The post-Loeffler-Frosch era: contribution of German virologists

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Summary. This presentation dealt with the contributions of German virologists in the rapid development of virology following the Loeffler-Frosch era. Thereby, only research was included which was undertaken within German institutions, even though guest scientists from other countries or international cooperative efforts have in some cases contributed to the work. Contributions to the field of veterinary virology were not considered here, since this topic was treated separately during this centennial symposium.

The overview includes contributions of the very early period when interest was focussed mainly on the determination of the physicochemical properties of the fast growing number of newly detected viruses, and of the pioneering period when fundamental discoveries of the nature of viruses were made. The concepts that derived from those studies made the development of modern virology possible. Some highlights of the present period were presented describing the findings of selected virus families. This part was followed by a description of the results which were relevant to problems of how viruses become pathogens, and the role of the immune response to virus infections. Finally, attention was drawn to the contributions of molecular studies which became important not only for the field of virology but also for life sciences in general.

Introduction

A century of virology is cause enough to remember with respect those tasks successfully completed in this field, to recognise ongoing work and to express wishes for success in the future. These aims reflect the spirit that inspired the organisers of this anniversary celebration to deal separately with the contributions of German virologists to the breathtakingly rapid development of our scientific discipline world-wide. This kind of inspiration, however, tends to be difficult to interpret correctly. In light of the international co-operation in science and the corresponding cosmopolitan behaviour of many scientists, it may even be questionable as to whether it is at all feasible to speak truly of national contributions. In the following, I will focus on research that was undertaken in German institutes, even though guest scientists from other countries and all other kinds of international co-

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operation may have contributed to the work in many cases. However, it will only be possible to recollect a selection of these achievements. These will be limited to work that either resulted in the discovery of previously unknown phenomena, or develop into new ideas, or created new methods that opened the pathways and defined the final objectives which led to the current knowledge and present understanding of our discipline in modern science. Repetitions or mere improvements upon imports of knowledge from other countries will not be considered.

In taking the freedom to express my own personal view on the contributions of German virologists on an international scale and without pretending a historical-biographical professionalism, I fully realise that my selection and evaluation will appear to be subjective. In doing so, I may offend certain individuals and would therefore like to apologise in advance. In any case, I will not sing a hymn of praise but will try to follow the admonishment of Baco von Verulam as quoted by Immanuel Kant in his 'Kritik der reinen Vernunft' (2ndedition): *De nobis ipsis silemus*. This will be somewhat less difficult for me since the topic of veterinary virology will be treated separately by Marian C. Horzinek.

The early post-Loeffler-Frosch period

The history of virology is a particularly good example of how scientific achievements are directly related to the prevailing way of thinking, and to the state of technical development, and to the methods available at that time. It also shows how the past 100 years of virology have forged new concepts and provided new insights into the history of life.

The first era of virology was dominated by bacteriologists or even hygienists who took advantage of the experimental procedures already used so successfully by the discoverers of foot and mouth disease virus. Even at the beginning of the 1950's I was told that a "good bacteriologist is also a good virologist". This attitude therefore clearly shows why virology did not become a separate discipline at German universities until the 1960's.

During that early period the viral aetiology of a large number of infectious diseases was recognised and even viruses which cause tumours were identified. It is remarkable however, that German scientists have taken comparatively little interest in the primary discovery of human pathogenic viruses. Herpes simplex virus (HSV) was among the few exceptions. The unequivocally infectious nature of HSV was recognised 1919 by A. Löwenstein. He demonstrated that virus retrieved from vesicles of herpes labialis produced lesions on the cornea of the rabbit [1]. Forty years later K.E. Schneweis found that 2 serotypes of HSV, HSV-1 and HSV-2, can be differentiated, which are associated with differences in the clinical manifestation of infection [2]. Whereas HSV-1 predominates in infections "above the belt", HSV-2 is associated with genital disease. This discovery breathed new life in herpes virus research. Marburg disease virus, discovered in 1967 by R. Siegert and W. Slenczka [3], was substantially characterised in Marburg and became the first representative member of a new virus family, the *Filoviridae*. In 1979 H. zur Hausen and L. Gissmann [4] discovered in a B-lymphoblastoid cell

line derived from an African green monkey a widely distributed B-lymphotropic polyomavirus. In this context Borna disease virus might also be mentioned. It was originally identified by W. Zwick (1926) as a pathogen of horses [5]. However, during the last few years it has attracted much wider attention since growing evidence indicates that Borna virus causes behavioural alterations or psychiatric disorders in humans and animals [6]. Finally, the laboratories of H. zur Hausen and H. Pfister (for [7]) contributed significantly to the world-wide efforts to identify new papillomaviruses. Presently no fewer than 80 different types have been identified, and in addition, more than 50 partial sequences are known, pointing to still more types of such viruses.

During this first period of virology interest focused primarily on the determination of size and shape of the bewildering variety of viruses detected, their sensitivity to chemical and physical agents, their host range and their differences in the manifestation of diseases caused by infection. Application of physicochemical and chemical techniques used in biochemistry and their continuous improvements helped to define the nature of viruses. By ultrafiltration, ultracentrifugation and electron microscopy, developed and applied by H. Bechhold and M. Schlesinger in Frankfurt, and Helmut Ruska in Berlin, in particular, the size and morphology of many viruses was determined. Mrowka, a veterinarian at the former leasehold German naval base at Tsingtao, China, was one of the first to use chemical procedures for the isolation of viruses, as early as 1912. He succeeded in precipitating fowl plague virus from infectious blood serum by means of tannin, without destroying infectivity. He concluded that the virus behaved in all respects like a colloid globulin and should be regarded as such [8]. Twenty years later differential centrifugation and ultrafiltration allowed M. Schlesinger

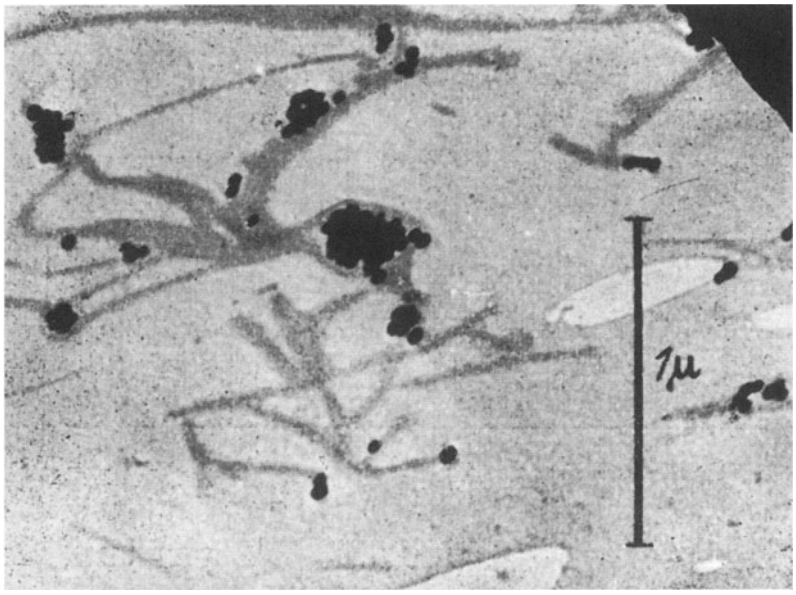

Fig. 1. First electron micrograph of tobacco mosaic virus [10]

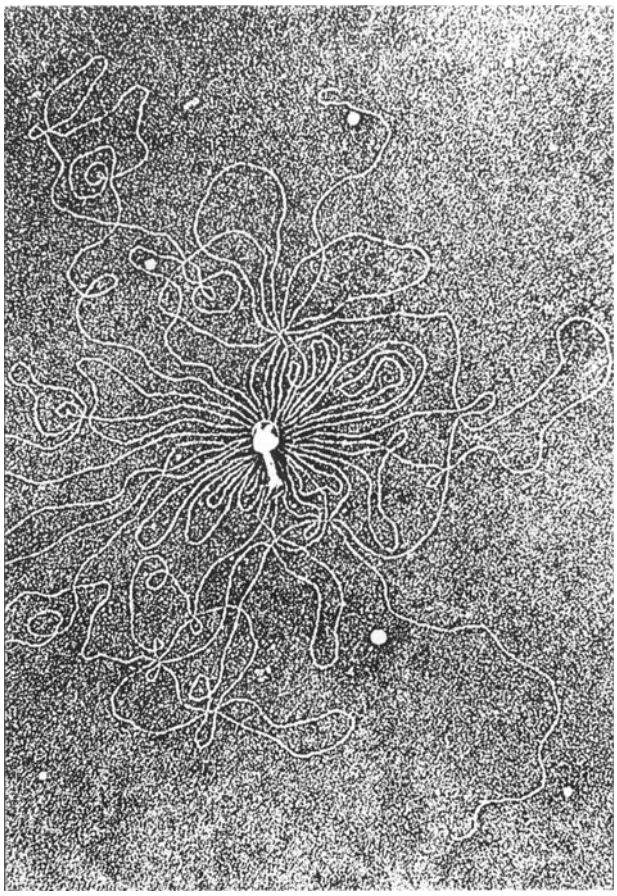

Fig. 2. The DNA of bacteriophage T2, liberated from the head of the phage by osmotic shock [12]

(1933) to purify bacteriophage particles in sufficient amounts for various further analyses. He not only obtained important information about the dimensions of such viruses but showed simultaneously that pure phage material consists only of protein and DNA in roughly equal amounts [9]. This led for the first time to the suggestion that viruses in general may be composed of nucleoprotein. In 1939, H. Ruska and co-workers presented the first electron micrograph of any virus, the tobacco mosaic virus (Fig. 1), using a microscope built by his brother Ernst [10]. Two years later he was the first to show how bacteriophages are adsorbed to the surface of their bacterial host [11]. In this context I would like to recall the aesthetic electron micrographs presented in 1962 by A. K. Kleinschmidt and his colleagues [12] which show the DNA molecule of bacteriophage T2 being liberated from the head of the phage particle by osmotic shock, published in several text books (Fig. 2). The Kleinschmidt spreading technique allowed the correct determination of lengths as well as the determination of higher order structures of nucleic acids. Brownian movement brings phage particles into random collision with their host cell which is, as originally described by M. Schlesinger (1932),

the first step leading to phage adsorption [13]. In 1954, W. Weidel presented the first evidence for the nature of a corresponding bacterial receptor [14]. G. Koch (1958) characterised as a lysozyme the enzyme responsible for phage release from a bacterial cell by lysis-from-within [15].

From all studies it became clear that viruses are autoreproductive particles ranging in size from the smallest bacteria to the largest known biologically active macromolecules. The most intriguing question remaining at that time was: Do viruses represent the transition from inanimate nature to the typical life? This question, intensively discussed by vitalists as well as by mechanists, became even more accentuated when in 1935 Wendell M. Stanley (Princeton) published the "Isolation of a crystalline protein possessing the properties of tobacco mosaic virus" [16]. However, if we adhere to the principle of the "whig interpretation of history", which evaluates the past on the standard of its significance for the present, today all these questions appear to be of minor interest.

The pioneering period

Immediately realising the utmost significance of Stanley's discovery, Adolf Butenandt, who at that time worked successfully on oestrogen, made a far-reaching decision. Together with F. von Wettstein and A. Kühn in 1938 he established a working group for virus research at the Kaiser-Wilhelm Institut für Biochemie in Berlin-Dahlem. G. Schramm was nominated to head its biochemical section and G. Melchers to be responsible for the genetic part. After the war that working group, which was later joined by H. Friedrich-Freksa, G. Bergold and W. Schäfer, continued with their investigations in Tübingen. The Max-Planck-Institut für Virusforschung, which emerged from this initiative in the 1950's became a focal point for virus research and was prominently involved in the development of molecular biology in Germany. More than that: Tübingen institutes became also the elite school for virology in Germany, which influenced the development of our discipline enormously. Thus, for example, more than 20 of Schäfer's descendants received prominent positions in national and international institutions.

On a par with the establishment of molecular virology in Tübingen, Richard Haas in Freiburg (Fig. 3) put considerable emphasis on medical virology, thereby promoting virology as a new field of research and application in medicine. He really was the forerunner of modern medical virology in Germany. His spirit was carried on by R. Thomssen, who has contributed enormously in tying together medical and molecular virology. He was often ahead of the time, e.g. when he developed the radioimmune assay before the Nobel prize was awarded for this technique [17]. It should also be mentioned that H. J. Eggers, K.-E. Schneweis, and R. Kandolf in particular also played a large part in the bringing together of basic and applied virology. They became particularly known for their work on antiviral agents (for [18]) and on herpes simplex virus pathogenesis (for [19]) or on picornavirus-induced myocarditis (for [20]), respectively.

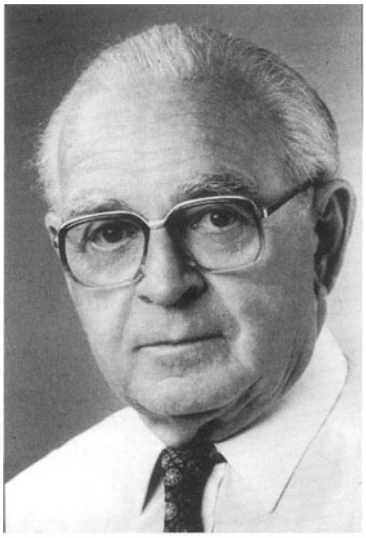

Fig. 3. Richard Haas (1910–1988)

Fig. 4. Gernot Bergold (∗ 1911)

Gernot Bergold (Fig. 4), who left Tübingen in 1948 for a leading position in Canada, can be very rightly regarded as the founder of biochemical insect virology. After a long period of errors in the research on inclusion body diseases of insects, in the 1940's he was able to elucidate the viral aetiology of the polyhedrosis disease of *Bombyx mori* and of another caterpillar disease, the granulosis disease. In both cases, he biochemically characterised the rod-like, DNA-containing viruses and discovered that they were embedded in protective, non-infectious protein structures, the so-called polyhedra [21, 22]. He also showed that infectious virus was released from polyhedra by treatment with diluted alkaline solution (Fig. 5).

Plant viruses, in particular tobacco mosaic virus (TMV), proved to be suitable as a model to elucidate the structural properties of viruses, since they could be obtained quite easily. It was found that up to 90% of the protein present in infected plant juice might consist of TMV and reliable methods to quantify virus particles then became available. Gerhard Schramm (Fig. 6), in Berlin, had already detected that treatment with slightly alkaline solution caused TMV to dissociate into subunits with defined size and shape. The isolated subunits could be reaggregated to TMV-like rods, while infectivity was lost [23]. The amino acid sequence of TMV protein later was resolved by A. Anderer (1960), as the first primary structure of any viral protein [24]. Subsequent determination of the protein sequences from different TMV strains and mutants helped H. G. Wittmann (1962) to contribute to the codon assignment for the genetic code, which of course added evidence to the universality of the genetic code [25]. Of exceptional importance was the finding by A. Gierer and G. Schramm [26] in 1956 that the genetic information of TMV resides in its RNA. From this discovery a most important conclusion was drawn that RNA could also be genetic material, a property previously thought to

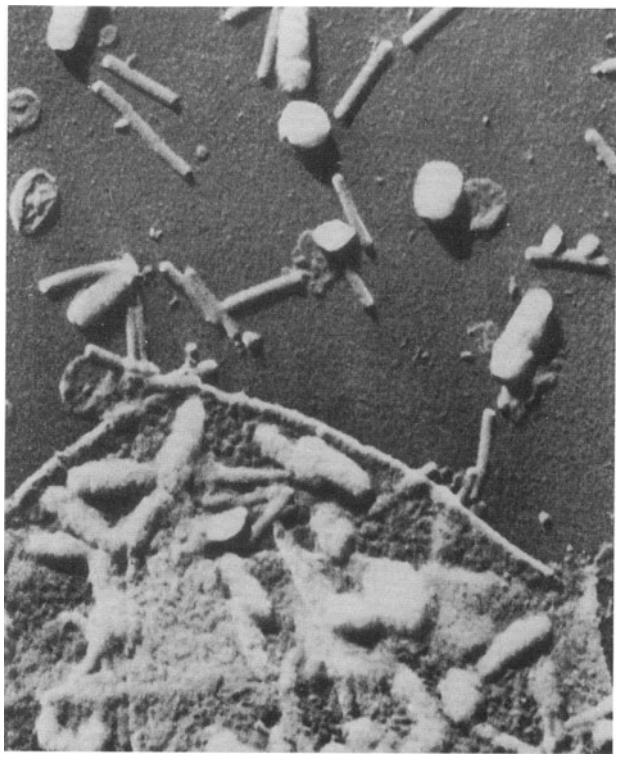

Fig. 5. Polyhedra obtained from *Lymantriz dispar*, dissolved with alkaline solution (Photograph by G. Bergold)

be restricted to just DNA. The possibility of isolating biologically intact RNA by the phenol method has contributed enormously to many facets of molecular biology. A modification described by E. Wecker [27], the "hot phenol method", also allowed the extraction of infectious RNA from enveloped positive stranded viruses. The analytical studies on TMV-RNA by H. Schuster provided the basis for an elucidation of the mechanisms of mutagenicity caused by nitrous acid and hydroxylamine treatments [28, 29]. Based on these results in 1958 A. Gierer and K. W. Mundry succeeded for the first time in generating specific virus mutants [30]. Treatment with chemical mutagens enhanced the mutation frequency, which became a useful tool for genetic studies in general. In 1963 Anderer was the first to demonstrate that an isolated hexapeptide of the TMV-protein forms the minimal structure for an epitope capable of inducing virus-specific antibodies [31].

Werner Schäfer (Fig. 7), the successor to Bergold in the field of animal virology in Tübingen, became acknowledged world-wide for his studies on fowl plague virus (FPV), Newcastle disease virus and encephalomyocarditis virus, as well as RNA tumour viruses. FPV proved to be an excellent paradigm to study structural and functional relationships of enveloped viruses and served as a feasible agent for tracing virus replication, particularly of orthomyxoviruses. Without any doubt FPV was for a long time one of the best known animal viruses, with respect to its physical, chemical, architectural and biological properties [32]. If

Fig. 6. Gerhard Schramm (1910–1969)

Fig. 7. Werner Schäfer (∗ 1912)

one is tempted to give testimony to a fair spirit of competition in the work of G. Schramm in Tübingen and H. Fraenkel-Conrat in Berkeley, one may also similarly recognise a competitive parallelism in the way Schäfer and Leslie Hoyle (Northampton) dealt with FPV and human influenza viruses, respectively. This became particularly evident when Schäfer found in 1955 that FPV is in fact an influenza virus [33], and that it might, perhaps through a process of recombination, exchange host specificity with other influenza A viruses which might contribute to the frequent occurrence of previously unencountered strains. We know today that this assumption was close to reality. The model of influenza virus structure developed by Schäfer showed a filamentous ribonucleoprotein surrounded by a lipid-containing envelope, into which a glycoprotein, the haemagglutinin (HA) is incorporated. The HA serves as a ligand during adsorption of the virus to a cellular receptor, the determinant of which was identified by E. Klenk (1955) as neuraminic acid [34]. In addition, the HA turned out to be the immunogen which induces the production of protective neutralising antibodies in the infected host [35]. Schäfer's proposal to use only the immunogenic glycoprotein for the vaccine production has meanwhile been realised via subunit vaccines also used for immunisation against other virus infections. Worth mentioning are the results obtained by the Tübingen group on the participation of the cell nucleus in the replication of influenza viruses [36], first indication that the virus envelope is a virus-specific altered host cell membrane [37], the first indication of the segmented nature of influenza virus RNA [38] and – already largely forgotten – the first evidence that the production of viral proteins is possible in subcellular fractions, i.e. in an in vitro system without employing intact cells [39].

Schäfer's scientific descendants in Giessen later extended the knowledge about structure and biology of orthomyxo- and parainfluenza viruses, when the

arsenal of methods had been expanded and refined. Recognition of the exceptional segmented structure of influenza viral RNA allowed new insights into viral genetics, into the emergence of new influenza viruses, and into molecular epidemiology (for [40]). Certainly, the results obtained by the Giessen team (mainly H. Becht, W. Garten, H.-D. Klenk, M. Orlich, R. Rott, M. F. G. Schmidt, C. Scholtissek and R. T. Schwarz) on structure, production and biological properties of influenza and parainfluenza viral glycoproteins have set a precedent for subsequent investigations with other viruses (for [41]). This includes post-translational modification of the glycoproteins by the different steps of glycosylation, by employment of new glycosylation inhibitors (for [42]), by palmitoylation and myristoylation (for [43]), and by proteolytic cleavage [44]. In this way the dominant role of these glycoproteins in the initial process of viral replication and their significance as determinants for pathogenicity have been resolved. Though the presence of receptor destroying enzyme of influenza C virus was demonstrated already in 1950, it was characterised only in 1985, by G. Herrler, as a neuraminate-9-O-acetyl esterase [45].

In 1953 Arnold Graffi isolated in Berlin-Buch the causative virus of murine myeloid leukemia of mice [46], named after him the "Graffi virus", which he identified later as a type D retrovirus. It was again W. Schäfer, who, together with Heinz Bauer, introduced basic retrovirus research in Germany. Following the previous experience with the myxoviruses that elucidation of the correlation between structure and function will yield the deepest insight into the nature of viruses, their groups made important contributions to retrovirus research. Characterisation of the different structural compounds of murine and chicken oncornaviruses was without doubt among the highlights of the diverse studies performed in Tübingen and later by several other groups in Germany. The fundamental insights achieved led to the world-wide understanding of the structure of these viruses, and the production of globally employed monospecific antibodies, some of which have been suggested for use in tumour therapy [47]. Completion of our knowledge on the action of the enzyme reverse transcriptase came from Karin Mölling (1971), who discovered RNase H activity and the mechanism of its function as a processively acting exonuclease [48].

The present period

Since the beginning of the 1960s the establishment of virology as a separate discipline at German universities, the possibility of study periods abroad, the continuous development and application of new techniques, but also the frequent use of viruses to study general biochemical and molecular biological aspects have all contributed late, but not too late, to the boost in virology in Germany.

It is interesting to note that in the early 1960's several virologists held the view that the golden age of virology was already over. With all major foundations of molecular biology elucidated no more spectacular results were expected; apparently the "eighth day of Creation" came to an end. Of course, this assumption turned out to be inaccurate. Even though no Nobel-prize awarded discoveries were

made in virology in Germany, a number of impressive results have significantly contributed to the mosaic of our current knowledge of the nature of viruses and of their properties as causative agents of infectious diseases. Since a large number of these *tesserae* should be common knowledge, I shall only expand upon a few areas in which German virologists have substantially contributed.

Viroids

Certainly, one of the most remarkable discoveries in plant virology in the post-Schramm era was the simultaneous and independent finding by Theodor O. Diener in the USA and Heinz L. Sänger in Giessen of "naked" small RNA molecules as a new kind of autonomously replicating subviral plant pathogens known today as viroids. Previously Sänger had successfully studied the structural and genetic interactions of the two particles of bipartite tobacco rattle virus whose unique helper mechanism he could elegantly explain [49]. Based on this experience he characterised the causative agent of exocortis disease of citrus as a viroid [50]. He then succeeded in isolating and purifying several other viroids, resulting in a detailed biochemical, physicochemical and morphological characterisation. Thus, in collaboration with G. Klotz, D. Riesner, H. J. Gross and A. K. Kleinschmidt [51] he was able to demonstrate in 1976 that "viroids are single-stranded covalently closed circular RNA molecules existing as highly base-paired rod-like structures" with a molecular weight of 120,000 corresponding to ca. 360 nucleotides. In 1978 both the nucleotide sequence and secondary structure of the first viroid RNA was published [52]. His subsequent studies later undertaken in Martinsried on the relation between viroids structure and function and on viroid replication rendered viroids the best characterised class of small RNA molecules next to tRNAs.

Hepadnavirus

It is generally agreed that German virologists around H. Schaller, P.-H. Hofschneider, W. Gerlich, and H. Will, contributed enormously to our current knowledge on hepadnaviruses, particularly hepatitis B virus (HBV). Schaller and Hofschneider were involved in cloning and sequencing the whole HBV-genome [53, 54], through which it became possible not only to understand this virus' structure but eventually also to produce the first anti-cancer vaccine. It was H. Will in Schaller's laboratory who obtained the first cloned infectious DNA [55]. Characterisation of viral transcripts by Schaller's group and study of the viral DNA polymerase revealed the full replication strategy of the hepadnaviral genome [56]. W. Gerlich deserves credit for elucidating the structure-function relationship of HB-S and HBe proteins [57, 58]. Hofschneider's group showed that the HBx protein acts as a transactivator, stimulating a striking variety of promoters, which do not share any known cis-regulator element [59]. This group also showed that HBx is frequently present in liver carcinomas. Gerlich's group demonstrated that HBx is in fact able to transform immortalised hepatocytes [60]. Most surprising was the observation by Hofschneider that the pre-S-domain of the HBV

genome also possesses a transactivating effect [61]. Finally, some indications on the pathogenesis of HBV-infection was obtained with virus variants isolated by H. Will.

Papillomaviruses

Since the beginning of this century, viruses had been known to be the causative agent of human skin warts, genital warts and laryngeal papillomas. For decades, wart viruses had been barely characterised due to the lack of in vitro systems for viral propagation and it was generally believed that there would be only a single type of human papilloma virus. Stimulated by the tendency of certain types of human warts to malignant conversion, H. zur Hausen, L. Gissmann, and H. Pfister started a systematic analysis of virus isolates from individual warts in the mid-1970's and soon established the heterogeneity of papilloma viruses by characterising HPV1 and HPV4. With the advent of recombinant DNA technology, these investigations led to cloning and characterisation of papillomaviruses from different sources. For instance HPV6 and HPV11 from *condylomata acuminata* and laryngeal papillomas, HPV8, 19, 20, and 25 from patients with *epidemodysplasia verruciformis*, viruses known to correlate with increased risk of developing skin cancer, HPV13 from Heck's disease of the oral mucosa, and HPV16 and 18 from cervical cancers. HPV 16 or 18 can be detected in up to 70% of carcinomas of the cervix uteri, and both are now recognised by the WHO as the major cause of cervical cancer [62–65].

Seroepidemiological studies in Pfister's laboratory during the 1980's indicated that HPVs, originally assumed to be restricted to patients with *epidermodysplasia verruciformis* (EV), are widespread in the general population. This was most recently confirmed by the demonstration – in plucked hairs – of EV-virus-specific and related HPV DNA sequences in a considerable proportion of asymptomatic controls. Such sequences were similarly found in more than 50% of cutaneous squamous cell carcinomas in the general population [65].

In the past decade zur Hausen's group discovered a number of intracellular and intercellular signalling pathways that regulate cell differentiation but that also influence HPV oncogene activity [66]. Similarly, Pfister and colleagues identified the cellular transcription factor YY1 as a repressor of HPV16 oncogene transcription, and showed frequent deletion of YY1 binding sites from extrachromosomal HPV16 DNA within cervical cancers [67]. This likely leads to increased activity of the oncogene promoter and suggests another important step in tumour progression.

Viral oncogenes

Germans were involved in other innovative studies on viral oncogenes. T. Graf and H. Beug [68] are particularly known as the discoverer of the retroviral erb B oncogene. K. Mölling [69, 70] found the first retroviral oncogene products, Myc and Myb, located in the cell nucleus, and their DNA-binding ability in vitro. She also discovered the first serine/threonine protein kinase encoded by the oncogene

mil/raf [71]. B. Fleckenstein in co-operation with W. Haseltine, Boston, identified the tax-gene product of human T-cell leukaemia virus type 1 as the T-cell transforming protein [72]. In his highly acknowledged studies on herpesvirus saimiri, Fleckenstein described new transforming genes. Thus, in a subgroup A strain an oncogene, stpA, which is responsible for peripheral T-cell lymphomas in transgenic mice, was detected, mapped and characterised [73]. At the homologous position in the genome of a subgroup C-strain the information is localised for two oncogenes stpC and tip. The first strongly transforms rodent fibroblasts while the product of tip interacts specifically with T-cell specific tyrosine kinase Lck, which might explain the T-cell tropism of transformation by herpesvirus saimiri [74].

Finally I emphasise Wolfgang Deppert's analysis of the interaction of the SV40 T antigen with the cellular regulator protein p53 [75]. The p53 protein is the most famous protein in tumour biology, as it is a tumour suppressor whose gene is genetically altered in about 50 to 60% of all human cancers. Deppert's finding that p53 exhibits 3′- to 5′-exonuclease activity substantially extended our view concerning its role as a "guardian of the genome" such as control of homologous recombination and the possibility that p53 might act as an external proof-reader for polymerase alpha in SV40 DNA replication [76].

Pathogenesis of virus infections

During the last 30 years, we have obtained more and more results that are relevant to the questions as to how viruses become pathogens. First demonstrated with influenza viruses and then confirmed for an increasing number of other viruses, pathogenicity is of polygenic nature. However, in addition to the necessity of an optimal gene constellation [77] the Giessen virologists demonstrated the importance of the structure of the cleavage site of the HA glycoprotein of influenza viruses and the F protein of parainfluenza viruses in determining pathogenicity of these viruses, and also pointed to the potential of the proteases secreted from co-infecting bacteria for enhancing viral invasiveness (for [78]). There is now evidence for an analogous effect with *Filoviridae* as shown by Heinz Feldmann in Marburg [79].

The Würzburg group, in cooperation with Martin Billeter from Zürich, has shown that in infected brain cells from patients with subacute sclerosing panencephalitis (SSPE) induced by measles virus the viral envelope glycoproteins are markedly underexpressed or even absent. This is apparently caused by the presence of a mutated stop codon in the corresponding genes. In addition, in measles virus cloned from infected brain tissue, a biased hypermutation has been demonstrated in the M gene, which leads to an exchange of up to 50% of a particular C residue to U, possibly caused by the action of a cellular duplex RNA-dependent adenosine deaminase activity found in human neural cell extracts [80, 81]. Thus, measles virus formation in brain cells seems to be associated with an abrogation of M protein function, as has also been suggested for abortive infection of influenza virus in brain cells [82].

In some virus-induced diseases of the central system (CNS), the lesions very much resemble the neuropathological changes observed in experimental allergic encephalitis. Based on these observations, the virologists around V. ter Meulen and H. Wege in Würzburg have established two interesting animal models in which a coronavirus or a measles virus infection leads to an autoimmune inflammatory disease process in the CNS. Both virus infections induce the activation of CD4+ T-cells against brain specific antigens, which become perpetuated after virus replication has ceased. Similarly, as shown by the Giessen group, vesicular stomatitis virus, when grown in brain cells, causes demyelination, too. In this case, myelin basic protein was found incorporated into the envelope during virus maturation [83–85]. These results suggest that unmasking of CNS membrane components and/or incorporation of host-specific antigens into the viral envelope and subsequent priming of self-reactive immune response might be a common pathogenic mechanism underlying the post-infectious encephalitis syndrome as already hypothesised in 1969 [86].

Otto Haller, when coming from Zürich to Freiburg, continued his studies on the Mx family of interferon-induced antiviral proteins, particularly the human MxA protein. Investigations on MxA transgenic mice have shown that MxA has a powerful antiviral effect also in vivo. In the Thogoto virus model he demonstrated for the first time a mechanism by which MxA exerts its protective activity: MxA binds to the incoming viral RNP in the cytoplasm of infected cells, thus preventing its import into the nucleus and consequently viral genome amplification and transcription [86a].

Virus interaction with the immune system

In the mid 1970's U. Koszinowski and R. Thomssen reported on lysis mediated by T-cells and restricted by H-2 antigen of target cells infected with vaccinia virus [87]. This was the first virological confirmation of the fundamental work on MHC restriction of virus-specific T-cells published only shortly before by Zinkernagel and Doherty. Furthermore in determining the requirements for generation of virus-specific cytotoxic T-cells, Koszinowski [88] and others [89] found for example, that fusion of Sendai or fowl plague virus with target cell membranes is required for T-cell recognition.

It is due to Fritz Lehmann-Grube that attention was drawn to the role of T-cell mediated cytotoxicity in the elimination of viruses in the infected organism. He contributed a great deal to our understanding of the mechanism of the immunopathogenesis of lymphocytic choriomeingitis of mice. This disease, which was originally studied by Erich Traub, became the paradigm for virus diseases, in which the infecting virus by itself does not affect vital functions but the outcome of the disease is caused by T-cell-dependent immunopathological reaction [90, 91]. A similar mechanism of immunopathogenesis was found underlying Borna disease [92] by a research group in Giessen and hepatitis A by A. Vallbracht and B. Fleischer [93].

Viruses are true survival artists and have invented different tricks to escape the immunological defence. Suppression of the host immune system was first documented 90 years ago by the German paediatrician Clement von Pirquet who observed that the tuberculin skin test of immune individuals was depressed during the course of acute measles virus (MV) infection [94]. A breakthrough in the understanding of this important phenomenon, regarded as a major cause of the high mortality of MV infection, came from V. ter Meulen's group [95]. They convincingly demonstrated that – in direct contrast to commonly held opinion – responding lymphocytes do not themselves need to be infected in order to be suppressed but rather that the contact with both viral glycoproteins triggers immune suppression.

Cytomegaloviruses (CMV) manipulate the immune system on several levels. U. Koszinowski and his co-workers have delivered important contributions on the disturbance of formation and transport of MHC molecules in CMV-infected cells, which prevent or reduce their expression on the cell surface. Recently, they found three new CMV proteins that interact with this process (for review see [96]).

Contributions to molecular biological studies

Obviously World War II and restrictions imposed by the Allies on particular fields of research, such as genetics, prevented German scientists in the post-war period to participate in development of molecular genetics as initiated by the "phage group" around Luria, Delbrück, Hershey and others. Unfortunately, German scientists also missed the boat in the beginning of recombinant DNA revolution where viruses again played a central role. Nevertheless, a few impressive contributions in the further development of that area which became important not only for molecular virological studies, but also for life sciences in general are mentioned here. Thus, I would like you to remember that R. Jaenisch was definitely the first to produce transgenic animals. In 1975, while still in Hamburg, he was able to show that after infection of early mouse embryos with Moloney leukaemia virus the viral genome became incorporated into cells to the germ line and that the integrated genetic information was inherited in accordance with Mendel's rules [97]. He found eight years later that the integration can lead to recessive lethal mutation of the cellular gene which carries the provirus. With this discovery Jaenisch was also the first who described the phenomenon of insertion mutagenesis in mammals (for [98]). In 1960 Hofschneider isolated infectious DNA from phages for the first time [99] and described in 1974 the isolation and properties of the replicative form of phage M12 RNA which is relevant for the replication of many RNA viruses [100]. With phage ϕX174 first evidence was obtained for genetic recombination of single stranded DNA. Once suitable selective genetic markers had been developed, D. Pfeifer (1961) found that recombination could be detected at a level of 10^{-4} and 10^{-5} recombinants per progeny virus [101]. It was much later in Giessen that non-homologous RNA recombination was detected with influenza viruses [102]. In the field of virus evolution the group of Manfred Eigen, who developed the "quasispecies" concept, has made important contri-

butions concerning the experimental coupling of mutation and selection [103]. As early as the 1970's M. Sumper, a member of Eigen's group, had shown that genomic RNA could be recognised and reproduced by Qß-replicase, the RNA polymerase of phage Qß, and that during replication under certain environmental conditions defined phenotypical properties may be selected [104]. H. Schaller studied the prokaryotic promoter structure in 1977 and defined the minus ten region, the "Schaller-box", as the polymerase binding site [105]. This was exemplified on phage fd, the first known filamentous, circular single-stranded DNA Ff phage, isolated by Hoffmann-Berling (1961). In 1977 J. Messing introduced part of the lac regulatory region into the genome of M13 [106], another Ff phage, which converted this phage to a most suitable cloning vehicle and made Sanger-sequencing with this single-stranded DNA vector genome quite easy.

M13 vectors soon became the most important vehicles for shotgun cloning and sequencing, and continue to be so today. It should also be mentioned that the first information about the significance of the baculovirus vector came from Giessen [107]. The strong promoter of cytomegalovirus is also widely used as the driving force in eukaryotic expression constructs in several aspects of gene technology. It is, as found in 1985 by B. Fleckenstein's team constitutively active and is not controlled by transactivating or other viral factors but can be regulated by cellular transcription factors [108]. In 1978 G. Hobom was the first to describe structure and function of the bacteriophage lambda origin of replication [109]. H. Lehrrach and A. Frischauf developed from the phage lambda the so-called EMBL phages [110], which proved to be a most suitable basis for the construction of gene banks.

Reverse genetics was extended to negative stranded RNA viruses by K.-K. Conzelmann (1994). He succeeded in molecular cloning an infectious cDNA of rabies virus, which proved useful as a new vector system [111]. Hobom (1994) was able to construct a cDNA system for in vivo expression of the segmented influenza viral RNA by RNA polymerase I [112]. Cloning the whole, up to 230 Kbp containing infectious genomes of herpes viruses by U. Koszinowski and W. Hammerschmidt, promises important results for these viruses in the future [113, 114].

In a follow-up study of his discovery that adenovirus 12 (Ad12) DNA persists in transformed hamster cells in an integrated state, Walter Doerfler (1978) found that integrated Ad12 DNA becomes modified by methylation and that integration also changes the methylation pattern of cellular DNA sequences (for [115]). These observations stimulated further studies on the role of DNA methylation in the regulation of eukaryotic gene expression.

Epilogue

It is certainly possible to criticise concerning the development of virology in Germany and also to point out reasons as to why our research may perhaps have had certain shortcomings, when compared to research carried out in some other countries. I must admit, however, that what had seemed first like a major burden

to me, then gained a touch of personal pride even after realizing that not only it would be impossible to mention every important contribution from our country to virus research but it would also be impossible to do justice to these contributions. The chosen examples – personally biased – can therefore neither be regarded a complete nor a truly representative selection of all innovative virus research carried out in Germany over the last century. Many of the studies not mentioned here, have made equally reputable contributions to international virology.

This is particularly true concerning clinical virology. On a daily basis, fundamental discoveries are rare events. Their true significance comes to light when sensitive methods, which in part have been developed by clinical virologists, are applied and as consequence, precise and definitive results are obtained, enabling a diagnosis to be made and a clinical alarm or all-clear to be sounded, or when epidemiological relationships can be established and infection chains thus uncovered. Moreover, through the development and testing of vaccines and methods for virus inactivation and also in the evaluation of the effect of chemotherapeutics – albeit to-date not yet as successful – clinical virology has proven itself to be invaluable. It is obvious that some areas of basic virological research have their roots in clinical observation. Furthermore, although German clinical virologists often tend not to make headline news, new discoveries they have made are highly regarded by their international community. For example, I might recall the standardisation of diagnostic methods or the ease with which new findings have repeatedly been qickly introduced into general praxis. The *Deutsche Vereinigung zur Bekämpfung der Viruskrankheiten* has no doubt played a significant role in this. Ultimately as a result of these successes, virology's reputation has not only been boosted in the eyes of the general public but more importantly it is also regarded in a different light by those institutions who provide substantial support for research. Although I could recount the names of many noteworthy clinically orientated virologists, I do not believe that I am wrong in choosing Gisela Ruckle-Enders from Stuttgart as an example. With a background in basic research, she earned special recognition in the area of epidemiology of intrauterine and perinatal virus infections whilst running a virus-diagnostic laboratory in a truly exemplary manner. This kind of fruitful juxtaposition of theory and practice or of more basic and more applied research, something that can also be seen in the transdisciplinary makeup of the *Gesellschaft für Virologie*, will become even more important in the future. This is especially evident if we think of the origins of virology and thereby address the questions, which again increasingly come to the forefront, concerning the mechanisms by which viruses become pathogenic agents and with which means we can better confront the problems of virus infection.

Virology is sometimes regarded as one of the jewels of German research. All the same, it would be dangerous, even on this 100th anniversary, to be too high handed in this regard in such a review. In recollecting such contributions I hope that our young adept scientists will be guided by the desire to equal the achievements made by their predecessors, even to outdo them in the future.

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