REFLECTIONS ON *IMAGE AND LOGIC*: PHILOSOPHY AND THE HISTORY OF EXPERIMENTATION ON THE ROUS SARCOMA VIRUS IN THE EARLY TO MID 1900s

by

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A THESIS

Presented to the Department of Philosophy
and the Robert D. Clark Honors College
in partial fulfillment of the requirements for the degree of
Bachelor of Science

June 2016

An Abstract of the Thesis of

Inina Kachelmeier for the degree of Bachelor of Science
in the Department of Biology to be taken June 2016

Title: Reflections on *Image and Logic*: Philosophy and the History of Experimentation on the Rous Sarcoma Virus in the Early to Mid-1900s

Approved: 

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When Rous published his experiments on transmissible chicken sarcomas at the beginning of the 20th century, laboratory research was just starting to grow more independent from the clinic. Later changes in the field and the growing emphasis on genetics meant that cancer came to be characterized not just as a physical phenomenon but also as a chemical and genetic condition within the body. I will begin my thesis by using Peter Galison's *Image and Logic* to introduce the topics of intercalated periodization, experimental image and logic traditions, and the utility of machines as loci of and participants in cross cultural exchange. I will then use the history of early experimentation on RSV as a model of scientific change. The interaction of virologists, cancer biologists, pathologists, bacteriophage researchers, experimental techniques, and technicians within experiments on RSV negotiated the importance of tumor virology to cancer research. Ultimately, I will show the emergence of distinct image and logic traditions within the early history of RSV, and I will discuss how images and logic represent fundamentally different modes of knowledge acquisition.

Acknowledgements

I would like to thank Professors Nicolae Morar, Alan Kelly, and Daniel Rosenberg for their patience and help throughout the thesis process. I came to each of them as an eager CHC student and now leave, thanks to their guidance, as a thesis survivor. In particular I would like to thank Nicolae Morar for the continued support and philosophical knowledge which he imparted upon this biology student. Lastly, I would like to sing the praises of my parents, friends, and roommates for continually supporting me (and for dealing with my sleep addled brain). I could not have switched into the biology major at the end of my sophomore year and still graduated within 4 years without the support of a community.

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Introduction

What are the roles of experiment, theory, and technology in scientific change? At any one moment within scientific history we can identify a set of predominant theories (or systems of explanation which aim to provide a description of reality), methods, and technologies which are broadly utilized in or accepted by the scientific community. Peter Galison discusses the role that the disparate traditions of experiment, theory, and technology play in scientific change within his 1997 publication *Image and Logic*. Galison proposes a model of intercalated periodization as a schematic of scientific change. The term *intercalated* suggests the interaction of scientific groups without homogenization ("Reflections on" 1). In this model, scientific change is structured by the distinct rhythm of experimentation, theory, and technology. Overall, Galison argues that the interaction of these differing traditions is responsible for the strength and sense of progress within science.

In *Image and Logic* Galison argues that machines are physical and ideological points of contact at which disparate scientific traditions interact. He organizes the history of particle physics around the tradition of image and logic devices. The image and logic tradition had distinct theories, technologies, and experimentation methods. Image and logic are also fundamentally different ways of looking at information. Images are immediate. They attempt to preserve information by maintaining form while logic seeks to locate meaning by analyzing the logical relationship between objects or ideas. In my thesis, I will explore the emergence of image and logic traditions within biology by analyzing the early history of the Rous Sarcoma Virus (RSV). I will also

show that this history is intercalated by discussing how the interaction of distinct groups of scientists led to the progression of research on RSV.

In my thesis I will use RSV as a model of scientific change. The story of RSV begins in the early 1900s when Peyton Rous published two articles on sarcomas in Plymouth Barred Rock Hens. In the first, published in 1910, he characterized the isolated sarcomas and demonstrated that the tumor was transplantable to other chickens. In his second publication, Rous ground up isolated tumor cells and passed them through a Berkefeld filter, which prevents bacteria and cells from passing through. Rous found that he could induce tumor formation in subsequent chickens by injecting them with the filtrate. This indicated that cancer could be inducible by a still unknown "filterable agent."

Despite the later significance of RSV to our understanding of cancer etiology, Rous' research was considered to be false or insignificant by mainstream cancer researchers for the majority of the early to mid-1900s. In 1966 Rous was recognized for his work on RSV when he was awarded a Nobel Prize in Physiology or Medicine alongside Charles Huggins. The 55 year period between when Rous discovered RSV and received the Novel Prize is the longest "incubation period" in Nobel Prize history (Weiss and Vogt 2353). I will analyze how scientific ideas about RSV changed over the course of a portion of those 55 years: the early to mid-20th century. I will show that the interaction of theory, experimentation, and technology within broader image and logic traditions was fundamental to research on RSV and to the development of a microbiological definition of cancer.

Overview of Thesis Organization

In chapter 1 of my thesis I will discuss *Image and Logic*. In particular, I will focus on Galison's conception of image and logic traditions within the history of particle physics, and the idea of intercalated periodization. In chapter 2 I will discuss how the relationship between bacteriology and (emerging) virology in the late 19th century led to the first definition of viruses and the extrinsic theory of cancer etiology. Then, in chapter 3, I will characterize the reaction of different groups of scientists to Rous' experiment and to the extrinsic theory of cancer. I will explore how the ever increasing divide between mainstream oncologists and experimental pathologists led these two groups to different interpretations of Rous' experiment. In this section I will also discuss how these groups developed unique image and logic traditions for researching cancer etiology. In chapter 4 I will focus on mid-1900s advances in experimentation practices (the focus and plaque assays) which led to further research on RSV. Finally, in chapter 5 I will return to *Image and Logic* and discuss the applicability of Galison's philosophical framework to the history of RSV.

Chapter 1: Peter Galison's *Image and Logic* and the Philosophy of Scientific Change

Introduction

Peter Galison explores the history of instrumentation in particle physics within his 1997 publication *Image and Logic*. In *Image and Logic* explores the social, theoretical, and experimental dynamics of particle physicists. Galison acknowledges that practitioners existed in a shifting scientific and social world. The Cold War would have a lasting impact on physics. In Galison's words: "Statistics, weapons design, mathematics, nuclear physics all realign during the Cold War to form a new subject, simulations—at the same time a new category of physicist emerges, not quite experimenter and not quite theorist" ("Reflections on" 255). During this time, the structure of physics would also undergo a fundamental change. The 20th century would see the development of a collaborative scientific culture in which the teamwork of hundreds of physicists comes to replace individualistic experimentation (Zimer 289). Within this changing environment, Galison hones in on points of contact between disparate scientific subgroups..

The philosophical importance of *Image and Logic* is in Galison's recognition that physics is not one monolithic whole, but a conglomeration of diverse cultures and subcultures interacting with each other. Galison is interested in the relationship of scientific subsets to each other at points where they interact, and the relationship between these points of contact and culture at large. He ultimately argues that the partial autonomy of these separate groups (experiment, theory, and technology) is a strength of

the scientific community. His ideas concerning trading zones, intercalated periodization, and machines as cultural entities provide a relevant context for understanding the ways in which different sciences interacted within the development of experimentation on RSV.

1.1 Scientific Change Through Intercalated Periodization

Galison conceives of scientific change as a process of intercalated periodization. Different scientific subcultures such as technology, experimentation, and theory have their own rhythms. In Galison's conceptual framework, big shifts or advancements in one subculture often do not coincide with changes in another. He uses the analogy of a brick wall in which the whole is made up of irregular breaks in the brick in order to illustrate this point and also to suggest that the incongruity between these subsets accounts for the strength and sense of progression within a science.

Disparate scientific traditions interact at points of exchange which Galison calls trading zones. A central aspect of Galison's intercalated periodization model is the interaction of differing scientific traditions. While groups have their own autonomy and differing rhythms of change, they also interact at points which Galison calls trading zones. Galison borrows the idea of trading zones from anthropology as a way of characterizing spatial, temporal, and symbolic regions where subcultures interact. Coordinate rules of exchange allows groups to trade despite vast global and local differences. Thus, groups will be able to trade despite differing conceptions of the value of the object in question. The ways in which these subcultures interact, what value they bring to each other, how they situate language, and how they relate to broader scientific culture is important to determining the outcome of trade.

1.2 The Importance of Technology

Technology is important in these trading zones because it mediates the relationship between diverse subcultures. To technology, Galison endows the gift of aggregation: "By the material culture of science I have in mind the study of instruments as accretion points, loci where new worlds emerge through the recombination of physics, engineering, warfare, industry, philosophy, chemistry, and mathematics" ("Reflections on" 1). In section 6.1 of *Image and Logic*, Galison likens the central role of machines in cultural exchange to pidgins and creoles. He writes: "Objects draw together clusters of cultural practices the way pidgins and creoles bind languages" (*Image and Logic* 436). Thus, technology has a central role in bringing together different subcultures and binding these cultures to one another. Due to its localizing properties, technology itself acts as a trading zone.

Machines also participate in cross-cultural exchange by means of their own histories. They are not neutral units of exchange. On this Galison writes:

> "while it would be an error to suppose that machines can be plucked cleanly from their context, it would be equally distorted to assume that objects carry the totality of their culture embedded within them. One of the central arguments of this book is that there is a partial peeling away, an (incomplete) disencumberance of meaning that is associated with the transfer of objects" (*Image and Logic* 436).

To borrow from his previous analogy, like a pidgin or creole machines are units of cross-cultural exchange both in the process of their creation (which may draw technologies or scientists from various fields) and in their use (which, again, often deviates from their original context and spans different contexts). This concept, that of a "partial peeling away," will be important when thinking about experimentation in RSV. In my thesis I will show the transfer of experimentation techniques between

bacteriology, bacteriophage biology, and virology. In each of these iterations, the definition of microbes and of cancer will undergo substantial revision.

1.3 Image and Logic Traditions

Lastly, as is evident from the title of the book, in *Image and Logic* Galison explores the unique history of image and logic devices. He differentiates between instrument makers who produce image-making devices and makers whose devices would produce logic. These are two fundamentally different methods of knowledge transmission. Galison labels these as "homomorphic," or preserving form, and "homologous," or preserving logical relations. Historically, the antipositivists viewed images as emblems of the "ineffable, the tacit, the non-rational," emblems of rebellion against the "science-as-rules" of a positivist scientific view ("Reflections on" 1). The idea here is that pictures are in some way pure because they attempt to capture the whole reality of a situation which is then interpreted in a variety of ways. Galison deviates from this argument and instead postulates that images are never unmediated. In his own words "by attending on anti-imaging alongside imaging, I aimed precisely to *de*-naturalize the visual, to make the production of visual data as historically, philosophically, and practically problematic as the generation of digital data" (1). A main focus of my thesis will be in analyzing the emergence of distinct image and logic traditions within the early history of RSV, and analyzing how these functioned as fundamentally different modes of knowledge acquisition.

1.4 *Image and Logic* and the History of Particle Physics

The distinction between image and logic devices is central to the history of particle physics within the book *Image and Logic*. Galison traces the distinct lineage of image devices from C.T.R. Wilson's 1911 cloud chambers, to photographic emulsion devices, to bubble chambers. These experimenters characterized the passage of particles at visualizable "golden moments" (Ziman 291). In contrast, another set of experimenters focused on "counters," or quantifying the frequency of moments of passage (292). These two traditions would meet in the technological innovations of the 1970s. Time projector cables (TPC) provided a three dimensional record of electronic passage such that the passage of particles was both imaged and statistically analyzed.

By focusing on image and logic devices, Galison highlights the central role that experimentation has in scientific history. As John Ziman writes in his review of *Image and Logic*, the history of "twentieth century microphysics has been dominated by theoreticians, who write the story inwards from unifying theories" (289). Galison organizes history around experimentation instead of theory. Thereby, he is able to elucidate the ways in which experiments and machines create and engage with knowledge. By analyzing the lineage of image and logic traditions, Galison shows that different groups of scientists have fundamentally different ways of presenting, analyzing, and creating data. Thus, Galison's analysis of image and logic represents a new way of thinking about scientific history. This methodology acknowledges the importance of looking at the ways in which knowledge is constructed and presented to the social and epistemic history of science.

Conclusion

In my thesis I will explore how technological/experimental advancements in the 20th century were sites at which disparate traditions in bacteriology, bacteriophage biology, genetics, and oncology established the importance of RSV to cancer research, where the definition of cancer and viruses were mediated, and where ultimately a unique scientific group focused on tumor virology established itself. To this end, I will use a historical approach. Galison wrote that histories must be "be dense and specific enough to understand the limits of the malleability of objects and meanings as they travel from domain to domain" (*Image and Logic* 357). I will use this approach because it will allow me to analyze the temporal configuration of differing scientific traditions, to exemplify the relationship between scientific experiments and social climate, and to analyze the lineage of experimental traditions through time. Ultimately, this approach also allows me to discuss the fundamental differences and applications of the image and logic divide.

Chapter 2: Cancer and Microbes in the Late-19th Century

Introduction

In this section of my thesis I will explore the intersection of bacteriology, virology, and cancer research in the latter part of the 19th century. The extrinsic theory of cancer (also called the parasitic, exogenous, or germ theory) is the idea that cancer is caused by microbes. The extrinsic theory became popular in the latter part of the 19th century after the recent clinical triumphs of bacteriology research opened the minds of mainstream scientists to the possibility that microbes could cause a variety of diseases, including cancer. Virology also emerged during this time period. Plant bacteriologists were the first to conceive of viruses when they found that certain cell-free filtrates remained infectious. The convergence of early work on bacteriology and virology formed the basis of the extrinsic theory of cancer etiology, and established the first definition of viruses as infectious organisms.

2.1 The Chamberland-Pasteur Filter

The development of the Chamberland-Pasteur filter in the latter part of the 19th century allowed scientists to separate bacteria and cells from filtrates and eventually led to the discovery of viruses. The Chamberland-Pasteur filter was developed by Charles Chamberland, then an assistant to Louis Pasteur, with the intention of "freeing the water in waterworks from micro-organisms" ("Chamberland's" 410). The filter works by removing bacteria and cells that are too large to fit through the natural pores in the porcelain from the remaining liquid. According to reports from the time period, while this filter was impractical for use in the household due the arduous process of heating

and purifying the porcelain filtering-rods it did succeed in blocking the passage of bacteria, cells, and fungi (“Chamberland’s” 410).

Viruses were first conceptualized in plant bacteriology experiments that stumbled upon the capability of cell-free filtrates to remain infectious. An 1892 experiment by Ivanovskij was the first to demonstrate that the filtrate could induce disease in tobacco plants. However, it was Martinus Beijerinck in 1898 who hypothesized that the cause of the disease was a disease-inducing organism, which he named *contagium vivum fluidum* (contagious living fluid). In an effort to prove the existence of a living microbe, Beijerinck conducted diffusion experiments. At the time filtration experiments were still “open to criticism” (Beijerinck 35). By showing that the infectious material had the ability to diffuse through an agar plate and infect subsequent plants, Beijerinck validated his assertion that the infectious material was a living and not static entity. He concluded the following about what he alternatively called *contagium vivum fluidium* and a virus: they were soluble, they were infectious, they could replicate only on living tissues, and they were small. His experiment was soon followed by the identification of the first animal and human viruses: foot-and-mouth disease virus and yellow fever virus (Javier and Butel 7694).

The emergence of research on viruses happened at a time when scientists were reconceptualizing the relationship between microbes and humans. The germ theory of disease, the idea that diseases are caused by microbes, was put forth by Louis Pasteur in 1867. Pasteur, a chemist by training, had previously shown that different microorganisms are associated with different kinds of fermentations and that a disease in silkworms was caused by microorganisms. Robert Koch’s subsequent work on

anthrax established the experimental framework of isolation, infection, and re-isolation for determining that a germ was responsible for a disease. Though initially rejected by many physicians, the germ theory proved useful as it eventually led to the identification of agents responsible for dysentery, cholera, and rabies among other diseases (Weinberg 42; Tsoucalas et al 519). These developments were recognized by doctors who, though wary of the benefit of laboratory science, acknowledged the clinical significance of these findings. In 1883 doctor William T. Belfied wrote in a review that: "Illness may be caused by the not living products of putrefactions, as well as by the living organisms which abound in and probably produce putrefactions. But in the latter case the disease may be farther expended to fresh, healthy individuals by infection: in the former it cannot be" (Belfied 134). Belfied's quote exemplifies the recognition of clinicians that "living" and "infectious" organisms could cause disease. At the same time, Belfied's emphasis on the pathology of viruses that infect "fresh, healthy individuals" suggests that bacteriologists and virologists had a fundamentally different relationship to microbial research. While clinicians and mainstream oncologists were interested in the relevance of such research to medicine, researchers were interested in determining characteristics of and defining these microscopic organisms.

2.2 Origins of the Extrinsic Theory of Cancer Etiology

The success of early experiments on bacteria and their applicability to real disease cases opened the possibility that cancer, which was just beginning to become prominent in public consciousness, could also be caused by microbes. One of Pasteurs followers, Etienne Bernet, wrote in his 1907 publication *La Lutte contre le microbes* (The fight against microbes) that: "It is long discussed for cancer that heredity is a

legend that will vanish when contagion will be proved" (Tsoucalas et al 520). To support his adamant vote of confidence for the extrinsic theory and for the causality of all disease by germs, Bernet likens cancer to tuberculosis. He writes: "Cancer is almost to the point where tuberculosis was, when Villemin demonstrated contagion and inoculability….Cancer had its Villemin and waits for the discovery of its microbe; it waits for Robert Koch" (519). Bolstered by the recent success of Jean-Antoine Villemin, the scientist credited with showing that tuberculosis was infectious, and Robert Koch, who identified the causative agent of tuberculosis, it was not difficult to imagine that cancer could also be caused by a microbe.

A number of subsequent experiments looked at the relationship between microorganisms and cancer. Initially, these experiments focused on bacteria and fungi and not on viruses. Such experiments included Scheuerling and Rapin's 1887 and 1889 experiments on intracellular microorganisms and the development of tumors; the microorganisms identified by Professor Charles Richet and (separately) Eugene Doyen; experiments on the influence of fungi on carcinogenesis; and eventually the sporozoite theory which postulated that sporozoites (a spore like stage in the lifecycle of some organisms) contaminated the air and food chain could explain cancer incidence in rural regions (520). In 1876 an experiment on canine venereal sarcomas by M.A. Novinsky showed that venereal canine sarcomas were transmittable across histocompatible barriers to foxes ("100 Years" 2352). These experiments largely failed to identify a definitive link between cancer and microbes, and their failures only served to suggest to other scientists that there was no link between the two.

In later experiments, scientists used filtration devices to show that cell-free filtrates could induce cancer in select organisms. In 1908 Ellerman and Bang conducted research on erythro–myeloblastic leukemias in chickens. They found that these leukemias were transmittable via cell free filtrates. Further research did not pick up because leukemias were not recognized as cancers until the 1940s and because the two researchers abandoned the project (Weiss and Vogt 2352; Van Epps 2013). In 1914 Fujinami and Inamoto (working independently of Rous) isolated another chicken sarcoma which they later found was also transmittable by filtrate (Martin 7910). The most famous of these experiments was Rous' 1911 experiment which, though not the only experiment to establish the transfer of cancer via filtrate, later identified the first virus recognized as a cause of cancer.

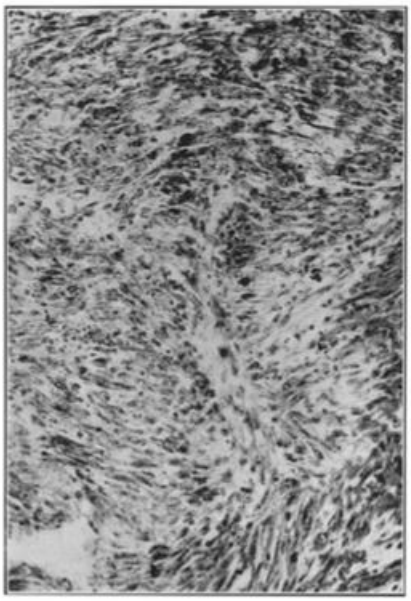
2.3 Rous' 1910 and 1911 Experiments

2 years after graduating medical school, Peyton Rous became head of a cancer research laboratory at the Rockefeller Institute (Rubin 14389). In 1910 and 1911 he published articles on research he had done concerning sarcomas within barred Plymouth Rock hens. In this section I will discuss the various imaging and logic techniques which Rous' used to characterize RSV. I will also analyze the utility of these two modes of knowledge retention within these early RSV experiments.

In his 1910 experiment, Rous used imaging techniques to characterize the original and transplanted chicken tumors. In this experiment, Rous conducts transplantation studies by using a large trocar to implant bits of the tumor rim into the left breast muscle and peritoneal cavity of the same fowl ("A Tranmissible" 697). In the beginning of his 1910 publication, Rous notes that "macroscopically, the growth suggested a sarcoma" (697).

However, Rous was unable to definitively confirm that the tumor was a spindle-cell sarcoma until he took microscopic images of sections of the tumor.

(a)



(b)

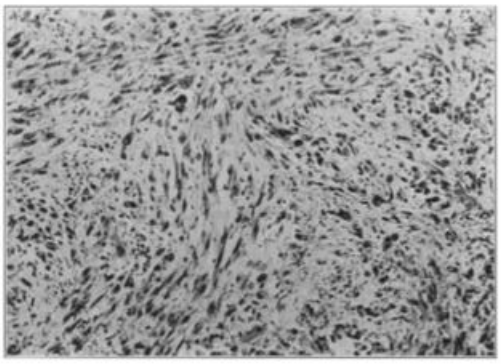


Figure 1a-b. Microscopic images of the original chicken sarcoma (a) and of the intraperitoneal chicken sarcoma (b)

These images (the first taken from the original tumor and the second from the transplanted growth) show that both tumorous growth are composed of narrow, elongated cells otherwise known as spindle cells ("A Transplantable" 707).

Spindle-cell sarcomas are a type of cancer localized to the connective tissue in which the cells are "spindle-shaped," or elongated. In figures 1a and 1b above, we see that microscopic images of the original and transplantable sarcoma both reveal that the

tumor is composed of narrow, elongated cells. These images confirmed that the original tumor was a spindle-cell sarcoma and that transplanted tumors retain spindle-cell characteristics.

In his 1910 report, Rous also uses microscopy to demonstrate that the original tumor cells infiltrated surrounding tissue. Tumors are defined as cancerous if they have the ability to metastasize (or invade distant sites). Rous noted that the original tumor appeared to be benign (it was stationary or not invading surrounding tissue), however within tumor sections he identified points at which the tumor had begun to invade the surrounding muscle tissue.

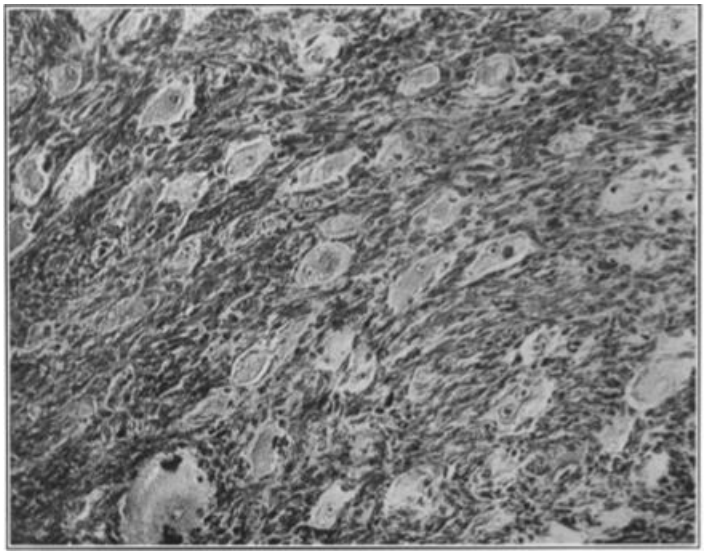


Figure 2. Invasions of muscle by tumor cells of the original chicken sarcoma

In this image one can see that the original sarcoma cells are beginning to invade muscle cells.

The infiltration of muscle cells by sarcoma cells can be seen in figure 2 above. The ability of these cells to metastasize means that Rous' chicken sarcomas were cancerous and not benign.

In his 1911 publication, Rous expanded upon his earlier transplantation studies by demonstrating that the tumors were transplantable to other chickens, and used a Berkefeld filter to show that tumors were inducible via a cell-free filtrate. Rous had used imaging of the transplanted and original sarcoma to confirm that both were spindle-cell sarcomas. Following these results, Rous undertook an effort to determine the factor which made the cancer transplantable. He used the Berkefeld filter (a filtration device like the Chamberland-Pasteur filter but using a different type of porcelain) to isolate a cell-free filtrate. Rous later describes his use of the Berkefeld filter as a decision "made merely in line of scientific duty….Here was a new growth and in a new family and tests for its cause just had to be made" (Becsei-Kilborn 127-128). Rous was not expecting these filtration experiments to be successful. In the introduction to his 1911 publication, Rous writes:

> "In a careful study of the growth, tests have been made to determine whether it can be transmittable by a filtrate free of tumor cells. Attempts to so transmit rat, mouse, and dog tumors have never succeeded; and it was supposed that the sarcoma of the fowl would not differ from them in this regard, since it is a typical neoplasm" ("A Sarcoma" 397).

Though not expected to do so, Rous' found that RSV was transmissible by a cell-free filtrate. The results of Rous' transplantation and filtration experiments are summarized in figure 3 below.

Rous made several general observations from figure 3. First of all, successful transplantation largely depended on the character and condition of the host. Transplantation largely failed to grow in impure chickens (circles) except in later generations. Rous also observes in his report that the health of the chicken was a factor which influenced successful metastasis and tumor growth (404). Rous' writes that the

importance of the aspects of the host (individual species and health) to tumor success had been used as “evidence against a specific cause for the disease, extrinsic of the cells” (409). The success of Rous’ filtration experiments, however, incited Rous to write that: “Such evidence is void, now that a growth has been found possessing the traits mentioned, yet transmissible independently of the cells” (409).

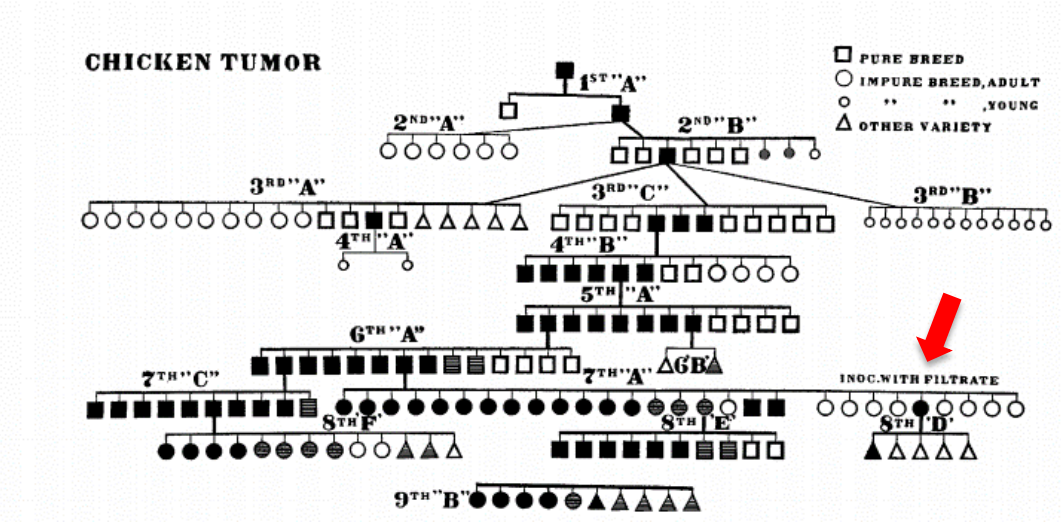


Figure 3. Summary of filtration and transplantation studies within the first eight generations of Rous' chicken sarcoma

The blackened symbols indicated tumors which grew in the host. The cross-barred symbols represent tumors which appeared but remained stationary or retrogressed. Clear symbols indicate tumors which did not grow at all. Rous used pure-bred (squares), impure bred chickens which were bought at random (circles), and other varieties of chickens of differing sort and appearance (triangles). The lines between generations indicate transplantation of the tumor from one chicken to others. Only the individuals of generation 7A (indicated by the red arrow) were not inoculated with a tumor segment but were inoculated by filtrate. The 8th E and D generations are therefore the only transplantations from the filtrate-inoculated tumor lines. The 9th B generation are chickens transplanted with tumor segments from the 7th or 8th generation which were omitted from this chart because conditions in them were "irregularly modified" ("A Sarcoma" 402).

The second important trend visible in figure 3 is the ability of a cell-free filtrate to induce cancer. The majority of chickens within generation 7A (those inoculated by a cell-free filtrate) developed tumors. Furthermore, these tumors developed in chickens of both pure and impure heritage. Microscopic imaging of tumors from generation 7A were able to show that filtrate-inoculated tumors retained spindle-cell sarcoma characteristics, and were invasive (Figure 4).

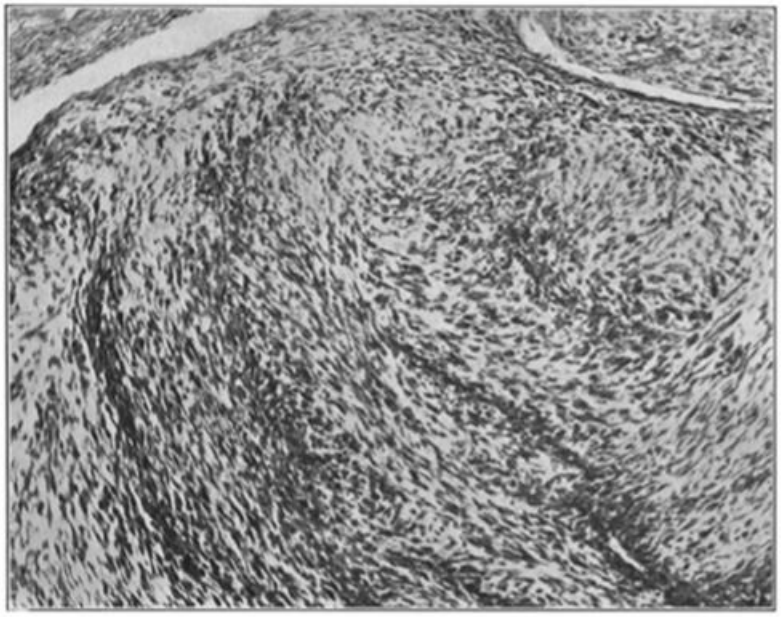


Figure 4. The margins of metastasis from filtrate inoculated chicken no. 116 from generation 7A

In this image once can see the invasion of the chicken sarcoma into muscle of the gizzard.

Upon the observation that tumorous growths were inducible via a cell-free filtrate, Rous made several attempts to identify a parasitic agent. He attempted to grow the microbe on a cell-culture, and tried to image it using dark-field microscopy. However, as Rous writes, "[n]either this nor the various histological procedures applied to the neoplastic tissue has disclosed anything which can be recognized as a parasitic organism" (407). What would later come to be characterized as a tumor virus would remain essentially invisible during this time period. As Rous notes, histological techniques were largely unable to characterize viruses and it would take later developments in cell-culturing techniques before viruses became quantifiable.

Conclusion

The early history of virology is highly intertwined with bacteriology. In the late 19th century, virology became conceived of as an offshoot of bacteriology. It was during this time period that the extrinsic theory of cancer origin was born, and that scientists began to use filtration devices. In the latter part of this chapter I analyze the techniques used by Rous in his 1910 and 1911 publications. Rous used a variety of imaging and logic techniques to characterize the nature and growth of chicken sarcomas. Imaging techniques were able to confirm that both the original, transplanted, and filtrate-induced tumors were spindle cell sarcomas (Figures 1a-b, 2, and 4). These images are homologous, they preserve form. Rous followed the growth of tumors through 8 generations of transplantation and filtrate-inoculation studies within figure 3. This chart is an example of homologous data, as it preserves logical relations and involves the accumulation of multiple data points. In this chart, Rous is able to show that the tumor was inducible by a filterable agent, possibly a microbe.

Though Rous used the Berkefeld filter "merely in the line of scientific duty," his results were born into a scientific culture defined by the divide between the extrinsic and intrinsic theory of cancer. In the next section of my thesis I will explore the reaction of laboratory scientists, mainstream oncologists, and clinicians to Rous' research. I will also look at how the continued association of the extrinsic cancer theory with the failure of early tumor virology experiments, the vague and unsatisfying definition of viruses, and the incongruity between the infectious nature of microbes and the non-infectiousness of cancer made mainstream cancer researchers skeptical of Rous' experiment.

Chapter 3: Initial Rejection: Reactions to RSV in the Early 20th Century

Introduction

In this section I will discuss the reaction of various members of the scientific community to experimentation on RSV. In the early 20th century, the majority of mainstream scientific researchers in the US considered Rous' research to be insignificant, irrelevant, or false. Many scientists doubted Rous' experiment on the basis that his induced chicken sarcomas were not "true" tumors. Others contended that the filterable agent was a chemical or small bacteria and not a virus. As Peter Vogt points out, Rous' filterable chicken sarcoma experiment was relatively irrefutable. Though many would acknowledge this, ultimately most mainstream scientists relegated Rous' experiment "to the realm of interesting but basically irrelevant scientific curios, of no significance to the understanding of human cancer" ("Peyton Rous" 1559). In the following section I will explore how the divide between scientists who supported the extrinsic and intrinsic cancer theory delineated two experimental traditions: one focused on image and the other on logic. The logic tradition is largely epitomized by laboratory scientists while two groups within the cancer community, oncologists and clinicians, were interested in the image tradition. I will also discuss how the state of theory, the ability of technology to characterize viruses and cancer, and the state of knowledge at the time limited the potential for further work on RSV.

3.1 Limitations of Technology and Knowledge

Becsei-Kilborn broadly classifies the main objections raised about the extrinsic theory and Rous' experiment within her publication "Scientific reputation and scientific discovery." Many scientists believed that the chicken sarcomas were not "true tumors" but were simply infectious growths called granulomas. In his 1911 report, Rous seems to anticipate this when he writes:

> "The above traits have figured largely in current discussions on cancer etiology, and most of them have been regarded as evidence against a specific cause for the disease, extrinsic of the cells. Such evidence is void, now that a growth has been found possessing the traits mentioned, yet transmissible independently of the cells. This fact, and not the problem of how to classify the growth, merits attention" ("A Sarcoma" 409).

Others believed that the cause of RSV was not a virus but a chemical component which had passed through the filter. Again, Rous makes himself open to this possibility in his paper. He writes: "an agency of another sort is not out of the question. It is conceivable that a chemical stimulant, elaborated by the neoplastic cells, might cause the tumor in another host and bring about in consequence a further production of the same stimulant" ("A Sarcoma" 410). It would be difficult to address these concerns without the advancement of more broadly accepted definitions of viruses and cancer.

These arguments were exacerbated by the limitations of knowledge about what viruses and cancer were. Rous recounts the reaction of one British oncologist who told him: "But, my dear fellow, don't you see, this can't be cancer because you know its cause" (Martin 2004). In a review published in *Science*, another pathologist writes that "cancer is probably caused by some change, perhaps a chemical change, which the human body undergoes in the course of years" (Wolff 1925). The extrinsic theory of

cancer is fervently rejected in the review, and Wolff says “There is no such thing as a cancer germ. There can be none” and he goes on to postulate that the lack of potassium suffered by “civilized human[s],” a theme belayed by racial undertones, “is one of the factors, if not the main factor, in the occurrence of cancer” (Wolff 1925). These reviews exemplify the pervasive lack of knowledge about cancer and virology. As Becsei-Kilborn writes, “[s]cientific publications on cancer were largely based on hypotheses and cancer research lacked a sense of direction” (Becsei-Kilborn 118). There was little knowledge about what viruses, much less cancer, were. Terms such as “parasite,” “germ,” and “virus” were used interchangeably (119). The ever shifting language made it difficult for scientists to hold concrete discussions about whether or not Rous’ sarcoma’s were “true” tumors and whether or not viruses could cause cancer.

The origins of virology within bacteriology also led to skepticism about whether or not viruses existed. In “The discovery of viruses: advancing science and medicine by challenging dogma,” Andrew Atenstein discusses the impact that the germ theory of disease and “Koch’s postulates” had on virology. Koch’s postulates were rules for proving the microbial etiology of a specific disease. Koch developed them during the time period in which he was working on tuberculosis (Artenstein 470). The postulates were as follows: *(1)* the pathogen can be identified in all cases of the disease; *(2)* the pathogen is not present in healthy individuals; *(3)* the pathogen must be able to induce the disease in animal models after isolation and passage through a pure culture (Byrd and Segre 224). Viruses did not fit Koch’s criteria. Biejerinck and Ivanovski were able to show that viruses could induce disease but, because viruses passed through filtration devices, researchers were unable to prove that they were not chemicals or small bacteria

(Artenstein 471). It was not until the 1950s that virology began to establish itself as a distinct and prominent science. Around this time, several virology journals established themselves and the first virology textbook was published ("When did" 142). It was not until the end of the first quarter of the twentieth century that scientists would definitively establish that viruses existed, and debate over the nature of viruses would continue long past that (472).

Still others were skeptical that chicken sarcomas could have any relevance to human cancer (Becsei-Kilborn 115). There were several incongruences between the idea that cancer was caused by microbes and what was known about cancer at the time. As Darwin Stapleton argues in *Creating a Tradition of Biomedical Research*, understanding the relationship between cell and virus was fundamental to debates about the extrinsic theory. While the filterable agent appeared to incite disease, "its behavior depended purely on the neoplastic cells themselves" (Stapleton 195). Experimentation on RSV had concluded that the virus did not spread epidemically among the chickens, and that metastasis was a result of migration of transformed cells and not a virus (195). In addition, experimentation done during the 1920s concluded that carcinogenic factors such as X-rays and coal tar could induce cancer. Until evidence that multiple exogenous and endogenous factors could contribute to cancer growth, scientists struggled to understand how carcinogenic factors and viruses could both lead to cancer. In this time period scientists assumed that for the extrinsic theory to be accurate, a virus would need to be present throughout the body in an inactive state. The virus would need to be activated upon association with another extrinsic element (Sankaran 193-200).

Rous and his coworker, Murphy, believed that the only way to show that RSV was alive would be in vitro propagation. However, this was impossible because cell culturing techniques were not yet advanced enough. As American virologist Thomas Rivers pointed out in 1932 that for the majority of the 20th century viruses were characterized by negative properties (Sankaran 192). As historians Waterson and Wilkinson write, viruses "were not retained by bacteriological filters; they could not be seen in the light microscope; and they could not be grown on artificial media" (Sanakaran 192; Rivers 78). Until further technological innovation happened in the 1940s and 1950s, viruses were un-capturable: they avoided both visibility and quantification.

3.2 A Move towards the Intrinsic Cancer Theory

Debates about the validity of Rous' experiment and work on tumor virology in general often centered on the divide between the intrinsic and extrinsic theory of cancer etiology. Mainstream cancer research began to move away from the extrinsic theory of cancer etiology in the early 20th century. H.G. Plimmer, a scientists working in the early 1900's who was a proponent of the extrinsic (or parasitic) theory, wrote in his 1903 report on the origins of cancer: "the battle around cancer still rages. Is it parasitic or not parasitic?" (Plimmer 1511). Though adamant that the "parasitic theory is by no means yet extinct," he concedes that "some would have us believe so" (1511). In reality, the theory was on its way out and by the end of 1910 Plimmer would have been one of a group of scientists that William Pusey identified as a "small but aggressive school of men which regard [cancer] as of infectious origin" (Becsei-Kilborn 122). Pusey goes on to suggest that as the extrinsic theory was losing validity with mainstream science, the

intrinsic theory was becoming more popular. He writes “the weight of opinion, however, is strong that it is a disease due to the intrinsic disturbance of the affected individual” (122). Pusey, like other mainstream cancer organizations and clinicians, was moving to the belief that cancer was caused by intrinsic disturbance, not extrinsic elements.

From a theory perspective, Rous’ experiment conflicted with the predominant focus of mainstream cancer biology on intrinsic or tissue-specific causes of cancer. Rous’ experiment was published one year after a correspondence from the 1910 International Conference on Cancer in Paris affirmed that the mass of evidence supported the view that “cancerous tissue is really a biological alternation of the tissue proper to the individual attacked by the disease, and thus its peculiar properties may be explained without assuming the intervention of extraneous agencies, such as a hypothetical cancer virus” (111-112). Rous’ publication put him on the losing side of a “battle” between extrinsic and intrinsic.

The distinction between extrinsic and intrinsic didn’t exist only in the vague realm of theory or paradigm. It also manifested itself physically in the divide between different subsets of scientists. Historian Neeraja Sankaran writes in her article “When viruses were not in style” that physicians tended to favor the intrinsic theory of cancer etiology because it was clear to them through personal experience that cancer was not infectious. In contrast, laboratory pathologists interested in infectious diseases tended to favor the extrinsic theory, or at least recognize it as worthy of further research (Sankaran 194).

In public discourse on Rous' experiments, laboratory scientists and clinicians were negotiating not only the value of Rous' experiment but also the nature of scientific research. The "lack of interface" between laboratory and clinic is a commonly emphasized area of tension for research in the early half of the twentieth century (Becsei-Kilborn 115). Scientists who supported research on RSV and the parasitic theory advocated for a more experimental approach to research, instead of the more common morphological approach. As British virologist Christopher Andrewes writes: "The virus theory has had its ups and downs, mostly downs, for pathologists in general have not regarded it favorably. On the other hand, many bacteriologists, and particularly virus workers, are impressed by arguments in its favor" (Sankaran 193). This fundamental difference is explored in Darwin Stapleton's work "Creating a Tradition of Biomedical Research." Erwin Smith, an experimental plant pathologist, is credited in the proceedings of the second Pan American scientific congress held in 1915 as writing:

> "I do not mean to condemn the study of sections, but only to suggest that there are also other ways of looking at this problem, which is one of growing things. There is too much reasoning in a circle on the part of many of these writers, too much argument basing one assumption on another assumption as if the latter were a well-established and solid fact, too little clear thinking of a biological sort, too little first-hand knowledge of living plants and animals, too much dogmatism, too much orthodoxy, and not enough experimentation. Hence the pessimism and the discouragement….These strong men, chiefly morphologists, have dominated the situation for a generation, but they have not explained cancer and they can not explain it, and they must now give way" (Stapleton 197-198)

Smith, whose scientific work established that the crown galls in plants retained tumor-like characteristics, was advocating for the usefulness of laboratory science not just in providing a cure for disease, but for understanding disease. On the other side of the divide were those like Victor Schmieden, a prominent surgeon, who said this of later

research on RSV: “It is alien to the clinician’s mode of thinking not to rely on conclusive evidence from human materials….[W]e must warn against attaching too much value to comparative observations from the animal or plant kingdom” (Stapleton 198). Though Erwin is an extreme example of what Becsei-Kilborn would classify as “one of those few clinical researchers who had strong reservations about the benefit of animal experimentation for medicine,” clinicians nevertheless often displayed distrust for bacteriology labs (Becsei-Kilborn 115).

In contrast to the virologist’s world of filtrates, a morphological approach to cancer research had developed its own experimental techniques. The intrinsic theory of cancer (otherwise called the biological view) grew in popularity over the first decade of the 20th century. Cancer “was increasingly regarded as a local condition due to chronic irritation and chemical carcinogenesis” (Becsei-Kilborn 121). Transplantation studies, in which tumors were isolated and transplanted to other places/organisms, and the study of tumor sections under a microscope were favored in mainstream research. In an essay from the proceedings of the Second Pan American Scientific Congress, Erwin Smith writes that “cancer morphologists have patiently cut and stained and studied hundreds of thousands of tumors, refining and refining their definitions and distinctions and building up high walls of separation where nature has made none” (Swiggett 487). In Smith’s view, these techniques captured but a snapshot in the life of a tumor and were unable to adequately depict the “plasticity of living, growing things” (487). On the other side of this debate, clinicians also did not understand the practicality of tumor virology experiments. As has been explored above, research on the microbial origin of cancer was considered to be insignificant.

The approach of mainstream cancer research in the early 20th century epitomizes what Galison calls morphological scientists, or those who (he quotes John Merz here) "look upon real things not as examples of the general and universal, but as alone possessed of that mysterious something which distinguishes the real and actual from the possible and artificial" (Galison 79; Merz 203). The divide between extrinsic and intrinsic delineated two definitions of cancer: one physical and directly observable, the other relegated to the vague and unseen realm of filtrates and viruses. Unlike bacteria, viruses were invisible to light microscopes and unable to be cultured outside of the body. They remained largely unseen until the later development of electron microscopy. The inability of researchers to "see" viruses meant that quantifiable or "logic" experiments were the only ways that virologists could continue research on them. The divide between clinician and an emerging group of laboratory scientists was not only between extrinsic and intrinsic, but also between different modes of knowledge acquisition: one homomorphic and the other homologous.

Conclusion

Peter Vogt, a molecular biologist, virologist, and geneticist, perhaps summarized it best when he wrote: in “the early 1900s, there was a lack of appropriate techniques for studying the fundamental aspects of viral oncogenesis, and the scientific attitudes prevalent during this time were not conducive for the kind of analysis that was needed” (Vogt 2010). Theory, experiment, and technology did not line up in such a way that Rous’ data was reconcilable with the search for cancer’s origin in intrinsic aspects of the cell. In the following section I will discuss the development of the plaque and focus assays. These quantification assays made it easier for virologists to count viruses, and led to the expansion of tumor virology.

Chapter 4: Mid-20th Century Quantification Techniques

Introduction

By the mid-1950s two of the major scientific concepts that were required to understand the behavior of RSV had already been established. First, research established the role of nucleic acids in the transfer of genetic information. Evidence for this was supported by work in genetics by Avery, MacLeod, and McCarthy as well as Watson, Franklin and Crick (who determined the structure of DNA). The second was the concept that viral genomes could become integrated into the cell genome, which was developed by Lwoff ("The DNA Provirus Hypothesis" 1075). This section of my thesis will look at the influence that quantification assays had on RSV research during the same time period.

Research in the 1930s and 1940s had also established that filtrates could induce cancer in other animals (Lucke 1938; Shope 1932; Shope 1933; Bittner 1936) and that various papillomas, warts, and other growths in humans and other animals were transmissible by filtrate (Gross 1962). The possibility that an extrinsic microbial agent had a role in cancer once again appeared plausible. Edward Shrigley, a microbiologist, wrote about the increasing interest in viral particles in a 1951 review: "appreciation of the fact that viruses or virus-like agents may possess the ability to elicit growths in animal tissues is entering the thinking of an ever-widening circle of modern oncologists" (Shrigley 241). Affinity for the microbe hypothesis was due, in part, to a newly emerging consensus that there was no one cause of cancer. A variety of intrinsic and extrinsic causes, some supported by experiments listed above, including "chemical

carcinogenic agents, heredity, hormones, milk factors, viruses, physical trauma, and precancerous conditions" had been associated with cancer (Stowell 286; Sankaran 195-6). Tumor virologists were no longer trying to prove that a microbe could be *the* cause of cancer, but simply that a microbe could be a factor in cancer etiology.

The 1940s and 50s also engendered a change in the definition of viruses. Though scientists had been working on characterizing viruses during preceding decades, in "When did virology start" Ton Van Helvoort argues that the "birth" of virology happened in the 1950s. Van Helvoort postulates that the emergence of virology as a promising discipline happened for two reasons. First, the identification of viral lysogeny (one of two modes of viral replication, it involves the integration of the viral genome into the host's DNA) definitively proved the difference between bacteria and viruses. Second, lysogeny experiments established a new definition of viruses that unified research on animal, plant, and bacterial viruses ("When did" 142). The new definition of viruses was important for confirming that sarcoma agents and bacteriophage were viruses and not chemicals or small bacteria. In a separate publication, "History of virus research in the twentieth century," Van Helvoort argues that the concept of filterable viruses "was *de*constructed" in the 1930s and 1940s ("History of" 189; Sankaran 194). The belief that sarcoma agents and bacteriophages were not viruses had been established only "in relation to the *paradigm of bacteriology*, which interpreted infectious agents as autonomous living microbes" ("History of" 186; Sankaran 194). As early as the 1930s, mainstream virology researchers began citing the sarcoma agent as a virus (Sankaran 194). The re-establishment of the sarcoma agent as

a virus and the increasing acceptance that cancer could be caused by a variety of extrinsic or intrinsic agents led to renewed interest in tumor virology.

In “When viruses were not in style” Neeraja Sankaran builds upon Van Helvoort’s analysis of lysogeny experiments. She makes a powerful argument for the importance of these experiments in establishing a link between virology and cancer. Sankaran demonstrates the pivotal role that experiments on lysogeny had in implicating bacteriophage (viruses that invade and replicate within bacterial cells) as agents of genetic change. In 1928 Burnet and Wollman proposed the idea that bacteriophages were genes that could be transmitted from cell to cell via an external nucleus. Though not initially popular, this theory was revived again in the 1950s by Lwolff (Sankaran 196). According to Sankaran, Lwoff’s research “opened up avenues of research by offering a new way of thinking about viruses and their relationship to their hosts” (Sankaran 196). In 1953 Lwolff proposed that the “potential power of a cell to become malignant may be perpetuated in the form of a genelike structure….and that carcinogenic agents induce the expression of the potentiality of this genetic material” (Lwolff 14). These experiments established a new molecular understanding of viruses, and proved that viruses could become part of the cellular genome.

While these theoretical advances pushed research on tumor virology towards a deeper understanding of the relationship between bacteriophage and genetics, there is a parallel history of technological advancement that further engendered the relationship between cancer research and tumor virology. This tradition begins, again, with bacteriophage research that established the first quantification assays for tumor viruses. The isolation of the bacteriophage by F.W. Twort and Félix d’Herelle, and the

subsequent “phage school,” was the technological and intellectual predecessor to later work on tumor microbiology.

4.1 The Phage School: Predecessor of Tumor Virology

Twort was the first to discover phage, or small agents that infect and kill bacteria. D’Herelle independently made the same discovery in 1917 while he was an unpaid intern at the Pasteur Institute. D’Herelle found an “invisible, antagonistic microbe of the dysentery bacillus” which formed clear spots in his cultures (Dublanchet 16; Summers 131). For d’Herelle the bacteriophage was a living entity; it was “a parasite, a virus which penetrated into the sensitive bacteria” (Lwolff 274). At that time, scientists were concerned with proving whether or not viruses were living entities. William Summers writes in his article “The strange history of phage therapy” that viruses and phage occupied a “murky position” in scientific discourse “at the borderline of life” (Summers 132). They could be crystalized, which according to organic chemistry would indicate that they were chemicals. However, they could also mutate and multiply which implied that they were “somehow beyond chemistry” (132).

Much like with RSV, bacteriophage had a complicated relationship to clinical biology. According to Lwolff, the response to d’Herelle’s work was “generally indifferent” with “opinions on d’Herelle ranging from visionary to fool” (Lwolff 16). Summers discusses three reports on bacteriophage done by the American Medical Association (AMA), writing that all three reports “exhibited the tension between laboratory study of phage therapy and its clinical applications, and between in vitro and in vivo action of phages on bacteria” (Summers 132). During the mid-1900s, medical practice was conducted by individual general practitioners and physicians who did not

have access to bacteriological labs. Though proven to be effective, bacteriophage therapy never became as widely used as antibiotics in mainstream medicine in the US. This was in part due to the easier shelf life of antibiotics, the association of bacteriophage with the Soviet Union and Germany during World War II (whose armies utilized phage therapy), and the advent of large pharmaceutical companies who wished to market "wonder drugs" instead of phage (Summers 131-132). The phage school did, however, pioneer experimental techniques which would prove useful for research on RSV.

D'Herelle's early experiments led to the development of a quantitative assay for bacteriophage. In 1917 d'Herelle originated the plaque assay technique. He writes:

> "if one adds to a culture of *Shiga* as little as a millionfold dilution of a previously lysed culture and if one spreads a droplet of this mixture on an agar slant, then one obtains after incubation a lawn of dysentery bacilli containing a certain number of circular areas….these points cannot represent colonies of the anatogonistic microbes: a chemical substance cannot concentrate itself over definite points" (Goldman 93)

The plaque assay quantifies viruses by exploiting the ability of viruses to lyse (kill) bacteria. It is used to determine the concentration (titer) of viruses in a sample. A virus is introduced to a monolayer (or sparse layer) of bacterial cells. Then, the plate is covered with a nutrient medium and incubated. The titer of viruses is estimated by counting the number of plaques (or circular zones of clearing resulting from the phage lysing the bacterial cells) (Panec and Katz 2006). Following its introduction, the technique was refined by Gratia, Hershey, and colleagues. Using this technique, bacterial researchers were for the first time able to calculate the titers of bacteriophage stocks.

Further research done on bacteriophage by Mack Delbruck and Salvador Luria (among others) helped pioneer a biochemical approach to virology research. In 1940, Delbrück started the Cold Spring Harbor Phage Course. Delbrück, trained as a physicist, is described as a researcher with a "missionary devotion to bacteriophage biology" who believed that "the only biology was 'quantitative biology'" (Susman 1101). Peter Vogt characterizes the new phage school as delineating a new quantifiable approach to virology research:

> "[t]he idea of viruses as model biological organisms, the challenges to understand viral replication, to define the role of the host cell, the strictly reductionist strategy that concentrated on the single cell and the single viral particle—all of these marked a new way of thinking about biological problems" (Vogt 7)

The phage school and the new quantitative assays exemplified a new laboratory-based methodology for isolating and characterizing viruses, and was part of an emerging experimental culture that further delineated itself from clinical research.

4.2 Quantitative Assays for Tumor Virology

The creation of a plaque assay for animal virology happened after further developments in cell culturing techniques enabled scientists to grow tumor cells in vitro (outside of the body). In 1955 Harry Eagle described the first defined media that could support the growth of animal cells. While it is relatively simple to culture bacteria and yeast, animal cells require a more complex culture media. The development of new cell culturing techniques was an important step in biomedical research as it allowed researchers to study mammalian tumor cell growth in vitro under a variety of controlled conditions (Herzenberg and Herzenberg 687-688). Renate Dulbecco modified the bacteriophage assay for use in animal virology (Panec and Katz 2006). Peter Vogt

describes the development of the animal plaque assay as “an almost direct transfer of phage technology to animal cells and animal viruses” (Vogt 7). He also credits the plaque assay as “the origin of experimental cellular virology and, ultimately, of today’s molecular virology” (7). The animal plaque assay made it possible for the first time to study neoplastic transformation and carcinogenesis in vitro.

In 1958, Temin and Rubin developed a cell culture-based assay for virus-induced transformation called the focus assay (Vogt 8). To do this they used the Bryant strain of RSV, which cannot produce infectious progeny. Though methodologically related to the plaque assay, the focus assay was an important development as it did not quantify cell killing but rather the ability of a virus to induce cell aggregation (later this would be identified as oncogenic transformation). Work done with the focus assay inherently dealt with the ability of tumor viruses to induce cancerous transformation. In a focus assay, the transformative potential of a virus is determined by the accumulation of foci that develop on a cell culture upon introduction of a virus. When a tumor virus is applied to a cell culture, cells which become oncogenically transformed will replicate faster and form a distinguishable “focus” (or pile of cells). The correlation between viral concentration and foci accumulation visible in figure 5 below indicates that there is a positive relationship between the number of viral particles and the number of transformed cells (Figure 5).

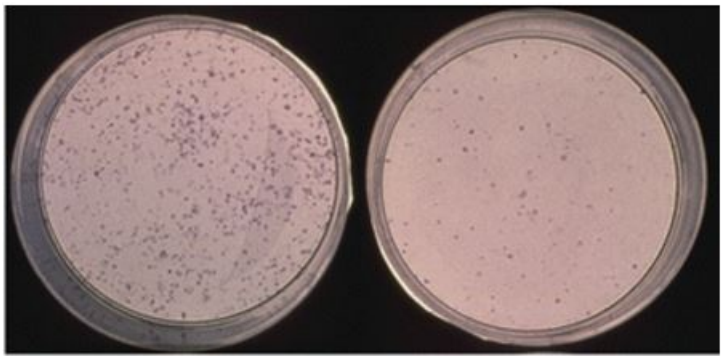


Figure 5. An RSV focus assay showing a 1:100 and 1:1000 dilution of virus stock

Experiments used a chick embryo fibroblast monolayer. The plate on the left had a 1:100 dilution of virus stock while the plate on the right was diluted by 1:1000. Each dot on the plates represents a focus of transformed cells ("100 Years" 2353).

Temin and Rubin used the focus assay in order to model the relationship between virus concentration and number of foci as well as virus concentration and number of infected cells. In figure 6a below we see that there is a positive linear relationship between viral concentration and number of foci. This figure is an extension of the results we saw in figure 5 but presented in graph form. In Figure 6b, the researchers also quantify the relationship between foci development and cell transformation. They indicate that there is a positive relationship between the virus concentration and the number of infected cells until the upper threshold of 0.5% of infected cells is reached (Figure 6b). While Temin and Rubin acknowledge that their method for determining the number of infected cells is not highly accurate, nevertheless this experiment shows that early experiments on the foci assay linked RSV to cancerous transformation ("Characteristics of" 681).

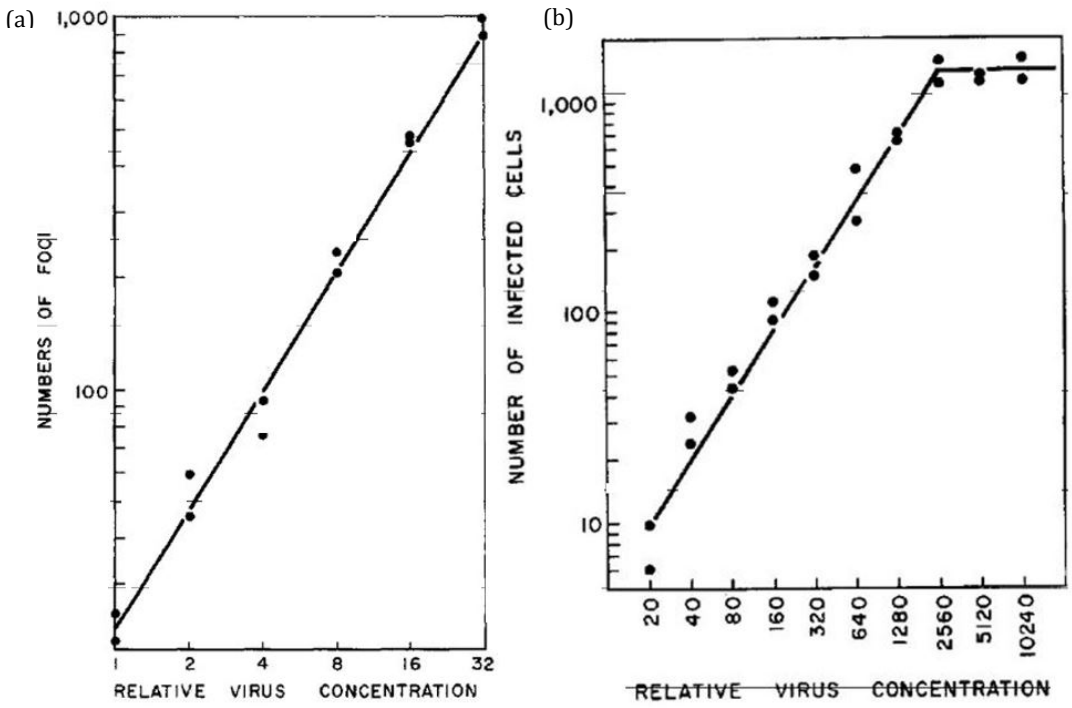


Figure 6a-b. The relationship between relative viral concentration, foci number (a) and number of infected cells (b) using RSV

Graph 6 (a) Virus concentration ranged from 1/80 and 1/2560 of stock. Foci were counted 5 days after infection. (b) Virus concentrations ranged from undiluted to 1/512 of stock. Virus stock was placed on a culture for 30 minutes. Cultures were washed, trypsinized, and layer with various dilutions of infected cell suspensions. After 16 hours, agar was added. Plates were counted 6 days after infection ("Characteristics of" 680-681).

Philosophically, the focus assay is a large departure from previous work on RSV. The focus assay was able to definitively establish the link between RSV and oncogenic transformation. In the words of Howard Temin, these cell culture assays "established that RSV by itself could transform cells and that it transformed fibroblastic cells" ("Neoplastic Transformation" 15). The focus assay was a turning point for tumor virology. Originally scientists were trying to determine whether or not tumor viruses

were living, whether or not they caused "true" cancerous growths, and how they were significant to human cancer cases. These were concerns expressed about RSV. With the introduction of the focus assay, the question now becomes: *how* do tumor viruses induce cancerous growths?

As Howard Temin writes, ultimately the quantification assays "opened the way to the research that has now defined reverse transcription, oncogenes, and proto-oncogenes and has shown that the fundamental connections between retroviral carcinogenesis and all other carcinogenesis" (15). Now, I will briefly outline the contribution of later work on RSV to our current understanding of cancer. This later history outlines the legacy of RSV research after the focus and plaque assays, and the role of RSV in a microbiological understanding of cancer.

4.3 A Brief Overview of Subsequent work on RSV

In their article "100 years of the Rous sarcoma virus," scientists Robin Weiss and Peter Vogt discuss the fundamental role that research on RSV has had on our current understanding of cancer growth. In particular, they look at how research on retroviruses (viruses which use the reverse transcription of RNA to DNA in order to incorporate their DNA into a host's genome) led to the discovery of oncogenes. Oncogenes are genes that have the potential to cause cancer. In human cancers, they are often mutated or overexpressed versions of normal growth regulatory genes (also called proto-oncogenes). When mutated they cause uncontrolled cell growth/division and lead to tumor formation. For example, *ErbB2, P13KCA, MYC,* and *CCND1* are genes often deregulated in human breast cancer cases (Lee and Muller 2). The inactivation of tumor-suppressor genes, or genes that limit cell growth, can also lead to tumor

formation. To again use breast cancer as an example, in hereditary breast cancer cases mutations in the now well-known tumor suppressor genes *BRCA1* and *BRCA2* are associated with an elevated risk of breast and ovarian cancer (Lee and Muller 2). These are now commonly accepted causes of tumorigenesis.

Research on RSV was fundamental to the discovery of retroviruses and oncogenes, and to the importance of genetic mutations in cancer growth. RSV was the first retrovirus to be recognized as a cause of cancer, and *src*, the mutated gene within RSV that is responsible for tumor formation, was the first identified oncogene. Research on RSV began to pick up after the introduction of the plaque and focus assays. RSV, once just a "filterable agent," became recognized as a retrovirus in the 1960s. RSV stimulates uncontrolled cell division within its host, thereby inducing tumor formation through the incorporation of the *src* gene. In 1975 Michael Bishop and Harold Varmus showed that a normal (non-mutated) version of *src* (called *c-src*) is found in the genome of many species besides chickens, and promotes cell growth and cell division within normal cells. The viral *src* led to tumor formation because it was expressed at abnormally high levels within host cells (Chial 33). Research on RSV and *src* was pivotal for our understanding of oncogenes, and later research on DNA tumor viruses led to the identification of tumor suppressor proteins (proteins that suppress cell growth) (Weiss and Vogt 2352). Subsequent work has revealed the importance of somatic mutations (mutations which are not inherited but are later acquired by the cell) in genes which have the potential to become oncogenes and tumor suppressor genes to induce cancer growth. Research on RSV has thus had a pivotal role in the development of a molecular understanding of the mechanisms of cancer growth.

Conclusion

I mention these later developments in research in order to show the importance that RSV studies have had on our understanding of cancer. In this section of my thesis I have discussed the importance of viral quantification techniques to tumor virology. The interaction between the phage school and tumor virology was essential for the development of the plaque and focus assays. Furthermore, these quantification methods are the legacy of the logic tradition of research established by early laboratory scientists in bacteriology and virology. These techniques are in large part responsible for the importance of research on RSV because they proved that cancer and RSV were intimately linked and provided a way for researchers to elucidate this interaction. By looking at the impact of later research on RSV, one can see the lasting impact that these research traditions have had on our current understanding of tumorigenesis and cancer etiology.

Chapter 5: Revisiting *Image and Logic*

Introduction

Thus far I have organized my thesis by looking at the progression of thought, knowledge, and experimentation techniques in the early history of RSV. At the beginning of the 20th century, viruses existed as ineffable entities within filtrates. They were characterized by some as germs, as enzymes or as protein products. In the cell culture assays of the 1950s they became agents of oncogenic transformation and quantifiable microscopic entities. I have shown the lineage of work done on tumor virology. Tumor virology began with early work on bacteriology, progressed through the technological and theoretical advancements of the bacteriophage school, and eventually grew to occupy its own profession. In this section I will discuss the importance of the theoretical, experimental, and technological progression of RSV to our understanding of scientific change, and the theoretical underpinnings of image and logic devices.

5.1 RSV and Trading zones

The differing trajectories of experiment, theory, and technology within RSV suggest the applicability of the intercalated periodization framework that Galison presents within *Image and Logic.* A continuing theme within the history of RSV is the inability of technology, theory, and experiment to coordinate with the other. Often one subunit is "ahead" of another. For example, in the early 20th century filtration devices showed that viruses could induce cancer. The ability of RSV to cause tumors was not accepted, however, until the 1950s. Furthermore, the progression of thought on RSV

was a direct result of the interaction between different scientific groups. For example, the progression of knowledge on viruses and cancer, as well as the advent of viral quantification techniques, were contributing factors to the renewal of interest in RSV. Similarly, the plaque and focus assays for tumor virology were largely borrowed and amended from previous work by bacteriophage scientists.

Experiments done concerning viruses and RSV were physical and metaphorical sites at which laboratory scientists and clinicians interacted. Often, these groups did not have the same understandings of the value of different developments. For example, while to bacteriophage scientists understanding the ability of phage to lyse bacteria was interesting unto itself, clinicians would largely be interested in these experiments for the potential of phage to be used as a therapy for bacterial illnesses. However, the value of concepts changed for different scientific subgroups. For example, research on RSV did not come to hold clinical significance until the advent of further advances in microbiological and genetic techniques showed the importance of mutation to cancer growth. In conclusion, the history of RSV affirms Galison's notion that the interaction of different scientific subsets is important to the progression of scientific ideas and that scientists would negotiate the value of experiments at moments of exchange.

5.2 Image and Logic Devices

A central theme within the history of RSV has been the disparate history of image and logic devices among opponents and proponents of Rous' experiments. The question is: why did some groups of scientists favor logic devices while others did not? Initially, logic devices were the only form of analysis available to laboratory scientists interested in RSV. Viruses escaped all forms of capture. Unlike bacteria they were too

small to be visualized by light microscopy, and could not be grown in culture. The advent of the plaque and focus assay allowed scientists to quantify and characterize tumor viruses, and to link their existence with cancer occurrence. The prominent role of logic devices in early RSV studies suggests the importance of such devices to the early history of microbiological research. Logic devices allowed scientists to go where the eye could not: inside the world of microscopic interaction.

Throughout the history of RSV, image and logic experiments have provided researchers with differing ways to characterize cancer and tumor viruses. Microscopy was integral to Rous' early experiments on RSV. Images of Plymouth Rock hen tumor sections proved that both transplantable, filtrate-induced, and original RSV tumors could be classified as sickle-cell sarcomas with invasive characteristics (Figures 1a-b, 2, and 4). However, it was only in logic experiments that Rous was able to preserve the existence and movement of RSV as a tumor virus. In logic experiments (Figure 3) Rous demonstrates the ability of a filterable agent to induce tumor formation in a majority of chickens. These data led Rous to postulates that cancer was inducible by a filterable agent. The development of quantification techniques in the 1950s continued the lineage of early logic experiments. The plaque and focus assays allowed scientists to quantify viruses and oncogenic transformation for the first time. The focus assay also established a definitive link between RSV and cancerous transformation. A close analysis of the way in which images and logic are used in these experiments reveals the utility of early logic experiments to the development of tumor virology.

The divide between image and logic represents two fundamentally different methods of knowledge retention. Images, for example of tumor sections, are meant to

retain the form of an object in all its complexity. Images are instantaneous, they represent the physical features of an object at a given moment in time. Images of tumors defined early concepts of what cancer was and what it was not. Clinicians and oncologists used images to define whether or not a tumor was cancerous and to determine the cellular origin of cancer metastasis (tumors that have spread outside the boundaries of the original tumor site). The history of RSV also suggests some of the limitations of images. In research on RSV, images were associated with a biological concept of what cancer was but were unable to define cancer in a biomedical or microbiological way.

Unlike image, logic sacrifices form and replaces it with metaphor. Types of logic experiments done on RSV included the use of early filtration devices, quantification assays, and surveys concerning cancer occurrence. Logic relies on the accumulation of data, and on working with available knowledge and techniques to determine characteristics of the unknown. Research on RSV has shown that logic experiments were fundamental to the development of microbiology.

However, later research on RSV also suggests that the delineation between image and logic experiments is not a clear divide. Though mainstream oncologists tended to study tumors by use of images, they were also interested in forms of logic experiments. For example, clinicians were also involved in quantifiable studies which looked at trends in carcinogenesis. Similarly, experimental pathologists extended a culture of quantification mechanisms and favored these over the course of early tumor virology research. However, it was the later development of electron microscopy imaging techniques that allowed laboratory researchers to confirm that viruses were

infectious organisms and not chemicals (Fawcett 740). In practice, researchers may utilize a combination of image and logic devices within one experiment. This has never been as evident as it is now. With the advent of imaging technology, it has become increasingly easier to analyze images in quantifiable ways.

Image and logic traditions represent unique modes of knowledge transmission. However, as image and logic traditions relate to the existing scientific community, different groups of researches did not rely purely on one or the other technique. Rather, we can think about disparate image and logic traditions as being actors in trading zones. Each experimental tradition brings with it its own traditions, forms of knowledge retention, and utility. When researchers use a technique/technology they are engaging with the history and mode of knowledge acquisition inherent to the device. The contact between researcher and device can thus also be characterized as a form of trade.

Conclusion

Galison argues that the differences between scientific subsets adds to the strength and resilience of science. There is enough contact between different scientific traditions such that science at large and differing scientific groups can undergo large shifts in theory and experimentation without losing a sense of the overarching unity of science. This means that points of contact between these groups, or trading zones, are important for the overall strength of science. In my thesis I have shown the importance of interactions between scientific subsets to the history of RSV, that this history can be characterized by image and logic traditions, and that image and logic devices represent different modes of knowledge acquisition and retention.

Conclusion

In my thesis I have explored the history of early experimentation on RSV and the development of image and logic traditions among scientists who were proponents and opponents of the extrinsic theory of cancer etiology. Cancer was first defined as a physical and biological condition. Experiments dealt broadly with who had it and who did not, how it appeared, and whether it was transplantable. Research on RSV, research on other tumor viruses, and the development of logic traditions within tumor virology eventually led to a molecular understanding of cancer etiology. In this conclusion I will elaborate upon the arguments I have made earlier in this thesis, discuss the importance of *Image and Logic* to RSV, and propose possible future directions for this research.

Analyzing the interaction of distinct image and logic traditions is an important development for our current understanding of the history of RSV. The divide between extrinsic and intrinsic has already been explored by multiple historians, including Eva Becsei-Kilborn, Neeraja Sankaran, and Darwin Stapleton. However, what these histories lack is a detailed account of the relationship between scientific groups and the way that they look at data. By looking at the differences between the intrinsic and extrinsic groups along the lines of image and logic experimentation techniques, we begin to get at a deeper understanding of the fundamental difference in the way that clinicians and laboratory experimenters were collecting and communicating scientific data.

In this thesis, the early history of experimentation on RSV has revealed inherent differences in the way that scientific data is organized in an image and logic system. Images work instantaneously. An image establishes information about cancer by

capturing one moment within cancer progression. For example, images are able to show whether or not a cancer has metastasized at a given moment in time. In contrast, logic experiments work through the accumulation of data and the establishment of a complex relationship of symbols and logical relationships. For example, in Rous' 1911 publication he conveyed the ability of filtrates to induce cancerous growths by looking at the frequency of cancerous tumors within chickens inoculated by filtrate. The difference between image and logic as modes of knowledge transmission indicate that clinicians and laboratory scientists developed not only a theoretical divide (extrinsic vs intrinsic) but were also organizing data in a fundamentally different way.

While a distinct lineage of image and logic traditions emerged in the early part of the 20th century, the divide between the two is imperfect. Rous, for example, used both image and logic techniques. Many clinicians also engaged in forms of data accumulation or logic experiments like carcinogenesis studies. Furthermore, as John Ziman points out in his review of *Image and Logic*, image and logic traditions may not be as epistemologically distinct as Galison presents in *Image and Logic* (Ziman 292). For example, he argues that there may be multiple logical prepositions which are used to analyze an image. In RSV studies, the conclusion that RSV tumor segments were examples of sickle-cell sarcomas required the pre-establishment of what sickle-cell sarcomas were and then the identification of tumor segments as such. Furthermore, these images were used in order to show that filtrate inoculated tumors were "true" tumors which, one could argue, is the continuation of a logical argument.

These discrepancies certainly complicate the epistemological distinction between image and logic. However, Galison's discussion of image and logic as two

significantly different modes of knowledge transmission is ultimately useful to our understanding of the early history of RSV. In his review, Ziman concludes that in the history of physics, image and logic experiments produce essentially the same type of knowledge just in differing forms. I would argue that there is a fundamental difference between the wasy in which images and logic are used within early experimentation on RSV. There is a central difference between images of tumor segments, which rely on intuition and interpretation to produce meaning, and Rous' charts, which build and lay out knowledge through the accumulation of multiple data points. The history of RSV does suggest that the image/logic divide may have been, in practice, a less distinct boundary than the image and logic traditions in *Image and Logic*. Furthermore, later developments in electron microscopy would largely integrate the image and logic traditions by allowing for the logical processing of images. However, ultimately images and logic did represent two fundamentally different modes of knowledge transmission that had different utilities to clinicians and experimental biologists within early 20th century research on RSV.

Historically, I have discussed the importance of different image and logic traditions to the development of tumor virology and microbiology. Philosophically, I explore how images and logic represent different forms of knowledge transmission. In my thesis I have discussed how the types of experiments which scientists engaged in were related to the questions they asked, the type of data they used, and the theories they proposed. Instead of focusing simply on theoretical advancements, this type of historical analysis suggests that the relationship between scientists and experimentation techniques has important philosophical, historical, and theoretical implications.

There are many aspects of experimentation on RSV in the early 20th century still to be elucidated. I chose in this thesis to focus on the intrinsic interaction of scientific subgroups. Further analysis may link these intra-scientific interactions to society at large. Another direction for further research would be to continue our discussion of intercalated periodization. The differing rhythms of experimentation, theory, and technology has been a theme throughout the history of RSV. However, there is room to delve further into analyzing the benefits and characteristics of these differing rhythms. Ultimately, I hope to have shown that by understanding how the image and logic framework relates to the extrinsic/intrinsic divide, we gain an understanding about differences in the way in which differing scientific groups were collecting and communicating scientific data. While *Image and Logic* has been discussed in physics and economics, it has not often been applied to a biology. In this thesis I hope to have provided an argument for the utility of Galison's philosophical framework to a biological history of scientific change, and to provide a baseline for further research on *Image and Logic* in biology.

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