human cells, just like bacteria, have the ability to have a decisive influence on metabolism and to produce effective secret substances of the most varied kinds" (Virchow 1888: 18).

[38] Jordan considered in 1939 that the contradiction between the two concepts could be eliminated: "Since serological experience has shown a relationship between the viruses of avian tumors and components of normal chicken cells, it is obvious that between the two competing interpretations of the cancer problem, mutation theory and virus theory, a synthesis in the sense of exploiting the similarities between virus elements and genes could be considered" (1939: 12).

inoculated - which maintained their growth capacity even after 24 hours of anaerobic treatment - and that the cell-free fluid was not sufficient for tumour transmission, were sufficient reasons for Haaland and Haaland to reject the assumption of cell-free transmission of mouse carcinoma and the associated ideas about the revived virus.

The cell-oriented interpretation of disease could still be asserted despite those transmission attempts, which were explained by the filterable, "cell parasitic" virus. On the one hand, it could be argued that the pathological anatomy had not found any parasites during microscopic examination (see Pentimalli 1927: 348)[39], that there was no clinical evidence for the effective presence of a specific microorganism and its transmissibility, for example from person to person, and thus the vaccination of the disease caused by it. Ideas that parasites were responsible for the growth of tumours have always been rejected: Researchers who believed that they had microscoped protozoa, nematode eggs, mites or something else as pathogens of certain tumours were countered by the argument that they had in fact come across cork cells, canvas fibres or other particles. Or what researchers, who understood cancer to be an infectious disease, thought they had found as the cause, was reinterpreted in a cell theoretical sense: What was presented as bacteria could be understood as secondary elements that had penetrated the tumours, or the protozoa and blastomycetes seen in the carcinoma cells could be seen as degeneration products of a granular kind in the nucleus and cellib (see Roncali 1914: 152), as degeneration products of the living cell substance or as atypical cell nuclear divisions, "cell inclusions" discovered in carcinomas and attributed to invading parasites as degenerated leukocytes, as regressive metamorphosis (see von Leyden 1904: 308 f.) or as a secretion of hyaline (glassy solidified) substances of the protoplasm (see Honda 1903).

On the other hand, the concept of an infectious development of malignant tumours was already vulnerable as long as it was not possible to overcome the great difficulties of making a cancer agent effective outside the tumours. According to Koch's postulates, it was not possible to separate an active tumour-generating agent from the tumour cell, to isolate the parasite completely from the host body and to re-cultivate it in pure culture sufficiently often, thus causing cancer anew. And this explains to a large extent "why it was possible to maintain the dogma that only the intact cancer cell in mammalian cancers is capable of producing tumours again", according to Blumenthal et al (1927: 231). Researchers have also repeatedly come up

[39] However, the question arose whether such methods were at all suitable for this purpose. "The problem of the relationships between impaired regenerative processes and tumour formation is a biological problem, for the solution of which, in my opinion, histological methods have so far proved to be virtually insufficient, since such methods can never teach us what happens and how and why it happens when a regenerative element transforms into a neoplastic, i.e. malignant element. The cell physiological methods, in particular the energy-supplying chemical reactions that have been developed in recent years and successfully applied to the carcinoma problem," said Pentimalli (1927: 348).

with findings which they believed proved the effect of cell or cell nucleus residues in the filtrates claimed to be cell-free (see Lewin 1925: 455; see also ders.1928: 466 ff.). Claims by tumour researchers that they could have excluded the presence of cells in the experiment could be questioned by referring to inadequacies of the means used for filtering, pulverising the tumour material or other techniques. The fact that the effective filtrates could have led to cell transfer - even when using filtration techniques which had proved to be particularly effective - could be reasonably assumed with reference to some experiments.[40] In 1924, Jung reported that the filtrate contained at least cell debris, nuclei to which fragments of plasma were still attached. A little later (1925), he reported that the filtrates or tumor powder, which was intended to dissolve all cell structures, still contained isolated cells or at least cell debris and germs. The theory that carcinogenesis should depend on cells was also supported by experiments, the results of which suggested that the reduction of cell material in the vaccination fluid delays tumour formation or increases the uncertainty that such formation will occur. And it was also reported time and again that only negative results could be achieved with cell-free filtrates. For example, Loeb reported that in his investigations of rat sarcomas he had not succeeded in achieving tumour formation by filtration after tumour cells had been eliminated, whereas control experiments had always produced positive results. According to all experiments, "it can be ruled out with a high degree of probability that any microorganism which is able to exist outside a cell and which can be filtered through Berkefield filters is the cause of these sarcomas... "“ (1903: 352 f.). Königsfeld and Prausnitz (1914), who had experimented with mouse tumours, came to the same conclusion; they too could never observe tumour formation when Berkefeld filters were used. Haaland and Haaland (1927) also believed to have proved the ineffectiveness of cell-free material (see above).

In support of the idea that the filterable agent of such tumours as chicken tumours could originate from the tissues of the tumour-affected animals themselves, reference was made to the pronounced tissue specificity of the transmission. With the idea that it is an independent, autonomous agent, the pathogens must be assumed to be ubiquitous, which is "the best proof for the *weakness of the theory of infection*", according to Teutschlaender (1927: 247). "This embarrassing hypothesis seems all the more absurd to us as we do not need it at all if we *do not see* the *specific moment of cancer development in a specific factor coming from outside, but in a specific factor located in the affected body itself*, which is already present in any organism in some form or can be formed in any organism".

[40] Deutschlaender argued that "anyone who is familiar with these things has experienced that quite considerable amounts of cancer cells have to be injected in the form of tumour mash in order to induce tumour formation ...". With new observations it has been possible "after subcutaneous injection with spleen mash to get 3 times real tumours, 2 of which could be further cultivated by transplantation" (ibid., 229).

It was considered not unlikely that tumour formation was due to a ferment in the filtrates or to toxins.[41] In 1935, when chemical studies of the Stanley virus in 1935 succeeded in isolating a crystalline protein with the properties of the tobacco mosaic virus, the assumption was strengthened that the virus was an autocatalytic protein, an assumption which also referred to the nature of cancer viruses. Fuchs, who had attempted to detect the agent of a type of cancer with the same methods Stanley had used, reported at a microbiologists' meeting in London in 1936 that he had obtained a crystalline substance from cell-free extract of a rabbit carcinoma with which he could again produce histologically similar tumours in rabbits (note from: Seiffert 1938: 28; no source cited). It should be added that there had been indications several decades before that such a substance could be obtained from tumours. In an essay by Novell (1913: 682) it is stated that a chemical, crystalline substance characteristic of tumours has been isolated from human carcinomas, which leads to multiple cancer formation in a rabbit after vaccination. Novell had prepared extracts from carcinoma tissue, from which he believed he had obtained the crystalline substance by confining it in a water bath and shaking it with ether. However, other researchers, such as Fränkel and Klein in 1916,[42] questioned this statement.
A further factor in favour of an endogenous specific factor was the fact that cancer formation could not be equated with normal reactions against infectious agents. The malignant tumours could not be interpreted as defensive symptoms against external stimuli, as is the case with changes in infectious diseases. For Doerr it was proven that exogenous infection as a specific cause of the spontaneously occurring, cell-free transmissible chicken sarcomas, for example, did not exist at all (1938: 45 ff.). Cancer, according to Teutschlaender (1927: 247, 248), was impressive as more or less derailed tissue formations which were only triggered by specific external factors, whether parasitic or non-parasitic (for example, tar and pitch were used to regularly produce cancer[43]). Parasites did not have a direct carcinogenic effect, "specifically" in the usual sense of the word, but only indirectly and under special conditions dependent on the organism itself (ibid., 249).

However, it was possible to object to the idea of an endogenously produced chemical substance as tumour pathogen that, as in the case of chicken tumours, inoculation with filtrates of cell emulsions hardly produces a considerably worse inoculation result than the transfer of the cell pulp usually used for inoculation, although, even if the filtrates are not cell-free, only very few

[41] According to Lewin (1925: 455), some tumours have been removed from the group of malignant tumours over time as undoubtedly toxic and infectious.

[42] They expressed their doubts in an essay in the Zeitschrift für Krebsforschung, Vol.15, 1916 (note from: Lewin 1925: 463; no title given).

[43] Carrel reported (1925: 1083) that the injection of embryo mash, which he had mixed with tar, indole and arsenic, could cause tumours in chickens whose virus could be transmitted further through cell-free filtrates. A.Fischer (1926: 1217; note from Seiffert 1938: 9, no title given) believed that by treating tissue cultures with arsenic he had created a filtrable, tumour-producing virus.

cells could be contained in them (see Lewin 1925: 461). In recognition of the fact that it is not the tumour cell, but the virus that causes the tumour to be inoculable, it could also be argued, for example, that ultraviolet light kills the tumour cells, but not the transmissibility of the tumour (see Rous 1913). Another argument: Because the tumour-exciting agent in Rouss' chicken sarcoma was not only found in the primary tumour but also in the metastases, it should be considered possible that a foreign chemical substance could proliferate in the organism, for which no example was known.

Barnard (1925) reported that he was able to image the agent of Roustumor on agar plates by ultraviolet light and with the wavelengths 275 µµ with the help of a combined illuminator as a round or spherical body on the photographic plate. This would indicate a corpuscular nature of the agent. Evidence that the tumor pathogens are particles of considerable and uniform size made it difficult to imagine them as an endogenous agent. After the results of a series of investigations it could be assumed that the viral elements extracted from tumour material (in the infectious sap of the Rous sarcoma) were of equal size and had a particle diameter of about 60 to 70 mµ (see Elford/Andrewes 1935 and 1936), which could be centrifuged at the appropriate speed of rotation and presented as granules in stained preparations of the ejected sediment (see Ledingham and Gye 1935).

To justify the thesis that a living agent is present in the filtrate, it could also be argued that the addition of chloroform significantly impairs or completely cancels out the virulence of the virus, so that it no longer has a tumour-forming effect. Or it was possible to refer to experiments according to which the agent was still detectable even in extreme dilution of the starting material. A chemical substance should have been gradually depleted.

With regard to the tumour virus, it can therefore also be stated that controversies on understanding of its nature by experimental means. The doubts about filtrability did not disappear, partly because individual authors achieved negative results in attempts to achieve cell-free transmission of such tumours as chicken sarcoma, and partly because positive results could be mistrusted with reference to possible sources of error. However, the same can also be said with regard to the other party.

## THE DECONSTRUCTION OF THE BACTERIOLOGICAL PARADIGM OF THE FORMER VIRUS RESEARCH AS A RESULT OF PROCESS DEVELOPMENT

The filterable, invisible agents did not immediately lead to the development of a new theory for their understanding. Initially, the predominant effort was to adapt the new phenomenon to the outdated explanatory pattern of bacteriology. Even in the 1930s, most virus researchers were

not inclined to attribute a biological characteristic to viruses. It seemed to them that investigations of a bacteriological nature could establish a continuous, seamless transition to virus research and vice versa. Virus research was conducted as "bacteriology without a microscope", or the dividing line between the two areas seemed to result only from the physical limitations of the microscope.[44] Filterable agents were usually seen as something like "minimal bacteria", "microbacteria" or "ultra-microbes" (Schuurman 1927: 136 ff.; Levinthal 1930), although they could not be treated like ordinary bacteria without difficulty. But it was believed that the difficulties could be overcome at some point. It was foreseeable that one day the virus could be made visible with improved microscopes or staining methods and separated from liquids with finer filters. Moreover, observations could be made that there seemed to be similarities between filterable agents and tiny bacteria in certain viral diseases. According to Burnet and Andrewes, referring to photographs (1933: 166), the small corpuscles of various viruses - the vaccine, the mouse and the canary virus - showed a structure and possibly even a mode of reproduction "that is essentially similar to that found in common bacteria. In vaccines, the particles also release a characteristic, soluble substance that is similar in many respects to that secreted by bacteria.

There was also the conviction that at some point filterable agents could also be cultivated on inactive culture media. And there were also researchers who claimed that they had cultivated virus on cell-free culture media (see Eagles and McClean 1931, who claimed to have cultivated the vaccine virus in such media; see also Eagles 1935). Reports of this kind, however, could not be confirmed by other virus researchers (lengthy verification experiments on the cultivation of the vaccine virus on artificial culture media were carried out by Haagen in 1933 and by Rivers and Ward in 1933, among others). The fact that success in this respect had not yet been achieved was interpreted as meaning that suitable soils had not yet been found or that the knowledge of the physiology and metabolism of the cell was not yet sufficient to artificially create the milieu conditions necessary for the growth and reproduction of viruses (see Burnet/Andrewes 1933: 162). The search for suitable soils continued undaunted until the early

[44] „There is no obvious dividing-line between Bacteriology and the study of viruses; in fact, it appears to me that the study of the one leads continuously and without break to the study of the other. The only demonstrable dividing-line, if such there be, seems to be one originally imposed by the available methods of study – arbitrarily imposed indeed by the physical limits of the micro-scope itself. When disease agents were discovered which were too small to be seen and resolved by the best microscopes then existing, morphology could offer no guidance as to the nature of these very small agents. They were thought to be something quite different from ordinary bacteria. There is, I think, no doubt now that had the microscope been more highly developed in those days, much so-called virus work would have been but a natural development of bacteriology. It is a fact that the dividing-line between virus and bacteria is placed just where the existing microscope failed, so far as the observation of a visual image is concerned“ (Barnard 1939: 2).

1930s.[45]The emerging thesis that the virus could only be made to multiply in the presence of living cells outside the animal or plant body, the assumption of an "obligate intracellular parasitism" as the essential viral characteristic, whereas filterability and invisibility were no longer to be regarded as decisive characteristics - , immediately spurred on to decisive opposition (see Gildemeister 1939a: 9). Only a few suspected that the presence of cells was a condition of viral replication. At that time, non-cultivability was a thoroughly contestable criterion for distinguishing the class of filterable viruses from other "microbes" as long as it was not possible to decide whether it was caused by essential features of the virus metabolism or only by unsuitable breeding techniques. The assumption that this was only a temporary problem was supported by the fact that it was possible to refer to certain bacteria which could only be propagated on artificial culture media if a certain substrate was added to the nutrient as a growth factor (for example haemoglobin).[46] Analogous to the fact that there were bacteria that needed special media to grow, in the case of the virus it seemed to be only a matter of finding the right substrate that allowed the agent to be cultured in vitro. There was no reason to believe that the ability of a bacterium to multiply in artificial media could depend on its size, so why should there be deeper reasons than just technical deficiencies for the failure to grow viruses, understood as ultra-microbes, in the established way (see M'Fadyan 1908: 240 f.), especially since there were also filterable agents for which this seemed to have been successful, agents which at that time were still classified as viruses (see Ruska 1950b: 6). Thus, the pathogen of pleuropneumonia in cattle, which had been described in 1898 by Roux, Nocard et al. in the form of tiny, fringed and mobile points of extreme thinness, was counted among the few types of virus that could be bred on lifeless breeding grounds (see Roux/Nocard et al. 1898: 244; Haagen 1939: 176; Barnard 1939: 8), as well as the pathogen of agalactia.[47] They made it appear possible that with further knowledge of the physiology and metabolism of the cell, i.e. with a more intimate familiarity with the physical-chemical processes within the living cell, the milieu conditions required for the growth and reproduction of viruses could be created artificially.

With the perfection of filtration technology (especially with graduated membrane filters), the separation of the infecting agent from liquids was finally successful. Filter types with standardized pore sizes were developed so that the size of different virus types - depending on

[45] „In the literature of twenty years ago it is not uncommon to encounter reports in which it was claimed that viruses had been successfully cultivated on lifeless media. These reports have not been confirmed and at present such claims are rarely made", so Rivers (1932: 429).

[46] According to Fildes, a substance or a chemical group which is involved in the synthesis chains necessary for bacterial growth as an essential factor, but which cannot be synthesized by the bacterial cell itself - factors of this kind he called "essential metabolites" - gains the significance of a "growth agent" which must be added to the nutrient medium if reproduction is to be made possible (1940). Apart from growth-promoting substances, attention was also paid to growth-inhibiting substances (see also Doerr 1944b).

[47] Frequent disease of mother sows as a result of infections of the suckling pigs at birth.

whether the pores were passed or not - could be measured comparatively.[48] However, with these improvements, it also became clear that the filterability of a pathogen is largely dependent on filter type and filtration conditions (e.g. pressure, duration) and not only on the size and surface area of the virus. Nor could collodion membranes be regarded simply as sieves that would retain particles whose diameter is larger than their pore size. As early as 1908, Prowazek had emphatically rejected the idea, which in his opinion had already hardened into a dogma, that it was possible to gain insights into the nature of the virus on the basis of filtration experiments, because every filter was dependent on particular fluctuations in relation to its stiffness (1908: 166). A few years later, Doerr had also taken a critical stance on problems of viral filtration at a meeting of microbiologists in Dresden and had discussed the nature of the medium (the nature of the liquid used for suspension), the forces of molecular attraction, capillarity, duration and pressure of filtration (1911). With the further refinement of filtration techniques, the process dependence of the facts obtained became increasingly obvious. "The difficulties become insurmountable when the success of vaccination with the filtrates is completely uncertain and fluctuates, as in the case of influenza... All filters... physically follow Poiseuille's law of filtration through capillaries, whose average width is thus determined... The retention of the pathogens occurs by surface adsorption, partly by real blockage of the 'bottlenecks'...The requirement of 'isoporosity' remains practically a pious wish" (Schmidt 1935: 1661). Moreover, difficulties arose in differentiating viruses from other agents on the basis of their filterability because some pathogens had been found that could pass through ultra-filters but were classified as bacteria (such as Pfeiffer's influenza bacillus), while at the same time it turned out that some ("larger") viruses were impermeable to these filters. These difficulties could not be overcome by the construction of new filters (membrane filters made of collodion and other materials) and the approximate determination of their "effective pore size".

And just as the property of filterability as a criterion for assessing viral nature lost its value to the extent that the improvement in techniques made it difficult to separate the empirical results from the nature of the observation conditions, the property of invisibility also proved to be unreliable for the identification of infectious agents as viruses with the perfection of techniques, as will be shown below.

Originally, it was widely believed that the biological uniformity of viruses could be derived from their dimensional coherence. Even in texts of the late 1930s, one still occasionally comes

[48] The average pore size of a given membrane was determined by the rate at which a certain amount of water flowed through a membrane surface of known size under standard conditions, taking into account the water content of the membrane (see Burnet/Andrewes 1933: 165).

across sentences which express a connection between differences in the size of the agents and the biological characteristics of the same. Thus, for example, Haagen asserted in an essay published in 1937: "The dimensional limitation upwards simultaneously represents a biological separation of the viruses from the other microorganisms, insofar as the Rickettsiae already clearly differ from the former in their cultural claims" (1937: 465). [55] However, some ("small") bacteria had already been encountered that could hardly be made visible, whereas there were infectious filtrates ("large" viruses) that could be observed by light microscopy.

For certain diseases where filterable viruses appeared to be involved, the[49]microscope revealed the existence of so-called "inclusion bodies". In 1904, Borrel reported the presence of minute copuscular elements in sheep pox and poultry pox, which he considered to be the pathogens of these diseases. Similar observation results were reported by Paschen (1906), who had examined human pox material, which led to the assumption that at least some viruses could be made visible using ordinary microscopic techniques. This discovery was followed by a lively search for morphological elements. Such findings were discovered, for example, in a viral disease of canaries (see Burnet 1933), in Molluscum contagiosum[50] (Goodpasture/Woodruff 1931), in psittacosis (Levinthal 1930) and in Ectromelia, a viral disease of the mouse (Barnard/Elford 1931: 530). To name such elements, von Prowazek (1911) introduced the term "elementary corpuscles", which is still used today. Lipschütz pleaded in 1930 for naming them "Chlamydozoae" and "Strongyloplasmas". However, this proposal did not prevail. The "elementary corpuscles" gave rise to a debate lasting several years, in which it was disputed whether these bodies were identical with the real pathogens. Some researchers suspected that the various cell inclusions were nothing more than special morphological virus forms, which in this way met their intracellular reproduction needs. The virus particles attacked the cell, injured it, and as a result inclusions were formed from the cell material. Other researchers regarded this as just a cellular reaction substance. The particles would penetrate the cell, which would react by forming a plastic material that would coalesce around the virus and partially or completely envelop it. Later, as a result of modern staining differentiation and tissue engineering, the view spread that virus and cell changes (inclusion bodies) should be strictly separated from each other (see Haagen 1937: 468).

[49] Rickettsiae were initially classified as bacteria. However, because they passed filters and only developed intracellularly, they were later regarded as a virus species with specific characteristics (for the history of the classification of Rickettsia, see Weindling 1995: 81 f.). This classification is no longer valid today, because Rickettsiae differ from viruses by their DNA/RNA content and their cell wall containing muramic acid. They are determined as a group of obligatory cell parasites which cannot be cultivated outside living cells and belong to the class of Gram-negative eubacteria (see Scherf 1997: 405).

[50] Contagious skin polyp.

The visibility of virus species has been further improved by the further development of optical devices, the use of ultraviolet light[51] and special staining methods. In the 1920s and 1930s, new techniques such as dark field illumination and UV microscopy became available. It was possible to make virus particles indirectly visible by working in the dark field of the microscope, i.e. by using the indirect illumination possibility to reflect the light rays hitting the sides of the microscope. A number of more strongly refractive particles could be detected in a less strongly refractive matrix. Thus objects could be perceived as bright points or spots of light. The use of UV microphotography also made smaller particles visible earlier than was possible with normal light microscopic techniques, because the resolution of a microscope depends on the wavelength of the light.[52] But with these means the size of the particles could only be detected indirectly. As a result of the increased resolving power, impurities in the cultures became much more disturbing than in photographs taken in ordinary light. Any other morphological control was not feasible because of the "ultravisibility" of the agent, so that it was not possible to decide with certainty whether what was seen was the pathogen or an impurity. The claim, for example, that the deep black formations that could be discovered on the photograph of the filtrate obtained from the infectious material of foot and mouth disease, which was obtained with UV rays, were the pathogens (see Frosch/Dahmen 1924 and Frosch 1924; Hinweis aus: Pfeiler/Simons 1925: 255, 256) and not the bright formations on the plate, could not be traced beyond doubt. "According to Pfeiler and Simons (ibid.), "The greatly increased capacity for resolution, however desirable it may be to the morphologist, can under certain circumstances be disastrous for the aetiological research of filterable virus species ... With the current state of bacteriological culture technology, it is completely impossible to produce pure cultures which do not contain any other particles at the colloidal boundary apart from the pathogen in their medium, let alone 'optically empty'; rather, such cultures inevitably contain more or less large particles of dust and culture medium, possibly also other living filterable microorganisms. Whereas, moreover, it could not be ruled out that the micro-organisms might undergo morphological changes as a result of the chemical effects of ultraviolet rays, that they might be largely damaged or killed during uptake

In the early 1930s, Zweibaum had used these techniques to investigate Rous sarcoma cells and had seen something completely different than Barnard had claimed to have seen in 1925 (see

[51] For early attempts to use ultraviolet light, see Köhler 1904.

[52] To obtain clear images of virus particles, a light source was used whose wavelength is taken from the ultraviolet part of the spectrum and which is not too large in relation to the size of the particle to be measured. "In addition to a monochromatic ultraviolet light source, a quartz lens and quartz prism system is of course required, as well as a device that allows the objects sought to be found in visible light. After the object has been adjusted with visible light, it is brought to the focus of the ultraviolet rays selected for capture by calculated fine adjustment, and then the image invisible to the human eye is photographed with ultraviolet-light darkfield illumination" (Burnet/Andrewes 1933: 164).

above), namely abundant amounts of filaments in which tiny round granules were embedded and which could be stained and blackened during osmosis.[53] He perceived the filaments as certain cell organelles (cell structures which perform certain functions in the cell), namely as mitochondria (mostly rod-shaped organelles which occur in all eukaryotic cells, multiply by division and possess their own genetic material and which carry out substance transformations and forming processes).[54] As he reported, the cell organelles would, when viewed in the dark field, very soon disintegrate into individual, smallest, illuminated granules as a result of the influence of light, and after this disintegration they could not be distinguished optically from the Rous gene corpuscles at all, which would suggest that the filamentous elements and the corpuscles in question are very closely related or identical with regard to their chemical structure and probably also in a genetic relationship (Zweibaum, 1933: 359). In some of his illustrations one could get the impression that the filamentous mitochondria originated from these small granules by stringing the latter together. According to Zweibaum, the mitochondria of the Rous sarcoma cells show differences from those of the homologous normal cells, which can be seen in their staining behaviour (behaviour towards vital dyes) and their rapid disintegration into small single granules already under the influence of light in dark field observation. In the same way as the Rous agent (high-speed centrifugation), Amies also found in the fraction of normal chicken tissue (leucocytes, spleen tissue) smallest corpuscles, which could not be distinguished from the Rous agent corpuscles neither in darkfield nor with regard to their staining behaviour (Amies, loc. cit., p. 141; see also Graffi, loc. cit., 520).

The diffraction images produced by dark-field illumination did not allow the size of the particles causing them to be determined directly. To determine the real size of the virus particles, even the observation in the stained specimen could not provide exact values. It was known, for example, that in a Giemsa-stained smear the infectious agents seem to have a much larger diameter than in the unstained specimen. The surrounding colour envelope only brings the pathogens into the visibility range of the light microscope. Thus, the only conclusion that could be drawn from a stained specimen was that the size of the particles is smaller than that of the stained specimen.

[53] Osmium is a precious metal belonging to the group of platinum metals, and "osmic acid" is a compound used in microscopy for staining and hardening biological preparations.

[54] This means that they show genetic continuity, i.e. they reproduce exclusively by self-division and possess autocatalytic growth capacity. It is assumed that they have developed in the course of evolution from bacteria that have migrated into the cell.

The fact that in 1939 an immediately visible detection of viruses was achieved with the aid of electron microscopy (Kausche, Pfankuch, Ruska 1939)[55], in which very fast electron beams replace light beams, did not at all eliminate the difficulties in determining the nature of the virus. Damage was already observed in the first attempts to image biological objects by electron microscopy. And changes in the objects were described. This had caused many biologists to be very sceptical about the results of the "over-microscope". And so Ruska et al. also had reasons, when presenting their "over-microscopic" images, to take a precautionary approach to the possible objection that "our newly found structures were artificial products that were created by the vacuum or electron beams. In particular, such an objection is obvious if hitherto unknown shells or capsules appear on the bacteria" (von Borries/Ruska/Ruska 1938: 923 f.). The following difficulties arose for the examination of biological objects with radiation: "1. the preparation must be in a high vacuum; this excludes the examination of life processes from the outset. 2. the preparation is heated slightly too much in the strong radiation and is destroyed by the radiation. 3. after passing through the object, the electrons have lost different amounts of speed, depending on the thickness of the layer or density of the preparation being irradiated. However, electron beams of different speeds behave similar to light beams of different colours in optics. They are deflected by the lens to different degrees, so that the chromatic aberration of the lens prevents good imaging" (Rüchardt 1938: 1836).

The use of electron microscopy seemed to cloud rather than sharpen the picture of the virus' nature. The results obtained with the new method, as Ruska explained in 1950, led to the insight "that the virus species show no biological coherence. They proved to be partly macromolecular infectious agents, partly very small organisms, and partly structures for which only the indefinite term virus is available for the time being. "Virus" is therefore not a term of biological systematics, but a "collective term" for various agents. Until the advent of electron microscopy, forms of the smallest microbes would have been classified under the collective term "virus". But 10 years after the beginning of electron microscopic work, all criteria based on methodological peculiarities and considered to be fundamental limits had become obsolete (Ruska 1950a: 223).[56]

[55] Bacteria and viruses were among the first objects of electron microscopy. Because the ability of electrons to penetrate is extremely low, the usefulness of the electron microscope was first demonstrated on such small and thin biological objects (see Hoppe 1991: 330).

[56] However, according to Ruska in another publication, although "virus" is not a concept of biological systematics, "there is still a need for an order of the manifold manifestations. The summary of all filterable virus types in a single order "viral" and their further subdivision into suborders, families, genera and species is "unbiological" in its present form. But it satisfies the practical need for a general possibility of communication (Ruska 1950b: 57).

The introduction of the tissue culture technique was particularly significant, which initially did not necessarily mean that one knew about the intracellular location of virus replication, which could be taken into account with this technique. Initially, this technique served only to preserve the virus in the tissue, and at best it allowed the virus to continue in an infectious form in a few culture passages. The further development of the method finally enabled the continuous breeding of virus, which was first succeeded by researchers who were completely familiar with cell research, said Carrel (1925), who had proved that the virus of Rouss' chicken sarcoma could be quantitatively multiplied in tissue explants and continuously continued in culture passages. However, the perfection of breeding techniques also caused problems. The differentiation of viruses from bacteria according to whether or not an artificial cultivar could be successfully cultivated proved to be unreliable because some bacteria required special culture media for growth, whereas some filterable pathogens such as mycoplasmas could be cultivated without direct contact with living cells. It was also found that some types of virus lost pathogenicity if cultured continuously and that tissues suppressed some viral characteristics. In general, it was still largely unclear what role tissue plays in virus replication. It was known that a classification according to the affinity of the pathogens to the various tissues of the organism and according to the clinical manifestations they cause could only be a makeshift one (see Seiffert 1938: 15). It was also one of the first attempts to systematize the virus types, guided by the experience that the colonization of the virus types in the organism seemed to obey a tissue specificity (Herzberg 1939: 17). The fact that virus replication was only possible in the explant confirmed the insight gained even before virus breeding that very close relationships must exist between host and virus. However, it remained open whether intra- or extracellular virus replication took place. Two mutually exclusive interpretations were still possible, either that the virus feeds on the cell in the manner of an animated pathogen and reproduces autonomously or that the virus is an enzyme-like substance whose regeneration is only possible through the living cell (see Hallauer 1938: 368). The uncertainty continued in virus research for several years. In a work published in 1950, Bedson argued that the different types of viruses were not uniform in nature. "Where is one to draw the line which is to separate the microbial midgets from the unorganized, nonliving, autocatalytic infective agents ? It is impossible to say be-cause, from the very smallest up to the largest virus, there is an unbroken series, not only of particle size, but also of complexity of structure; on merges into the next with no clear indication of a gap suggesting division of the group" (1950: 18-19).

In 1938, Doerr attributed what in his opinion was an unjustifiable adherence to the understanding of the virus as a biologically homogeneous entity to the fact that methods had to be applied "which have only little contact with the research means of microbiology; this must finally have an effect on the idea that the special and uniform methodology must also include

a special and uniform object (i.e. biologically identical or related objects of a special kind - K.L.) corresponds to the special and uniform methodology", a conclusion that is all the less admissible since the methods used are initially almost always those that are to be characterized negatively, such as the omission of (light) microscopic examination and the exclusion of larger dimensions by filtration (Doerr 1938: 98; 13). And a few years later: the object of virus research is uniform only by the means necessary for its scientific penetration, "i.e. in methodological-technological respect", although it is understandable to a certain extent "if the constant application of identical research means finally unintentionally leads to the idea of a not only technical but also a scientific and technological research..." (Doerr 1938: 98; 13) not only technically, but especially biologically homogeneous, an idea which, once it has taken root, one endeavours to justify afterwards, however well that may go" (Doerr 1944a: 7). In this essay, Doerr criticizes that either conclusions are drawn from considerations valid for individual virus types to an allegedly intrinsically connected totality, or that one looks around for more or less hypothetical characteristics which could be attributed to all virus types and which seem suitable as starting points for considerations about their nature. In any case, one would deviate from the facts in order to make general statements (ibid., 7 f.).

From the fact that the groupings of virus types as they were formed at the time were ultimately anchored in the applied research resources, it follows quite imperatively that the classification could not have remained unaffected by changes in methods and procedures. The methodological-technical uniformity of the object of virus research, which Doerr illuminated in 1944, was dissolved with the further development and application of new techniques. With changes in the conditions of fact production, specialisation, improvement, modification and the introduction of new experimental conditions or procedures, these could no longer act as coherence conditions - as conditions for the establishment of similarity relationships between the agents under investigation. In the [57]1930s, therefore, more and more judgments were made on the state of virus research, according to which the development of these and other procedures had moved even further away from a general understanding of the nature of the virus rather than approaching it. In 1932 Rivers expressed the suspicion that the "virus" was only a collective term for very different things, a term which would include both "microbes" and very small inanimate agents. "The dividing lines (according to which viruses could be separated

[57] According to Buchwald, it strengthens the robustness of a taxonomy if it is not only due to the application of a specific instrument, but is consistent with many other instruments used to pursue the classification objective, but in different ways (1992: 44). However, as our case study shows, the opposite may initially occur. If viruses could initially be described as filterable, light microscopically invisible agents that cannot be cultivated on cell-free culture media, the coherence of the above-mentioned characteristics was subsequently weakened. For example, there were submicroscopic pathogens that could not be filtered, agents visible under the light microscope that could not be cultivated on cell-free culture media, etc. It took a long time for results obtained by different techniques to confirm each other and thus help to establish a satisfactory classification.

from bacteria, protozoa, etc. - K.L.) are even more blurred now than they were at the turn of the century," Doerr said in 1938 (1938: 25 f.). And Seiffert in the same year: "Virus is not a scientifically founded biological term, as is sometimes believed, but only a methodologically conditioned collective term" (1938: 1). Kausche 1939: "At the present state of our knowledge, the refinement of research methods seems to have dissolved this collective term, 'virus', to the effect that we now have to distinguish between species which are similar to a living being with the characteristics of reproductive ability, respiration and its own metabolism, and those which apparently lack these characteristics and which, due to their mode and conditions of action, are to be assigned to the active substances of chemically inanimate nature" (1939: 9f.). And Blumenberg (1943: 629): "The term virus is only given the unity it lacks by its name; the question of the nature of a virus must be asked and answered anew in each individual case. The validity of the concept was put to the test, because the individual types of filterable viruses differed greatly in their chemical nature, which could be demonstrated thanks to improved methods (for example, the perfection of centrifuges made it easier to separate viruses from accompanying substances and thus make chemical analyses accessible). It was found that many plant viruses could be characterized as relatively simple nucleoprotein molecules, whereas animal viruses seemed to have a complex structure, i.e. they evaded a molecular concept for their understanding, as was shown by the results of chemical and physicochemical investigations (see Smadel/Hoagland 1942: 96). Nevertheless, the thesis that plant and animal viruses differed from each other in the above-mentioned respect did not only meet with approval. The fact that it was not possible, for example, to recognise characteristics of the flu virus in leaf extracts of diseased plants could, according to Pirie, also be due to the methods used at the time (1946: 575).

Attempts to focus virus phenomenology on further invariant characteristics in order to develop a more stable classification approximating the "natural order" have failed time and again. The "similarity relationships" that were gained repeatedly fell apart with further empirical progress: Among other things, it was tested whether invariant characteristics could be obtained from the analysis of the immunity conditions, immunity against virus infections, antigen functions (whether viruses have a certain antigen structure that gives rise to the formation of specific antibodies) and the serological reactions of the virus species, which differ substantially from the conditions that could be observed with other transmissible agents. In 1928, Schultz had assumed that no type of virus was capable of forming "complement-binding" antibodies or "precipitins", and that the so-called "virolicid" immune substances were the only type of antibody characteristic of the virus types (1928; quoted after Doerr 1938: 90 f.). However, it has been established that the immunizing power of the infection process does not depend on the fact that the agent is one of the virus types. Doerr (1938: 86) judged the efforts to gain

general aspects of a biological nature from the study of immunity conditions to be unsuccessful in that it was not possible to establish radical differences between virus types and other infectious agents. The antigen functions of the virus types did not allow fundamental deviations from the antigen functions of other infectious agents or microbes to be detected.

It was also tested whether viruses can be differentiated from other pathogens on the basis of preferred hosts. However, no fundamental differences could be identified in this respect either. It was not possible to classify viruses according to host affinity. Some viruses could be propagated in several hosts, which led to the difficulty that different names were often used for the same virus (see Ruska 1950b: 16), while others could also lose the ability to infect a particular host. In the same way, the same plant or animal host could also be infected by numerous types of virus, which differed greatly in other respects dimensionally, morphologically, chemically and serologically (see Fraenkel-Conrat 1974: 11).

Another attempt was to identify viruses as a separate category of infectious entities. Thus, in 1928 Rivers argued that viruses produce pathogenic effects in their host which, although not entirely different from other diseases, "yet sufficiently different from them in regard to phenomena related to proliferation and degeneration to warrant placing such agents in a group by themselves". Based on the changes assumed to be consistent, he came to the conclusion that an "intimate type of parasitism exists in viral diseases" (1928: 111). Later Bedson could counter this view by saying that what is common to the virus types cannot be found on the level of virus-related diseases: "...there is no fundamental difference in the clinical and epidemiological be-haviour of the diseases caused by these viruses which might lead one to think that some viruses were of an essentially different nature from others" (Bedson 1950: 19). Classifications based on symptomatology were rejected by Andrewes with the argument that viral properties such as virulence, mobility and persistence are largely unsuitable for establishing a classification simply because of their variability (Andrewes 1950: 165; quoted in van Helvoort 1994a: 216). Ruska emphasized that what was obtained in this way were not "systematic groups". "The similar or dissimilar disease symptoms caused by different types of viruses cannot, in our opinion, serve to group together larger virus groups, nor can they separate individual species into widely differing groups. Only where morphologically identical virus forms are present can the dissimilar disease patterns caused by them serve to separate closely related virus types" (1950a: 389). Even before this, symptomatology had been denied an essential role in explaining the nature of the virus because, according to it, it could only be a matter of looking for common characteristics of how infected organisms react to the viruses (see Gsell 1967).

## TO SOME CONDITIONS, UNDER WHICH THERE IS A CHANGE FROM BACTERIOLOGICAL TO MOLECULAR GENETIC UNDERSTANDING OF VIRUSES

The history of virus research in the 20th century is usually described as a continuous process, a history of progressive revelation of the nature of the virus (see Waterson 1978: xii; Hughes 1977: 75 ff.; for a critique of this concept see van Helvoort 1994a: 187). Our analysis of the case study material has, however, revealed many things that lead away from such a historical picture. In particular, it has been shown that the refinement and expansion of experimental means and procedures, which are generally seen as a guarantee for uninterrupted progress in the knowledge of nature, had tended to lead to setbacks in the period under consideration (for example, in the development of virus classification) and had widened the gap between the conflicting parties in virus research. With the "filterable" virus, something had been discovered which, according to the traditional concepts, which after all had mostly proved their worth in research into infectious diseases, could not be described in a way that all researchers could have shared. Very different interpretations of the nature of this phenomenon arose, which were put forward against each other. No experimental evidence for this or that concept, which all researchers should have accepted, could be presented by any side. In other words, the decision as to whether this or that explanation most accurately expresses the "true" nature of the virus could not be "objectified" empirically. Every version of the interpretation of the phenomenon remained open to attack, facts presented to the expert public could often be reinterpreted into fictions by opponents, who brought into play the dependence of the findings on the conditions of observation, the local situation of the experiments, the research-related nature of the attributions of characteristics, etc. as sources of error. For example, findings often reported by certain virus researchers at the time were not confirmed by other researchers as a result of their own experiments, or the observations could not be reproduced by all scientists working with the virus. Often, findings to the contrary were reported, or the findings that had been examined were considered artefacts. As with justification, reasons of various kinds could be invoked to reject the positions debated. Findings that were used to empirically confirm a suspected connection were often soon joined by negative findings reported by other researchers. However carefully and deliberately the techniques used in the experiments were employed, and despite the fact that each party could offer credible reasons for defending their respective positions and provide empirical evidence - which explains why "the various opponents 'constructed' widely diverging research objects which they identified as the 'virus'" (van Helvoort 1994a: 202) - at no time did they offer compelling reasons that would have led the other party to finally abandon artifact accusations.[58] We will illustrate this with a few examples:

[58] Scientific facts that Knorr-Cetina (1984, 1985a, 1985b), Collins/Pinch (1982) and others have emerged from processes of social construction can be "deconstructed" again. The transformation of fictions into facts or of

In defence of the concept that viruses originate endogenously, findings were frequently presented with the claim that in organisms that were protected against exogenous infections and were therefore free of virus in all parts, virus could usually be detected in abundance after a few weeks. Against the concept of endogenous virus formation it could again be argued that exogenous infections could not be completely ruled out due to technical inadequacies in the experiments carried out and that laboratory infections had to be expected (see Seiffert 1938: 9). There were sufficient grounds for suspecting that the virus had been present in the cultures from the very beginning, but in such weak concentrations that it had escaped identification (see Smith 1936). Researchers who thought the virus was a microbe could not do without such answers: With the understanding of the virus as an animate agent, the theorem of the continuity of all life must also apply to it.

The failure of attempts to prove respiration processes in viruses was attributed by researchers who believed the virus to be a living being only to still existing experimental deficiencies or to the fact that under the given artificial experimental conditions the virus might have been damaged (see Seiffert 1938: 7). Opponents, on the other hand, saw in the failure something that spoke against a living nature of the agent.

The claim that numerous phages were obtained from phage-free cultures (dysentery, typhus, coli, etc.) after a few passages, which was supposed to prove that the bacteria-dissolving phenomenon is caused by bacteria alone (that the dissolution is caused by an autolysin produced by the bacteria themselves), could always be countered by the fact that many cultures contained bacteriophages from the outset, which were often difficult to detect. Even the complete dissolution of bacteria as claimed by d'Herelle was not unanimously confirmed. For example, Gildemeister, who - as stated above - added the phenomenon discovered by d'Herelle to the variability of the bacteria, was unable to reproduce this result either by microscopic observation or by using histological techniques (1923: 184 f.).[59]

disputed points of discussion into undisputed facts refers Latour to a process of "modalization". Through the addition of modalities to factual assertions, the latter gain the character of personal opinions or speculations or of ideas that are bound to local or temporal peculiarities of opinion formation. A sentence loses its factual character if the readers go back to where the sentence was written, to the mouths and hands of those who wrote it (Latour, 1987: 25). Latour speaks of a sentence added "negative modalities" when an assertion is attributed to the conditions of production. In contrast, he calls those sentences "positive modalities" that lead an assertion away from its conditions of production, whereby the assertion gains the status of a fact (ibid., 23; see also Latour/Woolgar 1980: 79 ff.).
„Scientists in current controversies construct and employ histories of medicine, technology, and science to support their arguments or to deconstruct opponent's arguments ... This is more than a debating strategy. Constructing history is one means by which scientists (re)construct rules for verifying facts and findings; that is, constructing history is part of the verification process in science" (Fujimura 1996: 53).

[59] Later he expressed in a lecture that he had to be convinced of the complete dissolution of bacteria after all (1923: 184 f.).

Time and again, the standards by which the reliability of the exclusion of cell residues from tumour filtrates was measured, the reliability of such methods as filtration, pulverisation or the use of (cell-dissolving) glycerine in the treatment of tumour material prior to its overvaccination on healthy animals, have been made the subject of dispute. Researchers who saw the origin of the virus in the cell could object that, even if cancer nests or areas that could be identified as suspected tumours under the microscope could not be found in the filtrates, it could not be excluded that individual cancer cells were still present in the circulation and that these had changed their character within the possible limits. Or one referred to experiences that considerable amounts of cancer cells in the form of tumour mash have to be injected to cause tumour formation. There have always been occasions to attack or defend claims that tumour transmission was initiated by cell-free filtrates and thus the viral nature of cancer was demonstrated.

Statements about the fact that virus elements obtained by centrifugation from infectious juice of the Rous sarcoma are of the same size as one another and show up as granules in dyed preparations of the ejected sediment were doubted, among other things, with the argument that the fact that all particles are of the same size or approximately the same size as one another is a natural consequence of the technique of fractional centrifugation. That the assumed morphological homogeneity of the virus elements would have been produced by the centrifugation experiments was justified, for example, with the following arguments: From normal tissue extracts by centrifugation (15,000 revolutions per minute), it is possible to obtain tiny corpuscles of the same size which are similar in every respect to the elementary corpuscles obtained from an active cell-free tumour juice (Rous sarcoma) using the same technique. These carriers of the specific viral action were in no way different from other contaminating particles of the same dimension (see Fraenkel/Mawson 1937).

These examples may suffice to illustrate that the riddles that the nature of the virus posed to researchers during the period under consideration could not be progressively unravelled in accordance with empirical successes (in bacteriology, plant pathology, etc.). The improvement of the technical conditions for research, the accumulation of empirical data, the growing number of virus discoveries - by the end of the 1930s well over 100 diseases caused by pathogens that could be filtered but could not be detected by light microscopy (Heilmann 1940: 65) were already known - rather led to the uncertainty of what was already believed to be known about the nature of the virus. With the further development of the methods used, it seemed less and less possible to say how viruses should be understood in a very general sense, regardless of whether they were animal or plant, "large" or "small" viruses. Controversies on the

understanding of viruses were not defused by the empirical successes, not gradually reduced, but rekindled again and again.[60]

The question now is how modern (molecular genetic) understanding of viral nature came about when it could not have arisen from the empirical advances in virus research alone. The author of these lines is not yet in a position to provide an exhaustive answer to this question, which has been tested on the basis of scientific and historical material. Further extensive studies are required for this. However, it can at least be said that the development of a modern understanding of the virus has been helped by a process in which virus researchers have used terms from other disciplines (heredity research, biochemistry and other fields) to overcome the problems of interpretation and to consolidate the positions they have adopted in the debates. They included the "gene", the "macromolecule" or the "nucleic acid" in the debates. This also made the virus phenomenon interesting for geneticists, chemists, etc., and the dispute about its true nature extended beyond the circle of virus researchers.[61] Thus a development was initiated at the end of which the borrowed terms were found in a theoretically ordered relationship to each other, as expressed in the modern version of the virus term, a relationship which, however, was the *result of* a longer development process and not its precondition, of which the researchers would only have become aware step by step. At first, individual researchers only suspected that the "virus" was something *similar to* the "gene", the "macromolecule" or something else, and it was a matter of free judgement whether or not to be guided [62]by such similarity relationships constructed solely on a conceptual level.

The motivation for such action arose from the insight, born in the almost endless debates, that a generally accepted understanding of the nature of the virus would hardly emerge from the traditional practice of research into viral infections. With experimental results and observations structured according to this or that concept, the various parties created their own particular areas of experience from which they then drew evidence to justify their concept. As each side

[60] Konsens geht im Verständnis der neuen Wissenschaftssoziologie aus einem Konstruktionsprozeß hervor. „Since the settlement of a controversy is the cause of Nature‘s representation, not its consequence, we can never use this consequence, Nature, to explain how and why a controversy has been settled“ (Latour 1987: 258).

[61] "To fathom the origin and essence of life was and remains the last and highest goal of science, and the properties of the virus-like infectious substances, especially the minimal and in the minimum but again limited dimensions of their units, justify the expectation to come closer to this goal. Only in this way is it understandable that the results achieved by the specialist were able to arouse the interest of the widest circles so quickly, and that not only biologists, but also chemists and physicists began to concern themselves with the "true nature of the virus species" (Doerr 1944a: 1).

[62] This is in contrast to the similarity relationships in the early classifications. For example, certain diseases of humans, cows, horses, sheep and pigs were grouped under the term "smallpox" because they are similar in that they are all characterized by rashes. From today's point of view, it appears to be incorrect. "Several of these diseases were indeed caused by pox-viruses, but the deficiencies of this symptomatological classification are highlighted by the inclusion of chickenpox and the 'great pox' (syphilis) in the same category", as Fenner points out (1988: 3).

perfected its approaches, the dividing line between the parties became sharper and the controversies more radical. But at the same time, this process also enriched conditions that encouraged researchers to look for new reference aspects of research that would allow the virus phenomenon to be observed and evaluated differently from what was usual in conventional activities. The change of perspective - the observation of the virus phenomenon from the point of view of "outsiders" (geneticists, chemists, physicists, etc.) - was linked to the expectation that this would put an end to the controversies about whether viruses should be regarded as living beings or as a soluble substance or enzyme.

The fact that virus researchers consulted terms from this or that discipline in order to overcome problems of explanation cannot be seen as an inevitable consequence that they should have drawn from the results of their empirical work (otherwise there could be no question of a change of perspective). [69] These were terms that had arisen independently of the context of virus research. "...our knowledge of viruses," Darlington said in a review in the early 1950s, "has grown up in the same half century as genetics. But the concepts used have been quite independent until recently" (1951: 321). The fact that the equation of the virus with the gene as well as with the macromolecule and other terms did not result directly from the empirical experience gained when dealing with the virus is supported by the following:

These are terms that were still very controversial. The answer to the question of whether viruses are "organisms or... or chemical molecules... (is) very difficult, since there is no generally accepted definition of these two basic concepts in either chemistry or biology", said Schramm (1942b: 791). [70] There was no unanimous opinion on the applicability of the concept of molecule, which was derived from the behaviour of simple chemical compounds, to high-polymer organic natural substances and especially to colloidally soluble proteins. According to Doerr, it was left to "free discretion" whether one wanted to speak of giant molecules or molecular aggregates in the case of proteins, "especially since nothing more is known about the bonds that hold the units together than that they seem to be rather loose and can easily be broken" (Doerr 1944a: 11). Neither was there a generally accepted definition of the term gene, so that in this respect, too, it was left to every researcher to certify or deny that the virus is similar to the gene. „ ... depending on the aprioristic or professional attitude, the common and sometimes the differing moments were brought to the fore ..." (eben-

69 The reception of the Fleck heritage is very helpful for the analysis of such a process, which was initiated by the creation of new points of reference for research - which owed their existence to the borrowing of foreign disciplinary concepts - and which subsequently led to a new coherent knowledge. He describes the establishment of relationships between concepts from different disciplines, which he examined using the example of syphilis research, as "active couplings". In explaining why precisely these and not other couplings had arisen, he refers to the cultural-historical context that determined the biographies of the researchers

involved at the time. With "active couplings" it is expressed that interdisciplinary connections that initiate the formation of a new discipline or a new scientific specialty are characterized by indeterminacy. Fleck draws attention to such indeterminacy with regard to the interdisciplinary history of the development of serology. He explains that the modern concept of syphilis was not the only logical possibility. If the pioneers of this field had fallen for other links than those they had then realized, one could have come up with completely different classifications of disease, so that other disease units, among which syphilis as a disease unit could not be found at all in the demarcations as they apply today (<1935> 1980: 32 f.). Fleck explains these couplings as a "knot point in the developmental lines of some collective conceptions...". Furthermore, they functioned as conditions of the work of cognition, which consists in determining the "inevitable results" that can be determined under the given conditions. In order to make it plausible that the reference to concepts of other disciplines is one of the necessary preconditions for fulfilling the research objective and adequately grasping the objects of research, the subsequent assumptions that can be derived from them - the "passive couplings", as they can be called after Fleck (ibid., 56) - must be empirically substantiated. The preconditions "correspond to the active couplings and form the collective part of recognition. The inevitable results resemble the passive couplings and form what is perceived as objective reality" (ibid.).

70 Staudinger, who is considered the founder of macromolecular chemistry, was initially denied general recognition. Neither organic chemists nor colloid chemists addressed his ideas on polymer structures, especially as it did not seem very attractive at the time to deal with "smear chemistry" (see Staudinger 1961: 77). At the beginning of the 1920s, an attempt was probably made to apply a new physical method to problems of structure elucidation of organic high polymers. However, the application of this method, X-ray structure analysis, led to contradictory results which spoke for and against Staudinger's ideas.

da, 63). "It is (only) certain that genes cannot be 'seen'," as Geitler was forced to state at the end of the 1930s (1939: 144), and so, of course, all the characteristics in which one wanted to see analogies to the types of virus had to be hypothetical. It was still questionable whether genes were real at all or mere fictions or entities without substance (see Morgan <1933> 1965: 315), especially since one was not sure about the paths to their empirical research, which could have been taken. " ... the material used by genetics in the first half of this century (allowed) neither to study the substance (the genes K.L.) nor to investigate its mechanism of action", says Jacob (1972: 278). And Schrödinger (1951: 13): "After the rediscovery of Mendel's rules the science of classical genetics had emerged, which... had learned, so to speak, everything about the capacities of genetic material, but knew nothing about the nature of the genes themselves."

That is why there were of course a number of researchers who denied that the virus was similar to the gene or macromolecule. For example, Darányi thought it was absurd to see only macromolecules in viruses, "because molecule is a chemical term and not a life unit. The protein molecule is not alive. In order to live, it must also contain other substances (lipoids, salts, carbohydrates etc. - K.L.), although this does not change its size significantly" (Darányi 1937: 1267). Doerr (1944a: 49) also turned against the giant molecule concept. It was absurd to interpret the pathogen of psittacose as a giant molecule, for example. "Not only the size of these elements... would be inconsistent with such a concept, but also the size of the psittacose... but also the high-grade pleomorphism. And proponents of equating the virus with the gene, among other things, were cited: Genes are "found in every living organism that reproduces and passes its characteristics on to its offspring. Viral proteins... are only found in diseased organisms. Asked in this way, the question of the analogy of these two elementary units is therefore wrong", says Kausche (1939: 73). Doerr reproached researchers who held on to the

presumed similarity of the virus with the gene, saying that they had "tried to bridge all objections that oppose the identification of virus particles and gene by unrestrainedly piling up hypotheses" (1944a: 69).

Supporters of the microbial virus concept saw recourse to the genetic concept of heredity research as a way to refute the argument put to them by their opponents that the minuteness of filterable viruses was incompatible with the complexity and quality of organisation, which were generally regarded as characteristics of living organisms. How could such a tiny particle as the virus contain all those partial structures that are the carriers of the manifold vital functions (respiration, assimilation and dissimilation, reproduction, inheritance)? Burnet and Andrewes pointed out in 1933 that the individual virus particle of foot-and-mouth disease could not be larger than 10-20 haemoglobin molecules. They found it difficult to understand how a particle consisting of so few molecules could be organized in such a way "to be able to perform all the complex functions of a living, independent organism" (1933: 167).[63] The thesis that the virus was similar to the gene now seemed to render such questions meaningless: as small as genes are, heredity researchers ascribed them the rank of life units. They were presented not only as mere components of cell substance, but as a fundamental property of living matter. In the[64] early 1930s, suitable objects (gametes of Drosophila melanogaster) were used to determine the diameter of the volume of genes that corresponded to the dimensions of the smallest to medium-sized virus elements, thus providing a point of contact. According to Bail in 1925, certain peculiarities of the bacteriophage, which had caused difficulties for the supporters of the theory of living beings, could also be explained in the light of the concept of genes: Genes "really take a very independent position in the newer heredity research, they appear almost like organisms in the organism", as he wrote (referring to an essay written by Muller in 1922). "This makes it possible to understand the peculiarities of the bacteriophage, which on the one hand make it appear similar to an organism, but on the other hand make important characteristics of such an organism missing", such as the lack of independent reproduction (1925: 15). "It seems", according to Darányi in 1937, "as if such a unit as gene, virus, phag is generally the smallest unit of life" (1937: 1267). The analogizing recourse to the gene concept was promoted by the fact that heredity research attributed a high degree of autonomy and stability to genes, which was accompanied by a certain plasticity characterizing all organisms. The genes could be induced to vary under artificial conditions (e.g. by irradiation), just as they would spontaneously vary (mutation). And from a physiological point of view, the growth of the genes

[63] At the same time, however, they expected insurmountable difficulties in an attempt "to interpret all the complicated phenomena of viral diseases as being caused by a non-corpuscularly organized agent" (ibid.).

[64] „... a gene is a minute organic particle“, wie Demerec wenige Jahre vorher ausführt (1935: 271), „probably a single large molecule, possessing the power of reproduction, which power is one of the main characteristics of living matter. Changes in gens (mutations) are visualized as changes or re-arrangements within molecular groups of a gene molecule.“

in the cells appeared to be the proliferation of individual units, in which something very similar to viral replication could be seen. And the fact that, in the course of propagation - judged by their phenotypic effect - the genes showed considerable tenacity in the preservation of their properties, combined with a certain degree of variability, as expressed in the spontaneous and experimentally induced mutations, helped virus researchers to understand the connection between constant properties of the virus and the stability or variability of the symptoms (see Melchers 1960: 97).[65] Kausche saw the reason for this in the fact that the viruses, just like the genes in the organism, "are able to initiate a chain of reactions, at the end of which a manifested characteristic, i.e. the symptom, comes into being" (Kausche 1940: 362).

Developments in experimental heredity research also had an impact on cancer research. Initially, the classical ideas of genetics came into play: one of the dominant themes was the idea that pathological cell division can lead to cells that are still viable and capable of proliferation and possess the properties that can be observed in tumour cells, that it is possible that a factor exists within the cell that is substantially involved in tumour formation. At the beginning of this century this factor was called "chromosome" (structures observed during nuclear division). And so cancer was interpreted as being dependent on malformed chromosomes in the cell nucleus (see Boveri 1914; ders.,1929). In detail, this approach (described as the "somatic theory of cell mutation") says something like the following: Chronic irritation causes a certain change in the chromosome content of the cells, which is supposed to explain the abnormal proliferation, the emancipation of the tumour cells from the other cells of the body, the change in cell function, the inheritance of the new properties to all cells newly formed from such cells. When later the genes located on the chromosomes were to be the carriers of the hereditary dispositions instead of conceiving of the whole chromosome as a single entity (see Sutton 1902; note from: Jahn et al.1982: 465 f., 737; Boveri 1909), cancer formation could now be seen as a mutation of genes, based on the general idea that it was an irreversible change in the hereditary characteristics of a cell. A genetic transfer of tumour characteristics was envisaged. [74]

Those researchers who were inclined to the view that the virus was not a living organism but an enzyme-like substance and that one day it would be possible to obtain a chemically pure virus, hoped above all that progress in macromolecular chemistry would lead to an increase in knowledge of the nature of the virus (see Schmidt-Lange 1943: 711). Although it was a debatable consideration that the viral proteins, like those of other proteins, were composed of a number of identical subunits, there was no agreement on the structure, size and mutual relationship of the units. In the first decades of the 20th century, biochemistry was largely based

[65] "The mutations of the viruses manifest themselves in altered disease symptoms" (Melchers 1960: 97).

on the colloid and aggregate theory of living proteins, which stated that proteins and proteases in the protoplasm of living cells were aggregates of small molecules. It was widely held that the colloidal stage of protein compounds was to be regarded as a specificity of living cells to which the chemical laws were not fully applicable. And thus, at that time there was no justifiable reason to consistently attribute the physiological processes of the cell, the intracellular phenomena and the function of the cell nucleus or its material components to chemical laws (see Olby 1974: 19). For the theory of endogenous virus production, a gain in plausibility could be expected if it were actually possible to represent some virus types in the form of macromolecular proteins, i.e. proteins whose large molecules in the solution state can be identified with the virus elements. The assumption that viruses occur spontaneously in host bodies without exogenous infection became more attractive after Stanley succeeded in 1935 in presenting the tobacco mosaic virus in crystalline form. The virus presented itself to him as something that behaved like a chemically pure protein in all its properties, which was contrary to the understanding of the virus as a living being. Isolated protein molecules could be denied the ability to feed, reproduce, inherit and adapt The ability to crystallise was generally denied to organisms. It was pointed out that the structure of a crystal lattice presupposes a large degree of agreement and a great regularity in the structure of the individual particles, but if the living organism theory were correct, the chemical composition of the agent would have to be characterised by a certain variability or the virus particles would have to be characterised by a certain heterogeneity.

The borrowing of terms found outside the field of virus research did not immediately lead to a levelling of the gap between the various groups in this research field. The fronts rather hardened, there was a clash of genetic and biochemical "areas of experience" in the interpretation and research of the

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74 However, as Hildebrand objected in 1939, this concept could not be reconciled with the long latency period in tumour formation after contact with chemicals. How could a mutation, which was an immediate change, be consistent with the slow development of tumors? Hildebrand did not attribute the transformation of a normal cell into a tumour cell to a somatic mutation, but to a permanent modification, a change in the cytoplasmic cell components caused by a stimulus that attacks the cell plasma rather than the cell nucleus. With the assumption that the malignant transformation of a cell is based on a somatic mutation, i.e. a gene change, evidence was not compatible that carcinoma development in the skin when the mouse is brushed with a tar solution takes place in such a way that the uppermost cell layers (epithelia) of the deeper epidermal layers *gradually* assume the character of malignancy over numerous cell generations and that the transformation to a carcinoma cell takes place simultaneously, i.e. multicellularly and multicentrically, in many epithelia. Gene mutations, however, always take place by leaps and bounds. This is precisely what Hildebrand was never able to determine in the malignant transformation of the epidermal cells (Hildebrand 1939: 395).
In this context, the debate held at the time on the question of whether there might not possibly be relationships between the agents of the filterable chicken sarcomas and the genes of the nuclei of certain chicken cells, i.e. whether the Rous agent could be genetically derived from the nuclear genes of the chicken cell, i.e. whether the Rous agent is a malignantly modified (mutated) gene of the chicken cell. On the other hand, the incompatibility of the results of the above-mentioned duck experiment of Gye with Fujinami sarcoma could be argued. According to Graffi and referring to certain experiments, the nucleus and thus also the individual

> nucleus genes are autonomous with regard to species specificity; the genetic material of a nucleus retains its original species specificity in the plasma of an alien species under all circumstances. If the agent of the chicken sarcomas were to be genetically derived from the genetic material of the cell nucleus (genes), one would have to expect that the Fujinami sarcoma would also maintain its chicken specificity in the duck cell. However, according to Gye's experiment, the serologically ascertainable species specificity of the agent has changed from chicken specificity to duck specificity (Graffi, loc. cit., 545).

Virus origin and effect.[66] And yet this initiated a development that rendered the controversial questions irrelevant. With the turn to the "macromolecule", the question of whether the virus is a "contagium fixum" or something soluble appeared in a different light. In the light of advanced colloid-chemical ideas, both versions had something to offer. If it could be said that the virus was in a molecularly disperse state, then the alternative - liquid infectious agent or corpuscular pathogen - could be seen as a consequence of the now overcome state of development of colloid chemistry in the 19th century. Neither the equation of the tobacco mosaic virus with enzymes (Woods 1899) nor the understanding of the virus as a pathogen external to tobacco plants (Ivanovskij 1902) can be judged in retrospect to be absolutely wrong (see Wegmarshaus 1985: 78 f.): In material terms, both enzymes and viruses are proteins, albeit with different molecular weights, and viruses are proteins with an RNA or DNA content, but not a plant-specific enzyme. The virus is actually a corpuscular agent. Based on colloidal chemical considerations, Beijerinck's theory of a liquid infectious agent also had something to offer - the virus was in a molecularly disperse state. In the light of changed conceptual guidelines, neither the organism nor the molecular hypothesis could be fully supported any longer. [76] "The word organism demands," according to Bawden (1964: 12; note from: van Helvoort 1994a: 217), "a wealth of independent metabolic activities there was never any reason to assume viruses possess, and the word molecule implies a precise knowledge of chemical composition impossible to get with particles as large as viruses, and demands an unchangeable structure that conflicts strikingly with the great mutability of viruses.

With the aforementioned equations, initially only symbolically mediated transformation relations between different areas were created, which, however, uncovered a new development potential for empirical processes, for processes that led to operational coherence of previously independent areas of experience. A transfer of methods and procedures took place (see Kay 1993: 5), a transfer with which the previously only suspected equivalence of, for example, virus and macromolecule was to be practically established. [77] The fact that the convergence of research directions of various disciplines, which was initiated in the case study dealt with at the level of text language, was intended to be continued at the practical level of research, becomes clear, for example, in an essay by Kausche from 1940: He wrote that if one is already looking

[66] "The biologist who regards the viruses as living studies them in living hosts where they behave as organisms; the chemist who considers them chemicals studies them in the test tube where he sees only their chemical and physical properties", so Chester 1947 zur Lage in der Virusforschung (1947: 313, Hinweis aus: van Helvoort 1993: 24).

for analogies of a general and special kind between genes and virus proteins, then such considerations must also lead "according to the strict definition of genetics" to the experimental consequence "that one must 1. that one has to try to causally link the specific properties, i.e. the mode of action or the success of action of a viral protein with its defined physicochemical constants; 2. studies of analogies between genes and viruses aim to change the effect of the viral protein by means of overseeable interventions in such a way that it can be physicochemically and chemically proven. For this purpose, the final link in the reaction chain, i.e. the symptom picture, must first be manifested in a modified form and coupled with a change in the physico-chemical properties of the active body. Given the relatively high plasticity of the test objects in relation to the viral infection, such artificially induced modifications must be so firmly induced that they meet the strict requirements of genetics in terms of mutations, i.e. they must largely

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76 And in phage research, neither d'Herelles' position nor that of his opponents could be maintained without restriction. Virus replication was not comparable with the growth of a bacterium in a culture medium or with the direct conversion of an inactive "precursor" into an active enzyme, which Northrop had assumed. When it was possible to demonstrate that the substrates being handled were free of the lytic agent, and when it was possible to produce admixture-free, concentrated phage suspensions after using high-speed centrifuges, improved methods of turbidity measurement, isolation of viruses as the offspring of a single virus particle, and other means, after the phage had become a molecular genetic object (at the beginning of the 1940s) and was studied independently of therapeutic objectives - to an object that could not have been treated as a molecular genetic object either by lysis experiments or by simple genetic experiments (Doermann 1972: 95) -, a starting point was gained independently of the positions held in that controversy, which m.E. in the following quotation from Delbrück: "In d'Herelle's view the bacteriophages are small cells, in Bordet's view they are modified bacterial proteins. The issue is one which can only be settled by a clearer understanding of what actually goes on when the bacteriophage is reproduced. The experiments which have been devised in the attempt to settle this argument have not yet led to a clearer understanding of the mechanism of phage reproduction" (1942: 2). Ellis, who had worked with Delbrück for a time, seemed to come remarkably close to d'Herelle's description of the phage reproduction process "the picture we have today" (1972: 62). But it was not d'Herelles' intention to study the reproduction process itself, which was necessary to clarify the molecular basis of reproduction. Thus, a different picture had emerged, obtained by investigating the phage reproduction process separately from the reproduction of the phage hosts and from questions of antibacterial therapy (see Delbrück 1946: 174 f.). The organismic approach to bacteriophages developed by d'Herelle (phages equal parasitic microorganisms) was radically changed by one which treated "a phage particle as a package of genetic information", "which is encoded in the length of a nucleic acid molecule housed in a complicated infection apparatus", wrote Doermann in the 1950s (1972: 88). "The phages could no longer simply be regarded as extremely small intracellular parasites, as d'Herelle, who preferred analogy considerations," did. The "weakness of the analogy was that it could not explain the lack of metabolism in the particles...", said Hershey (1972: 108).

77 Following Stichweh, the interaction of heterogeneous knowledge systems - he refers to the developmental relationship between physics and electrical engineering - can be characterized as an interpenetration process, for which instrumental or experimental technology functions as an "interpenetration zone" (Stichweh 1988: 702). The different knowledge cultures connect to events in this zone in different ways and transport them into divergent horizons of meaning. Finally - as a result of the development of interdisciplinary traffic - the difference disappears in the new objects.
Die Molekularbiologie „would borrow methods not only from physics, mathematics, and chemistry but also from other fields of life science - genetics, embryology, physiology, immunology, mi-crobiology. The new biology aimed to transcend disciplinary boundaries and employ whatever tolls the problem at hand demanded. Although the transfer of techniques between fields was certainly not new, the design of a large-scale program based on interdisciplinary research encom-passing several disciplines was unprecedented“ (Kay, 1993: 5; siehe auch 136 ff.).

remain constant" (Kausche 1940: 362 f.). The fact that borrowing leads to consequences in methodological and other respects of the borrowing research field can also be shown with regard to the consequences that were conjured up when it was agreed to equate viruses with macromolecular nucleoproteins: For example, in the efforts to make the hypothesis of endogenous viral origin plausible, we could no longer be content with assuming that a nucleoprotein structure of the host cell would be converted directly, i.e. without chemical transformation, into a viral element. The idea was biologically unacceptable "that a particle belonging to the host cell is transformed by the influence of this very cell, directly or without changing its dimensions, its colour reactions and its chemical constitution, into a reproducible, transferable and specific agent with all the qualities of a pathogenic germ... As things now stand, the hypothesis of endogenous virus formation cannot be substantiated morphologically, but only by arguments of a different kind" (Doerr 1944a: 25).

By borrowing concepts from other disciplines, the associated research problems also gained an impact in their own field, and there was pressure to orient their own investigations to the procedures and questions of the foreign discipline. For a convincing presentation of concepts of foreign communities as something that belongs to the preconditions, the guidelines of one's own fact production, the research results must be presented as something that can also be evaluated and reconstructed in the reference system of the respective community. And this means in consequence that one's own experimental and observational findings must be translatable into those of the community whose concepts were used. Only in this way can it be made plausible that such a reference was the necessary prerequisite for the achievement of the research goals and belonged to the conditions of observation of the objects of research treated. One may assume - which must, however, be verified by further analysis of the case study in the history of science - that the controversies in virus research became irrelevant to the extent that conclusions were drawn from an empirical-practical point of view from the similarity relationships between the virus on the one hand and the gene, the macromolecule, etc. on the other hand, which were initially only suspected and considered in the debates.

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