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PREFACE

A microbiologist aspires to do for microbiology what Degas did for ballet. The admired beauty lies beyond the artist's discipline as he or she illuminates exquisite fragments of a whole that cannot be captured. Selection of exquisite fragments of microbiology is the pleasurable task of the editorial committee, which was assisted in its deliberations for this volume by guests Irving Crawford and E. Peter Greenberg. Richard Blakemore retired from the committee after the planning of Volume 40, and we shall miss his wise counsel.

The editorial committee for Volume 40 welcomed a new member, George Cross, whose presence reflects increased emphasis on parasitology. An innovation in this volume, already welcomed by the editors, is the addition of article titles in the references that follow each review..

Andrea Perlis joined us as Production Editor for this volume, which benefited from her organizational flair and sharp editorial eye. As always, the continuing functioning of my own office depends upon the extraordinary abilities of my administrative assistant, Anne Harrison.

L. NICHOLAS ORNSTON
EDITOR

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SPECIAL PUBLICATIONS

Annual Reviews Reprints: Cell Membranes, 1975–1977
Immunology, 1977–1979

Excitement and Fascination of Science, Vols. 1 and 2

Intelligence and Affectivity, by Jean Piaget

Telescopes for the 1980s

For the convenience of readers, a detachable order form/envelope is bound into the back of this volume.

OTHER REVIEWS OF INTEREST TO MICROBIOLOGISTS

From the *Annual Review of Biochemistry*, Volume 55 (1986)

Mutants in Glucose Metabolism, Dan G. Fraenkel
Transcription Termination and the Regulation of Gene Expression, Terry Platt
Double-Stranded RNA Replication in Yeast: The Killer System, Reed B. Wickner
Bacterial Periplasmic Transport Systems: Structure, Mechanism, and Evolution, Giovanna Ferro-Luzzi Ames
Biological Catalysis by RNA, Thomas R. Cech and Brenda L. Bass
Nonviral Retroposons: Genes, Pseudogenes, and Transposable Elements Generated by the Reverse Flow of Genetic Information, Alan M. Weiner, Prescott L. Deininger, and Argiris Efstratiadis
Discontinuous Transcription and Antigenic Variation in Trypanosomes, Piet Borst

From the *Annual Review of Biophysics and Biophysical Chemistry*, Volume 15 (1986)

Halorhodopsin: A Light-Driven Chloride Ion Pump, Janos K. Lanyi
Active Transport in Escherichia coli: *Passage to Permease*, H. Ronald Kaback

From the *Annual Review of Cell Biology*, Volume 2 (1986)

Cotranslational and Posttranslational Protein Translocation in Prokaryotic Systems, Catherine Lee and Jon Beckwith
Control of Synthesis and Assembly of Ciliary Proteins, Peter Lefebvre and Joel Rosenbaum
Proton-Translocating ATPases, Qais Al-Awqati

From the *Annual Review of Entomology*, Volume 31 (1986)

Microbial Control of Black Flies and Mosquitoes, Lawrence A. Lacey and Albert H. Undeen

From the *Annual Review of Genetics*, Volume 20 (1986)

The Early Days of Yeast Genetics: A Personal Narrative, Herschel Roman
Genetics of Endospore Formation in Bacillus subtilis, Richard Losick, Philip Youngman, and Patrick J. Piggot
Ribosomal Genes in Escherichia coli, Lasse Lindahl and Janice M. Zengel
Genome Reorganization in Ciliated Protozoans, Elizabeth H. Blackburn and Kathleen M. Karrer

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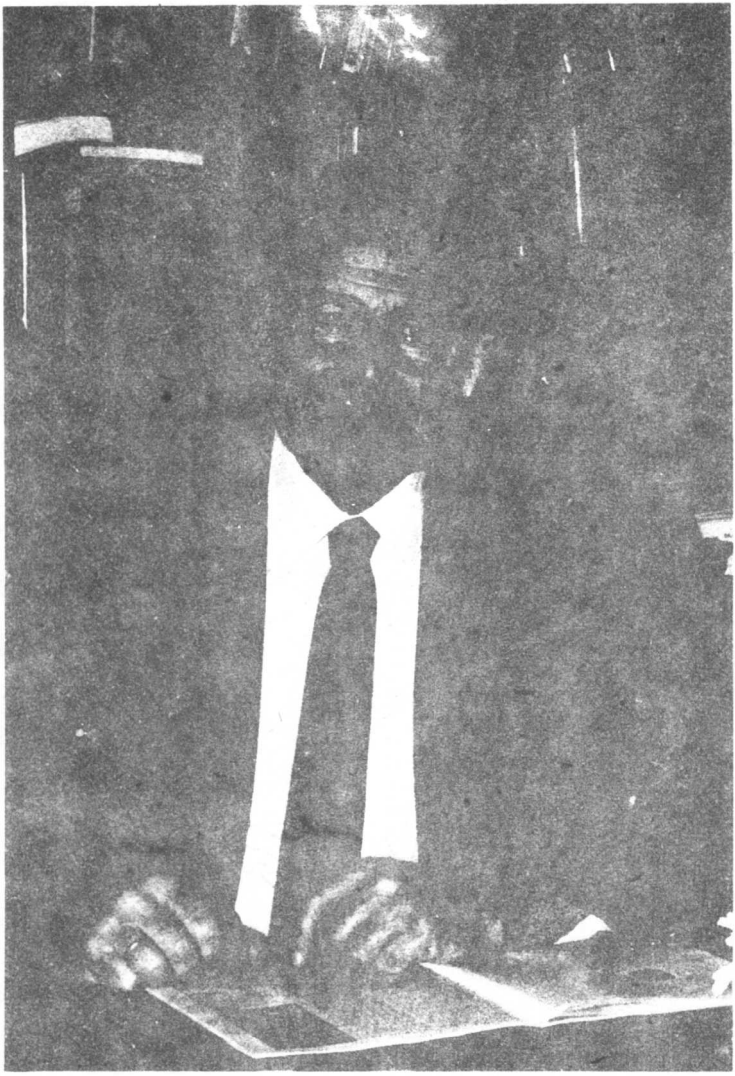
From the *Annual Review of Immunology*, Volume 4 (1986)

From the *Annual Review of Medicine*, Volume 37 (1986)

From the *Annual Review of Phytopathology*, Volume 24 (1986)

From the *Annual Review of Plant Physiology*, Volume 37 (1986)



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MY LIFE WITH YEASTS

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To Marinka, 1916–1985

EARLY DAYS IN THE NETHERLANDS

I was born on May 30, 1913 in the small city of Winschoten in the northeastern province of Groningen where I attended grammar school and high school. My mother came from a farming family and my father was one of five sons of my grandfather who operated a fruit winery and vinegar factory. When he retired one of his sons took over the vinegar and distilled beverage operation while my father and his brother Jacob started and developed a separate business for the production of fruit wines, mainly from apples and berries. My

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father was interested in the trade and sales aspects and my uncle Jacob handled the technical and production end of the business. Both of my parents died at an early age while I was still in the beginning of the five-year high school curriculum. My brother and I were very fortunate to be adopted by my uncle Jacob's family.

I was happy in my new environment, which had a profound influence on my later life. I thoroughly enjoyed observing and participating in the operations of the winery, especially during the crushing, pressing, and fermentation stages. Long before I received my first formal training in microbiology, I learned how to use a microscope (a monocular, of course, in those days of the mid 1920s), to observe the budding and growing of the yeast cells, and to determine the alcohol content of the fermenting fruit juices. I also witnessed for the first time the spontaneous and massive development of acetic acid bacteria in a batch of berry juice pressed from overripe fruit that had begun to ferment by the indigenous yeast flora on the berries. Through microscopic examination of the spoiled juice I observed the coexistence of yeast and acetic acid bacteria.

My high school years also contributed in a major way to another aspect of my life, namely my interest in music. After I unsuccessfully attempted to study piano our family doctor, who was very active in the local symphony orchestra, suggested that I learn to play the cello as there was always a shortage of cellists in the orchestra. Fortunately, there was an excellent teacher in my hometown whose major instrument was the cello. I soon fell in love with my new activity and often practiced several hours each day. I have maintained this interest up to the present and have played in many symphony orchestras and numerous chamber groups. I might add that through musical activities I have probably made a greater diversity of friends than through scientific contacts.

The Dutch high school system with its five-year curriculum was considerably more rigorous in its requirements than its American counterpart, as I learned later on. Five years of German and French and four years of English were mandatory (there were no elective courses). These studies, combined with in-depth training in mathematics, chemistry, and physics and a modest amount of biology, culminated in a nationally administered final examination consisting of both written and oral parts. Passing of this examination automatically conferred eligibility to enter any of the national universities of the Netherlands.

In retrospect, considering my later interest in mycology, I would have had more useful training had I attended the local gymnasium with its six-year curriculum. This system offered, in addition to the subjects taught in high school, in-depth training in Latin and Greek. These languages would have been useful to me because new taxa of yeasts and fungi (falling under the Botanical Code) require descriptions in Latin for their publication to be valid.

At any rate, when in 1932 the time came to go to college I chose the Technical University of Delft, which offered various curricula in engineering. I majored in chemical engineering, mainly because of the eminence of A. J. Kluyver, Professor of General and Applied Microbiology, which was at that time a subdivision of chemical engineering. Kluyver's prominence and that of his famous predecessor Martinus Beyerinck had already come to my attention. Their reputations, combined with the possibility that I might later enter the family winery, made the training offered at Delft seem particularly appropriate. It was not, however, until I had taken numerous courses in chemistry, mathematics, physics, and engineering that I was allowed to choose an area of specialization within the field of chemical engineering. I chose technical microbiology so that I could benefit from Kluyver's leadership in that field.

After being advanced to candidacy at the end of four years of study, each student was required to carry out a research project and prepare a thesis (somewhat comparable to the American MS thesis). I worked on the elaboration of extracellular pectin-hydrolyzing enzymes by fungi. This subject was of interest to me because such enzymes (of commercial origin) were used in my family's winery to obtain higher juice yields during pressing and to facilitate filtration as a result of hydrolysis of the viscous fruit pectins. Although the scientific results of my study were limited, the work was important in that it stimulated my scientific curiosity. A significant factor was the direct personal interest Kluyver took in the research of all of his students and postdoctoral visitors. I also had the privilege of becoming acquainted with Charles Clifton, Robert Starkey, and Ben Volcani, who were guests in the laboratory at that time.

DEPARTURE TO CALIFORNIA

Kluyver was a great admirer of microbiological research carried out in the United States; his enthusiasm and my own curiosity about life in that country were instrumental in my decision to do postgraduate work there. The Division of Fruit Products (later known as the Department of Food Science and Technology) at the University of California, Berkeley appeared to be most appropriate for my area of interest and was willing to accept me as a visitor. Kluyver suggested that someone from Delft, a well-known center for research on the systematics and physiology of yeast, better obtain some training in the taxonomy of yeasts before going to California, so I spent several months in the fall of 1938, after having earned my degree of Chemical Engineer, learning something about the principles and practical aspects of identifying yeasts.

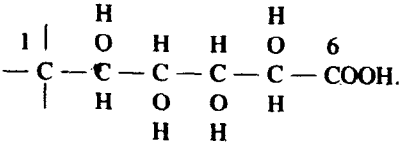
Upon my arrival in Berkeley in early 1939, I met Professors Cruess, Mrak, and Joslyn and other faculty in the department and I began participating in

some of the ongoing research projects. Maynard Joslyn soon suggested that to get the most out of my stay at the University of California I should continue my formal education and obtain a PhD in microbiology to supplement my earlier training in chemistry. I was fortunate to have entered the United States with an immigration visa, which was easy to obtain at that time, because the Second World War broke out the same year and the Netherlands was occupied a year later. I was able therefore to remain in California and to continue my education in the fall semester of 1939. Most of my supplementary course work was in biology and biochemistry, subjects in which my previous training had been deficient.

During my graduate years I had the privilege of becoming Emil Mrak's research assistant. The position was funded by the Agricultural Experiment Station, of which the department was a part. We isolated and identified the yeasts responsible for the spoilage of figs and dates in orchards of central and southern California (27, 28). This was my first foray in the area of yeast ecology and taxonomy.

University regulations stipulated at that time that Experiment Station research done under a research assistantship could not be credited for the PhD dissertation, so I chose Maynard Joslyn to guide my thesis research, which dealt with the elaboration of pectic enzymes by *Penicillium chrysogenum*. Midway in the research Professor H. A. Barker became my mentor because of Joslyn's absence during his service in the Army. "Nook," as he was affectionately called by his friends, became one of my lifelong friends. He had also spent a year in Kluyver's laboratory, but before I began my research there.

My thesis research showed that *P. chrysogenum* excreted in the medium two inducible hydrolytic enzymes required for the breakdown of pectin; pectin esterase (PE) removed methanol from pectin and polygalacturonase (PG) cleaved the glycosidic linkages of the polygalacturonic acid chain formed by the esterase. For their synthesis both enzymes responded simultaneously to a small number of inducers that included pectin, polygalacturonic acid, D-galacturonic acid, mucic acid, and L-galactonic acid, but not D-galactose, galactitol, or L-galacturonic acid. I concluded that the following configuration is essential for the synthesis of the two enzymes:



Substitutions on carbon 1 (aldehyde, alcohol, or carboxyl) did not change its specificity but the remainder of the stereochemical configuration was essential

for induction (32). At that time little was known about the induction of enzymes (then referred to as "adaptation") but evidently compounds containing the above configuration were able to bind to a repressor and to inactivate it, making transcription and elaboration of the enzymes possible by the derepressed system.

Among the highlights of my graduate career was the annual spring seminar at the home of Professor C. B. van Niel in Carmel, where students in microbiology from the Berkeley campus met with van Niel's students of the Hopkins Marine Station at Pacific Grove and presented informal talks about their research. Professor van Niel, the first and most prominent student of Kluyver, was a great inspiration to the graduate students as Barker had so aptly described earlier (4). There I also met for the first time the late Michael Doudoroff, who later became one of my best friends. These seminars were important not only because of their scientific content, but also because they taught us how to approach and analyze scientific problems critically.

By 1943 I completed my research for the PhD and I began applying to various universities for a faculty position (postdoctoral training was not common in those days). During my graduate student years I had already realized that my early plan to return to the Netherlands, even after the end of the war, and enter the family winery business was no longer attractive and challenging to me. I saw much greater opportunities for a scientific career in the United States. During the period of job applications, however, Professor W. V. Cruess, then chairman, offered me a position in the Department of Food Technology. This practice of hiring someone in the same department where he had obtained his PhD degree, without extensive advertising of the position, was not uncommon in those days. I gladly accepted the offer and have remained with the department until the present.

A FACULTY POSITION AT BERKELEY, 1943–1953

My early research years were rather heavily devoted to the development of improvements in the dehydration of fruits and vegetables, activities necessitated by the war and sponsored by the Departments of the Army and Navy. Under the leadership of Emil Mrak we developed a steam-blanching process whereby apricots, peaches, and pears could be efficiently dried in dehydraters (31) rather than by the traditional slow and sometimes unsanitary sun-drying process. Advances were also made in the dehydration of vegetables, particularly in the effective inactivation of oxidative enzymes that could cause early discoloration and off-flavor production in storage at elevated temperatures (33). We also developed a process for sterilizing dried prunes, figs, and dates of high moisture content with ethylene or propylene oxide so that the fruit could be consumed directly from plastic packages. Although these epoxides

are excellent germicidal compounds and decompose rapidly into their respective glycols, the Food and Drug Administration decided after a number of years that potentially harmful products could be formed by epoxides reacting with fruit components. While we were testing the killing action of ethylene oxide on a strain of *Saccharomyces cerevisiae* we discovered that when most cells had been killed the residual population contained a high proportion of cells that upon plating produced smaller colonies than those produced by the parental strain. The variant differed from the parental strain only in its inability to respire glucose or ethanol and its lack of cytochrome oxidase (62). We thus discovered nonrespiratory mutants of *S. cerevisiae*, and can only blame ourselves for not following up on this observation as Ephrussi and co-workers did later on (9a).

In 1948 I married Marinka Boratynski after failure of an earlier marriage from before my immigration to the United States. In the fall of 1951 I took my first six-month sabbatical leave and returned with my wife and young stepdaughter Sasha to my alma mater in Delft. The reason for this choice was that Mia Lodder and Nel Kreger–van Rij were preparing the first comprehensive treatise on the taxonomy of all yeasts known at that time (18a). The extensive collection of yeast strains at the Yeast Division of the Centraalbureau voor Schimmelcultures (CBS) stored at the Delft Laboratory for Microbiology and the inspiring leadership of Professor A. J. Kluyver made this enormous task possible. For me, it was a valuable and rewarding experience to review the manuscript in preparation and to update my knowledge of the advances and status of yeast taxonomy. I also started a small project on the reasons for the delayed fermentation of sucrose by osmophilic, haploid species of the genus *Saccharomyces (Zygosaccharomyces),* which I later published with D. Pappagianis (29), then a student in the Department of Food Technology at Berkeley. Besides providing scientific value, my leave gave me the opportunity to reestablish family ties that had almost been totally disrupted during the Second World War, which unfortunately had claimed a number of casualties among members of my family as a result of their activities in the resistance movement.

While in the Netherlands I received a letter from the dean of the College of Agriculture in Berkeley with the announcement that the Department of Food Technology was going to be transferred to the Davis campus of the University of California. Although space would be immensely improved in the new building, I had considerable misgivings about the move to a small campus with very little cultural activity and few strong science departments. In addition we had just built a house in Berkeley in an attractive location and we would have to go through this process once more in the flatlands of the Sacramento Valley.

Nevertheless, upon our return to Berkeley in 1952 we began planning for

the move to Davis. Through my wife Marinka's initiative we obtained the service of architect Rowan Maiden, a protégé of Frank Lloyd Wright, who designed a most interesting house which was completed in July of 1953. Since the official departmental move was made on July 1, 1952, I commuted to Davis by train three times weekly to fulfill my teaching obligations while continuing my research in Berkeley in my old departmental laboratory.

Several years before the move to Davis, Emil Mrak had become chairman of the department, succeeding William V. Cruess; as a result the time available to Mrak for laboratory work became progressively less, although his interest in yeast ecology and taxonomy remained strong. In 1948 and 1949 Mrak and I had published two extensive reviews on yeast dealing with broad biological aspects and with sexual reproduction and ascosporulation (26, 43, 43a). Together, we had been teaching a one-semester upper-division course with laboratory at Berkeley that was totally devoted to the taxonomy, ecology, and physiology of yeasts—one of the few or perhaps the only course of this kind in the United States. We transferred the course to Davis, made it a graduate course, and continued to be its instructors.

CONTINUATION OF MY CAREER AT DAVIS

At the time of my transfer to Davis I obtained tenure and decided to concentrate my research efforts in the broad area of yeasts and to disassociate myself from the applied research pertaining to dried fruits. Although the latter with its field work was enjoyable and productive, it virtually eliminated the summer for basic research and writing.

I was fortunate in my final years in Berkeley to attract three outstanding graduate students for the PhD program in microbiology: Bor Shiun Luh, a native of China, Moshe Shifrine, a native of Israel, and Arnold Demain from Michigan. Luh and I began a survey of various yeast species for the production of pectic enzymes. Because many species of filamentous fungi were known to produce these enzymes (including *Penicillium chrysogenum*, which I had used in my own doctoral research) we were interested to determine if there might be yeasts endowed with this ability and, if so, to characterize such enzymes. Screening was based on the ability of the various yeast species to clarify a pectin medium containing 0.2% glucose. *Saccharomyces fragilis* (later reclassified as *Kluyveromyces fragilis*) was one of the very few yeasts with this ability but the pectin molecule was only very slightly degraded. Polygalacturonic acid was hydrolyzed more extensively but not to completion. The reason for the very limited pectin hydrolysis was found to be the inability of *K. fragilis* to synthesize pectin esterase. Not only did the yeast polygalacturonase require de-esterified pectin as substrate, but we found that the end products consisted of tri-, di-, and monogalacturonic acids rather than