PROFESSOR STEFAN S. NICOLAU (1896–1967) A FOUNDER OF VIROLOGY IN ROMANIA*

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Forty years have passed since Professor Stefan S. Nicolau deceased. He was the founder of the Institute of Virology in to Romanian Academy' structure. As assistant of outstanding scientist Constantin Levaditi (1874–1953), Nicolau was trained at the Pasteur Institute. Their collaboration was exceptionally fruitful, producing new concepts in virology. Between their most cited contributions was the concept of viral oncolysis. Oncolytic viruses are capable of selective replication in malignant cells and therefore offer levels of potency and specificity that are potentially far higher than conventional treatments for tumour. Viral oncolytic therapy is today under intense investigation as a novel anticancer strategy, both alone and in combination with other conventional treatment modalities. Nicolau was among the first to observe a possible analogy between viruses and gene, and hence to anticipate the future importance of molecular biology. In Romania, it was in his Institute were people of different specialities, biologists, biochemists, and doctors debated the implications of genetic engineering long before it was possible in practice that science fiction became fact, as the use of DNA synthesis, restriction enzymes and plasmids.

Key words: Stefan S. Nicolau; Ultravirus; Viral oncolysis; Borna disease.

INTRODUCTION

In the history of virology, circumscribed to the first half of a twentieth century sequence, the contribution of Romanian scientists Constantin Levaditi and Stefan S. Nicolau played an important role. As faithful followers of Pasteur they assumed that new filterable agents of infectious diseases discovered by botanists (D.I. Ivanovski – Tobacco Mosaic Disease), by veterinarians (F. Loeffler, and P. Frosch – foot and mouth disease) or by physicians (W. Reed, and J. Carroll – yellow fever; K. Landsteiner, and C. Levaditi[1] – poliomyelitis) were small bacteria unable to isolate and cultivate as true microbes. Levaditi and Nicolau coined the term of ultravirus instead the label of "filterable viruses" or "invisible viruses", the label used throughout the 19th century in France as a blanket term for new, unorthodox, infectious agents. In fact, Levaditi and Pierre Lepine published the first textbook of virology in 1938: "Les ultravirus des maladies humaines".[2] It is to be noted that the term "virology" became established in its own right only after the World War II with the apparition of the S. E. Luria's classic manual "General Virology" (1953) and of the first issue of the journal Virology (1955).

Nicolau was a natural organizer and, together with Radu Portocală and Nicolae Cajal, he began to recruit people to work on isolation and cultivation of human and animal viruses. He developed a group of devotees and students who adopted his view points on the important problems of virus diseases of humans and domesticated animals in parallel with research in theoretical and experimental virology.

* The 40 years anniversary of Ştefan S. Nicolau's passing away.

Fig. 1. Jubilant "Nicolau" Medal awarded for excellence in PhD works at the Institute of Virology, Bucharest (a craftwork of outstanding Romanian sculptor Ion Jalea).

Stefan S. Nicolau founded the Institute of Inframicrobiology of the Romanian Academy. The Institute has its origin in the nucleus of the Chair of Virology, which began its activity in 1942 in the framework of the Faculty of Medicine in Bucharest. For the first time in the world, in the higher medical education, a separate discipline was devoted to the study of the ultrafiltrable infectious agents. Afterwards, a team of inframicrobiology of the Academy was created, which developed into the actual "Nicolau" Institute of Virology. The "Stefan S. Nicolau" Institute of Virology, which chronologically is one of the first institutions in this speciality in Europe, has celebrated in 1999 the 50th anniversary of its foundation. Many disciples of the school created by Professor Nicolau distinguished themselves by significant contributions: Adelina Derevic (respiratory viral infections), Elisabeta Nastac (virus-cancer relationships), Elena Oprescu (respirtory viral infections), Radu Portocala (biochemistry of the viral nucleic acids), Dan Sarateanu (ornithoses), Constantin Surdan (Rickettsia), Nicolae Cajal – his succesor at the Institute and Chair of virology direction. The Institute bridges the gap between clinical investigation and the application of basic science in order to understand the pathogenesis of the infectious diseases, their diagnosis and optimal management, prevention and control.

SHORT BIOGRAPHIC NOTE

Stefan S. Nicolau obtained in 1920 the MD degree at the Faculty of Medicine in Cluj, where he began this collaboration with professor Moldoveanu. From his student years in Cluj, Nicolau showed his tendency towards laboratory research. After having spent one year in Cluj, he began to work at Levaditi's laboratory in the Pasteur Institute. Nicolau studied for two decades at the Pasteur Institute in Paris (1920–1939). During this interval he passed his PhD at Sorbonne (1925) and visited National Institute for Medical Research – London (1927–1931). He received many awards from Paris Academy of Sciences and also important prizes: Prize Bellion, 1926, Prize Breant, 1930, Prize Montyon, 1935. Out of the country until 1939, Nicolau authored pioneering studies regarding viral diseases such as: herpes[3], rabies[4], poxvirus infections (especially vaccinia) [5, 6], aphthous fever, Borna disease[7], Aujeszky's disease, "looping illness" and others. Among the worldwide acknowledged priorities we mention: the first detection of inclusions in the yellow fever – a diagnostic criterion of this arbovirus infection[7]; the introduction of the synchronophylaxis concept, a fact which promotes him as a, precursor of the interference phenomenon description[8]; the discovery of the photodynamic action of stains (which is nowadays widely used in viral mutagenesis research on); the description of the septic neuritis[9] and others.

In 1939, Nicolau returned in Romania as professor of Microbiology at Iasi Medical School, and in 1942, as professor of Inframicrobiology at Bucharest Medical School. In the recognition of his outstanding publications he was elected member of Romanian Academy of Medical Sciences and of Romanian Academy.

Fig. 2. The office block of "Nicolau" Institute of Virology (www.virology.ro).

He was director of the Institute of Inframicrobiology in Bucharest (to day, "Ştefan S. Nicolau" Institute of Virology), where he have the opportunity to create many independent laboratories in which his co-workers served national public health and medical school. As a teacher he was the editor of remarkable manuals translated in French, Russian, Chinese etc.

EARLY WORKS

In 1922, Levaditi and Nicolau published a series of articles in which new properties of the viral agents were demonstrated: ultrafiltrability (passage through the pores of a collodion membrane), antigenic properties of killed and replicative viruses etc. Nicolau and Levaditi elaborated experiments on the filterability of viruses with a very original and more constructive interpretation. They opposed to the concept of non-particulate infective organism. In the debate about whether the viruses were living or nonliving, or perhaps were the primordial stuff from which life itself originated, Nicolau underscored the macromolecular nature of viruses and the significance of their tiny component – the genomic nucleic acid. The structure of viruses was soon amply confirmed by direct observation of these objects by newly available electron microscope in the early 1940.

In a broad prospective view of the pathogenesis of viral infections, Levaditi and Nicolau underlined the cell-mediated character of the antiviral immunity. Beside the viral cytocidal effect, Nicolau revealed the participation of the cellular immune response of the host in the triggering of the symptomatology.

In a critical review of the literature on the antigenic properties of the ultraviruses, Nicolau

and co-workers (1928)[11] called attention to the fact that these agents of disease, as far as they have been studied, seem to exhibit antigenic properties which suggest placing them in the general group of bacteria and other more highly organized proteinaceous bodies. They observed that vaccinia virus inoculated into the brain of an immunized rabbit could not be demonstrated two hours after the injection. While vaccinia virus persisted in the circulation of a susceptible rabbit for as long as 8 days following intravenous infection, it disappeared from circulation of an immune rabbit within 4 to 6 hours. Nicolau and Kopciowska made a similar observation regarding herpes virus introduced into the brain of immunized rabbits[7].

The same as the classic of Romanian medicine – Victor Babeş – Nicolau' early research works were connected with rabies. He described extremely important lesions in the central nervous system of animal dead of rabies or of other neurotropic viruses. Although described by Victor Babeº as far back as 1886, the cytoplasmic inclusions in rabies were given the name of "Negri bodies".[11] The priority of Babeş was recognized owing to the well documented works of Ştefan S. Nicolau and Mircea Babeş. Nicolau was the editor of two volumes of V. Babeş selected works and of many monographs which created a true virology library in Romanian (see books at the references).

In regard to the immunological conditions existing in herpes disease, his experiments have reaffirmed that herpes virus can be neutralized with the serum of actively immunized animals and have offered an explanation for the irregularity of the results of others. Nicolau *et al*. found that brain extracts from inoculated rabbits possess some virus-neutralizing power, but considerably less than the serum of the corresponding control animals. It has been shown that active immunity can be attained only when some degree of reaction to the living virus has occurred. Rabbits which survived neutralized serum-virus mixtures did not acquire immunity nor did those treated with virus phenolized to the extent of actual destruction.[9]

THE MOST CITED WORKS

Between the most cited Nicolau' publications were papers referring the role of Borna virus infection in neurobehavioural diseases. *Borna disease virus* (BDV) is the prototype of the family *Bornaviridae*, genus *Bornavirus*, within the nonsegmented negative-strand ribonucleic acid (RNA) viruses (order *Mononegavirales*). Nicolau and Galloway were the firsts to conduct experimental study of Borna disease in sheep and cattle and describe the characteristics of this enzootic encephalo-myelitis.[13] This neurotropic virus appears to be distributed worldwide and has the potential to infect most, if not all, warm-blooded hosts. The name Borna refers to the city of Borna, Germany, the site of an equine epidemic in 1895–1896 that crippled the Saxon cavalry. Natural infection has been reported primarily in Europe, but recent reports also include North America and parts of Asia (Japan, Israel and Iran). Reports of asymptomatic, naturally infected animals suggest that the virus may be more widespread than previously appreciated. Reports of BDV nucleic acid and proteins in peripheral blood mononuclear cells also indicate a potential for haematogenous transmission.

Although there is consensus that humans are likely to be susceptible to BDV infection, the epidemiology and clinical consequences of human infection remain controversial. After 1990, German scientists reported that Borna infectious virus has been isolated from humans. [14, 15] There have been no large controlled prevalence studies but methods for diagnosis of human infection were reliable suggesting an association between BDV and neuropsychiatric disorders, including unipolar depression, bipolar disorder or schizophrenia. BDV has also been linked to chronic fatigue syndrome,

An innovative concept developed by Levaditi and Nicolau was viral oncolysis in studies where the neurovaccinal virus was inoculated into grafted tumours.[16] Sinkovics J.C., and Horwath J[17]. (Viruses and cancer. *Medical Hypotheses* 1995, 44, 359–368) quoted from the paper of Romanian scientists: "Tumours are more susceptible to viruses than normal cells and tumours act as a sponge attracting viral replication". This hypothesis is now in the centre of interest for many biotechnology companies who enlarge cancer virotherapy models.

Stefan S. Nicolau made obvious the theory of viral etiology of some cancers. With a remarkable intuition Nicolau promoted the hypothesis of the oncogenic potential of the viral nucleic acids. He developed the infravirus model – the autonomized form of viral nucleic acids which, after its integration in the host cell genome, contribute to the viral transformation and the occurrence of various morbid states.

The school of professor Nicolau provided significant arguments for the involvement of viruses in the other fields of general pathology. Thus, morphological data supported the viral etiology of some cardiovascular diseases (arteritides, myocarditides)[21] and the role of chlamydia, rickettsia and some herpesviruses in the etiopathogenesis of some congenital malformations and spontaneous abortions.[22]

I shall detail my remarks to Nicolau' papers on the relations between viruses and cancer, published in the period from 1960 to 1967, which I personally consider highly significant. The author summarises the general properties of human tumour viruses, reviews the development of causal thinking in microbiology and proposes guidelines that might help to determine the role of viruses in human cancer. The work on the role of viruses in the pathogenesis of human cancer was hampered by the fact that virus-associated cancer develops only in a small minority of infected subjects, which implies that, if the virus does play a role in the pathogenesis of the malignancy, other factors must be also involved. Naturally oncolytic viruses are replication-competent viruses that have an innate ability to selectively infect and kill tumour cells. Despite being used in the original attempts to treat cancer with live viruses five decades ago, interest in naturally oncolytic viruses has lagged behind the support for engineered adenoviruses and herpesviruses as cancer therapeutics. Recently, however, there has been renewed interest in the high potency and selectivity of these naturally occurring agents.

Levaditi and Nicolau were the first to study the action of a neurotropic strain of vaccinia virus on tumours of mice and rats. They found epitheliomas of these animals to provide an excellent culture medium for the virus in contrast to sarcoma. These findings were in accord with their previous investigations where neurovaccinia was shown to have ectodermal rather than mesodermal tropism.

Studying tumours infected with neurovaccinia in immune animals Levaditi and Nicolau showed that virus did not survive in the immune host. If a portion of the tumour were transplanted into non-immune animal, it would support growth of the infectious agent. The proliferative power of the transplantable tumour was also interfered and is not always possible to make successful transplants from tumours carrying the vaccinia virus.

Nicolau and his co workers developed investigations among many animal and human viruses that contribute to a variety of malignancies.[18] Between most studied viruses linked to cancers were human papillomaviruses (cervical carcinoma); human polyomaviruses (mesotheliomas, brain tumours); Epstein-Barr virus (B-cell lymphoproliferative diseases and nasopharyngeal carcinoma); hepatitis viruses (hepatocellular carcinoma); and animal T-cell Leukemia Viruses (bovine, murine T-cell leukemias).[19, 20] The criteria most often used in determining causality were consistency of the association, either epidemiologic or on the molecular level, and oncogenicity of the agent in animal models or cell cultures.[21, 22] Whereas for most of the tumour viruses the viral genome persists in an integrated or episomal form with a subset of viral genes expressed in the tumour cells, some agents (Hepatitis B virus) are not inherently oncogenic, but infection leads to transformation of cells by indirect means.[23, 24] For some malignancies the viral agent appears to serve as a cofactor (Burkitt's lymphoma-EBV; mesothelioma - SV(40)). For others the association is inconsistent (Hodgkin's Disease, breast cancer - EBV) and may either define subsets of these malignancies, or the virus may act to modify phenotype of an established tumour, contributing to tumour progression rather than causing the tumour. In many other cases the association with malignancy is less consistent or still emerging. For example, despite the potent oncogenic properties of some strains of human adenovirus in tissue culture and animals the virus has not been linked with any human cancers. Finally it is likely that more agents, most likely viruses, both known and unidentified, have yet to be implicated in human cancer. In the meantime study of tumourigenic infectious agents will continue to elucidate molecular oncogenic processes.

Viruses have been used for cancer treatment for over a century. From the early clinical studies with various wild-type viruses, to the modern trials with engineered viruses, virotherapy has emerged as a promising therapeutic strategy.[19] In a recent review, which also mentions Levaditi and Nicolau priority, japanese authors discuss the progress and challenges associated with oncolytic virotherapeutic agents, summarizing the data from clinical reports, and the implications of this data for future virotherapy development.[25] Beyond historical perspective on the development of oncolytic virus the reviewers present the encouraging results of recent clinical trials (*e.g.*, an modified herpesvirus has been tested in clinical trial of nearly 250 patients and approved for human use by the Chinese FDA).

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