METCHNIKOFF AT ROCKEFELLER: A LEGACY OF PHAGOCYTE- MICROBIAL INTERACTIONS

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Background.

I owe the following information to Carol L Moberg, a biographer of Dubos [1] and historian of Rockefeller [2], who remained associated with the laboratory under the subsequent direction of Hirsch, Cohn and Steinman. Figure 1 shows Elie Metchnikoff seated at a desk. The signed photograph was taken by the famous French photographer Nadar in 1908, the year of the Nobel Prize, shared with Paul Ehrlich. The original photograph was given to Simon Flexner when he visited the Pasteur Institute in 1909. Flexner, Rockefeller's first Scientific Director, was searching for new scientific fields to open at the Institute. He not only learned about phagocytosis there, but went on to Germany after this visit, presumably to visit Paul Ehrlich. In July 1909 he published an article "Natural Resistance to Disease" where he describes both the phagocytic and humoral doctrines of bacterial destruction. At the time Flexner was the sole editor of the Journal of Experimental Medicine (JEM) from its first Rockefeller issue in 1905, until he appointed as editors Peyton Rous in 1922, the second Scientific Director of the Institute, Herbert Gasser, in 1936, and Rene Dubos in 1946. Moberg speculates that the portrait must have hung in the Scientific Director's office or next door Board Room until it passed to Dubos on Gasser's retirement in the early fifties.

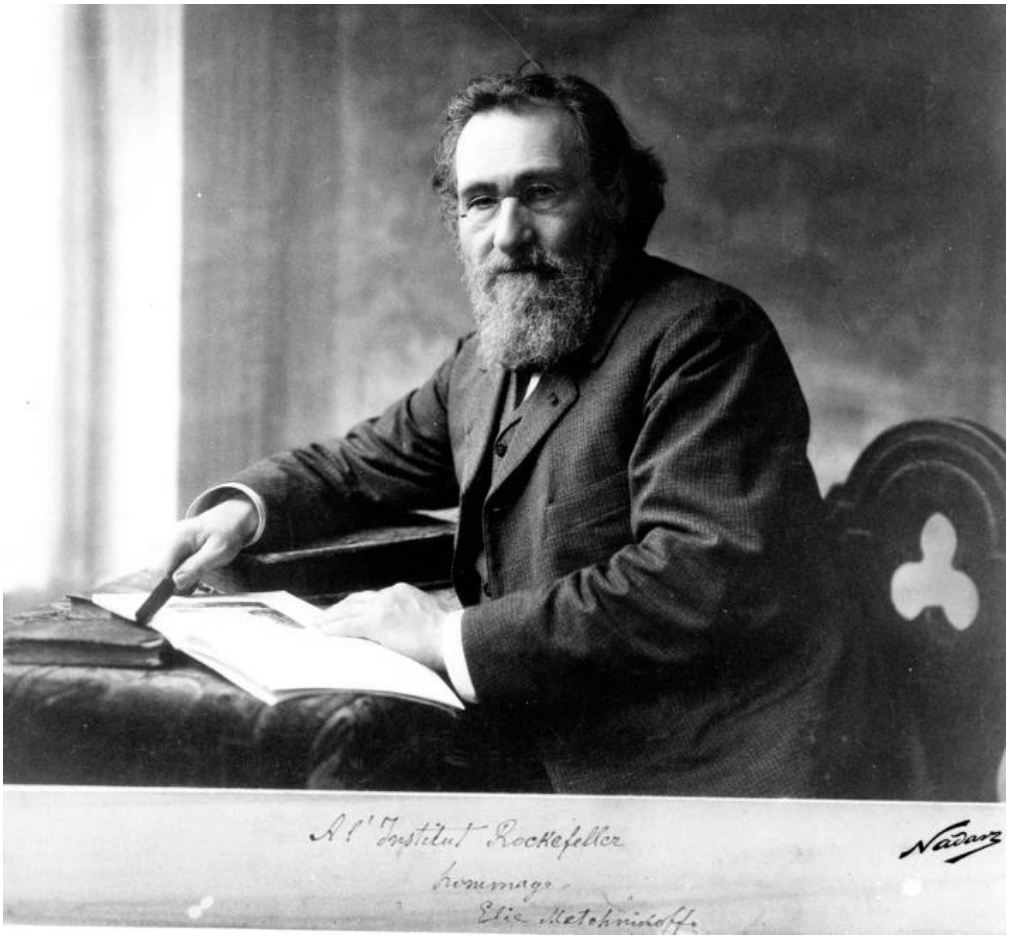

Fig 1. The portrait of Elie Metchnikoff, 1908, inscribed to Rockefeller Institute. Photograph by Nadar.

There are two significant articles on Metchnikoff, one by Dubos in 1955 [3], and another by the next JEM editor James Hirsch in 1959 [4]. They are interesting because they provide a rationale why Zanvil Cohn in 1960, the next JEM editor, began to study macrophages and phagocytosis. The Metchnikoff portrait passed to and was hung in Hirsch's office as Dean and then in Cohn's office until his death in 1993.

On an earlier occasion than Flexner, William Welch, a bacteriologist from John's Hopkins who championed the establishment of the Rockefeller Institute (RI), had also visited Metchnikoff at the Pasteur Institute. After its establishment, Rous, the discoverer of the sarcoma virus named after him, used magnetic particles to isolate phagocytic macrophages from liver (Kupffer cells) by an elegant method in 1934. Florence Sabin, also from John's Hopkins, who became the first female Member of the

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Institute in 1925, performed early studies on monocytes and macrophages in Tuberculosis. In his popular book Microbe Hunters, first published in 1926, Paul De Kruif who had worked briefly at RI, included a chapter on Metchnikoff, entitled "The nice phagocytes".

RENE DUBOS, born in 1901 and educated in Paris, migrated to the USA in 1924 and completed a doctorate at Rutgers University in New Jersey under Selman Waksman three years later (Figure 2). He had been inspired to become a soil microbiologist by Sergei Winogradsky, a Russian botanist and chemist, then at the Pasteur Institute. He was not eligible for an American postdoctoral fellowship, but was referred to Alexis Carrell a fellow Frenchman, at the RI. A chance meeting with Oswald T Avery led to a Fellowship in the RI Hospital, to study pneumococcal capsular polysaccharide, an important virulence determinant. He identified an induced bacterial enzyme that was able to degrade this complex saccharide, making the organism susceptible to phagocytic recognition. Dubos became an associate member of the RI, studying bactericidal agents from soil microbes, and by 1939 he was able to report the discovery of the first antibiotics, tyrothricin, tyrocidine and gramicidin, which unfortunately were too toxic for clinical use [5]. He progressed to membership at the Institute and was elected to the National Academy of Sciences by 1941. He left for 2 years at Harvard, but returned to the RI to establish his own laboratory in 1944, in order to study tuberculosis.

This choice of subject was partly in response to the death of his first wife from reactivation of this disease; he wrote a popular history, The White Plague, devised the widely used Dubos medium for in vitro growth of the organism, promoted standardisation of BCG vaccine and became interested in the host's contribution to reactivation of latent infection by environmental and other stressful stimuli. Dubos disagreed with Claude Bernard ("constancy of homeostasis") after his earlier demonstration of the ability of bacteria to adapt to altered circumstances by expressing novel properties, which he termed "adaptive enzymes". He changed the emphasis in his laboratory to the study of anaerobic gut organisms, and the microbiome of today, on the basis of his earlier experience that organisms should be studied in their natural habitat rather than in a culture dish. Experimental mouse studies explored the links between the microbial flora and the importance of early nutrition and other external influences on subsequent development and adult health.

In addition to an impressive career as microbiologist, Dubos wrote an historic biography of Louis Pasteur, and many books and other writings on environmental issues; his emphasis was on the ecology of landscape and human environment, natural and urban, rather than on pollution per se, well ahead of his time. He remained a strong champion of contributory host factors to infectious disease, including, rather than focussing on microbes as the sole cause.

Dubos became a professor in 1957 at the now Rockefeller University and an Emeritus in 1971, continuing as an international spokesman for environmental issues. He had rheumatic heart disease with life-long disability, contributing to his death in 1982; however, he was always outgoing, with a warm personality, as I personally experienced when joining the Cohn/Hirsch laboratory in 1966, by which time Dubos was a benign eminence, sequestered in his office, more concerned with his broader role as elder statesman.

There are many points of resemblance between Dubos and Metchnikoff. Apart from their shared admiration for Pasteur and personal brush with tuberculosis in the family, Dubos fulfilled Metchnikoff's enthusiasm for the importance of the intestinal flora as symbiotic organisms promoting health, rather than merely pathogenic invaders [6]. Dubos frequently warned clinicians that abuse of broad spectrum antibiotics would result in the emergence of drug-resistant pathogens. Reminiscent of Metchnikoff's own book on recovering optimism after depression, Dubos wrote a regular column in the American Scholar as a "Despairing Optimist". He fostered Metchnikoff's work on phagocytes in the laboratory by James Hirsch and Zanvil Cohn (Figure1); influential papers appeared in the Lancet in 1955 by Dubos "The micro-environment of inflammation or Metchnikoff revisited" [3] and by Hirsch, "Immunity to infectious diseases: review of some concepts of Metchnikoff " in 1959 [4].

The legacy of Metchnikoff was always apparent in the philosophy and experimental strategy in the laboratory, dealing with topics such as host-pathogen interactions, phagocytosis, inflammation, cell recruitment, and intracellular digestion. The approach was to study physiological and pathological processes in humans where possible, and to study experimental models of infection in situ and ex vivo. Research was supplemented with studies in cell culture, closely observed by phase contrast and electron microscopy, and biochemical analysis, as appropriate methods became available. The group benefited from the strong growth of cell biology at Rockefeller, described in detail by Moberg [2]. George Palade and Christian de Duve, Nobel laureates in cell biology, had particular impact through their cell fractionation and biochemical approaches. These were combined with the concurrent development of sensitive immunochemical and biochemical assays which could be applied to the limited amounts of material available from animal or human sources. The results shed light on endocytosis, receptor mediated phagocytosis, lysosomal digestion, membrane dynamics, cell secretion and degranulation. Perhaps most important was the nomenclature of the Mononuclear Phagocyte System, and discovery of the unique efficiency of Dendritic Cells. These highly specialised antigen presenting cells were able to activate naive T and B lymphocytes, bridging the gap between innate natural and acquired humoral and cell mediated immunity, and reconciling the schism between Ehrlich and Metchnikoff.

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René Dubos (1901-1982)

James Gerald Hirsch (1922-1987)

Zanvil A Cohn (1926-1993)

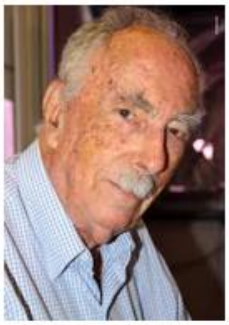
Ralph Steinman (1943-2011)

Fig 2. Directors of the microbial-host leukocyte laboratory at Rockefeller: honouring the life and work of Elie Metchnikoff 1944-2011.

From microbes to host leukocytes

The Hirsch-Cohn laboratory initiated a change in focus to the role of host leukocytes in infection, laying the groundwork for advances in myeloid cell immunobiology over the following half a century, and culminating in the 2011 award of a Nobel Prize to Ralph Steinman, their successor (Figure3).

Ralph van Furth



Michel Rabinovitch

Samuel Silverstein

Gilla Kaplan

Carl Nathan

Siamon Gordon

Fig. 3. Selected members of the Hirsch/Cohn laboratory, early 60's to late 70's.

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Apart from the laboratory heads, several of their colleagues continued to provide direct links to the Metchnikoff tradition (Figure3). Michel Rabinovitch, from Brazil, spent fruitful years at Rockefeller, before further spells at the Pasteur Institute and eventual retirement in San Paulo. He coined the term "professional phagocytes" to distinguish the superior ability of myeloid leukocytes to capture bacteria and other intracellular pathogens compared with amateur phagocytes. Samuel Silverstein performed elegant experiments with antibody and complement-coated erythrocytes to elucidate the role of opsonic receptors for Fc and C3bi in a "zipper" model of ingestion by mouse peritoneal macrophages. Hirsch and his collaborators Marcus Horwitz and Thomas Jones used electron microscopy to describe the remarkable coiling method of entry by Legionella, and inhibition of phagolysosomal fusion during uptake of Toxoplasma, respectively. Hirsch produced classic videos of how rabbit polymorphonuclear leukocytes hunted down bacteria and the explosive degranulation of eosinophils and other granulocytes during uptake of yeast particles.

Cohn studied the permeability of macrophage lysosomes to digestion products after endocytosis and with Steinman and students, the recycling of plasma membrane to the cell surface after ingestion. Cohn and Ralph van Furth, a Dutch visitor, used newly available Tritium isotopic labelling to study the production and tissue distribution of monocytes derived from the mouse bone marrow. Like many macrophage experimentalists, Cohn eventually turned to studies with Gilla Kaplan on human tuberculosis and leprosy, developing a taste for travel to exotic countries like India, to study these diseases in situ. Carl Nathan made important contributions to the activation of macrophage micobicidal activity through Interferon gamma, a lymphokine that induced NO radical production via inducible Nitric Oxide Synthase. When the AIDS epidemic struck, Cohn initiated studies on HIV-1 infection of human monocyte-derived macrophages, which remained an interest of the laboratory with Steinman. It was during the mid 80's when Steinman and Cohn described the unusual morphology of Dendritic Cells in mouse spleen and human mononuclear cells, and their potent role in antigen capture, processing and presentation, thus activating the adaptive immune system. After about a decade in the wilderness of scepticism by many immunologists, this discovery of DC grew exponentially and worldwide.

Tragically, Cohn did not live to share the Nobel award to Steinman. During its hayday, the Rockefeller group was the premier laboratory driving progress in the field, as can be gleaned from their output (7-9). The quadriennial meetings hosted by Van Furth in Leiden and elsewhere in Holland between the late 60's and early 90's were gatherings to review progress in the field, with fierce discussion at times, and published volumes to record the state of the field (10). As a young independent investigator, it was a privilege for me to be part of this community of friends and colleagues and to establish my own independent research group in Oxford in 1976. I owe my own life long interest in macrophages and in the life and legacy of Elie Metchnikoff to my apprentice years and mentors at Rockefeller University who welcomed me into their world.

Acknowledgement.
I dedicate this memoir to Fern Cohn, nee Dworkin (1926-2020).

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Metchnikoff at Rockefeller: a legacy of phagocyte-microbial interactions

Siamon Gordon, William Dunn

In 1909, Elie Metchnikoff presented a signed portrait of himself to Simon Flexner, the first scientific director of the newly formed Rockefeller Institute in New York. It was eventually passed on, at Rockefeller, to Rene Dubos, a French microbiologist, and then to his successors, James G Hirsch and Zanvil A Cohn; Metchnikoff's spirit continued to pervade the laboratory directed subsequently by Ralph M Steinman. Over 50 years, 1960-2010, this became one of the premier research laboratories in the world to investigate the immune interactions of microbes with granulocytes, monocyte-macrophages and dendritic cells, respectively, at all times under the benign presence of Metchnikoff. I was fortunate to join the laboratory as a student of Zanvil Cohn during 1966-1976, which initiated me into a life-long love affair with macrophages, lasting to the present day at Oxford. In this year of his 175th birthday, I would like to record the legacy that Metchnikoff left to my generation during that period at Rockefeller University.

Keywords: I.I.Mechnikov, Phagocyte-microbial interactions, history

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