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PLASTICS-IRRADIATED-ETCHED: THE NUCLEPORE® FILTER TURNS 45 YEARS OLD

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PROLOGUE

It has been said that progress in aquatic sciences is limited by the availability of methods that can be used to observe and describe characteristics, features and processes in freshwater, estuarine and marine habitats. Many key discoveries in limnology and oceanography can be traced to inventions of new tools, instruments or technologies that are often borrowed from unrelated disciplines. Some of these are used so often in our day-to-day research activities that we rarely think about their origins, or what life must have been like before they existed. This article will recount the important steps, from theory to practice, in the development of an indispensable tool of our trade, the Nuclepore® membrane filter. In a series of high-profile scientific publications during the period 1962-1965, including four front covers of *Science* magazine (Figure 1), a team of physicists and engineers from the General Electric Research Laboratory (GERL) in Schenectady, New York invented and perfected a novel Plastics-Irradiated-Etched ("PIE") membrane, the predecessor to the commercially available Nuclepore® filter. The introduction of this "precision" plastic sieve facilitated the development of many new methodologies, especially in the fields of aquatic chemistry and microbiology. I thank two of the inventors, Professors Robert L. Fleischer (Union College) and P. Buford Price (University of California, Berkeley), for sharing correspondence and unpublished reports that helped to capture the facts, excitement and importance of this remarkable achievement.

NUCLEAR TRACKS IN SOLIDS

Nuclear tracks in crystalline solids were first observed by Young (1958) and later investigated by Silk and Barnes (1959), all from the Atomic Energy Research Establishment at Harwell, U.K. The passage of heavily ionizing nuclear particles through most insulating solids creates a narrow path of intense radiation damage at the atomic scale. These damaged tracks can be enlarged by treatment with an appropriate chemical reagent that preferentially attacks the damaged materials. Young (1958) etched tracks in lithium fluoride crystals and mica and viewed them optically; then Silk and Barnes (1959) viewed fission tracks with transmission electron microscopy.

During the summer of 1961, Dr. Robert M. Walker, a physicist at GERL, calculated that it might be possible to observe cosmic

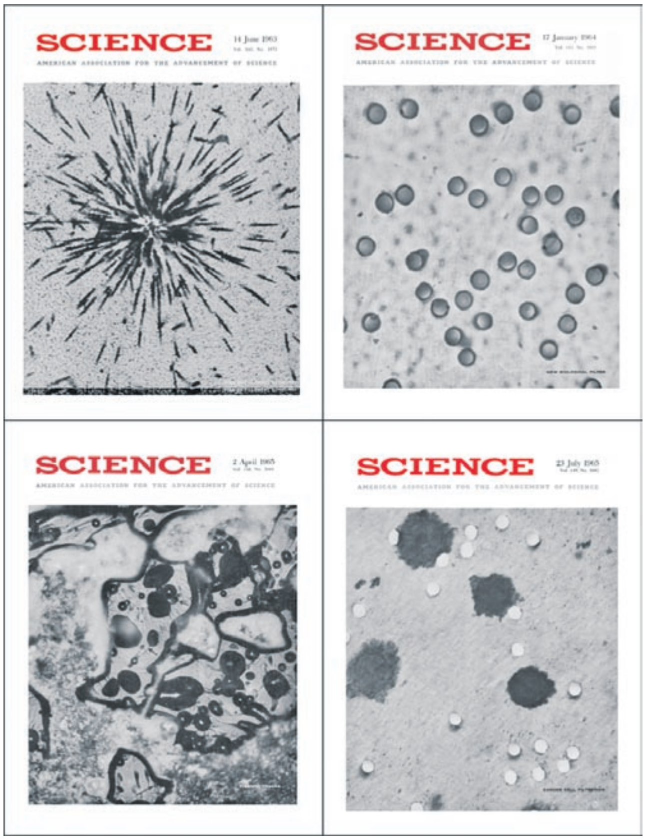

Figure 1. In a period of just two years (June 1963 – July 1965), the pioneering research on fission tracks in solids by the GERL group resulted in numerous "high profile" papers and four front covers of *Science*. From full cover of *Science,* 140(3572), 1963; 143 (3603), 1964; 148 (3666), 1965; and 149 (3682), 1965. Reprinted with permission from AAAS.

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Bulletin

The American Society of Limnology and Oceanography is a membership-driven scientific society (501(c)(3)) that promotes the interests of limnology (the study of inland waters), oceanography and related aquatic science disciplines by fostering the exchange of information and furthering investigations through research and education. ASLO also strives to link knowledge in the aquatic sciences to the identification and solution of problems generated by human interactions with the environment.

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ray damage tracks in extraterrestrial minerals by viewing lunar samples with an electron microscope. Walker visited Silk and Barnes at Harwell and, upon his return to GERL, persuaded Dr. P. Buford Price to join him in 1962 in searching for tracks of ancient energetic charged particles in meteorites and other extraterrestrial objects (Fleischer et al. 1975). They began a series of laboratory experiments using fission fragments as the energy source and mica as the target (Price and Walker 1962 a,b). They also discovered that the nuclear tracks could be chemically etched, leaving behind fine holes the dimensions of which could be controlled by etching conditions (Price and Walker 1962c). Curiously, Young's (1958) work at Harwell was overlooked until some years later. However, a major difference between the research at Harwell and the new work at GERL was a realization of the broad applications of fission track technology, and shortly thereafter the GERL team filed a disclosure letter that led to a patent on this process. When they realized that prolonged etching led to channels that could be quantified with a simple optical microscope they filed another patent for a radiation dosimeter based on the track etch principle.

In late 1962, Dr. Robert L. Fleischer joined the Walker-Price team and in early 1963, Fleischer discovered that the same fission track-etching procedure could be used to "drill" precise holes into a variety of other materials (up to this point, only mica had been used by the GERL group), including plastics such as General Electric (G.E.)'s bisphenolacetone carbonate, also known as Lexan® polycarbonate (Fleischer and Price 1963). Initially, ^{235}U was used to produce fission fragments, but the laboratory-based irradiation process was facilitated when a californium (^{252}Cf) source was obtained from the Lawrence Radiation Laboratory. ^{252}Cf undergoes spontaneous fission so a thin layer was deposited on a platinum disk and the Lexan® material was placed in an evacuated bell jar along with the radiation source (Figure 2). Much later the discovery of plastics etching was viewed, in retrospect, as the start of a "new ball game" (Fleischer 1998).

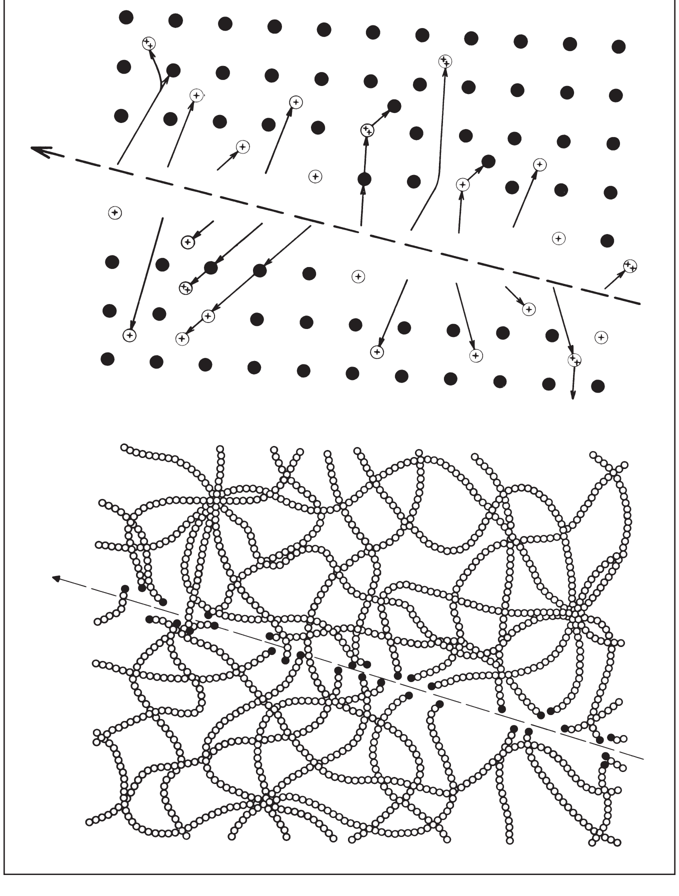
HOW TO MAKE A NUCLEPORE® FILTER

The method for producing a PIE membrane is essentially a two-step process. In step 1, which governs the number of holes per unit surface area, a sheet of Lexan® is irradiated with an

Figure 2. "PIE"-oneers P.B. Price (left), R. L. Fleischer (center) and R. M. Walker (right) at their laboratory at GERL circa 1963. Walker is holding a Lexan® strip that had been irradiated by ^{252}Cf fission fragments in the bell jar on the center of the table. From Fleischer (1998), courtesy of the author.

appropriate source of high energy particles which move through the target matrix altering the structure and leaving permanent tracks of damage. The atomic mechanism is referred to as "ion explosion" (Fleischer et al. 1969; Figure 3). The charged particle excites and ionizes molecules and, in the case of Lexan® irradiation, breaks the polymeric structure leaving exposed ends that are highly susceptible to subsequent chemical etching. A pore density of 1011 tracks per cm2 can be achieved by this process (Fleischer et al. 1963). In step 2, which governs the diameter of the pores, the irradiated Lexan® is chemically treated with a solution of 6M NaOH at 75°C which preferentially etches the nuclear track damage and then the surrounding undamaged material. The NaOH concentration and treatment conditions (time and temperature) are controlled to yield the desired pore dimensions. Individual holes as small as 25 Å or as large as a few millimeters in diameter can be achieved with great precision (Fleischer et al. 1963; R. L. Fleischer, pers. comm.). If an elastic matrix such as silicone-polycarbonate copolymer is used, it is even possible to construct a membrane filter with an "adjustable" pore diameter (Fleischer et al. 1972a). The 1963 invention of the PIE filter led to another patent, and to the publication of the now classic paper, "Novel Filter for Biological Methods" by Fleischer, Price and Symes that appeared in the 17 January 1964 issue of *Science* magazine (Figure 1).

Figure 3. Conceptual molecular model for the establishment of fission tracks in mica (top) and Lexan® (bottom). The process, termed "ion explosion," involves ionization of atoms and ejection of electrons along the path of the charged particle (right to left), followed by charge repulsion into the mica lattice to form a fission track of atomic disorder. In the case of polycarbonate and other plastics, the individual polymeric chains are broken, leaving a fission track in its wake. From Fleischer et al. (1965, 1969), courtesy of the authors.



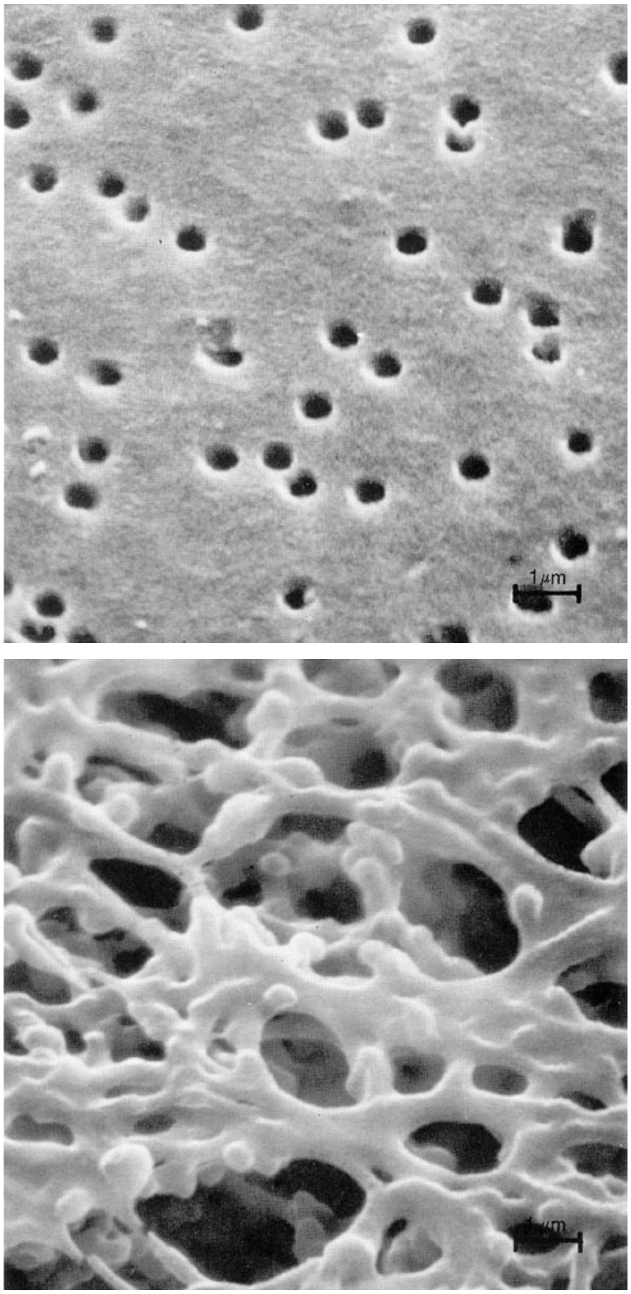
The "standard" Nuclepore® membrane is thin (6-10 μm) with a smooth flat surface that is punctuated with approximately 1 x 10^8 perpendicular cylindrical pores per cm^2 of membrane surface, and a resultant porosity of ~10% compared to ~80% for a cellulose-based filter (Porter and Schneider 1973). However, the Nuclepore® filter flow paths are shorter due to reduced tortuosity and to thinner membranes (Figure 4). The pores are randomly distributed and very uniform (±10% of stated diameter) in size (Heidam 1981; Figure 4). A unique characteristic of PIE membranes is their high tensile strength which permits filter folding and pleating to enhance filtration surface area per unit volume, for example, in filter cartridge applications. The polycarbonate material is also chemically pure, inert to most hydrocarbons, acids and alcohols, stable to autoclave conditions and non-hygroscopic. Because the filter surface is exceptionally smooth (Figure 4), PIE membranes are ideal for microscopic examination of particulate matter. Their high and uniform transparency permits the use of optical densitometry (Porter and Schneider 1973). DeBlois and Bean (1970) later devised a single pore plastic membrane that formed the basis for a novel particle counter based on changes in electrical conductivity as the particles pass through the hole, the same principle that is employed in Coulter® counters. The unipore "DeBlois/Bean counter" was used to enumerate particles as small as viruses (Fleischer et al. 1975), and this led to other unipore/oligopore devices such as the Microfiltrometer (Amoussou-Guenou et al. 2004). According to Fleischer et al. (1972b) Nuclepore® membranes have also been used to clarify and stabilize wine and beer by removing bacteria. This allows draft beer to be stored safely at room temperature without using pasteurization that would otherwise compromise its taste. I think most aquatic scientists would agree that this is a grand achievement above and beyond the use of Nuclepore® membranes in our research programs!

PIE IN THE SKY: THE ECONOMICS OF NUCLEAR TRACK MEMBRANES

While the basic engineering work was in progress, several applications of this new technology were initiated. In December 1962, Price provided Dr. Sam Seal of the Sloan-Kettering Institute several small mica filters for use in his ongoing experiments designed to separate live cancer cells from whole human blood, and to preserve them for study. These "glass" sieves were "made to order" with hole diameters designed to match the size differential between the cancer and non-cancer cells. The early attempts failed because the thin mica filters were too fragile to support the column of blood; however, after the PIE filters were available, the separation procedure was successful. Shortly thereafter, Seal published his results in the journal *Cancer* (Seal 1964), and predicted that up to 1 million filters per year might eventually be used in clinical research and cancer patient treatment. Dr. Charles A. Bruch, also of GERL, was tasked with the initial "mass production" of PIE filters (hundreds per week) for Seal's experiments, and to evaluate the market potential of this novel product.

On 14 January 1964, Bruch submitted a comprehensive "Technical and Economic Potential Analysis" on PIE filters to his supervisors at G.E. In addition to details governing the manufacture of these precision filters, Bruch also presented a variety of potential applications including the previously mentioned microfiltration of beer, water purification and even power generation using the process of "streaming potential." (Note: Very small holes in a membrane develop a surface charge. If an electrolyte solution is forced through the membrane under pressure, a charge differential and voltage is built up across the membrane.) In his economic analysis, Bruch calculated that 1 million filters could be produced at a cost of 5-10¢ per filter and could be sold at 18¢ per filter yielding an annual profit of $80-130 K per annum. A key requirement, of course, was the need for an irradiation facility. The use of ^{252}Cf, the fission fragment source in the pioneering experiments by Fleischer and Price (1963), was not commercially viable because of its rarity. According to Fleischer (1998), for the initial commercial production at G.E.'s nuclear test reactor at Vallecitos Atomic Laboratory in Pleasanton, California, plastic film was wound from one spool to another through the nuclear reactor which contained a plate with a thin layer of ^{235}U. The reactor neutrons induced fission in the ^{235}U, and the fission fragments bombarded the moving plastic film. The amount of ^{235}U, the neutron flux and the spooling speed combined to control pore density. A collimating honeycomb separating the fission plate from the film during reactor exposure, ensured that most of the fission fragments arrived close to normal to provide a complete track through the thin film.

Figure 4. Comparison of a slice of Lexan® PIE (top) and cellulose (bottom) membrane filter as viewed under high magnification using a scanning electron microscope. From Fleischer (1998), courtesy of the author.



The business potential appeared to be profitable but was considered small, at least by G. E. standards. Nevertheless, it was decided to move forward with plans for an "in house" commercial operation if interest was there; otherwise the process would be licensed to another company. In a 1967 National Academy of Sciences report on "Applied Science and Technological Progress," that was commissioned by Representative George Miller, then chairman of the Committee on Science and Aeronautics of the U. S. House of Representatives, the success story of the invention of PIE filters and its commercial applications was recounted by G. E. executives C. Guy Suits and Arthur M. Bueche. According to this report, the Nuclear Energy Division of G.E. under the leadership of Sidney C. Furman began to produce and market PIE filters under the trademark "Nuclepore®", short for nuclear-track pores, in early 1964. (NOTE: The Nuclepore filter is commonly misspelled Nucleopore (sic) in the scientific literature even by limnologists and oceanographers.) Based on information contained in the monograph Nuclear Tracks in Solids, "Nuclepore was first a G.E. product, then the business was separated into a new independent company – Nuclepore Corporation of Pleasanton, California, partly owned by G. E., and later sold" (Fleischer et al. 1975). In the December 1973 issue of *Fortune* magazine, Sharon Sabin provided an informative account of the origin and corporate organization of Nuclepore Corporation of Pleasanton, California (Sabin 1973). According to her report, G.E. invested "more than $7M into this venture during the first five years of operation (1965-1970)." By October 1971, G.E.'s Nuclepore sales were coming in at a meager $100 K per annum, and the operating losses were approximately $3 M (Sabin 1973). No one wanted the "Nuclepore division," but eventually a small group of G.E. employees decided to give it a try. This group attracted the interest of several investors, including Nomura Overseas Enterprise, a venture-capital firm allied with the Nomura Securities Co., Japan's largest brokerage house (Sabin 1973). Nomura Microscience Co., a new entity to market PIE filters in Japan, paid more than a half-million dollars for a 23% interest in the new company. An investment group of former G.E. employees and others had a 36% interest and G.E. retained

41% interest in the corporation based on prior investments. According to Sabin (1973), G.E. also threw in approximately $50 K worth of office equipment, "including file cabinets and pencils." Nuclepore Corporation was off and running. Today, Nuclepore Corporation is part of Corning, Separations Division, headquartered in Cambridge, MA.

ENVIRONMENTAL APPLICATIONS OF PIE MEMBRANES

The first environmental application of Nuclepore® filters was a size spectrum analysis of aerosols (Spurny et al. 1969 a,b). In 1972, Todd and Kerr compared the new G.E. Nuclepore® membrane to the more conventional Millipore® filter for the purpose of visualizing bacterial cells using scanning electron microscopy. The results were dramatic; "The Nuclepore membrane filter is preferred" (Todd and Kerr 1972). In a benchmark paper in aquatic sciences, Sheldon (1972) presented a comprehensive comparison of the characteristics of a variety of filters, including the new PIE membranes. In 1974, Zimmerman and Meyer-Reil reported higher bacterial counts using Nuclepore® filters compared to the standard mixed-ester cellulose membranes that were in common use; a lively debate ensued.

When compared independently, Daley and Hobbie (1975) concluded that the novel Nuclepore® polycarbonate filter technique was "inferior in several respects to the Francisco et al. (1973) technique as modified here," and presented comparative data from several freshwater and marine ecosystems to support their assertion. The preferred Francisco et al. (1973) method involved the use of ultraviolet epi-illumination, acridine orange staining and black Millipore® 0.45 µm cellulose-based filters. Ironically, two years later Hobbie and his colleagues published what is now considered to be a key paper in the history of marine microbiology, "Use of Nuclepore Filters for Counting Bacteria by Fluorescence Microscopy" (Hobbie et al. 1977). The sea change in their opinion of Nuclepore® filters was in large part due to several improvements that they introduced, namely pre-staining with irgalan black dye to eliminate filter autofluorescence, potential use of a surfactant, and a cellulose filter support beneath the Nuclepore® filter to ensure a uniform distribution of bacterial cells, and rapid processing of fresh wet mounts, for best results. Direct counts of bacteria were twice as high with Nuclepore® filters as with cellulose filters, in large part due to improved contrast between cells, the filter matrix and non-living particulate matter.

The Hobbie et al. (1977) method still is widely used; in 1990 the publication was designated a Citation Classic® by the Institute for Scientific Information (ISI) and for the period 1992-1995, this paper was deemed "the most influential paper in marine microbial ecology" based on citation rate (Duarte et al. 1997). In reviewing the history of this method, John Hobbie (past President of ASLO and 1983 recipient of the G. Evelyn Hutchinson Medal) claimed that "once perfected, the technique was absurdly easy. The bacteria glow with a green light against a dark background like stars in the sky" (Hobbie 1990).

After the paper by Hobbie et al. (1977) appeared, the Nuclepore® membrane became the filter of choice for all applications in marine microbiology because it facilitated, for the first time, accurate enumeration of bacterial cells and precise size fractionation of microbial assemblages (e.g., Azam and Hodson 1977). This quantitative estimation of sea microbes quickly led to the "picoplankton revolution" and to a comprehensive understanding of the quantitative role of small photoautotrophic microbial assemblages in the sea (Li et al. 1983; Platt et al. 1983). The only two possible disadvantages of PIE membranes are the fact that they contain carbon (polycarbonate) and therefore cannot be used directly for measurements of organic matter, and that they "load" more easily than other types of membrane filters due to their relatively low porosity. But compared to their numerous advantages, especially precision separation applications and microscopy, there are no substitutes.

EPILOGUE

When the G.E./Nuclepore Corporation patent expired, other companies began to market PIE filters under new trade names. Fleischer (1998) estimated that in 1993 the aggregate track-etched membrane production enterprise was at least a $50 M per annum industry, and since that time it has only grown. All this derived from a series of theoretical calculations, followed by careful laboratory experiments by a small team of basic research scientists (Price, Walker, Fleischer and Symes), and subsequent engineering for commercial mass production. It has been said that "like a vacuum, a hole is nothing, but holes of controlled geometry have many uses" (Fleischer 1998). Connections of one discovery to another are often unpredictable (Fleischer 1998), and there is perhaps no less obvious connection than among the detection of cosmic rays, nuclear track age dating of early hominid remains and the accurate estimation of bacterial abundance in lakes and oceans. In aquatic sciences, technology drives opportunity whether developed de novo for a particular application or borrowed from an unrelated discipline.

Just as we are all taught to respect our elders, we also need to honor those who ignited innovation and made enduring contributions to our discipline. If you use these filters in your research, as I do, please consider sending a brief note of thanks to Professors Fleischer and Price for their pioneering contributions to aquatic sciences (fleischr@union.edu and bprice@berkeley.edu). I know they would like to hear from you. In my opinion, the invention of the PIE filter ranks among the most significant technological advances of the past fifty years.

With many important discoveries, there are sometimes bruised egos, false claims of importance and a revisionist history of key events. In preparing this essay I have relied mostly on official GERL documents, information contained in two wonderful books – one dealing more with the science of nuclear tracks in solids (Fleischer et al. 1975) and the other a more personal account of people and events (Fleischer 1998) – and the archival scientific literature. This article was prepared, in part, to challenge ASLO members, both young and old, to think about other materials, equipment, instruments or methodologies that serve as the basic tools of our trade. Eventually, I would like to see these thoughts evolve into a regular column in the *L&O Bulletin*. Without a sense of the past, we have no future. I sincerely hope that my colleagues will rise up to this challenge. We may all learn something new, and it could even be a lot of fun.

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